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 $beta_1$ receptors. This specific group is probably blocked by both alpha and betaceptor antagonists separately, so phenylephrine becomes ineffective in presence of these antagonists.

Key words : phenylephrine, alphaceptors, betaceptors, alphaceptor antagonists, betaceptor antagonists

INTRODUCTION

Phenylephrine, an adrenergic agonist (1) has been grouped under direct acting amines (5, 19, 27), 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 its cardiostimulant effects by activation of alphaceptors, while others hold the opinion that it is a beta stimulant (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* $Cacl_2$ (anhydrous) 0.2 *g*, $Mgcl_2$ (anhydrous), 0.1 *g*, $NaH_2\ PO_4$ (anhydrous) 0.05 *g*, $NaHCO_3$ 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 effecton 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% O_2 and 5% CO_2. The pH and temperature of the tyrode solution were maintained at 7.4 and 37 ± 1°C respectively.

The spontaneous atrial contractions were recorded by a frontal lever on a smoked kymograph 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 afterabove 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 milli-

meters. Complete blockade was only labelled, when phenylephrine failed to increase the post antagonist rate and amplitude of atrial contractions. Statistically insignificant differences between post antagonist and post agonist rate and amplitude was taken as statistically significant blockade. The means of rate and amplitudes were compared using student's 't' test and statistical significance was assigned at $P < 0.05$. The dose response curve was not plotted in this study.

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 phentolamine 40 *μg/ml* and phenoxybenazmine, 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.

(A)

Replicates	*Control*	*PBZ* 40 *μg/ml*	*PE* 80 *μg/ml*	*Control*	*PBZ* 50 *μg/ml*	*PE* 80 *μg/ml*	*Control*	*PBZ* 60 *μg/ml*	*PE* 80 *μg/ml*
1	150	150	192	210	186	210	210	180	210
2	180	126	180	200	180	226	180	168	212
3	240	202	240	180	166	200	170	120	140
Mean	190	159.33	204	196.67	177.33	212	186.67	156	187.33
±SEM	±32.40	±27.47	±22.45	±10.76	±7.32	±2.92	±14.69	±22.45	±29.01

(B)

Replicates	*PBZ* 40 *μg/ml*	*PE* 80 *μg/ml*	*PBZ* 50 *μg/ml*	*PE* 80 *μg/ml*	*PBZ* 60 *μg/ml*	*PE* 80 *μg/ml*
1	128.5	171.29	60	79.98	55.6	66.72
2	100	114.2	74	88.8	60	76.8
3	67.1	110.12	82	107.42	63	69.3
Mean	98.53	131.87	72	92.07	59.33	70.94
±SEM	±21.74	±24.18	±7.87	±9.88	±5	±13.71

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

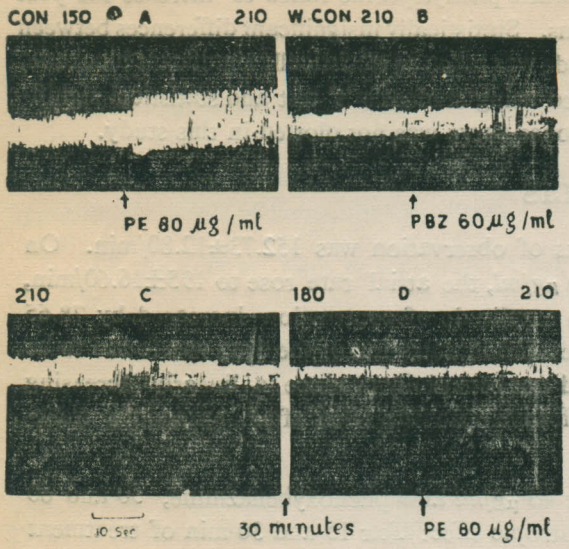

Fig. 1: Effects of phenylephrine (PE) on control (A) and phenoxybenzamine (PBZ) treated atria (D) of reserpinised rabbit. W.Con.—washed control; Numerical values over figures represent atrial rates/ minute (Panel C is continuation of panel B).

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

	(A)				(B)		
Replicates	*Control*	*PNT* 40 µg/ml	*PE* 80 µg/ml	*Replicates*	*PNT* 40 µg/ml	*PE* 80 µg/ml
1	180	120	140	1	38.5	38.5
2	120	88	108	2	20	20
3	120	72	85	3	50	50
4	170	120	140	4	50	50
Mean	143	100	18.85	Mean	39.62	39.62
±SEM	±21.88	±13.86	±15.48	±SEM	±8.19	±8.19

Phenylephrine-phenoxybenzamine :

There was a statistically significant blockade of inotropic but not of chronotropic effects of 80 *ug/ml* of phenylephrine by phenoxybenzamine in concentrations of 40,50 and 60 *ug/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 phentolamine for 15 min (Table II, A & B).

