THE RESPONSE OF ISOLATED FROG HEARTS TO MINIMUM EFFECTIVE CONCENTRATIONS OF CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE

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Summary: The action of increasing concentration of adrenaline, noradrenaline and 5-hydroxytryptamine on cardiac output, arterial pressure and rate of isolated perfused frog hearts working against a fixed resistance was investigated. The minimum effective concentration varied from heart to heart and ranged from $10^{-9}$ to $10^{-5}$ for catecholamines and from $10^{-6}$ to $10^{-3}$ g/ml for 5-hydroxytryptamine. The least effective concentration of 5-hydroxytryptamine ($10^{-9}$) was 100 times more than the least effective concentration of catecholamines ($10^{-11}$). Both inotropic and chronotropic responses were simultaneously affected at the least effective concentration and at greater concentrations. However the first effect on inotropic response was more common than on chronotropic response.

Noradrenaline produced stimulation in 100% cases while adrenaline and 5-hydroxytryptamine produced excitatory as well as inhibitory effects. Biphasic effects were observed in the forms of stimulation and inhibition occurring separately at different concentrations in the same heart or simultaneously at some concentration. Direct biphasic effect of 5-HT may explain complex changes in blood pressure observed in intact animals by previous workers. Inhibitory and biphasic effects of 5-HT are reported for the first time.

Key Words: 5-hydroxytryptamine isolated frog heart catecholamines biphasic cardiac actions cardioinhibition by 5-HT

A high degree of sensitivity of isolated frog hearts to acetylcholine has already been reported, the minimum effective concentration in some hearts being as small as $10^{-19}$ g/ml. (4,5,30). The detection of very small changes in several parameters i.e. cardiac output, arterial pressure, heart rate and venous pressure was made possible with the help of a specially designed experimental set up, a four channel recorder and other electronic assemblies. It was considered desirable to investigate the sensitivity of the frog hearts to other neurohumoral transmitters. This paper reports the response of isolated frog hearts to widely separated but serially increasing concentrations of adrenaline, noradrenaline and 5-hydroxytryptamine.

MATERIALS AND METHODS

Full details of experimental arrangement have been published elsewhere (4,5). The heart of pithed frog was perfused through the posterior vena cava with Ringer’s solution (NaCl, 108.7 mM; KCl, 3.8 mM; CaCl₂, 1.6 mM; NaHCO₃, 4.3 mM; NaHPO₄, 0.22 mM; Glucose, 5.0 mM) of pH 7.8 to 8.0. The outflow from the two aortae was lead through a common artificial resistance of a desired value to give arterial pressures roughly equivalent to
Adrenaline was supplied by B.D.H. in sealed ampoules of 1 ml of 1 in 1000 concentration. Noradrenaline bitartrate was obtained from Bayer Products Ltd. in sealed ampoules of 2 ml of 1 in 1000 concentration. The supply of 5-hydroxytryptamine was obtained from Roche Products Ltd. in a powder form as serotonin creatinine sulphate. Serial dilutions of these substances in Ringer’s solutions were prepared in 100 ml flask by a standard dilution procedure in steps of 1 in 100, starting from $10^{-3} \text{g/ml}$ and working up to $10^{-11} \text{g/ml}$ or still lower concentration when considered necessary. The test solutions were administered by perfusion, starting with ineffective concentration, each for a period of 2 minutes and the test perfusions were bracketed between control perfusions from the same vessel. Perfusion pressure was also monitored on a sensitive meter and with special motor carriage system it could be kept at a constant value within 0.1 mm of water so as to avoid cardiodynamic changes due to change in perfusion pressure (26, 27, 28). The temperature of the laboratory was controlled thermostatically.

RESULTS

Chronotropic responses were estimated from the record of heart rate and inotropic responses, from the amplitude of pulse pressure and cardiac output. If the response of a substance was inhibitory at one concentration and excitatory at another, or if complex changes occurred at any concentration with evidence of both inhibitory and excitatory components, the action was termed ‘biphasic’. The term threshold sensitivity means responsiveness to the minimum effective (threshold) concentration.

The observations of experiments on 26 hearts are summarised in Table I. Because of involvement of different recorded parameters in positive and/or negative direction quantitation of net effect was difficult. In Table I each + or − sign represents a 10% change above control values. The percentages of hearts affected at various concentrations of adrenaline (ADR), noradrenaline (NOR), and 5-hydroxytryptamine (5-HT) are shown in Fig. 1. Table II gives the number of tests in which the inotropic or chronotropic response was first affected irrespective of concentration and the nature of change in the first affected parameter. Fig. 2 gives the percentage of hearts which showed frank stimulation or inhibition or clear biphasic effect at different concentration of the three substances. The observations on the effect of each substance are considered individually.

