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The effects of changes in ionic environment and modification of adrenergic function on the vascular responses to sympathomimetic amines

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The responses to each of four sympathomimetic amines: noradrenaline 200 ng, octopamine 50 μg, metaraminol 20 μg and tyramine 100 μg were studied in the perfused rat mesentery preparation. Perfusion with Ca^{2+}- and Mg^{2+}-free solutions potentiated the responses to all four amines compared with control responses obtained during normal Krebs perfusion. Under perfusion conditions using either normal or Ca^{2+}- and Mg^{2+}-free Krebs solution, nialamide and reserpine retained their characteristic effects on the responses to each amine. Cocaine and desipramine abolished the responses to tyramine but potentiated those to noradrenaline and metaraminol under all perfusion conditions. The responses to each of the amines were only antagonized by ouabain when Ca^{2+} ions were present in the perfusion solution. It is concluded that perfusion with Ca^{2+}- and Mg^{2+}-free solution interferes with the normal uptake mechanisms occurring in the adrenergic neuron.

The role of Ca^{2+} and Mg^{2+} in the physiology and biochemistry of the sympathetic neuron is complex. Rubin (1970) has shown that the presence of extracellular Ca^{2+} is essential for the release of neurotransmitter substances and hormones. However, Thoenen, Huerlimann & Haefely (1969) and Keen & Bogdanski (1970) have described an increase in spontaneous catecholamine release from sympathetically innervated tissues in the absence of extracellular Ca^{2+}. The demonstration that extracellular Ca^{2+} is essential for the uptake of noradrenaline into the sympathetic neuron (Titus & Dengler, 1966; Dostal & Crout, 1967) is at variance with the reported absence of effect on noradrenaline uptake resulting from variation in the extracellular Ca^{2+} concentration (Horst, Kopin & Ramey, 1968). Bohr (1964) has demonstrated that smooth muscle is still able to respond to catecholamines in the absence of extracellular Ca^{2+} and Mg^{2+}. Rubin & Jaanus (1966) have shown that the presence of extracellular Ca^{2+} is essential for the action of indirectly acting sympathomimetic amines in releasing adrenaline from the adrenal medulla.

It was decided, therefore, to compare the responses of rat mesenteric arterial vessels to four sympathomimetic amines: tyramine, octopamine, noradrenaline and metaraminol, during perfusion with either normal Krebs solution or with solutions in which the concentration of either Ca^{2+} or Mg^{2+} or both ions had been altered. Any change in the nature of the response to sympathomimetic amines during perfusion with solutions of modified ionic composition could then be evaluated and the effects of Ca^{2+} and Mg^{2+} on the action of different sympathomimetic amines could thus be demonstrated.

METHODS

Male, albino Sprague-Dawley rats 300–350 g, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and given heparin 1000 units (i.p.).

The mesenteric arterial vessels were prepared for perfusion with either normal or modified Krebs solution according to McGregor (1965) and George & Leach (1971). Depolarizing Krebs solution containing K^+ at a concentration of 115 mM was prepared as described by Evans, Schild & Thesleff (1958).

Pretreatments

One group of rats was pretreated 10 h before experimentation with reserpine (10 mg kg^{-1}, i.p.) prepared in a lyophilized solution. One group of rats was given nialamide (5 mg kg^{-1}, i.p.) dissolved in warm saline and another group disulfiram (50 mg kg^{-1} i.p.) 5 h before the experiment. For comparison, three groups of animals were injected with volumes of saline corresponding to those used in injecting the three pretreatment drugs and used as controls.

The effects of noradrenaline 200 ng, octopamine 50 μg, metaraminol 20 μg, and tyramine 100 μg on the resting perfusion pressure of the rat mesenteric artery preparation were studied as described by George & Leach (1971). In the experiments in which cocaine, desipramine or ouabain were added to the actual perfusion solutions no change was observed in the mean resting perfusion pressure of the mesentery preparation.

Drugs

The drugs used were: (−)-noradrenaline hydrochloride (Sigma Laboratories); (−)-metaraminol bitartrate (Merck, Sharpe & Dohme Ltd.); (±)-octopamine hydrochloride (Sigma Laboratories); tyramine (Koch-Light); reserpine (lyophilized) (Halewood Chemicals Ltd.); disulfiram (Dumex Ltd.); nialamide (Pfizer); ouabain (strophanthin G) (BDH); desipramine (Geigy); cocaine hydrochloride (BDH).

