

分类号: Q965; Q966; Q7
密 级: _____

单位代码: 10335
学 号: 11116082

浙江大学

博士学位论文



菜青虫 5-羟色胺受体的药理学性质及其调控血细胞吞噬的研究

**Pharmacological characterization of serotonin receptors from the
small white butterfly, *Pieris rapae* and their roles in modulating
hemocyte phagocytosis**

申请人姓名: 齐易香

指导老师: 叶恭银 教授

黄 佳 副教授

专业名称: 农业昆虫与害虫防治

研究方向: 昆虫神经生物学与毒理学

所在学院: 农业与生物技术学院

论文提交日期 二〇一六年六月



菜青虫 5-羟色胺受体的药理学性质及其调控血细胞吞噬的
研究



论文作者签名: _____

指导老师签名: 叶恭银

论文评阅人: 双向隐名评阅

答辩委员会主席: 刘树生 教授 浙江大学昆虫科学研究所

委员: 万方浩 教授 中国农业科学院植物保护研究所

冯明光 教授 浙江大学生命科学学院

俞晓平 教授 中国计量大学生命科学学院

张传溪 教授 浙江大学昆虫科学研究所

陈学新 教授 浙江大学昆虫科学研究所

叶恭银 教授 浙江大学昆虫科学研究所

答辩日期: 二〇一六年六月九日

**Pharmacological characterization of serotonin receptors from the
small white butterfly, *Pieris rapae* and their roles in modulating
hemocyte phagocytosis**



Author signature: Qi Yixiang

Supervisor signature: Gongyin Ye

Huang Jia

Thesis reviewer: Anonymous

Chair: Prof. Shusheng Liu, Zhejiang University

**Committeeman: Prof. Fanghao Wan, Chinese Academy of
Agricultural Sciences**

Prof. Mingguang Feng, Zhejiang University

Prof. Xiaoping Yu, China Jiliang University

Prof. Chuanxi Zhang, Zhejiang University

Prof. Xuexin Chen, Zhejiang University

Prof. Gongyin Ye, Zhejiang University

Date of oral defence: 9 June, 2016

浙江大学研究生学位论文独创性声明

本人声明所呈交的学位论文是本人在导师指导下进行的研究工作及取得的研究成果。除了文中特别加以标注和致谢的地方外，论文中不包含其他人已经发表或撰写过的研究成果，也不包含为获得 浙江大学 或其他教育机构的学位或证书而使用过的材料。与我一同工作的同志对本研究所做的任何贡献均已在论文中作了明确的说明并表示谢意。

学位论文作者签名: 签字日期: 年 月 日

学位论文版权使用授权书

本学位论文作者完全了解 浙江大学 有权保留并向国家有关部门或机构送交本论文的复印件和磁盘，允许论文被查阅和借阅。本人授权 浙江大学 可以将学位论文的全部或部分内容编入有关数据库进行检索和传播，可以采用影印、缩印或扫描等复制手段保存、汇编学位论文。

(保密的学位论文在解密后适用本授权书)

学位论文作者签名： 导师签名：

签字日期： 年 月 日 签字日期： 年 月 日

本研究承蒙

国家重点基础研究发展计划(973 计划,批准号: 2013CB127600)

国家自然科学基金 (批准号: 31572039)

国家高技术研究发展计划(863 计划,批准号: 2011AA10A204)

国家自然科学基金创新研究群体基金 (批准号: 31321063)

资助

目录

致谢	I
摘要	III
Abstract	IV
第一章 昆虫 5-羟色胺及其受体的研究进展	1
1 引言	1
2 5-HT 的合成和分解	1
3 5-HT 的分布	3
4 5-HT 的再吸收	3
5 5-HT 的生理功能	4
6 5-HT 受体	7
6.1 5-HT 受体的克隆	7
6.2 5-HT 受体的信号转导途径	12
6.3 5-HT 受体的药理学性质	14
6.4 5-HT 受体的功能	15
7 小结和展望	16
第二章 5-羟色胺在免疫系统中的作用	18
1 引言	18
2 5-HT 和脊椎动物免疫系统	18
2.1 5-HT 在免疫细胞的合成和存储	18
2.2 5-HT 和先天免疫	20
2.3 5-HT 和获得性免疫	20
3 5-HT 和无脊椎动物免疫系统	22
第三章 菜青虫 5-羟色胺受体的克隆与药理学性质分析	23
1 引言	23
2 材料与方法	24
2.1 供试昆虫与药剂	24
2.2 RNA 抽提	24
2.3 基因克隆	25
2.4 序列分析	27
2.5 质粒构建	27
2.6 细胞培养及转染	28
2.7 cAMP 检测	28
2.8 组织分布	29
3 结果与分析	29
3.1 Pr5-HT _{1A} 、Pr5-HT _{1B} 和 Pr5-HT ₇ 的 cDNA 的克隆及序列分析	29
3.2 Pr5-HT _{1A} 、Pr5-HT _{1B} 和 Pr5-HT ₇ 的药理学性质	33
3.3 Pr5-HT _{1A} 、Pr5-HT _{1B} 和 Pr5-HT ₇ 的表达模式	36
4 讨论	37
第四章 一种新型 5-羟色胺受体 5-HT ₈ 的功能研究	39
1 引言	39
2 材料与方法	40

2.1 供试昆虫和试剂.....	40
2.2 基因克隆.....	41
2.3 序列分析.....	42
2.4 真核表达载体构建.....	42
2.5 细胞培养及转染.....	42
2.6 cAMP 检测.....	42
2.7 Ca^{2+} 检测.....	42
2.8 数据分析.....	42
3 结果与分析.....	43
3.1 Pr5-HT ₈ cDNA 的克隆及序列分析.....	43
3.2 Pr5-HT ₈ 受体的配体特异性检测.....	48
3.3 激动剂和拮抗剂对受体的偶联的 Ca^{2+} 信号的影响.....	50
4 讨论.....	53
第五章 5-羟色胺参与调控昆虫血细胞吞噬的研究.....	56
1 引言.....	56
2 材料与方法.....	57
2.1 供试昆虫与药剂.....	57
2.2 果蝇品系的杂交.....	57
2.3 血细胞的分离、培养和免疫诱导.....	58
2.4 5-HT 的检测.....	58
2.5 菜青虫 TRH 和 TPH 基因的克隆.....	59
2.6 荧光定量 PCR 和 RT-PCR.....	59
2.7 单头黑腹果蝇基因组 DNA 提取和 PCR.....	61
2.8 吞噬实验.....	62
2.9 菜青虫血细胞 RNAi.....	63
2.10 菜青虫 Pr5-HT _{1B} 和 Pr5-HT _{2B} 抗体制备.....	63
2.11 Western blot 和免疫荧光.....	64
2.12 Ca^{2+} 检测.....	65
2.13 果蝇生存率实验.....	65
2.14 细菌在果蝇体内扩增效率实验.....	66
2.15 数据统计.....	66
3 结果与分析.....	66
3.1 激活的血细胞自身合成 5-HT 的含量.....	66
3.2 5-HT 调控血细胞吞噬.....	67
3.3 5-HT 通过 Pr5-HT _{1B} 和 Pr5-HT _{2B} 调控血细胞吞噬.....	71
3.4 免疫诱导后血细胞中 5-HT _{1B} 和 5-HT _{2B} 的表达量变化.....	76
3.5 5-HT _{1B} 和 5-HT _{2B} 调控黑腹果蝇的吞噬和抵抗病原菌.....	77
4 讨论.....	84
总讨论.....	86
1 小结.....	86
2 本研究的创新之处.....	88
3 本研究的不足之处.....	88
4 今后的研究方向.....	88
参考文献.....	90

致谢

时间过得真快，转眼五年的博士学习生涯就要结束了。依然清楚的记得五年前我被叶老师精彩的学术报告所打动，决心在叶老师的指导下走上科研之路。在浙江大学的这五年里，我笑过，哭过，更多的是开心幸福的回忆，虽然也有难过的经历。这些都是我在这里最大的收获，我不仅培养了对科研的兴趣，学会了怎样做好科学研究，也学到了很多为人处事的道理。真心的感谢所有关心我，帮助过我的家人、朋友、老师和同学。

首先，我要特别感谢恩师叶恭银教授和黄佳副教授。叶老师在我博士论文课题设计，实验开展以及论文写作等方面给予了悉心的指导。他以严谨求实的态度、宽厚仁慈的胸怀和严于律己的作风为我树立了一辈子学习的典范。叶老师经常早上第一个到实验室工作。晚上我们回寝室了，他还在改论文、看文献。就算是节假日，叶老师也在孜孜不倦的工作。叶老师勤恳务实的态度为我们树立了一个榜样。叶老师对待科研一丝不苟，他总会提醒我要认真对待实验中的每个细节。叶老师不仅是我们的学习科研上的良师，也是我们生活中的益友。叶老师非常关心学生，他帮助我们树立正确的人生观和价值观。当我们生活上遇到问题时，他会尽全力帮助我们。叶老师虽然表面严肃，但是内心却很温暖。黄老师是我的另一个导师，这五年博士生涯中，黄老师在我实验方案，实验技能以及论文写作上给予了很大的帮助。我们会经常交流实验中出现的问题，探讨新的科研思路以及论文写作技巧等。我很佩服黄老师的智慧和创新意识，他积极乐观的生活态度也影响着我。我觉得非常幸运有两位这么好的博士生导师，培养了我应有的科研能力和综合素质。同时，我也特别感谢姚洪渭老师和方琦老师对我的教诲、关心和栽培。我要由衷的说声：谢谢你们！感谢你们无私的付出！

本论文的顺利完成得到很多老师的帮助。非常感谢浙江大学医学院俞云松教授惠赠致病菌金黄色葡萄球菌。美国 Leslie Vosshall 教授提供 5-HT_{1B} 果蝇突变体。加拿大 Shelley Adamo 教授以及美国农业部 David Stanley 教授在英文论文写作上提供的建议。感谢浙江大学分析测试中心李云琴老师帮忙拍摄电镜照片。谢谢昆虫所全体老师在我实验实验过程中提供的便利及帮助。同时也要非常感谢参与本论文评阅和答辩委员会的专家和教授们。

博士课题的顺利完成也得到了本实验室成员的支持与帮助。首先要特别感谢王飞师兄在实验技术上提供的指导和实验方案提供的建议。也要非常感谢神经生物学组的师妹夏仁英、武亚苏、李萌琦、毛芬、顾桂香等在我实验过程中提供的帮助。谢谢我同级的严智超、徐刚、刘玉娥等人的陪伴。感谢已经毕业的师兄师姐慎小晶、陈洋、王磊、常雪、吴顺凡、卢增斌、钱岑、王伟、郭燕、孙方达、姚尧、程璐、蒋鹏凌、朱宇等和师弟师妹巨青松、韩乃顺、刘洋等的关心和照顾，以及还在努力奋斗中的汪芳、王前进、腾子文、贾文茜、杨蕾、肖山、阿力、杜玉萍、陈利鹏、宁铎、党聪、王蓓蓓、陈晓昂、李晶晶、王嘉乐、王磊、王龙、常学飞、黄天宇等人在我的生活和实验中给予的关怀和帮助。同时，我也要特别感谢我的好朋友陈红丹，陪我从华中农业大学进入浙江大学，一起度过七年的校园时光，在生活和科研上给了我很多的关心和帮助。非常感谢好友冯莲、李娜、钟春燕在生活上对我的关心。

寒窗苦读二十余年，非常感谢家人的支持和关心。感谢父母无私的奉献，辛勤劳动，省吃俭用供我上学以及对我巨大的关爱和支持。感谢哥哥姐姐对我的关心、鼓励和照顾。感谢老公对我的爱护、理解和包容，不仅给了我美好，最幸福的校园恋爱，也给了我一个温暖的家，让博士这五年时间成为我人生中最难忘的时光。

谢谢所有关心我的人，因为你们的帮助和支持，我才能顺利完成博士论文。你们的关爱也是我以后努力奋斗的动力。

齐易香

2016年5月28日于浙大紫金港校区

摘要

5-羟色胺 (5-hydroxytryptamine, 5-HT) 是昆虫体内一种重要的生物胺。5-HT在昆虫神经组织和非神经组织中均可合成, 通过结合特异性的G蛋白偶联受体在昆虫体内发挥不同的神经调控作用, 调节昆虫主要的行为活动, 比如取食、生物钟、聚集、学习和记忆等。昆虫体内5-HT受体有5种, 分别为5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} 和 5-HT₇。其中5-HT_{1A}和5-HT_{1B}偶联G_i蛋白引起胞内cAMP的降低, 5-HT_{2A}和5-HT_{2B}偶联G_q蛋白引起胞内Ca²⁺的释放, 5-HT₇偶联G_s蛋白引起胞内cAMP的升高。近年来, 昆虫5-HT及其受体的研究有了很大的进展, 越来越多的5-HT受体被克隆, 并进行了功能和药理学性质分析。由于不同昆虫5-HT受体药理学性质存在差异, 这将为以5-HT受体为靶标, 设计新型特异性杀虫剂提供理论基础。鉴于此, 本文以十字花科上的重要害虫菜青虫*Pieris rapae* (鳞翅目Lepidoptera, 粉蝶科Pieridae) 为研究对象, 综合应用基因克隆与表达、生物信息分析、RNA干扰和药理学技术等手段, 对其体内的5-HT受体的药理学性质和5-HT信号通路参与调控昆虫血细胞吞噬做了较系统研究。

1. 菜青虫5-羟色胺受体的克隆与药理学性质分析

从菜青虫体内克隆了3个5-HT受体基因的全长cDNA分别为Pr5-HT_{1A}、Pr5-HT_{1B}和Pr5-HT₇。将这些受体分别表达于HEK 293细胞后, 用5-HT刺激时, Pr5-HT_{1A}和Pr5-HT_{1B}能够抑制forskolin引起的胞内cAMP浓度的升高, EC₅₀值分别为63.90 nM和2.59 nM; 而Pr5-HT₇能够引起胞内cAMP的增加, EC₅₀值为10.71 nM。药理学实验表明, 5-carboxamidotryptamine、5-methoxytryptamine 和 (±)-8-Hydroxy-2-(dipropylamino) 均对Pr5-HT_{1B}和Pr5-HT₇有激动剂作用。拮抗剂Methiothepin可拮抗5-HT对Pr5-HT_{1B}受体的作用, IC₅₀值为2.49 μM。Methiothepin也可拮抗5-HT对Pr5-HT₇受体的作用, IC₅₀值为899.16 nM。SB 216641是人5-HT_{1B}受体的特异性拮抗剂, 该药物能部分拮抗5-HT对Pr5-HT_{1B}受体的反应; 而10 μM SB-269970能完全阻断500 nM 5-HT激活的Pr5-HT₇受体反应。

2. 一种新型5-羟色胺受体Pr5-HT₈的功能研究

昆虫中已经鉴定出来5类5-HT受体,分别跟哺乳动物5-HT_{1A}、5-HT_{1B}、5-HT_{2A}、5-HT_{2B}和5-HT₇同源。我们从菜青虫神经组织中克隆了一种未知的G蛋白偶联受体cDNA序列,该基因跟5-HT受体序列相似,但是不属于任何已知的5-HT受体家族。将该受体基因在HEK 293细胞表达后,低浓度的5-HT (< 10 nM)能够激活该受体,并引起胞内Ca²⁺浓度升高。而其它的生物胺,包括多巴胺、章鱼胺和酪胺都不能激活该受体。所以我们命名该基因为Pr5-HT₈,以区别目前已知的7类5-HT受体。Pr5-HT₈受体能被多种5-HT受体激动剂激活,包括5-carboxamidotryptamine,5-methoxytryptamine和(±)-8-Hydroxy-2-(dipropylamino)-Methiothepin是一种5-HT受体的非选择性拮抗剂,我们发现它却能够激活Pr5-HT₈。SB-269970、SB-216641和RS-127445均不能够拮抗5-HT对Pr5-HT₈受体的作用,而WAY-100635对Pr5-HT₈受体有拮抗效应。综上,我们推测Pr5-HT₈受体代表了一类新的5-HT受体,并且跟已知的5-HT受体的药理学性质都不一样。我们在一些害虫和媒介昆虫的基因组中找到了Pr5-HT₈受体的同源基因,如赤拟谷盗和蚊子,但是在蜜蜂,寄生蜂和果蝇的基因组中没有找到该基因的同源序列。我们在哺乳动物的基因组中没有找到5-HT₈受体基因的同源基因,表明它可能是一类无脊椎动物特异性的受体。

3. 5-羟色胺经两种不同的5-HT受体调控昆虫血细胞吞噬

5-羟色胺能够调控脊椎动物的神经系统以及免疫反应,但是在昆虫免疫中的作用机制还尚不明确。我们以菜青虫血细胞为研究对象,发现菜青虫血细胞被脂多糖(LPS)激活后能够在色氨酸羟化酶(TPH)的作用下合成5-HT。通过药物阻断TPH的功能,或者通过RNAi干扰TPH基因的表达都能显著降低血细胞的吞噬能力。RT-PCR、细胞免疫组化以及钙离子成像实验表明血细胞主要表达5-HT_{1B}和5-HT_{2B}受体。用拮抗剂阻断5-HT_{1B}的功能会显著降低血细胞的吞噬能力。而用拮抗剂阻断5-HT_{2B}的功能可以提高血细胞的吞噬率。通过RNAi分别干扰这两个受体的表达后再做吞噬实验,得到类似的结果。我们通过模式生物黑腹果蝇的5-HT_{1B}突变体进一步验证了在5-HT_{1B}缺失的情况下,由于吞噬能力降低,果蝇被病原菌感染后更容易死亡。通过果蝇遗传学操作,发现血细胞上5-HT_{1B}或者5-HT_{2B}受体被特异性干扰的果蝇被感染后,也比对照果蝇更脆弱,更快死亡。综

合以上结果，我们可以知道5-HT通过5-HT_{1B}或者5-HT_{2B}受体调控血细胞吞噬。

关键词：菜青虫，G蛋白偶联受体，5-羟色胺受体，激动剂，拮抗剂，果蝇，吞噬，血细胞，免疫

Abstract

5-hydroxytryptamine (5-HT) is an important biogenic amine in insects. 5-HT is synthesized in both neuronal and peripheral tissues and it can be reuptaken by serotonin transporter into presynaptic neuron. 5-HT plays various important physiological roles in insects through specific G protein-coupled receptors, such as feeding, circadian behavior, aggregation, learning and memory. There are five types of 5-HT receptors in insects, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} and 5-HT₇. 5-HT_{1A} and 5-HT_{1B} inhibit intracellular cAMP production. 5-HT_{2A} and 5-HT_{2B} increase Ca²⁺ level, and 5-HT₇ induce cAMP production. In recent years, great progress have been made in the research of 5-HT in insects, especially their receptors. More and more 5-HT receptor genes have been cloned, and their function and pharmacological properties have been analyzed. The pharmacological differences of 5-HT receptors from different insects will provide fundamental basis for designing and developing new specific insecticides for pest management. In this study, we investigate the pharmacological properties of serotonin receptors in the small white butterfly, *Pieris rapae*, and the role of serotonergic signaling pathway in regulating insect hemocyte phagocytosis.

1. Molecular cloning and pharmacological characterization of serotonin receptors from the small white butterfly, *Pieris rapae*

The full length cDNA of three 5-HT receptors (Pr5-HT_{1A}, Pr5-HT_{1B} and Pr5-HT₇) were obtained from the small white butterfly, *Pieris rapae*. Receptors were stably expressed in HEK 293 cells and their ligand responses have been examined. Pr5-HT_{1A} and Pr5-HT_{1B} receptor activation with 5-HT can inhibit intracellular forskolin stimulated cAMP level, with an EC₅₀ value of 63.90 nM and 2.59 nM respectively. Pr5-HT₇ induces intracellular cAMP level, with an EC₅₀ value of 10.71 nM. Experiments with agonist and antagonist revealed that 5-carboxamidotryptamine, 5-methoxytryptamin and (±)-8-Hydroxy-2-(dipropylamino) tetralin can stimulate

Pr5-HT_{1B} and Pr5-HT₇. Methiothepin is a full antagonist for both Pr5-HT_{1B} (IC₅₀=2.49 μ M) and Pr5-HT₇ (IC₅₀=899.16 nM). SB 216641 is a human 5-HT_{1B} selective antagonist. It can partially inhibit Pr5-HT_{1B}. 10 μ M SB-269970 can fully inhibit 500 nM 5-HT stimulated cAMP level in HEK 293 cells stably expressing Pr5-HT₇.

2. Larvae of the small white butterfly, *Pieris rapae*, express a novel serotonin receptor.

The biogenic amine serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter in vertebrates and invertebrates. It acts in regulation and modulation of many physiological and behavioral processes through G protein-coupled receptors. Insects express five 5-HT receptor subtypes that share high similarity with mammalian 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} and 5-HT₇ receptors. We isolated a cDNA (*Pr5-HT₈*) from larval *Pieris rapae*, which shares relatively low similarity to the known 5-HT receptor classes. After heterologous expression in HEK-293 cells, Pr5-HT₈ mediated increased [Ca²⁺]_i in response to low concentrations (<10 nM) of 5-HT. The receptor did not affect [cAMP]_i even at high concentrations (>10 μ M) of 5-HT. Dopamine, octopamine and tyramine did not influence receptor signaling. Pr5-HT₈ was also activated by various 5-HT receptor agonists including 5-methoxytryptamin, ($\pm$)-8-Hydroxy-2-(dipropylamino) tetralin and 5-carboxamidotryptamine. Methiothepin, a nonselective 5-HT receptor antagonist, activated Pr5-HT₈. WAY 10635, a 5-HT_{1A} antagonist, but not SB-269970, SB-216641 or RS-127445, inhibited 5-HT-induced [Ca²⁺]_i increases. We infer that Pr5-HT₈ represents the first recognized member of a novel 5-HT receptor class with a unique pharmacological profile. We found orthologs of Pr5-HT₈ in some insect pests and vectors such as beetles and mosquitoes, but not in the genomes of honeybee or parasitoid wasps. This is likely to be an invertebrate-specific receptor because there were no similar receptors in mammals.

3. Serotonin modulates insect hemocyte phagocytosis via two different serotonin receptors

Serotonin (5-HT) modulates both neural and immune responses in vertebrates, but its role in insect immunity remains uncertain. We report that hemocytes in the caterpillar, *Pieris rapae* are able to synthesize 5-HT via TPH following activation by lipopolysaccharide. The inhibition of TPH with either pharmacological blockade or RNAi knock-down impaired hemocyte phagocytosis. Biochemical and functional experiments showed that naive hemocytes primarily express 5-HT_{1B} and 5-HT_{2B} receptors. The blockade of 5-HT_{1B} significantly reduced phagocytic ability, however the blockade of 5-HT_{2B} increased hemocyte phagocytosis. Silencing of 5-HT_{1B} and 5-HT_{2B} by RNAi generated similar results. We further found that the 5-HT_{1B}-null *Drosophila melanogaster* mutant showed higher mortality than controls when infected with bacteria, and this was assumed to be due to decreased phagocytotic ability. Flies expressing 5-HT_{1B} or 5-HT_{2B} RNAi in hemocytes also showed similar sensitivity to infection. Combined, these data demonstrate that 5-HT mediates hemocyte phagocytosis through 5-HT_{1B} and 5-HT_{2B} receptors and serotonergic signaling performs critical modulatory functions in immune systems of animals separated by 500 million years of evolution.

Key words: *Pieris rapae*, G protein-coupled receptor, 5-hydroxytryptamine receptor, agonist, antagonist, *Drosophila melanogaster*, phagocytosis, hemocyte, immune.

第一章 昆虫5-羟色胺及其受体的研究进展

1 引言

5-羟色胺 (5-hydroxytryptamine, 5-HT) 是一种生物胺, 在大多数动物中作为一种信号物质, 控制和调节很多重要的生理和行为过程, 如秀丽隐杆线虫 *Caenorhabditis elegans* 的运动和繁殖, 软体动物加州海兔 *Aplysia californica* 的取食, 美洲螯龙虾 *Homarus americanus* 的进攻行为, 哺乳动物的睡眠、食欲和心情等 (Weiger, 1997)。5-HT 最早于 1946 年被 Erspamer 发现, Erspamer 从肠道提取一种可引起平滑肌收缩的物质时, 发现一种源于肠道 Kultschitzky 细胞的血清血管收缩剂和体液性因子, 因此将其命名为小肠胺 (enteramine) (Erspamer, 1946)。此后, Rapport 等 (1948) 为寻求导致高血压的体液性因子时, 从血清中分离到一种缩血管物质, 并命名为血清素 (serotonin)。该物质后被 Erspamer 和 Asero (1952) 通过化学鉴定确定为 5-HT。近年来, 由于 5-HT 在昆虫生理功能中的重要性, 越来越多的人研究昆虫体内 5-HT, 特别是对模式昆虫黑腹果蝇 *Drosophila melanogaster* 和西方蜜蜂 *Apis mellifera* 的研究相对深入。为此, 在前期报道 (齐易香等, 2013) 的基础上, 结合新进展再做概述。

2 5-HT 的合成和分解

氨基酸色氨酸 (tryptophan, Try) 是 5-HT 的生物合成前体, 在色氨酸羟化酶 (tryptophan hydroxylase) 的作用下生成 5-羟色氨酸 (5-hydroxy tryptophan, 5-HTP), 然后在 5-羟色氨酸脱羧酶 (aromatic L-amino acid decarboxylase, AADC) 的作用下, 脱羧基生成 5-HT (Fitzpatrick, 1999)。色氨酸羟化酶是 5-HT 合成过程中的限速酶, 黑腹果蝇中色氨酸羟化酶由两个基因编码, 即 *dTPH* 和 *dTRH* (Coleman and Neckameyer, 2005)。两个基因的产物色氨酸羟化酶在体外都有活性, 但 *dTPH* 在非神经组织中表达, *dTRH* 基因在神经组织中表达 (Neckameyer *et al.*, 2007)。AADC 在中枢神经系统中的 5-HT 和多巴胺神经元中都有表达, 不仅

参与5-HT的合成, 也参与多巴胺的合成 (Bowsher and Henry, 1986)。

在已研究的一些后生动物, 包括软体动物、昆虫和哺乳动物体内, 5-HT的分解途径各有不同。昆虫体内, 5-HT主要在N-乙酰基转移酶 (N-acetyltransferase, NAT) 的作用下生成N-乙酰羟色胺 (N-acetyl serotonin, NAS)。有研究表明, 昆虫体内也存在哺乳动物5-HT的主要代谢产物5-羟吲哚乙酸 (5-hydroxyindole acetic acid, 5-HIAA) (Rubio *et al.*, 1983; Squires *et al.*, 2010)。哺乳动物体内, 5-HT先在单胺氧化酶a和b (monoamine oxidase forms a and b, MAOa and MAOb) 的作用下生成5-羟乙醛 (5-hydroxyindole acetaldehyde, 5-HIAL), 再在乙醛还原酶2 (aldehyde reductase 2, ALDH2) 的作用下形成5-HIAA (Squires *et al.*, 2010)。但是目前还没有在昆虫中克隆到MAO, 昆虫体内是否存在MAO这条代谢途径还存在争议。有研究表明昆虫神经组织内极少或没有MAO的活性 (Sloley, 2004)。5-HT在昆虫体内的具体代谢过程见图1.1。哺乳动物体内5-HT另一条代谢途径主要产生NAS、褪黑激素 (melatonin)、5-羟基噻唑烷羧酸 (5-hydroxyindole thiazolidine carboxylic acid), 某些情况甚至产生5-羟色胺硫酸 (5-HT sulfate) (Squires *et al.*, 2006, 2007)。而在软体动物中, 5-羟色胺硫酸和 γ -谷氨酰基-羟色胺 (γ -glutamyl-serotonin) 是5-HT分解的主要产物 (Stuart *et al.*, 2003; Stuart *et al.*, 2004; Hatcher *et al.*, 2008)。

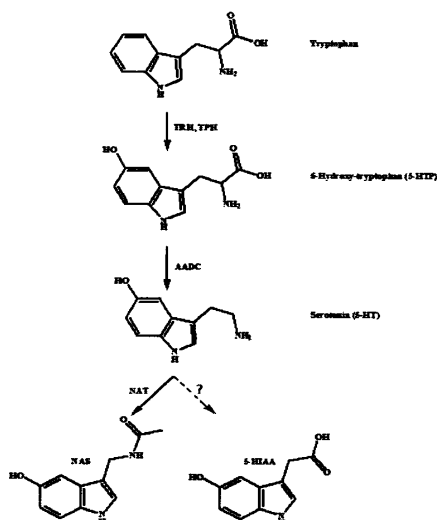


图1.1 昆虫5-HT合成和分解代谢简图(修改自Squires *et al.*, 2010; Watanabe *et al.*, 2011)
Fig 1.1 Major biosynthetic and catabolic pathways of 5-HT in insects (adapted from Squires *et al.*, 2010; Watanabe *et al.*, 2011)

Tryptophan: 色氨酸; TRH: 色氨酸羟化酶 Tryptophan hydroxylase; TPH: 色氨酸-苯丙氨酸羟化酶

Tryptophan- phenylalanine hydroxylase; 5-HTP: 5-羟色氨酸5-Hydroxy tryptophan; AADC: 5-羟色氨酸脱羧酶
Aromatic L-amino acid decarboxylase; 5-HT: 5-羟色胺5-Hydroxytryptamine; NAT: N-乙酰基转移酶
N-acetyltransferase; NAS: N-乙酰羟色胺N-acetylserotonin; 5-HIAA: 5-羟吲哚乙酸5-Hydroxyindole acetic acid.

3 5-HT 的分布

研究5-HT在昆虫体内的分布主要是通过免疫组织化学方法。5-HT在昆虫中枢神经系统（central nervous system, CNS）和外周神经系统中都有分布。腹神经索中，很多的5-HT神经元是中间神经元。在感觉神经元（Lutz and Tyrer, 1988）、咽下神经节（Helle *et al.*, 1995）、中间神经元（Settembrini and Villar, 2004）、神经分泌细胞（Nässel and Elekes, 1985）等部位都有5-HT的分布。对厩螫蝇 *Stomoxys calcitrans* 5-HT的分布研究发现，很多对5-HT有免疫活性的物质在成虫的CNS和胸腹部神经节中都有发现（Liu *et al.*, 2011）。Haselton等(2006)确定了5-HT存在雌马虻 *Tabanus nigrovittatus* 的CNS和中肠中。Siju等（2008）发现5-HT存在于埃及伊蚊 *Aedes aegypti* 的中央和外周化学感受系统中，在主要的嗅觉神经纤维和触角中都有5-HT的免疫活性物质存在。

5-HT在昆虫体内的分布有一定的特点，CNS分布很多，从幼虫期到蛹期，头部5-HT含量升高。成虫期5-HT含量也会随着日龄的增加而改变（Taylor *et al.*, 1992）。对黑腹果蝇各发育阶段和成熟后的神经系统中的5-HT神经元进行跟踪实验，发现幼虫期约84个神经元，成虫期多于100个（Vallés and White, 1988）。用5-HT单抗在黑腹果蝇成虫单个脑中鉴定出了38到41个5-HT神经元，其中一些已经被Vallés和White（1988）描述，主要是咽下神经元和腹部神经元。在黑腹果蝇幼虫和成虫的脑中，5-HT免疫反应的神经元与昼夜节律神经元的树突和背侧末端都相距很近（Hamasaka and Nässel, 2006）。

4 5-HT 的再吸收

5-HT在昆虫体内引起突触后细胞发生反应并产生相关信号后，需要通过其他途径终止它的反应。这个途径主要就是5-HT转运体将5-HT重吸收进入突触前结构中（Rudnick, 2006）。

5-HT转运体（serotonin transporter, SERT）、多巴胺转运体和去甲肾上腺素转运体都属于依赖 Na^+ 和 Cl^- 的神经递质转运体大家族（Rudnick and Clark, 1993）。

这些转运体有12个跨膜结构域 (transmembrane domain, TMD), 并且氨基端和羧基端都位于细胞质内。通过对人 (Ramamoorthy *et al.*, 1993)、老鼠 (Blakely *et al.*, 1991)、昆虫等生物中克隆到的SERT序列分析, 预测在TMD III和TMD IV之间有一个大的胞外环。

从黑腹果蝇中克隆的SERT是昆虫体内第一个确定的生物胺转运体 (Corey *et al.*, 1994), 后来Donly和Caveney (2005) 在克隆节肢动物单胺转运体的过程中, 克隆出了番茄天蛾 *Manduca quinquemaculata*、欧洲玉米螟 *Ostrinia nubilalis* 和马铃薯甲虫 *Leptinotarsa decemlineata* 的SERT。克隆出来的所有节肢动物单胺转运体中, SERT保守性最高, 氨基酸序列同源性达66%~96% (Donly and Caveney, 2005)。对黑腹果蝇SERT的功能分析显示 (Corey *et al.*, 1994; Demchyshyn *et al.*, 1994), 它的药理学性质跟哺乳动物SERT不同, 对拮抗剂三环类抗抑郁药的敏感性比哺乳动物的低很多。对烟草天蛾 *Manduca sexta* 中枢神经中克隆到的SERT研究表明TMD1和TMD2是影响其对拮抗剂特别是可卡因敏感性的结构域, 该转运体对可卡因相对不敏感 (Sandhu *et al.*, 2002)。通过克隆家蚕 *Bombyx mori* 的SERT, 并针对其与可卡因的亲合性分析表明SERT内保守的丝氨酸残基导致了该转运体与可卡因的高亲和性 (Gu *et al.*, 2006)。总的来说, 目前对昆虫SERT的研究不多, 主要集中在鳞翅目、鞘翅目和双翅目的少数昆虫。

昆虫和其他动物体内SERT选择性地 将5-HT与 Na^+ 和 Cl^- 一同运入神经细胞, 同时将 K^+ 运出细胞。5-HT的重吸收还与5-HT的合成有关。释放的5-HT来源一方面是通过合成, 另一方面则是通过转运体对5-HT的重吸收。SERT在调节5-HT信号方面发挥了重要的作用, 它将5-HT从细胞外清除, 并将5-HT回收至细胞内, 从而被囊泡吸收 (Murphy *et al.*, 2004)。通过研究黑腹果蝇幼虫中枢神经系统腹神经索中的5-HT信号发现, 当重吸收被抑制后, 合成是补充5-HT的必须途径, 表明控制5-HT的合成能够调节5-HT重吸收抑制剂的影响 (Borue *et al.*, 2010)。

5 5-HT 的生理功能

人体 5-HT 系统的紊乱跟一些疾病有关, 如精神分裂症、偏头痛、抑郁症、自杀行为、自闭症、饮食失调和强迫症等 (Jones and Blackburn, 2002)。目前人的 SERT 已经作为靶标来治疗精神障碍病人。在美国, 每年用选择性的

5-HT 重吸收抑制剂 (selective serotonin reuptake inhibitors, SSRIs) 来治疗抑郁症的人大约有 5%~10% (Bauer *et al.*, 2007)。昆虫体内 5-HT 也能够作为神经递质、神经调质或者神经激素发挥重要的生理功能, 调节主要的行为活动。5-HT 通过 5-HT_{1B} 受体调控黑腹果蝇昼夜节律, 5-HT_{1B} 在黑腹果蝇生物钟神经元中表达, 改变 5-HT_{1B} 的表达量能够从分子水平和行为上改变黑腹果蝇对光的节律反应 (Yuan *et al.*, 2005)。5-HT 能调控蜚蠊 *Periplaneta* 的唾液腺, 用 5-HT 激活唾液腺能够产生蛋白丰富的唾液 (Walz *et al.*, 2006)。5-HT 还能够调控吸血昆虫长红猎蝽 *Rhodnius prolixus* 取食相关行为, 它能够促进唾液腺的分泌, 增加跟消化相关的肌肉收缩, 增强心率, 引起角质层的塑化, 从而可以容纳更多吸食的血 (Orchard, 2006)。而且 5-HT 通过激发长红猎蝽 *R. prolixus* 胞内 cAMP 和 Ca²⁺ 的浓度促进马氏管内 Na⁺ 和 K⁺ 的运输 (Gioino *et al.* 2014)。通过药物增加黑腹果蝇脑部 5-HT 的含量, 能够增强它们的好斗行为, 另外通过遗传学手段增加 5-HT 的含量也能产生同样的效果 (Dierick and Greenspan, 2007)。Alekseyenko 等人的研究还发现果蝇脑部一对对称的 5-HT-PLP 神经元在好斗行为中起了关键作, 激活该神经元会促进雄性好斗行为, 而沉默该神经元会减弱好斗行为。5-HT-PLP 神经元跟 5-HT_{1A} 神经元之间相互影响。激活 5-HT_{1A} 神经元会降低果蝇好斗行为 (2014)。Bubak 等对达氏曲突眼蝇 *Teleopsis dalmanni* 的研究表明, 增加 5-HT 合成的前体物能够增加突眼蝇脑部 5-HT 的含量, 而 5-HT 含量高的蝇好斗能力更强, 获胜的几率更大 (2014)。同样通过遗传学和药理学手段表明 5-HT 对黑腹果蝇的记忆起了很重要的作用 (Sitaraman *et al.*, 2008)。沙漠飞蝗 *Schistocerca gregaria* 群居和散居行为也受 5-HT 系统的调控, 通过 5-HT 合成酶抑制剂, 以及 5-HT 受体激动剂和拮抗剂对沙漠飞蝗散居型和群居型的影响发现, 5-HT 系统能够促进沙漠飞蝗由散居变为群居型 (Anstey *et al.*, 2009)。5-HT 也调控东亚飞蝗 *Locusta migratoria* 群居和散居行为, 把群居型东亚飞蝗隔离后, 脑部 5-HT 含量会大幅度上升, 给群居型东亚飞蝗脑部注射 5-HT 并不能使其转变为散居型, 但是对隔离的群居型东亚飞蝗注射同样的 5-HT, 能够加快由群居型转变为散居型 (Guo *et al.*, 2013)。另外, 通过药物处理、RNAi 等对 5-HT 是否参与黑腹果蝇运动的研究结果表明, 5-HT 在幼

虫的运动中起了很重要的作用 (Silva *et al.*, 2014)。5-HT 通过调控果蝇幼虫的竖起动作而调控幼虫方向的改变能力, 这一过程主要是 5-HT 通过调节下游的腹部 LK 神经元的活性和白细胞激肽的分泌实现的(Okusawa *et al.*, 2014)。通过对果蝇的研究还发现 5-羟色胺神经元能够对外界营养条件作出反应, 从而调控甾类激素的合成 (Shimada-Niwa and Niwa, 2014)。目前已知的昆虫体内 5-HT 的主要功能见表 1.1。

表 1.1 昆虫体内 5-HT 的功能
Table 1.1 Functions of 5-HT in insects

功能 Function	昆虫种类 Insect species	参考文献 References
取食 Feeding	伏蝇 <i>Phormia regina</i>	Haselton <i>et al.</i> , 2009; Liscia <i>et al.</i> , 2012
	红头丽蝇 <i>Calliphora vicina</i>	Trimmer, 1985
	红尾粪麻蝇 <i>Neobellieria bullata</i>	Dacks <i>et al.</i> , 2003
	厩螫蝇 <i>Stomoxys calcitrans</i>	Liu <i>et al.</i> , 2011
	弓背蚊 <i>Camponotus mus</i>	Falibene <i>et al.</i> , 2012
	西方蜜蜂 <i>Apis mellifera</i>	French <i>et al.</i> , 2013
	埃及伊蚊 <i>Aedes aegypti</i>	Kinney <i>et al.</i> , 2014
繁殖 Reproduction	飞蝗 <i>Locusta migratoria</i>	Orchard and Lange, 1985; Lange, 2004
	沟额虻 <i>Tabanus sulcifrons</i>	Cook and Meola, 1978
	美洲大蠊 <i>Periplaneta americana</i>	Bamji and Orchard, 1995
	厩螫蝇 <i>S. calcitrans</i>	Liu <i>et al.</i> , 2013
学习和记忆 Learning and memory	黑腹果蝇 <i>Drosophila melanogaster</i>	Sitaraman <i>et al.</i> , 2008
	西方蜜蜂 <i>Apis mellifera</i>	Wright, 2011
嗅觉 Olfactory	烟草天蛾 <i>Manduca sexta</i>	Kloppenburg <i>et al.</i> , 1999; Kloppenburg and Mercer, 2008
昼夜节律 Circadian	黄斑黑蟋蟀 <i>Gryllus bimaculatus</i>	Page, 1987; Tomioka <i>et al.</i> , 1993
免疫反应 Immunity response	甜菜夜蛾 <i>Spodoptera exigua</i>	Kim and Kim, 2010
运动 Locomotor	黑腹果蝇 <i>D. melanogaster</i>	Neckameyer <i>et al.</i> , 2007; Silva <i>et al.</i> , 2014; Okusawa <i>et al.</i> , 2014
好斗行为 Aggression	黑腹果蝇 <i>D. melanogaster</i>	Dierick and Greenspan, 2007; Alekseyenko <i>et al.</i> , 2014
	达氏曲突眼蝇 <i>Teleopsis dalmanni</i>	Bubak <i>et al.</i> 2014
心率 Heart rate	黑腹果蝇 <i>D. melanogaster</i>	Dasari and Cooper, 2006; Majeed <i>et al.</i> 2013; Majeed <i>et al.</i> , 2014
	冈比亚按蚊 <i>Anopheles gambiae</i>	Hillyer <i>et al.</i> 2015
发育 Development	黑腹果蝇 <i>D. melanogaster</i>	Neckameyer, 2010
群居 Gregarization	沙漠蝗 <i>Schistocerca gregaria</i>	Anstey <i>et al.</i> , 2009
	东亚飞蝗 <i>Locusta migratoria</i>	Guo <i>et al.</i> , 2013

6 5-HT 受体

5-HT在昆虫体内发挥不同的生理作用，很多都是通过其受体实现的。目前有关昆虫5-HT受体类型的知识是有限的，昆虫体内鉴定出来的5-HT受体都是G蛋白偶联受体（G protein-coupled receptors, GPCR），这种受体是整合膜蛋白，有7个跨膜区域。

6.1 5-HT 受体的克隆

从脊椎动物中已克隆出14个5-HT受体基因，其中一个编码离子通道（5-HT₃），其余13个基因编码GPCR。根据序列同源性、基因的分布、跟第二信使的偶联路径以及药理学性质，将脊椎动物体内GPCR型的5-HT受体分为6大类。5-HT₁（5-HT_{1A/B/D/E/F}）和 5-HT₅（5-HT_{5A}）受体跟G_{i/o}蛋白偶联，抑制胞内cAMP的合成。5-HT₂（5-HT_{2A/B/C}）受体跟G_{q/11}偶联，调节肌醇磷酸盐的水解，从而使胞内Ca²⁺量升高。5-HT₄、5-HT₆和5-HT₇受体跟G_s偶联，促进胞内cAMP的形成（Hannon and Hoyer, 2008）。5-HT₅的另一个亚型5-HT_{5B}在人体内不编码功能性蛋白，但在小鼠和大鼠体内有表达，目前对5-HT_{5B}的了解相对较少（Nelson, 2004）。无脊椎动物5-HT受体的分类跟脊椎动物一样复杂。根据已经克隆到的ser-1, ser-4, ser-7受体以及基因组测序后的序列分析发现，秀丽隐杆线虫体内6种GPCR型的5-HT受体均存在（Carre-Pierrat *et al.*, 2006）。目前昆虫体内克隆到的5-HT受体基因并不多（表1.2），主要属于5-HT₁，5-HT₂和5-HT₇受体基因。例如黑腹果蝇至少有5种5-HT受体即5-HT_{1A}，5-HT_{1B}（Saudou *et al.*, 1992），5-HT_{2A}（Colas *et al.*, 1995），5-HT_{2B}（Gasque *et al.*, 2013）和 5-HT₇（Witz *et al.*, 1990），分别跟哺乳动物5-HT_{1A}，5-HT_{1B}，5-HT_{2A}，5-HT_{2B}和5-HT₇受体同源（Blenau and Thamm, 2011）。

