

BLOCKADE BY BURIMAMIDE OF THE EFFECTS OF CLONIDINE ON CARDIAC CONTRACTILITY PHOSPHORYLASE ACTIVATION AND CYCLIC ADENOSINE MONOPHOSPHATE IN ISOLATED GUINEA PIG HEART

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Summary: Clonidine in a dose range of 2.5 μg to 80 μg caused positive inotropic effect, which was accompanied by increase in the cyclic AMP levels and phosphorylase-activation of the isolated perfused guinea pig heart. Clonidine-induced biochemical and mechanical effects were blocked by burimamide, an H_2 -receptor antagonist. Propranolol ($1 \times 10^{-6}\text{M}$), phentolamine ($1 \times 10^{-6}\text{M}$) or reserpine pretreatment, did not affect the clonidine responses on the perfused guinea pig heart. Clonidine reduced the 4-methyl-histamine (H_2 -agonist) responses of guinea pig heart. Our data suggest that the cardiac effects of clonidine may be due to stimulation of H_2 -type of receptors.

Key words: H_2 -receptors myocardial contractility phosphorylase-*a* cyclic AMP

INTRODUCTION

Clonidine is an antihypertensive agent whose effects are believed to be centrally mediated via the stimulation of alpha-receptors (7). It has been reported that clonidine also possesses direct cardiostimulant properties (2). Recently Csongrady and Kobinger (2) suggested that clonidine-induced effect on the guinea pig heart is due to the stimulation of histamine receptors. Karppanen *et al.* (4), McNeill and Verma (5,6) suggested that histamine stimulates H_2 -receptors in the gastric mucosa and guinea pig heart. The present investigations were undertaken to study the direct cardiac effects of clonidine and to further characterise the type of histamine receptor involved.

MATERIALS AND METHODS

Langerdorff guinea pig preparation

Guinea pigs of either sex (500-700 g) were injected with heparin sodium (8 mg/kg, sc). The animals were killed by a blow to the head. The hearts were perfused with Chenoweth-Koelle solution (1). The composition of the Chenoweth-Koelle solution in milliequivalents was: NaCl, 119; KCl, 5.6; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3.2; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0; dextrose, 10; and NaHCO_3 , 25. The hearts were perfused through the aorta and the flow rate was maintained by means of a Hotler microinfusion roller pump as per the method described by Verma and McNeill (11). The cardiac force of contraction was recorded on Grass polygraph. Diastolic tension was adjusted to 2.0 g. The hearts were allowed to equilibrate for 15 min prior to injection of drugs.

Clonidine was injected by the side arm cannula. The dose-response experiments were conducted in the presence of antagonists like propranolol ($1 \times 10^{-6}\text{M}$) phenolamine ($1 \times 10^{-6}\text{M}$) and burimamide ($3 \times 10^{-5}\text{M}$). The results are expressed as percent increase in force over the control.

The biochemical experiments were conducted by injecting clonidine (20 μg) at one time in the side-arm cannula. The hearts were frozen at various time-intervals by the method of Verma and McNeill (12). The hearts were stored at -80°C , until analysed for phosphorylase activation and cyclic AMP.

The phosphorylase- α was measured by the method of McNeill and Verma (5). The cyclic AMP was measured by the competitive protein binding assay (3) using commercial kit from Ammersham and Searl. Some experiments were also conducted with reserpinized animals. Such hearts were perfused and frozen as described before. In another set of experiments, the interaction between 4-methylhistamine and clonidine was studied.

Statistical analyses were carried out using *t*' test for unpaired data for cyclic AMP and phosphorylase experiments and *t*' test for paired data for the contractile force experiments. Significance ($P < 0.05$).

RESULTS

Fig. 1 illustrates a dose-dependent increase in cardiac force of contraction produced by clonidine. Burimamide the H₂-receptor antagonist shifted the inotropic dose-response curve of clonidine to the right and in a parallel manner. Clonidine (20 µg) produced 75 ± 3% increase in the cardiac force of contraction. Neither propranolol (1 × 10⁻⁶M) nor phentolamine (1 × 10⁻⁶M) caused any significant changes in clonidine-induced increases in contractile force.

