5-HT stimulation of heart rate in *Drosophila* does not act through cAMP as revealed by pharmacogenetics

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Majeed ZR, Nichols CD, Cooper RL. 5-HT stimulation of heart rate in *Drosophila* does not act through cAMP as revealed by pharmacogenetics. *J Appl Physiol* 115: 1656–1665, 2013. First published October 3, 2013; doi:10.1152/japplphysiol.00849.2013.—The fruit fly, *Drosophila melanogaster*, is a good experimental organism to study the underlying mechanism of heart rate (HR) regulation. It is already known that many neuromodulators (serotonin, dopamine, octopamine, acetylcholine) change the HR in *Drosophila melanogaster* larvae. In this study, we investigated the role of cAMP-PKA signaling pathway in HR regulation and 5-HT positive chronotropic action. In order to obtain insight into the 5-HT mechanism of action in larvae cardiomyocytes, genetic and pharmacological approaches were used. We used transgenic flies that expressed the hM4Di receptor [designer receptors exclusively activated by designer drugs (DREADDs)] as one tool. Our previous results showed that activation of hM4Di receptors (modified muscarinic acetylcholine receptors) decreases or arrests the heart from beating. In this study, it was hypothesized that the positive chronotropic effect of serotonin [5-hydroxytryptamine (5-HT)] are mediated by serotonin receptors coupled to the adenylyl cyclase pathway and downstream cAMP and PKA activity. Activation of hM4Di by clozapine-N-oxide (CNO) was predicted to block the effects of serotonin by inhibiting adenylyl cyclase activity through Gαi pathway activation. Interestingly, we found here that manipulation of adenylyl cyclase activity and CAMP levels had no significant effect on HR. The ability of hM4Di receptor activation to slow or stop the heart is therefore likely mediated by activation of GIRK channels to produce hyperpolarization of cardiomyocytes, and not through inhibition of adenylyl cyclase.

**5-HT; M4D receptor; CNO; adenylyl cyclase; PKA**

**THE HEART IN THE FRUIT FLY, *DROSOPHILA MELANOGASTER*, IS A MODEL FOR INVESTIGATING THE UNDERLYING MECHANISM OF CARDiac DISORDERS**

For investigating the underlying mechanism of cardiac diseases which are related to human cardiac disorders, such as congenital heart diseases (39) and dilated cardiomyopathy (25). Although, the *Drosophila* heart is simple and consists of a heart tube at the caudal region and anterior aortic region (9), it has been established as a model system to study the ionic basis of myocardiacyttes (15). The myogenic heart of *Drosophila* larvae also is useful to examine modulators and their mode of actions, because modulators directly act on the myocardiocytes as there is no direct innervation on the pacemaker region. It has been previously shown that Ca2+ and K+ ions play a crucial role in generation of the cardiac action potential. Inhibition of L-type calcium channels or potassium channels by specific blockers significantly reduce the heart rate (HR) whereas the Na+ channel blocker, tetrodotoxin, does not have any effect on HR in *Drosophila* (18). To investigate the underlying mechanisms of modulation, ionic and second messenger cascades need to be determined.

Some neuromodulators and neurotransmitters, like serotonin, dopamine, octopamine, and acetylcholine are known to modulate heart performance in *Drosophila melanogaster* larvae (11, 20, 37). 5-Hydroxytryptamine (5-HT) is an essential neuromodulator that has many behavioral and physiological functions in the fly, such as learning and memory (22), courtship and mating (3), cardiac rate modulation (11, 41), and modulation of sensory-motor circuits (13). Moreover, 5-HT is assumed to be a modulator with an old evolutionary history because it is found in simple as well as in complex animals and even plants (2). There are four characterized 5-HT receptor genes in the *Drosophila melanogaster* genome, 5-HT1ADro, 5-HT1BDro, 5-HT2Dro, and 5-HT7Dro (3, 22, 24, 30). Recently, another 5-HT2Dro receptor subtype, 5-HT2BDro, has been identified in *Drosophila* (17). 5-HT1ADro, 5-HT1BDro inhibits adenylyl cyclase (AC) activity (36); 5-HT7Dro increases AC activity (40). Exogenous 5-HT application in semi-intact larvae increases the HR, but the underlying signaling mechanism has yet to be elucidated. However, we have shown that activation of 5-HT2Dro receptor mediates the positive chronotropic effect of 5-HT in *Drosophila* larval heart (Majeed ZR, Stacy A, Cooper RL, unpublished observations).

