ACTIONS OF DIBENAMINE ON ADRENERGIC $\beta$-RECEPTORS IN CAT'S PAPILLARY MUSCLE (MOLECULAR PHARMACOLOGICAL STUDIES ON DRUG-RECEPTOR INTERACTION SYSTEMS IN DRUG ACTION IX)

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Recently, the function of the myocardium has been explained by means of the classification of adrenergic receptors proposed by Ahlquist (1). The presence of beta adrenergic receptors in the myocardium was already established by Nickerson and Chan (2), Moran and Perkins (3), and Moran et al. (4), while that of alpha adrenergic receptors was reported on the isolated rabbit's atria by Govier et al. (5-7). Hence, this investigation has been prompted by interests to re-evaluate the existence of myocardial alpha and beta adrenergic receptors in the isolated papillary muscle of the cat, and to study the location of these two receptors. During the experiments of the action of dibenamine on adrenergic receptors, it was unexpectedly found that dibenamine has two different actions, either a short-reversible effect or a long-lasting effect, according to the duration of the administration.

METHODS

Isolated papillary muscle from the right ventricle of cats anesthetized by 30 mg/kg of pentobarbital was suspended in a bath containing 30 ml of Rock's solution having the following composition: NaCl 9 g, KCl 0.42 g, CaCl$_2$·2H$_2$O 0.32 g, NaHCO$_3$ 0.15 g, glucose 1 g in 1 l of distilled water. The bath was aerated slowly and maintained at 37°C. The strength of contraction was recorded by means of an essentially isometric strain gauge and polygraph. Throughout the experiment, the muscle was excited constantly with an electric stimulus of the following condition: 10 volts, 0.8 millisecond and 0.4 cycle per second, and then the strength of contraction was allowed to become constant before the addition of any drug. Dose-response curves were obtained for the cumulative effect of doses in the bath and were expressed in terms of percent of maximal increase. Antagonists were usually applied for 5 minutes before the administration of the agonist in the bath. Dibenamine in the long-lasting effect was applied for 20 minutes and then washed out for the following 20 to 30 minutes before the administration of the agonist. A control curve and curves using blocking agents were obtained from the same papillary muscle with repeated testing.

The following drugs used: adrenaline hydrochloride, noradrenaline hydrochloride,
isoprenaline hydrochloride, dichloroisoprenaline hydrochloride, pronethalol hydrochloride, dihydroergotamine methane sulfonate and dibenamine hydrochloride.

RESULTS

1. Comparison of the response of papillary muscle to adrenaline, noradrenaline and isoprenaline

The responses of papillary muscle of cats to adrenaline, noradrenaline and isoprenaline were tested to compare on the same preparation of papillary muscle. The positive inotropic effects of three catecholamines are apparent in the cumulative dose-response curves presented in Fig. 1. The order of their potencies was isoprenaline > adrenaline > noradrenaline. These data clearly indicated that the positive inotropic effect of catecholamines on the cat's papillary muscle is dependent more on the beta effect than on the alpha effect in the adrenergic receptors.

![Cumulative log dose-response curves](image)

**Fig. 1.** Cumulative log dose-response curves for the positive inotropic effects of the isolated right papillary muscle of the cat to the following catecholamines: isoprenaline, —○--; adrenaline, —◇--; and noradrenaline, —□—. Note the order of potency (isoprenaline > adrenaline > noradrenaline) produced with the same preparation of muscle.