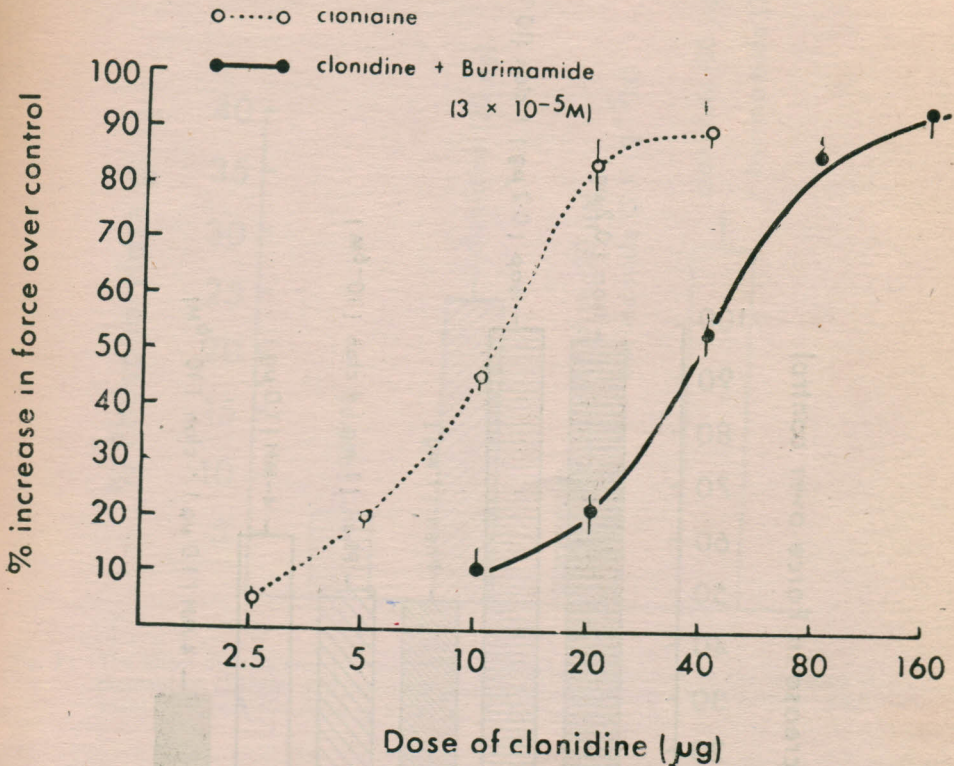


Fig. 1 : The effect of clonidine on the inotropic dose-response curve in the isolated guinea-pig heart, in the presence and in the absence of burimamide. Each point represents the mean ± SEM, of 4 hearts. ($P < 0.05$).

Clonidine (1 × 10⁻⁶M) had no significant effect on either isoprenaline or phenylephrine-induced increase in the force of contraction of the guinea pig heart, but it significantly reduced the positive inotropic effect of 4-methylhistamine, the H₂-receptor agonist (Fig.2).

In the isolated perfused guinea pig heart single dose of 20 µg clonidine caused 25% increase in phosphorylase-a activation (Fig.3). Burimamide (3 × 10⁻⁶M) significantly reduced the clonidine-induced phosphorylase-a activation.

Propranolol or reserpine pretreatment had no significant effect on phosphorylase levels. By increasing the dose of clonidine to 80 μg , the blockade of burimamide was overcome (Fig.3). Clonidine (20 μg) caused 3-fold increase in cyclic AMP. Neither propranolol nor reserpine pretreatment significantly affected the clonidine-induced increase in cyclic AMP level. Burimamide ($3 \times 10^{-5}\text{M}$) significantly reduced the tissue levels of cyclic AMP. Burimamide could not block the effect of 80 μg of clonidine. (Fig.4).