It is obvious that the *Drosophila* and vertebrates are different morphologically; notwithstanding, *Drosophila* and vertebrates use similar molecular pathways underlying cardiac development (7). Further, *Drosophila* and vertebrate hearts share crucial physiological and dynamic aspects, for example, cardiac output, and rate and duration of systole or diastole (10). The rationale for *Drosophila* larval heart study is to decode the undiscovered aspects of cardiac physiology and pathophysiology which in turn can be extended to the pathology of hearts in other animals, including humans.

In this study, we sought to elucidate the signaling pathway underlying the positive chronotropic effects of 5-HT in *Drosophila melanogaster* larvae by utilizing classical pharmacology with incorporation of a pharmacogenetic approach using designer receptors exclusively activated by designer drugs (DREADDs) receptors (4). DREADDs are powerful new tools that allow a high degree of spatial and temporal control of neuronal and effector pathway activity. Significantly, DREADD control is reversible, and requires no specialized equipment. We used the UAS-Gal4 binary expression system (8) to express hM4Di receptors, which are positively coupled to Gαi in muscle fibers. The hM4Di is a modified human muscarinic acetylcholine M4 receptor, mutated such that it no longer has affinity for the native ligand, acetylcholine. Instead, this engi...
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neered receptor has high affinity for a chemical that is considered physiologically inert, clozapine-N-oxide (CNO) that has final agonist efficacy at DREADD receptors (1, 4, 29). With this approach one can rule out off-target effects of the natural ligand to specifically and remotely control effector pathway activity in defined target tissues that the DREADD receptor is expressed in.

In the pupal stage of Drosophila forskolin does not produce a change in the HR (21), indicating that cAMP levels are not important in the pupa; however, in the pupal stage significant alterations in endocrine function accompanied by significant morphological changes are occurring, and mechanisms of cardiac function may be different than in the larva. We previously demonstrated that 5-HT increases HR in larvae; therefore, we examine here the potential mechanisms of action of 5-HT in the larval stage heart. Our working model was that 5-HT activates GoS, which activates adenylyl cyclase that in turn increases the level of cAMP. Increased cAMP leads to an increase in active PKA signaling and positive chronotropic modulation of the heart. In this scenario, blockade of adenylyl cyclase activity with pharmacological methods, or activation of GoS through activation of hM4Di receptors expressed in the heart, would be predicted to decrease cAMP levels and PKA activation and block the positive chronotropic effects of 5-HT on the heart. Our data, however, indicate that cAMP levels do not contribute to either modulation of HR or the positive chronotropic effects of 5-HT in the Drosophila heart. It has been reported that forskolin does not noticeably change the HR in P1 pupal stage of Drosophila (21). This confirms that Drosophila HR in larvae also might not be markedly changed by activation of AC-PKA pathway; although, AC-PKA pathway might increase the contractility of heart.

MATERIALS AND METHODS

Transgenic fly strains. In this study, two fly strains were used, UAS-hM4Di and 24B-GFP (a strain heterozygous for the 24B-GAL4 driver element that expresses in all larval muscle, and is also homozygous for the UAS-GFP element; from Dr. Charles Nichols, Louisiana State University Health Sciences Center). The UAS-hM4Di strain was crossed with 24B-GFP strain in order to express hM4Di (Goi) coupled receptor in muscle fibers, including cardiomyocytes. In the F1 generation, half the flies contained the 24B-GAL4 expression element, detected by GFP expression, and the other half did not. The GFP-positive, hM4Di-expressing larvae were used as the experimental animals, and the non-GFP-positive, non-hM4Di-expressing (but UAS-hM4Di containing) ‘littermates’ were used as controls (background). The flies were reared at room temperature (22°C) in vials containing cornmeal-agar-dextrose-yeast medium.

Heart rate measurement. Third instar larvae were dissected in the ventral side up position to expose the dorsal vessel (heart) in HL3 (saline solution) (NaCl 70 mM, KCl 5 mM, MgCl2.6H2O 20 mM, NaHCO3 10 mM, trehalose 5 mM, sucrose 115 mM, BES 5 mM, and CaCl2.2H2O 1 mM with the pH adjusted to 7.1). Because heart performance is very sensitive to pH change, the pH was tightly regulated and adjusted as needed. Recently, the effects of various buffers have been probed to optimize the saline for monitoring heart rate (14). Drugs were applied at various concentrations as indicated in the Results. The preparation was left for 1 min in saline after dissection, and then heart beats were counted for the following minute. Exceptions are indicated on graphs. The difference in the HR before and after application of drugs was used to measure the effects of the various compounds.

