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Effect of phenmetrazine, aminorex and (±) *p*-chloramphetamine on the motor activity and turnover rate of brain catecholamines

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Summary

1. The minimal doses (μmol/kg i.v.) of phenmetrazine, (±)-*p*-chloramphetamine, and aminorex which increase motor activity are 5·6, 3·5, and 1·5, respectively. We detected stereotype behaviour neither in rats receiving intravenous doses 3 times greater nor in animals injected intraperitoneally with 44, 62 and 112 μmol/kg of (±)-*p*-chloramphetamine, aminorex and phenmatrazine, respectively.

2. The latter doses of the three amphetamine congeners were tested for their action on tissue monoamine content. Only (±)-*p*-chloramphetamine decreased the concentration of tel-diencephalon 5-hydroxytryptamine (5-HT) and this decrease lasted longer than 24 hours. This and the other two amphetamine congeners failed to affect the concentration of noradrenaline (NA) in brain, heart and lung.

3. Aminorex (1·5 μmol/kg i.v.) and (±)-*p*-chloramphetamine (3·5 μmol/kg i.v.) decreased the turnover time of striatum dopamine (DM) but failed to change the turnover time of tel-diencephalon and brainstem NA. Phenmetrazine (5·6 μmol/kg i.v.) changed neither the turnover time of striatum DM nor that of NA in the two brain areas assayed.

Introduction

Release of neuronal noradrenaline (NA) mediates many actions of (+)-amphetamine, including psychomotor stimulation (Trendelenburg, Muskus, Flemming & Alonso de la Sierra, 1962; Stein, 1964; Rech, 1964; Weissman, Koe & Tenen, 1966; Sulser, Owens, Norvich & Dingell, 1968; Rech & Stalk, 1970). However, a dose of (+)-amphetamine (2 μmol/kg i.v.), which increases the motor activity of rats, neither accelerates the turnover rate nor reduces the concentration of NA in several brain areas (Groppetti, Naimzada & Costa, 1970). This dose of (+)-amphetamine increases the turnover rate of striatum dopamine (DM) (Groppetti *et al.*, 1970). These results are consistent with studies by Van Rossum, van der Schoot & Hurkmans (1962) and Smith (1963, 1965) in disputing that (+)-amphetamine's action on motor activity involves a release of brain NA.

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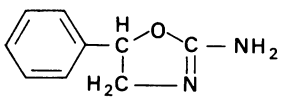
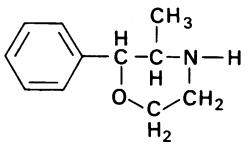
Aminorex, phenmetrazine and (±)-*p*-chloramphetamine are chemically similar to (+)-amphetamine (Fig. 1), and increase the spontaneous motor activity of rats. The aim of these experiments was to evaluate the action of these drugs on storage and metabolism of brain catecholamines. The results show that neither a decrease of brain NA concentrations nor an acceleration of its turnover rate are associated with the increase of motor activity elicited by these three amphetamine congeners. (A preliminary report was presented at the Annual Meeting of the Federation of the American Societies for Experimental Biology, 1971.)

Methods

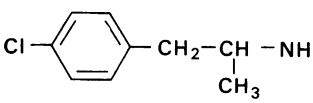
Male, Sprague-Dawley rats, 180–220 g body weight, were purchased from Zivic Miller (Pittsburg, Pa.) and kept in our animal quarters, housed ten per cage at 20° C for at least 4 days. The experiments were carried out in a room thermoregulated at 20° C. On the day of the experiments rats were placed one in each of the eight compartments (21 × 33 cm) of an I.R. Electronic Motility Meter (Metron Co., Sweden). The rectangular floor of each compartment of the instrument is illuminated by a red light and is provided with infrared photocells spaced 4 cm apart. Any movement of the animal, including grooming, is recorded by the activity meter. Rearing is detected by the instrument and can be discriminated by using a series of photocells placed on the side of the instrument 7 cm from the floor. Thus, this apparatus avoids many limitations discussed by Kršiak, Steinberg & Stolerman (1970) for the photocell activity cages described by Siegel & Steinberg (1949). The rats were left in this new environment for about 1 h, then they received intravenously either 0·5 or 1 mCi/kg of 3,5-^{3}H-L-tyrosine (31 Ci/mmol) and 10 min later they were either decapitated or injected in the tail vein with saline or with one of the three amphetamine congeners (Fig. 1). Immediately after the injections the spontaneous motor activity of each rat was recorded for 15 or 20 min, after which the animals were killed. Each experimental run included two rats receiving each of the drugs tested and two rats receiving saline. C.p.m. recorded by our activity meter from



Aminorex



Phenmetrazine



pCl Amphetamine

FIG. 1.

each animal receiving identical injections 10 min after ^{3}H tyrosine were averaged and the significance between the means estimated by Student's t test.

