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Postjunctional Origin of the Indirect-Like Sympathomimetic Effect of Metanephrine and Normetanephrine on Blood Pressure

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The effects of metanephrine and normetanephrine have been compared with those from equiactive doses of the origin compounds, adrenaline and noradrenaline, on the pressor responses in rat, in order to determine whether their effects are owed, at least partially, to a releasing presynaptic action of the catecholamines in normal animals as well as those pretreated with reserpine, guanethidine and 6-OH-dopamine. Their effects have likewise been studied in isolated perfused renal arteries both in normal and reserpinized rats.

None of the adrenolytic agent used were able either to reduce the duration of the hypertensive response or to accelerate tachyphylaxis. Identical results were obtained in renal artery preparations.

It is thus concluded that the catecholamines stored in presynaptic endings are not involved in the observed phenomena and it is suggested that they might depend on the high doses required to produce effects equiactive to those of the origin substances.

Key words: Metanephrine, Normetanephrine, Indirect-like Sympathomimetic effect, Blood pressure, Mechanism of action.

Although the effect of the methoxylated metabolites of catecholamines (CA), metanephrine (M) and normetanephrine (MN), on blood pressure has been repeatedly studied (1-13), certain details still merit further attention. Thus, the mechanism of their relatively long lasting hypertensive effect (7) as well as that of tachyphylaxis induced by them (8) have not been clarified. In general, substances exhibiting features like these act, at least in part, by releasing noradrenaline (NA) stored in adrenergic nerve endings (the so-called «indirect sympathomimetic agents»).

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The only literature data regarding this subject dealt with marginal observation in reserpinized preparations (10, 14, 17) from which a conclusion could not be drawn. From a theoretical point of view, agents possessing either indirect or mixed action must be taken up by the nerve endings. This requirement seems not to be fulfilled by the methoxy metabolites of CA (15), which might be a reason for the lack of investigations on this topic. In spite of that, the possible involvement of prejunctionally stored CA in the development of the above mentioned phenomena has been deemed worth. To this end, a systematic study of the pressor effect of M and NM was perfomed in rats pre-treated with adrenergic neuron blocking agents as well as in isolated perfused arterial preparations from normal and reserpinized animals, all the results being compared with those produced by equipressor doses of the origin compounds under the same circumstances.

Materials and Methods

Blood pressure. Male Sprague-Dawley rats, weighing 300-350 g, were used. The animals were divided into groups of six. All animals were anesthetized (sodium pentobarbital, 50 mg/kg, by intraperitoneal route), subsequently tracheotomized, and their blood pressure recorded through a carotid cannula connected to a P23Db Statham pressure transducer. The heart rate was monitored by means of a Gould Brush Bio-Tach computer from the blood pressure signal. The body temperature was maintained at a constant level (37 ± 0.1° C) by radiant heat, the temperature being controlled by means of a Panlab electronic rectal thermometer. All compounds were administered by intravenous route, the volume to be injected never exceeding 0.2 ml. Once the animals were prepared, a period of 30 min was allowed for blood pressure stabilization before starting administration of compounds.

In those experiments including reserpinized animals, a single dose of reserpine (Serpasol, Ciba), 1 mg/kg by intramuscular route, was given 18 hr before hand. To block catecholamine release, guanethidine (Guanethidine sulfate, Ciba) was administered at a dose of 5 mg/kg by intravenous route, 45 min before the experiments. One group of animals received 6-OH-dopamine (6-hydroxy-dopamine hydrobromide, Sigma) to destroy sympathetic nerve endings. A solution of this drug was prepared in 1 mM HCl under N_2 bubbling and injected into a caudal vein according to the following schedule: eight days before the experiments, the animals received for two days a daily dose of 50 mg/kg of the drug; four days later that dose was increased to 100 mg/kg for another two days.

The following treatment groups were formed: I. Metanephrine (dl-metanephrine hydrochloride, Sigma) was administered by i.v. route in series of five repeated doses of 400 μg/kg in such a way that each dose was given as soon as the effect of the previous one disappeared. II. Metanephrine, 800 μg/kg, was administered in a way similar to that mentioned in I. III. Normetanephrine (dl-normetanephrine hydrochloride, Sigma) was administered at a dose of 400 μg/kg in the way indicated in I. IV. Normetanephrine, 800 μg/kg, in the way indicated in I. V. Adrenaline equiactive to metanephrine 400 μg/kg. The dose of adrenaline (l-epinephrine bitartrate, Sigma) equiactive to metanephrine was individually determined for eachanimal and administered in the way indicated in I. VI. Adrenaline equiactive to metanephrine 800 μg/kg. As mentioned in V. VII. Noradrenaline equiactive to

normetanephrine 400 μg/kg. The dose of noradrenaline (l-norepinephrine bitartrate, Sigma) was determined and administered as mentioned in V. VIII. Noradrenaline equiactive to normetanephrine 800 μg/kg. As mentioned in V.

