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Exploration of Mechanism of Action of Ephedrine on Rat Blood Pressure.

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Research Article

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ABSTRACT

The aim of the study is to study mechanism of action (direct, indirect or mixed) of ephedrine on rat blood pressure by using agents affecting synthesis, storage and release of noradrenaline. 30 male Wistar albino rats divided into 6 groups (n=5). A: ephedrine control, B: tyramine control, C: reserpine + metyrosine + ephedrine, D: reserpine + metyrosine + tyramine, E: desipramine + ephedrine, F: desipramine + tyramine. In A and B: blood pressure responses of ephedrine and tyramine were taken for control, C and D: reserpine was injected 18 hrs. before and metyrosine was injected 2 hrs before taking responses of ephedrine or tyramine respectively. For E and F: desipramine was given 10 min. before taking response of ephedrine or tyramine respectively. Rat mean blood pressure was measured by using student's physiograph. Reserpine and metyrosine, inhibits the vesicular uptake and synthesis of noradrenaline respectively decrease the pressor responses of tyramine significantly (p=0.000) but not of ephedrine (p=0.893).Prior administration of desipramine which inhibits axonal uptake of noradrenaline also significantly decreases the effect of tyramine (p=0.000) but do not affect the effect of ephedrine significantly (p=0.893).Study concludes the pressor effect of ephedrine is not mediated by release of noradrenaline from neurons, indicating that ephedrine act directly on adrenergic receptors.

INTRODUCTION

Ephedrine is the principal alkaloid that is responsible for the physiological effects of herb ephedra. This herb is found in literature of India and China since ancient times because of its effectiveness as an antiashmatic. Chen and Schmidt in 1924 isolated primary alkaloid from ephedra plant. Since then ephedrine has been used for various conditions like bronchial asthma, nasal congestion, Stokes-Adams attacks with complete heart block, as a CNS stimulant in narcolepsy and depressive states, urinary incontinence, during spinal anesthesia, as psychostimulant, in losing weight etc. in all these conditions ephedrine has been replaced by better selective drugs. Currently, Ephedrine is recommended as the first-line vasopressor to treat hypotension associated with spinal anesthesia in obstetrics [1]. Report shows that ephedrine produces serious cardiovascular toxicities when used for therapeutic use in humans and can be used in the illicit manufacture of the powerful stimulant methamphetamine (crystal meth). So, it has been banned recently in many countries [2].

Ephedrine is classified as sympathomimetic drug. Since long it is considered that ephedrine acts by mixed action by releasing noradrenaline and by acting directly on receptors. Recently it has been reported that ephedrine has direct effects in isolated tissues; however the pressor response is mediated completely in indirect way in vivo in the rat [3]. Contrary to this in a recent study it has been shown that pressor responses of ephedrine were similar in dopamine β-hydroxylase knockout Dbh -/- and Dbh +/-

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mice and pretreatment of phentolamine and propranolol inhibits the increase in blood pressure and heart rate produced by ephedrine respectively. This finding indicates pressor responses to ephedrine are directly mediated by α and β adrenergic receptors [4].

Marked controversy exists whether ephedrine is directly, indirectly or mixed acting drug. So, this study was done to find out mechanism of pressor effect of ephedrine on rat blood pressure using drugs which affect the synthesis, storage and release of noradrenaline from the presynaptic nerve terminal in rats.

MATERIALS AND METHODS

Animals

Male *Wistar* Albino rats of 200-280 g were procured from the central animal house of the Institute. They were housed in polypropylene cages, and kept under controlled room temperature (24±2^{O}C) in 12-12 hour light-dark cycle. The animals were given a standard laboratory diet, and water *ad libitum.* Food was withdrawn 12 hour before the experiment. All experiments were performed after the prior permission of the Institutional Animal Ethics Committee, Government Medical College, Surat (Gujarat) constituted in accordance with guidelines of the Committee for the Purpose of Control and Supervision on Experiment on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Government of India.

Chemicals

Urethane and reserpine were purchased from Himedia laboratories, Mumbai, India. Tyramine and α-methyl-p-tyrosine (metyrosine) were purchased from Sigma-Aldrich, Mumbai, India.Ephedrine was purchased from Vikas Pharmaceutical, Mumbai India and desipramine was purchased from John Baker Inc. All the chemicals used were of analytical grade.