After decapitation the heart was immediately removed, rinsed with saline, blotted dry, frozen in dry ice and kept frozen for subsequent analysis. The brain was dissected as described earlier into tel-diencephalon, striatum and brain stem (Costa, Groppetti & Revuelta, 1971). In some experiments the tel-diencephalon was dissected into hypothalamus and telencephalon as described by Bapna, Neff & Costa (1971).

The specific activity of tyrosine, NA and DM was determined in each tissue sample according to Neff, Spano, Groppetti, Wang & Costa (1971). The fractional rate constant (k) for the efflux of the amines from various brain structures was estimated according to equation (1).

$$k \equiv \frac{\dfrac{Ct_2 - Ct_1}{\Delta t}}{\dfrac{(T - Ct_1) + (T - Ct_2)}{2}} \tag{1}$$

where C=specific radioactivity of the catecholamine, and T=specific radioactivity of the tyrosine. The derivation of equation (1) was reported elsewhere (Neff *et al.*, 1971); here we wish to note that equation (1) gives an approximation of k. In our experiment Δt was equal to 15 min (Tables 2, 3 and 4) or 20 min (Tables 5, 6 and 7).

Turnover time of the catecholamine contained in a given tissue was estimated from equation (2).

$$T_t = \frac{1}{k} \tag{2}$$

where k has the dimension of h^{-1}.

Dose-response relationships of the effect on motor activity exerted by the various doses of the three amphetamine congeners were investigated by injecting the animals intravenously, placing them in the electronic motility meter and recording the activity at 15, 30 and 60 min after treatment.

In other experiments the effect of these drugs on tissue monoamine content was studied. The maximal tolerated dose of each drug was injected intraperitoneally and the rats were decapitated at various times after the drug injection. Monoamine tissue concentrations were assayed according to the method described by Neff *et al.* (1971).

Changes in body temperature were recorded in groups of rats kept one per cage by measuring the temperature rectally with a tele-thermometer (Yellow Spring Instrument Co.).

Radioactive 3,5-^{3}H L-tyrosine (31 Ci/mmol) was purchased from New England Nuclear (Boston, Mass.), phenmetrazine was donated by Dr. Tedeschi from Ciba-Geigy, USA, (+)-amphetamine was obtained through the courtesy of Dr. A. Misher from Smith, Kline & French Laboratories, aminorex was received from McNeil Labs. and (±)-*p*-chloramphetamine was a gift of Lovens Kemiske Fabrik, Copenhagen.

Results

Effect of aminorex, (±)-p-chloramphetamine and phenmetrazine on the motor activity of the rat

We recorded for 1 h the motor activity of rats injected intravenously with either various doses of aminorex, (±)-*p*-chloramphetamine, phenmetrazine or an equal volume of vehicle (saline). The results of these experiments are reported in Table 1 and show that the threshold dose of aminorex to increase motor activity is smaller than that of (±)-*p*-chloramphetamine, while phenmetrazine appears to be the least active of the three drugs. In our experiments, the minimal effective dose (μmol/kg i.v.) that increases motor activity for at least 60 min was 1·5 for aminorex, 3·5 for (±)-*p*-chloramphetamine and 5·6 for phenmetrazine. At the highest dose reported in Table 1, none of the three drugs elicited stereotyped behaviour; such behaviour appeared after (+)-amphetamine (5 μmol/kg i.v.). Aminorex (1·5 μmol/kg i.v.) and phenmetrazine (5·6 μmol/kg i.v.) were without effect on body temperature but (±)-*p*-chloramphetamine (1·7 μmol/kg i.v.) lowered the rectal temperature of the rats for about 30 minutes.

Effect of aminorex, (±)-p-chloramphetamine and phenmetrazine on tissue concentrations of catecholamines and 5-hydroxytryptamine

We injected various doses of the three drugs intraperitoneally and found that the maximal tolerated doses (μmol/kg) were 44, 62 and 112 for (±)-*p*-chloramphetamine, aminorex and phenmetrazine, respectively. Three groups of twenty rats each received the above mentioned dose of each drug; groups of five rats were decapitated 1, 2, 4 and 24 h after the injections. Aminorex and phenmetrazine failed to change the concentrations of 5-HT, NA and DM in tel-diencephalon NA and 5-HT in brainstem, NA in lung and NA in heart. The results of the experiments with (±)-*p*-chloramphetamine are reported in Fig. 2. These data show that (±)-*p*-chloramphetamine reduces the 5-HT concentrations in tel-diencephalon by more than 50% from 2 h to 24 h but the NA concentrations in brainstem, tel-diencephalon and heart are not depleted.