表 1.2 昆虫 5-HT 受体的功能、表达谱和药理学性质
Table 1.2 Functions, expression patterns and pharmacological properties of insect 5-HT receptors

物种 Species	受体名 Receptor name	GenBank 登录号 GenBank accession number	功能 Function	细胞反应 Cellular response	表达模式 Expression pattern	激动剂 Agonists (EC ₅₀ or K _i)	拮抗剂 Antagonists (EC ₅₀ or K _i)	表达 细胞系 Cells	文献 References
黑腹果蝇 <i>Drosophila melanogaster</i>	5-HT _{1A}	Z11489	攻击行为 Aggressive behaviors; 光反应 Response to light; 促进睡眠 Sleep-promoting; 调节胰岛素 分泌细胞 Regulating Insulin-producing cells;	降低胞内cAMP浓 度 Inhibition of [cAMP] _i	腹侧不成对中间神经元 Ventral unpaired median neurons	Dihydroergocryptine (1.1×10 ⁻⁸ mol/L) > serotonin (1.6×10 ⁻⁵ mol/L) > (±)-8-hydroxy-2-(diethylamino) (8-OH-DPAT) (4.3×10 ⁻⁵ mol/L)	Prazosin (0.25×10 ⁻⁶ mol/L) > d-butacclamol (0.33×10 ⁻⁶ mol/L) > methysergide (1.4×10 ⁻⁶ mol/L) > l-butacclamol (1.4×10 ⁻⁵ mol/L) > yohimbine (1.8×10 ⁻⁵ mol/L)	COS-7	Saudou <i>et al.</i> , 1992; Yuan <i>et al.</i> , 2006; Johnson <i>et al.</i> , 2009; Rodriguez Moncalvo and Campos, 2009; Luo <i>et al.</i> , 2012
	5-HT _{1B}	Z11490	昼夜节律 Circadian entrainment	降低胞内cAMP浓 度 Inhibition of [cAMP] _i	幼虫腹神经节 Larval ventral ganglion	Dihydroergocryptine (3.8×10 ⁻⁹ mol/L) > serotonin (2.1×10 ⁻⁶ mol/L) > 8-OH-DPAT (2.7×10 ⁻⁵ mol/L)	d-Butacclamol (0.64×10 ⁻⁷ mol/L) > prazosin (0.18×10 ⁻⁶ mol/L) > methysergide (0.72×10 ⁻⁶ mol/L) > yohimbine (9.8×10 ⁻⁶ mol/L) > l-butacclamol (3.0×10 ⁻⁵ mol/L)	COS-7	Saudou <i>et al.</i> , 1992; Yuan <i>et al.</i> , 2005
	5-HT _{2A}	X81835	调控进攻行为 Modulate aggressive behaviors; 表皮形 成 Cuticular formation; 昼夜 节律 Circadian behaviors	提高胞内Ca ²⁺ 浓 度 Stimulation of [Ca ²⁺] _i	与 <i>fz</i> 在胚胎中共表达 Coexpression with pair-rule gene <i>fz</i> in embryo	Serotonin > dihydroergocryptine > 8-OH-DPAT	Ritanserin > pizotifen > methiothepin = methysergide > yohimbine > mianserin	COS-1	Colas <i>et al.</i> , 1995; Schaerlinger <i>et al.</i> , 2007; Nichols, 2007; Johnson <i>et al.</i> , 2009
	5-HT _{2B}	KC852205	-	提高胞内Ca ²⁺ 浓 度 Stimulation of [Ca ²⁺] _i	-	Serotonin (2.93 ×10 ⁻⁷ mol/L)	Metitepine	HEK 293T	Gasque <i>et al.</i> , 2013
	5-HT ₇	M55533	求偶和交配 Courtship and mating	提高胞内cAMP浓 度 Stimulation of [cAMP] _i	成虫头部 Adult head; 幼 虫腹神经节 Larval ventral ganglion	Serotonin (1.6×10 ⁻⁶ mol/L) > 8-OH-DPAT (1.06×10 ⁻⁴ mol/L)	d-Butacclamol (0.32×10 ⁻⁷ mol/L) > methysergide (1.2×10 ⁻⁶ mol/L) > prazosin (9.8×10 ⁻⁶ mol/L) > l-butacclamol (3.1×10 ⁻⁵ mol/L) > yohimbine (3.2×10 ⁻⁵ mol/L)	Sf9, NIH 3T3	Witz <i>et al.</i> , 1990; Saudou <i>et al.</i> , 1992; Obosi <i>et al.</i> , 1996; Becnel <i>et al.</i> , 2011
西方蜜蜂 <i>Apis mellifera</i>	5-HT ₇	AM076717	-	提高胞内cAMP浓 度 Stimulation of [cAMP] _i	内在蘑菇体神经元 Intrinsic mushroom body neurons; 外周器官 Peripheral organs	Serotonin (1.06×10 ⁻⁹ mol/L) > 5-carboxamidotryptamine (2.43 ×10 ⁻⁹ mol/L) > AS 19 > 8-OH-DPAT	Methiothepin (1.305 ×10 ⁻⁷ mol/L) > WAY100635	HEK 293	Schlenstedt <i>et al.</i> , 2006
	5-HT _{1A}	FN645449	趋光行为 Phototactic behavior	降低胞内cAMP浓 度 Inhibition of [cAMP] _i	脑 Central brain; 触角叶 Antennal lobes; 视叶 Optic lobes; 咽下神经 节 Subesophageal ganglion; 腹神经索 Ventral nerve cord	Serotonin (1.69×10 ⁻⁸ mol/L) > 5-carboxamidotryptamine (7.005 ×10 ⁻⁷ mol/L) > 5-methoxytryptamine (3.630×10 ⁻⁶ mol/L)	Prazosin (1.5×10 ⁻⁶ mol/L) > WAY100635 (4.8×10 ⁻⁶ mol/L) > methiothepin (1.06×10 ⁻³ mol/L)	HEK 293	Thamm <i>et al.</i> , 2010

	5-HT _{2A}	FR727107		提高胞内Ca ²⁺ 浓度 Stimulation of timulation of [Ca ²⁺] _i	中枢神经系统 CNS; 咽 下腺Hypopharyngeal Glands; 唾液腺Salivary glands; 马氏管 Malpighian tubules	Serotonin (7×10 ⁻⁸ mol/L) > 5-methoxytryptamine (7.0×10 ⁻⁸ mol/L) > 8-OH-DPAT (5.59×10 ⁻⁵ mol/L)	SB200646 = mianserin > cyproheptadine > methiothepin > clozapine > methysergide	HEK 293	Thamm <i>et al.</i> , 2013
	5-HT _{2B}	FR727108		提高胞内Ca ²⁺ 浓度 Stimulation of [Ca ²⁺] _i	中枢神经系统CNS; 咽 下腺Hypopharyngeal Glands; 唾液腺Salivary glands; 马氏管 Malpighian tubules	Serotonin (3.25×10 ⁻⁸ mol/L) > 5-methoxytryptamine (6.04×10 ⁻⁸ mol/L) > 8-OH-DPAT (5.615×10 ⁻⁷ mol/L)	Cyproheptadine > ketanserin > mianserin > clozapine	HEK 293	Thamm <i>et al.</i> , 2013
黄斑黑蟋蟀 <i>Gryllus bimaculatus</i>	5-HT _{1A}	AB618098	-	-	脑The central brain; 咽 下神经节 Suboesophageal ganglion; 睾丸 Testicle; 卵巢 Ovary; 马氏管 Malpighian tubule	-	-	-	Watanabe <i>et al.</i> , 2011
	5-HT _{1B}	AB618099	-	-	脑The central brain; 咽 下神经节 Suboesophageal ganglion; 睾丸 Testicle; 卵巢 Ovary	-	-	-	Watanabe <i>et al.</i> , 2011
	5-HT _{2A}	AB618100	-	-	唾液腺Salivary gland	-	-	-	Watanabe <i>et al.</i> , 2011
	5-HT ₇	AB618101	-	-	唾液腺Salivary gland; 中肠Midgut; 马氏管Malpighian tubule	-	-	-	Watanabe <i>et al.</i> , 2011
烟草天蛾 <i>Manduca sexta</i>	5-HT _{1A}	DQ840515	-	降低胞内cAMP浓 度 Inhibition of [cAMP] _i	触角Antennae; 胸和腹 神经节Thoracic and abdominal ganglia	Serotonin (2.95×10 ⁻⁸ mol/L), 5-methoxytryptamine, 2-methyl-5-hydroxytryptamine	Methysergide, WAY-100635	HEK 293,	Dacks <i>et al.</i> , 2006; Dacks <i>et al.</i> , 2013
	5-HT _{1B}	DQ84051	-	降低胞内cAMP浓 度 Inhibition of [cAMP] _i	触角Antennae; 胸和腹 神经节Thoracic and abdominal ganglia	Serotonin (5.70×10 ⁻⁸ mol/L), 5-methoxytryptamine	WAY-100635, methiothepin	HEK 293	Dacks <i>et al.</i> , 2006, 2013
	5-HT ₂	JX891652		提高胞内Ca ²⁺ 浓 度 Stimulation of [Ca ²⁺] _i	触角Antennae	Serotonin (2.54×10 ⁻⁸ mol/L)		HEK 293	Dacks <i>et al.</i> , 2013
	5-HT ₇	JX878498		提高胞内cAMP浓 度 Stimulation of [cAMP] _i	触角Antennae	Serotonin (1.05×10 ⁻⁸ mol/L),	Methysergide	HEK 293	Dacks <i>et al.</i> , 2013
埃及伊蚊 <i>Aedes aegypti</i>	5HT ₇	AF296125		提高胞内cAMP浓 度 Stimulation of [cAMP] _i	马氏管Malpighian Tubules; 气管细胞 Tracheolar cells	Serotonin(3.95×10 ⁻⁸ mol/L) > 5-carboxamidotryptamine 8-OH-DPAT > pimoziide	=	CHO-K1	Pietrantonio <i>et al.</i> , 2001; Lee and Pietrantonio, 2003

家蚕 <i>Bombyx mori</i>	5-HT ₁	X95604	-	-	-	-	-	-	Von Nickisch-Roseneck <i>et al.</i> 1996
烟芽夜蛾 <i>Heliothis virescens</i>	5-HT _{1A}	X95605	-	-	-	-	-	-	Von Nickisch-Roseneck <i>et al.</i> 1996
美洲大蠊 <i>Periplaneta americana</i>	5-HT ₁	CAX65666.1	-	降低胞内cAMP浓度 Inhibition of [cAMP] _i	脑Brain; 唾液腺 Salivary glands; 中肠 Midgut	Serotonin > 5-methoxytryptamine	WAY100635 > methiothepin	HEK 293	Troppmann <i>et al.</i> , 2010
红头丽蝇 <i>Calliphora vicina</i>	5-HT _{2A}	HE657271, HE856266	-	提高胞内Ca ²⁺ 浓度 Stimulation of [Ca ²⁺] _i	脑Brain; 唾液腺 Salivary glands	Serotonin (2.4×10 ⁻⁸ mol/L) > 5-methoxytryptamine (6.7×10 ⁻⁸ mol/L) > 5-carboxamidotryptamine (5.1×10 ⁻⁵ mol/L) > 8-OH-DPAT (6.2×10 ⁻⁵ mol/L)	Mianserin (7.3×10 ⁻⁷ mol/L) > methiothepin (1.2×10 ⁻⁶ mol/L) > cyproheptadine (1.6×10 ⁻⁶ mol/L) > yohimbine (2.9×10 ⁻⁶ mol/L) > ketanserin (1.4×10 ⁻⁵ mol/L) > clozapine (1.5×10 ⁻⁵ mol/L) > Cinanserin (3.0×10 ⁻⁵ mol/L)	HEK 293	Röser <i>et al.</i> , 2012
	5-HT ₇	HE657272	-	提高胞内cAMP浓度 Stimulation of [cAMP] _i	脑Brain; 唾液腺 Salivary glands; 飞行肌 Flight muscles; 马氏管 Malpighian tubules	R(+)-lisuride (2.0×10 ⁻⁹ mol/L) > serotonin (4.0×10 ⁻⁹ mol/L) > AS 19 (3.0×10 ⁻⁷ mol/L) > 5-carboxamidotryptamine (4.3×10 ⁻⁷ mol/L) > methysergide (7.7×10 ⁻⁷ mol/L) > 5-Methoxytryptamine (9.5×10 ⁻⁷ mol/L) > 8-OH-DPAT (7.9×10 ⁻⁶ mol/L)	SB-269970 (9.0×10 ⁻⁹ mol/L) > mianserin (6.7×10 ⁻⁸ mol/L) > cyproheptadine (7.3×10 ⁻⁸ mol/L) > clozapine (8.0×10 ⁻⁸ mol/L) > spiperone (2.8×10 ⁻⁷ mol/L) > cinanserin (4.0×10 ⁻⁷ mol/L) > ketanserin (1.5×10 ⁻⁵ mol/L) > phentolamine (1.5×10 ⁻⁵ mol/L)	HEK 293	Röser <i>et al.</i> , 2012
赤拟谷盗 <i>Tribolium castaneum</i>	5-HT ₁	KC196076	-	降低胞内cAMP浓度 Inhibition of [cAMP] _i	脑 Brain; 视叶Optic lobes; 肠道Gut	5-HT (9.5×10 ⁻⁵ mol/L) > am-5-HT (1.074×10 ⁻² mol/L) > 5-carboxamidotryptamine (2.472×10 ⁻² mol/L) > 5-methoxytryptamine (9.184×10 ⁻² mol/L) > 8-OH-DPAT (5.51×10 ⁻¹ mol/L)	Prazosin (1.39×10 ⁻³ mol/L) > methiothepin (1.638×10 ⁻² mol/L) > methysergide (3.397×10 ⁻² mol/L) > butaclamol (2.041×10 ⁻¹ mol/L) > SB-269970 (2.054×10 ⁻¹ mol/L)	CHO-WTA1 1	Vleugels <i>et al.</i> , 2013
	5-HT ₇	XM_961484	-	提高胞内cAMP浓度 Stimulation of [cAMP] _i	脑Brain; 视叶Optic lobes; 生殖系统 Reproductive system; 肠道 Gut	5-HT (2.73×10 ⁻⁵ mol/L) > am-5-HT (5.19×10 ⁻³ mol/L) > 5-carboxamidotryptamine (2.228×10 ⁻² mol/L) > 5-methoxytryptamine (3.028×10 ⁻² mol/L) > 8-OH-DPAT (3.875×10 ⁻² mol/L)	Ketanserin (1.95×10 ⁻³ mol/L) > Methysergide (6.98×10 ⁻³ mol/L) > Methiothepin (7.91×10 ⁻³ mol/L) > Butaclamol (1.365×10 ⁻² mol/L) > Prazosin (1.482×10 ⁻² mol/L) > SB-269970 (1.582×10 ⁻² mol/L) > Yohimbine (3.255×10 ⁻² mol/L) > WAY-100635 (7.680×10 ⁻² mol/L)	CHO-WTA1 1	Vleugels <i>et al.</i> , 2014

柞蚕 <i>Antheraea pernyi</i>	5HTR _A	-	-	-	脑Brain	-	-	-	Wang <i>et al.</i> , 2013
	5HTR _B	滞育 Diapause	-	-	脑Brain	-	-	-	Wang <i>et al.</i> , 2013
吸血椿象 <i>Rhodnius prolixus</i>	5-HT _{2B}	提高胞内Ca ²⁺ 浓度 Stimulation of [Ca ²⁺],	马氏管 Malpighian tubules; 唾液腺Salivary glands; 中枢神经系统 CNS; 前肠 Foregut; 后 肠 Hindgut; 背血管 dorsal vessel	5-HT(EC ₅₀ = 201 nM); alpha-methyl serotonin (EC ₅₀ = 3.2 μM)	propranolol, spiperone, ketanserin, mianserin, and cyproheptadine	CHO-K1	Paluzzi <i>et al.</i> , 2015		

EC₅₀: 有效中浓度 Median effective concentration; K_i: 抑制常数 Inhibition constant; COS-7: 起源于 CV-1(simian)且携带 SV40 遗传物质的细胞 Cell being CV-1 (simian) in origin, and carrying the SV40 genetic material; NIH-3T3: 小鼠胚胎成纤维细胞系 NIH 3T3 cells are established from a NIH Swiss mouse embryo; sf9: 草地贪夜蛾 *Spodoptera frugiperda*; CHO cell: 中国仓鼠细胞 Chinese hamster ovary cell; HEK-293 cell: 人胚肾细胞 Human embryonic kidney.

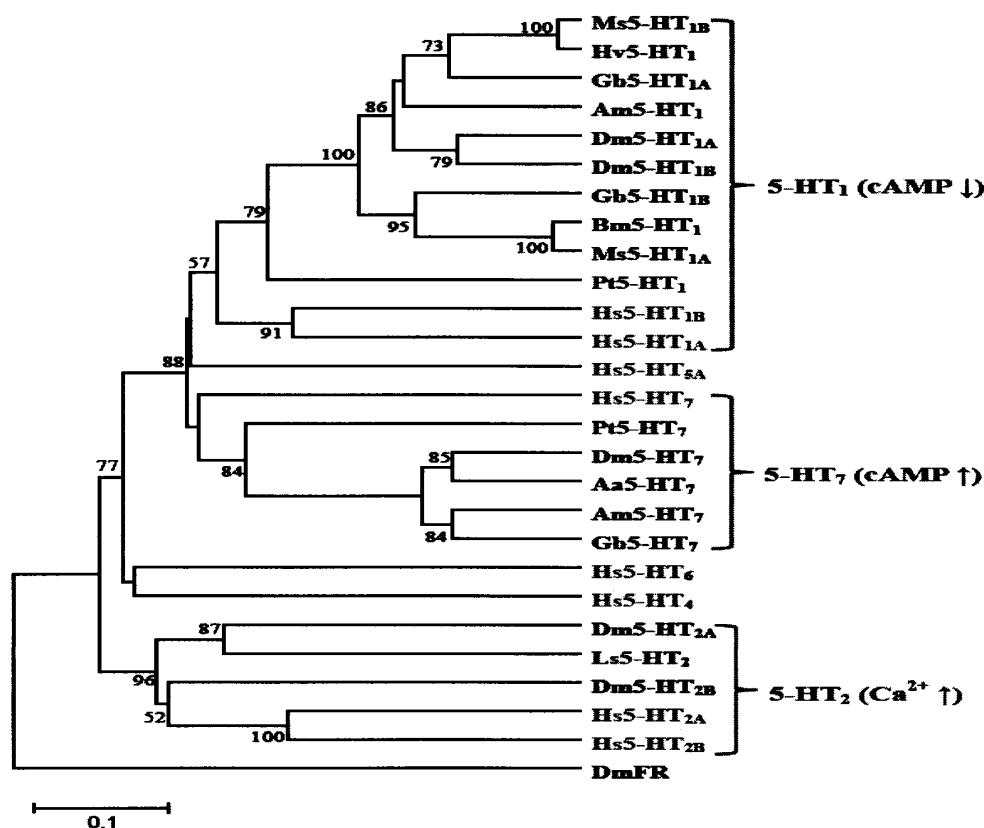


图1.2 不同物种5-HT受体的系统发育分析

Fig 1.2 Phylogenetic tree of 5-HT receptors from different species

黑色、绿色和蓝色字母分别代表昆虫、脊椎动物和软体动物种类。The black, green and blue letters represent insect, vertebrate and mollusc species, respectively. 5-HT 受体来源及 GenBank 登录号 The origin of 5-HT receptors and their GenBank accession numbers: 冈比亚按蚊 *Aedes aegypti* (Aa5-HT₇: AG49292); 西方蜜蜂 *Apis mellifera* (Am5-HT₁: NP_001164579; Am5-HT₇: NP_001071289); 家蚕 *Bombyx mori* (Bm5-HT₁: NP_001037502); 黑腹果蝇 *Drosophila melanogaster* (Dm5-HT_{1A}: CAA77570; Dm5-HT_{1B}: CAA77571; Dm5-HT_{2A}: AAF52113; Dm5-HT_{2B}: NP_649806; Dm5-HT₇: AAF57104; DmFR: AAF47700); 黄斑黑蟋蟀 *Gryllus bimaculatus* (Gb5-HT_{1A}: BAJ83479; Gb5-HT_{1B}: BAJ83480; Gb5-HT₇: BAJ83482); 烟草夜蛾 *Heliothis virescens* (Hv5-HT₁: CAA64863); 人 *Homo sapiens* (Hs5-HT_{1A}: CAH03197; Hs5-HT_{1B}: EAW48722; Hs5-HT_{2A}: AAM21129; Hs5-HT_{2B}: AAB31827; Hs5-HT₄: NP_000861; Hs5-HT_{5A}: AAM21132; Hs5-HT₆: AAH74996; Hs5-HT₇: BAG70296); 烟草夜蛾 *Manduca sexta* (Ms5-HT_{1A}: ABI33827; Ms5-HT_{1B}: ABI33826); 静水椎实螺 *Lymnaea stagnalis* (Ls5-HT₂: AAC16969); 蜗牛 *Planorbella trivolvis* (Pt5-HT₁: AAQ95277; Pt5-HT₇: AAQ84306)

6.2 5-HT 受体的信号转导途径

GPCR信号转导过程中的共同特点是GPCR被激活后会引引起细胞内第二信使浓度的改变。跟脊椎动物一样，昆虫5-HT受体被激活后，根据其偶联的G蛋白的不同，会使细胞内环腺苷酸cAMP或Ca²⁺浓度的改变，从而引起两种不同的信号

转导途径（图1.3）（Blenau and Baumann, 2001; Tierney, 2001）。

一种途径会使细胞内环腺苷酸cAMP浓度改变。受体结合到G_s蛋白（stimulatory G protein）上，被激活的G_{as}亚基会跟质膜上的腺苷酸环化酶（adenylyl cyclase, AC）发生反应，导致该环化酶的活性提高，使ATP转化为cAMP，cAMP浓度的升高会激活依赖cAMP的蛋白激酶A的活性（protein kinase A, PKA）。PKA能够将底物分子中的丝氨酸和苏氨酸磷酸化，这些底物包括细胞溶质蛋白、配体门控或电压门控的离子通道以及一些转录因子如CREB, CREM, 和ATF-1（De Cesare *et al.*, 1999）。5-HT受体也能抑制AC的活性，5-HT受体跟抑制性蛋白（inhibitory G proteins, G_i）结合，AC跟激活的G_{ai}亚基互动，就会跟激活的G_{as}亚基竞争性结合，从而影响AC的活性（Katada *et al.*, 1986; Stryer and Bourne, 1986）。

另一种途径会使细胞内Ca²⁺浓度改变。受体结合到G_{q/o}家族类蛋白上（Gudermann *et al.*, 1996, 1997），被激活的G_{q/o}亚基结合水解磷脂酶C（phospholipase C, PLC）并激活该酶的活性（Rhee and Bae, 1997），PLC能够水解一种膜结合底物——磷脂酰肌醇二磷酸（phosphatidylinositol 4, 5-bisphosphate），产生两种第二信使即三磷酸肌醇（inositol triphosphate, IP₃）和二酰基甘油（diacylglycerol, DAG）。IP₃自由扩散并结合于内质网膜上特定的IP₃受体上，从而使Ca²⁺通道打开，Ca²⁺释放到细胞质中。由于PLC不仅导致Ca²⁺的产生，也导致了DAG的产生，因此受体跟G_{q/o}蛋白偶联可能激活除Ca²⁺释放通道之外的另一条信号途径。与IP₃相比，DAG能够激活膜上的蛋白激酶C（protein kinase C, PKC）。PKC全酶的活性需要有Ca²⁺和DAG的存在以及激酶与膜的协作。与PKA一样，PKC能够使很多蛋白的丝氨酸和苏氨酸残基磷酸化，从而改变这些蛋白的功能。总之，GPCR激活后，依靠第二信使产生细胞内信号级联反应，不同的胞内信号通道可能在同一个细胞中同时激活，只要对应的受体和偶联的蛋白同时存在。这种共激活反应的产生可能导致细胞反应的放大或减小，从而提供了一个“巧合检测”的细胞理论基础（Blenau and Baumann, 2001）。

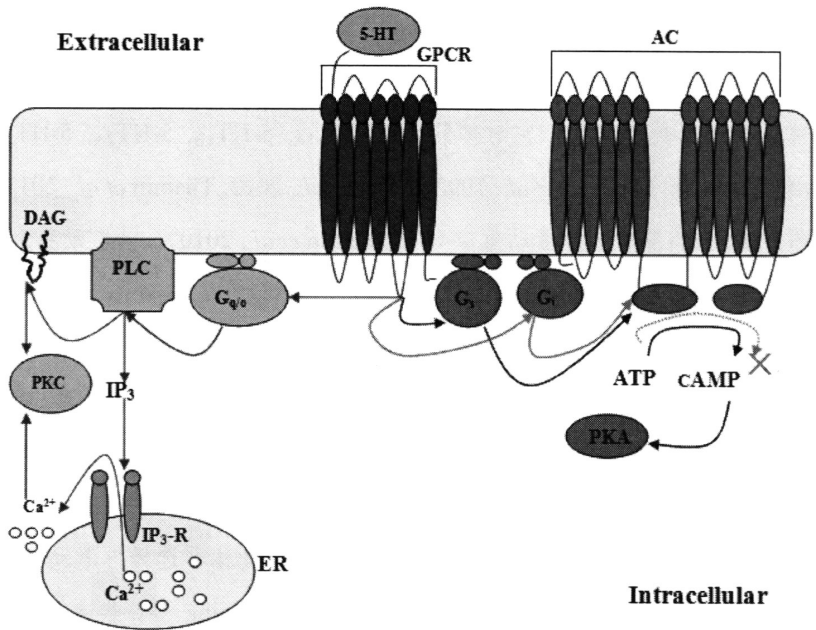


图1.3 昆虫5-HT受体偶联胞内环腺苷酸和三磷酸肌醇/二酰基甘油的信号转导途径（修改自 Blenau and Baumann, 2001）

Fig 1.3 Insect 5-HT receptors coupled to intracellular cAMP and IP3 /DAG signaling pathways (adapted from Blenau and Baumann, 2001)

AC: 腺苷酸环化酶 Adenylyl cyclase; ATP: 三磷酸腺苷 Adenosine triphosphate; cAMP: 环腺苷酸 Cyclic adenosine monophosphate; ER: 内质网 Endoplasmic reticulum; DAG: 二酰基甘油 Diacylglycerol; GPCR: 5-羟色胺G蛋白偶联受体 5-HT G protein coupled receptor; G_s: 激活性G蛋白 Stimulatory G protein; G_i: 抑制性G蛋白 Inhibitory G proteins; G_{q/10}: G_{q/10} 类G蛋白 G proteins of the G_{q/10} family; IP₃: 三磷酸肌醇 Inositol 1, 4, 5-trisphosphate; IP₃-R: 三磷酸肌醇偶联受体 Inositol 1, 4, 5-trisphosphate coupled receptor; PKA: 蛋白激酶A Protein kinase A; PLC: 磷脂酶C Phospholipase C; PKC: 蛋白激酶C Protein kinase C. 图中黑色箭头路径是5-HT受体偶联G_s蛋白的信号转导途径；红色箭头表示该5-HT受体偶联G_i蛋白的信号转导途径；蓝色箭头路径是5-HT受体偶联G_{q/10}蛋白的信号转导途径。The black arrow represents the signaling pathway of 5-HT receptors coupling to G_s protein, the red arrow represents the signaling pathway of 5-HT receptors coupling to G_i protein, and the blue arrow represents the signaling pathway of 5-HT receptors coupling to G_{q/10} protein.

6.3 5-HT 受体的药理学性质

昆虫体内5-HT受体的药理学性质跟哺乳动物不尽相同，不同昆虫之间5-HT受体药理学性质也存在很大异同。例如WAY100635是美洲大蠊 *Periplaneta americana* 5-HT₁受体的反向激动剂（Troppmann *et al.*, 2010），但是在大多数的哺乳动物5-HT_{1A}受体研究中，WAY100635都是作为中性拮抗剂（Martel *et al.*, 2007），只在某些情况下作为反向激动剂（Cosi and Koek, 2000）。Methiothepin是哺乳动物5-HT₁受体的反向激动剂（McLoughlin and Strange, 2000; Martel *et al.*, 2007），但是它是美洲大蠊5-HT₁受体的中性拮抗剂，能够拮抗5-HT和反向激动剂WAY100635对5-HT₁受体的激动效应（Troppmann *et al.*, 2010）。8-OH-DPAT

是哺乳动物5-HT_{1A}选择性激动剂 (Hjorth and Magnusson, 1988), 后来研究发现它对5-HT₇也有一定的激动效果 (Sprouse *et al.*, 2004), 但是该激动剂却是昆虫5-HT受体的非选择性激动剂, 它对部分昆虫5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}和5-HT₇均有激动效果 (Saudou *et al.*, 1992; Röser *et al.*, 2012; Thamm *et al.*, 2013), 但对美洲大蠊5-HT₁没有明显激动效应 (Troppmann *et al.*, 2010)。哺乳动物5-HT₇选择性拮抗剂SB 269970 (Hagan *et al.*, 2000), 对丽蝇*Calliphora vicina* 5-HT₇受体有很强的拮抗效应 (Röser *et al.*, 2012, Vleugels *et al.*, 2014), 但是对赤拟谷盗*Tribolium castaneum* 5-HT₇受体拮抗效应很弱 (Vleugels *et al.*, 2014) (表2)。目前对5-HT受体药理学性质的研究主要集中在黑腹果蝇和西方蜜蜂上。表2中列出了目前昆虫中克隆出来的5-HT受体的一些激动剂和拮抗剂, 从中可以看出, 不同昆虫受体药理学性质存在差异, 而主要使用的5-HT受体非选择性激动剂包括5-carboxamidotryptamine, 8-OH-DPAT和5-methoxytryptamine 等, 非选择性的拮抗剂主要包括 Methysergide和Methiothepin 等。不同昆虫5-HT受体跟5-HT的结合能力也不一样, 如意大利蜜蜂Am5-HT_{1A}的5-HT的EC₅₀值是16.9 nmol/L, 与其他昆虫的比较接近, 黑腹果蝇Dm5-HT_{1A}和Dm5-HT_{1B}分别为30和18 nmol/L (Saudou *et al.*, 1992), 但是美洲大蠊的Pea5-HT₁为130 nmol/L (Troppmann *et al.*, 2010)。虽然目前还没有针对5-HT受体开发的农药, 但是不同类型5-HT受体特异性激动剂和拮抗剂可作为选择性杀虫剂的潜在资源。

6.4 5-HT 受体的功能

5-HT的功能主要是通过其受体实现的, 对昆虫5-HT及其受体在生理和行为上的功能的了解主要是依靠药理学实验, 但是随着遗传学和分子生物学技术的不断进步, 通过基因敲除来研究5-HT及其受体的功能越来越常见。冈比亚按蚊*Aedes aegypti*的5-HT₇受体主要在微气管细胞和后肠中表达, 5-HT在呼吸过程中起了作用, 同时在利尿过程中, 5-HT₇受体可能协助肠道对5-HT作出反应 (Pietrantonio *et al.*, 2001)。西方蜜蜂5-HT参与了趋光行为, 其5-HT_{1A}受体在脑部参与视觉信息处理的部位高表达, 并且通过体内药理学实验证明5-HT_{1A}对单头蜜蜂光反应行为有很大的影响, 从基因表达水平到行为过程证明5-HT_{1A}参与调节蜜蜂对光的反应过程 (Thamm *et al.*, 2010)。黑腹果蝇5-HT_{1A}参与调控

睡眠过程,通过对黑腹果蝇3种5-HT受体突变体($d5-HT_{1A}$, $d5-HT_{1B}$, $d5-HT_{2A}$)基线睡眠值进行分析,发现 $d5-HT_{1A}$ 果蝇有短暂和片段睡眠现象,该现象可以通过成虫脑部蘑菇体部位表达5-HT_{1A}而得到补救。而 $d5-HT_{1B}$ 和 $d5-HT_{2A}$ 对果蝇基线睡眠值没有影响。通过药理学和遗传学的方法增加5-HT的表达量,能促进野生型果蝇的睡眠(Yuan *et al.*, 2006)。虽然5-HT_{1B}不参与调控果蝇睡眠,但是前面已经提到果蝇5-HT_{1B}受体调控昼夜节律(Yuan *et al.*, 2005)。同样通过遗传学和药理学的方法,利用非选择性5-HT受体拮抗剂metitepine对黑腹果蝇5种5-HT受体突变体($d5-HT_{1A}$, $d5-HT_{1B}$, $d5-HT_{2A}$, $d5-HT_{2B}$, $d5-HT_7$)取食的影响分析,发现5-HT_{2A}是metitepine抑制取食唯一需要的受体(Gasque *et al.*, 2013)。黑腹果蝇5-HT_{2A}和5-HT_{2B}受体参与其幼虫阶段心率调控作用(Majeed *et al.*, 2014)。5-HT₂还参与调控东亚飞蝗群居和散居型转变,对散居型飞蝗作拥挤处理,飞蝗脑部的5-HT含量不会变化,但是5-HT₂受体的含量在处理1小时后1小时会上升,之后维持稳定状态。用激动剂激活5-HT₂受体会抑制作拥挤处理的散居型飞蝗转变为群居型(Guo *et al.*, 2013)。在所有的昆虫中,黑腹果蝇5-HT及其受体的功能相对研究的比较多(表1.2)。总体而言,这方面还需要大量深入的研究。

7 小结和展望

5-HT 是昆虫和哺乳动物中枢神经系统中一种重要的神经信号调节物。了解5-HT 信号调节系统的先决条件是对5-HT 受体的分子特征和功能特性的了解,以及它们在CNS中的分布。与哺乳动物一样,不同的5-HT受体调节昆虫体内5-HT的反应。目前昆虫体内鉴定出来的5-HT受体都是GPCR类型。昆虫和哺乳动物对药物反应,行为以及基因反应的相似性,有可能使某些昆虫如果蝇和蜜蜂作为研究人类某些疾病的工具,从而可以快速、高通量、低成本的去了解CNS中5-HT的功能(Becnel *et al.*, 2011)。但是,有关昆虫体内5-HT及其受体的研究还远远不够,近几年才有相对比较大的进展,并且研究的昆虫种类还不多。随着越来越多的人关注5-HT,昆虫体内5-HT的研究将会更加深入。

对昆虫体内5-HT受体研究的另一个意义在于开发高效杀虫剂,目前化学农药是防治害虫的主要手段,但是传统的杀虫剂带来了一些不可避免的问题,如害

虫的抗药性，因此，开发新的作用方式的杀虫剂非常必要。5-HT在无脊椎动物的各种生命活动中发挥了重要的作用，很多的5-HT受体已经从不同物种中克隆出来。有趣的是，除了从寄生性线虫—捻转血矛线虫*Haemonchus contortus*体内克隆出来的5-HT_{1Hc}受体外，其它的受体与5-HT的亲合力都相对低，而且5-HT_{1A}的一种激动剂即1-[(4 -氨基苯基)乙基] -4-[3- (三氟甲基)苯基]哌嗪 (PAPP, 1-[(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine) 跟这个受体的亲合力很高。有人预测这种化合物可能成为一种新的杀虫剂。通过对PAPP衍生物的设计，合成和生测，发现它们能够抑制粘虫*Pseudaletia separata* 的生长和幼虫的活性，这个结果更加确定了5-HT受体作为新农药靶标的可能性 (Cai *et al.*, 2009)，但还需要进一步的研究。最近，人的5-HT_{1B} (Wang *et al.*, 2013) 和5-HT_{2B} (Wacker *et al.*, 2013) 受体的晶体结构已有报导，这可以帮助我们更深入的研究5-HT这类受体的结构特点，从而为将来设计和开发杀虫剂奠定了基础。

脊椎动物体内已发现13种GPCR类型的5-HT受体(Blenau and Baumann, 2001)，而目前昆虫里面只发现5种，所以未来昆虫5-HT的研究可能会致力于新受体的发现以及受体新功能的研究。基于不同昆虫5-HT受体药理学性质存在差异，筛选对害虫5-HT受体高选择性，而对非靶标生物无害的药物作为新型杀虫剂是另一个努力的目标

第二章 5-羟色胺在免疫系统中的作用

1 引言

5-羟色胺 (5-HT) 不仅是一种经典的神经递质, 它还可以在中枢神经系统以外的免疫信号传导中发挥重要作用。自 1984 年 5-HT 具有免疫调节效应首次被提出以来(Slauson *et al.*, 1984), 5-HT 在免疫系统中的作用逐渐引起人们关注。近年来一些研究表明 5-HT 主要通过结合表达在免疫细胞上的受体, 调节免疫细胞的功能。

2 5-HT 和脊椎动物免疫系统

2.1 5-HT 在免疫细胞的合成和存储

血小板能够储存肠嗜铬细胞合成的 5-HT。此外, 啮齿动物 (不包括人) 的肥大细胞也能够吸收和合成 5-HT。Kushnir-Sukhov 等 (2007) 的实验表明人的肥大细胞上有少量外周 5-HT 合成酶 TPH1 (tryptophan hydroxylase 1) 的表达。但这是否会导致 5-HT 的大量合成还尚不清楚。有报道表明 mastocytosis 病人的全血中 5-HT 的含量异常高 (Kushnir-Sukhov *et al.*, 2008)。mastocytosis 病人跟正常人比, 肥大细胞会大量增加, 这就表明肥大细胞可能导致了 5-HT 含量的升高。O'Connell 等 (2006) 的研究表明小鼠树突状细胞 DCs (dendritic cells) 表达 5-HT 转运体 SERT (serotonin transporter), 但是不表达 TPH1 和 TPH2。DCs 的激活物比如 LPS 和 anti-CD40 能够促进 SERT 的表达, 同时 5-HT 的主要降解酶单胺氧化酶 A 和 B 的含量降低, 因此激活的 DCs 主要储存 5-HT 而不是降解 5-HT。

与 DCs 一样, B 淋巴细胞被激活后 SERT 的表达量显著上升 (Meredith *et al.*, 2005)。而在休息的状态下扁桃体 B 细胞中几乎检测不到 SERT, 但有丝分裂原刺激的 B 细胞中 SERT 蛋白的表达量会急剧升高。值得注意的是, 多种 B 细胞起源的恶性肿瘤中检测到了高水平的 SERT 的表达, SERT 的表达跟基底细胞的生长成正相关。5-HT 在 T 淋巴细胞里也能合成, 说明 5-HT 在 DCs 和 B 细胞的摄入是十分相关联的 (Leon-Ponte *et al.*, 2007; O'Connell, 2006)。虽然初始脾脏 T 细胞能表达少量

TPH-1（不表达TPH-2），但是激活6小时的T细胞内TPH-1的转录量会升高30倍，5-HT的合成量增加5倍(Leon-Ponte *et al.*, 2007)。有趣的是，尽管T细胞可以缓慢摄取5-HT(O'Connell, 2006)，但未能检测到T细胞上有SERT的表达(Leon-Ponte *et al.*, 2007)。5-HT在T细胞上的摄取可能是通过对5-HT有低亲和力的多巴胺转运DAT（dopamine transporter）实现的。

有研究发现肠道巨噬细胞也表达TPH-1(Nakamura *et al.*, 2008)，但是还没有直接的数据证明巨噬细胞可以合成5-HT。5-HT在免疫细胞上的合成，转运以及5-HT受体表达情况见表 2.1。

表 2.1 5-HT在免疫细胞上的合成，转运以及5-HT受体表达情况
Table 2.1 Summary of 5-HT synthesis, 5-HT transport and 5-HT receptor subtypes in different immune cells.