ADRENALINE

Threshold concentrations: The minimum effective concentration of adrenaline varied...
April, 1972.
Ind. J. Physiol. Pharmac.

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were administered by per-
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Fig. 1. Table II gives
as first affected irrespec-
meter. Fig. 2 gives the
or clear biphasic effect at
the effect of each substance

<table>
<thead>
<tr>
<th>Heart No.</th>
<th>Action of Adrenaline</th>
<th>Action of Noradrenaline</th>
<th>Action of 5-HT</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>+</td>
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<tr>
<td>2</td>
<td>0</td>
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<tr>
<td>22</td>
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<td>0</td>
<td>+</td>
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</table>

For all three variable parameters: —O, no effect; +, increase and —, decrease. S, transient stoppage; R, Heart Rate. Signs (+ or —) prefixed to R indicate change in blood pressure and output associated with change in R, as shown by suffixed signs.

Note:— The table does not include actions of 5-hydroxytryptamine (5-HT) 10^{-3} g/ml.

The least effective concentration of adrenaline under present experimental conditions was 10^{-11} g/ml equivalent to 4.5 \times 10^{-11} M. At this concentration the heart showed frank excitatory
effect involving both the inotropic and chronotropic responses (Table I, heart 9 & Fig. 1). At higher concentrations greater number of hearts were affected (Fig. 1) till at $10^{-5}$ g/ml hearts, in which this concentration was tested, were affected following a sigmoid relationship between involvement-incidence and concentration.

**Fig. 1:** Percentage of hearts affected at various concentrations in g/ml of adrenaline (ADR), noradrenaline (NOR) and 5-hydroxytryptamine (5-HT).

**Nature of action:** Fig. 2A shows the incidence of stimulatory, inhibitory or biphasic responses to increasing concentration of adrenaline and confirms the sigmoid type of relationship of frank excitatory effect. The curve for the incidence of inhibitory effect was linear definite progressively as the concentration was raised till $10^{-5}$ g/ml the inhibitory effect was replaced by stimulation in all hearts. Significant inhibitory effects occurred in 4 out of 22 (18%) hearts at low concentration of adrenaline out of these 4 hearts 3 showed the inhibitory effect threshold concentration of $10^{-9}$ g/ml while 1 heart showed inhibitory effects at $10^{-8}$ g/ml. Clear biphasic effect was seen only in heart 26 in which adrenaline at $10^{-7}$ g/ml initially produced profound stimulation followed by repeated periods of asystole at intervals. For 4 hearts which showed inhibition at low concentrations and stimulation at high concentrations are included in the category of heart showing biphasic effects the overall incidence of biphasic effect would be 22% (5 out of 22).
April, 1972.

Ihd. J. Physiol. Pharmac.

Effects (Table I, heart 9 & Fig. 2A) affected (Fig. 1) till at $10^{-5}$ g/ml all following a sigmoid relationship

-7

10

10

10

10

CONCENTRATIONS

10

10

10

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TABLE II : Involvement of inotropic and chronotropic responses.

<table>
<thead>
<tr>
<th>First affected response</th>
<th>Noradrenaline Total trials=30</th>
<th>Adrenaline Total trials=32</th>
<th>5-Hydroxytryptamine Total trials=25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total + -</td>
<td>Total + -</td>
<td>Total + -</td>
</tr>
<tr>
<td>Inotropic</td>
<td>2 2 0 3 1 2 13 4 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronotropic</td>
<td>0 0 0 1 1 0 1 1 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both together</td>
<td>28 28 0 28 25 3 11 6 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30 30 0 32 27 5 25 11 14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Stimulation+, Inhibition—.

Table II shows that the action on inotropic and chronotropic responses was dissociable in some trials and in case of adrenaline the inotropic response was more frequently affected first than the chronotropic response. When affected first, the inotropic response changed positively or negatively. The negative inotropic effect was predominantly responsible for the incidence of inhibitory effect. Perhaps the diffusion of adrenaline to pacemaker site is restricted in comparison to the contractile myocardium so that threshold concentration is more likely to involve the contractile musculature. The chronotropic response was positive when it was first affected at the threshold concentration otherwise it was effected in the same way as the inotropic response.