Measurement of Rat Blood Pressure

Male *wistar* albino rats were anesthetized with intraperitoneal injection of Urethane 150 mg/kg. When the corneal reflexes lost, inverted 'T' shaped incision was made in front of neck to expose the trachea, a nick was placed in trachea and polythene cannula of appropriate size was inserted in to trachea to ensure free airway. Right external jugular vein was carefully cannulated with polythene cannula attached to a 26-gauge needle. This cannula of vein was utilized for injecting drugs and saline after each injection. Left common carotid artery was carefully separated from adhering tissues, vagosympathetic nerve and a fine blood vessel found parallel to the common carotid artery. A bull-dog clamp was applied on the proximal end of common carotid artery. A polythene cannula attached to a 26-gauge needle was inserted into the common carotid artery and tied tightly to the artery with threads. The arterial cannula was inserted to measure the blood pressure. This arterial cannula connected via a three-way stopcock to the pressure bottle of Condon mercury manometer filled with 0.9% NaCl solution containing heparin 1000 units per ml and to the channel to the pressure transducer filled with 0.9% NaCl solution containing heparin 1000 units per ml. Pressure transducer is connected to student physiograph (INCO Biodevice Ambala,India) through strain gauge coupler. Student Physiograph was standardized each time and calibration done by giving positive pressure up to 120 mm Hg. Then, the bull-dog clamp was removed and baseline blood pressure was taken. After this the responses of the drugs were taken. Normal saline was injected after each intravenous injection of drug. Student physiograph was set at the sensitivity of 50 μV and the speed of the paper was 0.25 mm/sec. After completion of each experiment animals were sacrificed by intraperitoneal pentobarbital (200mg/kg).

Groups

30 male Wistar albino rats were randomly divided in to 6 groups (n=5).

Group A: (control of ephedrine) blood pressure responses of ephedrine (10 mg/kg, i.v.) were taken.
Group B: (control of tyramine), blood pressure response of tyramine (100 μg/kg, i.v.) were taken.
Group C and D: Reserpine (2.5 mg/kg, i.p.) was injected 18 hrs. before and metyrosine (200 mg/kg, i.p.) was injected 2 hrs. before taking responses of ephedrine (10 mg/kg, i.v.) and tyramine (100 μg/kg, i.v.).
Group E and F: All rats were injected desipramine (100μg/kg, i.v.) 10 minutes before taking the responses of ephedrine (10 mg/kg, i.v.) and tyramine (100 μg/kg, i.v.) respectively.

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Statistical analysis

Distribution of data is checked by skewedness, kurtosis, histogram, Q-Q plot, Kolmogorov-Smirnov test and Shapiro Wilk test. Data were found to be normally distributed. Descriptive statistic is shown as mean and standard deviation. Comparison between groups for mean blood pressure was done by one way ANOVA test followed by Tukey and Scheffe post hoc tests. Analysis was done by statistical package for social science (SPSS) version 17 (trial version).

RESULTS

The result obtained shows that ephedrine and tyramine produced rise in systemic arterial blood pressure of rat as seen in the control groups.(Table 1) The mean increase in BP by ephedrine was 39.26 mmHg and by tyramine was 26.42 mmHg.

Comparison of mean blood pressure responses of ephedrine control group, reserpine + metyrosine + ephedrine group and desipramine + ephedrine group:

Result shows that there is no significant difference in rise in mean blood pressure produced by ephedrine control group, reserpine + metyrosine + ephedrine group, and desipramine + ephedrine group. (One way ANOVA P value = 0.893) (Figure 1,Table 2)

Comparison of mean blood pressure responses of tyramine control group, reserpine + metyrosine + tyramine group and desipramine + tyramine group:

Result shows that there is significant difference in rise in mean blood pressure produced by tyramine control group, reserpine + metyrosine + tyramine group, and desipramine + tyramine group. (One way ANOVA P value = 0.000). There was significant decrease in rise of mean blood pressure in reserpine + metyrosine + tyramine group in comparison to tyramine control group. (Tukey post hoc test P value =0.000, and Scheffe's post hoc test *P value* = 0.000). (Figure 2, Table 2) Similarly, there was significant decrease in rise of mean blood pressure in desipramine + tyramine group in comparison to tyramine control group. (Tukey post hoc test *P value* =0.000, and Scheffe's post hoc test *P value* = 0.000).