	细胞功能 Cell function	5-HT的合成 5-HT synthesis	5-HT的转运 5-HT transport	5-HT受体表达情况及功能 5-HT receptors and signaling	参考文献 Reference
先天免疫					
嗜酸性粒细胞 Eosinophil	抗寄生虫Antiparasitic, 抗病毒Antiviral, 分泌颗粒Secretory granules	-	-	5-HT _{2A} 趋化作用Chemotaxis	Boehme <i>et al.</i> , 2008
肥大细胞 Mast cell	抗寄生虫Antiparasitic, 分泌颗粒Secretory granules	TPH-1	SERT	5-HT _{1A} 趋化作用Chemotaxis	Kushnir-Sukhov <i>et al.</i> , 2006
自然杀伤细胞 Natural killer cell	自然细胞毒性 Innately cytotoxic	-	SERT	- 增强细胞溶解作用 Increase cytolytic function	Evans <i>et al.</i> , 2008
巨噬细胞 Macrophage	吞噬Phagocytotic, 抗原 呈递Antigen presentation	TPH-1		5-HT _{1A} 增强吞噬作用Phagocytosis; 5-HT _{2C} , 趋化作用Chemotaxis	Mikulski <i>et al.</i> , 2010; Nakamura <i>et al.</i> , 2008
树突状细胞 Dendritic cell	抗原呈递给初始T细胞 Antigen presentation to naive T cells		SERT	5-HT _{1B} , 5-HT _{1E} , 5-HT _{2B} 趋化作用 Chemotaxis; 5-HT ₄ , 5-HT ₇ 影响细胞 趋化因子分泌	Idzko <i>et al.</i> , 2004; Mueller <i>et al.</i> , 2009; O'Connell, 2006
获得性免疫					
T细胞 T cell	细胞介导的免疫 Cell mediated immunity	TPH-1		5-HT ₇ , 5-HT _{1B} , 5-HT _{2A} 促进T细胞增 殖Proliferation	Inoue <i>et al.</i> , 2011; Leon-Ponte <i>et al.</i> , 2007; Nakamura <i>et al.</i> , 2008; O'Connell, 2006; Yin <i>et al.</i> , 2006
B细胞 B cell	分泌抗体Secrete antibodies		SERT	5-HT ₇ 功能未知unknown; SSRIs 调亡Apoptosis	Ek <i>et al.</i> , 2002; Klein <i>et al.</i> , 2003; Meredith <i>et al.</i> , 2005; Rinaldi <i>et al.</i> , 2010

2.2 5-HT 和先天免疫

表1总结了5-HT参与先天免疫的一些研究。Kushnir- Sukhov等（2006）的研究表明5-HT能够诱导老鼠和人的肥大细胞的趋化性和粘附性，不会诱导肥大细胞脱颗粒性。通过药理学和遗传学的方法表明5-HT_{1A}在这些过程中起了关键性的作用，而且5-HT能够诱导肥大细胞到达炎症处。从皮肤注射5-HT，野生型老鼠能够导致肥大细胞的聚集，而5-HT_{1A}敲除的老鼠没有此反应。

同样，Boehme等（2008）的研究表明5-HT是嗜酸性粒细胞的趋化因子，过敏性哮喘的特征就是嗜酸性粒细胞的浸润，而5-HT能够促进嗜酸性粒细胞的聚集，有哮喘症状的样本血浆5-HT的量会比没有此症状的高。Boehme等人的研究首次揭示了5-HT在嗜酸性粒细胞迁移和聚集到肺部发挥了重要作用。药理学研究表明该过程主要受5-HT_{2A}调控。

巨噬细胞最主要的功能的就是吞噬，Nakamura等（2008）的研究发现5-HT能够调控小鼠巨噬细胞吞噬能力，5-HT_{1A}起主要作用。此外，Mikulski（2010）等表明肺泡巨噬细胞表达5-HT_{2C}受体。

自然杀伤细胞是一种先天免疫细胞，具有重要的细胞溶解作用。Evans等（2008）表明选择性5-羟色胺再摄取抑制剂(selective serotonin reuptake inhibitors, SSRIs)能够增强自然杀伤细胞在体内的溶解作用。并且，Hernandez等（2010）报道人长时间使用SSRI会增加自然杀伤细胞的增殖能力。然而，其中的信号传导机制还尚不清楚。

树突状细胞(DCs)是专职抗原递呈细胞，并且有诱导激活初始T细胞的能力。一些研究鉴定了5-HT在DCs功能中的作用。Idzko等（2004）描述了人单核细胞源性DCs上表达多种5-HT受体。未成熟DCs在mRNA水平表达5-HT_{1B}, 5-HT_{1E}, 5-HT_{2B}, 而成熟DCs表达5-HT₄和5-HT₇。

2.3 5-HT 和获得性免疫

近20年来，5-HT被认为是一种T细胞调制器。早期的研究表明5-HT能够促进T细胞的增殖，并且基于药理学方法证明了5-HT_{1A}起关键性作用。Leon-Ponte等（2007）对初始和激活的小鼠脾T细胞上5-HT受体的表达做了全面的分析。研究

表明初始T细胞选择性的表达5-HT₇受体。虽然初始T细胞有5-HT_{1B}的mRNA表达,但是在蛋白水平只检测到了5-HT₇受体。T细胞激活后,5-HT₇、5-HT_{1B}和5-HT_{2A}的mRNA表达量会上升。为了确定5-HT₇在初始T细胞信号传导中发挥作用。作者使用5-HT₇选择性拮抗剂SB 269970 (10-100 nM)检测到该药剂能完全阻断5-HT诱导的ERK的激活和I κ Ba的磷酸化,而5-HT_{1B}的选择性拮抗剂SB 216641 (100 nM)没有效果。并且,5-HT₇受体的激动剂AS-19能够恢复T细胞由于5-HT合成受阻而导致的增殖降低现象。因此,5-HT至少在T细胞上通过5-HT₇受体行使自分泌信号传导作用。

还有一些研究证明5-HT_{1B}和5-HT_{2A}在激活的T细胞上有表达。Yin等(2006)人的研究表明5-HT_{1B}的拮抗剂抑制CD4⁺辅助性T细胞的增殖。Inoue等(2011)人的研究则证明5-HT_{2A}激动剂能够增强Concavalin-A对T细胞的激活作用,而5-HT_{2A}拮抗剂能够阻断T细胞受体调控的白介素2和interferon-gamma的生产。Akiyoshi等(2006)的结果也表明5-HT_{2A}的拮抗剂能够增强小鼠心脏移植的存活率。

有一些数据证明5-HT信号通路在B细胞也发挥了作用。SERT在1990年中期被发现。Serafeim等(2002)证明5-HT的重吸收能促进伯基特淋巴瘤(BL)细胞的凋亡。拮抗SERT后会减弱这个效果。Hernandez等(2010)发现人长期服用SSRIs会使体内B细胞数量增加30%左右。5-HT可以诱导细胞凋亡的原理尚不清楚,它可能是独立的氧化信号,但胞内的5-HT信号通路会参与其中。有趣的是,Serafeim等(2003)的研究发现高剂量SSRIs通过抑制DNA的合成,并且将细胞锁定在G₀/G₁阶段,可以直接加强BL细胞的凋亡,而正常的外周和扁桃腺B细胞对SSRIs诱导的细胞凋亡有抗性。因而推测这种选择性为使用SSRIs对BL进行治疗,而正常B细胞功能不受影响打开了一扇门。Meredith等(2005)还发现SERT在不同的B细胞系有表达,预示着SERT将作为B细胞恶性肿瘤的潜在治疗靶标。

有一些研究报道了B细胞上有5-HT₃受体的表达。胚胎阶段检测到B细胞上5-HT₃受体在mRNA水平高表达,而在初始B细胞和套细胞淋巴瘤里mRNA的表达量低很多(Ek *et al.*, 2002; Klein *et al.*, 2003)。Rinaldi等(2010)在蛋白水平证实了这些发现。

3 5-HT 和无脊椎动物免疫系统

目前,有关无脊椎动物5-HT参与免疫的研究还很少。Kim等人(2009)的研究表明5-HT能够促进甜菜夜蛾*Spodoptera exigua*血细胞的吞噬和结节,以及细胞数量的增加(Kim and Kim, 2010)。章鱼胺和5-HT能够调节美洲大蠊*Periplaneta americana*血细胞的吞噬和结节(Baines *et al.*, 1992)。总体而言,5-HT在无脊椎动物免疫系统中的作用及其机理的研究几乎空白,有待进一步加强。

第三章 菜青虫 5-羟色胺受体的克隆与药理学性质分析

1 引言

5-羟色胺 (5-hydroxytryptamine, 5-HT) 在生物体内发挥重要的作用, 它可以调控各种重要的生理和行为过程(Weiger, 1997; Blenau and Thamm, 2011)。5-HT 发挥作用主要通过对应的5-HT受体。脊椎动物中已克隆出14个5-HT受体基因, 其中一个编码离子通道 (5-HT₃), 其余13个基因编码G蛋白偶联受体 (G protein-coupled receptors, GPCR) (Blenau and Baumann, 2001)。根据序列同源性、基因的分布、与第二信使的偶联路径以及药理学性质, Hoyer等将脊椎动物体内GPCR型的5-HT受体分为6大类(Hoyer and Clarke, 1994; Hoyer and Martin, 1995; Hoyer and Martin, 1997)。其中: 5-HT₁ (5-HT_{1A/B/D/E/F}) 和 5-HT₅ (5-HT_{5A}) 抑制胞内cAMP的合成; 5-HT₂ (5-HT_{2A/B/C}) 调节胞内Ca²⁺量升高; 5-HT₄、5-HT₆和5-HT₇促进胞内cAMP的形成 (Hannon and Hoyer, 2008)。5-HT₅的另一个亚型5-HT_{5B}在人体内不编码功能性蛋白, 但在小鼠和大鼠体内有表达, 目前对5-HT_{5B}的了解相对较少 (Nelson, 2004)。

5-HT₁, 5-HT₂和5-HT₇早在7.5亿年前从最原始的5-HT受体进化而来, 5-HT₅和5-HT₇约6.5亿到7亿年之前从5-HT₁受体进化而来 (Peroutka and Howell, 1994; Walker *et al.*, 1996)。原口动物和后口动物约在6亿到6.5亿年前出现分化(Ayala *et al.*, 1998), 而5-HT受体的进化早于原口动物和后口动物的分化, 表明无脊椎动物和脊椎动物5-HT系统可能有大致相同的主要5-HT受体类型 (Peroutka and Howell, 1994; Hauser *et al.*, 2006)。但是无脊椎动物和脊椎动物5-HT受体不同亚型是独立进化的, 因而存在差异 (Peroutka and Howell, 1994; Tierney *et al.*, 2001)。目前昆虫中仅发现5种5-HT受体, 且均为GPCR。黑腹果蝇中5种5-HT受体即5-HT_{1A}(Saudou *et al.*, 1992)、5-HT_{1B} (Saudou *et al.*, 1992)、5-HT_{2A}(Colas *et al.*, 1995)、5-HT_{2B}(Gasque *et al.*, 2013)和 5-HT₇(Witz *et al.*, 1990), 分别与哺乳动物5-HT_{1A}、5-HT_{1B}、5-HT_{2A}、5-HT_{2B}和5-HT₇受体同源 (Blenau and Thamm, 2011)。目前无脊椎动物5-HT受体的分类主要以已有的脊椎动物5-HT受体分类系统, 基于受体氨基酸序列的同源性以及激活的第二信使作为分类依据。而包括昆虫在内

的无脊椎动物5-HT受体的药理学性质跟哺乳动物5-HT受体的药理学性质存在差异 (Hauser *et al.*, 2006)。由于目前有关无脊椎动物5-HT受体的药理学性质数据不多, 就无法以药理学性质差异为依据对无脊椎动物5-HT受体进行分类。这就表明还需要更多有关昆虫以及其它无脊椎动物5-HT受体的药理学研究数据。

本研究中, 我们从菜青虫中克隆得到了5-HT_{1A}、5-HT_{1B}、5-HT₇的完整开放阅读框序列以及5-HT_{2A}和5-HT_{2B}的部分cDNA序列, 并对这些受体的序列进行了分析。将5-HT_{1A}、5-HT_{1B}、5-HT₇分别表达于HEK 293细胞系中, 对其偶联的信号通路和药理学性质做了分析。通过药理学检测发现这三种受体能被5-HT这种生物胺特异性的激活, 并引起胞内cAMP浓度的变化。几种5-HT受体的广谱性激动剂和拮抗剂也对菜青虫5-HT受体起作用。这些数据将为研究5-HT受体参与调控菜青虫的生理和行为过程提供理论基础,

2 材料与方法

2.1 供试昆虫与药剂

菜青虫种群最初采自杭州市浙江大学卷心菜实验田。田间采来的虫子饲养于人工气候室, 饲养条件为 $25 \pm 1^\circ\text{C}$ 、R.H. = 70%、Light: Dark = 14:10 h。并饲以新鲜的卷心菜叶片。

多巴胺盐酸盐 (Dopamine hydrochloride), 酪胺盐酸盐 (Tyramine hydrochloride), 章鱼胺盐酸盐 (Octopamine hydrochloride), 5-羟色胺盐酸盐 (5-Serotonin hydrochloride), forskolin, G 418 二硫酸盐 (G418 disulfate salt), SB-269970 盐酸盐 (SB-269970 hydrochloride), 5-甲氧基色胺 (5-Methoxytryptamine), 8-Hydroxy-DPAT hydrobromide (8-OH-DPAT) 和 Methiothepin mesylate salt购买自 Sigma-Aldrich (St Louis, MO, USA)。SB 216641 和 RS 127445购买于Tocris Bioscience (Bristol, UK)

2.2 RNA 抽提

取30头菜青虫5龄幼虫的神经组织, 用TRIzol法 (Invitrogen, Carlsbad, CA, USA) 提取样品的总RNA, 具体操作步骤如下:

- (1) 在样品中加入 1 ml TRIzol和1颗不锈钢珠,用组织研磨仪TissuLyser II 匀浆 1 min, 室温静置 5 min;
- (2) 4°C, 12000 g 离心 10 min, 吸取上层液体至新的 1.5 ml 离心管;
- (3) 加入 200 μ l 氯仿剧烈振荡 15 sec, 室温静置 5 min;
- (4) 取上清到新的 1.5 ml 离心管中, 加入500 μ l 异丙醇, 室温静置 10 min 后, 放入离心机 (4°C, 12000 g) 离心 10 min;
- (5) 去除上清, 加入1 ml 75% 的乙醇, 涡旋混匀, 离心 (4°C, 7500 g) 5 min;
- (6) 去除上清, 室温干燥后将提取的RNA样品溶解在DEPC水中;
- (7) 用NanoDrop分光光度计 (Thermo Scientific) 检测RNA纯度及浓度。样品保存于-70°C。

2.3 基因克隆

2.3.1 编码菜青虫 5-HT_{1A} 的 cDNA

以1.0 μ g 总RNA为模板, 采用反转录试剂盒ReverTra Ace-kit (Toyobo, Osaka, Tapan)进行反转录合成第一链cDNA。采用Takara公司的3'- Full RACE Core Set合成3' RACE (Rapid amplification of cDNA ends) 模板和 5'- Full RACE Core Set试剂盒合成5' RACE 模板。依据本实验室得到的菜青虫转录组中5-HT_{1A} 基因的序列片段, 设计基因特异性3' RACE和5' RACE 引物。具体序列见表3.1。采用Takara LA Taq酶进行巢式PCR反应, 以扩增5-HT_{1A}的5'和3'端的序列。5' RACE 的具体反应条件如下, 第一轮PCR, 94 °C, 3 min; 94 °C, 30 sec, 60 °C, 30 sec, 72 °C, 1 min, 30个循环; 72 °C, 10 min。以1 μ l第一轮PCR产物为模板进行第二轮PCR。条件为, 94 °C, 3 min; 94 °C, 30 sec, 65°C, 30 sec, 72 °C, 1 min, 35个循环; 72 °C, 10 min。3' RACE 的具体反应条件如下, 第一轮PCR, 94 °C, 3 min; 94 °C, 30 sec, 55 °C, 30 sec, 72 °C, 1 min, 30个循环; 72 °C, 10 min。以1 μ l第一轮PCR产物为模板进行第二轮PCR。条件为, 94 °C, 3 min; 94 °C, 30 sec, 63°C, 30 sec, 72 °C, 1 min, 35个循环; 72 °C, 10 min。PCR 反应完成后, 进行1%的琼脂糖凝胶电泳检测, 将目的条带切下, 并用Axygen凝胶回收试剂盒回收。将回收的产物克隆到pGEM[®]-T Easy Vector (Promega,

Madison, WI, USA)后, 转入到Trans-T1感受态细胞中, 将菌液涂在含有Ampicillin和X-gal的LB固体培养基上, 过夜培养后进行蓝白斑筛选, 做菌落PCR, 将插入片段大小正确的菌株送到上海博尚测序公司测序。通过RACE得到5'和3'端的序列后, 设计全长引物克隆得到了*Pr5-HT_{1A}*的全长序列(表3.1)。

表 3.1 本研究中所用到的引物
Table 3.1 Primers used in this study

Primer name	Primer sequence (5'-3')
RACE PCR for <i>P. rapae</i>	
5-HT _{1A} -3'outer	CCAACGTACAAGGGCCGAAACAATTGC
5-HT _{1A} -3'inner	CCACGGTAGACACGTTAGACGAAGAACCA
5-HT _{1A} -5'outer	CGCTTCTCATTCTTATGTGGATGTAGTCG
5-HT _{1A} -5'inner	AATCCAACCCTGGCTAACCTCATAAACAGC
5-HT ₇ -5'outer	CGCTGTGGAACCTCTTGTAGGGTTC
5-HT ₇ -5'inner	GCTGCCCTCCATTTTTCACATTTA
Complete sequence	
5-HT _{1A} -compF	CGATTTTGAGGAGAGGAGC
5-HT _{1A} -compR	AACACATACCGTATCACAGAGC
5-HT _{1B} -compF	GATTGTGAGTGAGTGCGATGTGC
5-HT _{1B} -compR	GACAGATAACGCCTATTGCCTAC
5-HT ₇ -compF	TCAAACGGAGAAACCTGATGTA
5-HT ₇ -compR	CTGGGGTATTGACAATCATAGAAAG
Construction of expression vector	
5-HT _{1A} - Kpn I	TTGGTACCACCATGAACCTATCAGGGACA
5-HT _{1A} - EcoR I	CCGAATTCTCAGTATTTTTATT
5-HT _{1B} - Kpn I	TTGGTACCACCATGGAGGGCGTGTACC
5-HT _{1B} - EcoR I	TTTGAATTCTAGGGCGGAGCCCCGA
5-HT ₇ - Kpn I	TTGGTACCACCATGGCGTCTCAAAAT
5-HT ₇ - Xho I	CCCTCGAGTCATAGAAAGCTCTC

2.3.2 编码菜青虫 5-HT_{1B} 的 cDNA

以2.3.1节中的第一链cDNA为模板, 依据本实验室得到的菜青虫转录组中5-HT_{1B}基因的全长序列设计引物(表3.1)克隆得到*Pr5-HT_{1B}*的全长序列。

2.3.3 编码菜青虫 5-HT₇ 的 cDNA

依据本实验室得到的菜青虫转录组中5-HT₇基因的部分序列, 以2.3.1节中5' RACE 模板为模板进行5' RACE PCR。具体反应条件如下, 第一轮PCR, 94 °C, 3 min; 94 °C, 30 sec, 58 °C, 30 sec, 72 °C, 2 min, 30个循环; 72 °C, 10 min。以1 μl第一轮PCR产物为模板进行第二轮PCR。条件为, 94 °C, 3 min; 94 °C,

30 sec, 64°C, 30 sec, 72 °C, 2 min, 35个循环; 72 °C, 10 min。PCR反应结束后参照2.3.1节中的步骤, 对产物进行测序鉴定。然后设计全长引物克隆得到了 *Pr5-HT₇* 的全长序列 (表3.1)。

2.4 序列分析

获得了编码 *Pr5-HT_{1A}*、*Pr5-HT_{1B}*、*Pr5-HT₇* 的全长序列cDNA序列后, 用 DNASTar 软件包 (Version 7.1, DNASTar Inc) 对其ORF进行预测, 并对其等电点和分子量大小进行预测。利用NCBI网站的GenBank数据库, 通过BLASTX方法对其进行同源序列比对分析。用TMHMM Server v.2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>) 对跨膜结构域进行预测。用Clustal X 对从NCBI上下载的序列进行多重序列比对。通过MEGA 5.05 软件, 采用邻位相连法 (Neighbour-joining) 进行系统发育和分子进化分析。

2.5 质粒构建

2.5.1 *Pr5-HT_{1A}* 真核表达载体构建

用Premier primer 5 设计真核表达引物, 正向引物加上 *Kpn I* 酶切位点序列以及Kozak序列 (Kozak, 1987), 反向引物加上 *EcoR I* 酶切位点序列 (引物见表3.1)。采用KoD- Plus- Neo高保真性PCR酶进行PCR扩增。反应条件为: 94 °C, 2 min; 98 °C, 10 sec, 58 °C, 30 sec, 68 °C, 1 min, 40个循环; 68 °C, 7 min。PCR完成后用1%的琼脂糖凝胶电泳检测, 将目的条带切下, 并用Axygen凝胶回收试剂盒回收。利用限制性内切酶 *Kpn I* 和 *EcoR I* 分别对回收后的产物以及pcDNA3 (Invitrogen) 质粒进行双酶切处理。双酶切完成后, 用1%的琼脂糖凝胶电泳检测, 将目的条带切下, 并用Axygen凝胶回收试剂盒回收。用T4 DNA 连接酶 (宝生物) 将回收后的目的产物与5'端具有HA标签的载体产物连接。得到 pcDNA3-*Pr5-HT_{1A}* 融合质粒, 通过测序验证插入序列的正确性。

2.5.2 *Pr5-HT_{1B}* 真核表达载体构建

用2.5.1中的方法构建融合质粒pcDNA3-*Pr5-HT_{1B}*。

2.5.3 Pr5-HT₇真核表达载体构建

用2.5.1中的方法构建融合质粒pcDNA3-Pr5-HT₇。正向引物加上 *Kpn I* 酶切位点, 反向引物加上 *XhoI* 酶切位点序列(引物见表3.1)。双酶切所用酶为 *Kpn I* 和 *XhoI*。

2.6 细胞培养及转染

人胚肾细胞(Human Embryonic Kidney 293, HEK293)购买自上海中科院细胞库。HEK 293细胞培养于D-MEM培养基(Gibco BRL, Gaithersburg, MD), 培养基中还添加10% 胎牛血清(Fatal bovine serum, FBS) 和1%双抗(青霉素和链霉素)。细胞放在恒温的二氧化碳培养箱(37 °C, 5% CO₂)培养。参照说明, 用脂质体 2000 (Invitrogen) 分别将8 µg的质粒pcDNA3-Pr5-HT_{1A}, pcDNA3-Pr5-HT_{1B} 和pcDNA3-Pr5-HT₇ 转入5cm培养皿(Nunc)内呈指数生长的HEK 293细胞中(~4×10⁵个细胞)。转染24小时后按细胞数量1: 10传代。待传代细胞贴壁后, 用0.8 mg/ml的G418筛选两个星期左右, 最终获得稳定转染的细胞系。用RT-PCR和免疫细胞化学检测转染效率。

2.7 cAMP 检测

将稳定转染的细胞系传代至12孔板(Nunc)上, 至细胞长满(~1×10⁶/孔)。去除培养基, 用PBS洗两次。每孔加入450 µl用PBS配制的浓度为100 µM的3-isobutyl-1-methylxanthine (IBMX, Sigma-Aldrich), IBMX可以抑制磷酸二酯酶水解cAMP。室温孵育20 min。加入50 µl用PBS溶解的待测试剂, 轻轻混匀后在室温下孵育20 min。去除上层溶液, 加入250 µl细胞裂解液, 在-80 °C反复冻融两次, 使细胞完全裂解。收集裂解液于1.5 ml离心管, 12000 g, 4°C 离心10 min。吸取上清到另一个1.5 ml离心管, 继续做cAMP检测或将样品冻存于-80 °C冰箱。cAMP浓度检测所用试剂盒为cAMP Parameter Assay Kit (R&D Systems, Minneapolis, MN, USA), 具体操作步骤参照该试剂盒的说明书进行。

2.8 组织分布

在解剖镜下分别从5龄菜青虫幼虫体内提取脂肪体，表皮，马氏管，肠道，脑和神经索。取出各组织后，均放入PBS中漂洗三遍。采用TRIzol试剂(Invitrogen, CA)按照说明书提取以上各组织的总RNA。提取好总RNA后，采用DNase (RQ1-Rnase-Free DNase, Promega)消化去除基因组DNA。用反转录试剂盒 the ReverTra Ace qPCR kit 将1 μg RNA 反转录成 cDNA。用于荧光定量PCR分析的特异性引物见表3.2。用 $2^{-\Delta\Delta CT}$ 的方法 (Livak and Schmittgen, 2001) 对 *Pr5-HT_{1A}*、*Pr5-HT_{1B}*、*Pr5-HT₇* 的表达量定量。用18s RNA 作为内参基因。

表 3.2 荧光定量PCR所用到的引物
Table 3.2 Primers used for real-time PCR

Primer name	Primer sequence (5'-3')
18s-q-F	CCTGCGGAAGGATCATTAAAC
18s-q-R	AACGAAAGAAAGCGTCCAAA
5-HT _{1A} -q-F	TTTGCAGCTCAGCATCAATC
5-HT _{1A} -q-R	TTGGCTTACGAGGCACTTCT
5-HT _{1B} -q-F	AACCTGGTGGGAGACATCTG
5-HT _{1B} -q-R	CGGCCAGAGAAAGTATGAGC
5-HT ₇ -q-F	CTCCGCATCAGAAAAAGCTC
5-HT ₇ -q-R	ACGGCGTCAGGAATTGTATC

2.9 数据分析

药理学数据均采用单因素的方差分析，平均数之间的比较采用Tukey's multiple comparison test。用星号表示差异显著性，其中* $p<0.5$ ；** $p<0.01$ 。

3 结果与分析

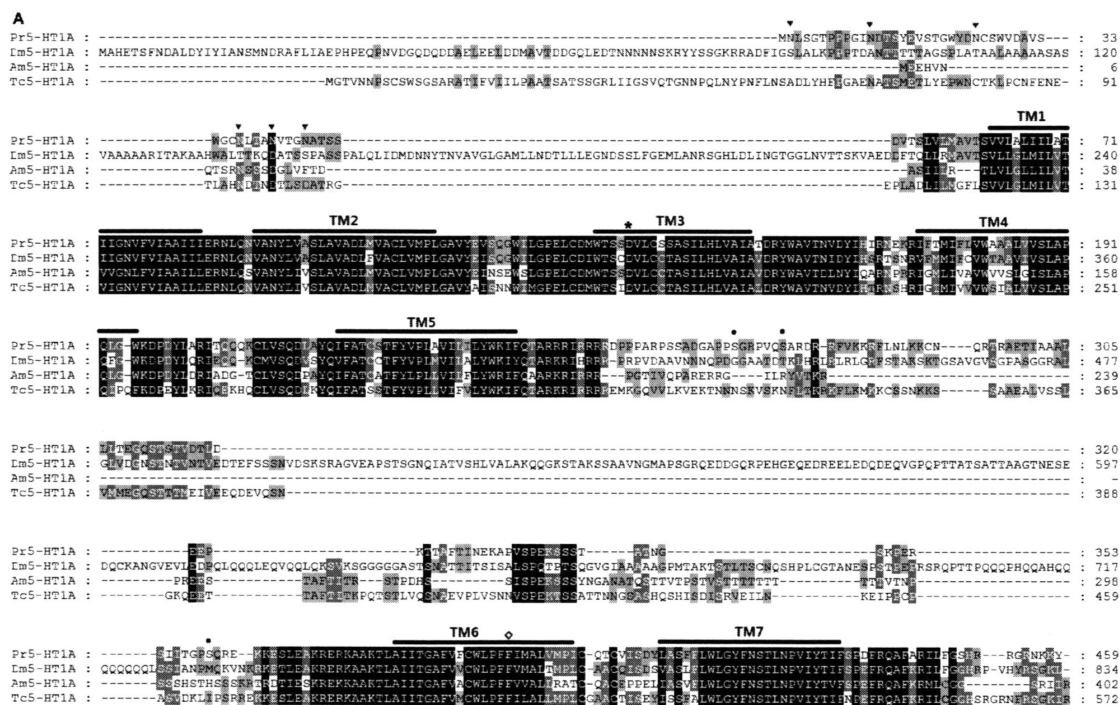
3.1 *Pr5-HT_{1A}*、*Pr5-HT_{1B}* 和 *Pr5-HT₇* 的 cDNA 的克隆及序列分析

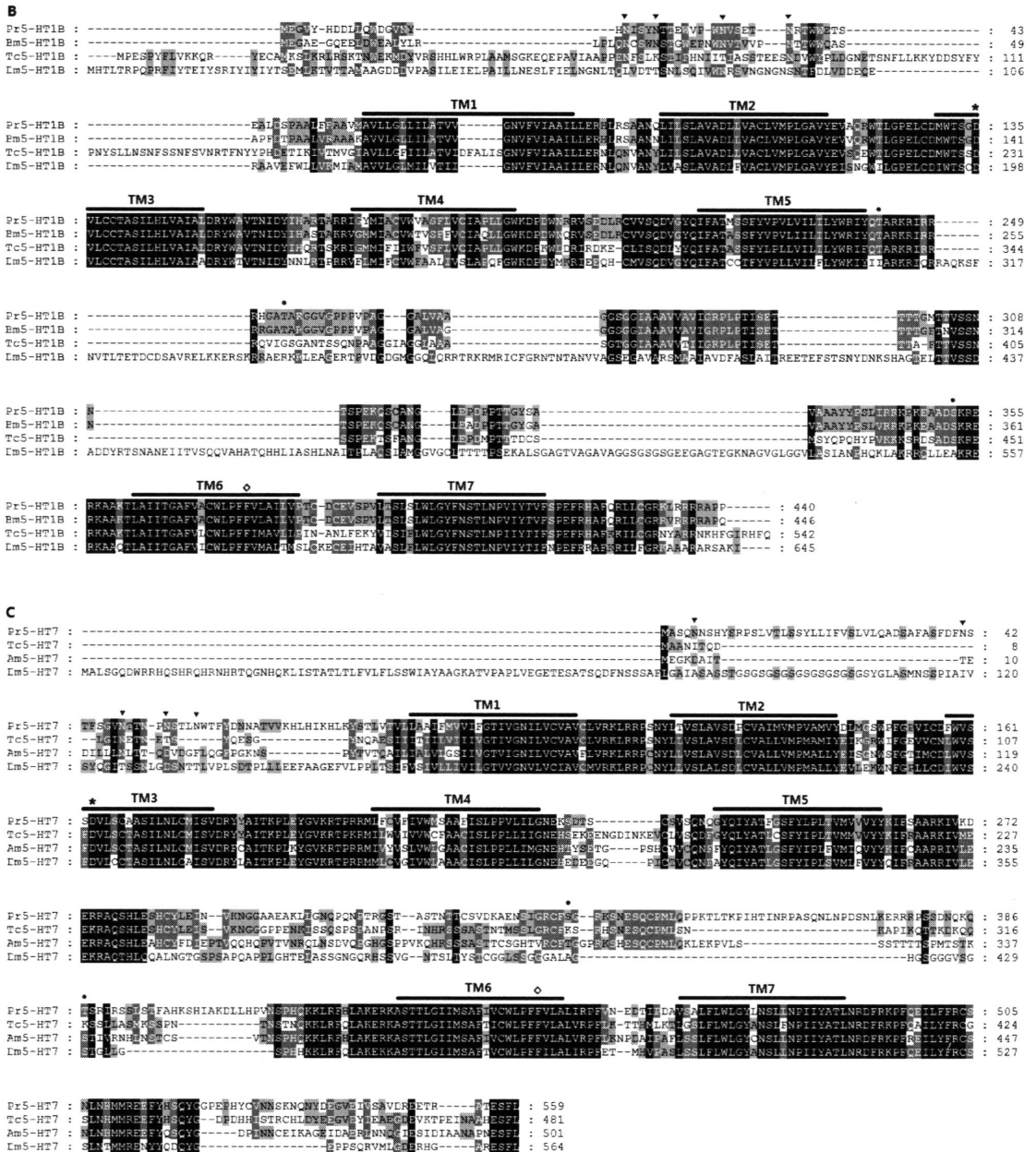
*Pr5-HT_{1A}*的Genbank登入号为KT946786。序列分析结果表明，编码*Pr5-HT_{1A}*的ORF大小为1380 bp，共编码459个氨基酸，预测分子量大小为50.98 kDa，等电点为9.09；*Pr5-HT_{1B}*的Genbank登入号为KT946787，编码*Pr5-HT_{1B}*的ORF大小为1323 bp，共编码440个氨基酸，预测分子量大小为48.31 kDa，等电点为8.57；

Pr5-HT₇ 的 Genbank 登入号为 KT946790, 编码 *Pr5-HT₇* 的 ORF 大小为 1680 bp, 共编码 559 个氨基酸, 预测分子量大小为 62.82 kDa, 等电点为 8.99。

通过 TMHMM 和 TMpre 预测发现这三个基因编码的蛋白均具有七个跨膜结构域 (图 3.1), 属于典型的 G 蛋白偶联受体。在跨膜 3 (TM3) 后胞内 2 区 (ICL2) 上存在典型的 D-R-Y 结构域, 在 TM7 上存在 N-P-x-x-Y 结构域, 这两个特征都在视紫红质类 G 蛋白偶联受体体内高度保守。Pr5-HT_{1A} 的 N 端有 6 个糖基化位点 N-x-[S/T], 第三内膜区有 3 个磷酸化激酶 C 位点 [S/T]-x-[R/K]。Pr5-HT_{1B} 的 N 端有 4 个糖基化位点, 第三内膜区有 3 个磷酸化激酶 C 位点。Pr5-HT₇ 的 N 端有 5 个糖基化位点, 第三内膜区有 2 个磷酸化激酶 C 位点。

对人和昆虫的 5-HT 受体基因构建系统发育进化树, 以邻近法 (Neighbor-joining) 重复 1000 次, 并以 DmFR 为外源基因, 分析结果表明 Pr5-HT_{1A}、Pr5-HT_{1B} 和 Pr5-HT₇ 分别跟人和昆虫的 5-HT_{1A}、5-HT_{1B} 和 5-HT₇ 受体聚在一起 (图 3.2)。



图3.1 *Pr5-HT_{1A}*、*Pr5-HT_{1B}*、*Pr5-HT₇*氨基酸序列跟其他昆虫同源基因的多重序列比对分析。Fig 3.1 Sequence alignments of *Pr5-HT_{1A}*、*Pr5-HT_{1B}*、*Pr5-HT₇* and orthologous receptors from other insects.

(A) *Pr5-HT_{1A}*氨基酸序列跟其他昆虫中同源基因的多重序列比对分析；(B) *Pr5-HT_{1B}*氨基酸序列跟其他昆虫中同源基因的多重序列比对分析；(C) *Pr5-HT₇*氨基酸序列跟其他昆虫中同源基因的多重序列比对分析。黑色线条表示跨膜区 (TM1-TM7)，黑色倒三角形表示糖基化位点，黑色圆圈表示磷酸化的蛋白激酶C的位点，星形表示配体结合相关位点，菱形表示第六个跨膜区中FXXXWXP位点中的第二个丙氨酸。

(A) Sequence alignments of *Pr5-HT_{1A}* and orthologous receptors from *Drosophila melanogaster* (Dm5-HT_{1A}, GenBank accession number CAA77570), *Apis mellifera* (Am5-HT_{1A}, accession number NP_001164579) and *Tribolium castaneum* (Tc5-HT_{1A}, accession number EFA10707). (B) Sequence alignments of *Pr5-HT_{1B}* and

orthologous receptors from *Bombyx mori* (Bm5-HT_{1B}, accession number NP_001037502), *T. castaneum* (Tc5-HT_{1B}, accession number XP_008197542) and *D. melanogaster* (Dm5-HT_{1B}, GenBank accession number CAA77571). (C) Sequence alignments of Pr5-HT₇ and orthologous receptors from *T. castaneum* (Tc5-HT₇, accession number NP_001280535), *A. mellifera* (Am5-HT₇, accession number NP_001071289) and *D. melanogaster* (Dm5-HT₇, accession number NP_524599). The seven transmembrane regions are predicted by TM1-TM7. Potential N-glycosylation sites and potential phosphorylation sites for protein kinase C are labelled by filled triangles and filled circles respectively. Residues are predicted to be involved in ligand binding are labeled with asterisks. The second phenylalanine after the FxxxWxP motif in TM6, which is a unique feature of aminergic receptors, is indicated by a rhombus.

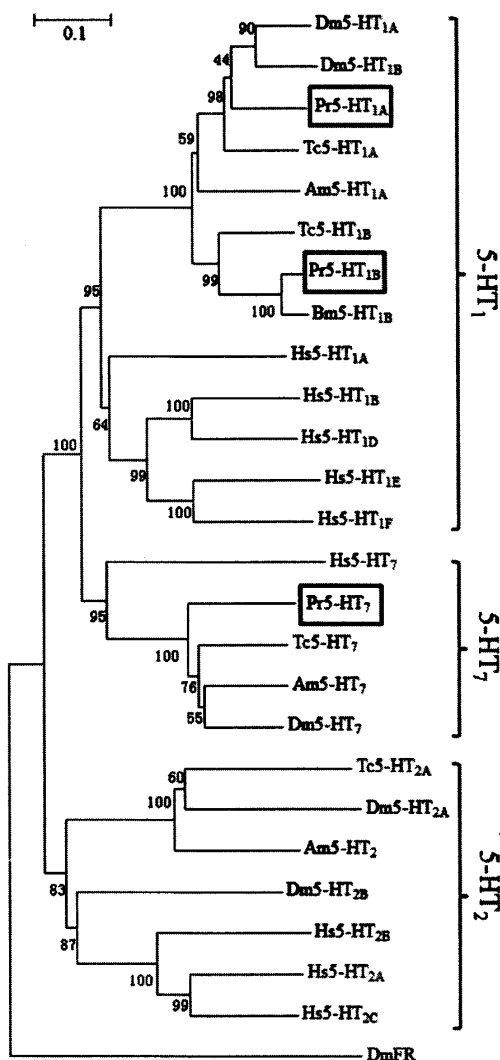


图3.2 Pr5-HT_{1A}、Pr5-HT_{1B}、Pr5-HT₇跟其他昆虫5-HT受体的进化树分析

Fig 3.2 Phylogenetic analysis of Pr5-HT_{1A}, Pr5-HT_{1B}, Pr5-HT₇ and various 5-HT receptors. *Apis mellifera* (Am5-HT_{1A}, NP_001164579; Am5-HT₇, NP_001071289). *Bombyx mori* (Bm5-HT_{1B}, NP_001037502). *Drosophila melanogaster* (Dm5-HT_{1A}, CAA77570; Dm5-HT_{1B}, CAA77571; Dm5-HT_{2A}, AAF52113; Dm5-HT_{2B}, NP_649806; Dm5-HT₇, AAF57104; DmFR, AAF47700). *Tribolium castaneum* (Tc5-HT_{1A}, EFA10707; Tc5-HT_{1B}, XP_008197542; Tc5-HT₇, NP_001280535). *Homo sapiens* (Hs5-HT_{1A}, CAH03197; Hs5-HT_{1B}, EAW48722; Hs5-HT_{2A}, AAM21129; Hs5-HT_{2B}, AAB31827; Hs5-HT₄, NP_000861; Hs5-HT_{5A}, AAM21132; Hs 5-HT₆, AAH74996; Hs5-HT₇, BAG70296).

3.2 Pr5-HT_{1A}、Pr5-HT_{1B} 和 Pr5-HT₇ 的药理学性质

为了研究Pr5-HT_{1A}、Pr5-HT_{1B}和Pr5-HT₇受体的信号转导及药理学性质,我们分别获得了稳定表达Pr5-HT_{1A}、Pr5-HT_{1B}和Pr5-HT₇受体的细胞系。10 μ M 的5-HT能够显著抑制表达Pr5-HT_{1A}受体细胞内forskolin引起的cAMP升高(图 3.3A)。5-HT有一定的剂量效应,EC₅₀值为15.49 nM(图 3.3B)。而章鱼胺(OA)、酪胺(TA)和多巴胺(DA)均不能引起类似的反应。

同样,5-HT能够显著抑制表达Pr5-HT_{1B}受体细胞内forskolin引起的cAMP升高(图 3.4A)。剂量反应实验表明5-HT的EC₅₀值为2.59 nM(图 3.4B)。5-HT受体的激动剂8-OH-DPAT也对Pr5-HT_{1B}受体有一定的激动效果(图 3.4C)。激动剂5-Methoxytryptamine (5-MT)对Pr5-HT_{1B}受体的激动效应有明显的剂量效应,EC₅₀值为1.44 μ M(图 3.4D)。拮抗剂Methiothepin可拮抗5-HT作用,IC₅₀值为2.49 μ M(图 3.4E)。SB-216641是人5-HT_{1B}的特异性拮抗剂,该试剂能部分拮抗5-HT对Pr5-HT_{1B}受体的反应(图 3.4F)。

四种生物胺中,5-HT能够显著诱导表达有Pr5-HT₇受体的细胞内产生cAMP(图 3.5A)。5-HT剂量效应实验表明其EC₅₀值为10.77 nM(图 3.5B)。激动剂8-OH-DPAT(图 3.5C)和5-MT(图 3.5D)对Pr5-HT₇受体的激动效应均有明显的剂量反应,EC₅₀值分别为3.02 μ M和4.29 μ M。Methiothepin也可拮抗5-HT对Pr5-HT₇受体作用,IC₅₀值为899.16 nM(图 3.5E)。10 μ M SB-269970能完全拮抗500 nM 5-HT引起的cAMP反应(图 3.5F)。

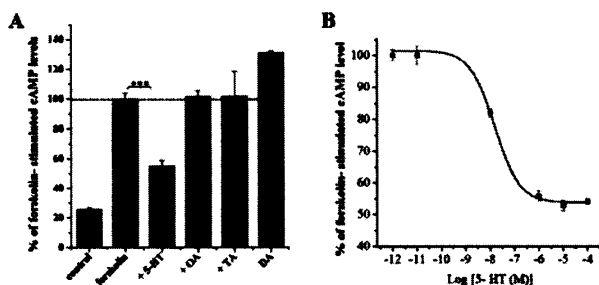


图3.3 不同生物胺、激动剂和拮抗剂对pcDNA3-Pr5-HT_{1A}/HEK 293的[cAMP]_i影响。

Fig 3.3 cAMP levels in HEK 293 cells expressing Pr5-HT_{1A} and treated with various aminergic ligands.

The amount of cAMP is given as the percentage of the value obtained with 10 μ M forskolin. (A) Effects of 10 μ M biogenic amines on 10 μ M forskolin-stimulated cAMP production in Pr5-HT_{1A}-expressing cells. (B) Dose-dependent effect of 5-HT (10⁻¹⁰ to 10⁻⁵ M) on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT_{1A} receptor. Data represent the mean $\pm$ SEM of four values.

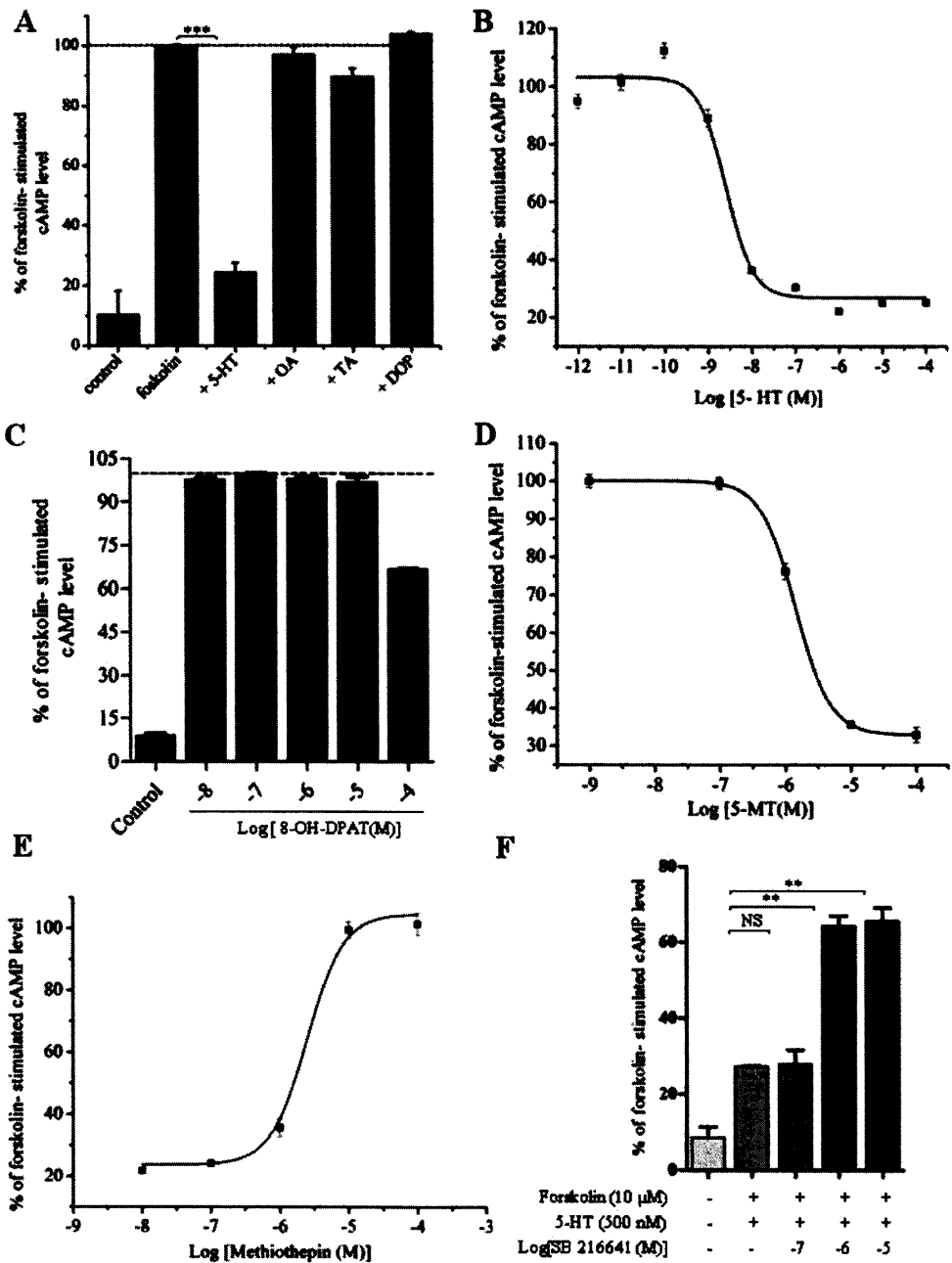


图3.4不同生物胺、激动剂和拮抗剂对pcDNA3-Pr5-HT_{1B}/HEK 293的[cAMP]_i影响。
Fig 3.4 cAMP levels in HEK 293 cells expressing Pr5-HT_{1B} and treated with various aminergic ligands.