**Noradrenaline**

**Threshold concentration**: The threshold sensitivity to noradrenaline varied from $10^{-11}$ to $10^{-5}$ g/ml. Only 1 out of 21 hearts responded to $10^{-11}$ g/ml and 2 hearts responded to $10^{-9}$ g/ml. In 15 hearts the minimum effective concentration was $10^{-7}$ g/ml while the first effective concentration in the remaining 3 hearts was $10^{-5}$ g/ml. Thus 18 out of 21 (about 86%) hearts responded to concentration ranging from $10^{-9}$ to $10^{-7}$ g/ml. The least effective concentration $10^{-11}$ g/ml equivalent to $2.7 \times 10^{-11}$ M producing frank stimulation of both inotropic and chronotropic responses.

**Nature of action**: Tables I & II and Figs. 1 and 2 show that the effect of increasing concentrations of noradrenaline was similar to that of adrenaline with the following exceptions:

(a) The chronotropic response was not involved alone nor it was affected first in any trial.

(b) Only stimulation of both chronotropic and inotropic responses was observed. Unlike adrenaline no inhibitory or biphasic effects were seen.
5—HYDROXYTRYPTAMINE

Threshold concentration: From Table I and Fig. 1 it is at once clear that the minimum effective concentration of 5-HT was 100 times greater than that of catecholamines. The frog hearts were relatively less sensitive to 5-HT than to catecholamines. The threshold sensitivity to 5-HT ranged from $10^{-9}$ g/ml to $10^{-3}$ g/ml. Only one out of 18 hearts showed threshold sensitivity at $10^{-9}$ g/ml. The minimum effective concentration in 8 hearts was $10^{-7}$ g/ml while 7 hearts responded to a concentration as high as $10^{-6}$ g/ml. The remaining 2 hearts responded to $10^{-5}$ g/ml. Thus 16 out of 18 hearts (about 88%) responded to concentrations ranging from $10^{-7}$ to $10^{-5}$ g/ml.

The least effective concentration was $10^{-9}$ g/ml equivalent to $4.2 \times 10^{-10}$ M which produced frank inhibitory effect (Table I, heart 19). The incidence of involvement of hearts at increasing concentrations increased in a linear fashion unlike catecholamines which showed sigmoid pattern (Fig. 1).
Nature of action: Fig. 2 shows that the stimulatory action of 5-HT followed a sigmoid curve as in case of catecholamines. The incidence of inhibitory effects was maximum at 10^{-5} g/ml but occurred at all concentrations. Biphasic effects were common with 5-HT at concentrations above 10^{-5} g/ml and the incidence of biphasic effects was almost equal to the incidence of excitatory effects at 10^{-5} g/ml. Complex changes in blood pressure and output were seen due to biphasic effects. Graded inhibitory effects at all concentration were recorded in 10% hearts. Table II shows that in almost half the tests with 5-HT the inotropic response was first affected. The incidence of negative inotropic effects was twice more common than the incidence of positive inotropic effect when the inotropic was first involved. The incidence of involvement of inotropic and chronotropic response individually paralleled that observed in case of adrenaline. However when both inotropic and chronotropic responses were simultaneously affected, half the tests showed excitation and the remaining half were characterized by inhibitory effects unlike adrenaline which produced stimulation in 25 out of 18 hearts. The chronotropic response was affected first only in heart 14 (Table I) in which case acceleration occurred. The positive chronotropic action of 5-HT was quite prominent in many hearts (Table I).

From the above results it is clear that:

1. The least effective concentration of catecholamines was 10^{-11} g/ml but the threshold concentration varied from heart to heart. The least effective concentration of 5-HT was 100 times more i.e. 10^{-9} g/ml.
2. Involvement of chronotropic response alone or first involvement of chronotropic response was not common and wherever the chronotropic response was so involved, acceleration was observed.
3. Noradrenaline produced only excitatory effect at all concentrations. Adrenaline and 5-HT produced either excitatory or inhibitory or biphasic effects. The incidence of biphasic effects with adrenaline at the same concentration was rare but about 10 to 15% hearts showed inhibition at low concentrations and excitation at high concentrations. In case of 5-HT, the incidence of biphasic effect increased with concentration.
4. The incidence of inhibitory effects with adrenaline and 5-HT was less at high concentration.
5. The commonest effect with all three substances was involvement of inotropic and chronotropic responses together. Negative inotropic effects largely contributed to the inhibitory component of biphasic effects.
6. The incidence of stimulation was 100% in case of noradrenaline, very high in case of adrenaline while with 5-HT stimulation or inhibition plus biphasic action occurred with almost equal frequency.
The solutions of progressively increasing concentration were tested in steps of dilution of 1 in 100. This procedure permitted the testing of a wide range of concentrations without subjecting the heart to too many tests. The tests solutions were administered by perfusion for 2 minutes. The cardiac output was about 2 ml per min. Hence about 4 ml of test solution reached the heart under normal conditions. The total dose can be calculated fairly accurately by multiplying: Cardiac output, testing time and concentration. In usual pharmacological work the doses are administered by injecting test solutions in the perfusion system and it is very difficult to know the actual acting concentration.

