STIMULANT ACTION OF METHOXAMINE IN THE ISOLATED ATRIA OF NORMAL AND 6-PROPYL-2-THIOURACIL-FED RATS

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Abstract—Methoxamine showed positive inotropic and chronotropic actions in the rat atria. These actions were blocked by phentolamine, but not by propranolol. PTU treatment sensitized the animals to the positive inotropic and chronotropic actions of methoxamine. These results verify the findings of previous research that there are α adrenergic receptors in rat atria, that stimulation of these receptors shows positive inotropic and chronotropic action and that α receptors are sensitized by PTU treatment.

In previous papers from this laboratory (1, 2), it was reported that phenylephrine exerted positive inotropic and chronotropic actions on the isolated atria of 6-propyl-2-thiouracil (PTU)-fed rats, mainly by stimulation of α receptors. This indicates 1) involvement of the α receptor mechanism in the cardiostimulant action of catecholamines, and 2) exaggeration in the hypothyroid status. To further confirm these ideas, the present experiment was conducted with methoxamine which has a pure α stimulant activity.

METHODS

Male Wistar rats (170 to 200 g) were used. The rats were divided into two groups. One group was fed a normal diet while the other group was placed in a hypothyroid state following of 0.15% PTU diet for 6 to 8 wk. This group developed dry fur, slow heart rate and a considerable growth retardation.

Each rat was sacrificed by stunning. The excised, isolated left atrium was secured to a hook in a bath and suspended from a force displacement transducer (Nihon Kohden SB-1T). A resting tension of 0.5 g was placed on each atrium. The preparation was stimulated by a unipolar electrode (150/min, 3 msec. duration, voltage twice threshold). The isometric tension was recorded by an ink writing oscillograph (Nihon Kohden WI-130). The bath contained 25 ml Ringer's solution of the following composition (mM): NaCl 148, KCl 5.6, CaCl₂ 2.2, NaHCO₃ 15.0 and glucose 5.6. The solution was maintained at a temp. of 30±0.5°C and bubbled with 95% O₂ and 5% CO₂.

The spontaneously beating right atrium was connected via a thin thread, to a semi-conductor strain gauge. The amplified input from the strain gauge was used to drive a tachometer (Nihon Kohden RT-2). The beating rate was continuously recorded on

an ink writing oscillograph. Drugs were dissolved in 0.9% saline. Before the drugs were added to the bath, the preparation was washed several times and allowed to stabilize for approx. 30 min. Methoxamine hydrochloride (kindly provided by Nihon Shinyaku, Ltd.), phentolamine mesylate (Regitin, Ciba-Geigy) and DL-propranolol hydrochloride (Inderal, ICI) were used in the present study. Phentolamine or propranolol was allowed to interact with the tissue for 30 min.

Results were analyzed statistically by Student's *t* test.

RESULTS

In the present experiment, methoxamine showed positive inotropic and chronotropic actions in both non-treated and PTU-fed rats.

Fig. 1 shows dose response curves for the inotropic action of methoxamine on atria from non-treated and PTU-fed rats, which were obtained by the cumulative dose method. The magnitude of changes was expressed as percent change to the amplitude existing just prior to the addition of the drugs. The increases in contractile force produced by methoxamine in PTU-fed rats were greater than those obtained in non-treated rats. These positive inotropic actions of methoxamine were blocked by 3×10^{-7} M phentolamine both in non-treated rats (Fig. 2A) and in PTU-fed rats (Fig. 2B). The responses to methoxamine

FIG. 1. Dose response curves for the inotropic action of methoxamine on atria from non-treated (●) and PTU-fed (○) rats.

Each point represents a mean value.

The standard error of mean is indicated by vertical bars.

Ordinate : percent changes of contractile amplitude relative to those existing prior to addition of methoxamine.

Abscissa : molar concentrations of methoxamine on a logarithmic scale.

FIG. 2. Effects of propranolol and phentolamine on dose response curves for the inotropic action of methoxamine in non-treated (A) and PTU-fed (B) rats.

- methoxamine alone
- ▲ methoxamine after propranolol 3×10^{-7} M
- × methoxamine after phentolamine 3×10^{-7} M

appeared to be diminished by propranolol, but the difference between control and propranolol pretreatment groups was not statistically significant (Fig. 2A, B). Fig. 3 depicts a typical example of these responses. The numbers of non-treated and PTU-fed rats used for these experiments were 5 and 13 respectively.

Fig. 4 shows the dose response curves for the chronotropic action of methoxamine in non-treated and PTU-fed rats. Absolute increase in spontaneously beating rate in PTU-fed rat was greater than that observed in non-treated rats ($P < 0.05$). These dose response curves were blocked by 3×10^{-7} M phentolamine but not by 3×10^{-7} M propranolol (Fig. 5). The numbers of non-treated and PTU-fed rats used for these experiments were 8 and 17 respectively.

FIG. 3. Effects of 3×10^{-7} M propranolol or 3×10^{-7} M phentolamine on the positive inotropic action of 3×10^{-5} M methoxamine in PTU-fed rats. Methoxamine was added at the point indicated by arrows.

FIG. 4. Influence of graded doses of methoxamine on spontaneously beating rate in atria from non-treated (●) and PTU-fed (○) rats. Ordinate: absolute change of spontaneously beating rate.

FIG. 5. Effects of propranolol and phentolamine on dose response curves for the chronotropic action of methoxamine in non-treated (A) and PTU-fed (B) rats.

- methoxamine alone
- ▲ methoxamine after propranolol 3×10^{-7} M
- × methoxamine after phentolamine 3×10^{-7} M

DISCUSSION]

Many investigators (3-8) have reported that methoxamine, as one of pure α stimulants, has virtually no cardio-stimulant action. Methoxamine has also β blocking property, so that larger doses of methoxamine often produce negative inotropic and chronotropic responses in the mammalian heart (5, 7, 8).

In the present experiment, however, methoxamine showed positive inotropic and chronotropic actions in the rat atria. Both of these actions were blocked by phentolamine, but not by propranolol. Release of catecholamine from the heart was ruled out, since these actions of methoxamine were not depressed by reserpine pretreatment (unpublished observation). In previous experiments (1, 2), it was observed that phenylephrine exerts both positive inotropic and chronotropic actions on the rat atria, which are produced by stimulation of both α and β receptors. The present results support these previous suggestions that there are α receptors in the heart and stimulation of the α receptors produces positive inotropic and chronotropic effects.

It has been demonstrated that there are both α and β receptors in the hearts of

rabbit (9, 10), guinea pig (11), rat (12, 13) and frog (13) and that stimulation of the α receptors produces a positive inotropic response. Participation of α receptors in chronotropic response to phenylephrine, however, has been ruled out by some authors (10, 14). James et al. (8), reported that α receptor stimulation produces negative chronotropic action in dogs. For these reasons it is of interest that the experiments herein clearly showed the involvement of α receptor in the positive chronotropic response to phenylephrine and methoxamine in the rat atria.

In both previous (1, 2), and present experiments, it was also observed that positive inotropic and chronotropic responses mediated by α adrenergic receptor were potentiated after PTU treatment. Such a treatment may provide a useful method for the experimental analysis of the α adrenergic mechanism.

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