Phenylephrine-practolol :

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.

	(A)				(B)	
Replicates	*Control*	*Pract.* 20 *µg/ml*	*PE* 80 *µg/ml*	*Replicates*	*Pract.* 20 *µg/ml*	*PE* 80 *µg/ml*
1	108	150	135	1	75	82.5
2	90	85.8	138	2	85.8	85.8
3	120	116.6	135	3	116.6	116.6
4	110	91.7	120	4	91.7	91.7
Mean ±SEM	107 ±7.21	132 ±10.2	132 ±4.69	Mean ±SEM	92.27 ±10.20	94.15 ±8.92

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

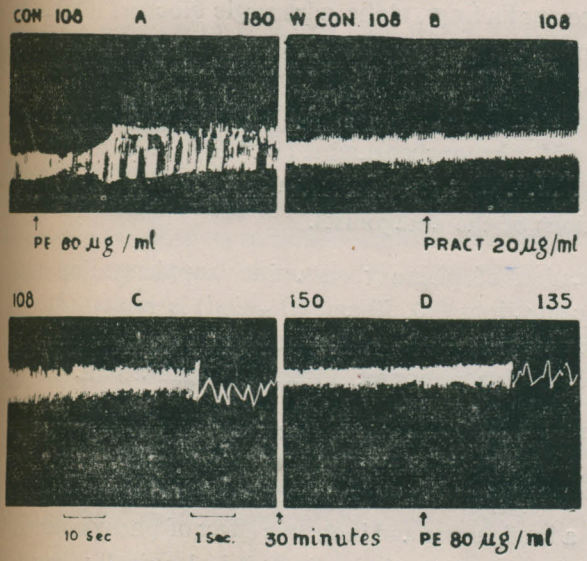

Fig. 2: Effects of phenylephrine (PE) on control (A) and practolol (PRACT) treated atria (D) of reserpinised rabbit. W.Con.—washed control; Numerical values over figures represent atrial rates/ minute. (Panel C is continuation of panel B)

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 feed back mechanism 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 tachyphylaxis 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 too, the phenylephrine responses were elicitable after complete depletion of myocardial norepinephrine stores, by reserpinisation. 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 smiilar depression of contractile amplitude of isolated perfused rabbit heart has been demonstrated by phentolamine and 5-10 $\mu g/ml$ of phenoxybenzamine (22). Practolol, however in concentration of 20 $\mu g/ml$ did not depress the myocardial contractility in the present study. This result is thus in agreement with the studies with practolol on isolated turtle 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 $\mu g/ml$) is remote; as in our another study (manuscript under preparation) on reserpinised atria, phenoxybenzamine 40 $\mu g/ml$ had not the least depressed the norepinephrine response; instead, potentiated it.

A complete blockade of phenylephrine responses by practolol (20 $\mu g/ml$) 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 blockihg drugs. A similar blockade of chrono and inotropic effect of phenylephrine was described with pindolol a betablocker 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 aets on both alpha and $beta_1$ 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-

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

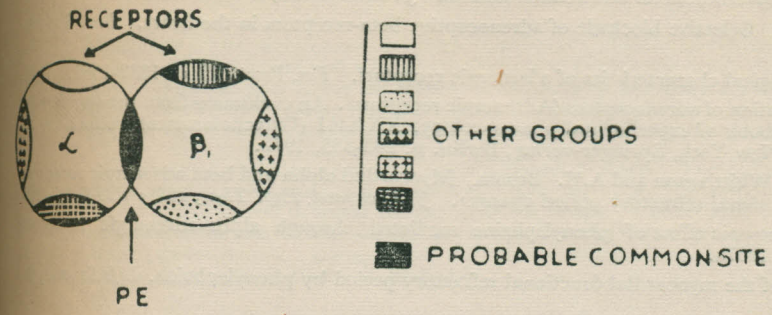

Fig. 3: A phenylephrine sensitive probable common group at receptors shown.

It has been hypothesized (5) that alphaceptors 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 reveal 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 betaceptors in pacemaker cells and in the myocardium. Although a preponderence of betaceptors in pacemaker cells is more likely as betacepotr antagonist was more effective in mitigating chronotropic response than alphaceptor antagonists, which inconsistently showed blockade. The suggestion that in lower concentration phenylephrine acts through alphaceptors and in higher concentration through betaceptors (24) is again not confirmed as both alpha and beta adrenoceptors antagonists were effective against a single fixed concentration of agonist used in our study.

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