Identical treatment groups were formed with the animals pretreated with reserpine, guanethidine and 6-OH-dopamine.

The reason for using 400 and 800 μg/kg of metabolites was that, these doses give rise to hypertensive responses of intermediate intensity, as was demonstrated in a previous work (7).

The doses of CA equiactive to those of the metabolites ranged from 1-2 μg/kg, approximately, for 400 μg/kg of metabolites, and from 2 to 4 μg/kg for 800 μg/kg.

Mean blood pressure, heart rate and duration of the hypertensive responses were studied in all animals.

Isolated perfused rat renal artery. Renal arteries were prepared according to the procedure described by HRDINA *et al.* (16). Male Sprague-Dawley rats, weighing 300-400 g were used. Two groups of animals (six animals each) were employed. The first consisted of normal animals. The second one consisted of animals pretreated with reserpine (1 mg/kg, by i.m. route) 18 h before the experiments. All animals were killed by a blow on the head, and after opening the abdominal cavity, the adrenal artery and one of the two end branches of the renal artery were ligated and distally sectioned. The renal artery was then removed together with a small aortic segment (5 mm large) in which the renal artery emerges. The preparation was placed in Krebs solution and cannulated through the aortic segment, the end orifice of the renal artery left free. Once prepared, the artery was transferred to a 5 ml organ bath and perfused with Krebs solution by means of a peristaltic Desaga pump at a rate of 7.5 ml/min. Krebs solution inside and outside the artery was continously bubbled with 5 % CO_2 and 95 % O_2, the temperature being maintained at 37° C. A Statham P23Db pressure/volume transducer was placed between the artery and the pump, the perfusion pressure being monitored through a Gould Brush dynograph. Five doses of both metanephrine and normetanephrine 10^{-5} M were used. Likewise, five doses of adrenaline equiactive to metanephrine were employed. All compounds were perfused in a volume of 5 ml at the rate before mentioned. The effect of M was also tested in arteries from reserpinized animals. In this case the concentration of the metabolites was reduced to 10^{-4} M due to the expected hypersensitivity in these preparations.

Statistical calculations. Student's t test was used for comparison between means. In the case of the experiments performed in the isolated renal artery, the Kruskal Wallis test for one quantitative variable was employed.

Results

The effects of the metabolites, and those of their origin compounds, on blood pressure are drawn in figure 1. The metabolites, at the two doses used, induced clear hypertensive responses, the peak effect being steadly lowered by repeated administration. The origin compounds, as expected, gave rise to consistent hypertensive responses, no changes being observed by subsequent administration of repeated doses. The effects of the metabolites in the animals pre-treated with adrenolytic agents are presented in tables I and II. In the reserpinized animals, the effects of the metabolites were quite similar to those

