A MECHANISM OF ACTION OF PHENYLEPHRINE ON HEART

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Summary: Phenylephrine exerted a positive chronotropic and inotropic effect on isolated, spontaneously beating, atria of reserpinised rabbits. Addition of phenoxybenzamine and phentolamine resulted in a depression of control contractile amplitude. Practolol, however, was devoid of this effect. The positive inotropic response to phenylephrine was significantly antagonised by all the three blockers used, while positive chronotropic response was annulled by phentolamine and practolol, but not with phenoxybenzamine. It is, therefore, suggested that phenylephrine exerts its cardiostimulant effects through mediation of both alpha and beta-1 adrenoceptors. A probable mechanism of action could be that phenylephrine acts on some specific chemical group, shared by alpha and beta, receptors. This specific group is probably blocked by both alpha and beta receptor antagonists separately, so phenylephrine becomes ineffective in presence of these antagonists.

Key words: phenylephrine alphaceptors betaceptors

INTRODUCTION

Phenylephrine, an adrenergic agonist (1) has been grouped under direct acting amines (19, 21), since prolonged depletion of myocardial norepinephrine stores fails to prevent its cardiostimulant effects. Regarding its action on specific adrenoceptors of heart, a series of work has been carried out on isolated tissue preparations like atria and ventricular strips, either spontaneously beating or electrically driven from animals like rabbit, guinea pig, or rat. On basis of results obtained from trials of phenylephrine against alpha and betaceptor antagonists, two opinions are forth coming. Few workers (4,5,12,13,14,15,30) suggest that phenylephrine exerts cardiostimulant effects by activation of alphaceptors, while others hold the opinion that it is a betastimulant (6,10, 16, 18, 19, 28, 29, 31).

Certain authors have attempted to differentiate the action of phenylephrine on right and left atrium separately; and they have pointed out that phenylephrine induced positive chronotropic response of right atrium is mediated through betaceptors and the positive inotropic effect on left atrium, through alphaceptors (23). On the contrary, others (29) have concluded that both positive chronotropic and inotropic effects of phenylephrine on right and left atrium respectively are mediated by betaceptors alone.

In view of afore mentioned controversy regarding mode of action of phenylephrine on heart, it was considered worthwhile to revaluate its specific receptor affinity in the heart.
MATERIALS AND METHODS

The present study was conducted on 11 rabbits of either sex weighing 1-1.5 kg. The animals were stunned by a blow at the back of the neck; their hearts quickly removed and placed in oxygenated cold tyrode solution of following composition (26): NaCl 8 g, KCl 0.2 g, CaCl₂ (anhydrous) 0.2 g, MgCl₂ (anhydrous) 0.1 g, NaH₂PO₄ (anhydrous) 0.05 g, NaHCO₃ 1 g, Dextrose 1 g; in double distilled water to make 1 litre. The tyrode solution was cooled to 4°C to produce hypothermic arrest of heart, thus facilitating dissection and isolation of the atria.

All the animals were reserpinised 24 hrs earlier by administration of 5 mg/kg reserpine phosphate (Serpasil, Ciba) subcutaneously in 10% ascorbic acid base. This vehicle was found to have no effect on atrial rate and amplitude (7); and this dose schedule of reserpine has been demonstrated to deplete the entire norepinephrine from the auricles (21).

After a careful dissection, the atria with intact sino-atrial node were isolated and placed in oxygenated tyrode solutions of graded rising temperatures till they resumed spontaneous beating. These preparations were then transferred to the muscle chamber of Dale's bath filled with 25 ml of fresh tyrode solution bubbled continuously with a gas mixture of 95% O₂ and 5% CO₂. The pH and temperature of the tyrode solution were maintained at 7.4 and 37 ± 0.2°C respectively.