2. Effect of beta adrenergic blocking agents

The mode of action of pronethalol on each dose-response curve for isoprenaline, adrenaline, or noradrenaline was tested. The appearance of shifting in the cumulative dose-response curves presented in Fig. 2 shows clearly that pronethalol has an inhibitory effect for three catecholamines and causes the parallel shift to the right in the curve for isoprenaline only. These data indicate that pronethalol acts as a competitive antagonist of isoprenaline. In contrast to isoprenaline, both adrenaline and noradrenaline give the appearance of non-competitive antagonists to pronethalol. These are indicated by the reduction of the maximal height after some parallel shifting in each dose-response curve for adrenaline or noradrenaline. Moreover, the same results as the effects of pronethalol on the three catecholamines were also obtained with dichloroisoprenaline. From these results, it is suggested that the declining slope in the dose-response-curves for adrenaline
Fig. 2. Cumulative log dose-response curves for isoprenaline (upper), adrenaline (middle) and noradrenaline (lower) in the absence and in the presence of various doses of the beta adrenergic blocking agent, pronethalol, obtained on the cat papillary muscle. Note the parallel shift in the curves for isoprenaline caused by pronethalol, indicating that this compound acts as a competitive antagonist of isoprenaline; and note the depression in the maximal height of the curves for adrenaline or noradrenaline caused by pronethalol at the higher doses, indicating a non-competitive component in the antagonistic action. The same effect is also observed with dichloroisoprenaline.

or noradrenaline caused by beta adrenergic blocking agents is dependent upon another effect other than the beta effect, likely an alpha effect.
3. Effect of alpha adrenergic blocking agents

The mode of action of dihydroergotamine on each dose-response curve for isoprenaline, adrenaline or noradrenaline was tested. The appearance of shifting of their curves presented in Fig. 3 shows clearly that dihydroergotamine inhibits the positive inotropic effects produced by every three catecholamines, though it was less potent than was the beta adrenergic blocking agent, and moreover shows that dihydroergotamine causes the parallel

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**Fig. 4.** Cumulative log dose-response curves for isoprenaline (upper), adrenaline (middle) and noradrenaline (lower) in the absence and in the presence of dibenamine in the short-reversible reaction for 5 minutes. Note the slight inhibition evidenced in the curves for catecholamines at doses lower than $10^{-6}$M caused by dibenamine, but never for catecholamine at higher doses.

**Fig. 5.** Cumulative log dose-response curves for isoprenaline (upper), adrenaline (middle) and noradrenaline (lower) in the absence and in the presence of dibenamine in the long-lasting effect for 20 minutes. Note that the parallel shift in the curves for isoprenaline caused by dibenamine is larger than that for adrenaline or noradrenaline, indicating that dibenamine increases the sensitivity to isoprenaline to a greater degree than to adrenaline or noradrenaline.
shift to the right in each dose-response curve for adrenaline or noradrenaline while it causes also the reduction of the maximal height of the dose-response curve for isoprenaline. These indicate that the dihydroergotamine behaves as a non-competitive antagonist against isoprenaline, in contrast to its action as a competitive antagonist against either adrenaline or noradrenaline in the same dose tested, and as a result, suggest that the site of action of dihydroergotamine is the alpha adrenergic receptor presented in the papillary muscle. At the same time, the parallel shift of the curves for adrenaline or noradrenaline caused by dihydroergotamine indicates that the alpha adrenergic effect also produced the positive inotropic response.

On the other hand, another one of the alpha adrenergic blocking agents, dibenamine, caused only a slight inhibition of the positive inotropic effect of the papillary muscle to three catecholamines, even at the higher doses $3.3 \times 10^{-6}$ M of dibenamine. It was observed that neither of the dose-response curves for catecholamines presented in Fig. 4 was caused to marked shift by dibenamine, but their slopes were increased.

4. Sensitizing action of dibenamine in the long-lasting effect

Prompted by the above-mentioned results, the action of dibenamine in the long-lasting effect, which is produced by washing out muscle incubated by dibenamine for 20 minutes, was tested on each dose-response curve for catecholamines. A larger parallel shift to the left in the dose-response curve for isoprenaline was caused by dibenamine than for either adrenaline or noradrenaline, presented in Fig. 5. These data indicate that dibenamine in the long-lasting effect increases to a greater degree the positive inotropic sensitivity to isoprenaline than to adrenaline or noradrenaline.

