BETA-ADRENERGIC BLOCKADE IN THE ISOLATED TOAD HEART

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The chronotropic and inotropic effects of catecholamines on the mammalian heart are well known. The effects are now believed to be mediated via the activation of beta-receptors in the myocardium as proposed by Ahlquist (1948) since they are blocked by specific beta-receptor anatagonist drugs such as pronethalol (Black & Stephenson, 1962) and propranolol (Black et al, 1964). The present study was carried out in order to determine the action of the catecholamines and anatgonist drugs on an amphibian heart; the local toad Bufo melanostictus was used.

METHODS

Toads weighing from 40 to 70 grams and of either sex were used. The toad was pithed, a Syme's cannula tied into the sinus venosus and the heart removed. The perfusion fluid was Clark-Ringer solution of the following composition (grams per litre): NaCl, 6.5; KCl, 0·14; $CaCl_2$, 0·12; NaH_2PO_4, 0·01; $NaHCO_3$, 0·2. The perfusion pressure was 3 cm. of water above the tip of the cannula. All experiments were conducted at room temperature (28° to 29°C).

Heart rates were determined by counting the beats over half minute periods. The amplitude of myocardial contraction was recorded by a Gimbal lever writing on a smoked kymograph drum. This lever applied a relatively constant load to the heart and the work done by the contraction was indicated by the degree of excursion of the writing point.

Injections of catecholamines appropriately diluted with Ringer solution were made into the opening above the cannula. The volume injected in all cases was 0·1 ml. Two Marriotte bottles were used as reservoirs of the perfusion fluid and were connected by a three-way tap to the cannula. This permitted the perfusion fluid to be changed without interruption to the experiment. Antagonist drugs introduced into the perfusion fluid were allowed to act for 10 minutes prior to recording.

The following compounds were used: noradrenaline bitratrate, adrenaline bitartrate, isoprenaline bitartrate, pronethalol (nethalide, Alderlin), propranolol hydrochloride (Inderal), phentolamine methanesulphonate (Regitine), tolazoline hydrochloride (Priscoline), piperoxan hydrochloride and yohimbine hydrochloride. Doses are expressed as weight of salt used.

RESULTS

Controls

The initial heart rate varied from preparation to preparation in the range of 60 to 100 per minute; most were within the range of 80 to 100. The addition of noradrenaline in doses of 0·1 to 3 micrograms had no effect on the rate; at higher doses from 4 micrograms upwards, it produced a slight slowing of the heart (Fig. 1) Adrenaline

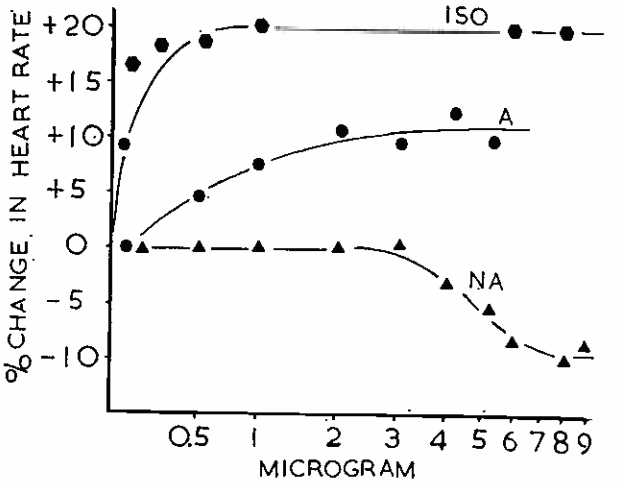

Fig. 1. Changes in heart rate produced by catecholamines. Abscissa percentage change in rate. Ordinate dose of amine in microgram added to the cannula. Noradrenaline (NA); adrenaline (A); isoprenaline (ISO).

in similar doses, on the other hand tended to increase the heart rate slightly while isoprenaline even at low doses accelerated the heart markedly and with a dose of 1·0 microgram, the heart showed its maximum response. The inotropic effect was very definite and all three amines produced positive responses. In no case was a negative inotropic effect observed. The approximate doses that doubled the amplitude of contraction were: noradrenaline 6 microgram, adrenaline 1 microgram, isoprenaline 0·1 microgram.