The spontaneous atrial contractions were recorded by a frontal lever on a smoked mograph at medium and fast speeds. For stabilisation of physiological conditions, before starting the control recordings an hour’s interval was allowed. When a fairly constant rate and amplitude of atrial contraction was attained, phenylephrine hydrochloride (SIGMA), freshly prepared from stock solution (50 mg in 100 ml of 0.9% w/v NaCl), was added to the muscle chamber in increasing concentrations, till a positive chronotropic and inotropic response was recorded. Since increasing concentrations could not linearly augment the magnitude of response, the effective minimal dose was used in all the test trials. The atrial preparations were then thoroughly washed, control recordings retaken, and different doses of alpha and beta antagonists phenoxybenzamine hydrochloride (S.K. & F.), phentolamine hydrochloride (CIBA) and practolol (CIPLA) added for varying periods of time, and their effects registered in different sets of observations. Phenylephrine (minimal effective concentration) was retested after above mentioned antagonist treatment. The stock solutions for all the drugs were prepared and diluted in 0.9% w/v NaCl on the day of experiment and the drugs were added in volume of 0.2-0.5 ml after withdrawing similar volume of fluid from muscle chamber.

Analysis of data:

Phenylephrine induced positive chronotropic and inotropic responses were compared with their preceding control, and the effects of antagonists were compared with their preceding washed controls and subsequent post agonist rate and amplitude. A similar method of analysis of data was employed in another study on spontaneously beating isolated rabbit atrium (19). The control amplitude was mentioned in our study as hundred percent irrespective of excursions in mill
sex weighing 1.5 kg. The animals quickly removed and placed in NaCl 8 g, KCl 0.2 g CaCl₂ (anhydrous) 0.05 g, NaHCO₃ 1 g, Dextrose 1 g in water was cooled to 4°C to produce a solution of the atria.

In another experiment, 5 mg/kg reserpine was administered to the rabbits (21). The atrial rate and amplitude of atrial contractions were found to be equal to that of reserpine treated rabbits. The atrial rate and amplitude of atrial contractions were then thoroughly examined in different sets of observation (Table I and II). Practolol 20 μg/ml, however, was without any myocardial depressant effect (Table III, Fig.2).

RESULTS

The mean control atrial rate of 11 sets of observation was 152.73±12.80/min. On addition of phenylephrine in concentration of 80 μg/ml, the atrial rate rose to 195±16.60/min, with a percentage increase of 39.36±6.85. The amplitude of contraction increased by 78.65±14.49% over normal control level of 100%. A lesser concentration of the drug was found to be ineffective and concentrations above 80 μg/ml did not essentially produce a linearly increasing magnitude of response, therefore, the minimum effective dose was tried after antagonist treatment also.

The alphaceptor antagonists phenolamine 40 μg/ml and phenoxybenzamine, 50 and 60 μg/ml exhibited a significant (P<.05) negative inotropic effect after 15 and 30 min of treatment (Table I and II). Practolol 20 μg/ml, however, was without any myocardial depressant effect (Table III, Fig.2).

**Table I**: Effect of phenylephrine (PE) on rate (A; per minute) and amplitude (B) of contraction of phenoxybenzamine (PBZ) treated atria of reserpinised rabbits.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Control</th>
<th>PBZ</th>
<th>PE</th>
<th>Control</th>
<th>PBZ</th>
<th>PE</th>
<th>Control</th>
<th>PBZ</th>
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<td></td>
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<td>80 μg/ml</td>
<td>50 μg/ml</td>
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<td>202</td>
<td>189</td>
<td>166</td>
<td>200</td>
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<tr>
<td>Mean</td>
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<td>159.33</td>
<td>204</td>
<td>196.67</td>
<td>212</td>
<td>186.67</td>
<td>156</td>
<td>187.33</td>
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<tr>
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<td>±32.40</td>
<td>±27.47</td>
<td>±22.45</td>
<td>±10.76</td>
<td>±7.32</td>
<td>±2.92</td>
<td>±14.69</td>
<td>±22.45</td>
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</tbody>
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**Table II**: Effect of phenylephrine (PE) on rate (A; per minute) and amplitude (B) of contraction of phenoxybenzamine (PBZ) treated atria of reserpinised rabbits.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>PBZ</th>
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<th>Control</th>
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<td>128.5</td>
<td>171.29</td>
<td>60</td>
<td>79.98</td>
<td>55.6</td>
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<td>114.2</td>
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<tr>
<td>3</td>
<td>67.1</td>
<td>110.12</td>
<td>82</td>
<td>107.42</td>
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<tr>
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<td>131.87</td>
<td>72</td>
<td>92.07</td>
<td>59.33</td>
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<td>±21.74</td>
<td>±24.18</td>
<td>±7.87</td>
<td>±9.88</td>
<td>±3</td>
<td>±13.71</td>
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<td></td>
</tr>
</tbody>
</table>