The amount of cAMP is given as the percentage of the value obtained with 10 μ M forskolin. (A) Effects of 10 μ M biogenic amines on 10 μ M forskolin -stimulated cAMP production in Pr5-HT_{1B} -expressing cells. (B) Dose-dependent effect of 5-HT (10^{-12} to 10^{-4} M) on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT_{1B} receptor. Data represent the mean $\pm$ SEM of three values. (C) The effect of 8-OH-DPAT on [cAMP]_i. (D)

Dose-dependent effect of agonist 5-MT on 10 μ M forskolin-stimulated cAMP production in Pr5-HT_{1B}-expressing cells. (E) Dose-dependent effects of Pr5-HT_{1B} receptor antagonists (10^{-8} to 10^{-4} M) on 5-HT-mediated (500 nM) inhibition of forskolin-stimulated cAMP production in Pr5-HT_{1B}-expressing cells. Data represent the mean $\pm$ SEM of four values. (F) Effects of 5-HT and the antagonist SB 216641 on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT_{1B}. Data represent the mean $\pm$ SEM of three values.

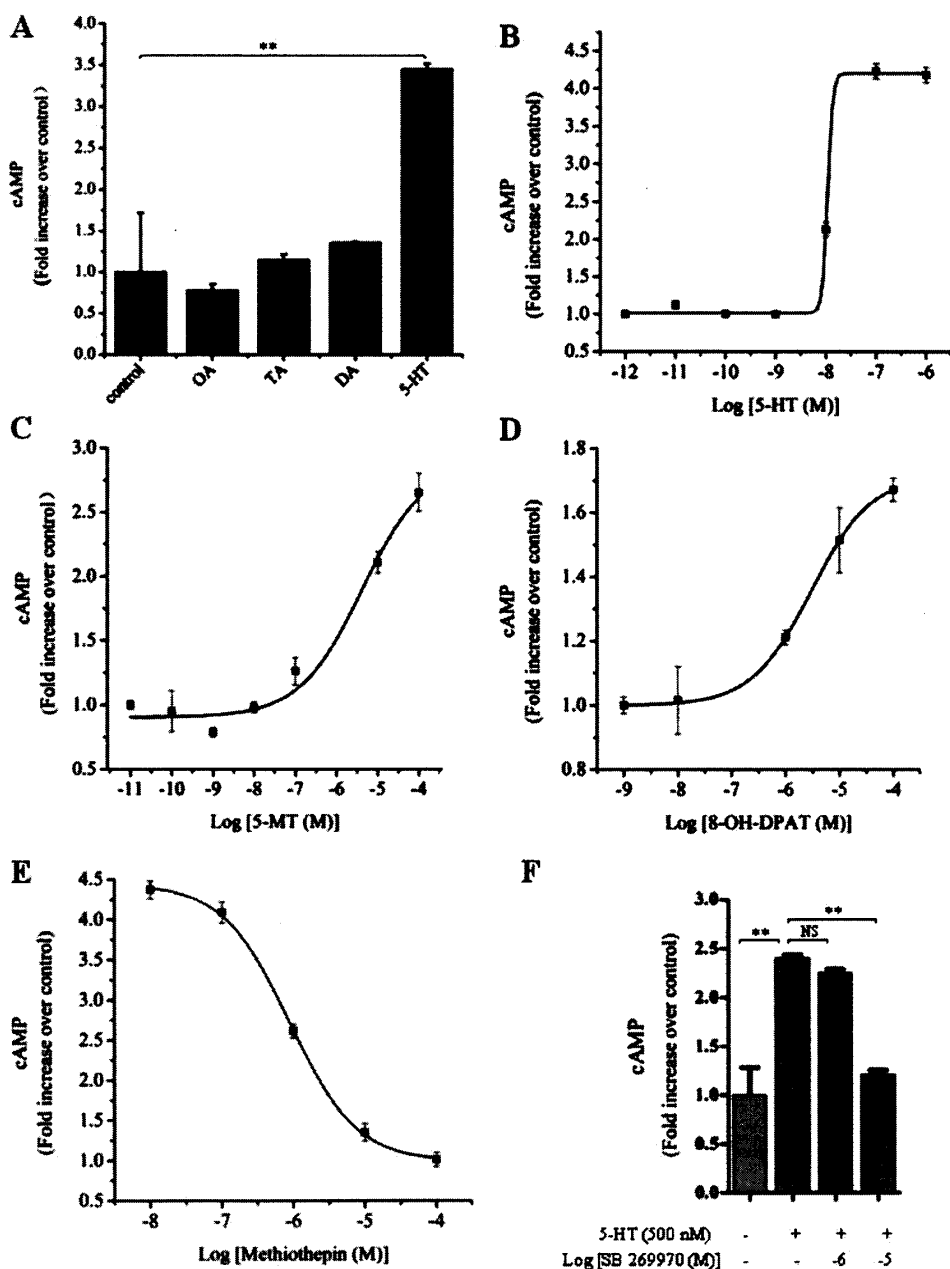


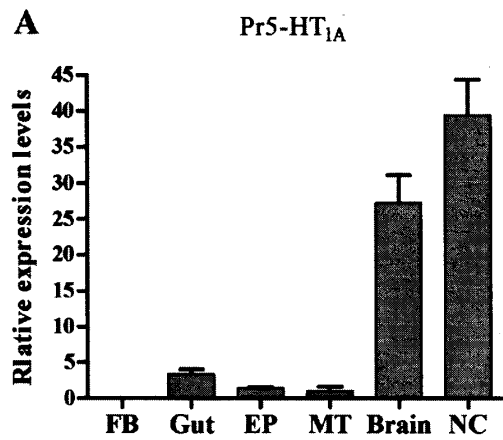
图3.5 不同生物胺、激动剂和拮抗剂对pcDNA3-Pr5-HT₇/HEK 293的[cAMP]_i影响。

Fig 3.5cAMP levels in HEK 293 cells expressing Pr5-HT₇ and treated with various aminergic ligands.

(A) Effects of 10 μ M biogenic amines on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT₇. (B) Dose-dependent effect of 5-HT (10^{-12} to 10^{-6} M) on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT₇ receptor. (C-D) Dose-dependent effect of 5-HT agonist 5-MT and 8-OH-DPAT on [cAMP]_i. (E) Dose-dependent effect of 5-HT antagonist (10^{-8} to 10^{-4} M) on 5-HT-mediated (500 nM) stimulation of cAMP production in Pr5-HT₇ -expressing cells. Data represent the mean $\pm$ SEM of four values. (F) Effects of 5-HT and the antagonist SB 269970 on intracellular cAMP levels in HEK 293 cells stably expressing Pr5-HT₇ receptor. Data represent the mean $\pm$ SEM of three values.

3.3 Pr5-HT_{1A}、Pr5-HT_{1B} 和 Pr5-HT₇ 的表达模式

用 qPCR 的方法分别对 Pr5-HT_{1A}、Pr5-HT_{1B} 和 Pr5-HT₇ 的表达谱进行了分析。Pr5-HT_{1A} 在脂肪体中不表达，但是在其它受测的组织中均有表达，包括肠道，表皮，马氏管，脑和神经索（图 3.3A）。其中，该基因在神经组织包括脑和神经索中表达量最高，非神经组织中肠道表达量最高。Pr5-HT_{1B} 在所有检测的组织中均有表达，但是在神经组织中表达量最高，其次是肠道和表皮，脂肪体和马氏管中仅有微量表达（图 3.3B）。Pr5-HT₇ 也在所有检测的组织中均有表达，但是肠道表达量最高，其次是马氏管，这两个组织中该基因的表达量显著高于神经组织，而在脂肪体和表皮中表达量最低（图 3.3C）。这些数据显示三个基因的表达模式存在异同，预示着这三个基因可能参与调控菜青虫不同的生理功能。



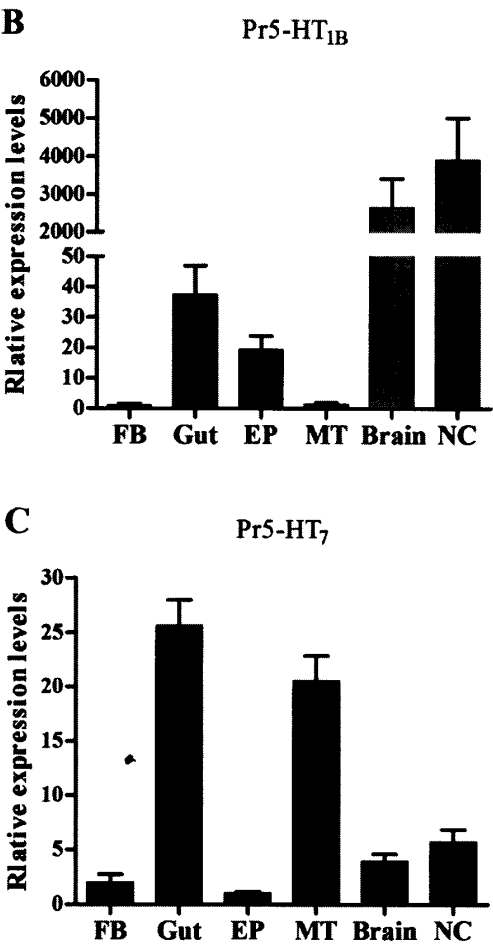


图 3.3 Pr5-HT_{1A}、Pr5-HT_{1B} 和 Pr5-HT₇ 的组织分布
Fig 3.3 Expression pattern of Pr5-HT_{1A}, Pr5-HT_{1B} and Pr5-HT₇ mRNA levels in tissues of fifth-instar larvae were quantified by qPCR.
Tissues tested are fat body (FB), Gut, epidermis (EP), Malpighian tubules (MT), Brain and nerve cord (NC). Data represent means± S.E. (n= 3 repetitions)

4 讨论

本研究中,我们从菜青虫体内克隆到了三个5-HT受体基因,分别为Pr5-HT_{1A}、Pr5-HT_{1B}和Pr5-HT₇。对这三个受体的序列和药理学性质进行了分析。这三个受体都有7个典型的跨膜区以及GPCR受体的保守结构域,其中Pr5-HT₇有八个跨膜域,这个特点在其它的GPCR类型的生物胺受体中也存在,比如黑腹果蝇Dm5-HT₇ (Witz *et al.*, 1990), DmTyR (Saudou *et al.*, 1990), 埃及伊蚊Aa5-HT₇

(Pietrantonio *et al.*, 2001) 以及牛蜚虫体内一个酪胺受体也有八个跨膜结构域 (Baxter and Barker, 1999)。5-HT_{1A}和5-HT_{1B}受体偶联G_i, 能够抑制胞内cAMP的生成。5-HT₇受体偶联G_s, 可以促进胞内cAMP的升高 (Hannon and Hoyer, 2008)。我们将这三个受体分别稳定表达于HEK 293细胞中, 药理学实验结果表明这三个受体的药理学性质存在一定差异。5-methoxytryptamine 和(±)-8-Hydroxy-2-(diethylamino) (8-OH-DPAT) 均对Pr5-HT_{1B}和Pr5-HT₇有激动作用。其中8-OH-DPAT对Pr5-HT_{1B}和Pr5-HT₇的激动效果相对较弱。8-OH-DPAT对*Apis mellifer*和*Periplaneta americana*的5-HT₁受体激动效果也较弱 (Thamm *et al.*, 2010; Troppmann *et al.*, 2010)。拮抗剂Methiothepin可拮抗5-HT对Pr5-HT_{1B}的cAMP影响, IC₅₀为2.49 μM (图 3.4E)。Methiothepin也可拮抗5-HT对Pr5-HT₇受体作用, IC₅₀为899.16 nM。Methiothepin (Monachon *et al.*, 1972) 是昆虫5-HT_{1A}(Thamm *et al.*, 2010)、5-HT_{2A}(Röser *et al.*, 2012)和5-HT₇(Schlenstedt *et al.*, 2006)的完全拮抗剂。SB-216641是哺乳动物5-HT_{1B}受体的选择性拮抗剂 (Price *et al.*, 1997), 该试剂能部分拮抗5-HT对Pr5-HT_{1B}受体的反应。SB-269970是哺乳动物5-HT₇受体的选择性拮抗剂 (Lovell *et al.*, 2000) 对昆虫的5-HT₇受体也有拮抗效果 (Schlenstedt *et al.*, 2006; Röser *et al.*, 2012)。10 μM SB-269970 能完全阻断500 nM 5-HT 引起的cAMP反应。荧光定量PCR结果表明Pr5-HT_{1A}和Pr5-HT_{1B}主要在神经组织中表达。而5-HT₇却主要在肠道和马氏管中表达, 其次是神经组织, 表明5-HT₇可能参与菜青虫的消化和排泄。通过RACE PCR未能克隆到Pr5-HT_{2A}和Pr5-HT_{2B}的5端cDNA序列, 因而无法得到这两个基因的开放阅读框, 所以不能对这两个受体进行药理学检测。随着转录组测序继续的发展, 希望接下来通过对菜青虫神经组织转录组测序能够获得Pr5-HT_{2A}和Pr5-HT_{2B}基因的全长, 从而构建真核表达载体, 检测这两个受体的药理学性质。

第四章 一种新型 5-羟色胺受体 5-HT₈ 的功能研究

1 引言

5-HT调节和调控脊椎动物和无脊椎动物很多的生理功能和行为, 比如调控七鳃鳗的运动(Harris-Warrick and Cohen 1985), 龙虾的进攻行为(Kravitz, 2000), 以及蠕虫的取食(Sze et al., 2000)。5-HT影响昆虫的繁殖(Lange, 2004), 聚集(Anstey et al., 2009)以及学习和记忆(Sitaraman et al., 2008)等。5-HT还调节人的一些疾病, 比如抑郁症、精神分裂症、饮食失调和强迫症都跟5-HT系统紊乱有关(Jones and Blackburn, 2002)。

5-HT主要通过受体行使功能。5-HT受体都是G蛋白偶联受体(GPCR), 除了5-HT₃是离子通道。目前, 已知脊椎动物体内有14个基因编码5-HT受体。脊椎动物中将GPCR型的5-HT受体分为6类, 即5-HT₁(包括5-HT_{1A}、5-HT_{1B}、5-HT_{1D}、5-HT_{1E}和5-HT_{1F}), 5-HT₂(包括5-HT_{2A}、5-HT_{2B}和5-HT_{2C}), 5-HT₄, 5-HT₅(包括5-HT_{5A}和5-HT_{5B}), 5-HT₆和5-HT₇。5-HT₁和5-HT₅跟G_i偶联, 抑制cAMP升高; 5-HT₄、5-HT₆和5-HT₇跟G_s偶联, 促进cAMP的生成; 5-HT₂跟G_q偶联, 可以升高胞内Ca²⁺浓度(Nichols and Nichols, 2008)。目前, 昆虫中已知的5-HT受体有三类, 即5-HT₁(5-HT_{1A}、5-HT_{1B})、5-HT₂(5-HT_{2A}、5-HT_{2B})和5-HT₇(Blenau and Thamm, 2011)。

5-HT受体的功能已在不同昆虫中得到研究。黑腹果蝇 *Drosophila melanogaster* 中, 5-HT_{1A}和5-HT₂通过不同的方式调控攻击行为(Johnson et al., 2009)。5-HT和5-HT₂受体在胚胎期表达量达到最大, 调控发育(Colas et al., 1995)。埃及伊蚊 *Aedes aegypti* 的5-HT₇受体在排泄和呼吸器官中有表达(Pietrantonio et al., 2001), 在马氏管和后肠中也有表达(Lee and Pietrantonio, 2003)。西方蜜蜂表达5-HT₇受体(Schlenstedt et al., 2006)以及5-HT_{1A}、5-HT₂(5-HT_{2α}和5-HT_{2β})受体(Thamm et al., 2010), 其中5-HT_{1A}能够调控视觉行为(Thamm et al., 2010)。Dacks等(2013)从烟草天蛾 *Manduca sexta* 的触角叶和大脑中克隆了四个5-HT受体。他们的研究发现5-HT_{1A}表达在GABAergic亚群的中间神经元, 因而推测5-HT受体表达在触角叶中。美洲大蠊 *Periplaneta americana* 中

的一个5-HT受体在唾液腺等多个组织有表达，并且该受体调控唾液腺的功能（Troppmann *et al.*, 2010）。蟋蟀*Gryllus bimaculatus*中至少表达四个5-HT受体，并且在中枢神经系统，咽侧体和唾液腺中均有表达（Watanabe *et al.*, 2011）。绿头苍蝇的唾液腺长期被用作研究5-HT功能的一个模型（Dalton, 1977）。丽蝇*Calliphora vicina*在唾液腺中表达5-HT_{2α}和5-HT₇两个受体（Roser *et al.*, 2012）。Vleugels等（2013）从赤拟谷盗中克隆了一个5-HT₁受体基因，并将该基因在中国仓鼠卵巢细胞WTA11 cells中表达，能显著抑制NKH-477激活胞内cAMP升高。

目前尚不清楚昆虫体内是否表达跟脊椎动物不同类型的5-HT受体。基于昆虫种类繁多，可推测其可能表达迄今未被发现的5-HT受体。本文揭示了菜青虫体内表达的一种未知5-HT受体，该受体基因跟目前已知的5-HT受体基因相似度不高，推测该受体应该属于一类新的5-HT受体，并将它命名为Pr5-HT₈。

2 材料与方法

2.1 供试昆虫和试剂

本研究中菜青虫的饲养第三章 2.1 节，所用的化学试剂来源见表 4.1。

表 4.1 本研究中所用试剂
Table 5.1 Compounds used in this report. S-A = Sigma-Aldrich; AS = Ascent Scientific; TB = Tocris Bioscience.

OMPOUND	ABBREV-IATION	ACTION
Isopropyl β-D-1-thiogalactopyranoside, Sigma-Aldrich, St. Louis, MO, USA	IPTG	Lactose analog
(±)-Octopamine hydrochloride (S-A)	OA	Biogenic amine
Tyramine hydrochloride (S-A)	TA	Biogenic amine
Dopamine hydrochloride (S-A)	DA	Biogenic amine
Serotonin hydrochloride (S-A)	5-HT	Biogenic amine
Forskolin (S-A)		Adenylyl cyclase activator
(±)-8-Hydroxy-2-(dipropylamino)tetralin hydrobromide (S-A)	8-OH-DPA T	5-HT _{1A} agonist
5-Carboxamidotryptamine maleate salt (S-A)	5-CT	5HT receptor agonist
G418 disulfate salt (S-A)		Antibiotic
Sumatriptan succinate (S-A)		5-HT ₁ agonist

WAY 100635 maleate salt (S-A)	5-HT _{1A} antagonist
NKH477 (S-A)	Forskolin analog
5-Methoxytryptamine (S-A)	5-HT agonist
3-Isobutyl-1- methylxanthine (S-A)	Inhibits cAMP phosphodiesterase
Adenosine (S-A)	
Histamine (S-A)	Biogenic amine
Melatonin (S-A)	Hormone
Tryptamine (S-A)	Biogenic amine
Methiothepin mesylate salt (S-A)	5-HT receptor antagonist
Pramipexole dihydrochloride, Ascent Scientific, Avonmouth, UK (AS)	Dopamine receptor agonist
Bromocriptine mesylate (AS)	Dopamine receptor agonist
6,7-ADTN hydrobromide (AS)	Dopamine receptor agonist
AS-19, Tocris Bioscience, Bristol, UK (TB)	5-HT ₇ receptor agonist
SB-269970 (TB)	5-HT ₇ receptor agonist
SB 216641 (TB)	5-HT _{1B} receptor antagonist
RS 127445 (TB)	5-HT _{2B} receptor antagonist

2.2 基因克隆

菜青虫神经组织RNA提取以及cDNA模板的获得参考第三章第2.2节和2.3节。通过本地BLAST分析本实验室已有的菜青虫神经组织转录组数据。找到一条可能为5-HT受体基因的序列，用EditSeq (version 5.02; DNASTar, Madison, WI, USA)找到该基因的ORF。用Premier primer 5设计全长引物，正向引物位于起始密码子上游Pr5HT₈-compF (5'-TTATGTGAGTTACCGAAATG-3')，方向引物位于终止密码子下游pr5HT₈-compR (5'-TAGTCCAATACGGATTACCA-3')。用TransStart_ Taq DNA Polymerase (TransGen, Beijing, China)根据操作手册进行PCR。PCR反应条件为：94℃, 3 min; 94℃, 30 s, 55℃, 30 s, 72℃, 2 min, 35个循环; 72℃, 10 min。PCR反应完成后，进行1%的琼脂糖凝胶电泳检测，将目的条带切下，并用Axygen凝胶回收试剂盒回收。将回收的产物克隆到pGEM®-T Easy Vector (Promega, Madison, WI, USA)后，转入到Trans-T1感受态细胞中，将菌液涂在含有Ampicillin和X-gal的LB固体培养基上，过夜培养后进行蓝白斑筛选，做菌落PCR，将插入片段大小正确的菌株送到上海博尚测序公司测序。

2.3 序列分析

参考第三章2.4节。

2.4 真核表达载体构建

参考第三章2.5.1中的方法构建融合质粒pcDNA3-Pr5-HT₈。正向引物加上 *Kpn I* 酶切位点，反向引物加上 *XhoI* 酶切位点序列，引物为 Pr5-HT₈-*KpnI*, 5'-TTGGTACCACCATGGAAATAAATAAT-3'; Pr5-HT₈-*XhoI*, 5'-CCCTCGAGTTACCATTTCCCTT-3'。双酶切所用酶为 *Kpn I* 和 *XhoI*。

2.5 细胞培养及转染

参考第三章2.6节中的方法。

2.6 cAMP 检测

参考第三章2.7节中的方法。

2.7 Ca²⁺检测

将转染有 pcDNA3-Pr5-HT₈ 的 HEK-293 细胞种在 12 mm 玻片上 (Thermo Scientific)。次日取出预培养的细胞，除去培养基。用 PBS 洗两次后，加入 2 mL 用 PBS 稀释的 1 μ mol/L Fura 2-AM (Dojindo Laboratories, Kumamoto, Japan); 37°C 避光孵育 30 min，去除 Fura 2-AM 工作液，用 Ca²⁺ 成像过程使用的缓冲液 (152 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 1.8 mM CaCl₂, 0.8 mM MgCl₂, and 10 mM HEPES, pH 7.4) 洗细胞两次。后面的步骤与第四章2.12节中 Ca²⁺ 测定方法

2.8 数据分析

药理学数据均采用单因素的方差分析，平均数之间的比较采用 Tukey's

multiple comparison test。用星号表示差异显著性，其中* $p<0.5$ ；** $p<0.01$ 。

3 结果与分析

3.1 Pr5-HT₈ cDNA 的克隆及序列分析

Pr5-HT₈基因的最大ORF为1317 bp (GeneBank No. KF878930)，编码438个氨基酸 (图4.1)，预测分子量为49.4 kDa，等电点为9.62。

Pr5-HT₈基因推导的氨基酸序列包括7个跨膜区 (TM1-TM7)，由胞外区和胞内区交替连接，这是GPCR受体的典型特征 (Strader *et al.*, 1995)。Pr5-HT₈基因序列具有配体结合和信号传导的保守位点 (图4.1)。N端有两个糖基化位点 (N₅, N₁₁)，第二内膜区和第三外膜区有3个蛋白激酶C磷酸化位点 (T₁₄₀, S₂₄₄, S₂₄₇)。而位于Pr5-HT₈第三跨膜区TM3的天冬氨酸D^{3.32}被亮氨酸L₁₀₆替代，而在其它的生物胺受体序列中，这个位点非常保守，因为它跟配体结合相关。Pr5-HT₈基因的TM3区另一个在生物胺受体基因中非常保守的位点苏氨酸T^{3.37}被蛋氨酸M₁₁₁替代。Pr5-HT₈基因序列中这些被替换的位点预示着该受体的配体可能是其它化合物，比如肽。其它昆虫中，该基因的同源基因在这两个位置也被替换成了非极性氨基酸 (图4.1)。Pr5-HT₈基因TM3的C端有保守序列D-R-Y(D₁₂₃-R₁₂₄-Y₁₂₅)，这是G蛋白受体必需的位点 (Moro *et al.*, 1993)。TM5区的丝氨酸残基 (S₁₈₉, S₁₉₂, S₂₀₄) 通过形成氢键能够提高跟配体的结合能力。Pr5-HT₈基因的TM6区的F₃₅₉-X-X-X-W₃₆₃-X-P₃₆₅序列是很多非肽类受体共同的特点。这段序列后的第二个苯丙氨酸F₃₆₇是胺类受体的特征序列。Pr5-HT₈基因的TM7区的N₄₀₇-P₄₀₈-X-X-Y₄₁₁的序列对受体sequestration 和 internalization很重要 (Barak *et al.*, 1994)。Pr5-HT₈基因的C端有一个半胱氨酸残基，它是翻译后棕榈酰化的可能性靶标。通过对Pr5-HT₈基因的序列分析，我们推测该受体是一个膜蛋白受体，尽管目前还没有关于该受体的报道。

通过BLAST分析，没有在哺乳动物的基因组里面找到Pr5-HT₈基因的同源基因，但是在某些昆虫中有该基因的同源序列。鳞翅目和鞘翅目一些害虫中有Pr5-HT₈基因的同源基因，比如小菜蛾*Plutella xylostella*、赤拟谷盗*T. castaneum*以及冈比亚按蚊*Anopheles gambiae*。而模式昆虫黑腹果蝇*D. melanogaster*以及西

方蜜蜂*A. mellifera*和丽蝇蛹集金小蜂*Nasonia vitripennis*基因中没有*Pr5-HT₈*基因的同源基因。*Pr5-HT₈*基因序列跟目前已知的5-HT受体序列相似度不高，因此，推测该受体可能为一类新的5-HT受体。通过序列比对，*Pr5-HT₈*基因跟其它昆虫中该基因的同源基因的相同性和相似性如下：*Bombyx mori*相同性60%，相似性75%；*P. xylostella*相同性44%，相似性61%；*Aedes aegypti*相同性36%，相似性56%；*T. castaneum*相同性36%，相似性56%；*Culex quinquefasciatus*相同性36%，相似性61%；*A. gambiae*相同性32%，相似性53% (所用到的序列信息见表4.2)。

将*Pr5-HT₈*与人和昆虫已知的GPCR型的生物受体作进化树分析，结果表明该受体跟已知的生物胺受体家族在不同的进化枝上（图4.2），所以预测*Pr5-HT₈*为一类新的受体。

表4.2 进化树中所用基因序列信息

Table 4.2 Accession numbers and annotations for the sequences used in the phylogenetic analysis.

Gene ID	Accession nr and description
AmTA ₁	NP_001011594.1 tyramine receptor type1 [<i>Apis mellifera</i>]
AmTA ₂	XP_394231.2 PREDICTED: putative tyramine receptor 2 [<i>Apis mellifera</i>]
AmOA ₁	NP_001011565.1 octopamine receptor [<i>Apis mellifera</i>]
AmOA _{2B1}	XP_397139.2 PREDICTED: octopamine receptor beta-1R [<i>Apis mellifera</i>]
AmOA _{2B2}	XP_396348.4 PREDICTED: octopamine receptor beta-2R isoform 5 [<i>Apis mellifera</i>]
AmOA _{2B3}	XP_397077.3 PREDICTED: similar to Octopamine receptor beta-3R (DmOct-beta-3R) [<i>Apis mellifera</i>]
AmDOP ₁	NP_001011595.1 dopamine receptor, D1 [<i>Apis mellifera</i>]
AmDOP ₂	NP_001011567.1 dopamine receptor 2 [<i>Apis mellifera</i>]
AmDOP ₃	NP_001014983.1 D2-like dopamine receptor [<i>Apis mellifera</i>]
Am5-HT _{1A}	FN645449.1 <i>Apis mellifera</i> mRNA for serotonin receptor (5-HT ₁ gene)
Am5-HT _{2A}	XP_394798.1 PREDICTED: similar to Serotonin receptor 2 CG1056-PB, isoform B [<i>Apis mellifera</i>]
Am5-HT _{2B}	NP_001191178.1 serotonin receptor [<i>Apis mellifera</i>]
Am5-HT ₇	AM076717.1 <i>Apis mellifera</i> mRNA for serotonin receptor (5-HT ₇ gene)
BmTA ₁	BAD11157.1 tyramine receptor 1 [<i>Bombyx mori</i>]
BmTA ₂	BAI52937.1 tyramine receptor 2 [<i>Bombyx mori</i>]
BmOA ₁	NP_001091748.1 octopamine receptor [<i>Bombyx mori</i>]
BmOA ₂	BAJ06526.1 octopamine receptor [<i>Bombyx mori</i>]
BmDOP ₁	NP_001108459.1 dopamine receptor-1 [<i>Bombyx mori</i>]
BmDOP ₂	NP_001108338 dopamine receptor 2 [<i>Bombyx mori</i>]
Bm5-HT _{1B}	CAA64862.1 serotonin receptor [<i>Bombyx mori</i>]
Bm5-HT ₈	XP_004923410.1 PREDICTED: alpha-1A adrenergic receptor-like [<i>Bombyx mori</i>]
BiOA ₁	GU074418.1 <i>Balanus improvisus</i> alpha-like octopamine receptor mRNA, complete cds
BiOA _{2B1}	GU074419.1 <i>Balanus improvisus</i> beta-like octopamine receptor R1 mRNA, complete cds
BiOA _{2B2}	GU074420.1 <i>Balanus improvisus</i> beta-like octopamine receptor R2 mRNA, complete cds
BiOA _{2B3}	GU074421.1 <i>Balanus improvisus</i> beta-like octopamine receptor R3 mRNA, complete cds
BiOA _{2B4}	GU074422.1 <i>Balanus improvisus</i> beta-like octopamine receptor R4 mRNA, complete cds

DmTA ₁	NP_524419.2 Octopamine-Tyramine receptor, isoform A [<i>Drosophila melanogaster</i>]
DmTA ₂	NP_650652.1 tyramine receptor [<i>Drosophila melanogaster</i>]
DmOA _{1a}	NP_732541.1 octopamine receptor in mushroom bodies, isoform A [<i>Drosophila melanogaster</i>]
DmOA _{1b}	NP_524669.2 octopamine receptor in mushroom bodies, isoform B [<i>Drosophila melanogaster</i>]
DmOA _{2B1}	NP_651057.1 octopamine receptor 2, isoform A [<i>Drosophila melanogaster</i>]
DmOA _{2B2}	NP_001034049.1 Octbeta2R, isoform A [<i>Drosophila melanogaster</i>]
DmOA _{2B3}	NP_001034043.1 Octbeta3R, isoform D [<i>Drosophila melanogaster</i>]
DmDOP ₁	CAA54451.1 dopamine receptor [<i>Drosophila melanogaster</i>]
DmDOP ₂	NP_733299.1 dopamine receptor 2, isoform A [<i>Drosophila melanogaster</i>]
DmDOP ₃	AAX52464.1 dopamine 2-like receptor, isoform C [<i>Drosophila melanogaster</i>]
Dm5-HT _{1A}	CAA77570.1 5HT-dro2A receptor (serotonin receptor) [<i>Drosophila melanogaster</i>]
Dm5-HT _{1B}	CAA77571.1 5HT-dro2B receptor (serotonin receptor) [<i>Drosophila melanogaster</i>]
Dm5-HT _{2A}	NP_524223.2 serotonin receptor 2, isoform A [<i>Drosophila melanogaster</i>]
Dm5-HT _{2B}	NP_001262373.1 CG42796, isoform E [<i>Drosophila melanogaster</i>]
Dm5-HT ₇	NP_524599.1 serotonin receptor 7 [<i>Drosophila melanogaster</i>]
DmFR	AAF47700.1 fmrf receptor [<i>Drosophila melanogaster</i>]
DmninaE	NP_524407.1 neither inactivation nor afterpotential E [<i>Drosophila melanogaster</i>]
HsA _{1A}	NP_000671.2 alpha-1A adrenergic receptor isoform 1 [<i>Homo sapiens</i>]
HsA _{1B}	NP_000670.1 alpha-1B adrenergic receptor [<i>Homo sapiens</i>]
HsA _{1D}	NP_000669.1 alpha-1D adrenergic receptor [<i>Homo sapiens</i>]
HsA _{2A}	NP_000672.3 alpha-2A adrenergic receptor [<i>Homo sapiens</i>]
HsA _{2B}	NP_000673.2 alpha-2B adrenergic receptor [<i>Homo sapiens</i>]
HsA _{2C}	NP_000674.2 alpha-2C adrenergic receptor [<i>Homo sapiens</i>]
HsB ₁	NP_000675.1 beta-1 adrenergic receptor [<i>Homo sapiens</i>]
HsB ₂	NP_000015.1 beta-2 adrenergic receptor [<i>Homo sapiens</i>]
HsB ₃	NP_000016.1 beta-3 adrenergic receptor [<i>Homo sapiens</i>]
HsD ₁	NP_000785.1 D(1A) dopamine receptor [<i>Homo sapiens</i>]
HsD ₂	NP_000786.1 D(2) dopamine receptor isoform long [<i>Homo sapiens</i>]
HsD ₃	NP_000787.2 D(3) dopamine receptor isoform a [<i>Homo sapiens</i>]
HsD ₄	NP_000788.2 D(4) dopamine receptor [<i>Homo sapiens</i>]
HsD ₅	NP_000789.1 D(1B) dopamine receptor [<i>Homo sapiens</i>]
Hs5-HT _{1A}	NP_000515.2 5-hydroxytryptamine receptor 1A [<i>Homo sapiens</i>]
Hs5-HT _{1B}	NP_000854.1 5-hydroxytryptamine receptor 1B [<i>Homo sapiens</i>]
Hs5-HT _{1D}	NP_000855.1 5-hydroxytryptamine receptor 1D [<i>Homo sapiens</i>]
Hs5-HT _{1E}	NP_000856.1 5-hydroxytryptamine receptor 1E [<i>Homo sapiens</i>]
Hs5-HT _{1F}	NP_000857.1 5-hydroxytryptamine receptor 1F [<i>Homo sapiens</i>]
Hs5-HT _{2A}	NP_000612.1 5-hydroxytryptamine receptor 2A isoform 1 [<i>Homo sapiens</i>]
Hs5-HT _{2B}	NP_000858.3 5-hydroxytryptamine receptor 2B [<i>Homo sapiens</i>]
Hs5-HT _{2C}	NP_000859.1 5-hydroxytryptamine receptor 2C isoform a precursor [<i>Homo sapiens</i>]
Hs5-HT _{5A}	NP_076917.1 5-hydroxytryptamine receptor 5A [<i>Homo sapiens</i>]
Hs5-HT ₄	NP_000861.1 5-hydroxytryptamine receptor 4 isoform b [<i>Homo sapiens</i>]
Hs5-HT ₆	NP_000862.1 5-hydroxytryptamine receptor 6 [<i>Homo sapiens</i>]
Hs5-HT ₇	NP_000863.1 5-hydroxytryptamine receptor 7 isoform a [<i>Homo sapiens</i>]

TcTA ₁	NP_001164311.1 tyramine/octopamine receptor [<i>Tribolium castaneum</i>]
TcTA ₂	XP_001811970.1 PREDICTED: similar to putative GPCR class a orphan receptor 4 (AGAP004034-PA) [<i>Tribolium castaneum</i>]
TcOA ₁	XP_970007.2 PREDICTED: similar to octopamine receptor [<i>Tribolium castaneum</i>]
TcOA _{2B1}	XP_974265.1 PREDICTED: similar to GA19956-PA [<i>Tribolium castaneum</i>]
TcOA _{2B2}	XP_974214.1 PREDICTED: similar to beta adrenergic receptor [<i>Tribolium castaneum</i>]
TcOA _{2B3}	XP_974238.2 PREDICTED: similar to Octopamine receptor beta-3R (DmOct-beta-3R) [<i>Tribolium castaneum</i>]
TcDOP ₁	XP_971542.2 PREDICTED: similar to dopamine receptor 1 [<i>Tribolium castaneum</i>]
TcDOP ₂	XP_972779.2 PREDICTED: similar to dopamine receptor 2 [<i>Tribolium castaneum</i>]
TcDOP ₃	XP_969037.2 PREDICTED: similar to Dopamine 2-like receptor CG33517-PA [<i>Tribolium castaneum</i>]
Tc5-HT _{1A}	XP_967449.2 PREDICTED: similar to serotonin receptor [<i>Tribolium castaneum</i>]
Tc5-HT _{1B}	XP_972856.2 PREDICTED: similar to putative serotonin receptor [<i>Tribolium castaneum</i>]
Tc5-HT _{2A}	XP_972327.1 PREDICTED: similar to putative serotonin 5HT-2a receptor (AGAP002232-PA) [<i>Tribolium castaneum</i>]
Tc5-HT ₇	XP_966577.2 PREDICTED: similar to serotonin receptor 7 [<i>Tribolium castaneum</i>]
Tc5-HT ₈	XP_001809893.1 PREDICTED: similar to serotonin receptor, putative [<i>Tribolium castaneum</i>]
Cq5-HT ₈	XP_001863625 serotonin receptor [<i>Culex quinquefasciatus</i>]
Ag5-HT ₈	XP_312245.4 AGAP002679-PA [<i>Anopheles gambiae</i> str. PEST]
Aa5-HT ₈	XP_001658546.1 serotonin receptor, putative [<i>Aedes aegypti</i>]
Px5-HT ₈	The sequence of Px5-HT ₈ was downloaded from diamondback moth genome database (http://www.iae.fafu.edu.cn/DBM/) Px010018 5-hydroxytryptamine receptor 1A-alpha [<i>Plutella xylostella</i>]

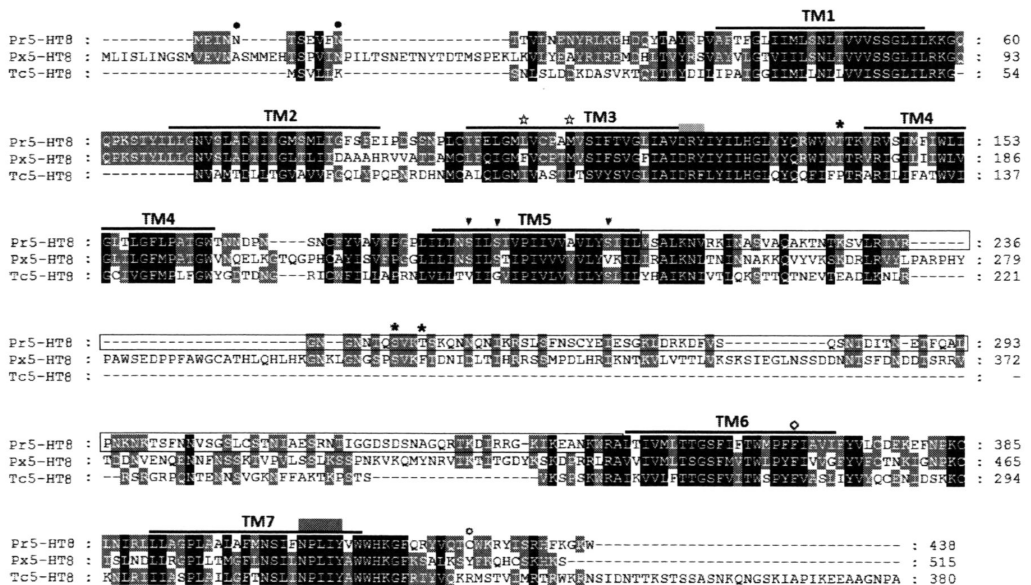


图4.1 *Pr5-HT₈*氨基酸序列跟其他昆虫中同源基因的多重序列比对分析。

Fig 4.1 Amino acid sequence alignment of Pr5-HT₈ and orthologous receptors from *Plutella*

xylostella (Px5-HT₈; accession no. Px 010018) and *T. castaneum* (Tc5-HT₈; accession no. XP_001809893). Putative transmembrane domains are indicated by TM1 to TM7. The shaded sequences highlight the identity level of amino acids between the receptors. Potential N-glycosylation and phosphorylation sites are labeled by filled circles and asterisks, respectively. Serine residues (S₁₈₉, S₁₉₂, S₂₀₄) predicted to be involved in agonist binding are labeled with filled triangles. The second phenylalanine after the FxxxWxP motif in TM6, is indicated by a rhombus. The cysteine residue (C₄₂₅) in the C-terminus, a potential target for post-translational palmitoylation, is labelled by a unfilled circle. The corresponding positions of the conserved Asp^{3.32} and Thr^{3.37} in other aminergic receptors are indicated by unfilled stars. Letters in the box represent the region used to raise Pr5-HT₈-specific antibodies. The amino acid position is indicated on the right.

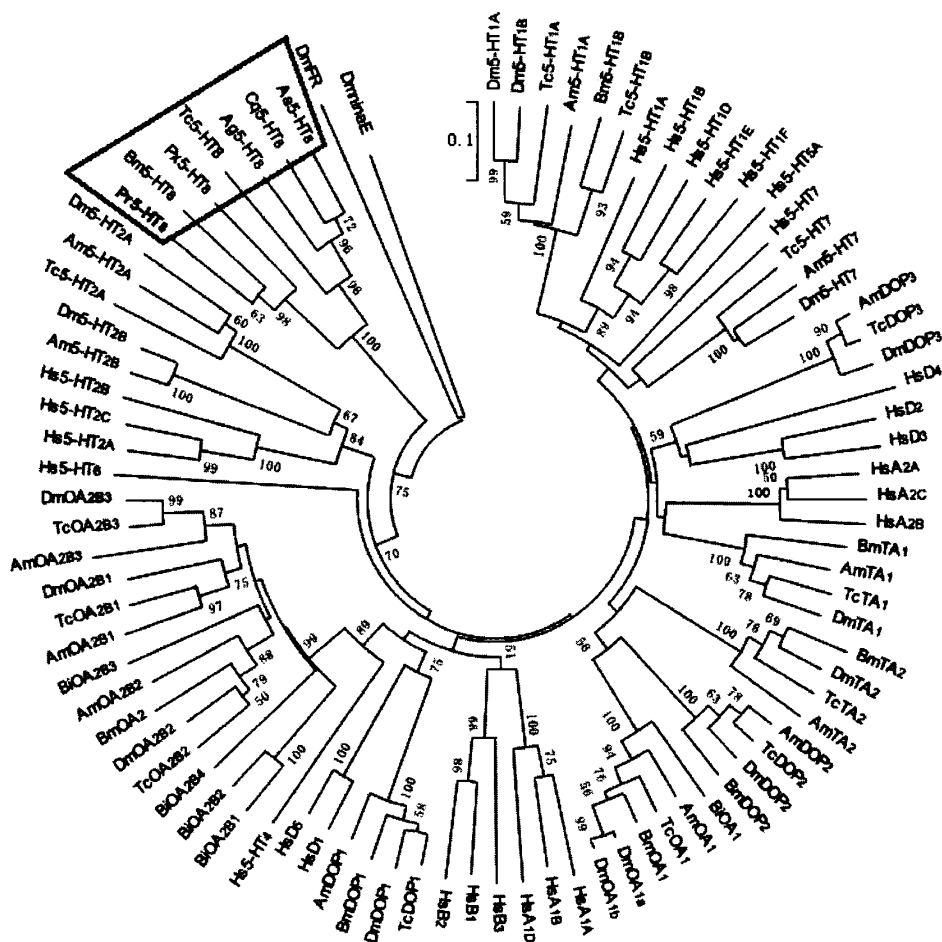


图4.2 Pr5-HT₈跟其他生物生物胺受体的进化树分析

Fig 4.2 Phylogenetic analysis of Pr5-HT₈ and selected biogenic amine receptors.

Neighbor joining trees were constructed using MEGA 5 software with 1000-fold bootstrap re-sampling. The numbers at the nodes of the branches represent the level of bootstrap support for each branch. DmFR and DmnaE were used as out-groups. The receptor sequences, followed by their GenBank accession numbers are list in Table 5.1.