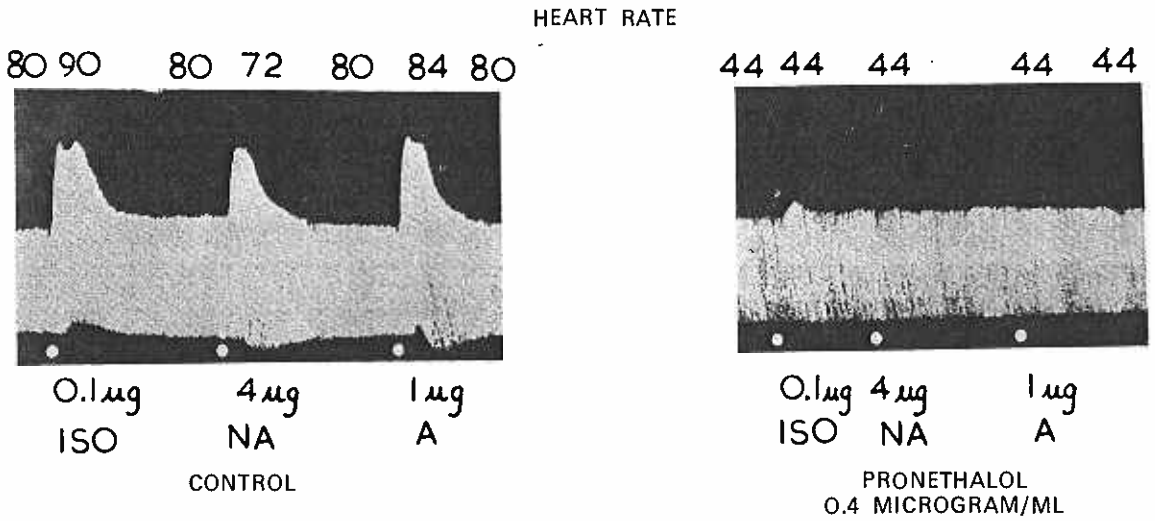

Fig. 2. Blockade by pronethalol on amplitude of contraction produced by catecholamines. At the white dots the doses of amines indicated, noradrenaline (NA), adrenaline (A), isoprenaline (ISO), were added to the perfusion cannula. The figures above the tracings are the heart rates in beats per minute.

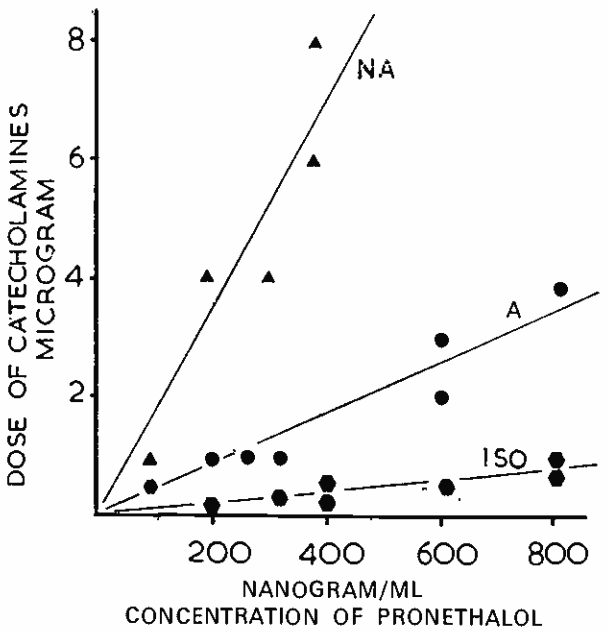

Fig. 3. A graph showing the minimal concentration of pronethalol that would just inhibit the inotropic response induced by a given dose of noradrenaline (NA), adrenaline (A), isoprenaline (ISO).

