

STUDIES ON THE ACTION OF BIOGENIC AMINES ON COCKROACH HEART

By C. COLLINS AND T. MILLER

*Department of Entomology,
Division of Toxicology and Physiology, University of California,
Riverside, California 92502*

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SUMMARY

1. Measurement of the beat of the semi-isolated cockroach heart, by impedance conversion, showed that the whole abdominal heart of large nymphs or adults responded to low concentrations of acetylcholine (ACh) or 5-hydroxytryptamine (5-HT) by increasing the rate of heartbeat.

2. Preparations, which included 2-chamber sections of the abdominal heart, showed vastly reduced responses to ACh compared to the responses of the whole abdominal heart. Responses to 5-HT were similar in both preparations.

3. Following assay with 5-HT, the cockroach heartbeat often developed marked regularity. ACh assays produced such regularity only rarely.

4. The sectioned cockroach heart preparation was decreasingly responsive to 5-HT > synephrine > octopamine > tryptamine > dopamine > tyramine.

5. Dose-response curves revealed that the antagonist 501 c interacted competitively with 5-HT on the cockroach heart preparation. 501 c appeared to be a non-competitive antagonist to octopamine, suggesting that 5-HT and octopamine act at separate receptor sites on the myocardium.

6. Experiments in which solutions of dibutyryl cyclic AMP, dibutyryl cyclic GMP, or aminophylline were continuously perfused on the cockroach heart, failed to establish that cyclic nucleotides mediate neurotransmitter or hormone action on this tissue.

7. Cockroach hearts were not responsive to prostaglandin E₁, F₁, picrotoxin, L-glutamate, or ATP below 10⁻³–10⁻⁴ M doses.

INTRODUCTION

5-hydroxytryptamine (5-HT) is known to have several effects on insect organs or physiological functions at low concentrations (Miller, 1975), and many of these organs are known not to be innervated. For example, 5-HT causes an increase in secretion by the non-innervated salivary glands of the blowfly, *Calliphora erythrocephala*, at thresholds of near 10⁻¹¹ M (Berridge, 1972). Despite this activity at such a low concentration, and the suggestion that 5-HT may act as a neurohormone in insects (Berridge & Prince, 1972), the actual physiological role of 5-HT in diuresis of the blowfly or in any other insect tissue has not been clarified.

Besides a clear-cut action of 5-HT and other biogenic amines on salivary glands and Malpighian tubules of some insects, biogenic amines have been known to increase the rate of heartbeat in semi-isolated heart preparations from several insects (cf. Jones, 1974). Indeed, the cockroach heart has been used for assay of cardio-accelerator factors, biogenic amines and acetylcholine for several years (Jones, 1974; Gersch, 1972; Richter & Stürzebecher, 1971; Hertel, 1971; Gardner & Rounds, 1969).

In view of the current interest in biogenic amines and cyclic AMP in the insect nervous system, it was decided to reinvestigate the pharmacological effects of 5-HT and other amines plus acetylcholine on the semi-isolated heart preparation of the American cockroach. Impedance conversion devices provided a vast improvement in the measurement of cockroach heart beat by simplifying the procedure involved.

METHODS AND MATERIALS

(a) *Cockroach heart preparation*

Under CO₂ anaesthesia, abdominal hearts of female adult *Periplaneta americana* were prepared and monitored as described elsewhere (Miller, 1973). Hearts were bathed in a carbonate-buffered saline composed of 200.17 mM-NaCl, 10.73 mM-KCl, 0.996 mM-MgSO₄, 3.40 mM-CaCl₂, 2.14 mM-NaHCO₃, 0.083 mM NaH₂PO₄ and adjusted to pH 6.9. Dorsal abdomens with hearts exposed were cradled in a simple wax trough. Saline perfusion was regulated by a flowmeter (Gilmont, No. F 1100) to maintain a constant saline level over the beating heart for the duration of each experiment. Saline was delivered to the caudal tip of the abdomen and spilled from the rostral end of the preparation. A 50–100 µl saline volume was contained in the average dorsal abdominal preparation.

A pair of insulated copper wires (100 µm diam.) were positioned for recording along one side of the heart spanning one chamber or two adjacent chambers; the wires did not touch the heart tissue. Often two pairs of electrodes were used to simultaneously monitor different chambers along the same heart. Heartbeat was recorded with a Biocom 2991 impedance convertor (Miller, 1973), and displayed on one channel of a Brush 220 recorder. The heartbeat signal also was integrated by an Ortec 4672 instantaneous frequency/time meter and a d.c. potential proportional to heartbeat rate was applied to the second channel of the Brush 220 recorder.

Two types of heart preparations were used: one with the dorsal vessel and lateral cardiac nerve cords intact; the other with nerve cords and heart sectioned at the ostia of the second, the fourth and the sixth abdominal chambers. The latter provided sections consisting of two semi-isolated heart chambers with attached portions of nerve cord. One heart was used for a single assay sequence or to obtain one dose-response curve for a given compound. Replicate assays on several hearts are represented in the data reported here.

(b) *Drop-on assays*

Test solutions were prepared at the designated concentrations in carbonate buffered saline and applied as single 35 µl drops on the surface of the heart; all were administered in increasing concentrations from the lowest dose providing a response. Each

drug solution was assayed only after single drops of saline caused no interruption in heartbeat when repeatedly applied. All dosages refer to the concentration of the stock solution applied. The heartbeat was monitored for several minutes preceding each drug application, to establish a control rate, and was recorded continuously following treatment until termination of the response to be measured. The preparation was left undisturbed after each test solution was applied while the drug was slowly diluted and washed off the heart by the perfusing saline. 0.5 ml saline was gently flushed from a dropper directly on the heart 3 or 4 times after recording each response and a normal heartbeat pattern allowed to resume before the experiment proceeded.