3.2 Pr5-HT₈ 受体的配体特异性检测

各种生物胺(包括5-羟色胺、章鱼胺OA、酪胺TA和多巴胺DA)均不能促进pcDNA3-Pr5-HT₈/HEK 293胞内cAMP浓度的升高(图4.3A),也不能抑制由NKH 477引起的胞内cAMP的增加(图4.3B)。但是,低浓度的5-HT能够引起pcDNA3-Pr5-HT₈/HEK 293胞内Ca²⁺浓度升高,并且有梯度效应,EC₅₀值为44.8 nM(图4.5)。5-HT不会引起未转染的HEK 293细胞的Ca²⁺浓度变化。腺苷、色胺和褪黑激素均不会引起pcDNA3-Pr5-HT₈/HEK 293胞内第二信使浓度的变化(图4.4)。而组胺所引起的pcDNA3-Pr5-HT₈/HEK 293胞内Ca²⁺浓度变化跟未转染的HEK 293细胞胞内Ca²⁺浓度变化没有差异,可能是由于HEK 293细胞有内源性组胺受体H1,该受体偶联G_q,能引起胞内Ca²⁺浓度升高(Iwata *et al.*, 2005)。但是组胺不会影响pcDNA3-Pr5-HT₈/HEK 293胞内cAMP浓度变化(图4.4)。

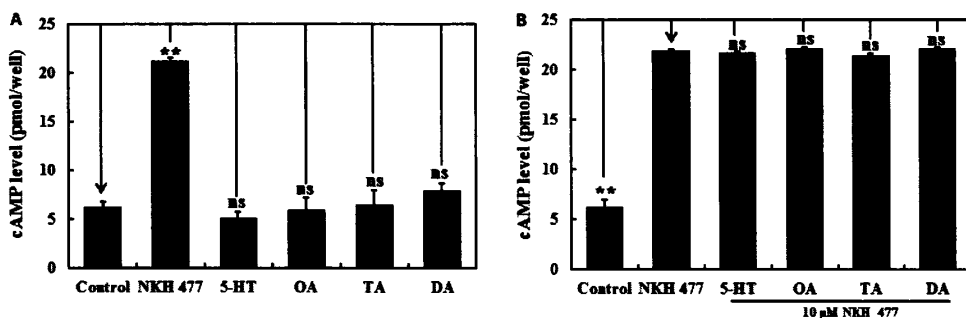


图4.3 不同生物胺对pcDNA3-Pr5-HT₈/HEK 293的[cAMP]_i影响.

Fig 4.3 Modulation of [cAMP]_i in HEK-293 cells stably expressing Pr5-HT₈. (A) Effects of various biogenic amines and 10 μM NKH 477 on [cAMP]_i in HEK-293. (B) Effects of various biogenic amines on [cAMP]_i stimulated by 10 μM NKH 477 in HEK-293 cells. Data represent means ± SE of three experiments. Asterisks indicate values significantly different from the control value using one-way ANOVA with the Tukey-Kramer multiple comparisons test (***P* < 0.001), and ns means no significant difference.

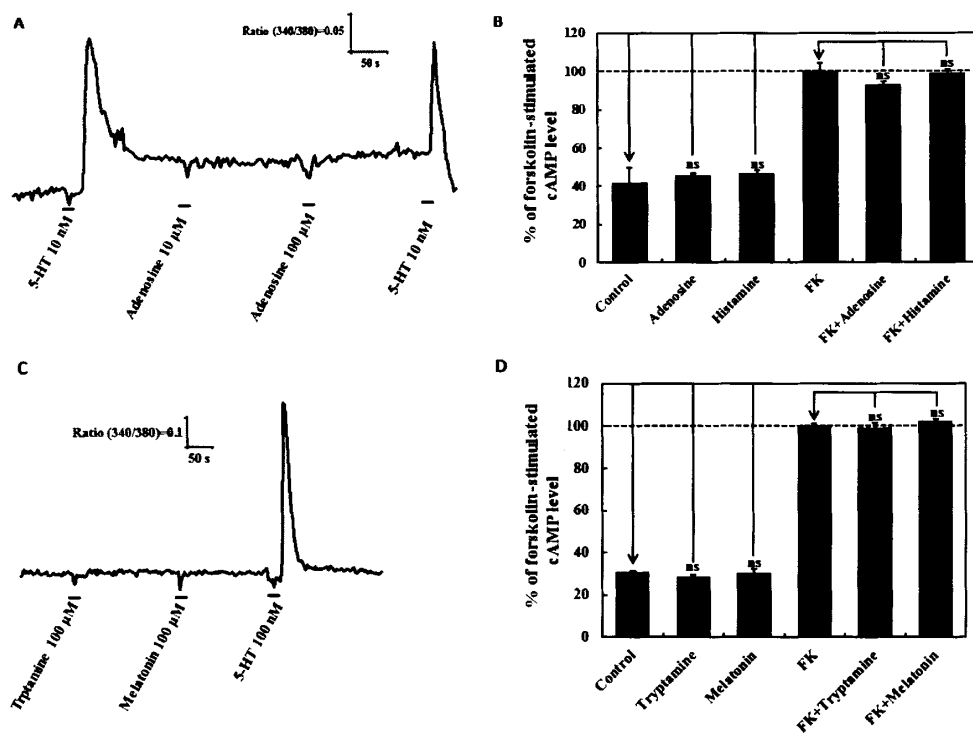


图4.4 其它一些配体对pcDNA3-Pr5-HT₈/HEK 293的[cAMP]_i和[Ca²⁺]_i影响。

Fig 4.4 Effects of histamine, adenosine, tryptamine and melatonin on [cAMP]_i and [Ca²⁺]_i in HEK-293 cells constitutively expressing Pr5-HT₈. Data represent means ± SE of three experiments. Asterisks indicate values significantly different from the control value using one-way ANOVA with the Tukey-Kramer multiple comparisons test and ns means no significant difference. (A) Effects of 100 μM adenosine on [Ca²⁺]_i in HEK-293 cells. (B) Effects of 10 μM histamine and adenosine on intracellular cAMP levels in HEK-293 cells in the presence and absence of 10 μM forskolin (FK). Data represent means ± SE of three experiments. ns means no significant difference. (C) Effects of 100 μM tryptamine and melatonin on [Ca²⁺]_i in HEK-293 cells. (D) Effects of 10 μM histamine and adenosine on intracellular cAMP levels in HEK-293 cells in the presence and absence of 10 μM FK.

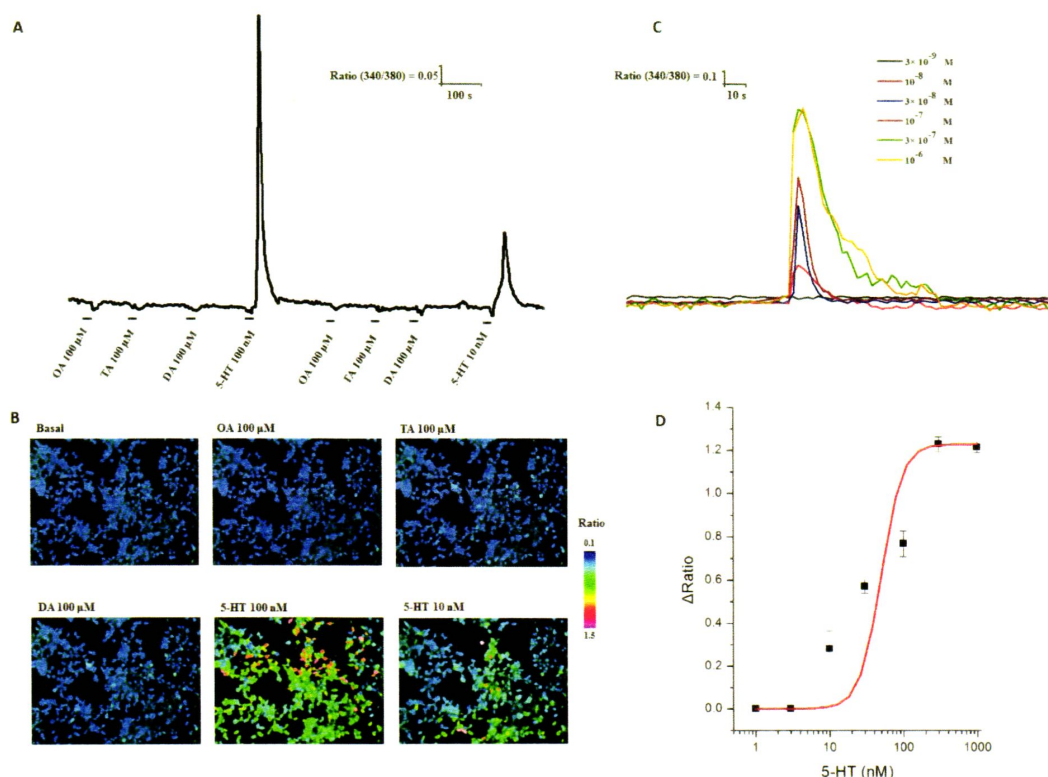


图4.5 不同生物胺对pcDNA3-Pr5-HT₈/HEK 293的[Ca²⁺]_i影响

Fig 4.5 Representative Ca^{2+} responses and dose-response profiles for 5-HT in HEK293 cells stably expressing Pr5-HT8. (a) Effects of 5-HT and the biogenic amines OA, TA, and DA on $[\text{Ca}^{2+}]_i$ in a representative single HEK293 cell. (b) Pseudocolored images of HEK293 cells following stimulations with a series of biogenic amines. (c) Increasing concentrations of 5-HT induced changes of fluorescence ratio in a dose-dependent way. (d) Dose-response curve for 5-HT in cells, obtained from Ca^{2+} imaging. We analyzed the data in terms of two binding models, a one-site and a two-site model. The resulting F statistic indicates a closer fit to the one-site binding model ($F = 2.6 \ 9 \ 10_{-11}$, $p = 1$). Each point represents the mean $\pm$ SE from 30 to 50 cells in a representative experiment from three to five replicates.

3.3 激动剂和拮抗剂对受体的偶联的 Ca^{2+} 信号的影响

5-HT 受体的激动剂 5-MT、8-OH-DPAT 和 5-CT 能够引起 pcDNA3-Pr5-HT₈/HEK 293 胞内 Ca^{2+} 浓度升高, 并且都有剂量效应。而 sumatriptan 和 AS-19 不能引起胞内 Ca^{2+} 浓度变化 (图 4.6A 和 B)。5-MT 的 EC_{50} 值为 14.1 nM, 5-CT 的 EC_{50} 值为 19.0 nM, 8-OH-DPAT 的 EC_{50} 值为 603.1 nM (图 4.6C)。DA 受体的激动剂包括 pramipexole、bromocriptine 和 6,7-ADTN hydrobromide 均不能引起 pcDNA3-Pr5-HT₈/HEK 293 胞内 Ca^{2+} 浓度变化 (图 4.6D)。Methiothepin, 一种 5-HT

受体的广谱性拮抗剂,能够激活Pr5-HT₈受体,并且有梯度反应,EC₅₀值为17.4 μM (图4.7)。5-HT₇受体的拮抗剂SB-269970, 5-HT_{1B}受体拮抗剂SB-216641和人5-HT_{2B}受体的拮抗剂RS-127445都不能抑制5-HT引起的pcDNA3-Pr5-HT₈/HEK 293胞内Ca²⁺浓度升高(图4.8A-C)。而5-HT_{1A}受体的拮抗剂WAY-100635能够拮抗5-HT引起的胞内Ca²⁺浓度变化(图4.8D)。

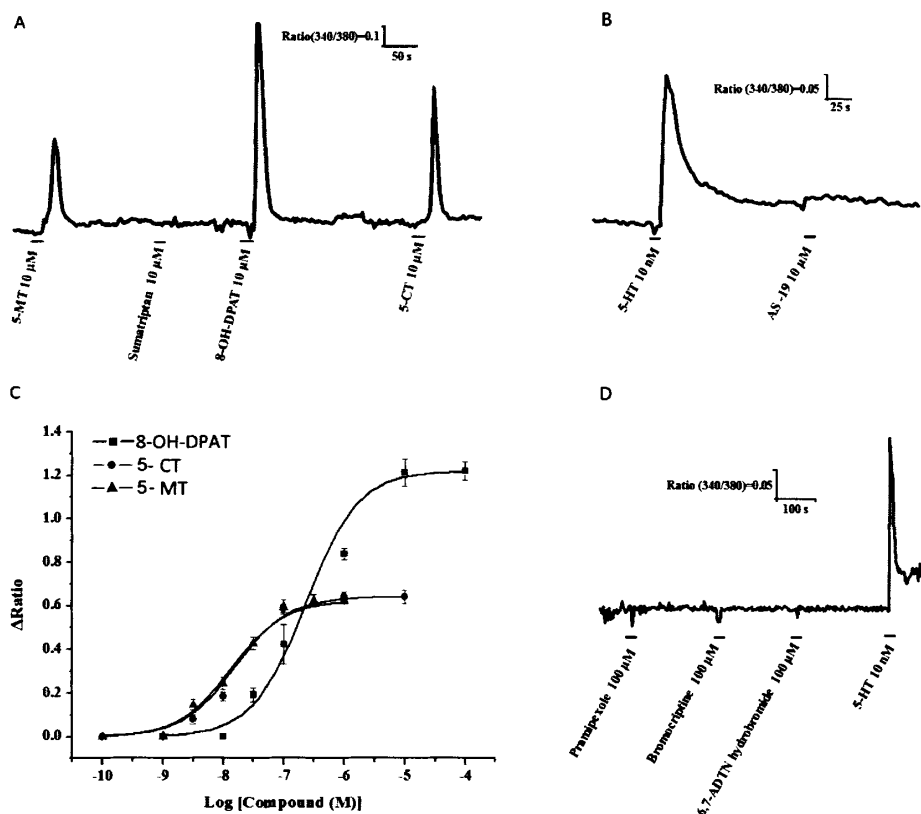


图4.6 5-HT受体激动剂对pcDNA3-Pr5-HT₈/HEK 293的[Ca²⁺]_i影响

Fig 4.6 Representative Ca²⁺ responses and dose-response profiles for various agonists in HEK-293 cells stably expressing Pr5-HT₈. (A) Effects of various 5-HT receptor agonists (10 μM) on [Ca²⁺]_i in a representative single HEK-293 cell. (B) Effects of 10 μM AS-19 on [Ca²⁺]_i in a representative single HEK-293 cell. (C) Dose-response curve for 5-MT, 5-CT and 8-OH-DPAT stimulating [Ca²⁺]_i in HEK-293 cells. Each point represents the mean ± SE from 30 to 50 cells in a representative experiment from 3 to 5 replicates. (D) Effects of various dopamine receptor agonists (100 μM) on [Ca²⁺]_i in a representative single HEK-293 cell.

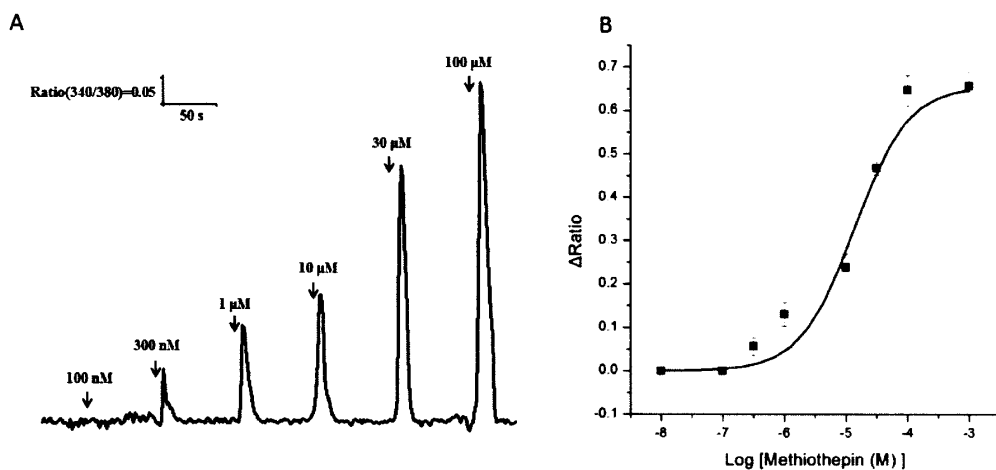


图4.7 Methiothepin对pcDNA3-Pr5-HT₈/HEK 293的 $[Ca^{2+}]_i$ 影响

Fig 4.7 Representative Ca^{2+} responses and dose-response profiles for methiothepin in HEK-293 cells stably expressing Pr5-HT₈. (A) Dose-response of methiothepin on $[Ca^{2+}]_i$ in a representative single HEK-293 cell. (B) Dose-response curve for methiothepin stimulating $[Ca^{2+}]_i$ in HEK-293 cells. Each point represents the mean $\pm$ SE from 30 to 50 cells in a representative experiment from 3 to 5 replicates.

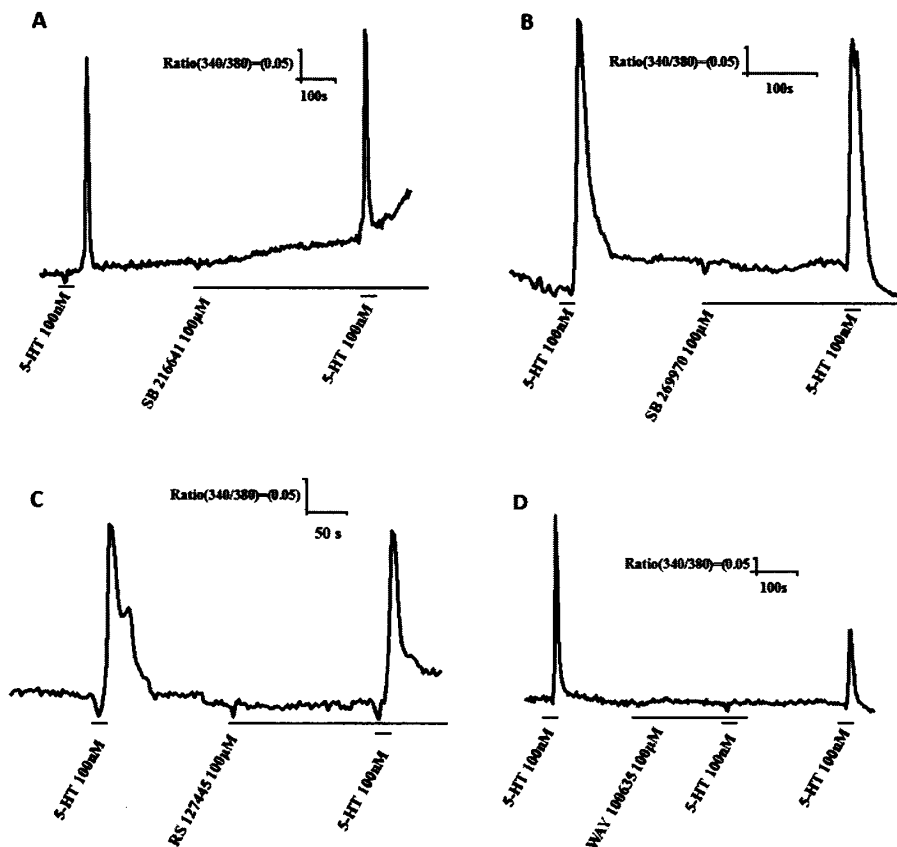


图4.8 5-HT受体拮抗剂对pcDNA3-Pr5-HT₈/HEK 293的[Ca²⁺]_i影响

Fig 4.8 Effects of SB 216641 (A), SB 269970 (B), RS 127445 (C) and WAY 100635 (D) on 5-HT induced [Ca²⁺]_i increase. Pr5-HT₈ transfected HEK-293 cells were first stimulated with 100 nM 5-HT, which was washed out for 2 min. Then cells were incubated with various antagonists for 2 min prior to the second application of 100 nM 5-HT.

4 讨论

本实验数据表明,昆虫表达一类新的5-HT₈受体。首先,序列分析结果表明菜青虫表达的这个受体基因跟目前已知的5-HT受体基因不一样;第二,进化树分析结果说明这个受体跟已知的生物胺受体家族在不同的进化枝上;第三,该受体在HEK 293细胞表达后,能够被5-HT受体的非选择性拮抗剂Methiothepin激活。除了以上几点,Pr5-HT₈跟菜青虫体内另外五个5-HT受体(Pr5-HT_{1A}、Pr5-HT_{1B}、Pr5-HT_{2A}、Pr5-HT_{2B}、Pr5-HT₇)的序列相似性低。该受体的mRNA可以在菜青虫

多个组织中检测到。综合以上几点,可以得出结论菜青虫表达一类新的5-HT₈受体,该受体在鳞翅目昆虫以及其它的一些昆虫均有表达。

通过序列分析,发现Pr5-HT₈有配体结合,受体激活以及G蛋白偶联的关键序列位点(Nichols and Nichols, 2008)。根据人的5-HT_{1B}和5-HT_{2B}受体-配体结合的晶体结构分析(Wacker et al. 2013; Wang et al. 2013), D^{3.32}和T^{3.37}在配体结合中起关键作用。但是这两个在大部分5-HT受体中均保守的氨基酸序列在Pr5-HT₈基因中被其它氨基酸残基替换。这可能导致了该受体跟其它5-HT受体药理学性质不一样。

被检测的几种生物胺均不能激活pcDNA3-Pr5-HT₈/HEK 293胞内cAMP的升高,也不能抑制NKH 477引起的胞内cAMP的升高,所以Pr5-HT₈既不偶联G_S也不偶联G_i。除了5-HT,其它几种生物胺都不能引起pcDNA3-Pr5-HT₈/HEK 293胞内Ca²⁺浓度升高,表明Pr5-HT₈偶联G_q,升高胞内Ca²⁺浓度。

Pr5-HT₈的药理学性质跟其它的5-HT受体存在异同。已知的一些5-HT受体的激动剂如5-CT、5-MT和8-OH-DPAT对Pr5-HT₈也有激动效果。Sumatriptan是5-HT₁受体的激动剂(Razzaque et al., 1999), AS-19是5-HT₇的一种选择性激动剂(Schlenstedt et al., 2006; Röser et al., 2012)。但这两个激动剂都对Pr5-HT₈受体没有影响。Methiothepin (Monachon et al., 1972)是昆虫5-HT_{1A}(Thamm et al., 2010)、5-HT_{2A}(Röser et al., 2012)和5-HT₇(Schlenstedt et al., 2006)的完全拮抗剂。它能激活Pr5-HT₈受体,引起胞内Ca²⁺浓度升高。Methiothepin的EC₅₀值为17.4 μM,比5-MT、5-CT和8-OH-DPAT的EC₅₀值要高很多。已有一些研究证明5-HT受体的拮抗剂会有激动剂的效果,如Yohimbine是人5-HT_{1A}、5-HT_{1B}、5-HT_{1D}和绿头苍蝇5-HT₂受体的拮抗剂(Röser et al., 2012),但是该试剂对人的5-HT_{1A}受体有部分激动剂效应(Millan et al., 2000)。由于Methiothepin是其它昆虫5-HT受体的拮抗剂,它有可能是Pr5-HT₈受体的特异性激动剂。

本章也检测了几种已知的5-HT受体拮抗剂对Pr5-HT₈受体的影响。SB-269970是哺乳动物5-HT₇受体的选择性拮抗剂(Lovell et al., 2000)对昆虫的5-HT₇受体也有拮抗效果(Schlenstedt et al., 2006; Röser et al., 2012)。SB-216641是哺乳动物5-HT_{1B}受体的选择性拮抗剂(Price et al., 1997),对菜青虫Pr5-HT_{1B}受体也有拮抗反应(图3.3F)。RS-127445是哺乳动物5-HT_{2B}受体的选择性拮抗剂

(Bonhaus *et al.*, 1999)。这几个药剂对 5-HT₈ 都没有拮抗效果。而 WAY-100635 能够拮抗 5-HT 引起的 pcDNA3-Pr5-HT₈/HEK 293 胞内 Ca²⁺ 浓度升高。WAY-100635 是哺乳动物 5-HT_{1A} 的选择性拮抗剂 (Fornal *et al.*, 1996)，也是多巴胺受体 D₄ 的完全激动剂 (Chemel *et al.*, 2006)。WAY-100635 是美国大蠊 Pea5-HT₁ 受体的反向激动剂 (Troppmann *et al.*, 2010)，也是西方蜜蜂 Am5-HT_{1A} 受体的部分拮抗剂 (Thamm *et al.*, 2010)。5-HT₈ 受体药理学性质跟其它 5-HT 受体的不同更加说明它代表了一类新的 5-HT 受体。

分析 5-HT 受体的组织分布情况能够帮助我们推测这些受体可能调节的生理功能。5-HT 受体的组织分布已在一些昆虫中得到研究。西方蜜蜂的 Am5-HT₇ 受体在光叶、大脑、飞行肌以及马氏管中均有表达 (Schlenstedt *et al.*, 2006)。作者推断该受体可能在处理感觉信号以及大脑信号方面发挥作用。在马氏管中有表达可能是该受体跟利尿相关。同样，埃及伊蚊 *Aedes aegypti* 的受体 Aae5-HT₇ 在中肠和跟马氏管相关的 tracheolar 细胞中有表达，因而推测该受体可能参与中肠肌肉的抑制性收缩 (Pietrantonio *et al.*, 2001)。

Pr5-HT₈ 受体在神经组织的高表达，这跟我们预测它在菜青虫中是一种神经递质、神经调制和神经激素相符。组织分布的结果表明神经索、血细胞、脂肪体、马氏管和中肠中都有 Pr5-HT₈ 受体的转录本。因此该基因在菜青虫各个组织均有分布，可能参与调控多种生理功能。

第五章 5-羟色胺参与调控昆虫血细胞吞噬的研究

1 引言

5-羟色胺是进化上古老的神经递质或神经激素之一(Turlejski, 1996)。它调控脊椎动物和无脊椎动物很多生理过程, 如调控线虫的新陈代谢(Sze *et al.*, 2000)和运动(Ranganathan *et al.*, 2000); 调节昆虫繁殖(Anstey *et al.*, 2009)以及学习和记忆(Sitaraman *et al.*, 2008); 调控人的生理状态和行为, 包括疼痛, 食欲, 心情和睡眠(Mössner and Lesch, 1998)。除了神经系统外, 5-HT 在免疫信号调控中也发挥重要作用。免疫细胞可以合成和储存5-HT。比如人的肥大细胞表达外周5-HT合成的关键酶TPH1(Kushnir-Sukhov *et al.*, 2007, 2008)。老鼠肥大细胞表达5-HT转运体SERT, 能够从微环境吸收5-HT(O'Connell *et al.*, 2006)。5-HT通过不同的受体调控免疫反应和炎症级联反应, 而不同的免疫细胞表达不同类型的5-HT受体(Baganz and Blakely, 2013)。5-HT_{2A}能促进嗜酸性粒细胞的趋化性(Boehme *et al.*, 2008)。肺泡巨噬细胞上的5-HT_{2C}受体能被5-HT激活(Mikulski *et al.*, 2010)。然而, 大部分的研究都是在体外用药物刺激免疫细胞完成的, 因而5-HT在生物体内的免疫调控作用和机制还尚未清楚。

脊椎动物血细胞, 如巨噬细胞, 已经进化出了各种处理颗粒和杂质的策略, 包括胞饮反应, 受体介导的内吞作用以及吞噬。吞噬是一种高度保守的先天免疫反应, 它能将大于0.5 μm 的大颗粒吞入细胞, 从而通过特定的吞噬细胞如巨噬细胞和中性粒细胞快速的吞没死亡细胞和病原菌(Aderem and Underhill, 1999)。血细胞吞噬也是昆虫抵抗病原菌和寄生物的一种非常重要的细胞防御反应(Lavine and Strand, 2002), 而鳞翅目昆虫的浆血细胞和颗粒血细胞则是主要的吞噬细胞(Kanost *et al.*, 2004)。被感染的昆虫中, 免疫系统和神经系统可能在调控吞噬过程中相互作用。然而, 跟脊椎动物相比, 5-HT调控昆虫血细胞吞噬的分子机制还没有完全清楚, 虽然已有研究表明5-HT能增强昆虫血细胞吞噬(Baines *et al.*, 1992; Kim *et al.*, 2009)。

昆虫体内很多调控免疫系统的信号通路跟哺乳动物的先天免疫很相似(Lemaitre and Hoffmann, 2007), 有些通路如Toll受体信号通路在昆虫中首次被发现(Hoffmann, 2003)。有间接证据表明昆虫和哺乳动物之间的这种相似性可以

扩展到研究神经内分泌系统调控免疫的分子机制 (Adamo, 2008)。由于昆虫的免疫系统相对简单, 研究5-HT介导的第二信使和免疫相关的胞内信号通路之间的关系会比较容易。本研究中, 我们首先通过药理学手段以及细胞RNAi技术研究菜青虫体内5-HT信号调控细胞免疫的过程。我们发现血细胞能自身分泌5-HT, 并且5-HT可以通过5-HT_{1B} 和 5-HT_{2B} 受体调控细胞吞噬, 而这两种受体通过不同的方式发挥作用。通过模式昆虫果蝇5-HT_{1B}突变体以及血细胞特异性的RNAi干扰发现5-HT_{1B} 和 5-HT_{2B} 受体在抵御病原菌的感染过程中发挥重要作用。

2 材料与方法

2.1 供试昆虫与药剂

菜青虫的饲养以及所用化学试剂来源参考第三章2.1节。

本实验所用的黑腹果蝇均来自于布卢明顿果蝇库中心 (Bloomington *Drosophila* Stock Center), 包括*HmlΔ -Gal4* (30139); *UAS-5-HT_{1B} RNAi* (27634, 25833); *UAS-5-HT_{2B} RNAi* (25874, 60488)。5-HT_{1B} control 和5-HT_{1B}^{AlII-V} 果蝇是 Leslie Vosshall (Gasque *et al.*, 2013) 赠送的。果蝇饲养于人工培养箱 (温度25°C, 湿度70%, 光周期为L:D=12 h:12 h)。所用饲料为标准的玉米粉培养基, 其组分为7%的白糖、7.3%的玉米粉、1.7%的酵母粉、1%的黄豆粉、0.5%的琼脂和0.25%的尼泊金甲酯。本实验所用的果蝇饲养密度均不可太大, 保证幼虫生长期不会拥挤。处理间所用果蝇的生长密度一致。每天取新羽化的果蝇到新的果蝇管, 用羽化后5到7天的果蝇做实验。

2.2 果蝇品系的杂交

杂交果蝇前, 收集好处女蝇是关键。收集处女蝇前, 先将果蝇瓶内羽化出来的成虫全部清理出来。果蝇在25°C人工培养箱内, 雌雄果蝇在羽化8小时内不会交配, 8 h内收集的雌性果蝇均为处女蝇。果蝇在18°C人工培养箱内, 果蝇的发育速率显著减慢, 新羽化的雌雄果蝇16小时内不会交配。因此可以将果蝇瓶白天放在25°C人工培养箱, 每6 h收集一次处女蝇。晚上放在18°C人工培养箱, 12 h

收集一次处女蝇。收集出来的处女蝇一般体色苍白，体型较大，腹部透明有黑点。为了确保收集的果蝇都是处女蝇，一般将果蝇放在25°C人工培养箱内2-3天，如果没有产卵，说明管内雌蝇均为处女蝇。一般杂交所用的果蝇为羽化后3-7天的处女蝇和雄蝇交配，雌雄比例为7:3。太老的果蝇交配能力和产卵能力都会下降。每管放入相同数量的果蝇，每3天将亲本果蝇倒入新鲜的果蝇管产卵。这样就可以基本维持每管幼虫密度一致以及F1代数足够多。

本实验用*UAS-5-HT_{1B} RNAi* (27634, 25833)两个品系分别跟*HmlΔ-Gal4*杂交，F₁代为干扰血细胞5-HT_{1B}的品系。用*UAS-5-HT_{1B} RNAi* (27634, 25833)两个品系以及*HmlΔ-Gal4*品系分别跟W¹¹¹⁸杂交，F₁代作为干扰血细胞5-HT_{1B}的对照果蝇。用*UAS-5-HT_{2B} RNAi* (25874, 60488)两个品系分别跟*HmlΔ-Gal4*杂交，F₁代为干扰血细胞5-HT_{2B}的品系。用*UAS-5-HT_{2B} RNAi* (25874, 60488)两个品系以及*HmlΔ-Gal4*品系分别跟W¹¹¹⁸杂交，F₁代作为干扰血细胞5-HT_{2B}的对照果蝇。

2.3 血细胞的分离、培养和免疫诱导

用70%的酒精将5龄菜青虫幼虫表面清洗。用手术剪将前足剪开一点点，等血淋巴流出来，用10 μl的枪头将血淋巴收集于Grace's Insect Medium (1:10, v/v; Invitrogen, Carlsbad, CA)。稀释的血淋巴加到12孔细胞培养板里 (Nunc, Roskilde, Denmark)，密度约为每孔 2×10^6 个细胞。每孔加入100 ng/ml LPS (*Escherichia coli* 0111:B4; Sigma Aldrich) 孵育15min, 1h, 2h 和 4h (Ngkelo *et al.*, 2012; Wu *et al.*, 2015)。用枪头将培养基收集于1.5 ml离心管，离心后取上清，用上清检测5-HT的含量。收集培养板上的细胞层提取RNA。

2.4 5-HT 的检测

收集第一天的菜青虫五龄幼虫血淋巴，轻轻混匀。30 μl血淋巴中加170 μl Grace's Insect Medium，培养基中加入2 μl 饱和的2-phenylthiourea (PTU)。稀释的血淋巴加到一个8孔细胞培养板中 (Lab-Tek™, Nunc, Thermo Fisher Scientific, Rochester, USA)，将细胞放在27°C细胞培养箱，贴壁20 min，形成单层细胞。去除培养基，用PBS缓慢的清洗细胞2次，加入4%的多聚甲醛固定20 min。去除固定液，PBS漂洗3次，每次5 min，用1%的BSA将细胞在室温下封闭1 h。用5-HT

抗体(Sigma)将细胞在4 °C孵育24小时。阴性对照的处理是将5-HT抗体提前用10 mM 5-HT在4°C孵育24小时。PBS漂洗细胞3次，每次5 min。用SA-Alexa Fluor 546 二抗（Invitrogen）室温孵育1 h。二抗孵育结束后，用PBS对细胞漂洗4次，每次5 min。用1 μg/mL的DAPI室温染细胞核5 min。在LSM 510型激光共聚焦荧光显微镜（Zeiss, Göttingen, Germany）下拍照。

用5-HT ELISA 试剂盒（Jiancheng, Nanjing, China）检测上清中5-HT的含量（Li *et al.*, 2014）,具体步骤参照试剂盒的说明书进行。

2.5 菜青虫 TRH 和 TPH 基因的克隆

使用第三章2.3.1中提取的RNA以及合成的3'RACE 模板做RCR。所用引物见表5.1。

表 5.1 本研究中所用到的引物
Table 5.1 Primers used in this study

Primer name	Primer sequence (5'-3')
RACE PCR for <i>P. rapae</i>	
TRH-3'outer	ATTGGCAACGCTATATTCTTCACG
TRH-3'inner	CACGGATTGTTTGAGGAAGCTAAG
Complete sequence	
TPH- compF	ACTCTTTCGACCTCTCCTCTC
TPH- compR	CACCAATTCACTTTAAACAGCT
TRH- compF	CGTCCTTACTCGTCAAATAACC
TRH- compR	CGCCGTTTGTATGTACTAAAAATT

2.6 荧光定量 PCR 和 RT-PCR

用小量RNA提取试剂盒high pure RNA isolation kit（Roche）提取菜青虫5龄幼虫血细胞的RNA，具体步骤参照试剂盒说明书进行。为了收集果蝇幼虫血细胞，将30头3龄幼虫的前端用手术镊子轻轻撕开，让血浆流入100 μl不含核酸酶的灭菌水中，将幼虫残体在水中放置1 min再取出。果蝇幼虫血细胞RNA的提取方法跟菜青虫血细胞RNA的提取方法相同。用Nanodrop 2000 spectrophotometer (Thermo Scientific Inc., Bremen, Germany) 检测提取出来的RNA的质量和浓度。荧光定量RCR的模板合成参照ReverTra Ace® qPCR RT kit (Toyobo, Osaka, Japan) 试剂盒说明书进行，所用RNA的量为1μg。RT-PCR的模板参照KOD- Plus- (Toyobo,

Osaka, Japan)试剂盒说明书合成。模板合成好后，实时荧光定量PCR所用试剂为 SsoFast Eva Green Supermix with Low Rox (Bio-Rad, Hercules, CA), 按照说明书步骤在荧光定量PCR仪Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems by Life Technologies, Carlsbad, CA)中进行。用于荧光定量PCR分析的特异性引物见表5.2。用 $2^{-\Delta\Delta CT}$ 的方法 (Livak and Schmittgen, 2001) 对不同基因的表达量定量。菜青虫用18s RNA作为内参基因，黑腹果蝇用RpL 11作为内参基因。

表 5.2 荧光定量PCR和RT-PCR中所用到的引物
Table 5.2 Primers used for real-time PCR and RT-PCR

Primer name	Primer sequence (5'-3')
RT-PCR and qRT-PCR for <i>P. rapae</i>	
18s-q-F	CCTGCGGAAGGATCATTAAC
18s-q-R	AACGAAAGAAAGCGTCCAAA
TPH-q-F	AAGATTGTACCGGGTTCACG
TPH-q- R	AACGATGCCAGTCCAATTTC
TRH-q-F	TACCAAAAACACGCCTGTGA
TRH-q- R	AGCCAAACCGGAAAGAAAAT
SERT-q-F	GCGGCATCACAAATCTTCTT
SERT-q-R	TCCGGGTAGACGATGAAAAC
5-HT _{1A} -q-F	TTTGCAGCTCAGCATCAATC
5-HT _{1A} -q-R	TTGGCTTACGAGGCACTTCT
5-HT _{1B} -q-F	AACCTGGTGGGAGACATCTG
5-HT _{1B} -q-R	CGGCCAGAGAAAAGTATGAGC
5-HT _{2A} -q-F	CCAGAGCTATGCGTCATCAA
5-HT _{2A} -q-R	TCCACATTGCCTTCTCTGTG
5-HT _{2B} -q-F	TCCAGAATGTGAAAAGAGAATACC
5-HT _{2B} -q-R	CAATACTGACACAAAAGCACCTT
5-HT ₇ -q-F	CTCCGCATCAGAAAAAGCTC
5-HT ₇ -q-R	ACGGCGTCAGGAATTGTATC
RT-PCR for <i>D.melanogaster</i>	
5-HT _{1B} -F	CGTATTCGTAATTGCCGCCA
5-HT _{1B} -R	CCGGTCCCAATATCCATCCA
5-HT _{2B} -F	ACACCACTGTACAGGAGAGC
5-HT _{2B} -R	AGCGGTCTGTCCCCAAAATA
TPH-F	TCGAGACCTGGGGCATTATC
TPH-R	CCGAGAGCTGAGTAGTCCAG
RPL11- F	CGATCCCTCCATCGGTATCT
RPL11- R	AACCACTTCATGGCATCCTC

2.7 单头黑腹果蝇基因组 DNA 提取和 PCR

配制提取果蝇基因组 DNA 的缓冲液 (Squash Buffer), 具体方法为, 称取一定量的 Tris, EDTA 和 NaCl 固体, 用双蒸水稀释, 并用浓盐酸调节 Tris 的 pH=8.0, 将 100 ul 1M Tris (pH = 8.0, 20 ul)、 0.5 M EDTA 和 50 ul 5 M NaCl 加入 15ml 灭菌的塑料管子内, 并加入 9.8 ml 双蒸水, 充分震荡混匀, 配成体积为 10 ml 的提取基因组 DNA 的缓冲液。

单头果蝇基因组 DNA 提取的具体操作方法如下:

- (1) 将单头果蝇放入 1.5 ml 离心管中。将冻死的果蝇从冰箱取出后, 每个离心管中加入 50 ul 的提取缓冲液 (缓冲液使用前, 每 50 ul 需加入 1ul 10 mg/ml 的蛋白酶 K)。
- (2) 将研磨棒伸入管底, 将果蝇在缓冲液中研碎。
- (3) 涡旋震荡离心管, 使形成匀浆。
- (4) 将上述匀浆转移入 PCR 管, 放置在 PCR 仪内, 程序为 37 °C 30 min, 95 °C 2 min, 4 °C 保存。
- (5) 将 PCR 产物 14000 rpm 离心 7 min。
- (6) 将上清即基因组 DNA 转入新的 PCR 管, 置于 4 °C 冰箱保存。

提取好的 DNA 用 Nanodrop 2000 spectrophotometer 检测质量和浓度。PCR 所用引物见表 5.3。采用 KoD- Plus- Neo 高保真性 PCR 酶进行 PCR 扩增。反应条件为: 94 °C, 2 min; 98 °C, 10 sec, 58 °C, 30 sec, 68 °C, 1.5 min, 40 个循环; 68 °C, 7 min。

表 5.3 果蝇基因组 PCR 所用引物
Table 5.3 Primers used for genomic PCR

Primer name	Primer sequence (5'-3')
Genomic PCR for <i>D.melanogaster</i>	
5-HT _{1B} -genomic-F	CTGCGCTCCTTCTTCAGC
5-HT _{1B} -genomic-R	CGTAATTGCCGCCATTATACTC

2.8 吞噬实验

2.8.1 菜青虫血细胞体外吞噬

菜青虫血细胞体外吞噬实验参照Cuttell *et al.* (2008), 略有改变。血细胞提取出来后, 将其培养在96孔细胞培养板上(CoStar, Washington, USA)。用20 μ M Cell Tracker Blue CMAC (Molecular Probes) (Life Technologies, Carlsbad, CA, USA) 27 °C孵育1 h之后, 用PBS洗三次。参照pHrodo *E. coli* (Invitrogen, Carlsbad, CA) 使用说明进行吞噬实验。pHrodo *E. coli*颗粒用pH为7.4的PBS悬浮起来至浓度0.5 mg/ml, 涡旋混匀。细胞先用10 μ M cytochalasin D处理1h后, 每孔加入45 μ l悬浮液和5 μ l药剂, 27 °C孵育90 min。在荧光显微镜(Nikon Eclipse TS100, Nikon, Japan)下拍照。200×放大倍数, 每个孔随机拍摄5个视野。

2.8.2 果蝇体内吞噬

准备8到10头羽化后5-7天的果蝇, 雌雄比例1:1。用显微注射仪FemtoJet microinjection system (Eppendorf)给每头果蝇注射大约0.2 μ l的1 mg/mL pHrodo[®] green *E. coli* 或者 pHrodo[®] red *S. aureus* (Invitrogen, Carlsbad, CA)。将果蝇放在25 °C培养箱1 h。然后立即将果蝇放于冰上, 在荧光显微镜(Nikon AZ100M, Nikon, Japan)下给每头果蝇拍照。用Image J软件计算荧光值, 果蝇吞噬量用相对荧光值表示, 即背血管的荧光值除以周围的荧光 $[\text{fluorescence}]_{\text{dorsal vein area}}/[\text{fluorescence}]_{\text{adjacent area}}$ 。

果蝇体内珠子的吞噬实验步骤如下: 用显微注射仪Drummond Scientific Nanoject II给每头果蝇注射36.8 nL PBS或者1 μ g/ μ l 5-HT, 25°C 培养30 min。然后再注射 27 nL 的1.0 μ m Red Fluorescent Carboxylate Modified FluoSpheres (Invitrogen), 用PBS按1: 2稀释。25°C 培养10 min后, 注射36.8 nL的0.4%台盼蓝 (Invitrogen)淬灭未吞噬的珠子。然后立即将果蝇放于冰上, 按照前面的方法给果蝇拍照。