RESULTS

No evidence of tachyphylaxis in the responsiveness of the preparation to tyramine (100 μg) was observed with repeated administration of six separate doses of tyramine (100 μg) injected at 15 min intervals (George & Leach, 1971); likewise there were no modifications in the response to repeated administration of the other three amines tested: noradrenaline 200 ng, octopamine 50 μg, or metaraminol 20 μg, when injected at 5 min intervals (George & Leach, 1971).

A marked potentiation was observed, however, in the responsiveness of the rat mesenteric arterial vessels to each of the four amines after changing to perfusion with a solution in which the Ca^{2+} and Mg^{2+} concentration had been modified (Figs 1 and 2).

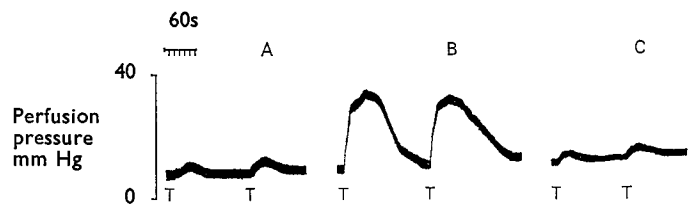

FIG. 1. The effect of perfusion with Ca^{2+}- and Mg^{2+}-free Krebs on the mesenteric responses to tyramine (T) 100 μg. (A) initial perfusion with normal Krebs; (B) perfusion with Ca^{2+}- and Mg^{2+}-free Krebs; (C) restoration of perfusion with normal Krebs. Time = 60s; T indicates addition of tyramine.

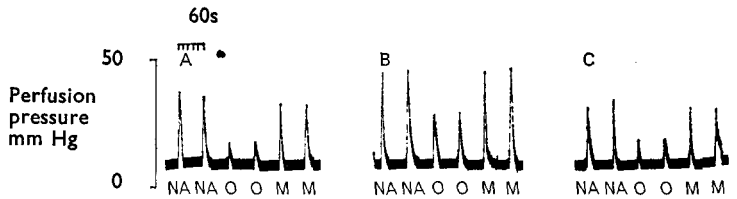

FIG. 2. Effect of alteration of ionic composition on sympathomimetic responses. Responses to each of three sympathomimetic amines: noradrenaline (NA) 200 ng, octopamine (O) 50 μg, and metaraminol (M) 20 μg. (A) initial perfusion with normal Krebs, (B) perfusion with Ca^{2+}- and Mg^{2+}-free Krebs, (C) restoration to perfusion with normal Krebs. Time = 60s.

This potentiation was rapidly reversible upon recommencement of normal Krebs perfusion, and the potentiation was seen to be maximal when both Ca^{2+} and Mg^{2+} were omitted from the perfusing medium. Perfusion with such modified solutions had no effect on the mean resting perfusion pressure of the mesentery.

Action of reserpine

After reserpine pretreatment (10 mg kg^{-1}, i.p.) the response to tyramine of the mesentery preparation was abolished during perfusion with either normal or Ca^{2+}- and Mg^{2+}-free Krebs. When compared with their appropriate untreated controls, the responses to noradrenaline (200 ng) were not significantly altered whilst the responses to metaraminol (20 μg) and octopamine (50 μg) were decreased by 20 and 60% respectively during normal Krebs perfusion and 22 and 70% respectively during Ca^{2+}- and Mg^{2+}-free perfusion (Table 1).

Action of nialamide

During perfusion with normal Krebs solution, the responses of the nialamide-pretreated (5 mg kg^{-1}) rat mesentery preparation to tyramine (100 μg) were potentiated by a factor of 65%. Responses to octopamine (50 μg) were similarly potentiated by 50%, but the response to noradrenaline and metaraminol were not significantly altered (Table 1). During perfusion with Ca^{2+}- and Mg^{2+}-free Krebs solution, the responses to tyramine (100 μg) in the nialamide pretreated preparation were seen to be potentiated by a further factor of 40% above the usual response increase obtained after transference to Ca^{2+}- and Mg^{2+}-free solution (George & Leach, 1971). Similarly,