TABLE 1. *Effect of aminorex, (±) p-chloramphetamine and phenmetrazine on spontaneous motor activity of rats*

		Min after drug injection (counts/min) (mean±S.E.)		
Drug	μmol/kg i.v.	15	30	60
Aminorex	0·61	43±18	23±11	14±5
Aminorex	1·5	67±19*	68±13*	56±6·5*
Aminorex	3·1	79±13*	87±14*	66±8·1*
Aminorex	6·1	118±12*	188±38*	151±24*
Saline	5 ml/kg	30±6	13±3	10±2
p-Chloramphetamine	0·6	46±18	10±4	13±2
p-Chloramphetamine	1·8	31±8	21±7·2	18±3·8
p-Chloramphetamine	3·5	66±6*	46±12*	31±6·9*
p-Chloramphetamine	7·1	80±15*	72±11*	56±6·7*
Saline	5 ml/kg	30±6	13±3	10±2
Phenmetrazine	2·8	29±10	30±15	23±13
Phenmetrazine	5·6	56±11*	49±9*	36±6*
Phenmetrazine	11·3	89±13*	75±11*	51±5*
Phenmetrazine	22·6	101±16*	91±15*	63±9*
Saline	5 ml/kg	30±6	13±3	10±1·6

Each group consisted of eight rats. * $P<0·01$ when compared to rats receiving saline.

Effect of aminorex (±)-p-chloramphetamine on the turnover time of catecholamines in various brain structures

We estimated the turnover time of NA and DM in various parts of brain tissue by an isotopic method and compared it in three groups of rats receiving aminorex, (±)-*p*-chloramphetamine and phenmetrazine, respectively. We administered each drug at the minimal effective dose for increasing motor activity as listed in Table 1. None of these drugs changed the specific radioactivity of tyrosine in striatum, brainstem and tel-diencephalon, when they were injected 10 min after the labelled amino-acid and the rats were killed 15 min after the drug injections. The data reported in Tables 2, 3 and 4 concern an experiment where we estimated the action of aminorex and (±)-*p*-chloramphetamine on motor activity and turnover time of tissue catecholamines. As reported in these tables the motor activity of rats receiving the two drugs was greater than that of rats receiving saline. Despite the increase of motor activity, the specific activity of NA in tel-diencephalon was equal in saline and drug treated rats (Table 2). We followed equation 1 to calculate the fractional rate constant (k) of NA stored in tel-diencephalon, and found that k values are comparable in the three groups of rats. Since the two drugs fail to change the concentration of NA in tel-diencephalon, the data listed in Table 2 indicate that the turnover time of tel-diencephalon NA is unaffected by doses of aminorex and (±)-*p*-chloramphetamine which increase the motor activity of rats for at least 1 h (Table 1).

Table 3 shows the results of our assay carried out in the brainstem of the same animals included in Table 2. These data show that the turnover time of brainstem NA is unaltered by doses of aminorex and (±)-*p*-chloramphetamine which increase the motor activity of rats.

The data reported in Table 4 show that not only is the specific radioactivity of striatum DM in the three groups of rats killed at 25 min after labelling greater than

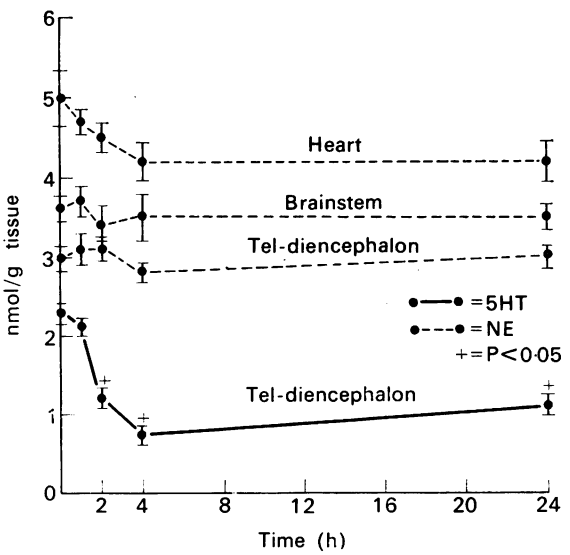


FIG. 2. Effect of (±)-*p*-chloramphetamine on the concentrations of monoamines in various tissues. Each point represents the mean value of four rats; vertical bars represent S.E.