Chemicals. All the chemicals, serotonin hydrochloride [5-hydroxytryptamine (5-HT)], forskolin, SQ 22,536, N°, 2′,O-Dibutyryl adenosine 3′,5′ cyclic monophosphate sodium salt (dbcAMP), 2′,5′-dideoxyadenosine were purchased from Sigma-Aldrich, St. Louis, MO. Clozapine-N-oxide was kindly synthesized by Dr. David Nichols (Purdue University). 5-HT stock solution (1 mM) was prepared with HL3 saline and immediately before use, a 1 µM serotonin solution was made from the stock solution. Forskolin was dissolved in 1% dimethyl sulfoxide (DMSO) to obtain a 1 mM stock solution. The final concentration of DMSO was less than 0.1% (roughly about 0.03%) in 30 µM forskolin and 0.5% in 100 µM forskolin. 300 µM and 1 mM of dbcAMP were also prepared with HL3 saline. 200 µM of SQ 22,536 was made with saline from a 1 mM stock solution. A 1-mM dideoxyadenosine stock solution was made in DMSO, then 50 µM (0.04% final DMSO concentration) and 500 µM (0.2% final DMSO concentration) with dilutions in saline.

Statistical analysis. All data are expressed as mean ± SEM. The paired t-test (before and after) was used to compare the difference of HR change after exchanging solution with saline containing chemicals. T-test was used to compare between background (UAS-hM4Di and hM4Di expressing (UAS-hM4DiX24B-Gal4) larval HRs (Sigma-Plot version 11.0). P ≤ 0.05 is considered as statistically significant. The number of asterisks are considered as P ≤ 0.05 (**), P ≤ 0.02 (***), and P ≤ 0.001 (***)

RESULTS

Effects of media change on heart rate. We previously found that the larval HR is sensitive to the physical exchange of the bathing media (9). In order to control for the exchange of the bathing media when exposing the heart to various compounds, a series of control experiments of saline bath exchanges was conducted. Also, because some of the drugs contained small concentrations of DMSO, the effect of low concentrations of DMSO alone and the effect of switching the DMSO bathing media were examined (Fig. 1). In addition, the HR was measured with an additional exchange of the bathing media twice for the same period as the first one, left for 1 min, and then examined for the following minute. Although the larvae were already exposed to saline during the dissection, there was still an average of 20 beats per minute (BPM) increase in HR with the first saline change. After another 2 min the heart was not as sensitive to the exchange of the saline, as only an average of 10 BPM was noted (Fig. 1B). Exchanging bathing media that contained DMSO did not produce as large of an increase in HR as saline. The second exchanges also resulted in a smaller alteration in HR (Fig. 1D).

CNO concentration dependently inhibits heart rate in hM4Di expressing larvae. Increasing concentrations of CNO (10 nM, 100 nM, and 1 µM) were applied to the exposed heart of semi-intact larval preparations. Application of CNO decreases the HR in a concentration dependent manner in the UAS-M4D X 24B-GFP larvae (Fig. 2). CNO application at the highest concentration does not significantly change the HR in the nonexpressing UAS-M4D (control) larvae (Fig. 2). In this study, 500 nM CNO was chosen to use for the primary experiments because 1 µM stopped the heart from beating in all UAS-M4D expressing preparations. We observed that most of the hearts started to beat again when the saline + CNO was exchanged with saline only (not shown).

The presence of CNO to activate hM4Di in expressing larvae blocks the positive chronotropic effect of 5-HT. In flies that do not express hM4Di receptors (background), 5-HT

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markedly increases the HR, even in the presence of CNO (Fig. 3, A and C). These results confirm the positive chronotropic effects of 5-HT and that CNO does not block the plasma membrane receptor binding site for 5-HT. Only when hM4Di DREADD receptor expression was induced did CNO have an effect on the positive chronotropic effect of 5-HT (Fig. 3, B and C). We observed that CNO by itself slightly decreased HR in background larvae (Fig. 3, A and C). This may be due to leaky expression of the UAS-GAL4 element present in the background genotype rather than a direct effect of CNO on the heart. When the CNO + 5-HT containing saline was exchanged with saline alone (4→5), the HR significantly increased in both background and hM4Di-expressed larvae. This might be due to the 5-HT residual effect on the HR. The increase in HR on this final wash was 2-fold greater in the hM4Di expressing larvae, suggesting a rebound effect occurring from the removal of the repressing CNO mediated hM4Di signaling.