In contrast to methods which required removal and replacement of the entire saline bath, the 'drop-on' assay caused little or no disturbance of heartbeat rhythm and minimized the variability in responses measured during replicate assays. The 'drop-on' assay provided a uniform dilution factor for each drop applied to a given heart preparation and exposed receptor(s) on the myocardium surface to a fairly uniform drug dose. It thus permitted quantitative comparisons of relative responses.

Responses of the myocardium to 5-hydroxytryptamine creatinine sulphate (Sigma) and acetylcholine bromide (Sigma) were recorded from sectioned heart chambers (prepared as described above with saline perfusion regulated at $0.015\text{--}0.03\text{ ml min}^{-1}$), and compared to responses measured under identical conditions when heart and lateral cardiac nerve cord remained intact. The same procedure was used to assay tryptamine HCl (Aldrich Chem. Co.), tyramine HCl (Calbiochem), synephrine, dopamine, octopamine, picrotoxin, dibutyryl cyclic AMP, dibutyryl cyclic GMP, aminophylline, adenosinetriphosphate (Sigma), L-monosodium glutamate (Nutritional Biochemicals Corp.), prostaglandins E_1 and F_1 (Upjohn) and ascorbic acid.

Particular care was taken with solutions of unstable, light sensitive amines. They were prepared fresh daily and used within 2–3 h. The low aqueous solubility of prostaglandins necessitated preparation of their stock solutions in ethanol and the use of saline dilutions containing 1% ethanol for heart assays. Saline combined with 1% ethanol was always administered to measure 'control' responses. If some experiments included 5% ascorbic acid as a possible activator in solution with prostaglandins, then ascorbic acid was added to the 'control' saline as well.

(c) Assays with antagonist

The xylamidine analogue 501 c (Wellcome) (Fig. 1), which is known to antagonize the peripheral action of 5-HT in mammals (Copp *et al.* 1967), was found to reduce the potency of 5-HT on the semi-isolated cockroach heart. Dose-response measurements were made for 5-HT and octopamine in the presence of this antagonist using sectioned cockroach heart preparations and the drop-on assay procedure described here.

To saturate non-specific receptors prior to assay, fresh heart preparations received preliminary doses of agonist and (when appropriate) antagonist. Doses of 10^{-2} M 5-HT or octopamine, and 501 c at the concentration to be applied for inhibition, were flushed over the hearts and allowed to remain approximately 1 min. This consistently induced systolic arrest and was followed by continuous saline perfusion ($0.12\text{--}0.22\text{ ml min}^{-1}$) until a regular heartbeat returned.

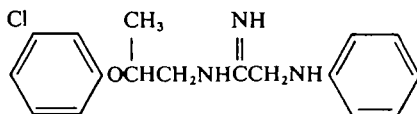


Fig. 1. 5-HT antagonist 501 c (Wellcome).

Hearts flushed with octopamine regained contractile activity earlier than those treated with similar doses of 5-HT and were stabilized for assay within 30–60 min. 5-HT perfused hearts required 60–90 min bathed in saline to recover sufficiently for assay to commence.

The control rates, which were recorded following the first dose and subsequent higher doses, were normally quantitatively similar. Thus, it was standard procedure during each assay to repeat the lowest dose and use the heart's second response for preparation of dose–response curves. When antagonist was applied, it was included with the agonist in solution at 10^{-6} M or at 10^{-5} M and administered in the same manner as the agonist alone. It was not soluble in saline above 10^{-5} M.

(d) *Long-term perfusion of drugs*

In some assays of cyclic nucleotides, aminophylline, or prostaglandins, these compounds were dissolved in the saline used for continuous heart perfusion. To detect possible long-term effects, sectioned and intact heart preparations were monitored for periods of 1–2 h while drugs remained in constant contact with tissues. The temperature and flow rate of the saline or drug solutions were regulated precisely and monitored throughout some experiments in an effort to stabilize the heartbeat rate.

After an initial equilibration period, following dissection, heartbeat rates were recorded in the absence of drugs at a perfusion rate of 0.04 – 0.05 ml min $^{-1}$, for a minimum of 20 min to establish a reference 'control' rate. The control heartbeat rate did not vary more than 3 beats min $^{-1}$ during a 10 min interval.

While the saline reservoir used for heart perfusion was replaced with one containing a saline solution of the test drug, the heart was gently flushed several times with the drug solution to displace the control bath. The heart remained undisturbed as recording continued for an additional 50 min or more and the flow rate of perfusing drug solutions varied no more than ± 0.02 ml min $^{-1}$ from the 'control' period. When monitored, temperature was recorded at 10–20 min intervals using a thermistor immersed in the saline bathing the heart. It ranged between 22 and 24 °C with less than 1 °C variation within each experiment.

Occasionally, slight changes in the electrode position on a heart preparation during prolonged periods of recording resulted in a change in amplitude of the recorded signals. Thus, in these lengthy experiments amplitude changes seen in the record were not considered significant unless they were also evident when the beating heart was observed under the microscope.