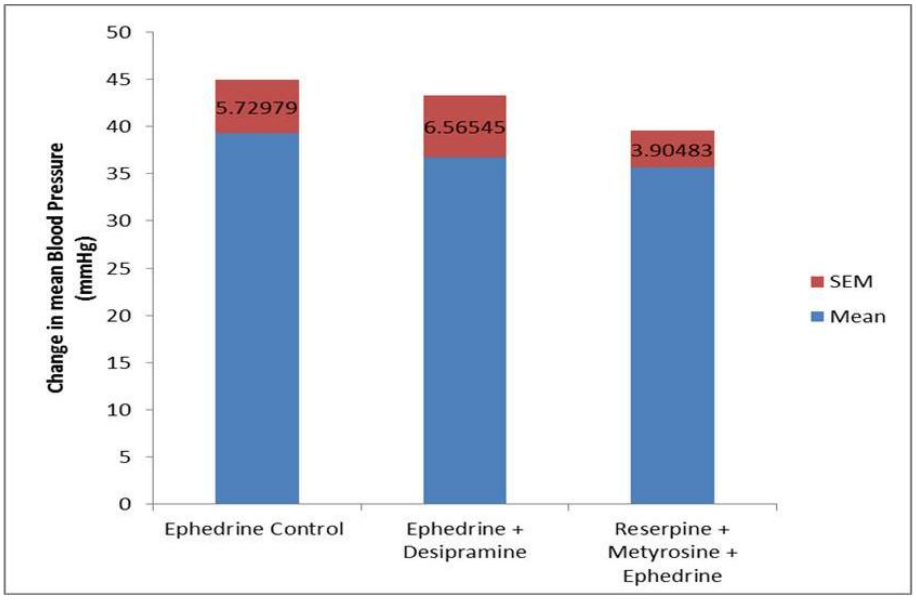

Figure 1: Comparison of the mean blood pressure responses of ephedrine (10 mg/kg i.v.) of the control group with the pressor response of ephedrine (10 mg/ kg i,v.) after prior injection of desipramine (100 µg/kg i.v.) and pressor response of ephedrine (10 mg/ kg i,v.) after the prior injections of reserpine (2.5 mg/kg i.p.) and metyrosine (200 mg/kg i.p.). *n*indicates number of experiments, and the asterisk indicates that the response is significantly different than control. (n = 5). (By one way ANOVA test, Degree of freedom = 2, F value = 0.114 and P value = 0.893).Data are expressed in Mean±SEM.

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Table 1: Baseline blood pressure and rise in blood pressure in each group. (n=6)

Group	Rat No.	Baseline blood pressure (mm hg)	Rise in blood pressure (mm hg)
Group A: Ephedrine Control	1	80	39.38
	2	92.5	55.87
	3	85.32	21.28
	4	112	34.58
	5	120	45.22
Group B: Tyramine Control	1	120	35.75
	2	72	26.66
	3	93.3	26.66
	4	90	20.98
	5	100	22.06
Group C: Reserpine + metyrosine + ephedrine	1	91	44.13
	2	100	40.67
	3	85.32	21.33
	4	105.8	34.9
	5	91.4	37.14
Group D: Reserpine + metyrosine + tyramine	1	62.66	2.66
	2	60	4.36
	3	90	5.42
	4	108.25	1.37
	5	119.25	11.03
Group E: Desipramine + ephedrine	1	114.4	39.38
	2	80	27.11
	3	100	61.33
	4	110.64	29.33
	5	90.64	26.6
Group F: Desipramine + tyramine	1	80	2.71
	2	74	14.03
	3	88.1	5.4
	4	76.5	5.51
	5	71.2	2.8

Table 2: Value of mean, standard deviation and standard error of each group.