Table 1 (*a*). *Effect of reserpine and nialamide pretreatments on mesenteric responses obtained to sympathomimetic amines in normal and Ca^{2+}- and Mg^{2+}-free Krebs.*

	A Control		B Reserpine		C Nialamide	
Amine	Normal	Ca^{2+}- & Mg^{2+}-free	Normal	Ca^{2+}- & Mg^{2+}-free	Normal	Ca^{2+}- & Mg^{2+}-free
Noradrenaline 200 ng	38·2 ± 3·5	50·2 ± 4·1	39·5 ± 3·2	52·1 ± 4·4	40·1 ± 3·4	52·8 ± 4·1
Metaraminol 20 μg	29·6 ± 2·2	49·3 ± 3·1	23·2 ± 2·1	39·5 ± 2·1	30·8 ± 2·6	51·7 ± 3·8
Octopamine 50 μg	12·3 ± 1·8	30·5 ± 3·0	4·1 ± 1·1	5·6 ± 1·4	16·4 ± 2·1	43·8 ± 2·9
Tyramine 100 μg	3·5 ± 1·3	30·2 ± 2·5	—	—	5·6 ± 0·9	44·6 ± 4·2

Mean mesenteric perfusion pressure responses (mm Hg ± s.e. 1 mm Hg = 1·33 m bar). A. saline treated control rats. B. rats pretreated with reserpine 10 mg kg^{-1}, i.p. C. rats pretreated with nialamide 5 mg kg^{-1}, i.p. Each result is the mean of at least 8 experiments.

Table 1 (*b*). *% change in perfusion pressure to each of the four amines: noradrenaline, octopamine, metaraminol and tyramine after pretreatment with (A) reserpine 10 mg kg^{-1}, (B) nialamide 5 mg kg^{-1}.*

	A		B	
	1	2	1	2
Noradrenaline	0	0	+2	+3
Metaraminol	−20	−22	0	0
Octopamine	−60	−70	+50	+48
Tyramine	—	—	+65	+44

(+) = % increase (−) = % decrease

Column 1: normal Krebs perfusion:

$$\frac{\text{mean change in perfusion pressure in pretreated rat}}{\text{mean control perfusion pressure}} \times 100$$

Column 2: Ca^{2+}- and Mg^{2+}-free Krebs perfusion:

$$\frac{\text{mean change in perfusion pressure in pretreated rat}}{\text{mean control perfusion pressure}} \times 100$$

the responses to octopamine (50 μg) obtained during Ca^{2+}- and Mg^{2+}-free perfusion of nialamide-pretreated mesenteric arteries were 48% greater than the usual response obtained using Ca^{2+}- and Mg^{2+}-free solution (Table 1).

During perfusion with Ca^{2+}- and Mg^{2+}-free solution, the responses to noradrenaline 200 ng, and metaraminol 20 μg, in mesentery preparations derived from nialamide-pretreated rats were not significantly altered compared to responses obtained during perfusion of mesenteric arteries from untreated rats with Ca^{2+}- and Mg^{2+}-free solution.

Pretreatment with disulfiram

Disulfiram pretreatment (50 mg kg^{-1}) had no effect on the responses to any of the four amines under any perfusion conditions when compared to saline treated control rats.

Action of phentolamine

Phentolamine (2×10^{-3} M), added to the perfusion fluid, reversibly antagonized the responses to all four amines under perfusion conditions using either normal or Ca^{2+}- and Mg^{2+}-free Krebs.

Action of cocaine

Cocaine (5×10^{-6} M), added to the perfusion fluid, reversibly antagonized the response to tyramine (100 μg) during perfusion with either normal or Ca^{2+}- and Mg^{2+}-free Krebs solutions. The responses to noradrenaline and metaraminol were potentiated by 40 and 20% respectively above their corresponding control values obtained during perfusion with normal Krebs solutions and a similar factor increase above the usual potentiated value was seen when tested after transference to Ca^{2+}- and Mg^{2+}-free solution (Table 2). The responses to octopamine showed a similar decrease in the presence of cocaine during perfusion with both normal and Ca^{2+}- and Mg^{2+}-free solutions.