*The control amplitude taken as hundred percent irrespective of excursions in millimeters.
Fig. 1: Effects of phenylephrine (PE) on control (A) and phenoxybenzamine (PBZ) treated atria (D) of reserpinised rabbit. W.Con.—washed control; Numerical values over figures represent atrial rate (Panel C is continuation of panel B).

**TABLE II:** Effect of phenylephrine (PE) on rate (Upper minute) and amplitude *B* of contraction of phentolamine treated atria of reserpinised rabbits.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Control</th>
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<th>PE 80 µg/ml</th>
<th>Replicates</th>
<th>PNT 40 µg/ml</th>
<th>PE 80 µg/ml</th>
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<td>120</td>
<td>140</td>
<td>1</td>
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<tr>
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<td>120</td>
<td>88</td>
<td>108</td>
<td>2</td>
<td>20</td>
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<td>120</td>
<td>140</td>
<td>4</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>


**Phenylephrine-phenoxybenzamine:**

There was a statistically significant blockade of inotropic but not of chronotropic effects of 80 µg/ml of phenylephrine by phenoxybenzamine in concentrations of 40, 50 and 60 µg/ml exposed for 30 min (Table IA & B; Fig. 1).

**Phenylephrine-phentolamine:**

The positive chronotropic and inotropic responses to phenylephrine (80 µg/ml) were significantly antagonised by pretreatment with 40 µg/ml phenotolamine for 15 min (Table II, A & B).
Pretreatment with 20 μg/ml of practolol for 30 min completely abolished the positive chronotropic and significantly abolished the inotropic responses of 80 μg/ml of phenylephrine (Table III, A & B, Fig. 2).

Table III: Effect of phenylephrine (PE) on rate (A; per minute) and amplitude (B) of contraction of practolol (Pract.) treated atria of reserpinised rabbits.

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Control (μg/ml)</th>
<th>PE (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>108</td>
<td>135</td>
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<tr>
<td>2</td>
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<td>3</td>
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<td>135</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>120</td>
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Mean ±SEM: 107 ±7.21 ±10.2 ±4.69

The control amplitude taken as hundred percent irrespective of excursions in millimeters.

Fig. 2: Effects of phenylephrine (PE) on control (A) and practolol (Pract.) treated atria (D) of reserpinised rabitt. W.Con. = washed control; Numerical values over figures represent atrial rates/minute. (Panel C is continuation of panel B).

Discussion

For settling the action of an amine or for characterisation of adrenoceptors in a particular organ, isolated tissue preparations have been said to be more desirable (11) as in intact animals many neural and humoral feedback mechanisms may modify the response of agonists and antagonists. The temperature, pH and oxygenation of the bathing fluid have been maintained at desired level, as advocated by above author. The tissue preparation under study was too small.
to take up sufficient amount of agonists and antagonists thus producing discrepancy in the calculated and real concentration of the drugs in the bath. To prevent the phenomenon of metphyaxis the atrial preparations were thoroughly washed after each dose of agonist and enough time was given before subsequent tests.

In the present study, elicitation of positive chronotropic and inotropic responses by phenylephrine, reveals its direct myocardial stimulant action. Other authors (5,19,27) have also attributed the myocardial stimulant effect of phenylephrine to its direct action; as in their studies the phenylephrine responses were elicitable after complete depletion of myocardial norepinephrine stores, by reserpination. However, an indirect action has also been suggested on account of phenylephrine evoked release of norepinephrine from the heart (8,15). It, therefore, seems that phenylephrine possesses both direct and indirect actions on the myocardium.