RESULTS

(a) The cockroach heart preparation

When abdominal heart preparations appeared to have damaged or unhealthy tissues, they were discarded. Over 90% of the dissected hearts recovered from the initial shock when perfused with carbonate buffered saline, and were found to beat with steady regularity over extended periods (up to 4–6 h). This was a significant improvement over the unbuffered salines used in the past (Miller, 1969) and provided a more uniform 'control heart rate' on which to base all measurements of drug responses. Hearts beat more rapidly following initial recovery from dissection than after 30–60 min bathed, undisturbed, in saline regulated at a constant flow rate.

Heartbeat rates for untreated preparations normally ranged between 55 and 95 beats min^{-1} (bpm). In whole hearts, each contraction was usually co-ordinated along the length of the vessel and variation in heartbeat rate between chambers, when present, usually did not exceed 2–3 bpm. In general, the contractions of isolated heart chambers (Fig. 2*b*) revealed a more uniform amplitude than preparations with the dorsal vessel intact (Fig. 2*a*). However, an isolated two-chamber section could beat at a rate 5–20 bpm different from an adjacent two-chamber section in the same abdomen. The rates of the sectioned two-chamber hearts were not only independent of one another, but more erratic through an assay sequence compared to the whole heart (see Figs. 3, 4).

(b) Action of ACh and 5-HT on cockroach hearts

To demonstrate a distinction between neurogenic and myogenic drug actions on the cockroach heart, initial comparison was made of the two heart preparations to 'drop-on' application of acetylcholine and 5-HT (Figs. 3, 4).

Acetylcholine, at 10^{-7} M, initiated a slight increase in rate when applied to the whole heart preparation. Lower concentrations were without effect. The greatest increases in heartbeat rates preceded brief periods of full or near systolic arrest and resulted from treatment with 5×10^{-6} or 10^{-4} M ACh (Fig. 3). The intact heart exhibited threshold responses to 5-HT as low as 5×10^{-8} M and maximum rate increases followed by first signs of cardiac arrest occurred at 10^{-6} M (Fig. 4). The responses of sectioned hearts to 5-HT were comparable to those of whole hearts within a range of 5×10^{-8} to 10^{-6} M (Fig. 4); however, the sectioned chambers were much less sensitive than whole hearts to applications of ACh (Fig. 3).

A typical response of the whole heart to either ACh or 5-HT included increased rate of heartbeat and decrease in amplitude (Figs. 5, 6). The decrease in amplitude at high heartbeat rates resulted from shortened diastole, the hearts no longer having sufficient time to relax completely to the larger diameters before the succeeding contraction occurred. This point is rather important since it stresses that the *rate of contraction* did not change significantly; rather the period of diastole or diastasis shortened, producing increased rate and decreased amplitudes (a similar response of the heartbeat to nerve extracts has been reported (Miller, 1969)).

Hearts treated with ACh often exhibited irregular heartbeat amplitudes (Fig. 5*a*, *b*, dots). This was more evident when more concentrated drug solutions were applied. By way of contrast, 5-HT treatment occasionally produced heartbeats with a more

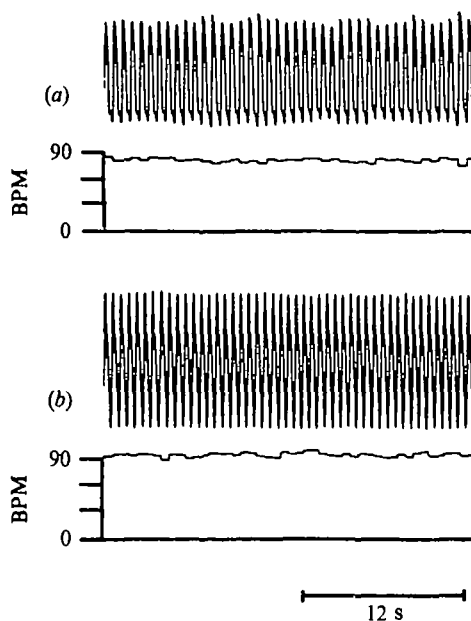


Fig. 2. Untreated intact (a) and sectioned (b) *P. americana* heart preparations. Recording (b) shows a uniform heartbeat amplitude typical of untreated sectioned hearts.