2.9 菜青虫血细胞 RNAi

根据菜青虫5-HT受体及TPH的核苷酸序列，用Invitrogen公司siRNA 设计软件 (<http://rnaidesigner.thermofisher.com/rnaiexpress/>)设计了相应的siRNA，具体序列见表5.4。siRNA由Invitrogen公司合成。血细胞按照2.2中的方法获得。用Grace’s培养基将细胞培养于96孔细胞培养板。细胞贴壁后，加入2.4 ng siRNA 和 0.7 μl siRNA 转染试剂 INTERFERin® (Polyplus-transfection SA, France)，具体操作步骤参照该试剂的说明书进行。细胞放在27℃培养箱培养。

表 5.4 siRNA 合成序列
Table 5.4 siRNA sequence

Gene name	Sense (5'-3')	Antisense (5'-3')
negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
TPH	GCUAGCGUCCGAGUAUUUTT	AAAUACUCGGAACGCUAGCTT
Pr5-HT _{1B}	GCUGACUCCAAGAGAGAAATT	UUUCUCUCUUGGAGUCAGCTT
Pr5-HT _{2B}	CCAGUUCACCAUCAUCAUTT	AUAGAUGAUGGUGAACUGGTT
Pr5-HT ₇	GGUUGUGAAGCAUCUCCAUTT	AUGGAGAUGCUUCACAACCTT

2.10 菜青虫 Pr5-HT_{1B} 和 Pr5-HT_{2B} 抗体制备

构建5-HT_{1B}原核表达载体所用引物：正向引物Pr5-HT_{1B}- *BamH I* 5'-CGGGATCCCAAACAGCTAGGAAAAGAAT -3' 和反向引物Pr5-HT_{1B}- *XhoI* 5'-CCCTCGAGTTATGTCTTTGCCGCTTTCC -3'。该引物扩增5-HT_{1B}受体第三胞内区序列，用pET-28a (+)作为原核表达载体。采用KoD-Plus-Neo高保真性PCR酶进行PCR扩增。反应条件为：94℃，2 min；98℃，10 sec，60℃，30 sec，68℃，30 s，40个循环；68℃，7 min。PCR完成后用1%的琼脂糖凝胶电泳检测，将目的条带切下，并用Axygen凝胶回收试剂盒回收。利用限制性内切酶*BamH I*和*XhoI*分别对回收后的产物以及pET-28a (+)质粒进行双酶切处理。双酶切完成后，用1%的琼脂糖凝胶电泳检测，将目的条带切下，并用Axygen凝胶回收试剂盒回收。用T4 DNA 连接酶（宝生物）将回收后的目的产物与载体产物连接。得到的融合质粒，通过测序验证插入序列的正确性。构建好的载体转入*Escherichia coli* BL21

(DE3)表达菌株中,将菌液涂在含Kanamycin抗性(50 mg/ml)的LB固体培养基上,过夜培养。挑出一个单菌落于5 ml LB Kanamycin抗性液体培养基。放入37°C摇床过夜培养,保存于4°C作为母液。按1:50的比例,将母液稀释到2L含卡那霉素抗性的LB培养基中,放入摇床培养(37°C, 220 rpm),至培养基的OD₆₀₀值为0.8。加入0.5 mM isopropyl- β -d-thiogalactopyranoside (IPTG) 28°C, 200 rpm 过夜诱导表达。离心,弃培养基上清,收集细胞,超声破碎细胞。在8M尿素的变性条件下,采用Ni-chelating affinity column (TransGen Biotech, Beijing, China)对与His-tag融合重组蛋白进行纯化。为了确定重组蛋白的正确性,取部分纯化后的样品跑SDS-PAGE电泳,然后转到PVDF膜上(Bio-Rad)。用抗His标签的多克隆抗体检测条带的正确性,条带大小约为17 kD。验证条带正确后,将纯化后的蛋白样品作为抗原送到华安生物公司,将500 μ g与福氏佐剂等体积混合的抗原首次注入新西兰雄兔中,每2-3周加强免疫一次(背部皮下注射)。加强免疫的剂量为120 μ g左右,且不加任何佐剂。第四次加强免疫后第七天,从耳缘静脉取血,制备血清,检测抗体效价。效价达到预期以后,从颈动脉取血,分离血清。采用Montage Antibody Purification kit with RPOSEP-A media进行抗体纯化。

Pr5-HT_{2B}抗体的制备采用多肽合成的方法,由杭州华安生物公司合成。用GenScript Optimum AntigenTM design tool预测Pr5-HT_{2B}表面抗原序列。抗原合成的序列为SAAAKTSKGTNISEC,多肽序列的最后一个半胱氨酸是用于载体蛋白结合。作为免疫抗原的多肽合成好后,按照Pr5-HT_{1B}抗体制备的方法进行抗体的制备和纯化。

2.11 Western blot 和免疫荧光

血细胞在冰冷的缓冲液(1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl pH 7.5, 5 mM EDTA, 1 mM sodium-molybdate)中裂解,缓冲液中加入蛋白酶抑制剂phenylmethyl sulfonylfluoride (PMSF, Sangon Biotech, P0754, Shanghai, China)。为了获得膜蛋白,离心(15 500g, 10 minutes, 4°C)沉淀细胞碎片。收集上清,用Bradford reagent (Sangon Biotech, Shanghai, China)方法检测蛋白浓度。样品中加入4 $\times$ Protein SDS PAGE Loading Buffer (Takara Biotechnology, Japan)稀释,沸水煮10 min,将蛋白变性。然后,采用12%的还原型SDS-PAGE对蛋白进行分离。

通过Bio-Rad半干转膜仪将蛋白转到硝酸纤维膜上（PVDF膜）。用TBST（5% Tris- buffered saline; pH 7.0; 0.1% Tween 20）配制的5%脱脂奶粉封闭液室温孵育2h。用TBST洗2次，每次5min。加入含有5-HT_{1B}多克隆抗体的封闭液或含有5-HT_{2B}多克隆抗体（1: 500）的封闭液，4°C，孵育过夜。用TBST洗4次，每次5min。加入含有羊抗兔的二抗封闭液（1: 5000）室温孵育1 h。用TBST洗5次，每次5min。用ECL (Thermo Scientific)方法显色，拍照。

细胞免疫荧光参照2.4节中的方法，除了所用抗体不一样。

2.12 Ca²⁺检测

细胞内Ca²⁺浓度检测采用Easy Ratio Pro钙成像分析系统（Photon Technology International, Birmingham, NJ）。菜青虫血细胞Ca²⁺成像步骤如下：

- 1) 将5龄菜青虫幼虫血细胞种在12 mm玻片上（Thermo Scientific），细胞放在27°C，贴壁半小时；
- 2) 细胞贴壁后，将培养基去除，用PBS洗两次细胞。加入用PBS稀释的1 μmol/L Fura 2-AM (Dojindo Laboratories, Kumamoto, Japan)；
- 3) 27°C 避光孵育30 min，去除Fura 2-AM工作液，用Ca²⁺成像过程中使用的缓冲液（152 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 1.8 mM CaCl₂, 0.8 mM MgCl₂, and 10 mM HEPES, pH 7.4）洗细胞两次。
- 4) 最后用Easy Ratio Pro钙成像分析仪检测细胞，激发波长380 nM（Fura 2）和340 nM（Ca²⁺-Fura 2），发射波长510 nM。

每个实验重复三次以上，用Origin Pro (Origin Lab, Northampton, MA)软件拟合S型曲线计算EC₅₀值。

2.13 果蝇生存率实验

具有四环素抗性的金黄色葡萄球菌*Staphylococcus aureus*来自于浙江大学医学院俞云松老师实验室。挑一个单菌落于5 ml含四环素抗性的LB培养基，放到摇床上过夜培养（4°C, 225 rpm）。将过夜培养的菌液放入离心机中5000 g离心3 min。去掉上清，用灭菌的PBS悬浮沉淀，至OD₆₀₀为0.4。每管放入约20头果蝇，雌雄分开。用Drummond Scientific Nanoject II给每头果蝇前腹部同一部位注射32.2 nL

细菌悬浮液。并以注射相同体积灭菌的PBS作为对照。注射后的果蝇放入25°C培养箱培养。注射后, 6 h之内死去的果蝇当做注射过程受伤而死, 将其从试管除去, 不计入总数。每隔12 h统计一次死亡数量。每天将果蝇倒入新鲜果蝇管。

2.14 细菌在果蝇体内扩增效率实验

参照2.9中的方法获得金黄色葡萄球菌悬浮液, OD_{600} 为0.4。每种基因型的果蝇准备24头, 每头果蝇注射23 μ l菌液。每种基因型果蝇注射完8头后, 单头放入1.5ml 离心管中, 离心管里加有100 μ l灭菌的PBS。立即放在冰上, 匀浆。每管按1:10稀释, 依次稀释两次。用10 μ l的排枪, 从低浓度到高浓度, 取10 μ l的样品依次滴在四环素抗性的固体LB培养基上, 作为注射后0 h的样品。其它注射完的果蝇放在25°C培养箱培养。分别在感染6 h和18h后取样, 每种基因型取8头。按照上面的方法, 将单头果蝇匀浆后, 每管按1:10稀释, 依次稀释五次。将LB培养板放在37°C培养箱培养10小时左右, 等细菌单菌落清晰可见时, 将培养板拿出来, 计数, 统计数量为30到300个菌落的浓度, 根据稀释比例计算原始匀浆中金黄色葡萄球菌的数量。

2.15 数据统计

成对数据用 Student t tests方法统计。细菌感染雄性果蝇的死亡率差异显著性分析用Log rank tests方法, 感染雌性果蝇用Gehan-Breslow-Wilcoxon test方法统计死亡率。其它数据采用单因素的方差分析, 平均数之间的比较采用Tukey-Kramer方法进行。所有图和数据分析都是通过Origin 8.0 (Origin Lab, Northampton, MA, USA)和GraphPad Prism 5.0 (San Diego, CA, USA)这两个软件完成的。

3 结果与分析

3.1 激活的血细胞自身合成 5-HT 的含量

用100 ng/ml LPS诱导菜青虫血细胞2 h后, 通过免疫荧光方法检测到细胞内有5-HT的合成。血细胞内可以检测到5-HT信号(图5.1A上), 而阴性对照没有

(图5.1A下)。5-HT的合成需要两种酶,即色氨酸羟化酶(TPH/TRH)和L-氨基酸脱羧酶(AADC)(图5.1B)。色氨酸羟化酶是5-HT合成过程中的限速酶,该酶可由两个基因编码,昆虫中编码色氨酸羟化酶的是TPH/TRH,哺乳动物是TPH1/TPH2。这两个基因在体内表达的蛋白产物都有色氨酸羟化酶活性。TPH/TPH1基因在非神经组织中表达,TRH/TPH2基因在神经组织中表达(Côté *et al.*, 2003; Neckameyer *et al.*, 2007; Watanabe *et al.*, 2011)。5-HT转运体SERT是一种膜蛋白,它能从胞外摄取5-HT(Rudnick, 2006; Torres *et al.*, 2003)。通过RT-PCR检测TPH, TRH和SERT基因在血细胞上的表达情况。图5.1C表明血细胞表达TPH的mRNA,但是TRH和SERT的转录本没有检测到。这些实验结果表明血细胞通过TPH自身合成5-HT,而不是从胞外吸收5-HT。接着,用ELISA的方法检测了血细胞释放到培养基中的5-HT含量。血细胞在LPS诱导1 h后5-HT含量显著上升,诱导2 h达到最大值,但诱导4 h后5-HT含量有所下降(图5.1D)。荧光定量PCR的结果显示血细胞在LPS诱导15 min和4 h的TPH的mRNA表达量是对照的6倍左右(图5.1E)。综合上述结果可知,血细胞能在体外合成和释放5-HT,这个反应在血细胞被LPS激活后得到加强。因此,推测5-HT参与调控血细胞免疫。

3.2 5-HT 调控血细胞吞噬

为了研究5-HT具体调控血细胞哪项免疫功能,阻断5-HT的合成后检测这是否会影响血细胞吞噬革兰氏阴性菌*E. coli*的能力。大肠杆菌*E. coli*被pHrodo标记,这是一种在中性环境不显色,而在酸性环境如细胞内会发荧光的染料。 α -methyltryptophan (AMTP)可以抑制5-HT的合成(Gal and Christiansen, 1975)。图5.2中的结果显示,血细胞中加入10 μ M AMTP后5-HT的含量显著下降。而且10 μ M AMTP会显著降低血细胞吞噬*E. coli*的能力。加入100 nM 5-HT后,血细胞吞噬*E. coli*的能力可以恢复(图5.3A-D)。然后,用siTPH干扰血细胞上TPH的表达,干扰效率约为80%(图5.3E)。干扰48 h后,血细胞吞噬*E. coli*的能力显著下降。而在干扰了TPH的血细胞里加入100 nM 5-HT后,血细胞吞噬*E. coli*的能力明显升高(图5.3F-I)。这些数据表明,血细胞合成的5-HT在血细胞吞噬中发挥重要作用。

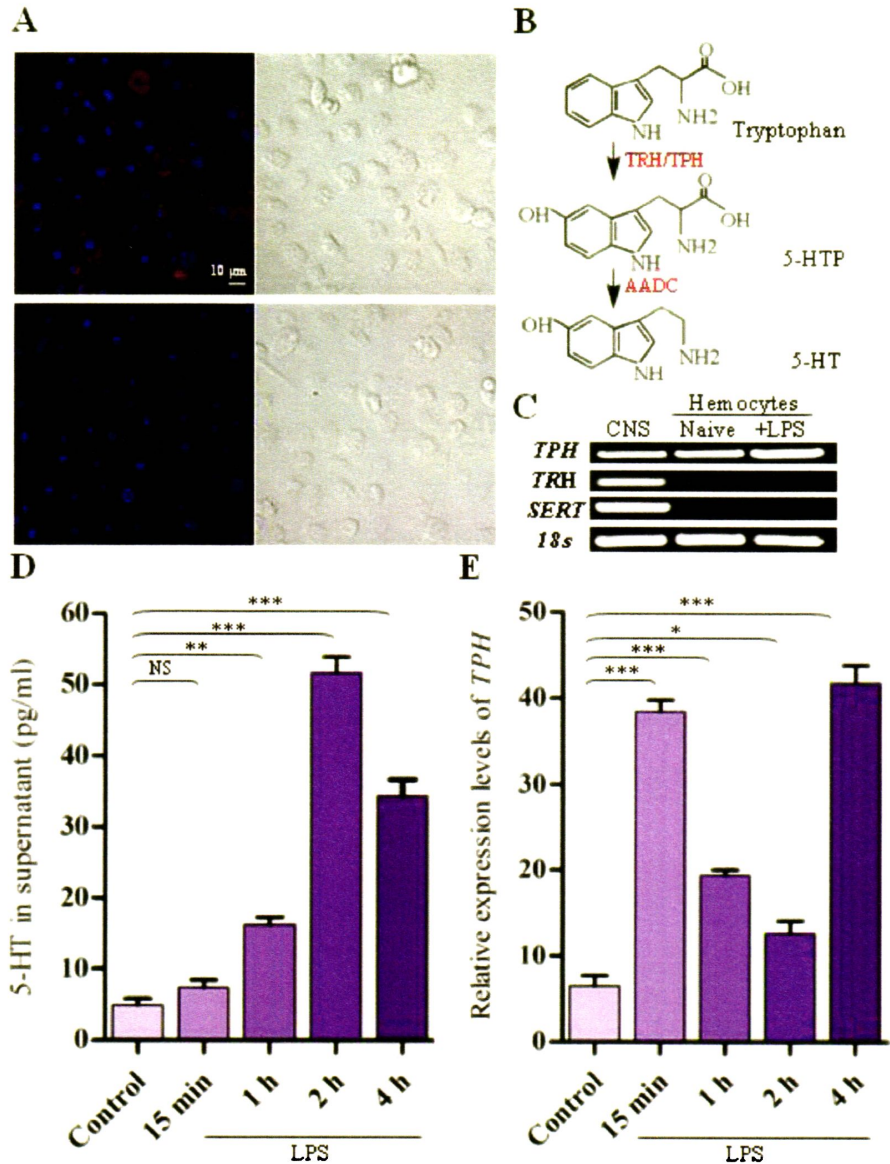


图5.1 激活的血细胞能够合成5-HT
Fig 5.1 Activated hemocytes are capable of 5-HT synthesis.

(A) 5-HT was visualized by confocal microscopy in hemocytes activated with 100 ng/ml LPS by labeling with 5-HT antisera (Alexa Fluor 546; red) (upper left). 5-HT antisera was preabsorbed with 5-HT as the negative control (lower left). Nuclei were counterstained with DAPI (blue). Scale bar represents 10 μ m. Data are *representatives* of two independent experiments. (B) Serotonin biosynthetic pathway. Tryptophan- phenylalanine hydroxylase (TRH/TPH), aromatic L-amino acid decarboxylase (AADC), 5-hydroxy tryptophan (5-HTP). (C) Expression of gene transcripts for *TPH*, *TRH*, and *SERT* were determined by RT-PCR from naive hemocytes and from hemocytes activated with 100 ng/ml LPS for 2h, Central nervous system (CNS) as positive control. Data are *representatives* of three independent experiments. (D) 5-HT concentrations in hemocytes supernatants were determined by ELISA. Hemocytes were activated with 100 ng/ml LPS. Naive hemocytes treated with PBS are as control ($n = 4$). (E) Relative expression of TPH was quantified by real time PCR. Hemocytes were activated with 100 ng/ml LPS. Naive hemocytes treated with PBS is as control ($n = 3$). One-way ANOVA followed by Tukey's multiple comparison test for D and E. Error bars indicate $\pm$ s.e.m., *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ and NS means no significant difference.

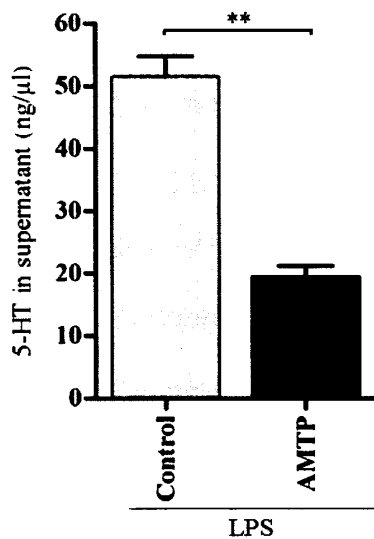


图 5.2 AMTP抑制5-HT的合成

Fig 5.2 Inhibition of 5-HT synthesis by AMTP. Both PBS treated hemocytes (control) and 10 μ M AMTP-treated hemocytes were incubated with 100 ng/ml LPS for 2 h. Error bars indicate $\pm$ s.e.m., $n = 3$, the statistical analysis is based on two-tailed t -test, $**P < 0.01$.

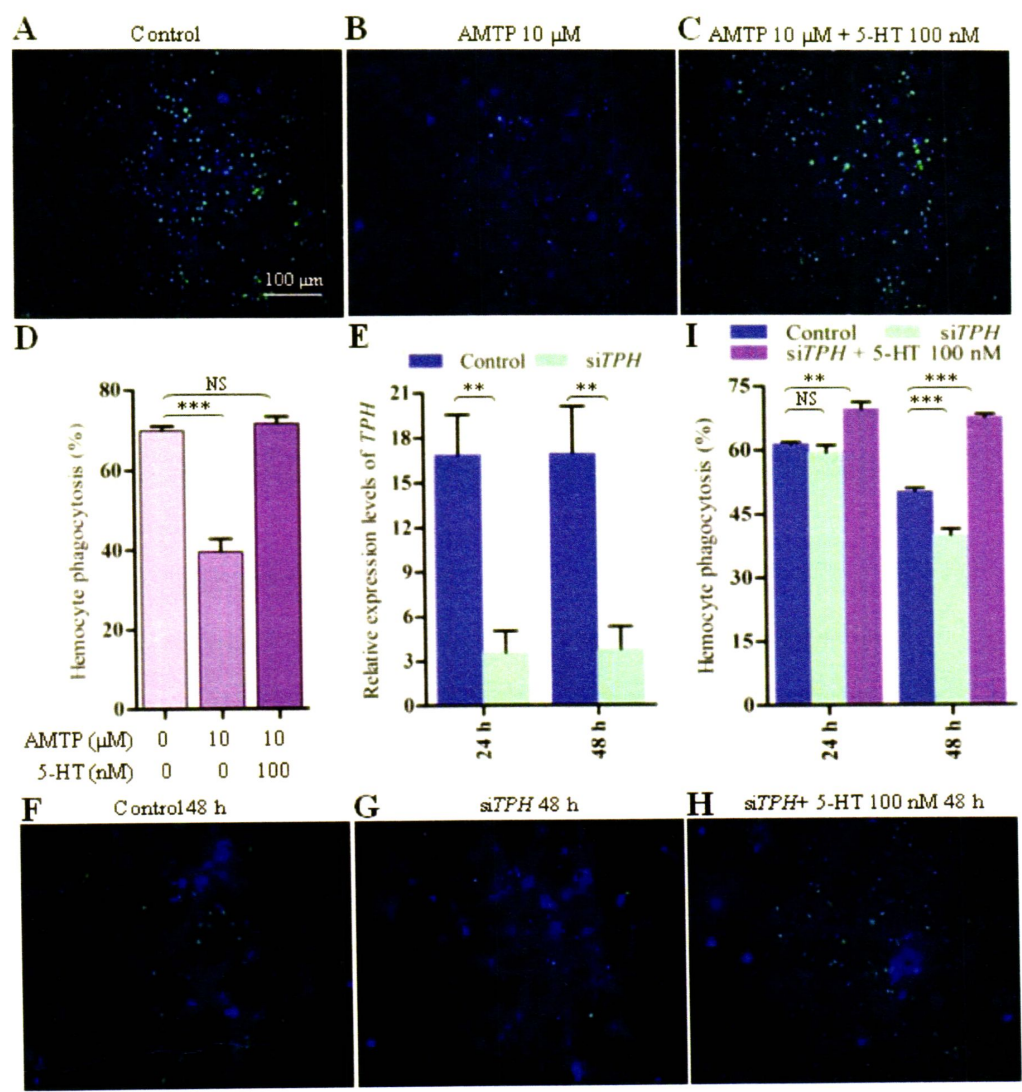


图 5.3 抑制5-HT的合成会减弱血细胞的吞噬能力

Fig 5.3 Inhibition of endogenous 5-HT synthesis impairs hemocyte phagocytosis. (A-C) Hemocyte phagocytosis was visualized by florescence microscope. Hemocytes were stained with Cell Tracker Blue CMAC (blue), green represent the phagocytosed pHrodo *E. coli*. (D) Quantification of phagocytosis of *E. coli* by hemocytes ($n = 3$). (E) Confirmation of knock-down effect of *TPH* by real-time qPCR. The *P. rapae18s rRNA* gene was used as an internal reference gene ($n = 4$). (F-H) Effect of siTPH on hemocyte phagocytosis was visualized by florescence microscope. (I) Quantification of siTPH effect on hemocyte phagocytosis ($n = 3$). One-way ANOVA followed by Tukey's multiple comparison test for D and I; two-tailed *t*-test for E. Error bars indicate $\pm$ s.e.m., *** $P < 0.001$, ** $P < 0.01$ and NS means no significant difference.

3.3 5-HT 通过 Pr5-HT_{1B} 和 Pr5-HT_{2B} 调控血细胞吞噬

我们已经知道5-HT参与调控血细胞吞噬,但是还不清楚5-HT具体通过哪个受体行使相应的功能。目前,昆虫里面已经鉴定出来5种5-HT受体,包括5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}和5-HT₇ (Blenau and Thamm, 2011; Gasque *et al.*, 2013)。5-HT_{1A}和5-HT_{1B}跟G_i偶联,降低胞内cAMP浓度。5-HT_{2A}和5-HT_{2B}跟G_q偶联,升高胞内钙离子浓度。5-HT₇跟G_s偶联,升高胞内cAMP浓度。(Blenau and Thamm, 2011; Gasque *et al.*, 2013)。我们用100 ng/ml LPS诱导血细胞2 h后,通过RT-PCR检测受体的表达情况,并以菜青虫中枢神经组织(CNS)作为阳性对照。图5.4A的结果表明未诱导的血细胞表达Pr5-HT_{1B}, Pr5-HT_{2B} 和 Pr5-HT₇受体的转录本。并且通过免疫组化进一步证明血细胞在蛋白水平表达Pr5-HT_{1B} (图5.4B) 和 Pr5-HT_{2B}(图5.4C)。Ca²⁺成像数据更加确认血细胞上有Pr5-HT_{2B}的表达。浆血细胞和颗粒血细胞是血细胞中最多的两大类细胞,添加5-HT均能引起这两种血细胞Ca²⁺浓度变化。5-HT的EC₅₀值为0.15 nM (图5.5)。

血细胞上存在多种受体,我们想通过药理学手段检测到底是哪些受体参与调控血细胞吞噬。实验中分别选用终浓度为10 μM三个受体特异性的拮抗剂(SB-216641, RS-127445, SB-269970)来阻断受体的功能。其中SB-216641对Pr5-HT_{1B}的拮抗作用和SB-269970对Pr5-HT₇的拮抗作用已在第三章的实验中验证。RS-127445是人5-HT_{2B}受体特异性拮抗剂。我们发现拮抗Pr5-HT_{1B}能显著降低血细胞吞噬*E.coli*的能力,而拮抗Pr5-HT₇对血细胞的吞噬没有明显影响。比较有趣的是,拮抗Pr5-HT_{2B}能显著升高血细胞吞噬*E. coli*的量(图 5.4D-H)。

我们进一步发现SB-216641对血细胞吞噬的影响呈剂量效应(图5.6A)。为了更加确认上述结果,我们用siRNA介导的干扰方法沉默血细胞上表达的对应受体。结果表明siPr5-HT_{1B}处理血细胞24 h和48 h后, Pr5-HT_{1B}的表达量在mRNA水平和蛋白水平显著降低(图5.6B-C)。跟用阴性对照处理的血细胞相比,siPr5-HT_{1B}处理的血细胞吞噬水平显著降低(图5.6D-F)。siPr5-HT_{2B}能在48 h显著降低Pr5-HT_{2B}在转录水平和蛋白水平的表达(图5.7A-B),但血细胞的吞噬能力反而增强(图5.7C-E)。而干扰血细胞5-HT₇的表达后并未影响血细胞的吞噬(图5.7F-G)。这些结果表明5-HT通过5-HT_{1B}和5-HT_{2B}调控血细胞的吞噬,而这两种受体的作用方式不同。

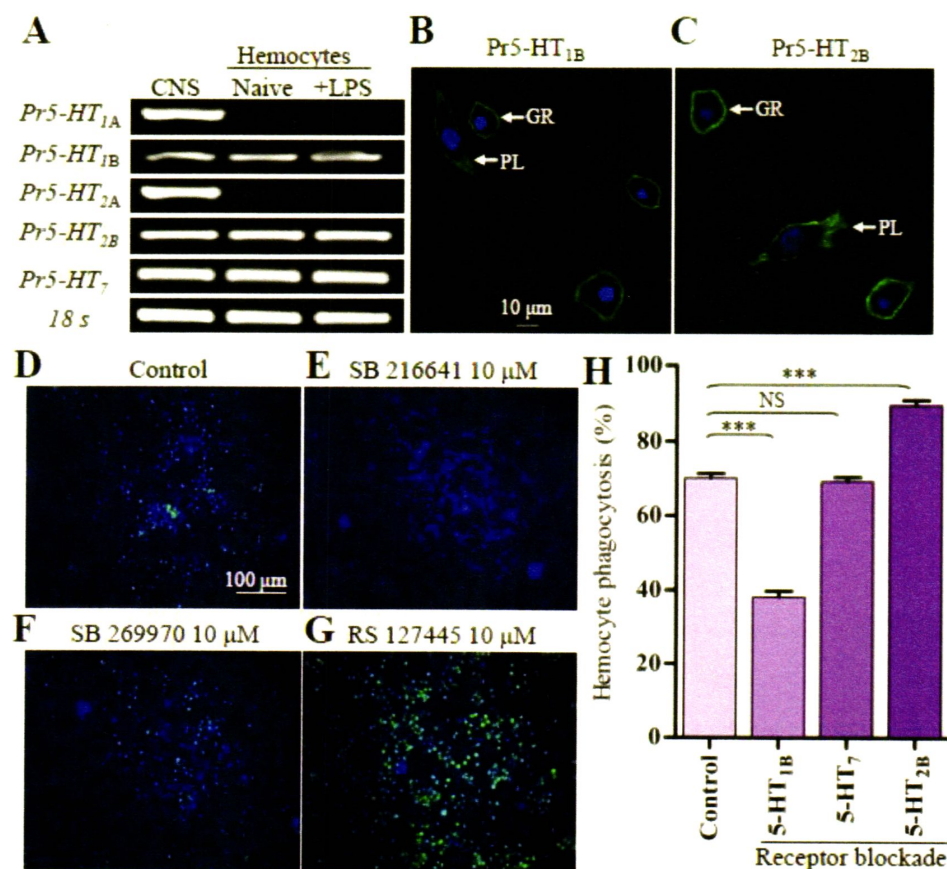


图 5.4 诱导和未诱导的血细胞上5-HT受体表达情况

Fig 5.4 5-HT receptor subtypes expressed in naïve and LPS-activated hemocytes. (A) Hemocytes were negatively purified and activated with 100 ng/ml LPS for 2 hours. The gene expression for 5-HTR subtype was examined by RT-PCR. Data are representatives of three independent experiments. (B) Gene expression for *Pr5-HT_{1B}* was examined by immunofluorescence. The scale bar represents 10 μ m. Data are representatives of two independent experiments. (C) Gene expression for *Pr5-HT_{2B}* was examined by immunofluorescence. PL, plasmatocytes; GR, granulocytes. Data are representatives of two independent experiments. (D-G) The effect of different antagonist on hemocyte phagocytosis was visualized by fluorescence microscope. SB216641 is an antagonist of 5-HT_{1B}. SB269970 is an antagonist of 5-HT₇ and RS127445 is a human 5-HT_{2B} antagonist. (H) Quantification of different antagonist on hemocyte phagocytosis. Data are from three independent experiments that each consists of cells from ten fifth-instar larvae. One-way ANOVA followed by Tukey's multiple comparison test for H. Error bars indicate $\pm$ s.e.m., *** P < 0.001, ** P < 0.01 and NS means no significant difference.

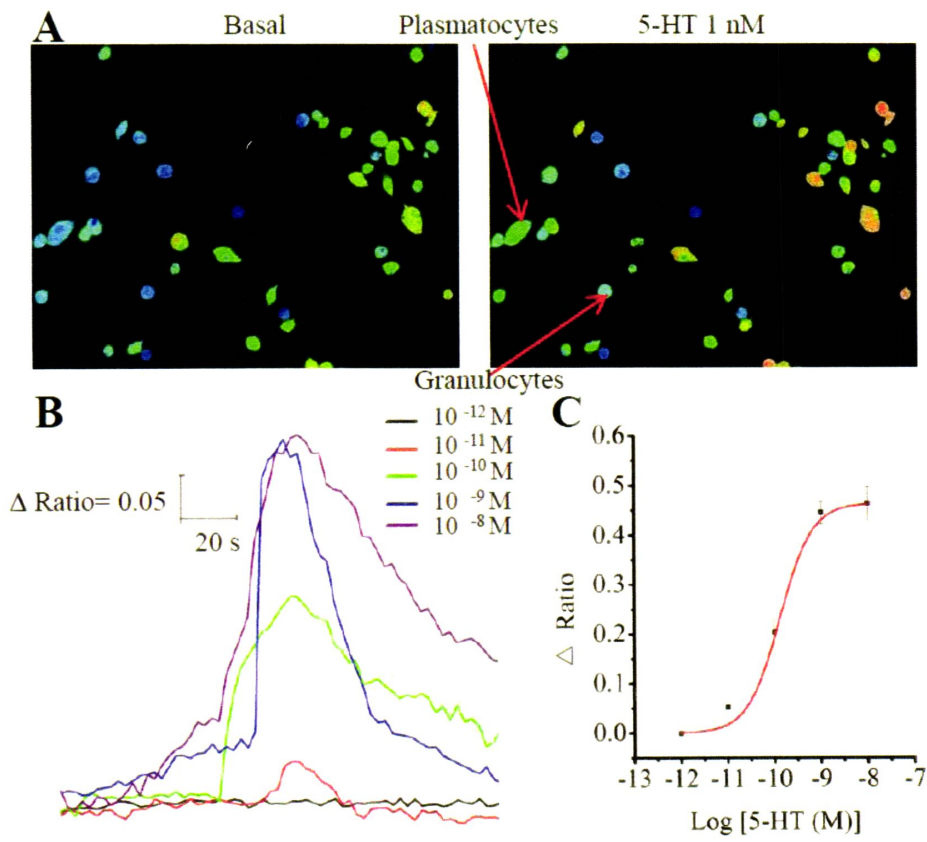


图 5.5 血细胞内5-HT引起的钙离子变化

Fig 5.5 Representative Ca^{2+} responses and dose-response profiles for 5-HT in hemocytes. (A) Pseudocolored images of hemocytes before and after application of 1 nM 5-HT. Red cells indicate high levels of intracellular Ca^{2+} measured by fluorescent ratio intensities, and blue cells represent the basal levels. Two major kinds of hemocytes, plasmatocytes and granulocytes, are indicated by arrows. (B) Increasing concentrations of 5-HT dose-dependently induced changes in the fluorescence ratio of hemocytes. (C) Dose-response curve for 5-HT in hemocytes, as obtained from Ca^{2+} imaging. Each point represents the mean $\pm$ s.e.m. from three to five replicates.

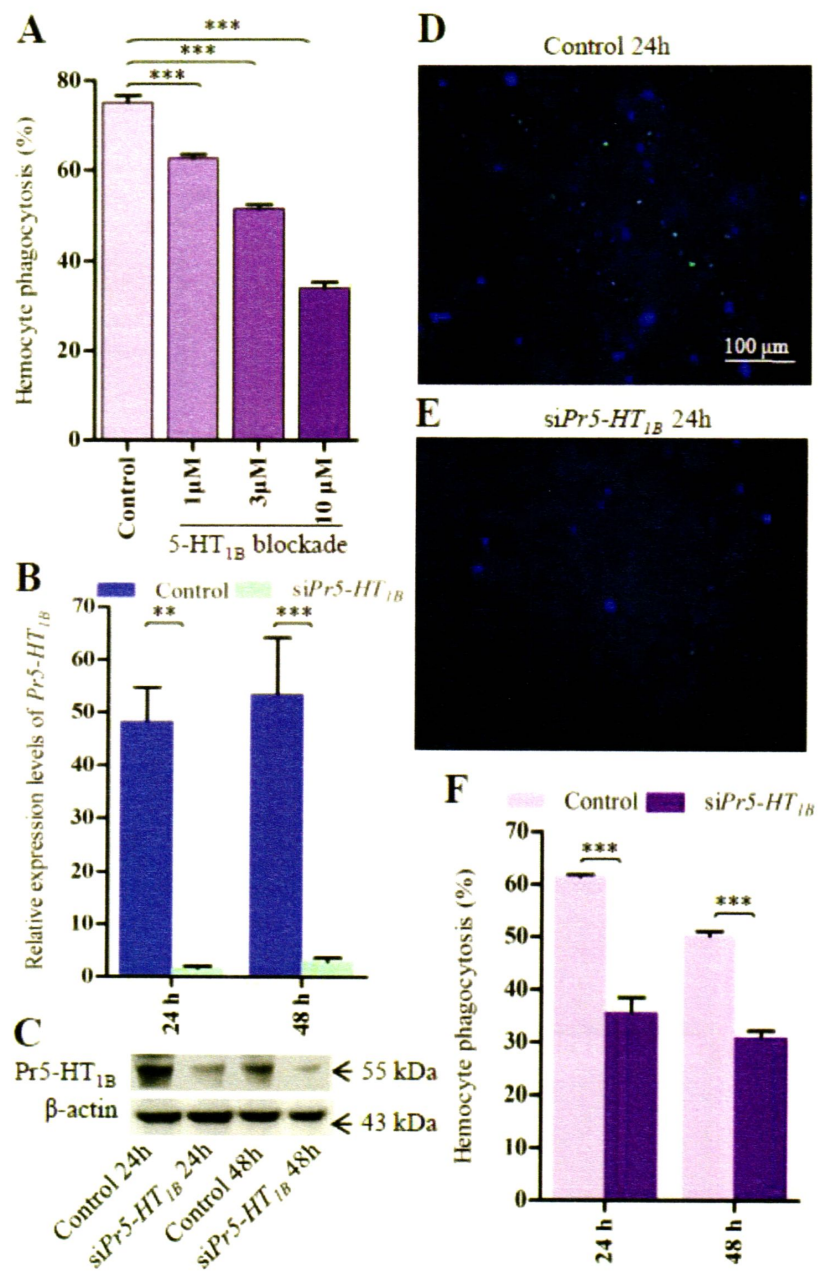


图 5.6 Pr5-HT_{1B}调控血细胞吞噬

Fig 5.6 Pr5-HT_{1B} mediates hemocyte phagocytosis. Dose-response profiles for the effects of Pr5-HT_{1B} blocker SB 216641 on hemocyte phagocytosis. Data are from three independent experiments that each consists of cells from ten fifth-instar larvae. (B) Confirmation of knock-down effect of *Pr5-HT_{1B}* by real-time PCR. The *P. rapae* 18s rRNA gene was used as an internal reference gene ($n = 4$). (C) Western blot analysis of knock-down effect of Pr5-HT_{1B}. β-actin was used to show equal protein loading. (D-E) Effect of *siPr5-HT_{1B}* on hemocyte phagocytosis was visualized by fluorescence microscope. (F) Quantification of

siPr5-HT_{1B} effect on hemocyte phagocytosis. Data are from three independent experiments that each consists of cells from ten fifth-instar larvae. One-way ANOVA followed by Tukey's multiple comparison test for A; two-tailed *t*-test for B and F. Error bars indicate $\pm$ s.e.m., ****P* < 0.001, ***P* < 0.01.

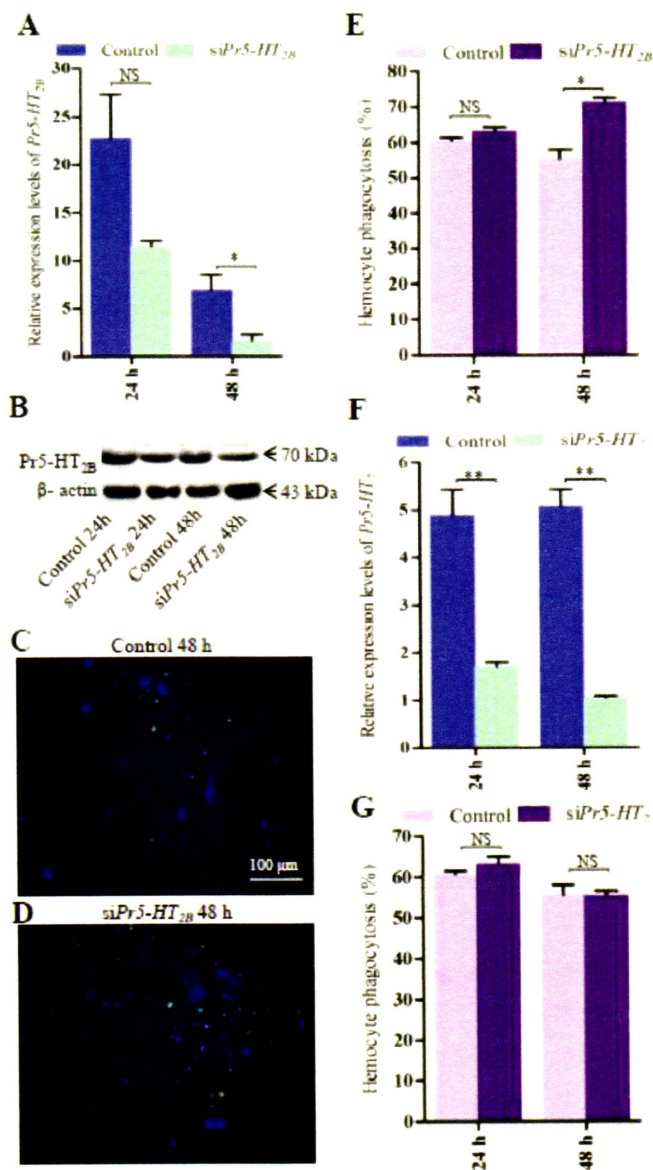


图 5.7 干扰Pr5-HT_{2B}和Pr5-HT₇对血细胞吞噬的影响

Fig 5.7 Effect of siPr5-HT_{2B} and siPr5-HT₇ on hemocyte phagocytosis. Confirmation of knock-down effect of Pr5-HT_{2B} by real-time PCR. The *P. rapae* 18s rRNA gene was used as an internal reference gene (*n* = 3). (B) Western blot analysis of knock-down effect of Pr5-HT_{2B}. β-actin was used to show equal protein loading. (C-D) Effect of siPr5-HT_{2B} on hemocyte phagocytosis was visualized by fluorescence microscope. (E) Quantification of siPr5-HT_{2B} effect on hemocyte phagocytosis (*n* = 3). (F) Confirmation of knock-down effect of Pr5-HT₇ by

real-time PCR. The *P. rapae* 18s rRNA gene was used as an internal reference gene ($n= 3$). (G) Quantification of siPr5-HT₇ effect on hemocyte phagocytosis ($n= 3$). Two-tailed *t*-test for A, E, F and G. Error bars indicate $\pm$ s.e.m., *** $P< 0.001$, ** $P< 0.01$, * $P< 0.05$ and NS means no significant difference.

3.4 免疫诱导后血细胞中 5-HT_{1B} 和 5-HT_{2B} 的表达量变化

虽然上面的数据显示激活的Pr5-HT_{1B}和Pr5-HT_{2B}受体对细胞免疫产生相反的影响，但是5-HT却能提高血细胞吞噬。为了弄清楚其中的原因，我们检测了血细胞受诱导后这两个受体的表达量变化。荧光定量PCR的结果表明血细胞被LPS激活15 min到4 h时，Pr5-HT_{1B}的转录量显著升高（图5.8A），激活2 h和4 h时，Pr5-HT_{1B}蛋白表达量明显升高（图5.8C）。有意思的是，血细胞Pr5-HT_{2B}的mRNA表达量在LPS诱导1 h和4 h时显著降低（图5.8B），蛋白表达量也在对应的时间点降低（图5.8D）。

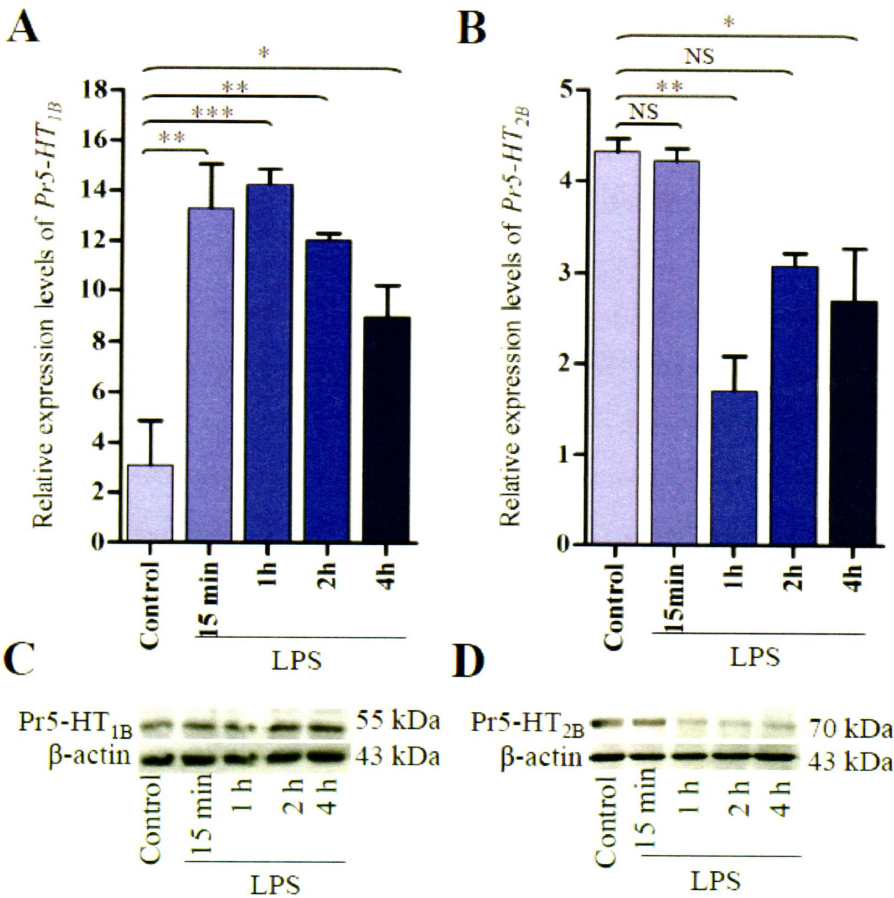


图 5.8 LPS诱导血细胞后Pr5-HT_{1B}和Pr5-HT_{2B}的表达量变化

Fig 5.8 Expression analysis of Pr5-HT_{1B} and Pr5-HT_{2B} in naïve and LPS-induced hemocytes. (A-B) Relative expression of Pr5-HT_{1B} and Pr5-HT_{2B} were quantified by q-PCR. The *P. rapae* 18s rRNA gene was used as an internal reference ($n = 3$). (C-D) Western blot analysis of Pr5-HT_{1B} and Pr5-HT_{2B} in naïve and LPS-induced hemocytes. β -actin was used to show equal protein loading. One-way ANOVA followed by Tukey's multiple comparison test for A and B. Error bars indicate $\pm$ s.e.m., *** $P < 0.001$, ** $P < 0.01$ * $P < 0.05$, and NS means no significant difference.