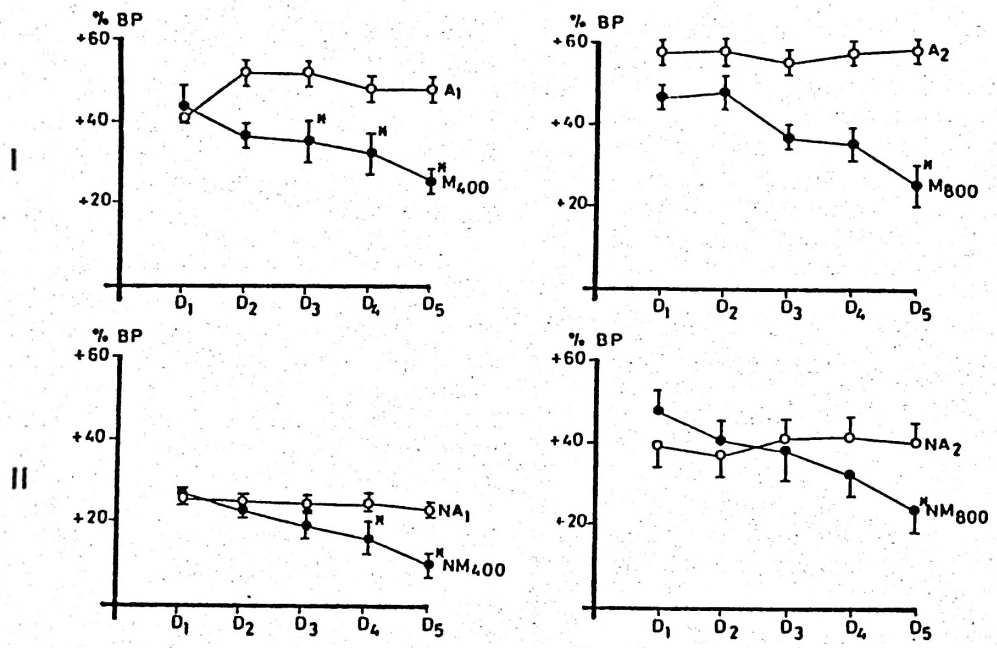

Fig. 1. *Effect of repeated administration of metanephrine and equiactive doses of adrenaline (I) and of normetanephrine and equiactive doses of noradrenaline (II).*

M_{400} and M_{800} = metanephrine, 400 and 800 μg/kg, respectively. A_1 and A_2 = adrenaline equiactive to M_{400} and to M_{800}, respectively. NM_{400} and NM_{800} = normetanephrine, 400 and 800 μg/kg, respectively. NA_1 and NA_2 = noradrenaline equiactive to NM_{400} and to NM_{800}, respectively. D_1-D_5 in abscissa indicate successive drug administration. Ordinate: percent of increase of mean blood pressure as compared with control. Base-line value of blood pressure was 118.5 ± 10.6 mmHg (x̄ ± SD). * $p < 0.05$, as compared with the response to D_1.

Table I. *Effects of metanephrine, and equiactive doses of adrenaline, on blood pressure of rats pretreated with reserpine, guanethidine and 6-OH-dopamine.*

Values indicate percent of increase as compared with base-line pressure (x̄ ± SE). Base-line values were (x̄ ± SD): 80.4 ± 10.6 mmHg, for reserpinized animals; 90.3 ± 10.1 mmHg, for animals pretreated with guanethidine; 86.9 ± 6.4 mmHg, for animals pretreated with 6-OH-DA. Remaining lettering and symbols as in figure 1.

	Reserpine	Guanethidine	6-OH-DA		Reserpine	Guanethidine	6-OH-DA
M_{400}	65.7 ± 4.2	52.4 ± 12.3	61.0 ± 4.9	A_1	77.2 ± 4.6	47.9 ± 2.9	64.5 ± 3.1
	50.8 ± 4.5*	52.2 ± 10.6	57.4 ± 4.4		73.9 ± 6.7	47.8 ± 5.6	67.2 ± 7.7
	43.4 ± 5.2*	48.1 ± 9.9	47.5 ± 2.6*		66.4 ± 6.4	40.5 ± 5.1	66.1 ± 8.5
	39.5 ± 4.8*	45.6 ± 11.1	48.8 ± 3.2		62.1 ± 6.5	39.1 ± 5.1	65.5 ± 6.3
	34.8 ± 4.2*	47.6 ± 11.1	45.4 ± 3.2*		63.1 ± 7.1	39.2 ± 5.3	68.4 ± 7.6
M_{800}	72.5 ± 6.4	71.9 ± 13.1	72.7 ± 4.9	A_2	86.4 ± 3.6	63.6 ± 5.6	76.4 ± 2.2
	66.9 ± 6.4	70.4 ± 10.5	67.2 ± 5.3		85.8 ± 4.8	78.3 ± 2.9	76.5 ± 2.5
	62.6 ± 5.9*	67.8 ± 10.6	58.9 ± 4.2*		86.6 ± 6.1	83.2 ± 8.8	79.1 ± 3.9
	57.0 ± 7.4*	64.9 ± 10.8	61.1 ± 7.2*		83.4 ± 7.3	83.9 ± 13.1	84.3 ± 10.1
	54.5 ± 7.6*	64.4 ± 2.3	56.8 ± 4.9*		85.5 ± 9.9	83.7 ± 10.3	85.2 ± 10.8

seen in normal animals, a certain degree of hypersensitivity being the only difference observed. Tachyphylaxis also occurred after repeated administration of the metabolites whereas the origin compounds did not induce such phenomenon. In the animals pretreated with guanethidine, the drug did not change the hypertensive responses to the metabolites but practically abolished tachyphylaxis. No changes were observed in the responses to adrenaline

Table II. *Effects of normetanephrine, and equiactive doses of noradrenaline, on blood pressure of rats pretreated with reserpine, guanethidine and 6-OH-dopamine.*

Values indicate percent of increase as compared with base-line pressure ($\bar{x} \pm SE$). Base-line values were the same indicated in table I. Remaining lettering and symbols as in figure 1.