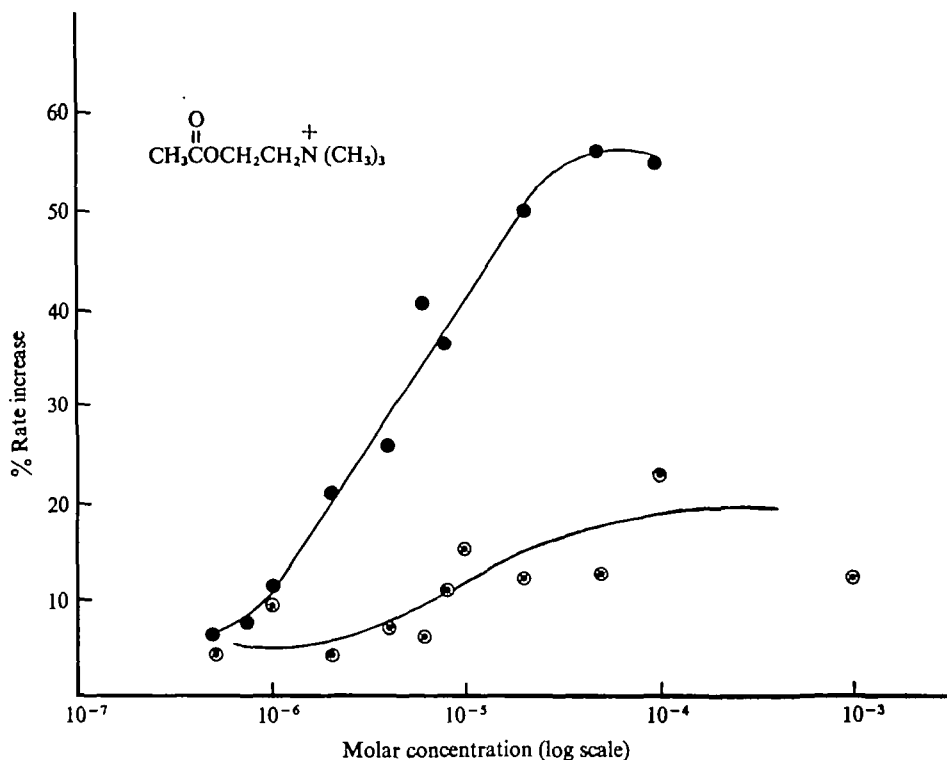


Fig. 3. Graph showing responses of intact ●—● and sectioned ○—○ *P. americana* heart preparations to increasing concentrations of ACh. Each curve represents the response of one heart and is typical of the 3 or more hearts assayed. Note that ACh applied to the sectioned heart induced relatively small heartbeat rate increases.

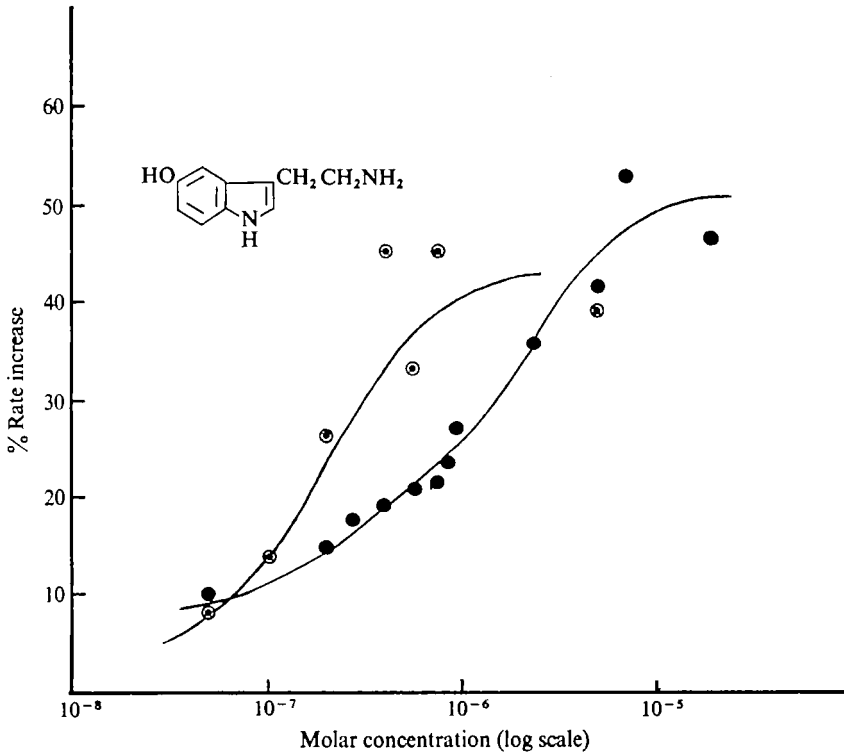


Fig. 4. Graph showing responses of intact ●—● and sectioned ○—○ *P. americana* heart preparations to increasing concentrations of 5-HT. Each curve represents the response of one heart and is typical of the 3 or more hearts assayed.

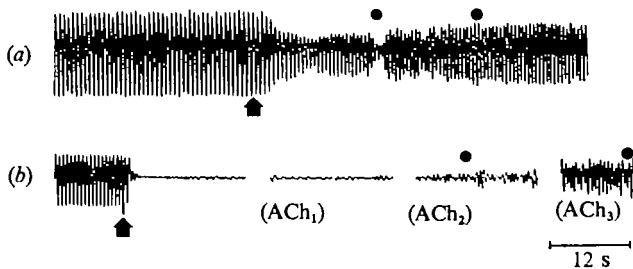


Fig. 5. Heartbeat changes induced by 5×10^{-8} M ACh (a) and 5×10^{-5} M ACh (b). Dots (●) mark regions of irregular heartbeat amplitude. In (b), recordings were made 1 min (ACh₁), 2 min (ACh₂), and 3 min (ACh₃) following application of ACh. Note amplitude variability during recovery (●).

uniform amplitude (Fig. 6a) or initiated a cyclic pattern of changing amplitude (Fig. 6b). Heartbeats which developed pronounced regularity in amplitude following 5-HT treatment were most evident with intact hearts (possibly because intact untreated hearts tended to beat with amplitudes of contraction which were more irregular than isolated chambers). The development of marked regularity following 5-HT treatment was seldom observed at concentrations below 10^{-6} M. Similarly, at the highest 5-HT doses, amplitude irregularities were quite pronounced (Fig. 6c, dots).



Fig. 6. Heartbeat changes induced by 5-HT. Dots (●) mark amplitude changes following treatment. (a) Heartbeat rate increase in response to 7.5×10^{-8} M 5-HT was accompanied by a reduced but more uniform amplitude (●). (b) Shows a pattern of regular amplitude changes (●) after 5×10^{-8} M 5-HT was applied. (c) Shows a gradual onset of systolic arrest following 2×10^{-4} M 5-HT.