3.5 5-HT_{1B} 和 5-HT_{2B} 调控黑腹果蝇的吞噬和抵抗病原菌

我们推测5-HT也调控其他昆虫的免疫，并且在抵抗病原菌方面发挥重要作用。由于鳞翅目昆虫体内RNAi很难成功 (Terenius *et al.*, 2011)，因此我们选择了模式生物黑腹果蝇*Drosophila melanogaster*来做体内的实验。通过RT-PCR，我们在果蝇幼虫血细胞里检测到了5-HT_{1B}和5-HT_{2B}的转录本。果蝇中TPH的同源基因Henna也在血细胞上有表达，并且mRNA的量相对较高（图5.9A）。我们检测了敲除5-HT_{1B}基因的突变体果蝇（5-HT_{1B}^{ΔIII-V}）和它相对应的对照果蝇的吞噬细菌能力以及病原菌感染后的生存率。5-HT_{1B}^{ΔIII-V}果蝇是将基因组DNA中5-HT_{1B}基因的第三跟第四跨膜区之间1344 bp的片段敲除掉了（图5.9B）。

果蝇体内吞噬能力的计算参照Kocks等介绍的果蝇成虫体内吞噬分析法（2005），计算背血管附近的荧光值。5-HT_{1B}^{ΔIII-V}果蝇体内吞噬*E.coli*的能力比对照果蝇弱很多（图5.9C-D）。吞噬革兰氏阳性菌*S. aureus*实验出现了类似的结果（图5.9E-F）。被致病菌*S. aureus*感染后，5-HT_{1B}^{ΔIII-V}果蝇比对照果蝇死的更快，雌雄果蝇抵抗*S. aureus*的能力均下降（图5.9G-H）。为了检测5-HT_{1B}^{ΔIII-V}果蝇较高的死亡率是由于抵抗病原菌的能力下降（defective resistance）还是由于对病原菌的耐受力下降（decreased tolerance）（Schneider and Ayres, 2008），我们检测了感染6 h和18 h的果蝇体内病原菌的扩增情况。结果显示5-HT_{1B}^{ΔIII-V}果蝇体内病原菌的扩增速率比对照果蝇扩增速率快（图5.9I）。

另外，我们通过RNAi的方法验证了5-HT_{1B}调控果蝇体内血细胞吞噬。UAS/GAL4系统是果蝇遗传学中一种常用的异位表达系统。UAS (upstream active sequence, UAS)上游激活序列，是酵母中一种类似高等真核生物增强子的序列。GAL4 (GAL actose-regulated upstream promoter element, GAL半乳糖调节上游启动子元件)，是一个酵母转录激活因子。与原核生物乳糖操纵子相似，在酵母中GAL4与UAS结合可以调节与半乳糖代谢相关基因的表达。Fischer等将GAL4在果

蝇的特定组织中表达,发现GAL4能以组织特异性的方式激活与UAS相连接的下游基因的转录(1988)。由于UAS-靶基因系中,其转录激活不存在,因而靶基因处于沉默状态,只有将GAL4转基因系与UAS-靶基因系进行杂交,才可能产生特异表达靶基因的后代。我们用*HmlΔ-Gal4*果蝇 (Sinenko *et al.*, 2004)作为驱动子分别跟*UAS-5-HT_{1B} RNAi* (Yuan *et al.*, 2005)和*UAS-5-HT_{1B} RNAi²⁵⁸³³*果蝇杂交,特异性干扰血细胞内5-HT_{1B}的表达。以*HmlΔ-Gal4*果蝇, *UAS-5-HT_{1B} RNAi*果蝇和*UAS-5-HT_{1B} RNAi²⁵⁸³³*果蝇分别跟野生型果蝇*W¹¹¹⁸*杂交的F1代为对照果蝇。*UAS-5-HT_{1B} RNAi*果蝇和*UAS-5-HT_{1B} RNAi²⁵⁸³³*果蝇分别跟*HmlΔ-Gal4*果蝇杂交的F1代果蝇吞噬*E. coli*和*S. aureus*的能力均比对照组低(图5.10A-D)。为了确定干扰血细胞内5-HT_{1B}的表达是破坏了血细胞本身的吞噬能力,还是导致了血细胞对病原菌的免疫诱导不再敏感,我们做了给果蝇体内注射PBS加珠子(latex beads)或5-HT加珠子的实验。给所有杂交品系的果蝇注射PBS不会导致吞噬珠子的差异,而给所有杂交品系的果蝇注射5-HT时,对照组果蝇吞噬珠子的量显著上升,而干扰了血细胞5-HT_{1B}表达的果蝇品系吞噬珠子的能力明显比对照果蝇低(图5.10E)。被病原菌*S. aureus*感染后,干扰了血细胞5-HT_{1B}表达的果蝇品系明显比对照果蝇死的快(图5.10F-I)。RNAi的数据表明敲除5-HT_{1B}基因的突变体果蝇免疫能力下降是由于血细胞内5-HT_{1B}的功能缺失引起的。

为了研究5-HT_{2B}在果蝇吞噬方面所起的作用,我们通过两个独立的RNAi干扰品系跟*HmlΔ-Gal4*果蝇杂交达到特异性干扰血细胞内5-HT_{2B}的目的。跟菜青虫中获得的5-HT_{2B}实验结果不同,血细胞内5-HT_{2B}表达量的降低导致了果蝇吞噬*E. coli*和*S. aureus*的能力减弱(图5.11A-D)。而且珠子吞噬实验表明,注射5-HT能使干扰了血细胞5-HT_{2B}表达的果蝇比对照果蝇吞噬珠子的能力低(图5.11E)。注射病原菌*S. aureus*到果蝇体内后,血细胞内5-HT_{2B}被干扰的果蝇死的比对照组快(图5.11F-G),而且病原菌在其体内的扩增速率也显著快于对照果蝇(图5.11I)。因此,我们的实验可以说明5-HT_{1B}和5-HT_{2B}在宿主抵抗病原菌的过程中起了非常重要的作用。

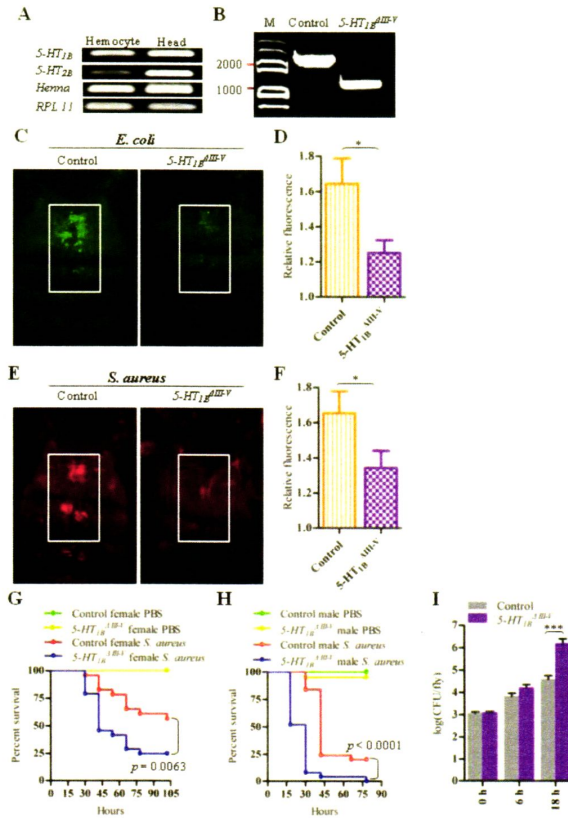


图 5.9 5-HT_{1B}在调控果蝇吞噬和抵抗病原菌的感染过程中发挥重要作用

Fig 5.9 5-HT_{1B} is required for microbial phagocytosis and plays an important role in the *Drosophila* defense against *S. aureus* infection. (A) 5-HT_{1B}, 5-HT_{2B} and TPH are expressed in *Drosophila* naïve hemocytes. RPL11 was used as an internal reference gene. Data are *representatives* of three independent experiments. (B) Genomic PCR of 5-HT_{1B} control and 5-HT_{1B}^{ΔIII-V} flies. Data are *representatives* of three independent experiments. (C) Representative pictures depicting phagocytosis in 5-HT_{1B} control and 5-HT_{1B}^{ΔIII-V} flies of fluorescein-labeled *E. coli* bioparticles. (D) Quantification of in vivo phagocytosis of *E. coli*. Approximately ten flies per genotype were used in each experiment. Data are *representatives* of three independent experiments. (E) Representative pictures depicting phagocytosis in 5-HT_{1B} control and 5-HT_{1B}^{ΔIII-V} flies of fluorescein-labeled *S. aureus* bioparticles. (F) Quantification of in vivo phagocytosis of *S. aureus*. Approximately eight flies per genotype were used in each experiment. Data are *representatives* of three independent experiments. (G-H) Representative survival curves of female (G) and male (H) 5-HT_{1B} control and 5-HT_{1B}^{ΔIII-V} flies after injection of *S. aureus* (optical density [OD] 0.4). *n* = 20-25 flies. Experiments were performed in triplicate. (I) Comparison of the *S. aureus* (OD 0.4) recovered in 5-HT_{1B} control and 5-HT_{1B}^{ΔIII-V} flies 0, 6, and 18 hr post infection. Bacterial load was measured in eight individual female flies per genotype at each time point in each experiment. Two-tailed *t*-test for D, F and I. Error bars indicate ± s.e.m., ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

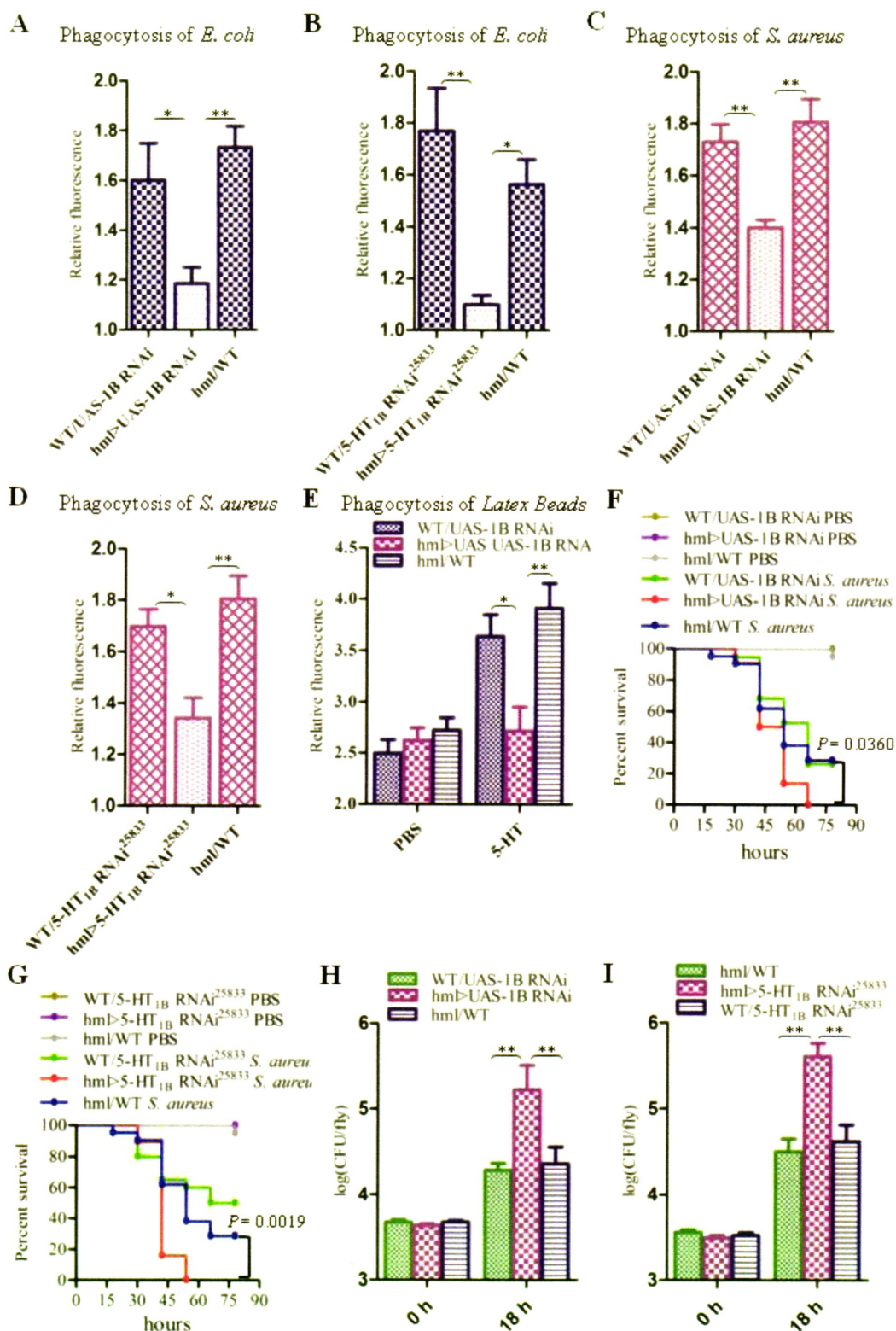


图 5.10 干扰果蝇血细胞中5-HT_{1B}的表达会影响吞噬和生存率

Fig 5.10 Knockdown of 5-HT_{1B} in hemocytes affects *Drosophila* phagocytosis and survival. (A) Quantification of in vivo phagocytosis of *E. coli* in WT/*UAS-1B RNAi*, *hml*> *UAS-1B RNAi* and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice, (B) Quantification of in vivo phagocytosis of *E. coli* in WT/*UAS-5-HT_{1B} RNAi*²⁵⁸³³, *hml*>*UAS-5-HT_{1B} RNAi*²⁵⁸³³ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice. (C) Quantification of in vivo phagocytosis of *S. aureus* in WT/*UAS-1B RNAi*, *hml*> *UAS-1B RNAi* and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice, (D) Quantification of in vivo phagocytosis of *S. aureus* in WT/*UAS-5-HT_{1B} RNAi*²⁵⁸³³, *hml*>*UAS-5-HT_{1B} RNAi*²⁵⁸³³ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice. (E) Quantification of in vivo phagocytosis of red fluorescently labeled latex beads in WT/*UAS-1B RNAi*, *hml*>*UAS-1B RNAi* and *hml*/WT flies after a 30 min preinjection of either PBS or 1μg/μl 5-HT. Approximately six flies per genotype were used in each experiment. Experiments were done twice, (F) Representative survival curves of WT/*UAS-1B RNAi*, *hml*>*UAS-1B RNAi* and *hml*/WT male flies after injection of *S. aureus*. *n*=19-22 flies. Data are *representatives* of three independent experiments. Each experiment was performed in triplicate. (G) Representative survival curves of WT/*UAS-5-HT_{1B} RNAi*²⁵⁸³³, *hml*>*UAS-5-HT_{1B} RNAi*²⁵⁸³³ and *hml*/WT male flies after injection of *S. aureus*. *n*=19-21 flies. Data are *representatives* of two independent experiments. Each experiment was performed in triplicate. (H) Comparison of the *S. aureus* (OD 0.4) recovered in WT/*UAS-1B RNAi*, *hml*>*UAS-1B RNAi* and *hml*/WT flies 0, and 18 hr post infection. Bacterial load was measured in eight individual male flies per genotype at each time point in each experiment. Experiments were performed in triplicate. (I) Comparison of the *S. aureus* (OD 0.4) recovered in WT/*UAS-5-HT_{1B} RNAi*²⁵⁸³³, *hml*>*UAS-5-HT_{1B} RNAi*²⁵⁸³³ and *hml*/WT flies 0 and 18 hr post infection. Bacterial load was measured in eight individual male flies per genotype at each time point in each experiment. Experiments were performed in triplicate. One-way ANOVA followed by Tukey's multiple comparison test for A, B, C, D, E, H and I. Error bars indicate ± s.e.m., ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

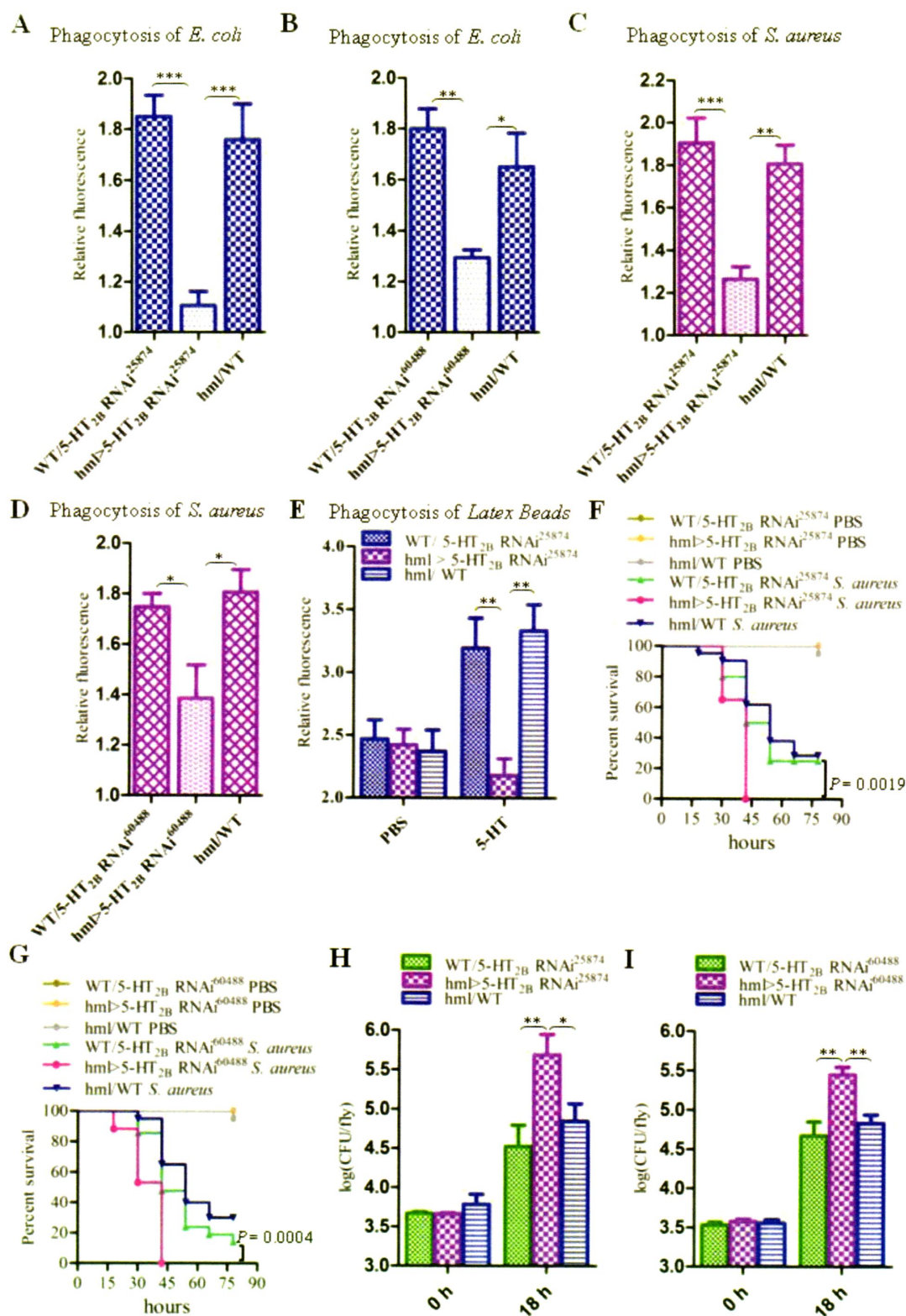


图 5.11 干扰果蝇血细胞中5-HT_{2B}的表达会影响吞噬和生存率

Fig 5.11 Knockdown of 5-HT_{2B} in hemocytes affects *Drosophila* phagocytosis and survival. (A) Quantification of in vivo phagocytosis of *E. coli* in WT/*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴, *hml*>*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice, (B) Quantification of in vivo phagocytosis of *E. coli* in WT/*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸, *hml*>*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice. (C) Quantification of in vivo phagocytosis of *S. aureus* in WT/*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴, *hml*>*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice, (D) Quantification of in vivo phagocytosis of *S. aureus* in WT/*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸, *hml*>*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸ and *hml*/WT flies. Approximately six flies per genotype were used in each experiment. Experiments were performed twice. (E) Quantification of in vivo phagocytosis of red fluorescently labeled latex beads in WT/*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴, *hml*>*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴ and *hml*/WT flies after a 30 min preinjection of either PBS or 1μg/μl 5-HT. Approximately six flies per genotype were used in each experiment. Experiments were done twice, (F) Representative survival curves of WT/*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴, *hml*>*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴ and *hml*/WT male flies after injection of *S. aureus*. *n*=20-21 flies. Data are *representatives* of two independent experiments. Each experiment was performed in triplicate. (G) Representative survival curves of WT/*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸, *hml*>*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸ and *hml*/WT male flies after injection of *S. aureus*. *n*=19-21 flies. Data are *representatives* of two independent experiments. Each experiment was performed in triplicate. (H) Comparison of the *S. aureus* (OD 0.4) recovered in WT/*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴, *hml*>*UAS-5-HT_{2B} RNAi*²⁵⁸⁷⁴ and *hml*/WT flies 0, and 18 hr post infection. Bacterial load was measured in eight individual male flies per genotype at each time point in each experiment. Experiments were performed in triplicate. (I) Comparison of the *S. aureus* (OD 0.4) recovered in WT/*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸, *hml*>*UAS-5-HT_{2B} RNAi*⁶⁰⁴⁸⁸ and *hml*/WT flies 0, and 18 hr post infection. Bacterial load was measured in eight individual male flies per genotype at each time point in each experiment. Experiments were performed in triplicate. One-way ANOVA followed by Tukey's multiple comparison test for A, B, C, D, E, H and I. Error bars indicate ± s.e.m., ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

4 讨论

我们的研究结果说明了生物体内普遍存在的5-HT受体信号通路在免疫中的作用。本研究首次探明了昆虫血细胞表达TPH,并且可以合成5-HT,这与人的巨噬细胞类似(Nakamura *et al.*, 2008)。被LPS激活的血细胞TPH的表达量升高,从而促进5-HT的合成。而且,血细胞分泌的5-HT似乎是一个重要的自分泌刺激因子。它能促进吞噬:通过药物抑制TPH的活性或者通过siRNA干扰TPH的表达均能显著降低血细胞的吞噬率。

神经系统中神经递质也能调控免疫(Meredith *et al.*, 2005)。5-HT就是其中一种经典的神经递质,它在昆虫和哺乳动物免疫调控中起了重要作用。近些年的研究在5-HT调控哺乳动物免疫方面有了一定的进展,特别是免疫细胞合成、储存、释放5-HT以及对5-HT的反应(Ahern, 2011)。已有研究表明5-HT调控昆虫一系列的免疫反应,比如血细胞吞噬、结节、血细胞数量等(Baines *et al.*, 1992; Kim *et al.*, 2009; Kim and Kim, 2010),但是具体的信号通路还不清楚。通过全基因组RNAi筛查影响血细胞吞噬白色念珠菌*Candida albicans*的基因发现,黑腹果蝇的Henne(TPH同源基因)基因就是其中一个(Stroschein-Stevenson *et al.*, 2006)。研究昆虫中5-HT受体调控的第二信使系统跟免疫相关的胞内信号通路之间的关系也许可以帮助我们更好的认识哺乳动物中这两者之间的作用和联系。

另外,我们的研究结果表明未被诱导的菜青虫血细胞内表达5-HT_{1B}、5-HT_{2B}和5-HT₇受体,但是只有5-HT_{1B}和5-HT_{2B}有功能。通过选择性的拮抗剂和RNAi的方法,我们发现,阻断5-HT_{1B}受体的功能会降低血细胞吞噬,而阻断5-HT_{2B}受体的功能可以促进血细胞吞噬。显然,这两个受体以相反的方式影响血细胞。有趣的是,我们发现血细胞被LPS激活后,5-HT_{1B}的表达量显著上升,而5-HT_{2B}的表达量却明显下降。因此,即便这两种受体在血细胞上的作用方式不同,但是血细胞被激活后,这两种受体都是有利于细胞吞噬的(图5.12)。哺乳动物树突细胞也表达5-HT_{1B}和5-HT_{2B}受体,5-HT通过这两个受体促进树突细胞的趋化性(Müller *et al.*, 2009)。虽然昆虫的浆血细胞类似于巨噬细胞,但是5-HT通过5-HT_{1A}(Nakamura *et al.*, 2008)和5-HT_{2C}(Mikulski *et al.*, 2010)激活巨噬细胞,这跟昆虫血细胞内激活的受体类型并不一致。5-HT₇在老鼠肠道免疫反应中起了关键性的作用(Kim *et al.*, 2013)。虽然我们的实验结果表明5-HT₇并不参与调控

血细胞吞噬，但是该受体可能调控其他的免疫反应。

哺乳动物免疫细胞上也表达一些神经递质受体，这些受体调控先天免疫行为。但是，这些受体在生物体内的具体功能并不清楚，因为大部分的研究都是在体外进行的（Sternberg, 2006），而在昆虫体内进行免疫实验相对容易（Lemaitre and Hoffmann, 2007）。我们发现敲除5-HT_{1B}受体的突变体果蝇由于吞噬能力下降，被病原菌感染后更容易死亡。定点干扰血细胞内5-HT_{1B}受体的果蝇出现类似的结果，表明5-HT_{1B}受体调控的血细胞吞噬在昆虫抵抗病原菌的过程中发挥了重要作用。干扰了血细胞5-HT_{2B}受体表达的果蝇感染病原菌后比对照果蝇更脆弱，说明激活果蝇体内5-HT_{2B}受体会促进吞噬，这跟菜青虫体外实验结果并不一致。

我们可以得出结论，昆虫血细胞能合成和释放5-HT，释放的5-HT通过血细胞内5-HT_{1B}和5-HT_{2B}受体调控吞噬。我们用模式生物黑腹果蝇进一步证明了5-HT_{1B}和5-HT_{2B}受体在体内调控血细胞吞噬的功能。这些结论说明5-HT这种古老的信号分子，可以调控不同物种的免疫。而由于昆虫的免疫系统相对简单，研究5-HT受体调控的信号通路跟免疫相关的通路之间的相互关系会更容易。

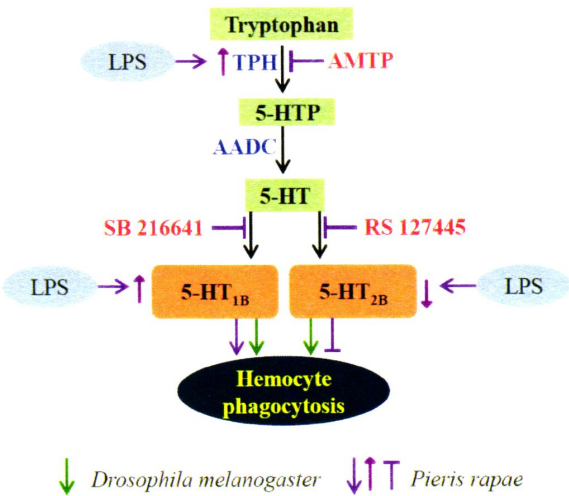


图 5.12 总结模式图

Fig 5.12 A schematic diagram of serotonin signaling on hemocyte phagocytosis. LPS enhances the expression of TPH, which catalyzes tryptophan into 5-HT via 5-HTP. 5-HT, which secreted from hemocytes, activates the hemocyte-membrane receptor 5-HT_{1B} and 5-HT_{2B}. The immune responses of *P. rapae* are labeled in purple: activation of 5-HT_{1B} promotes hemocyte phagocytosis and activation of 5-HT_{2B} lead to opposite effects. LPS increases 5-HT_{1B} expression but decreases that of 5-HT_{2B}. The immune responses of *Drosophila* are labeled in green arrows: activation of 5-HT_{1B} promotes hemocyte phagocytosis and activation of 5-HT_{2B} lead to the same effects.

总讨论

1 小结

5-羟色胺是一种古老的单胺类神经递质,它属于生物胺。生物胺来源于芳香族氨基酸,能作为神经递质和神经调制在中枢神经系统中发挥作用,也能释放到外周组织,作为神经激素发挥作用。生物胺调控生物体很多的生理和行为过程。昆虫体内有 5 种主要的生物胺,包括 5-羟色胺、多巴胺、酪胺、章鱼胺和组胺。5-HT 在很多物种中发挥重要作用,人体 5-HT 系统的紊乱跟很多的疾病相关,比如偏头痛、抑郁症、精神分裂症和焦虑等 (Jones and Blackburn, 2002)。5-HT 调控昆虫的取食 (Gasque *et al.*, 2013)、昼夜节律 (Yuan *et al.*, 2005)、聚集 (Anstey *et al.*, 2009)、攻击行为 (Dierick *et al.*, 2007) 以及学习和记忆 (Sitaraman *et al.*, 2008) 等。5-羟色胺主要通过偶联相应的受体,调控生物体的生理功能。脊椎动物中已克隆出 14 个 5-HT 受体基因,除了 5-HT₃ 为离子通道型受体,其余 13 个均为 GPCR 受体 (Blenau and Baumann, 2001)。Hoyer 等将脊椎动物体内 GPCR 型的 5-HT 受体分为 6 大类 (Hoyer and Clarke, 1994; Hoyer and Martin, 1995; Hoyer and Martin, 1997)。其中: 5-HT₁ (5-HT_{1A/B/D/E/F}) 和 5-HT₅ (5-HT_{5A/B}) 偶联 G_i 蛋白抑制胞内 cAMP 的合成; 5-HT₂ (5-HT_{2A/B/C}) 偶联 G_q 蛋白调节胞内 Ca²⁺ 量升高; 5-HT₄、5-HT₆ 和 5-HT₇ 偶联 G_s 蛋白促进胞内 cAMP 的形成 (Hannon and Hoyer, 2008)。目前昆虫中仅发现 5 种 GPCR 型 5-HT 受体,分别为: 5-HT_{1A}、5-HT_{1B}、5-HT_{2A}、5-HT_{2B} 和 5-HT₇, 分别跟哺乳动物 5-HT_{1A}、5-HT_{1B}、5-HT_{2A}、5-HT_{2B} 和 5-HT₇ 受体同源 (Blenau and Thamm, 2011)。尽管昆虫 5-HT 受体跟脊椎动物 5-HT 受体序列相似性高,且偶联的第二信使相同,但是昆虫 5-HT 受体的药理学性质跟脊椎动物 5-HT 受体的药理学性质存在差异。虽然哺乳动物 5-HT 受体的药理学性质得到很好的研究,但是哺乳动物 5-HT 受体的激动剂和拮抗剂并没有在昆虫 5-HT 受体上得到全面检测。并且深入研究 5-HT 受体药理学性质的报道不多。因而导致昆虫 5-HT 受体的药理学性质很多还未知。我们对菜青虫 5-HT_{1A}、5-HT_{1B} 和 5-HT₇ 受体进行了药理学检测,表 6.1 显示这三个受体的药理学性质存在差异。由于未能克隆到 5-HT_{2A} 和 5-HT_{2B} 两个受体的全长,因而无法对这两个受体的药理学性质进行解析。研究菜青虫 5-HT 受体的药理学性质将有

助于了解昆虫 5-HT 受体药理学性质跟脊椎动物 5-HT 受体药理学性质的差异，为昆虫 5-HT 受体的分类提供理论基础。

5-羟色胺信号通路参与调控哺乳动物的先天免疫和获得性免疫得到了广泛的研究 (Ahern, 2011)。但是 5-HT 调控昆虫免疫的研究并不多，少量文献报道 5-HT 可以调控昆虫血细胞吞噬、结节、血细胞数量等 (Baines *et al.*, 1992; Kim *et al.*, 2009; Kim and Kim, 2010)，而这些实验主要是在体外进行的，5-HT 调控昆虫免疫的具体信号通路并不清楚。我们的研究发现昆虫血细胞可以自身合成并分泌 5-HT。基于药理学手段和 RNAi 技术，我们发现 5-HT 通过 5-HT_{1B} 和 5-HT_{2B} 调控血细胞吞噬。模式生物黑腹果蝇体内的实验数据进一步证明 5-HT 可以通过 5-HT_{1B} 和 5-HT_{2B} 两个受体调控血细胞吞噬病原菌的能力。这些结果可为开发针对破坏害虫免疫系统的药剂提供理论基础。

表 6.1 菜青虫体内 5-HT 受体的药理学性质

Table 6.1 The pharmacological properties of serotonin receptors from <i>Pieris rapae</i>					
受体名称 Receptor	偶联信号 Transduction	激动剂 Agonist	致死中浓 度 EC ₅₀	拮抗剂 Antagonist	半抑制浓 度 IC ₅₀
Pr5-HT _{1A}	G _i , [cAMP] _i ↑	5-HT	63.90 nM		
Pr5-HT _{1B}	G _i , [cAMP] _i ↑	5-HT	2.59 nM	Methiothepin	2.49 μM
		8-OH-DPAT	-	SB-216641	-
		5-Methoxytryptamine	1.44 μM		
Pr5-HT ₇	G _s , [cAMP] _i ↓	5-HT	10.77 nM	Methiothepin	899.16 nM
		8-OH-DPAT	3.02 μM	SB-269970	-
		5-Methoxytryptamine	4.29 μM		
Pr5-HT ₈	G _q , [Ca ²⁺] _i ↑	5-HT	44.8 nM	WAY-100635	-
		8-OH-DPAT	603.1 nM		
		5-Methoxytryptamine	14.1 nM		
		5-Carboxamidotryptamine	19.0 nM		
		Methiothepin	17.4 μM		

基于昆虫种类繁多，目前尚不清楚昆虫体内是否表达跟脊椎动物不同类型的 5-HT 受体。我们克隆到了一个新的 5-HT 受体，该受体跟目前已知的 5-HT 受体序列相似性低。通过体外表达，并进行药理学分析发现，该受体能被广谱性的 5-HT 受体激动剂激活，激动剂效果为 5-Methoxytryptamine>5-Carboxamidotryptamine>8-OH-DPAT> Methiothepin（表 6.1）。Methiothepin (Monachon *et al.*, 1972) 是昆虫 5-HT_{1A}(Thamm *et al.*, 2010)、5-HT_{2A}(Röser *et al.*, 2012)和 5-HT₇(Schlenstedt *et al.*, 2006)的完全拮抗剂。它能激活 Pr5-HT₈ 受体，引起胞内 Ca²⁺浓度升高。WAY-100635 对该受体有拮抗作用。

我们在一些害虫和媒介昆虫的基因组中找到了 Pr5-HT₈ 受体的同源基因, 如赤拟谷盗和蚊子, 但是在蜜蜂, 寄生蜂和果蝇的基因组中没有找到该基因的同源序列。而且我们在哺乳动物的基因组中也没有找到 5-HT₈ 受体基因的同源基因, 说明它可能是一类无脊椎动物特异性的受体, 由于它主要在害虫的基因组中存在, 因而有可能成为一个新的农药靶标。

2 本研究的创新之处

- 1) 对菜青虫的 3 个 5-HT 受体 5-HT_{1A}、5-HT_{1B} 和 5-HT₇ 进行了体外功能性表达和药理学性质实验, 可以比较 3 个受体的异同。
- 2) 克隆得到一个新的 5-HT 受体基因 (Pr5-HT₈), 通过体外药理学实验证明该受体偶联 G_q, 能引起胞内 Ca²⁺ 浓度上升。我们在一些害虫如鳞翅目和鞘翅目昆虫的基因组数据中找到该基因的同源基因, 而在哺乳动物以及膜翅目昆虫和果蝇中则不存在。
- 3) 以菜青虫血细胞为研究对象, 通过体外诱导, 药物阻断以及 RNAi 等方法首次证明了昆虫血细胞能自身合成并分泌 5-HT。5-HT 通过血细胞上 5-HT_{1B} 和 5-HT_{2B} 受体调控吞噬, 这一结果在模式生物黑腹果蝇体内得到验证。

3 本研究的不足之处

- 1) 对菜青虫 5-HT_{1A}、5-HT_{1B} 和 5-HT₇ 受体的药理学实验中, 所用的激动剂和拮抗剂数量不多, 并且没有进一步研究这三个受体可能参与的生理功能。
- 2) 菜青虫 5-HT₈ 受体参与调控的生理功能不明确。
- 3) 虽然通过黑腹果蝇验证了 5-HT_{1B} 和 5-HT_{2B} 受体调控体内血细胞吞噬, 但是这跟菜青虫体外实验的结果有一点不同。由于鳞翅目昆虫体内 RNAi 实验不容易成功, 所以菜青虫体外的实验结果没有在自身虫体内得到验证。

4 今后的研究方向

- 1) 完善菜青虫 5-HT_{1A}、5-HT_{1B} 和 5-HT₇ 受体的药理学实验。
- 2) 改善菜青虫体内 RNAi 的效率, 或者通过受体选择性拮抗剂来完成体内实验。

3) 利用药物阻断或者 RNAi 的方法研究 5-HT₈ 受体可能参与的生理功能, 并研究其作为杀虫剂靶标的可能性。

参考文献

- 齐易香, 吴顺凡, 黄佳, 等. 2014. 昆虫 5-羟色胺及其受体的研究进展[J]. 昆虫学报, 57(7): 844-859.
- Adamo SA. 2008. Norepinephrine and octopamine: linking stress and immune function across phyla. *Invert. Surv. J.*, 5: 12-19.
- Aderem A and Underhill DM, 1999. Mechanisms of phagocytosis in macrophages. *Annu. Rev. Immunol.*, 17(1): 593-623.
- Ahern GP, 2011. 5-HT and the immune system. *Curr. Opin. Pharmacol.*, 11(1): 29-33.
- Akiyoshi T, Zhang Q, Inoue F, Aramaki O, Hatano M, Shimazu M, Kitajima M, Shirasugi N, Niimi M. 2006. Induction of indefinite survival of fully mismatched cardiac allografts and generation of regulatory cells by sarpogrelate hydrochloride. *Transplantation*, 82(8): 1051-1059.
- Alekseyenko O, Chan YB, Fernandez MP, Bülow T, Pankratz MJ, Kravitz E. 2014. Single serotonergic neurons that modulate aggression in *Drosophila*. *Curr. Biol.*, 24(15): 2700-2707.
- Anstey ML, Rogers SM, Ott SR, Burrows M, Simpson SJ, 2009. Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. *Science*, 323(5914): 627-630.
- Baganz, NL and Blakely RD, 2013. A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem. Neurosci.*, 4(1): 48-63.
- Baines D, DeSantis T, and Downer RG, 1992. Octopamine and 5-hydroxytryptamine enhance the phagocytic and nodule formation activities of cockroach (*Periplaneta americana*) haemocytes. *J. Insect Physiol.*, 38(11): 905-914.
- Bamji SX, Orchard I, 1995. Pharmacological profile of octopamine and 5-HT receptors on the lateral oviducts of the cockroach, *Periplaneta americana*. *Arch. Insect Biochem. Physiol.*, 28(1): 49-62.
- Barak LS, Tiberi M, Freedman NJ, Kwatra MM, Lefkowitz RJ, Caron MG, 1994. A highly conserved tyrosine residue in G protein-coupled receptors is required for agonist-mediated beta 2-adrenergic receptor sequestration. *J. Biol. Chem.*,

- 269(4) : 2790-2795.
- Bauer M, Bschor T, Pfennig A, Whybrow PC, Angst J, Versiani M, Möller HJ, 2007. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of unipolar depressive disorders in primary care. *World J. Biol. Psychiatry*, 8(2): 67-104.
- Baxter GD, Barker SC, 1999. Isolation of a cDNA for an octopamine-like, G-protein coupled receptor from the cattle tick, *Boophilus microplus*. *Insect Biochem. Mol. Biol.*, 29(5): 461-467.
- Becnel J, Johnson O, Luo J, Nässel DR, Nichols CD, 2011. The serotonin 5-HT₇Dro receptor is expressed in the brain of *Drosophila*, and is essential for normal courtship and mating. *PLoS ONE*, 6(6): e20800.
- Blakely RD, Berson HE, Freneau RT Jr, Caron MG, Peek MM, Prince HK, Bradley CC, 1991. Cloning and expression of a functional serotonin transporter from rat brain. *Nature*, 354: 66-70
- Blenau W, Baumann A, 2001. Molecular and pharmacological properties of insect biogenic amine receptors: lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arch. Insect Biochem. Physiol.*, 48(1): 13-38.
- Blenau W, Thamm M, 2011. Distribution of serotonin (5-HT) and its receptors in the insect brain with focus on the mushroom bodies. Lessons from *Drosophila melanogaster* and *Apis mellifera*. *Arthropod Struct. Dev.*, 40(5): 381-394.
- Boehme SA, Lio FL, Pandit TS, Lavrador K, Rao SP, Sriramara P. 2008. Cutting edge: serotonin is a chemotactic factor for eosinophils and functions additively with eotaxin. *J. Immunol.*, 173(6): 3599-3603.
- Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M, Van Natta K, Chang L, Pulido-Rios T, Webber A, 1999. RS-127445: a selective, high affinity, orally bioavailable 5-HT_{2B} receptor antagonist. *Br. J. Pharmacol.*, 127(5): 1075-1082.
- Borue X, Condrón B, Venton BJ, 2010. Both synthesis and reuptake are critical for replenishing the releasable serotonin pool in *Drosophila*. *J. Neurochem.*, 113(1): 188-199.