After 5-HT is applied to increase HR, the addition of CNO reverses the positive chronotropic effects of 5-HT. 5-HT was applied prior to exposure of CNO in order to see if CNO can also reverse the effects of 5-HT in addition to block, as demonstrated above. 5-HT markedly increases the HR (Fig. 4). The addition of CNO reversed the effects of 5-HT (Fig. 4). When CNO + 5-HT saline was exchanged with saline alone (4→5), the HR increased again, as observed in the experiments shown in Fig. 3, indicating a possible rebound effect from removal of the repressing hM4Di signaling.

CNO blocks the effects of forskolin. In order to know more about the effect of hM4Di expression on cardiac muscle fiber second messengers, forskolin, an AC activator was examined. In the presence of CNO forskolin increased the HR in background larvae (Fig. 5A). The presence of CNO blocked the positive chronotropic effect of forskolin in hM4Di-expressing larvae. Furthermore, not only was the HR completely arrested by CNO application, but forskolin was unable to overcome this blockade (Fig. 5, B and C).

Forskolin (30 μM) alone was observed to increase the HR (Fig. 6). The subsequent addition of CNO had no inhibiting effect in the background larva but dramatically halted the HR in the hM4Di expressing larvae (Fig. 6). Since forskolin was not able to overcome the effects of hM4Di activation this may indicate that cAMP levels alone are not the primary mediator of HR. The effect of forskolin did not show a significant difference between two genotypes (Fig. 6, C).

A higher concentration of forskolin (100 μM) was used to determine whether or not the 30 μM concentration was maximal or near maximal and thus producing a saturating effect. We also incubated with the higher concentration of forskolin for 10 min to observe if there was a time-dependent effect present on heart rate for forskolin exposure compared with an acute exposure. We observed that forskolin at 100 μM increased the HR at the beginning; however, it was not greater...
than that of 30 μM, and no further increase the HR occurred during the 10 min incubation time (Fig. 7, A and B), indicating that our original 30 μM concentration was likely saturating for forskolin.

To further probe the role of cAMP, we used the AC inhibitor SQ 22,536 (SQ). Application of SQ had no effect in the first minute (Fig. 8). Because no inhibitory effect of SQ on the HR was observed within the first few minutes, we prolonged the interval to 9 min. At the ninth minute of incubation the HR decreased significantly (P < 0.02), but it did not stop (Fig. 8). This decrement in HR might be due to the lengthy incubation period, rather than the drug. However, it was shown if the semi-intact heart preparation (Canton-S flies) that were incubated inside the saline (pH 7.00) for 10 min the HR do not show a marked change (data not shown). Taken together, these results show that SQ has a slightly inhibitory effect on the HR. When the SQ solution was exchanged with SQ + 5-HT solution, HR significantly increased (Fig. 8). These results indicate that SQ cannot override the positive chronotropic effect of 5-HT. Either the SQ is not effective in blocking Drosophila AC as it is for mammalian AC, or perhaps manipulating cAMP levels alone is not sufficient to mediate an increase in HR, in agreement with our results using forskolin.

We next tried another AC inhibitor, dideoxyadenosine. Application of dideoxyadenosine (50 and 500 μM) did not produce a noticeable change in HR in hM4Di expressing larvae

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**Fig. 3.** Effect of activation of hM4Di receptors on the action of 5-HT in larval heart. A: Background UAS-M4D, n = 10. B: UAS-M4D X 24B-GFP, n = 10. C: Comparison between background (dark grey) and M4D expressing group (light grey). The differences between genotypes are represented by NS (not significant) or asterisk(s) above the lines. The differences, which are due to the changing of one solution with another one in the same genotypes, are represented by NS or asterisk(s) above the bars. P ≤ 0.05 (*), P ≤ 0.02 (**), P ≤ 0.001 (***)

**Fig. 4.** A: Effect of 5-HT application plus hM4Di activation on larval heart rate (UAS-M4D X 24B-GFP); n = 9. B: The difference in heart rate before and after change of solution. P ≤ 0.02 (**), P ≤ 0.001 (****).
By the second minute HR showed a very slight decrease in hM4Di expressing larvae. However, when dideoxyadenosine (50 or 500 μM) solution was exchanged with dideoxyadenosine/5-HT, a marked increase in the HR occurred (Fig. 9 and 10).