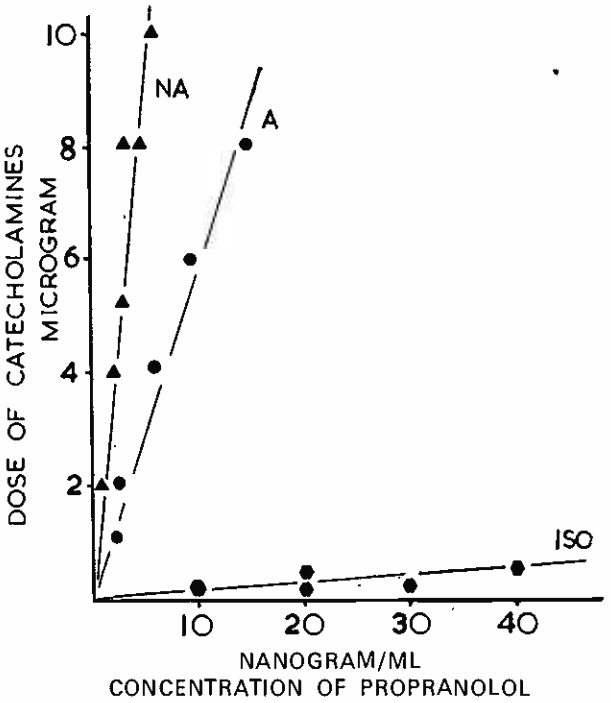

Fig. 4. A graph showing the minimal concentration of propranolol that would just inhibit the inotropic responses induced by a given dose of noradrenaline (NA), adrenaline (A), isoprenaline (ISO).

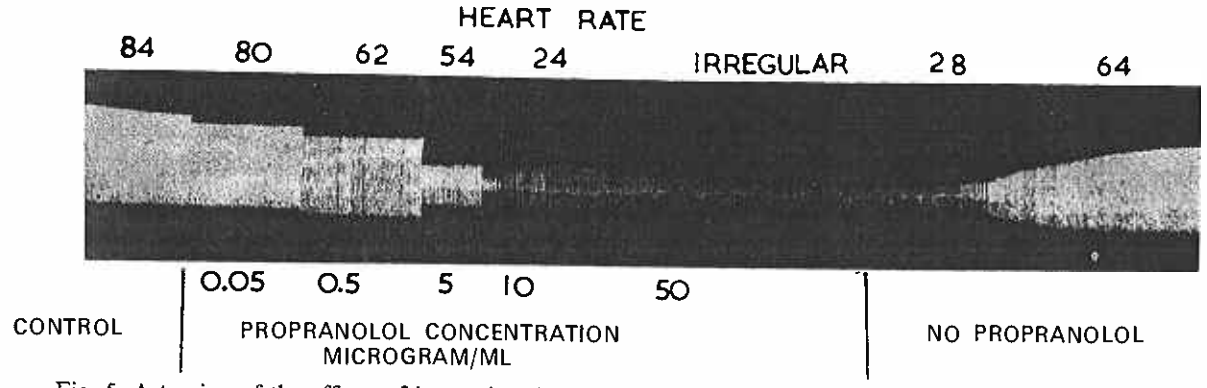

Fig. 5. A tracing of the effects of increasing the concentration of propranolol in the perfusion fluid. As the concentration was increased, the rate slowed down and the amplitude diminished. Thus at 50 microgram/ml the amplitude of contraction markedly decreased and the rate became very slow and irregular. On changing the perfusion fluid back to plain Ringer solution, the heart returned almost to its original state.

Beta-adrenergic blocking agents

The addition of low concentrations of pronethalol (below 100 nanogram/ml) or of propranolol (below 10 nanogram/ml) to the perfusion fluid blocked the effects produced by the catecholamines without themselves affecting the normal heart rate or the amplitude of contraction. Tracings illustrating the inhibition of the inotropic effect by pronethalol are shown in Fig. 2. The effects of the various doses of the catecholamines were determined while the heart was perfused with increasing concentration of the beta-receptor antagonist in order to determine the concentration which would just block off the inotropic response. The results are presented graphically for pronethalol in Fig. 3 and propranolol in Fig. 4. The weaker the inotropic effect of the amine the more readily was it blocked by the anatagonist and this is shown by a steeper slope of the graph; thus a concentration of 400 nanogram/ml of pronethalol would abolish the effect of 6 microgram noradrenaline or 1·5 microgram adrenaline but was effective against only 0·1 microgram isoprenaline. Compared with pronethalol, propranolol was much more potent on a weight basis. These two compounds were also capable of preventing cardioacceleration induced by the amines. Normally an injection of 0·1 microgram of isoprenaline accelerated the heart by 10% but in the presence of pronethalol (400 nanogram/ml) or propranolol (20 nanogram/ml) this phenomenon was not seen.