![Diagram](https://example.com/diagram.png)

Fig. 6. Cumulative log dose-response curves for isoprenaline sensitized by dibenamine ($3.3 \times 10^{-6}$ M) in the long-lasting effect for 20 minutes in the absence (---) and in the presence (-----) of dihydroergotamine ($3.7 \times 10^{-4}$ M, upper), pronethalol ($4 \times 10^{-6}$ M, middle), and both (lower), then the control curves for isoprenaline (-----). Note that the parallel shift in the curves for isoprenaline protected with dihydroergotamine is larger than the shift for isoprenaline protected with pronethalol or with both pronethalol and dihydroergotamine, indicating that pronethalol only protects the beta adrenergic receptor or its immediate area (which is acted upon by dibenamine in the long-lasting effect).
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5. The site of action of dibenamine for supersensitivity to isoprenaline

According to the protection method for receptors originated with Furchgott (8, 9), it was tested how the dose-response curve for isoprenaline protected with dihydroergotamine and/or pronethalol before the administration of dibenamine is shifted by dibenamine in the long-lasting effect. The parallel shift to the left in the curve for isoprenaline protected with dihydroergotamine was caused by dibenamine to be greater the shift for either the curve for isoprenaline protected with pronethalol or with both pronethalol and dihydroergotamine presented in Fig. 6. The data indicate that the long-lasting effect of dibenamine on isoprenaline is interfered clearly only by the beta adrenergic blocking agent and little by the alpha adrenergic blocking agent. These results suggest that the sensitizing action of dibenamine is dependent upon the beta adrenergic receptor or its immediate area.

DISCUSSION

In this paper, it has been reconfirmed that the papillary muscle of cats also possesses beta adrenergic receptors, because the general criteria were fulfilled by the results that isoprenaline produces a more potent contractile response than does adrenaline or noradrenaline (Fig. 1), and the contractile response is specifically antagonized by beta adrenergic blocking agents such as pronethalol (Fig. 2) and dichloroisoprenaline. On the other hand, alpha adrenergic blocking agent dihydroergotamine behaved as a competitive antagonist against adrenaline or noradrenaline, while it behaved as a non-competitive antagonist against isoprenaline in the same doses of dihydroergotamine tested (Fig. 3). These two non-discrepant results are clear-cut evidence that the alpha adrenergic receptor is present in the papillary muscle. This evidence is supported by studies that have shown that piperoxan, a classical alpha adrenergic blocking agent, acts as a competitive antagonist of noradrenaline while acting as non-competitive antagonist of isoprenaline and that the phenomenon of the depression in the maximal height of the curve for noradrenaline caused by pronethalol indicates a non-competitive antagonism, between noradrenaline and pronethalol, tested on the isolated vas deferens of the rat (10, 11). Hence, it is concluded that the effect of the alpha adrenergic receptor on the papillary muscle is a positive inotropic response similar to that of the contraction of the vas deferens.

About the effect of dibenamine, Hunt (12) already showed that the response of papillary muscle to adrenaline, noradrenaline or isoprenaline is not influenced by dibenamine. In the present paper it was shown that dibenamine does inhibit slightly the positive inotropic response to catecholamines in doses lower than approximately $10^{-5}$M. The observed fact agreed with the report of Serin (13) tested on the isolated rat heart. However, the fact that dibenamine has little or no inhibitory effect on catecholamines at higher doses has attracted our attention. This phenomenon is considered to be caused by some other effect, which is produced by the time course change of dibenamine during immersion in the bath while the cumulative administration of catecholamine is completed.

Thus it has been found that the long-lasting effect of dibenamine, that is an irreversible
effect produced by the administration of 20 minutes, increases the sensitivity of the papillary muscle to isoprenaline a greater extent than to adrenaline or noradrenaline. The reports on the sensitizing action of dibenamine to catecholamine is little except Furchgott's paper (14), though it is about the potenciation on the aorta of guinea pigs to noradrenaline. In order to determine the site of the sensitizing action of dibenamine, furthermore, it has been found that pronethalol protects the beta adrenergic receptor from dibenamine.

It is concluded therefore that the site of the sensitizing action of dibenamine is the beta receptor or its immediate area, which is called "the additional receptor area", proposed by Ariëns et al. (15), and at least is never connected with the alpha receptor. After all, the effect of dibenamine appears to consist of the dual action, which affects alpha and beta adrenergic receptors. The dual action of dibenamine, at the same time, suggests that the alpha and beta adrenergic receptor in the papillary muscle also are located independently of each other, like papers suggested by many workers (16–19).

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