Since solutions were tested in steps of 1 in 100 dilution, the minimum effective concentration observed was not the real threshold concentration, the value of which obviously lay between the ineffective and the first effective concentration. Repetitions of tests with six steps of dilution in order to define the real minimum effective concentration was not considered desirable, at this stage, in the middle of the experiment. However, the observed values appeared to be very close to the real threshold concentrations because the observed minimum effective concentration was associated with quite small effect.

**Threshold and least effective concentrations**: The sensitivity of frog heart to adrenaline and noradrenaline was almost equal, while the frog heart was 100 times less sensitive to 5-HT. The least effective concentration of adrenaline and noradrenaline was $10^{-11}$ g/ml while that of 5-HT was $10^{-9}$ g/ml. The variation in sensitivity from heart to heart may be due to different parameters, inactivating mechanisms, distribution of receptors or receptor characteristics like storage.

West (38,39) used frog hearts for assay of catecholamines only up to $10^{-9}$ g/ml dilution. Greater dilutions have been found effective on pressor response in rats (9), rat uterus and colon (15, 16). The sensitivity to 5-HT has been tested in a number of preparations. The isolated colon of rat (10,14), isolated rat uterus (3) and fundal strips of rat stomach (36) has been reported to be sensitive to 5-HT up to $10^{-9}$ g/ml. Hearts of marine invertebrates are not sensitive to 5-HT which stimulates them (12, 17, 35, 37) up to a dilution of $10^{-10}$ g/ml.

**Nature of action**: The excitatory actions of catecholamines are brought about presumably through adrenergic beta receptors (1,2) through the formation of cyclic nucleotide which activates phosphorylase to increase glycolysis (34). Adrenaline is the dominant neurohormone in hearts (13).

The inhibitory effects of adrenaline, however, have not received enough attention although many workers have reported inhibitory effects from adrenaline in the isolated heart of frog (18, 19, 33), fish (8) and molluscs (17). The present work further confirms the occurrence of definite inhibitory effects of adrenaline even at low concentration. These inhibitory effects cannot be attributed to artefacts or decomposition product as the tests were well controlled.

were tested in steps of dilution over a range of concentrations without being administered by perfusion for about 4 ml of test solutions, which were calculated fairly accurately in the perfusion system and it is imperative to perfume the solution. In usual pharmacological procedures, the minimum effective concentration was not considered even if the observed values appear to be the observed minimum effective concentration of frog heart to adrenaline. The occurrence of inhibitory effect at one concentration and excitatory effect at another concentration in the same heart with the same substance or the simultaneous occurrence of inhibitory and excitatory effect at one concentration could only occur when the acting substance involved common receptor mechanism both positively and negatively. Such biphasic effects could also occur even if there were separate excitation coupled and inhibition coupled receptors provided both are involved by the acting substance. In any case the substance has to have bidirectional affinity. This is why the apparently two phenomena, i.e. occurrence of inhibition and stimulation at different concentration in the same heart or simultaneous occurrence of inhibition and excitation leading to complex change at any concentration, both have been put under the category of biphasic effects.

Excitatory effects of 5-HT involving both inotropic and chronotropic responses in marine invertebrate hearts (12, 15, 35, 37) and several species of mammalian hearts (32) have been reported by previous workers. In the present study the excitatory action of 5-HT was observed to be more prominent on chronotropic response. This is consistent with the finding of other workers on mammalian hearts (23, 24).

Inhibitory or biphasic actions of 5-HT were not observed by previous workers either in vertebrate or invertebrate hearts. The complex changes in blood pressure observed by other workers (22, 23, 24, 31) in intact preparations were attributed to coronary chemoreflex (11, 19) or increased arterial resistance (20) or ganglionic blockade (25). The observation of complex changes in cardiac output and blood pressure in isolated hearts working against a fixed resistance rules out involvement of arterial resistance or ganglionic blockade. Coronary chemoreflex is not operative in frogs. Hence the complex changes in intact mammals observed by previous workers are attributable to direct biphasic action 5-HT on the heart.

REFERENCES