that of rats killed 10 min after labelling, but also that the specific radioactivity of striatum DM of rats receiving the two drugs and killed 25 min after labelling is greater than that of rats receiving saline. Since neither steady state concentrations of tyrosine and DM nor the specific activity of striatum tyrosine was changed by the drug injection, it follows that the turnover time of striatum DM in drug treated rats is faster than in rats injected with saline. We have assayed the concentrations of 5-HT in brainstem and tel-diencephalon of the drug treated rats listed in Tables 2, 3 and 4, and found that they were not significantly different from those of saline treated rats.

TABLE 2. *Effect of aminorex and* (±) p-*chloramphetamine on rat motor activity and on the turnover rate of tel-diencephalon noradrenaline* (*NA*)

Min after 3,5-^{3}H-L-tyrosine (33 nmol/kg i.v.)		Drug (μmol/kg i.v.) 10 min after ^{3}H L-tyrosine	Motor activity (events/min) during 15 min	NA (dpm/nmol ±S.E.)	Tyrosine (dpm/nmol ±S.E.)	k (h^{-1})	Turnover time (h)
10	(5)	—	—	396±30	5776±580	—	—
25	(4)	Saline	17±3	744±66	2500±230	0·39	2·6
25	(5)	Aminorex (1·5)	77±12*	765±45	2529±143	0·41	2·4
25	(5)	(±) *p*-Chlor-amphetamine (3·5)	57±16*	821±50	2785±103	0·46	2·2

* $P<0·05$ when compared to rats receiving saline. None of these drugs changed the steady state concentrations of NA (2·1±0·071 nmol/g) and tyrosine (103±5·4 nmol/g) in tel-diencephalon.

TABLE 3. *Effect of aminorex and* (±) p-*chloramphetamine on rat motor activity and on the turnover time of brainstem norepinephrine* (*NA*)

Min after 3,5-^{3}H-L-tyrosine (33 nmol/kg i.v.)		Drug (μmol/kg i.v.) 10 min after ^{3}H-L-tyrosine	Motor activity (events/min) during 15 min	NA (dpm/nmol ±S.E.)	Tyrosine (dpm/nmol ±S.E.)	k (h^{-1})	Turnover time (h)
10	(5)	—	—	252±56	5636±360	—	—
25	(5)	Saline	7·0±1·4	608±79	2867±156	0·37	2·7
25	(5)	Aminorex (1·5)	77±19*	652±84	2910±169	0·42	2·4
25	(5)	*p*-Chloramphet-amine (3·5)	57±16*	611±55	2796±169	0·38	2·6

* $P<0·05$ when compared to rats receiving saline. None of these drugs changed the steady state concentrations of NA (3·0±0·1 nmol/g) and tyrosine (108±6·4 nmol/g) in brainstem.

TABLE 4. *Effect of aminorex and* (±) p-*chloramphetamine on rat motor activity and on the turnover time of striatum dopamine* (*DM*)

Min after 3,5-^{3}H-L-tyrosine (33 nmol/kg i.v.)		Drug (μmol/kg i.v.) 10 min after ^{3}H L-tyrosine	Motor activity (events/min) during 15 min	DM (dpm/nmol ±S.E.)	Tyrosine (dpm/nmol ±S.E.)	k (h^{-1})	Turnover time (h)
10	(5)	—	—	549±20	5890±468	—	—
25	(5)	Saline	7·0±1·4	848±75	2642±353	0·34	2·9
25	(5)	Aminorex (1·5)	77±19*	1102±21*	2601±238	0·65	1·5
25	(5)	*p*-Chloramphet-amine (3·5)	57±16*	1069±61*	2621±348	0·60	1·7

* $P<0·05$ when compared to rats receiving saline. None of these drugs changed the steady state concentrations of DM (63±3·3 nmol/g) and tyrosine (102±16 nmol/g) in striatum.