If the concentration of pronethalol was increased above 100 nanogram/ml or of propranolol above 10 nanogram/ml, the resting heart rate fell; with propranolol at a concentration of 500 nanogram/ml, the rate fell by 26% (Fig. 5). Propranolol levels of 10 to 50 microgram/ml very markedly depressed cardiac function; the rate became very slow and irregular, sometimes ending in cardiac arrest. However even at this stage, perfusion with normal Ringer solution would restore the heart almost to its initial state; the heart would once again respond to injections of catecholamines.

Alpha-adrenergic blocking agents

The effect of 4 alpha-adrenergic anatgonists, phentolamine, tolazoline, piperoxan and yohimbine was similarly investigated. At concentrations of 0·5 to 5 microgram/ml, none of the four compounds antagonised the chronotropic and inotropic effects of 0·1 microgram isoprenaline.

The four alpha-blocking agents in concentrations from 0·5 to 5 microgram/ml themselves caused a decrease in heart rate, in three instances by nearly 20%. Even in these cases, the amplitude of contraction was not significantly affected. Concentrations above 5 microgram/ml tended to cause the heart to beat irregularly.

DISCUSSION

This study shows the chronotropic and inotropic response of the toad heart to catecholamines and their blockade by beta-adrenergic antagonists. The results show that the amines had the following order of potency: isoprenaline > adrenaline > noradrenaline. This is in agreement with the results of Ahlquist (1948) experimenting on mammalian hearts and Lands & Howard (1952) using the hearts of Rana pipiens. With all three amines, changes in amplitude were more readily induced than were changes in heart rate. The experiment also confirms the observation of Black et al (1964) that propranolol is more potent than pronethalol.

Erlij et al (1965) reported the blockade of the effects of adrenaline in the frog heart (Rana pipiens) with another beta-blocking compound, dichloroisoprenaline (DCI); however this agent has the disadvantage that it has some inherent sympathomimetic activity of its own (Black & Stephenson, 1962). Nonetheless the results of the two studies agree.

In contrast with the present experiments where the only effect produced by noradrenaline on the rate of beating was a reduction at high doses, Nayler (1956) found noradrenaline to cause an increase in rate with the heart of another toad, Bufo marinus. An explanation for the difference may lie in the experimental conditions, a higher ambient temperature in the present study and the species of toads used. As was anticipated from the mammalian investigations the alpha-adrenergic antagonists phentolamine, tolazoline, piperoxan and yohimbine failed to block the beta actions of isoprenaline.

High concentrations of the beta-blocking agents were found to directly depress the myocardium and resulted in a reduction in force and rate of beating. These effects were reversed on removal of the antagonist drug.

The similarity of the heart of Bufo melanostictus to the mammalian heart in its response to beta-adrenergic blockade together with the

simplicity, cheapness and robustness of the preparation suggest that it might well find use as a screening test for beta-adrenergic blocking compounds or as a laboratory demonstration for teaching purposes.

SUMMARY

The response of the isolated heart of Bufo melanostictus to noradrenaline, adrenaline and isoprenaline was investigated. The chronotropic and inotropic effects of these catecholamines were reversibly abolished by the beta-adrenergic blocking compounds, pronethalol and propranolol. The alpha-receptor antagonists, phentolamine, tolazoline, piperoxan and yohimbine did not effectively prevent the stimulatory activity of isoprenaline. These results suggest that the heart of Bufo melanostictus contains only beta-adrenergic receptors.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Prof. J. E. Gardiner for his help and advice in the preparation of this manuscript.

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