	Reserpine	Guanethidine	6-OH-DA		Reserpine	Guanethidine	6-OH-DA
NM_{400}	88.2± 5.4	76.5±10.5	66.9±4.1	NA_1	76.9±6.3	78.1± 4.9	59.9±2.9
	80.4±74.4	74.4± 8.2	46.5±5.7*		71.1±4.2	73.4± 2.8	56.2±2.8
	74.8± 4.2	64.8±15.2	47.6±5.9*		71.8±4.0	71.1±11.5	54.3±3.7
	69.4± 4.7*	68.9±13.1	41.2±3.2*		76.4±4.3	68.5± 4.8	56.5±3.3
	64.4± 5.6*	64.0±15.3	35.3±7.8*		75.4±3.9	68.6± 5.3	52.1±6.2
NM_{800}	102.5± 5.8	83.2±16.1	66.4±4.7	NA_2	83.8±4.1	77.5± 2.7	61.3±2.5
	91.1± 3.1	91.3±10.9	53.2±4.0		77.2±5.2	76.2± 3.4	55.5±2.6
	89.0± 5.5	83.9±16.0	55.5±6.7		79.9±5.9	76.2± 3.7	53.4±3.5
	89.1± 6.5	79.2±12.5	52.2±3.8*		82.0±6.1	75.9± 8.9	53.3±3.8
	90.8± 7.2*	76.2±2.6	51.3±3.6*		78 9±8.1	71.5± 3.4	52.9±4.1

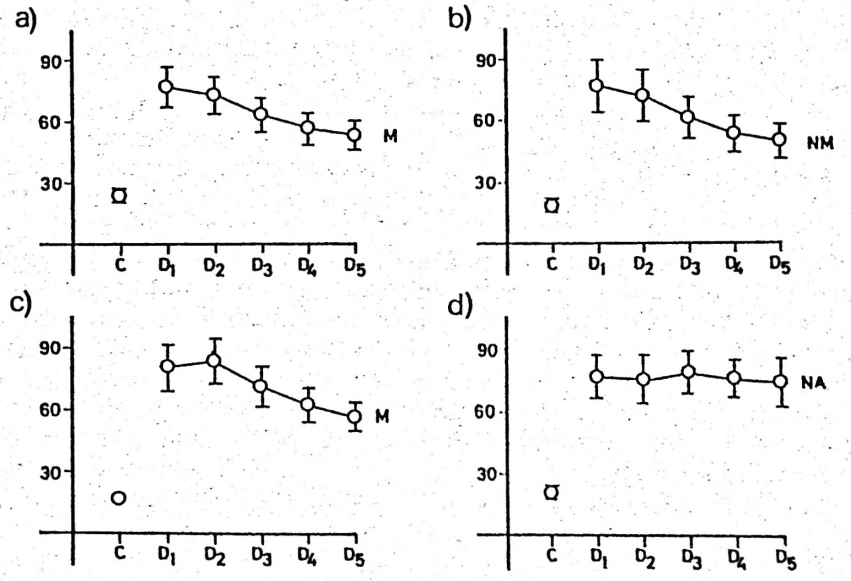

Fig. 2. *Effect of methoxy metabolites and noradrenaline on isolated perfused rat renal arteries.* Ordinate: flow resistance expressed as KPa.s.ml^{-1}. In abscissa, C: Control and D_1-D_5 = repeated drug administration. a) Metanephrine, 10^{-5} M. b) Normetanephrine, 10^{-5} M. c) Metanephrine, 10^{-4} M —arteries provening from reserpinized animals—. d) Noradrenaline, 3×10^{-7} M (equiactive to normetanephrine 10^{-5} M). A statistically significant difference was found between the responses to D_1 and to D_5 in a, b and c.

Table III. *Duration of the pressor responses induced by adrenaline, noradrenaline, metanephrine and normetanephrine.*

Value indicate seconds ($\bar{x} \pm SE$). Symbols and lettering as in figure 1. Duration of the responses induced by metanephrine and normetanephrine at the dose of 800 mcg/kg and by equiactive doses of adrenaline and noradrenaline was approximately 50 % higher than those presented in this table. It has been omitted to simplify presentation of results.