- Bowsher RR, Henry DP, 1986. Aromatic L-amino acid decarboxylase: biochemistry and functional significance. In: Boulton AA, Baker GB, Yu PH eds. *Neuromethods, Series 1: Neurochemistry, Neurotransmitter Enzymes*. Humana Press, Clifton, New Jersey. 33-77.
- Bubak AN, Renner KJ, Swallow JG. 2014. Heightened serotonin influences contest outcome and enhances expression of high-intensity aggressive behaviors. *Behav. Brain Res.*, 259(2): 137-142.
- Cai M, Li Z, Fan F, Huang Q, Shao X, Song G, 2009. Design and synthesis of novel insecticides based on the serotonergic ligand 1-[(4-aminophenyl) ethyl]-4-[3-(trifluoromethyl) phenyl] piperazine (PAPP). *J. Agric. Food Chem.*, 58(5): 2624-2629.
- Carre-Pierrat M, Baillie D, Johnsen R, Hyde R, Hart A, Granger L, Ségalat L, 2006. Characterization of the *Caenorhabditis elegans* G protein-coupled serotonin receptors. *Invert. Neurosci.*, 6(4): 189-205.
- Chemel BR, Roth BL, Armbruster B, Watts VJ, Nichols DE, 2006. WAY-100635 is a potent dopamine D₄ receptor agonist. *Psychopharmacology*, 188(2): 244-251.
- Colas JF, Launay JM, Kellermann O, Rosay P, Maroteaux L, 1995. *Drosophila* 5-HT₂ serotonin receptor: coexpression with fushi-tarazu during segmentation. *Proc. Natl. Acad. Sci. USA*, 92(12): 5441-5445.
- Coleman CM, Neckameyer WS, 2005. Serotonin synthesis by two distinct enzymes in *Drosophila melanogaster*. *Arch. Insect Biochem. Physiol.*, 59(1): 12-31.
- Cook BJ, Meola S, 1987. The oviduct musculature of the horsefly, *Tabanus sulcifrons* and its response to 5- hydroxytryptamine and proctolin. *Physiol. Ent.*, 3(4): 273-280.
- Corey JL, Quick MW, Davidson N, Lester HA, Guastella J, 1994. A cocaine-sensitive *Drosophila* serotonin transporter: cloning, expression, and electrophysiological characterization. *Proc. Natl. Acad. Sci. USA*, 91(3): 1188-1192.
- Cosi C, Koek W, 2000. The putative <<silent>> 5-HT_{1A} receptor antagonist, WAY 100635, has inverse agonist properties at cloned human 5-HT_{1A} receptors. *Eur. J. Pharmacol.*, 401(1): 9-15.

- Côté F, Thévenot E, Fligny C, Fromes Y, Darmon M, Ripoché MA., Bayard E, Hanoun N, Saurini F, Lechat P, Dandolo L, Hamon M, Mallet J, Vojdani G, 2003. Disruption of the nonneuronal *tph1* gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc. Natl. Acad. Sci. USA*, 100(23): 13525-13530.
- Cuttell L, Vaughan A, Silva E, Escaron CJ, Lavine M, Goethem EV, Eid JP, Quirin M, Franc NC, 2008. Undertaker, a *Drosophila* junctophilin, links draper-mediated phagocytosis and calcium homeostasis. *Cell* 135(3): 524-534.
- Dacks AM, Dacks JB, Christensen TA, Nighorn AJ, 2006. The cloning of one putative octopamine receptor and two putative serotonin receptors from the tobacco hawkmoth, *Manduca sexta*. *Insect Biochem. Mol. Biol.*, 36(9): 741-747.
- Dacks AM, Nickel T, Mitchell BK, 2003. An examination of serotonin and feeding in the flesh fly *Neobellieria bullata* (Sarcophagidae: Diptera). *J. Insect Behav.*, 16(1): 1-21.
- Dacks AM, Reale V, Pi Y, Zhang W, Dacks JB, Nighorn AJ, Evans PD, 2013. A characterization of the *Manduca sexta* serotonin receptors in the context of olfactory neuromodulation. *PLoS ONE*, 8(7): e69422.
- Dalton T, 1977. Threshold and receptor reserve in the action of 5-hydroxytryptamine on the salivary glands of *Calliphora erythrocephala*. *J. Insect Physiol.*, 23(5): 625-631.
- Dasari S, Cooper RL, 2006. Direct influence of serotonin on the larval heart of *Drosophila melanogaster*. *J. Comp. Physiol. B*, 176(4): 349-357.
- De Cesare D, Fimia GM, Sassone-Corsi P, 1999. Signaling routes to CREM and CREB: plasticity in transcriptional activation. *Trends Biochem. Sci.*, 24(7): 281-285.
- Demchyshyn LL, Pristupa ZB, Sugamori KS, Barker EL, Blakely RD, Wolfgang WJ, Forte MA, Niznik HB, 1994. Cloning, expression, and localization of a chloride-facilitated, cocaine-sensitive serotonin transporter from *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA*, 91(11): 5158-5162.
- Dierick HA, Greenspan RJ, 2007. Serotonin and neuropeptide F have opposite

- modulatory effects on fly aggression. *Nat. Genet.*, 39(5): 678-682.
- Donly BC, Caveney S, 2005. A transporter for phenolamine uptake in the arthropod CNS. *Arch. Insect Biochem. Physiol.*, 59(3): 172-183.
- Ek S, Högerkorp CM, Dictor M, Ehinger M, Borrebaeck CAK. 2002. Mantle cell lymphomas express a distinct genetic signature affecting lymphocyte trafficking and growth regulation as compared with subpopulations of normal human B cells. *Cancer Res.*, 62(15): 4398-4405.
- Erspamer V, 1946. Presenza di enteramina o di una sostanza enteraminosimile negli estratti gastrointestinali e splenici dei pesci e negli estratti gastroenterici delle Ascidie. *Experientia*, 2(9): 369-371.
- Erspamer V, Asero B, 1952. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature*, 169: 800-801.
- Evans DL, Lynch KG, Benton T, Dube B, Gettes DR, Tustin NB, Lai JP, Metzger D, Douglas SD. 2008. Selective serotonin reuptake inhibitor and substance P antagonist enhancement of natural killer cell innate immunity in human immunodeficiency virus/acquired immunodeficiency syndrome. *Biol. Psychiatry*, 63(9): 899-905.
- Falibene A, Rössler W, Josens R, 2012. Serotonin depresses feeding behaviour in ants. *J. Insect. Physiol.*, 58(1): 7-17.
- Fischer JA, Giniger E, Maniatis T, Ptashne M, 1988. GAL4 activates transcription in *Drosophila*. *Nature*, 332: 853- 856.
- Fitzpatrick PF, 1999. Tetrahydropterin-dependent amino acid hydroxylases. *Annu. Rev. Biochem.*, 68(1): 355-381.
- Fornal CA, Metzler CW, Gallegos RA, Veasey SC, McCreary AC, Jacobs BL, 1996. WAY-100635, a potent and selective 5-hydroxytryptamine_{1A} antagonist, increases serotonergic neuronal activity in behaving cats: comparison with (S)-WAY-100135. *J. Pharmacol. Exp. Ther.*, 278(2): 752-762.
- French AS, Simcock KL, Rolke D, Gartside SE, Blenau W, Wright GA, 2013. The role of serotonin in feeding and gut contractions in the honeybee. *J. Insect. Physiol.*, 61: 8-15.

- Gal EM and Christiansen PA, 1975. Alpha-methyltryptophan: effects on cerebral monooxygenases *in vitro* and *in vivo*. *J. Neurochem.*, 24(1): 89-95.
- Gasque G, Conway S, Huang J, Rao Y, Vosshall LB, 2013. Small molecule drug screening in *Drosophila* identifies the 5HT_{2A} receptor as a feeding modulation target. *Sci. Rep.*, 3: srep02120.
- Gioino P, Murray BG, Ianowski JP. 2014. Serotonin triggers cAMP and PKA-mediated intracellular calcium waves in Malpighian tubules of *Rhodnius prolixus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 307(7): R828-836.
- Gu HH, Wu X, Han DD, 2006. Conserved serine residues in serotonin transporter contribute to high-affinity cocaine binding. *Biochem. Biophys. Res. Commun.*, 343(4): 1179-1185.
- Gudermann T, Kalkbrenner F, Schultz G, 1996. Diversity and selectivity of receptor-G protein interaction. *Annu. Rev. Pharmacol. Toxicol.*, 36(1): 429-459.
- Gudermann T, Schöneberg T, Schultz G, 1997. Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu. Rev. Neurosci.*, 20(1): 399-427.
- Guo X, Ma Z, Kang L. 2013. Serotonin enhances solitariness in phase transition of the migratory locust. *Front. Behav. Neurosci.*, 7(11): 129-129.
- Hagan JJ, Price GW, Jeffrey P, Deeks NJ, Stean T, Piper D, Smith MI, Upton N, Medhurst AD, Middlemiss DN, 2000. Characterization of SB-269970-A, a selective 5-HT₇ receptor antagonist. *Br. J. Pharmacol.*, 130(3): 539-548.
- Hamasaka Y, Nässel DR, 2006. Mapping of serotonin, dopamine, and histamine in relation to different clock neurons in the brain of *Drosophila*. *J. Comp. Neurol.*, 494(2): 314-330.
- Hannon J, Hoyer D, 2008. Molecular biology of 5-HT receptors. *Behav. Brain Res.*, 195(1): 198-213.
- Harris-Warrick RM and Cohen AH, 1985. Serotonin modulates the central pattern generator for locomotion in the isolated lamprey spinal cord. *J. Exp. Biol.*, 116(1): 27-46.
- Haselton AT, Downer K, Zylstra J, Jr Stoffolano JG, 2009. Serotonin inhibits protein

- feeding in the blow fly, *Phormia regina* (Meigen). *J. Insect Behav.*, 22(6): 452-463.
- Haselton AT, Yin CM, Jr Stoffolano JG, 2006. Occurrence of serotonin immunoreactivity in the central nervous system and midgut of adult female *Tabanus nigrovittatus* (Diptera: Tabanidae). *J. Med. Entomol.*, 43(2): 252-257.
- Hatcher N, Zhang X, Stuart J, Moroz L, Sweedler J, Gillette R, 2008. 5-HT and 5-HT-SO₄, but not tryptophan or 5-HIAA levels in single feeding neurons track animal hunger state. *J. Neurochem.*, 104(5): 1358-1363.
- Hauser F, Cazzamali G, Williamson M, Blenau W, Grimmelikhuijzen CJ, 2006. A review of neurohormone GPCRs present in the fruitfly *Drosophila melanogaster* and the honey bee *Apis mellifera*. *Prog. Neurobiol.*, 80(1): 1-19.
- Helle J, Dircksen H, Eckert M, Nässel DR, Spöhrhase-Eichmann U, Schürmann FW, 1995. Putative neurohemal areas in the peripheral nervous system of an insect, *Gryllus bimaculatus*, revealed by immunocytochemistry. *Cell Tissue Res.*, 281(1): 43-61.
- Hernandez ME, Martinez-Fong D, Perez-Tapia M, Estrada-Garcia I, Estrada-Parra S, Pavón L. 2010. Evaluation of the effect of selective serotonin-reuptake inhibitors on lymphocyte subsets in patients with a major depressive disorder. *Eur. Neuropsychopharmacol.*, 20(2): 88-95.
- Hillyer JF, Estévez-Lao TY, Mirzai HE. 2015. The neurotransmitters serotonin and glutamate accelerate the heart rate of the mosquito *Anopheles gambiae*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 188: 49-57.
- Hjorth S, Magnusson T, 1988. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 338(5): 463-471.
- Hoffmann JA, 2003. The immune response of *Drosophila*. *Nature*, 426(6962): 33-38.
- Hoyer D, Clarke DE, Fozard JR, et al., 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.*, 46(2): 157-203.
- Hoyer D, Martin GR, 1995. Classification and nomenclature of 5-HT receptors: a

- comment on current issues. *Behav. Brain Res.*, 73(1): 263-268.
- Hoyer D, Martin G, 1997. 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacology*, 36(4): 419-428.
- Huang J, Hamasaki T, Ozoe F, Ozoe Y, 2008. Single amino acid of an octopamine receptor as a molecular switch for distinct G protein couplings. *Biochem. Biophys. Res. Commun.*, 371(4): 610-614.
- Idzko M, et al., 2004. The serotonergic receptors of human dendritic cells: Identification and coupling to cytokine release. *J. Immunol.*, 172(10): 6011-6019.
- Inoue M, Okazaki T, Kitazono T, Mizushima M, Omata M, Ozaki S. 2011. Regulation of antigen-specific CTL and Th1 cell activation through 5-Hydroxytryptamine 2A receptor. *Int. Immunopharmacol.*, 11(1): 67-73.
- Iwata K, Luo J, Penn RB, Benovic JL, 2005. Bimodal regulation of the human H1 histamine receptor by G protein-coupled receptor kinase 2. *J. Biol. Chem.*, 280(3): 2197-2204.
- Johnson O, Becnel J, Nichols CD, 2009. Serotonin 5-HT₂ and 5-HT_{1A}-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. *Neuroscience*, 158(4): 1292-1300.
- Jones BJ, Blackburn TP, 2002. The medical benefit of 5-HT research. *Pharmacol. Biochem. Behav.*, 71(4): 555-568.
- Kanost MR, Jiang H, Yu XQ, 2004. Innate immune responses of a lepidopteran insect, *Manduca sexta*. *Immunol. Rev.*, 198, 97-105.
- Katada T, Oinuma M, Ui M, 1986. Mechanisms for inhibition of the catalytic activity of adenylate cyclase by the guanine nucleotide-binding proteins serving as the substrate of islet-activating protein, pertussis toxin. *J. Biol. Chem.*, 261(11): 5215-5221.
- Kenakin T and Christopoulos A, 2013. Measurements of ligand bias and functional affinity. *Nat. Rev. Drug. Discov.*, 12(6): 483.
- Kim GS, Nalini M, Kim Y, Lee DW. 2009. Octopamine and 5-hydroxytryptamine mediate hemocytic phagocytosis and nodule formation via eicosanoids in the beet armyworm, *Spodoptera exigua*. *Arch. Insect. Biochem. Physiol.*, 70(3):

- 162-176.
- Kim GS, Kim Y, 2010. Up-regulation of circulating hemocyte population in response to bacterial challenge is mediated by octopamine and 5-hydroxytryptamine via Rac1 signal in *Spodoptera exigua*. *J. Insect Physiol.*, 56(6): 559-566.
- Kim JJ, Bridle BW, Ghia JE, Wang H, Syed SN, Manocha MM, Rengasamy P, Shajib MS, Wan Y, Hedlund PB, 2013. Targeted inhibition of serotonin type 7 (5-HT₇) receptor function modulates immune responses and reduces the severity of intestinal inflammation. *J. Immunol.*, 190(9): 4795-4804.
- Kinney MP, Panting ND, Clark TM. 2014. Modulation of appetite and feeding behavior of the larval mosquito *Aedes aegypti* (L.) by the serotonin-selective reuptake inhibitor paroxetine: shifts between distinct feeding modes and the influence of feeding status. *J. Exp. Biol.*, 217(6): 935-943.
- Klein U, Tu Y, Stolovitzky GA, Keller JL, Joseph H, Vladan M, Giorgio C, Andrea C, Riccardo DF. 2003. Transcriptional analysis of the B cell germinal center reaction. *Pro. Natl. Acad. Sci. USA*, 100(5): 2639-2644.
- Kloppenborg P, Ferns D, Mercer AR, 1999. Serotonin enhances central olfactory neuron responses to female sex pheromone in the male sphinx moth *Manduca sexta*. *J. Neurosci.*, 19(19): 8172-8181.
- Kloppenborg P, Mercer AR, 2008. Serotonin modulation of moth central olfactory neurons. *Annu. Rev. Entomol.*, 53: 179-190.
- Kocks C, Cho JH, Nehme N, Ulvila J, Pearson AM, Meister M, Strom C, Conto SL, Hetru C, Stuart LM, Stehle T, Hoffmann JA, Reichhart JM, Ferrandon D, Rämet M, Ezekowitz RA, 2005. Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila*. *Cell*, 123(2): 335- 346.
- Kozak M, 1987. At least six nucleotides preceding the AUG initiator codon enhance translation in mammalian cells. *J. Mol. Biol.*, 196(4): 947-950.
- Kravitz E, 2000. Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *J. Comp. Physiol. A*, 186(3): 221-238
- Kushnir-Sukhov NM, Brittain E, Scott L, Metcalfe DD. 2008. Clinical correlates of

- blood serotonin levels in patients with mastocytosis. *Eur. J. Clin. Invest.*, 38(12): 953–958.
- Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD. 2007. Human mast cells are capable of serotonin synthesis and release. *J. Allergy Clin. Immunol.*, 119(2): 498–499.
- Kushnir-Sukhov NM, Gilfillan AM, Coleman JW, Brown JM, Sandra B, Miklos T, Metcalfe DD, 2006. 5-hydroxytryptamine induces mast cell adhesion and migration. *J. Immunol.*, 177(9): 6422–6432.
- Lange AB, 2004. A neurohormonal role for serotonin in the control of locust oviducts. *Arch. Insect Biochem. Physiol.*, 56(4): 179–190.
- Lavine MD and Strand MR, 2002. Insect hemocytes and their role in immunity. *Insect Biochem. Mol. Biol.*, 32(10): 1295–1309.
- Lee DW, Pietrantonio P, 2003. In vitro expression and pharmacology of the 5-HT₇-like receptor present in the mosquito *Aedes aegypti* tracheolar cells and hindgut-associated nerves. *Insect Mol. Biol.*, 12(6): 561–569.
- Lemaitre B and Hoffmann J, 2007. The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.*, 25: 697–743.
- Leon-Ponte M, Ahern GP, O'Connell PJ. 2007. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT₇ receptor. *Blood*, 109(8): 3139–3146.
- Li B, Zhao J, Lv J, Tang F, Liu L, Sun Z, Wang L, Siwela SP, Wang Y, Song Y, Manchishi SM, Cui R, 2014. Additive antidepressant-like effects of fasting with imipramine via modulation of 5-HT₂ receptors in the mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 48:199–206.
- Liscia A, Solari P, Gibbons S, Gelperin A, Jr Stoffolano JG, 2012. Effect of serotonin and calcium on the supercontractile muscles of the adult blowfly crop. *J. Insect Physiol.*, 58(3): 356–366.
- Liu SS, Li AY, Witt CM, Pérez de León AA, 2011. Immunohistological localization of serotonin in the CNS and feeding system of the stable fly *Stomoxys calcitrans* L.(Diptera: Muscidae). *Arch. Insect Biochem. Physiol.*, 77(4): 199–219.

- Liu SS, Li AY, Witt CM, Pérez de León AA, 2013. Effects of reserpine on reproduction and serotonin immunoreactivity in the stable fly *Stomoxys calcitrans* (L.). *J. Insect Physiol.*, 59(9): 974–982.
- Livak KJ and Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(-Delta Delta C(T)) Method. *Methods*, 25(4): 402-408.
- Luo J, Lovell PJ, Bromidge SM, Dabbs S, Duckworth DM, Forbes IT, Jennings AJ, King FD, Middlemiss DN, Rahman SK, Saunders DV, 2000. A novel, potent, and selective 5-HT₇ antagonist:(R)-3-(2-(2-(4-methylpiperidin-1-yl) ethyl) pyrrolidine-1-sulfonyl) phenol (SB-269970). *J. Med. Chem.*, 43(3): 342-345.
- Becnel J, Nichols CD, Nässel DR, 2012. Insulin-producing cells in the brain of adult *Drosophila* are regulated by the serotonin 5-HT_{1A} receptor. *Cell. Mol. Life Sci.*, 69(3): 471-484.
- Lutz E, Tyrer N, 1988. Immunohistochemical localization of serotonin and choline acetyltransferase in sensory neurones of the locust. *J. Comp. Neurol.*, 267(3): 335-342.
- Majeed ZR, Nichols CD, Rl. C. 2013. 5-HT stimulation of heart rate in *Drosophila* does not act through cAMP as revealed by pharmacogenetics. *J. Appl. Physiol.*, 115(11): 1656-1665.
- Majeed ZR, Stacy A, Cooper RL, 2014. Pharmacological and genetic identification of serotonin receptor subtypes on *Drosophila* larval heart and aorta. *J. Comp. Physiol. B*, 184(2): 205-219.
- Martel JC, Ormière AM, Leduc N, Assié MB, Cussac D, Newman-Tancredi A, 2007. Native rat hippocampal 5-HT_{1A} receptors show constitutive activity. *Mol. Pharmacol.*, 71(3): 638-643.
- McLoughlin DJ, Strange PG, 2000. Mechanisms of agonism and inverse agonism at serotonin 5-HT_{1A} receptors. *J. Neurochem.*, 74(1): 347-357.
- Meredith EJ, Holder MJ, Chamba A, Challa A, Lee AD, Bunce CM, 2005. The serotonin transporter (SLC6A4) is present in B-cell clones of diverse malignant origin: probing a potential antitumor target for psychotropics. *FASEB J.*, 19(7):

U888-U911.

- Meredith EJ, Chamba A, Holder MJ, Barnes NM, Gordon J, 2005. Close encounters of the monoamine kind: immune cells betray their nervous disposition. *Immunology* 115(3): 289-295.
- Mikulski Z, Zaslona Z, Cakarova L, Hartmann P, Wilhelm J, Tecott LH, Lohmeyer J, Kummer W, 2010. Serotonin activates murine alveolar macrophages through 5-HT_{2C} receptors. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, 299(2): L272-L280.
- Millan MJ, Newman - Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP, Cogé F, Galizzi JP, Boutin JA, Rivet JM, 2000. Agonist and antagonist actions of yohimbine as compared to fluparoxan at α_2 -adrenergic receptors (AR) s, serotonin (5-HT)_{1A}, 5-HT_{1B}, 5-HT_{1D} and dopamine D₂ and D₃ receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. *Synapse*, 35(2): 79-95.
- Monachon M, Burkard W, Jalfre M, Haefely W, 1972. Blockade of central 5-hydroxytryptamine receptors by methiothepin. *Naunyn Schmiedeberg's Arch. Pharmacol.*, 274(2): 192-197.
- Moro O, Lameh J, Högger P, Sadee W, 1993. Hydrophobic amino acid in the i2 loop plays a key role in receptor-G protein coupling. *J. Biol. Chem.*, 268(30): 22273-22276.
- Mössner R and Lesch KP, 1998. Role of serotonin in the immune system and in neuroimmune interactions. *Brain Behav. Immun.*, 12(4): 249-271.
- Müller T, Dürk T, Blumenthal B, Grimm M, Cicko S, Panther E, Sorichter S, Herouy Y, Di Virgilio F, Ferrari D, Norgauer J, Idzko M, 2009. 5-hydroxytryptamine modulates migration, cytokine and chemokine release and T-cell priming capacity of dendritic cells *in vitro* and *in vivo*. *PLoS One* 4(7): e6453.
- Murphy DL, Lerner A, Rudnick G, Lesch KP, 2004. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol. Interv.*, 4(2): 109.
- Nakamura K, Sato T, Ohashi A, Tsurui H, Hasegawa H. 2008. Role of a serotonin precursor in development of gut microvilli. *Am. J. Pathol.*, 172(2): 333-344.
- Nässel D, Elekes K, 1985. Serotonergic terminals in the neural sheath of the blowfly

- nervous system: electron microscopical immunocytochemistry and 5, 7-dihydroxytryptamine labelling. *Neuroscience*, 15(1): 293-307.
- Neckameyer W, Coleman C, Eadie S, Goodwin S, 2007. Compartmentalization of neuronal and peripheral serotonin synthesis in *Drosophila melanogaster*. *Genes Brain Behav.*, 6(8): 756-769.
- Neckameyer WS, 2010. A trophic role for serotonin in the development of a simple feeding circuit. *Dev. Neurosci.*, 32(3): 217-237.
- Nelson DL, 2004. 5-HT₅ receptors. *Curr. Drug Targets CNS Neurol. Disord.*, 3(1): 53-8.
- Ngkelo A, Meja K, Yeadon M, Adcock I, Kirkham PA, 2012. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and G_{ia} dependent PI-3kinase signalling. *J. Inflamm.*, 9(1): 1
- Nichols CD, 2007. 5-HT₂ receptors in *Drosophila* are expressed in the brain and modulate aspects of circadian behaviors. *Dev. Neurobiol.*, 67(6): 752-763.
- Nichols DE, Nichols CD, 2008. Serotonin receptors. *Chem. Rev.*, 108: 1614-1641.
- Obosi LA, Schuette DG, Europe-Finner GN, Beadle DJ, Hen R, King LA, Bermudez I, 1996. Functional characterisation of the *Drosophila* 5-HT_{dro1} and 5-HT_{dro2B} serotonin receptors in insect cells: activation of a G_{as}-like protein by 5-HT_{dro1} but lack of coupling to inhibitory G-proteins by 5-HT_{dro2B}. *FEBS Lett.*, 381(3): 233-236.
- O'Connell PJ. 2006. A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. *Blood*, 107(3): 1010-1017.
- Okusawa S, Kohsaka H, Nose A. 2014. Serotonin and downstream leucokinin neurons modulate larval turning behavior in *Drosophila*. *J. Neurosci.*, 34(7): 2544-2558.
- Orchard I, 2006. Serotonin: A coordinator of feeding-related physiological events in the blood-gorging bug, *Rhodnius prolixus*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 144(3): 316-324.
- Orchard I, Lange AB, 1985. Evidence for octopaminergic modulation of an insect

- visceral muscle. *J. Neurobiol.*, 16(3): 171-181.
- Page TL, 1987. Serotonin phase-shifts the circadian rhythm of locomotor activity in the cockroach. *J. Biol. Rhythms.*, 2(1): 23-34.
- Paluzzi JP, Bhatt G, Wang CH, Zandawala M, Lange AB, Orchard I, 2015. Identification, functional characterization, and pharmacological profile of a serotonin type-2b recept in the medically important insect, *Rhodnius prolixus*. *Front Neurosci.*, 9(175):
- Peroutka SJ, Howell TA, 1994. The molecular evolution of G protein-coupled receptors: focus on 5-hydroxytryptamine receptors. *Neuropharmacology*, 33(3): 319-324.
- Pietrantonio P, Jagge C, McDowell C, 2001. Cloning and expression analysis of a 5HT₇-like serotonin receptor cDNA from mosquito *Aedes aegypti* female excretory and respiratory systems. *Insect Mol. Biol.*, 10(4): 357-369.
- Price G, Burton M, Collin L, Duckworth M, Gaster L, Göthert M, Jones B, Roberts C, Watson J, Middlemiss D, 1997. SB-216641 and BRL-15572- compounds to pharmacologically discriminate h5-HT_{1B} and h5-HT_{1D} receptors. *Naunyn Schmiedebergs Arch. Pharmacol.*, 356(3): 312-320.
- Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang FT, Chang AS, Ganapathy V, Blakely RD, 1993. Antidepressant-and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc. Natl. Acad. Sci. USA*, 90(6): 2542-2546.
- Ranganathan R, Cannon SC, Horvitz HR. 2000. MOD-1 is a serotonin-gated chloride channel that modulates locomotory behaviour in *C. elegans*. *Nature* 408(6811): 470-475.
- Rapport MM, Green AA, Page IH, 1948. Serum vasoconstrictor (serotonin) IV. Isolation and characterization. *J. Biol. Chem.*, 176(3): 1243-1251.
- Razzaque Z, Heald M, Pickard J, Maskell L, Beer M, Hill R, Longmore J, 1999. Vasoconstriction in human isolated middle meningeal arteries: determining the contribution of 5-HT_{1B} and 5-HT_{1F} receptor activation. *Brit. J. Clin. pharmaco.*, 47: 75-82.

- Rhee SG, Bae YS, 1997. Regulation of phosphoinositide-specific phospholipase C isozymes. *J. Biol. Chem.*, 272(24): 15045-15048.
- Rinaldi A, Chiaravalli AM, Mian M, Zucca E, Tibiletti MG, Capella C, Berton F. 2010. Serotonin receptor 3A expression in normal and neoplastic B cells. *Pathobiology*, 77(3): 129-135.
- Rodriguez Moncalvo VG, Campos AR. 2009. Role of serotonergic neurons in the *Drosophila* larval response to light. *BMC Neurosci.*, 10: 66.
- Röser C, Jordan N, Balfanz S, Baumann A, Walz B, Baumann O, Blenau W, 2012. Molecular and pharmacological characterization of serotonin 5-HT_{2α} and 5-HT₇ receptors in the salivary glands of the blowfly *Calliphora vicina*. *PLoS ONE*, 7(11): e49459.
- Rubio MC, Bonelli CG, Mastronardi IO, Rondina DC, Izquierdo JA, 1983. Presence of 5-hydroxy-indolacetic acid in *Diloboderus abderus* larvae without monoamine-oxidase-like activity. *Acta Physiol. Lat. Am.*, 33(3): 253-256
- Rudnick G, 2006. Serotonin transporters-structure and function. *J. Membr. Biol.*, 213(2): 101-110.
- Rudnick G, Clark J, 1993. From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim. Biophys. Acta.*, 1144(3): 249-263.
- Sandhu SK, Ross LS, Gill SS, 2002. A cocaine insensitive chimeric insect serotonin transporter reveals domains critical for cocaine interaction. *Eur. J. Biochem.*, 269(16): 3934-3944.
- Saudou F, Amlaiky N, Plassat JL, Borrelli E, Hen R, 1990. Cloning and characterization of a *Drosophila* tyramine receptor. *EMBO J.*, 9(11): 3611.
- Saudou F, Boschert U, Amlaiky N, Plassat JL, Hen R, 1992. A family of *Drosophila* serotonin receptors with distinct intracellular signalling properties and expression patterns. *EMBO J.*, 11(1): 7.
- Schaerlinger B, Launay JM, Vonesch J, Maroteaux L, 2007. Gain of affinity point mutation in the serotonin receptor gene 5-HT_{2Dro} accelerates germband extension movements during *Drosophila* gastrulation. *Dev. Dyn.*, 236(4): 991-999.
- Schneider DS and Ayres JS, 2008. Two ways to survive infection: what resistance and

- tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.*, 8(11): 889-895.
- Schlenstedt J, Balfanz S, Baumann A, Blenau W, 2006. Am5-HT₇: molecular and pharmacological characterization of the first serotonin receptor of the honeybee (*Apis mellifera*). *J. Neurochem.*, 98(6): 1985-1998.
- Serafeim A, Grafton G, Chamba A, Gregory CD, Blakely RD, Bowery NG, Barnes NM, Gordon J. 2002. 5-Hydroxytryptamine drives apoptosis in biopsylike Burkitt lymphoma cells: reversal by selective serotonin reuptake inhibitors. *Blood*, 99(7): 2545-2553.
- Serafeim A, Holder MJ, Grafton G, Chamba A, Yson MT, Luong QT, Bunce CM, Gregory CD, Barnes NM, Gordon J. 2003. Selective serotonin reuptake inhibitors directly signal for apoptosis in biopsy-like Burkitt lymphoma cells. *Blood*, 101(8): 3212-3219.
- Settembrini BP, Villar MJ, 2004. Distribution of serotonin in the central nervous system of the blood-feeding heteropteran, *Triatoma infestans* (Heteroptera: Reduviidae). *J. Morphol.*, 260(1): 21-32.
- Shimada-Niwa Y, Niwa R. 2014. Serotonergic neurons respond to nutrients and regulate the timing of steroid hormone biosynthesis in *Drosophila*. *Nat. Commun.*, 15(5): 5778-5778.
- Siju K, Hansson BS, Ignell R, 2008. Immunocytochemical localization of serotonin in the central and peripheral chemosensory system of mosquitoes. *Arthropod Struct. Dev.*, 37(4): 248-259.
- Sinenko SA, Kim EK, Wynn R, Manfrulli P, Ando I, Wharton KA, Perrimon N, Mathey-Prevot B, 2004. Yantar, a conserved arginine-rich protein is involved in *Drosophila* hemocyte development. *Dev. Biol.*, 273(1): 48-62.
- Silva B, Goles NI, Varas R, Campusano JM, 2014. Serotonin receptors expressed in *Drosophila* mushroom bodies differentially modulate larval locomotion. *PLoS ONE*, 9(2): e89641.
- Sitaraman D, Zars M, LaFerriere H, Chen YC, Sable-Smith A, Kitamoto T, Rottinghaus GE, Zars T, 2008. Serotonin is necessary for place memory in

- Drosophila*. *Proc. Natl. Acad. Sci. USA*, 105(14): 5579-5584.
- Slauson DO, Walker C, Kristensen F, Wang Y, Weck ALd. 1984. Mechanisms of serotonin-induced lymphocyte proliferation inhibition. *Cell. Immunol.*, 84(2): 240-252.
- Sloley BD, 2004. Metabolism of monoamines in invertebrates: the relative importance of monoamine oxidase in different phyla. *Neurotoxicology*, 25(1-2): 175-183.
- Sprouse J, Reynolds L, Li X, Braselton J, Schmidt A, 2004. 8-OH-DPAT as a 5-HT₇ agonist: phase shifts of the circadian biological clock through increases in cAMP production. *Neuropharmacology*, 46(1): 52-62.
- Squires LN, Jakubowski JA, Stuart JN, Rubakhin SS, Hatcher NG, Kim WS, Chen K, Shih JC, Seif I, Sweedler JV, 2006. Serotonin catabolism and the formation and fate of 5-hydroxyindole thiazolidine carboxylic acid. *J. Biol. Chem.*, 281(19): 13463-13470.
- Squires LN, Rubakhin SS, Wadhams AA, Talbot KN, Nakano H, Moroz LL, Sweedler JV, 2010. Serotonin and its metabolism in basal deuterostomes: insights from *Strongylocentrotus purpuratus* and *Xenoturbella bocki*. *J. Exp. Biol.*, 213(15): 2647-2654.
- Squires LN, Talbot KN, Rubakhin SS, Sweedler JV, 2007. Serotonin catabolism in the central and enteric nervous systems of rats upon induction of serotonin syndrome. *J. Neurochem.*, 103(1): 174-180.
- Sternberg EM, 2006. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat. Rev. Immunol.*, 6(4): 318-328.
- Strader CD, Fong TM, Graziano M, Tota M, 1995. The family of G-protein-coupled receptors. *FASEB J.*, 9(9): 745-754.
- Stroschein-Stevenson SL, Foley E, O'Farrell PH, Johnson AD, 2006. Identification of *Drosophila* gene products required for phagocytosis of *Candida albicans*. *Plos. Biol.*, 4(1): e4.
- Stryer L, Bourne HR, 1986. G proteins: a family of signal transducers. *Annu. Rev. Cell Biol.*, 2(1): 391-419.
- Stuart JN, Ebaugh JD, Copes AL, Hatcher NG, Gillette R, Sweedler JV, 2004.

- Systemic serotonin sulfate in opisthobranch mollusks. *J. Neurochem.*, 90(3): 734-742.
- Stuart JN, Zhang X, Jakubowski JA, Romanova EV, Sweedler JV, 2003. Serotonin catabolism depends upon location of release: characterization of sulfated and γ - glutamylated serotonin metabolites in *Aplysia californica*. *J. Neurochem.*, 84(6): 1358-1366.
- Sze JY, Victor M, Loer C, Shi Y, Ruvkun G, 2000. Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* 403(6769): 560-564.
- Taylor DJ, Robinson GE, Logan BJ, Laverty R, Mercer AR, 1992. Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. *J. Comp. Physiol. A*, 170(6): 715-721.
- Terenius O, Papanicolaou A, Garbutt JS, Eleftherianos I, Huvenne H, Kanginakudru S, Albrechtsen M, An C, Aymeric JL, Barthel A, 2011. RNA interference in *Lepidoptera*: an overview of successful and unsuccessful studies and implications for experimental design. *J. Insect Physiol.*, 57(2): 231-245.
- Thamm M, Balfanz S, Scheiner R, Baumann A, Blenau W, 2010. Characterization of the 5-HT_{1A} receptor of the honeybee (*Apis mellifera*) and involvement of serotonin in phototactic behavior. *Cell. Mol. Life Sci.*, 67(14): 2467-2479.
- Thamm M, Rolke D, Jordan N, Balfanz S, Schiffer C, Baumann A, Blenau W, 2013. Function and distribution of 5-HT₂ receptors in the honeybee (*Apis mellifera*). *PLoS ONE*, 8(12): e82407.
- Tierney A, 2001. Structure and function of invertebrate 5-HT receptors: a review. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, 128(4): 791-804.
- Tomioka K, Ikeda M, Nagao T, Tamotsu S, 1993. Involvement of serotonin in the circadian rhythm of an insect visual system. *Naturwissenschaften*, 80(3): 137-139.
- Torres GE, Gainetdinov RR, Caron MG, 2003. Plasma membrane monoamine transporters: structure, regulation and function. *Nat. Rev. Neurosci.*, 4(1): 13-25.
- Trimmer BA, 1985. Serotonin and the control of salivation in the blowfly *Calliphora*.

- J. Exp. Biol.*, 114(1): 307-328.
- Troppmann B, Balfanz S, Baumann A, Blenau W, 2010. Inverse agonist and neutral antagonist actions of synthetic compounds at an insect 5-HT₁ receptor. *Br. J. Pharmacol.*, 159(7): 1450-1462.
- Turlejski K, 1996. Evolutionary ancient roles of serotonin: long-lasting regulation of activity and development. *Acta. Neurobiol. Exp.*, 56(2): 619-636.
- Vallés AM, White K, 1988. Serotonin - containing neurons in *Drosophila melanogaster*: Development and distribution. *J. Comp. Neurol.*, 268(3): 414-428.
- Vleugels R, Lenaerts C, Baumann A, Broeck JV, Verlinden H, 2013. Pharmacological characterization of a 5-HT₁-type serotonin receptor in the red flour beetle, *Tribolium castaneum*. *PLoS ONE*, 8(5): e65052.
- Vleugels R, Lenaerts C, Vanden Broeck J, Verlinden H, 2014. Signalling properties and pharmacology of a 5-HT₇-type serotonin receptor from *Tribolium castaneum*. *Insect Mol. Biol.*, 23(2): 230-43.
- Von Nickisch-Rosenegk E, Krieger J, Kubick S, Laage R, Strobel J, Strotmann J, Breer H, 1996. Cloning of biogenic amine receptors from moths (*Bombyx mori* and *Heliothis virescens*). *Insect Biochem. Mol. Biol.*, 26(8): 817-827.
- Wacker D, Wang C, Katritch V, Han GW, Huang XP, Vardy E, McCorvy JD, Jiang Y, Chu M, Siu FY, 2013. Structural features for functional selectivity at serotonin receptors. *Science*, 340(6132): 615-619.
- Walker RJ, Brooks HL, Holden-Dye L, 1996. Evolution and overview of classical transmitter molecules and their receptors. *Parasitology*, 113(S1): S3-S33.
- Walz B, Baumann O, Krach C, Baumann A, Blenau W, 2006. The aminergic control of cockroach salivary glands. *Arch. Insect Biochem. Physiol.*, 62(3): 141-152.
- Wang C, Jiang Y, Ma J, Wu H, Wacker D, Katritch V, Han GW, Liu W, Huang XP, Vardy E, 2013. Structural basis for molecular recognition at serotonin receptors. *Science*, 340(6132): 610-614.
- Wang Q, Mohamed AA, Takeda M. 2013. Serotonin receptor B may lock the gate of PTTH release/synthesis in the Chinese silk moth, *Antheraea pernyi*; a diapause initiation/maintenance mechanism? *PLoS ONE*, 8(11): e79381.

- Watanabe T, Sadamoto H, Aonuma H, 2011. Identification and expression analysis of the genes involved in serotonin biosynthesis and transduction in the field cricket *Gryllus bimaculatus*. *Insect Mol. Biol.*, 20(5): 619-635.
- Weiger WA, 1997. Serotonergic modulation of behaviour: a phylogenetic overview. *Biol. Rev.*, 72(1): 61-95.
- Witz P, Amlaiky N, Plassat J, Maroteaux L, Borrelli E, Hen R, 1990. Cloning and characterization of a *Drosophila* serotonin receptor that activates adenylate cyclase. *Proc. Natl. Acad. Sci. USA*, 87(22): 8940-8944.
- Wright G, 2011. The role of dopamine and serotonin in conditioned food aversion learning in the honeybee. *Commun. Integr. Biol.*, 4(3): 318-320.
- Wu SF, Wang F, Huang J, Fang Q, Shen ZC, Ye GY, 2013. Molecular and cellular analyses of a ryanodine receptor from hemocytes of *Pieris rapae*. *Dev. Comp. Immunol.* 41(1): 1-10.
- Wu SF., Xu G, Stanley D, Huang J, Ye GY, 2015. Dopamine modulates hemocyte phagocytosis via a D1-like receptor in the rice stem borer, *Chilo suppressalis*. *Sci. Rep.*, 5: 12247.
- Yin J, Albert RH, Tretiakova AP, Jameson BA. 2006. 5-HT_{1B} receptors play a prominent role in the proliferation of T-lymphocytes. *J. Neuroimmunol.*, 181(1-2): 68-81.
- Yuan Q, Joiner WJ, Sehgal A, 2006. A sleep-promoting role for the *Drosophila* serotonin receptor 1A. *Curr. Biol.*, 16(11): 1051-1062.
- Yuan Q, Lin F, Zheng X, Sehgal A, 2005. Serotonin modulates circadian entrainment in *Drosophila*. *Neuron*, 47(1): 115-127.

作者简历

教育背景:

2011 年 9 月-2016 年 6 月 浙江大学农业昆虫与害虫防治专业 博士学位
2007 年 9 月-2011 年 6 月 华中农业大学植物保护专业 学士学位

博士期间发表的学术论文:

1. **Qi YX**, Huang J, Li MQ, Wu YS, Xia RY, Ye GY. Serotonin modulates insect hemocyte phagocytosis via two different serotonin receptors. *eLife* 2016, DOI: <http://dx.doi.org/10.7554/eLife.12241>
2. **Qi YX**, Xia RY, Wu YS, Stanley D, Huang J, Ye GY. Larvae of the small white butterfly, *Pieris rapae*, express a novel serotonin receptor. *Journal of Neurochemistry* 2014, 131(6): 767-777.
3. **Qi YX**, Teng ZW, Gao LF, Wu SF, Huang J, Ye GY, *et al.* Transcriptome analysis of an endoparasitoid wasp *Cotesia chilonis* (Hymenoptera: Braconidae) reveals genes involved in successful parasitism. *Archives of Insect Biochemistry & Physiology* 2015, 88: 203-221.
4. Wu SF, Sun FD, **Qi YX**, Yao Y, Fang Q, Huang J, *et al.* Parasitization by *Cotesia chilonis* influences gene expression in fatbody and hemocytes of *Chilo suppressalis*. *Plos One* 2013, 8(9): e74309-e74309.
5. Wu SF, Xu G, **Qi YX**, Xia RY, Huang J, Ye GY. Two splicing variants of a novel family of octopamine receptors with different signaling properties. *Journal of Neurochemistry* 2014, 129(1): 37-47.
6. 叶恭银, **齐易香**, 朱家颖, 方琦, 王磊. 金小蜂基因组学研究前沿. 应用昆虫学报 2011, 48(6): 1553-1558.
7. **齐易香**, 吴顺凡, 黄佳, 叶恭银. 昆虫 5-羟色胺及其受体的研究进展. 昆虫学报 2014, 57(7): 844-859.
8. 吴顺凡, 徐刚, **齐易香**, 夏仁英, 黄佳, 叶恭银. 昆虫多巴胺及其受体的研究进展. 昆虫学报 2013, 56(11):1342-1358.

专利:

叶恭银, 齐易香, 黄佳, 菜青虫 5-羟色胺受体基因 Pr5-HT₈ 及其应用, 专利号:
CN201310517959.8

博士期间参加会议:

- 1) 2013 年 6 月赴南京参加第四届国际昆虫生理与分子生物学会议 (The 4th International Conference Of Insect Physiology and Molecular Biology) 作英文口头报告, 获优秀口头报告奖。
- 2) 2014 年 11 月赴美国波特兰参加第 62 届美国昆虫学年会 (Entomological Society of America) 作 poster.