(c) Action of biogenic amines on cockroach hearts

Heartbeat responses to biogenic amines were similar in whole or sectioned cockroach heart preparations and were like the responses described for 5-HT. Hearts were decreasingly responsive to 5-HT > synephrine > octopamine > tryptamine > dopamine > tyramine (Table 1). It should be noted, however, that threshold responses to each of the last three compounds were induced by drug concentrations near 10^{-6} M (Fig. 7).

The heart exhibited greatest sensitivity to 5-HT, consistently responding to 5×10^{-8} M and sometimes 1×10^{-8} M doses. Synephrine induced threshold responses in the same concentration range as 5-HT, but the dose-response curves for synephrine were of slightly greater slope and hearts succumbed to systolic arrest at higher concentrations of synephrine compared to 5-HT.

Threshold for heartbeat responses to tryptamine was near 10^{-6} M. Tryptamine caused systolic arrest when applied in concentrations at or above 10^{-4} M.

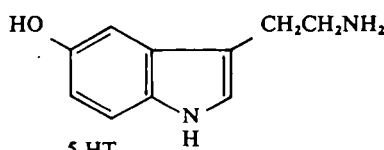
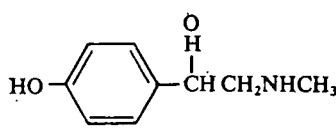
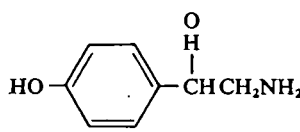
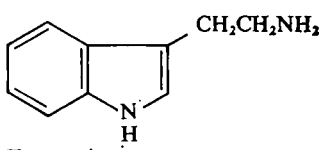
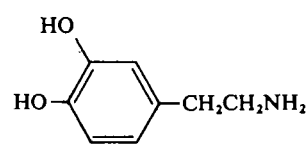
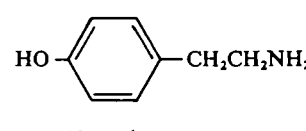
(d) 5-HT and octopamine interaction with an antagonist

A solution of 10^{-5} M 501 c (in carbonate buffered saline at pH 6.9) had negligible effect on heartbeat rate but induced slight irregularities in amplitude when applied alone, as a 35 μ l drop on the heart surface. However, larger quantities of the antagonist or prolonged exposure not only induced irregular contractions, but significantly slowed the heartbeat. This reaction was evident during assays of the agonist-antagonist combination following repeated doses of the drugs and limited the number of points one could determine for the dose-response curve from an individual heart.

Averages of the responses of sectioned heart to 5-HT (alone and in the presence of antagonist) revealed a parallel shift in the dose-response curves with no significant differences in the averaged maximal rate increases recorded (t test, $\alpha = 0.05$) regardless of the antagonist concentration (Fig. 8). The antagonist apparently competes with

Table 1. *Relative potency figures for these biogenic amines compare the amounts of each required to provide a response 50% of maximum (see Fig. 6)*

(These values are based on linear regression lines calculated for each drug from dose-response measurements. n = number of hearts tested.)

Compound	N	Relative potency
 5-HT	8	1
 Synephrine	4	2
 Octopamine	3	16
 Tryptamine	1	50
 Dopamine	3	75
 Tyramine	3	89

5-HT for its receptors, decreasing the affinity of agonist and receptor without altering the intrinsic activity of 5-HT. The uniformity of the hearts' maximum responses to 5-HT when treated with various amounts of antagonist then reflects the reversible nature of the 5-HT-antagonist interaction at receptor sites (Hubbard, Llinas & Quastel, 1969).

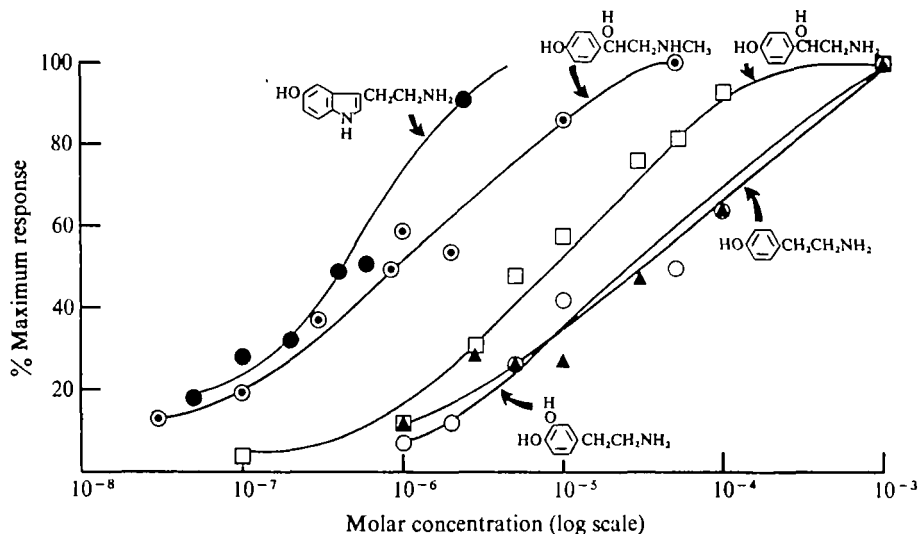


Fig. 7. Dose-response curves show the effect of biogenic amines applied to sectioned *P. americana* heart preparations. Each curve represents the averaged responses of 3 or more hearts. Calculations of % response in this graph are based on the maximum response attained with each compound. See Table 1 for relative potency values based on linear regression lines calculated from this data.