In a different approach, we attempted to manipulate cAMP levels by using the cAMP analog dbcAMP, which both inhibits phosphodiesterase and mimics the effects of cAMP on downstream effectors like PKA. dbcAMP is resistant to cleaving by phosphodiesterase (19). Application of dbcAMP (300 μM) does not change the HR in background larvae (Fig. 11, A and C). Because no effect was observed with the initial exposure of dbcAMP on the HR, the HR was measured for the following second minute. Again, there was no significant change in HR with a longer exposure to dbcAMP (Fig. 11, A and C). When the dbcAMP solution was exchanged with dbcAMP/CNO solution, HR slightly increased in background larvae (Fig. 11, A and C). Application of dbcAMP slightly decreased HR in (Fig. 9 and 10). By the second minute HR showed a very slight decrease in hM4Di expressing larvae. However, when dideoxyadenosine (50 or 500 μM) solution was exchanged with dideoxyadenosine/5-HT, a marked increase in the HR occurred (Fig. 9 and 10).

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hM4Di expressing larvae (Fig. 11, B and C). When the dbcAMP solution was exchanged with the dbcAMP + CNO solution, the HR dramatically decreased in the hM4Di expressing larvae (Fig. 11, B and C). dbcAMP did not have a significant difference between two genotypes.

Because there was no significant change after application of 300 μM dbcAMP, a higher concentration of dbcAMP (1 mM) was used. Application of dbcAMP at this higher concentration only slightly decreased the HR in the first and second minute (Fig. 12) to the same degree as with the lower concentration (Fig. 11). However, when the dbcAMP solution was exchanged with dbcAMP + CNO, the HR markedly decreased (Fig. 12). Changing dbcAMP + CNO solution with saline alone showed a marked increase in HR (Fig. 12).

**DISCUSSION**

Drosophila has been established to be a good model for the study of cardiac physiology in part because of malleable genetics and conserved development with mammalian heart (33). Moreover, fly and mammalian hearts use many of the similar signaling pathways during development (39). In mammals, 5-HT receptor dysfunction or overactivation leads to abnormal cardiac development and physiological disturbance (23, 28). Clearly, 5-HT and proper activation of relevant 5-HT receptors is critical for normal cardiac development and function in mammals (5). 5-HT receptors are all G-protein coupled receptors (GPCRs), with the exception of the 5-HT3 receptor that is a ligand gated ion channel (31). Modified DREADD GPCR receptors have been shown to be excellent tools to further understand the function of specific GPCRs, like serotonin receptors, and their signaling pathways in normal physiological processes in both fly and mammals (4, 29). For example, the hM4D1 DRAEDD receptor we have used here has also been used to understand the role of serotonin in respiratory control (34) and to determine key structural components of the native muscarinic M4 acetylcholine receptor itself (27). These pharmacogenetic approaches have opened up new avenues to further understand the roles of GPCR receptors in physiology and are promising tools to obtain additional insight into underlying molecular mechanisms of human diseases.

Here, we initiated studies to elucidate effector pathways underlying HR regulation in the Drosophila heart and to further explore the positive chronotropic role of serotonin on the Drosophila larva heart. Our original hypothesis was that Drosophila larva HR, like mammalian HR, was governed by adenylyl cyclase activity and its downstream effectors and second messengers. Further, that the positive chronotropic effect of 5-HT was due to positive modulation of adenylyl cyclase activity. To address this hypothesis, we used both pharmacological and pharmacogenetic approaches using a combination of drugs and the modified mammalian G-protein coupled receptor technologies.
coupled receptor (hM4Di, coupled to Goi) to provide an insight into potential signaling pathways underlying HR and the effects of 5-HT in Drosophila larval heart. We initially observed that activation of hM4Di can slow or stop the heart. Moreover, that hM4Di activation can override the positive chronotropic effect of 5-HT. These observations were consistent with our hypothesis, with the prediction that hM4Di activation would activate Goi, reduce adenyl cyclase activity, and slow the heart.