	A_1	M_{400}	NA_1	NM_{400}
Control	104.2 ± 17.2	245.6 ± 22.1*	136.2 ± 9.2	168.0 ± 10.3*
Reserpine	218.2 ± 18.6	268.2 ± 27.2*	195.3 ± 11.1	536.1 ± 15.6*
Guanethidine	148.0 ± 12.4	222.1 ± 41.0*	182.3 ± 32.4	322.6 ± 10.6*
6-OH-dopamine	154.2 ± 17.2	224.1 ± 7.4*	207.0 ± 26.2	304.2 ± 62.0*

and noradrenaline. In the animals pretreated with 6-OH-dopamine, the metabolites continued to produce pressor responses, which were steadily lowered by repeated administration. Once more the origin compounds exhibited normal hypertensive responses.

As to the duration of the pressor responses, table III shows clearly how the metabolites induced longer effects than those of the origin compounds.

Heart rate data were irrelevant. The metabolites caused a slight bradycardia without statistical significance, whereas the origin compounds gave rise to the normally expected tachycardia. Data are omitted to simplify presentation of results.

On perfused renal arteries, the metabolites gave rise to an increase in the flow resistance (fig 2), a steady decrease in the peak effect being observed by repeated administration. This phenomenon was not seen in the case of adrenaline. On the other hand, reserpine did not change the repeatedly mentioned metabolite effects.

Discussion

As compared with CA, their methoxylated metabolites produced slightly more durable hypertensive responses, which were, moreover, subject to tachyphylaxis, confirming other previously reported results (7, 8). On the other hand, the adrenergic neuron blocking agents neither decreased the duration of such responses nor accelerated the development of tachyphylaxis, two facts which permit us to rule out any action of the metabolites upon CA stored in the nerve endings. With guanethidine pretreatment tachyphylaxis was practically abolished, no satisfactory explanation being found for this action, which, nevertheless, does not invalidate the above mentioned statement.

In the perfused renal arteries, including those from reserpinized animals, the metabolites continued to induce tachyphylaxis, this phenomenon being not observed with the origin compounds. These data represent a further support for ruling out an action of the metabolites on presynaptic sites. However, the possibility of an action on presynaptic beta-receptors, which facilitate CA release (18), may be also considered, in so far as the metabolites would produce CA release without being taken up by the nerve endings. In this respect, a few marginal experiments were performed in three renal arteries in which the effect of the metabolites was tested in the presence of l-propranolol 10^{-6} M. It was observed that the drug did not modify tachyphylaxis.

The slight bradycardia noticed after metabolites administration may be

attributed to a reflex mechanism. Indeed, the rate of blood pressure increase induced by these compounds was slower than that observed with their origin compounds. Under such circumstances the bradycardic response by baroceptor activation might overwhelm the slight cardiac stimulation caused by the metabolites.

In view of all data the conclusion would be drawn that the features of the action of metabolites on blood pressure belong to compounds acting on postjunctional sites. Although the study of the mechanism by which these compounds altered these sites to induce longer lasting action and tachyphylaxis was not the aim of the present investigation, it would be proposed that, as the doses of metabolites required to produce effects similar to those of CA were several hundred times higher, a prolonged receptor stimulation (lenghthening in the response), and a receptor desensitization (tachyphylaxis) could be expected.

Resumen

Se estudian los efectos de la metanefrina y normetanefrina, comparándolos con los de dosis equiactivas de las sustancias de origen, adrenalina y noradrenalina, sobre la respuesta presora en rata, al objeto de determinar si sus efectos se deben, al menos en parte, a una acción presináptica liberadora de catecolaminas, tanto en animales normales como tratados con reserpina, guanetidina y 6-OH-dopamina. También se estudian sus efectos en arterias renales aisladas y perfundidas de ratas normales y tratadas con reserpina.

Ninguno de los agentes adrenolíticos utilizados reduce la duración de la respuesta hipertensiva de los metabolitos, ni acelera el desarrollo de taquifilaxia. Idénticos resultados se observan en las preparaciones de arteria renal.

Se concluye que las catecolaminas almacenadas en la terminación presináptica no participan de los fenómenos observados y se sugiere que las mismas pueden depender de las elevadas dosis que se precisan para conseguir efectos equiactivos con las sustancias de origen.

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