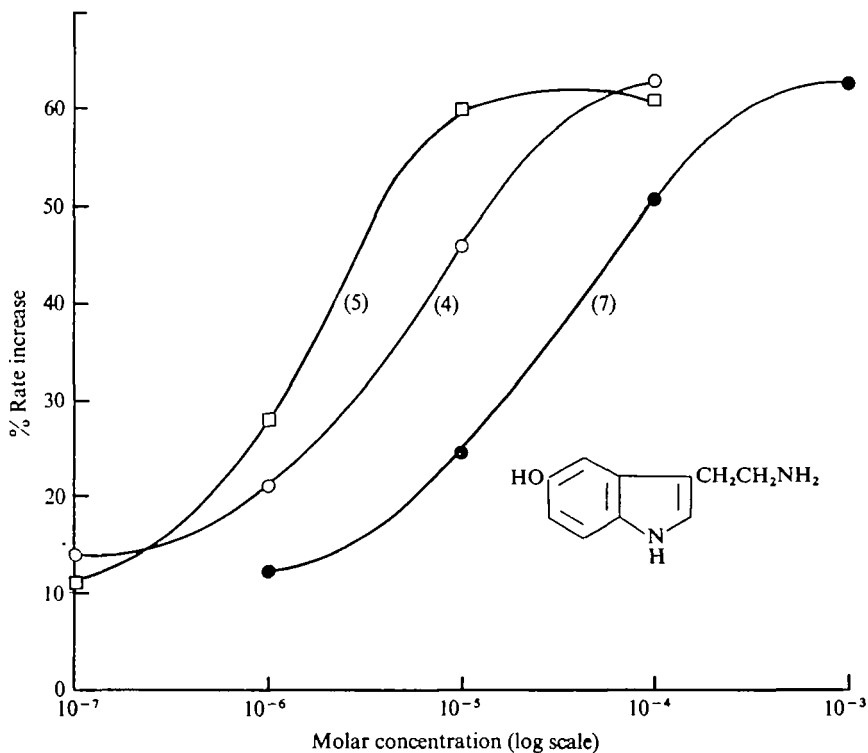


Fig. 8. Antagonist effect of 501 c (Wellcome) when applied with 5-HT to sectioned heart preparations. The number of heart assays averaged for each dose-response curve is given in parentheses. Graphs show the response to agonist alone \square — \square , with 10^{-6} M antagonist \bigcirc — \bigcirc , with 10^{-4} M antagonist \bullet — \bullet . Abscissa: concentration of agonist.

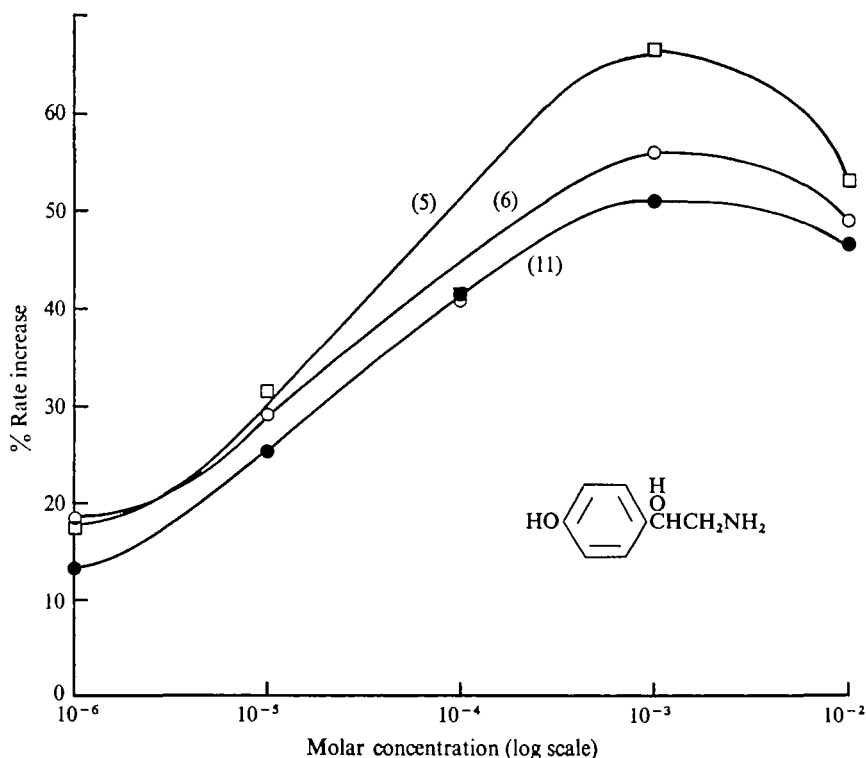


Fig. 9. Antagonist effect of 501 c (Wellcome) when applied with octopamine to sectioned heart preparations. The number of heart assays averaged for each dose-response curve is given in parentheses. Graphs show the response to agonist alone \square — \square , with 10^{-5} M antagonist \circ — \circ , with 10^{-4} M antagonist \bullet — \bullet . Abscissa: concentration of agonist.

The small but consistent decline in the averaged peak responses of the heartbeat to octopamine (Fig. 9) was statistically significant (t test, $\alpha = 0.15$) comparing treatments with no antagonist *v.* 10^{-5} M antagonist. This implies that, to some degree, 501 c irreversibly blocks the sites of octopamine attachment at the myocardium and reduces the intrinsic activity of this agonist (Hubbard *et al.* 1969).