Groups	Number (n)	Mean	Standard deviation	Standard Error
Group A: Ephedrine control group	5	39.2660	12.81221	5.72979
Group B: Tyramine control group	5	26.4220	5.82596	2.60545
Group C: Reserpine + Metyrosine + Ephedrine group	5	35.6340	8.73147	3.90483
Group D: Reserpine + Metyrosine + Tyramine group	5	4.9680	3.72808	1.66725
Group E: Desipramine + Ephedrine group	5	36.7500	14.68079	6.56545
Group F: Desipramine + Tyramine group	5	6.0900	4.63963	2.07490

Data expressed as Mean±SEM

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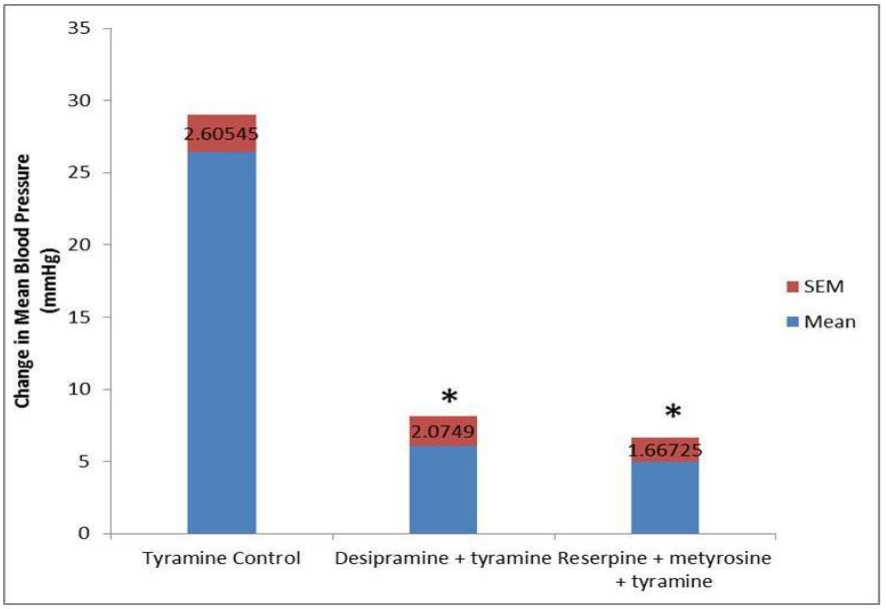

Figure 2: Comparison of the pressor responses of tyramine (100 µg/kg i.v.) of the control group with the pressor response of tyramine (100 µg/ kg i,v.) after prior injection of desipramine (100 µg/kg i.v.) and pressor response of tyramine (100 µg/ kg i,v.) after the prior injections of reserpine (2.5 mg/kg i.p.) and metyrosine (200mg mg/kg i.p.). *n*indicates number of experiments, and the asterisk indicates that the response is significantly different than control. (n = 5, * P <0.05). (By ANOVA test Degree of freedom = 2, F value = 31.533 and P value = 0.000). There is significant decrease in rise of mean blood pressure in reserpine + metyrosine + tyramine group in comparison to tyramine control group. (Tukey post hoc test P value =0.000, and Scheffe's post hoc test P value = 0.000). Similarly, there is significant decrease in rise of mean blood pressure in desipramine + tyramine group in comparison to tyramine control group. (Tukey post hoc test P value =0.000, and Scheffe's post hoc test P value = 0.000). Data are expressed in Mean±SEM.

DISCUSSION

In this study it was observed that, prior injection of reserpine and metyrosine, decreases the pressor responses of tyramine significantly; however the pressor responses of ephedrine were not significantly affected by prior treatment of these drugs. Similarly desipramine also significantly decreases the rise in blood pressure produced by tyramine but do not affect the rise in blood pressure produced by ephedrine significantly.These data provide support for the hypothesis that in rats increases in systemic arterial pressure in response to ephedrine are mediated by direct activation of α and β-receptors and that these responses are not dependent on the presence of endogenous noradrenaline stores.

Indirect action of ephedrine has been shown in cat's denervated pupil, dog heart-lung tissue preparation, cat's pupillary muscle tissue preparation, cat's nictinic membrane, dog's forelimb, and dog's coronary artery[5-10].Some authors suggested that the effect of ephedrine or tyramine is mediated exclusively by norepinephrine release[11,12].

In contrast, direct action of ephedrine has been proved by in vitro models of guinea pig tracheal ring, HEK (human embryonic kidney), Chinese hamster ovary cells, and guinea pig portal vein, where functional activity of ephedrine alkaloids at alA, a2A, and a2C adrenergic receptors has been established by using cell-based receptor gene assavs.P"[13-15].