Action of desipramine

Desipramine (1×10^{-7} M), added to the perfusion solution, antagonized the responses to tyramine obtained during perfusion with either normal or Ca^{2+}- and Mg^{2+}-free Krebs, and like cocaine also potentiated the responses to metaraminol and noradrenaline (Table 2). The action of desipramine on the responses to octopamine appeared to vary and at a concentration of 10^{-7} M, the octopamine response was antagonized in only 60% of experiments (Table 2) in both normal and Ca^{2+}- and Mg^{2+}-free perfusion.

Action of ouabain

Ouabain (1×10^{-5} M), added to the perfusion solution, antagonized the tyramine and octopamine responses obtained during perfusion with normal Krebs solution; during perfusion with either Ca^{2+}- and Mg^{2+}-free solution or Ca^{2+}-free solution the responses to tyramine and octopamine were seen to be maximally potentiated in the

Table 2 (*a*). *Effect of desipramine, cocaine and ouabain on mesenteric responses obtained to sympathomimetic amines in normal, Ca^{2+}- or Mg^{2+}-free Krebs.*

	A Control				B DMI		C Cocaine		D Ouabain		
Amine	Normal	Ca^{2+}- & Mg^{2+}-free	Ca^{2+}-free	Mg^{2+}-free	Normal	Ca^{2+}- & Mg^{2+}-free	Normal	Ca^{2+}- & Mg^{2+}-free	Normal	Ca^{2+}-free	Mg^{2+}-free
Noradrenaline 200 ng	38·2 ±3·5	50·2 ±4·1	44·9 ±3·8	41·7 ±3·6	56·9 ±4·7	76·3 ±5·2	55·1 ±3·9	74·4 ±4·1	53·6 ±4·9	45·7 ±3·2	58·5 ±5·4
Metaraminol 20 μg	29·6 ±2·2	49·3 ±3·1	41·6 ±3·0	37·1 ±2·9	41·2 ±3·8	71·4 ±6·3	36·2 ±3·5	56·5 ±4·9	38·9 ±3·4	43·0 ±3·8	48·2 ±4·1
Octopamine 50 μg	12·3 ±1·8	30·5 ±3·0	21·7 ±1·9	16·5 ±1·7	—	—	10·7 ±1·1	28·7 ±2·9	—	20·5 ±1·9	4·2 ±1·1
Tyramine 10 mg	3·5 ±1·3	30·2 ±2·5	20·6 ±3·1	14·8 ±1·6	—	—	—	—	—	21·7 ±2·2	10·2 ±1·6

Mean mesenteric perfusion pressure responses (mm Hg ± s.e.). A no drug addition to the Krebs perfusion solution. B in the presence of desipramine (DMI) (1×10^{-7} M), C in the presence of cocaine (5×10^{-6} M) and D in the presence of ouabain (1×10^{-5} M). Each result is the mean of at least seven experiments.

Table 2 (*b*). *% change in perfusion pressure response to each of the four amines noradrenaline, octopamine, metaraminol and tyramine in the presence of (A) cocaine 5×10^{-6} M, (B) desipramine 1×10^{-7} M, (C) ouabain 1×10^{-5} M.*

	A		B		C	
	1	2	1	2	1	2
Noradrenaline	+40	+30	+45	+50	+37	0
Octopamine	−18	−15	—	—	—	+2
Metaraminol	+20	+28	+32	+40	+27	−1
Tyramine	—	—	—	—	—	−2

(+) = % increase (−) = % decrease

Column 1: perfusion with normal Krebs:

$$\frac{\text{mean change in perfusion pressure in presence of A, B or C}}{\text{mean control perfusion pressure response}} \times 100$$

Column 2: perfusion with Ca^{2+}- and Mg^{2+}-free Krebs:

$$\frac{\text{mean change in perfusion pressure in presence of A, B or C}}{\text{mean control perfusion pressure response}} \times 100$$

presence of ouabain (Table 2). Conversely, ouabain potentiated the responses to noradrenaline and metaraminol in all experiments using normal Krebs; during perfusion with Ca^{2+}-free or Ca^{2+}- and Mg^{2+}-free solution the potentiating effect was abolished (Table 2).