The manner in which the antagonist alters heartbeat responses suggests that it interacts competitively with 5-HT and non-competitively with octopamine and that 5-HT and octopamine are acting at separate receptor sites (Arunlakshana & Schild, 1959).

(e) Effects of cyclic nucleotides and aminophylline on cockroach hearts

'Drop-on' application of 10^{-3} M dibutyryl cyclic AMP in 35 μ l drops slightly reduced the rate of heartbeat for the first minute following application. This diminished rate was not accompanied by pronounced changes in the character of the heartbeat. Continuous perfusion of heart preparations with 10^{-5} M dibutyryl cyclic AMP for 50 min or 10^{-4} M dibutyryl cyclic GMP for 1–2 h revealed no significant changes in heartbeat rate or character. (t test showed no significant difference in the rates of control *v.* treated hearts, $\alpha = 0.05$.)

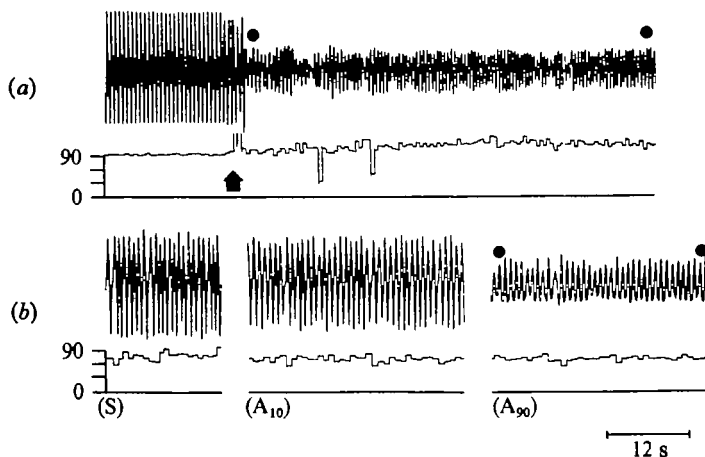


Fig. 10. Responses of *P. americana* heart preparations to aminophylline. Arrow in (a) indicates application of 10^{-3} M aminophylline as a $35\ \mu\text{l}$ drop to the surface of a saline bathed heart. Dots mark region of irregular amplitude typically induced by this procedure. Portions of recording (b) were made during continuous perfusion with saline (S), then with saline containing 10^{-4} M aminophylline. Recording (A_{10}) was made after 10 min and (A_{90}) after 90 min of drug perfusion. The reduced heartbeat amplitude (A_{90}) was also evident when viewed under the microscope.

Drops of 10^{-4} M aminophylline provided no immediate response when applied to the surface of saline perfused hearts. The contractile activity of both intact and sectioned hearts was increased for approximately 2 min immediately following application of the drug at 10^{-3} M. Fig. 10a illustrates the reduced and irregular amplitude accompanying this increase in heartbeat rate.

Hearts gradually developed an irregular heartbeat and reduced amplitude during perfusion experiments which continuously exposed them to 10^{-4} M aminophylline for a 90 min period (Fig. 10b). Whole and sectioned heart responded to 5×10^{-8} to 10^{-5} M 5-HT, when dropped on to preparations which were continuously perfused with saline containing 10^{-6} M aminophylline. The dose-response curves were similar to those obtained with 5-HT alone.

Hearts which were continuously perfused for 50 min with 10^{-6} M dibutyryl cyclic AMP reacted to $35\ \mu\text{l}$ drops of 10^{-6} M 5-HT with 16–18% increases in rate. This is within the range of heart responses to 5-HT alone (80% confidence interval for the mean response of 5 hearts to 10^{-6} M 5-HT; $0.16 < \bar{x} < 0.40$ bpm).

(f) Prostaglandins applied to cockroach hearts

The rates of heartbeat declined slightly during 45–60 min of continuous perfusion with 10^{-5} M prostaglandin F_1 , in saline containing 10^{-5} M ascorbic acid (a prostaglandin activator). Prostaglandin F_1 without ascorbic acid did not provide consistent rate changes during 1–2 h observation whether pooled on the heart preparation or present in perfusing saline. 10^{-4} M prostaglandin E_1 and 10^{-3} M prostaglandin F_1 (applied as $35\ \mu\text{l}$ drops on the whole heart) produced responses which were indistinguishable from those produced by saline alone.

Prostaglandin E_1 is thought to inhibit adenyl cyclase in humans and has been reported to reduce the effect of norepinephrine on Purkinje cells when they were

applied together (Siggins, Hoffer & Bloom, 1969). The lack of significant response in the cockroach heart suggests that prostaglandin F_1 and E_1 do not function in its regulation.

(g) *Miscellaneous drugs tested on cockroach myocardium*

ATP, picrotoxin, and L-glutamate each produced immediate responses when applied, dropwise, to heart preparations in relatively high concentrations. Hearts were not susceptible to these drugs at concentrations below 10^{-3} to 10^{-4} M, which suggests non-specific sites of action.

Picrotoxin at 4.7×10^{-4} M (a saturated solution in saline) reduced the heartbeat rates of both sectioned and whole heart preparations. Three heart preparations responded to 35 μ l drops of 10^{-2} M ATP with cardiac arrest and failed to recover heartbeat while monitored during the following 3 min. A dose of 10^{-4} M ATP elicited slight rate increases (approximately 10%).