It has been shown, however, that forskolin does not noticeably change the HR in P1 pupal stage of Drosophila (21). We extended the investigation in larvae and showed, in general, manipulation of adenyl cyclase activity and cAMP levels with the dbcAMP, SQ 22,536 and dideoxyadenosine did not have a noticeable impact on the HR. In rodents adenosine analogs can retard the positive chronotropic effect of norepinephrine (35). There was a positive effect of forskolin; however, the effect was only slight. Also, dbcAMP slightly decreased the HR. This may be due to activation of targets by cAMP that could bring about ion channel activation, such as K+ channels. This might result in depolarization of the pacemaker cell membrane potential. Further, adding dbcAMP is not the same as stimulating AC as there are various isoforms of AC that might be differentially activated by forskolin whereas addition of dbcAMP maintains a high level of cAMP, bypassing activation of AC.

Our findings indicate that cAMP signaling does not contribute to regulation of HR in Drosophila larvae as it does in mammals, and that CNO is not a component of the positive chronotropic effects of 5-HT in the fly heart. Supporting this conclusion is that 5-HT is still able to produce a large increase in HR in the presence of AC inhibitors. As an additional tool to probe mechanisms underlying HR, we employed the designer receptor hM4Di, which is positively coupled to Goi signaling in both mammals and the fly, as well as the G-coupled inwardly rectifying potassium channel (GIRK) to produce silencing. For example, CNO activation of hM4Di in mammalian systems results in the hyperpolarization and electrical silencing of hippocampal neurons in culture (1). We previously observed that CNO significantly slowed or stopped the heart when applied to Drosophila hearts expressing hM4Di receptors (4) and observe the same effect here. Significantly, pharmacological manipulation of AC or cAMP levels in the presence of activated hM4Di had no effect on the negative chronotropic effects of activated hM4Di. Forskolin was unable to override the repressing effects of CNO. Together, the responses likely indicate that the main effects of hM4Di activation on HR are probably mediated through Gβγ activation of GIRK channels with subsequent hyperpolarization and silencing of cardiomyocytes rather than through negative modulation of cAMP levels through Goi signaling.

When we applied 5-HT to the larvae preparations, we observed that 5-HT markedly increased the HR, in agreement...
with our earlier studies. Our original hypothesis was that 5-HT activates AC, which in turn lead to the activation of PKA and phosphorylation of Ca\(^{2+}\) channels that lead to inward Ca\(^{2+}\) currents and contraction. If this were correct, then the positive chronotropic effects of 5-HT action could be blocked by decreasing AC activity or levels of cAMP. Since our multiple attempts at manipulation of AC and cAMP levels had no or little effect on HR indicate that HR in the fly is in fact not substantially mediated by cAMP. The inhibitory effect of activation of hM4Di we observed is therefore likely mediated through G\(\alpha_i\) activation of GIRK channels and silencing of cardiomyocytes, rather than inhibition of AC through G\(\alpha_o\) to decrease cAMP levels and PKA activity.

Toward understanding the mechanism of action of serotonin on the heart, it needs to be determined if there may be different 5-HT receptors on heart muscle fibers acting through multiple signaling mechanisms. The known fly 5-HT receptors are all G-protein coupled receptors: 5-HT1ADro and 1BDro inhibit adenylyl cyclase through activation of G\(\alpha_i\); 5-HT7Dro activates adenylyl cyclase through G\(\alpha_s\) activation; 5-HT2Dro signaling pathway has not been identified yet (6, 32, 36). A remaining possibility for the positive chronotropic effect of 5-HT could be that 5-HT2Dro receptors are mediating HR. 5-HT2 receptors couple with G\(\alpha_q\) and activation of phospholipase C (PLC). PLC cleaves PIP2 to IP3 and diacylglycerol (DAG). IP3 leads to increases in intracellular Ca\(^{2+}\) (16), which could lead to contraction. However, we previously demonstrated that mutation or misexpression of the 5-HT2Dro receptor does not have a dramatic effect on Drosophila larval HR (12). A second 5-HT2Dro receptor has recently been reported (17), and it may be this receptor mediating the effects if
REFERENCES


AUTHOR CONTRIBUTIONS


REFERENCES


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