Ouabain, however, antagonized the tyramine and octopamine responses and potentiated those to noradrenaline and metaraminol in Krebs solutions which contained reduced amounts of Ca^{2+} ($1{\cdot}2 \times 10^{-3}$ M) and from which Mg^{2+} had been omitted (Table 2), but this action was not seen when the mesentery was perfused with a Krebs solution containing $1{\cdot}8 \times 10^{-3}$ M Mg^{2+} and from which the Ca^{2+} had been omitted. The action of ouabain on the amines responses was antagonized in Krebs solutions containing K^+ ($1{\cdot}2 \times 10^{-4}$ M).

Depolarizing Krebs solution

When the rat mesentery preparation was perfused with Krebs depolarizing solution containing K^+ (115×10^{-3} M) there was an immediate 100-fold rise in mean resting perfusion pressure which was succeeded by a slow decline to its resting level. The elevation in perfusion pressure lasted approximately 20 min. During perfusion with the K^+ depolarizing solution, responses to octopamine, tyramine or metaraminol could not be elicited and no significant response to noradrenaline could be detected upon recommencement of perfusion with normal Krebs solution. Normal responsiveness of the mesenteric arteries to each of the four amines was restored within approximately 25 min and throughout this time there was no further alteration in the mean resting perfusion pressure. Perfusion with Krebs solutions containing either 2 or 4 times the normal K^+ concentration reduced the response to each of the 4 amines compared to control responses obtained in normal Krebs.

DISCUSSION

During perfusion of rat mesenteric arteries, with Ca^{2+}- and Mg^{2+}-free solutions, the response to noradrenaline, octopamine, metaraminol and tyramine were potentiated compared with the control responses obtained in normal Krebs. The magnitude of the potentiation was different for each amine and this phenomenon has been shown not to occur as the result of a general increase in smooth muscle responsiveness (George & Leach, 1971). It would appear that the perfusion of rat mesenteric arteries with solutions containing reduced Ca^{2+} and Mg^{2+} concentrations does not cause a general depolarization of the arteriolar smooth muscle as responses to the four amines were not obtainable upon exposure of the preparation to complete K^+ depolarization by the method of Evans & others (1958) and partial K^+ depolarization caused a reduction in the responses to each of the four amines.

Each of the four amines exhibited the response characteristics appropriate to their proposed mechanism of action described by Trendelenburg (1963). The action of tyramine is thought to occur by the release of noradrenaline from intraneuronal stores and so to excite the α-adrenoceptors (Muscholl, 1966). Noradrenaline exerts a direct action on α-adrenoceptors, while octopamine and metaraminol are mixed-acting amines being able to both release noradrenaline and to act directly themselves at α-adrenoceptors (Muscholl, 1966; Iversen, 1967).

The results obtained with reserpine show that an intact store of intraneuronal noradrenaline is necessary for a tyramine-evoked response both in normal or Ca^{2+}-

and Mg^{2+}-free conditions and that the reduction in noradrenaline content caused by reserpine in the mesentery reduced the responsiveness to metaraminol and octopamine. It has been shown that reserpine causes a depletion of noradrenaline stores from sympathetic nerve endings (Burn & Rand, 1958) and thus noradrenaline release is essential for the tyramine response to occur during perfusion with either normal or modified Krebs solution.

Tyramine, octopamine and noradrenaline are all known to act as substrates for monoamine oxidase (MAO) (Sandler & Ruthven, 1969), though noradrenaline can be metabolized by other routes and tyramine may alternatively be converted to octopamine by the enzyme dopamine-β-hydroxylase (Goldstein & Contrera, 1962). Pretreatment with the MAO inhibitor nialamide, potentiated the responses of the perfused mesentery preparation to tyramine and octopamine under all perfusion conditions. Inhibition of MAO had no significant action on the responses to either noradrenaline or metaraminol. These results are consistent with the demonstrated substrate affinity of MAO (Blaschko, Richter & Schlossman, 1937) and they also show that the increase in responsiveness during perfusion with solutions containing reduced concentrations of Ca^{2+} and Mg^{2+} is not a result of MAO inhibition.

Disulfiram is an irreversible inhibitor of dopamine-β-hydroxylase (Goldstein, Anagnoste & others, 1964) and treatment of rats with this drug had no effect on the responses of the mesenteric vessels to any of the amines under any perfusion conditions. It is concluded that inhibition of the conversion of tyramine to octopamine has no effect on the tyramine response during perfusion with normal or modified Krebs solutions.