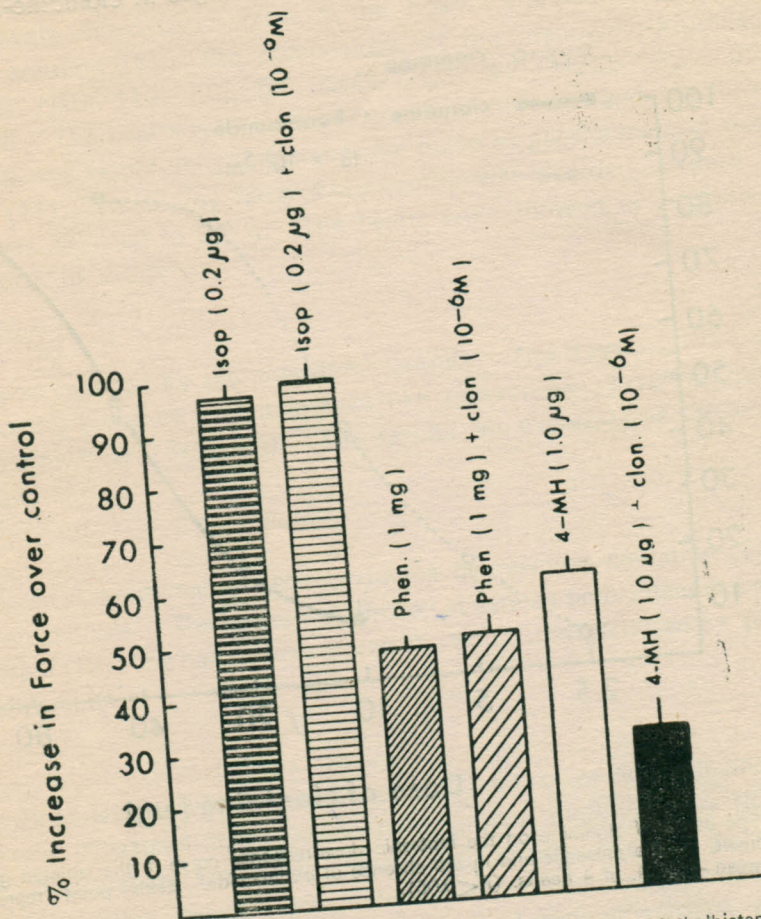


Fig. 2 : Interaction of clonidine with Isoproterenol, Phenylephrine and 4-Methylhistamine on the positive inotropic effect in the isolated perfused guinea pig heart. Each point represents the mean \pm SEM, of 4 hearts. ($P < 0.05$).

DISCUSSION

The results of the present study on the effect of clonidine on the isolated perfused

guninea pig heart indicate that clonidine possesses a positive inotropic effect, which was blocked by burimamide, the H₂-receptor antagonist. This confirms the previous findings of Csongrády and Kobinger (2).