The alphaceptor antagonists phenoxybenzamine and phentolamine, revealed a direct myocardial depressant action. A similar depression of contractile amplitude of isolated perfused rabbit heart has been demonstrated by phentolamine and 5-10 μg/ml of phenoxybenzamine (22). Phentolamine, however in concentration of 20 μg/ml did not depress the myocardial contractility in the present study. This result is thus in agreement with the studies with practolol on isolated turf heart (25), electrically driven left atrium of guinea pig (2) and anaesthetised dog (9).

Phenylephrine induced positive inotropic responses were significantly annulled by phentolamine and phenoxybenzamine. Akin to our findings, alpha adrenergic blockers inhibited the increase in contractility produced by phenylephrine in electrically driven rat ventricle (30), rabbit atrium (4) and guinea pig atrium (13, 15). These authors have, therefore, concluded that phenylephrine acts on alphaceptors. However, in another study (31) on spontaneously beating atria from rabbit, phentolamine (10⁻⁶ M) and phenoxybenzamine (10⁻⁶ M) could not block the inotropic action of phenylephrine. Our results are thus contrary to this observation probably because we have employed higher concentrations of the antagonists.

The probability of local anaesthetic action of phenoxybenzamine supplementing its alphaceptor antagonistic activity at these concentrations (40, 50 and 60 μg/ml) is remote; as in our another study (manuscript under preparation) on reserpinised atria, phenoxybenzamine at 20 μg/ml had not the least depressed the norepinephrine response; instead, potentiated it.

A complete blockade of phenylephrine responses by practolol (20 μg/ml) was sighted in our study. In conformity with this observation, the chronotropic effect of phenylephrine on the isolated rabbit atrium (20) and guinea pig atrium (17) has been reported to be inhibited by beta adrenoceptor blocking drugs. A similar block of chrono and inotropic effect of phenylephrine was described with pindolol a beta blocker on both spontaneously beating and electrically driven atria from rabbit and guinea pig (28), and with propranolol (31). A beta stimulant action of phenylephrine has thus been suggested by these workers. From our study it can be concluded that phenylephrine acts on both alpha and beta receptors. We would like to advance a hypothesis for explanation of blockade of phenylephrine by alpha and beta antagonists.

The so-called alpha and betaceptors are enzymatic chemical groups attached to cell memb-
Mechanism of Phenylephrine Action

It is not very inconceivable that they might possess some common chemical group which can be selectively stimulated by phenylephrine only. Now, if an alpha receptor antagonist is used, it will not only block the alphas but also that specific group at beta receptor; hence phenylephrine will not act in presence of alpha receptor antagonists. Similarly a beta antagonist will block the groups of the betas as well as the specific group at alpha receptor site, thus rendering phenylephrine ineffective. The following illustration (Fig. 3) will give a lucid picture of the hypothesis.

Fig. 3: A phenylephrine sensitive probable common group at receptors shown.

It has been hypothesized (5) that alpha receptors do not respond to phenylephrine when heart is under control of normal pace maker. However, we have not found this statement true, as alpha blockers have mitigated the responses to phenylephrine in spontaneously beating atrium. The other proposition (23) that pace maker receptors in rabbit atrium are of beta type and the receptors in myocardium of the left atrium are of alpha type, is not supported by our results, which revealed that alpha blocking agent phentolamine had significantly blocked the chronotropic effect and beta antagonist mitigated inotropic effect of phenylephrine. So there seems to be a mixed population distribution of alpha and beta receptors in pacemaker cells and in the myocardium. Although a preponderance of betas in pacemaker cells is more likely as beta antagonist was more effective in mitigating chronotropic response than alpha receptor antagonists, which inconsistently showed blockade. The suggestion that in lower concentration phenylephrine acts through alphas and in higher concentration through betas (24) is again not confirmed as both alpha and beta adrenergic antagonists were effective against a single fixed concentration of agonist used in our study.

ACKNOWLEDGEMENTS

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REFERENCES