The responses to each of the four amines were antagonized by phentolamine under all perfusion conditions and these results can satisfactorily be ascribed to antagonism at the α-adrenoceptor site (Nickerson, 1949) and offers further evidence that no change occurred in the nature of the individual sympathomimetic response of the four amines studied during perfusion with modified Krebs solutions.

A specific amine uptake process has been postulated to exist at the membrane of the sympathetic neuron (Carlsson & Waldeck, 1965; Titus & Dengler, 1966). The exact nature of this process is unknown, but it is thought to be sodium-dependent and similar to that postulated to exist in the intestine for the mucosal to serosal transport of sugars and amino-acids (Crane, 1965).

To elicit a response, tyramine must first enter the sympathetic neuron and displace noradrenaline (Nash, Wolff & Ferguson, 1968). A proportion of the response elicited by octopamine and metaraminol is also dependent on each of these amines entering the sympathetic neuron and releasing noradrenaline (Iversen, 1967). The uptake process can be inhibited directly by cocaine which competes with sympathomimetic amines for uptake sites on the carrier (Hertting, Axelrod & Patrick, 1961; Iversen, 1967) and in this way the tyramine response is inhibited or abolished (Burn & Tainter, 1931) as a result of the inhibition of tyramine uptake (Weiner & Trendelenburg, 1962). Similarly, desipramine competitively inhibits noradrenaline uptake (Axelrod, Hertting & Potter, 1962), metaraminol uptake (Leitz & Stefano, 1970) and tyramine uptake (Commarato, Brody & McNeill, 1969). Ouabain has also been shown to antagonize noradrenaline uptake (Leitz & Stefano, 1970), the ouabain inhibition being caused by an indirect effect as a result of inhibition of the carrier system, through the sodium pump (Glynn, 1964).

Our results show that noradrenaline responses were potentiated by and the tyramine responses antagonized by cocaine and desipramine, under all perfusion conditions. Cocaine and desipramine potentiated the action of metaraminol but had little action on octopamine again under all conditions of perfusion. This further demonstrates that the mode of action of each of the amines was unaltered by perfusion with Ca^{2+}- and Mg^{2+}-modified Krebs solution and that entry into the sympathetic neuron is necessary for tyramine action under all perfusion conditions. It appears, therefore, that the amine entry process still functions when Ca^{2+} and Mg^{2+} are absent from the perfusing solution.

Ouabain inhibited the action of tyramine and octopamine and potentiated the action of metaraminol and noradrenaline during perfusion with normal Krebs solution. In the presence of ouabain, the accumulation of noradrenaline and tyramine has been found to be inhibited (George and Leach, to be published). When Ca^{2+} was absent from the perfusing solution, ouabain failed to potentiate the noradrenaline or metaraminol responses and was not capable of inhibiting the effect of octopamine or tyramine. Since ouabain has no action on the amine responses when Ca^{2+} is absent from the solution, it would appear that the carrier process is not operating normally under conditions of reduced extracellular Ca^{2+}. Cocaine and desipramine may still be effective under these conditions because they directly inhibit amine binding to sites on the carrier. Such an explanation is unsatisfactory because it is also necessary to explain how tyramine can enter the neuron when the uptake process is inhibited. If it is assumed that all sympathomimetic amines enter the sympathetic neuron by a common uptake process (Titus & Dengler, 1966; Iversen, 1967), then the position of tyramine and octopamine is paradoxical since the responses to each of these amines should be abolished during zero Ca^{2+} and Mg^{2+} Krebs perfusion as those of noradrenaline are potentiated. However, it has been demonstrated that more than one uptake process for sympathomimetic amine exists in the sympathetic neuron (Sugrue & Shore, 1969), and some tyramine could enter the neuron by diffusion. A third alternative could be that removal of Ca^{2+} from the perfusing solution causes an allosteric change in the affinity of the uptake carrier for amines and would simultaneously reduce the affinity of noradrenaline for the uptake process, while increasing or leaving unaltered, the affinity of the carrier for tyramine. Since Nash, & others (1968) have demonstrated that ease of passage through the nerve membrane is the limiting factor in determining the quantity of noradrenaline released by an amine, this concept of alteration of carrier affinity could have an important bearing on the potency of indirectly and mixed acting sympathomimetic amines.

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