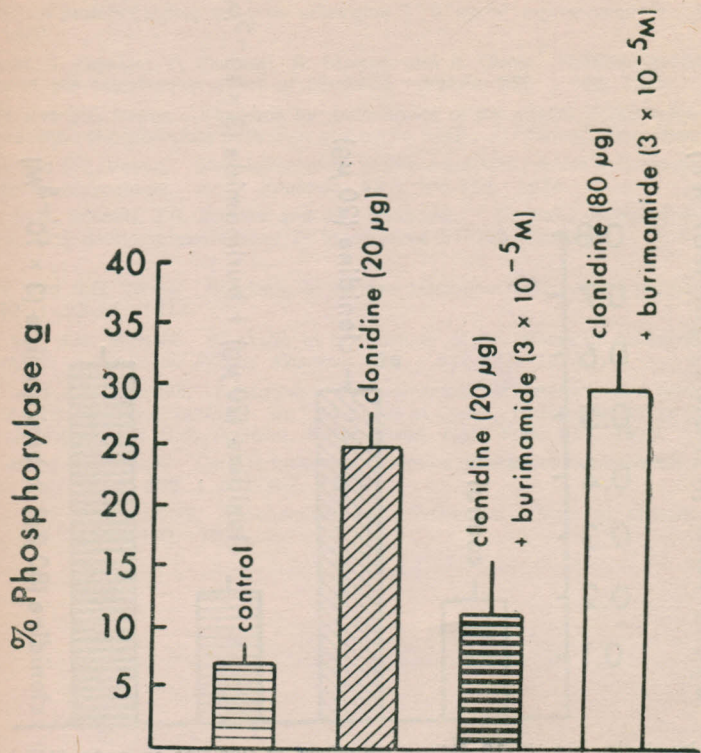


Fig. 3: The effect of clonidine (20 µg) on Phosphorylase activation in the presence of propranolol (10⁻⁶M) and reserpine pretreatment (5 mg/kg I.P. 24 hours). Each point represents the mean \pm SEM, of 4 hearts, ($P < 0.05$).

The other blocking agents, propranolol and phentolamine had no effect on clonidine-induced biochemical and mechanical responses. This confirms that neither beta nor alpha receptors are involved. Moreover, reserpine pretreatment did not alter the clonidine effects. This rules out the possibility of any indirect effect of clonidine. Promethazine an H₁-receptor antagonist does not competitively block the cardiac effects of histamine (8).

Clonidine reduced the effects of 4-methylhistamine, a specific H₂-agonist. This finding suggests that clonidine may be competing with the 4-methylhistamine for the same receptor sites i.e. the H₂-receptors.

Stimulation of H_2 -receptor by histamine in the guinea pig heart increases cyclic AMP and phosphorylase-*a* (9). Clonidine like histamine activated both the nucleotide and phosphorylase levels, further strengthening the contention that clonidine may activate H_2 -receptors. That clonidine may stimulate H_2 -receptors also finds support in the literature

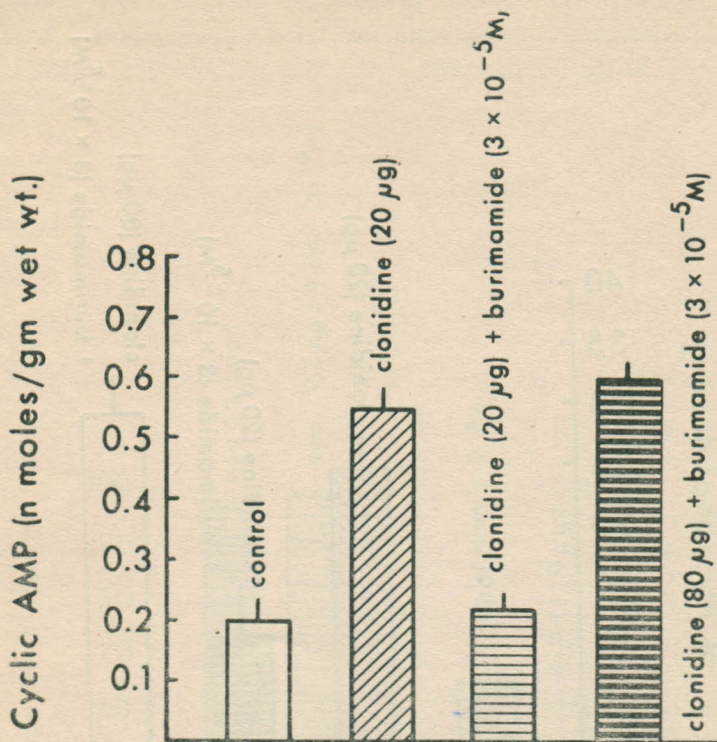


Fig. 4 : Blockade by burimamide ($3 \times 10^{-5} M$) of the effects of clonidine on cyclic AMP levels in the isolated perfused guinea pig heart. Each point represents the mean \pm SEM, of 4 hearts, ($P < 0.05$).

Clonidine, like histamine stimulates the gastric mucosa (10), which possesses H_2 -receptors. Recent study of Karpanann *et al.* (4) showed that clonidine-induced decrease in blood pressure may be due to the stimulation of H_2 -receptors in the central nervous system. The H_2 -receptor antagonist metiamide blocked the hypotensive effect of clonidine.

The results suggest that cardiac receptors for both the biochemical and mechanical effects of clonidine are histamine receptors of the H_2 -type.

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