Recently conducted study by Liles & Baber et al. also provides strong evidence for direct mechanism of action of ephedrine[16]. In this study pressor responses of ephedrine were similar in dopamine β-hydroxylase knockout Dbh -/- and Dbh +/- mice and pretreatment of phentolamine and propranolol inhibits the increase in blood pressure and heart rate produced by ephedrine respectively. This finding indicates pressor responses to ephedrine are directly mediated by α and β adrenergic receptors [16]. The recent study by Liles et al. showed that pressor responses to ephedrine are not diminished in rats that have markedly attenuated responses to tyramine after treatment with catecholamine depleting agents [16].

Our aim was to block the release of noradrenaline from presynaptic nerve terminal to see whether these drugs which inhibit noradrenaline release from presynaptic nerve terminal affect the pressor response of ephedrine and tyramine. So, we used reserpine to block the vesicular uptake of noradrenaline

and to deplete the vesicular pool of noradrenaline and to inhibit the cytoplasmic synthesis of the noradrenaline we used metyrosine. Several reports favour the fact that after reserpine treatment the catecholamine stores are not completely depleted. So, many studies have utilized combination of reserpine and metyrosine for completely depleting presynaptic catecholamine pool [17]. Reserpine depletes vesicular stores, and metyrosine depletes the cytoplasmic pool of noradrenaline. So, pretreatment with reserpine and metyrosine combination decreases the response of indirectly acting or mixed acting drugs by depleting adrenergic noradrenaline pool, but do not affect the responses of the directly acting drugs. Present study shows thatreserpine and metyrosine do not affect the pressor response of ephedrine in rat indicating that the ephedrine act directly on the adrenergic receptors.

Our study also shows that pressor responses of tyramine are significantly inhibited by noradrenaline depletion by reserpine and metyrosine pretreatment.This finding is in concordance with many studies showing indirect mechanism of action of tyramine.

The hypothesis that responses to ephedrine are not mediated by an indirect mechanism is supported by experiments with desipramine (inhibits axonal uptake of noradrenaline), showing that ephedrine induced pressor responses were not reduced significantly by desipramine pre-treatment. In contrast, the increase in systemic arterial pressure in response to tyramine was abolished significantly by pre-treatment with desipramine.

Similar study was done by Kobayashi et al. by using 6-hydroxydopamine which destroy adrenergic nerve terminal[3]. They showed that ephedrine has direct effects in isolated tissues and 6-hydroxydopamine reduces the pressor response of ephedrine, indicating that pressor response of ephedrine is completely indirectly mediated in vivo in the rat[3]. However, 6-hydroxydopamine causes non-specific depression of vascular responses and it could not be sufficient to prove indirect mechanism of action of ephedrine.

The inconsistency in the results of previous in vivo studies showing indirect mechanism of action of ephedrine and present study showing direct mechanism of action of ephedrine, can be explained by differences in the regimens and doses used. We have used the combination of reserpine and metyrosine for complete depletion of the axonal catecholamine stores as mentioned in discussion and we also confirmed our result by blocking axonal uptake by desipramine. The dose of ephedrine used in the present study is based on previous animal studies and dose used in ephedrine-dependent individuals [16].

Limitations

- Dose response curve could not be plotted because of the phenomenon of tachyphylaxis with ephedrine and tyramine. Therefore, we could not calculate area under curve and area under curve of all groups could not be compared.
- Here, in present study we explored the mechanism of action of ephedrine on rat blood pressure only. It can be possible that ephedrine can act directly at some sites and can act indirectly at some sites. So, further exploration of mechanism of action ephedrine on different systems is required to know the exact mechanism of action of ephedrine.
- Sample size was small, because we used minimum animals for our experiment as per the CPCSEA guideline and there are no fixed criteria for selecting sample size for such kind of experiments. So, we have taken the same sample size as previous similar study.

CONCLUSION

The pressure effect of ephedrine in ratmay be because of direct action of ephedrine on adrenergic receptors and not mediated by release of noradrenaline from presynaptic nerve terminal.Further studies with use of alpha and beta blockers are needed to confirm these findings.

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