At concentrations below 10^{-3} M, L-glutamate had no effect on the contractile activity of the heart, and at 10^{-3} M induced variable responses of short duration. During sequential exposure to doses of between 10^{-6} and 10^{-3} M, one heart continued rhythmic contractions without interruption, another was arrested by 10^{-3} M glutamate for approximately 1 min but resumed its pretreatment rate within 3 min. A third heart, receiving 10^{-3} M glutamate as its first dose of the drug, responded with a 30% rate increase than decrease to near the original rate within 1 min.

DISCUSSION

The semi-isolated heart preparations of cockroach respond to a group of biogenic amines which are considered to be neurohormone candidates (Pitman, 1971; Gerschenfeld, 1973; Hertel, 1975). It is not clear, however, whether biogenic amines are acting at synaptic receptors. Ultimately, a candidate for neurotransmitter or neurohormonal activity must be demonstrated to act at the same receptor sites as the endogenous agent.

Though the cockroach heart was most sensitive to 5-HT, the potency of the group of biogenic amines tested varied less than 100-fold. This implies that the myocardium has a general susceptibility to the ethylamine group. It seems clear that the parallel shift in dose-response curves for these amines reflects the different affinities of the myocardium receptor(s) for these various molecular structures. The presence of a hydroxyl group on the β carbon apparently enhances the activity of synephrine and octopamine over the other phenylamine analogues tested.

Octopamine and 5-HT evidently act at separate receptor sites on the myocardium. The heartbeat rate changes induced by 5-HT with increasing amounts of antagonist are in keeping with the characteristic pattern of competitive antagonism (Arunlakshana & Schild, 1959). The antagonist had a less pronounced but, nevertheless, consistent effect on the responses of the heart to octopamine which appeared to be a non-competitive antagonism (Arunlakshana & Schild, 1959). Though the differences in the dose-response curves for octopamine are small, they are typical of a non-competitive antagonism when the concentration of antagonist is low (Hubbard *et al.* 1969).

The cockroach apparently contains cyclic GMP at higher levels than cyclic AMP and the tissue concentrations of both have been increased 60–116% within 30 min following *in vivo* injection of 200 pmoles/g aminophylline into the Madagascar cockroach (A. Rojakovick & R. B. March, personal communication). The experiments documented here, however, have failed to reveal a clear association between cyclic nucleotides and the action of biogenic amines.

Increased susceptibility to 5-HT was not detected when it was applied in the presence of dibutyryl cyclic AMP or aminophylline. The changes in heartbeat induced by 5-HT and other biogenic amines were not characteristic of heartbeat responses to cyclic nucleotides or aminophylline.

Lengthy periods of continuous perfusion with cyclic nucleotides had no significant effect on the cockroach heartbeat; only perfusion with high concentrations of aminophylline produced any response. Relatively concentrated doses of dibutyryl cyclic AMP or aminophylline were required to induce heartbeat rate changes by drop-on application and these effects lasted only 1–2 min. The slight rate decreases initiated by dibutyryl cyclic AMP contrast with the rate increases induced by aminophylline.

Phosphodiesterase inhibitors or cyclic AMP induced similar responses in *Calliphora* salivary glands (Berridge & Prince, 1972; Prince & Berridge, 1972), *Carausius* Malpighian tubules (Maddrell, Pilcher & Gardiner, 1971; Pilcher, 1971), the cockroach hindgut (Cook, Holman & Marks, 1975) and the heart of *Locusta* (S.-Rozsa, 1974). The threshold concentrations reported to initiate responses in these tissues ranged from 10^{-8} to 10^{-2} M for cyclic AMP and 10^{-8} to 10^{-2} M for aminophylline or theophylline. The time intervals required for these drugs to induce peak responses, when reported, ranged to a maximum of 15 min. The heartbeat rate of *Locusta* heart preparations increased when the saline bath contained theophylline at 10^{-6} M or dibutyryl cyclic AMP at 10^{-7} M.

The cockroach heart showed some response to aminophylline or cyclic nucleotides at high concentrations. However, the presence of aminophylline or dibutyryl cyclic AMP in the bathing medium had no effect on the dose-response curves of 5-HT.

S.-Rozsa also noted the complexity of *Locusta* heart responses to 5-HT and cyclic AMP. She observed that they act by separate mechanisms involving ion transport across the cell membrane and that the action of one was antagonized rather than potentiated by the presence of the other, though they both increased heartbeat rates when applied separately.

The cyclic AMP molecule is said to penetrate the cell with difficulty (Berridge & Prince, 1972). The dibutyryl derivatives of cyclic nucleotides were selected for these experiments in an effort to achieve effective intracellular concentrations. However, to be effective, a cyclic nucleotide must be made available more rapidly than it is degraded by phosphodiesterase; in addition to cell penetration, the cyclic nucleotide is possibly non-functional before intracellular esterases remove its dibutyryl group (Berridge & Prince, 1972).

Besides the problems of penetration and metabolism of cyclic nucleotides, the responses of cockroach heart are not consistent. It is difficult to be sure that the responses observed are not non-specific effects. The responses to biogenic amines are more clear. Since there appears to be more than one site of action for 5-HT and octopamine, it may be presumptive to compare biogenic amines in a series. Except

for novel responsiveness of the cockroach heart to a variety of amines, there is no evidence that the amines have a neurohumour or neurotransmitter role in the heart.

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