Effect of phenmetrazine on the turnover time of hypothalamic NA and striatum DM

Rats received a dose of phenmetrazine (5·6 μmol/kg i.v.) which stimulates motor activity (Table 1); they were killed 25 min after radioactive tyrosine injections (33 nmol/kg i.v.) and 15 min after drug injections. A group of rats was killed 10 min after the radioactive tyrosine. The animals receiving this dose of phenmetrazine failed to show any change whatsoever in the turnover time of brainstem and teldiencephalon NA and of striatum DM. Before excluding that phenmetrazine affects catecholaminergic axons when given in doses that increase motor activity, we repeated this experiment and compared the turnover time of NA in hypothalamus and striatum of rats receiving either saline or one of the two doses of phenmetrazine (5·6 or 2·8 μmol/kg i.v.).

The data included in Tables 5 and 6 show that phenmetrazine (2·8 μmol/kg i.v.) fail to decrease the turnover time of striatum DM and hypothalamic NA and to elicit an increase of motor activity. A dose twice as large increases motor activity but still does not change the turnover time of DM and NA in the two brain structures assayed.

Effect of aminorex, phenmetrazine, and (±)-p-chloramphetamine on the turnover time of cardiac catecholamines

The turnover time of heart NA of the rats included in the experiments reported in Tables 2–5 and 6 was also measured. We found that none of the three drugs had changed the incorporation of radioactive tyrosine into the NA contained in heart tissue.

TABLE 5. *Effect of phenmetrazine on rat motor activity and on turnover time of hypothalamus noradrenaline (NA)*

Min after 3,5-^{3}H L-tyrosine (16 nmol/kg i.v.)		Phenmetrazine (μmol/kg i.v.) 10 min after ^{3}H-L-tyrosine	Motor activity (events/min) during 20 min	NA (dpm/nmol ±S.E.)	Tyrosine (dpm/nmol ±S.E.)	k (h^{-1})	Turnover time (h)
10	(5)	—	—	145±27	4630±340	—	—
30	(5)	Saline	23±6·8	422±162	1240±130	0·31	3·2
30	(5)	5·6	61±6·8*	486±122	1270±84	0·39	2·6
30	(5)	2·8	42±6·2	450± 65	1166±142	0·35	2·9

* $P<0·05$ when compared with saline treated rats. Neither dose of phenmetrazine changes the steady state concentration of NA (8·6±0·66 nmol/g) and tyrosine (110±7·4 nmol/g) in hypothalamus.

TABLE 6. *Effect of phenmetrazine on rat motor activity and on turnover time of striatum dopamine (DM)*

Min after 3,5-^{3}H-L-tyrosine (16 nmol/kg i.v.)		Phenmetrazine (μmol/kg i.v.) 10 min after ^{3}H L-tyrosine	Motor activity (events/min) during 20 min	DM (dpm/nmol ±S.E.)	Tyrosine (dpm/nmol ±S.E.)	k (h^{-1})	Turnover time (h)
10	(9)	—	—	107±18	4230±28	—	—
30	(5)	Saline	23±6·8	334±71	1160±91	0·28	3·6
30	(5)	5·6	61±6·8*	361±6·6	1190±67	0·31	3·2
30	(5)	2·8	42±6·2	399±5·2	1128±83	0·36	2·8

* $P<0·05$ when compared to rats receiving saline. Neither dose of phenmetrazine changed the stead state concentration of DM (64±2·2 nmol/g) and tyrosine (103±11 nmol/g) in striatum.

Discussion

Rats receiving maximal tolerated doses of phenmetrazine and aminorex, maintain normal concentrations of NA, DM and 5-HT in the tissues we assayed (various brain parts, heart and lung). Since the long lasting decrease of tissue NA concentrations elicited by (+)-amphetamine relates to the persistent localization in adrenergic neurones of an amphetamine metabolite, *p*-hydroxynorephedrine (Groppetti & Costa, 1969), it may be inferred that phenmetrazine and aminorex fail to form a metabolite which can tenaciously bind to adrenergic nerve terminals.

Rats and guinea-pigs receiving either (±)-*p*-chloramphetamine or *p*-chlormethamphetamine present a prolonged and simultaneous decrease in the concentrations of cerebral 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) (Pletscher, Bartholini, Bruderer, Burkard & Gey, 1964; Fuller, Hines & Mills, 1965; Lippman & Wishnick, 1965; Nielsen, Magnussen, Kampmann & Frey, 1967; Miller, Cox, Snodgrass & Maickel, 1970). Koe & Weisman (1966) and Jequier, Lovenberg & Sjoerdsma (1967) have suggested that *p*-chlorphenylalanine depletes brain 5-HT because it inhibits tryptophan-5-hydroxylase. The chemical structure and effects on brain 5-HT of *p*-chloramphetamine and *p*-chlorphenylalanine suggest the possibility of a similar mechanism of action. But chlorinated amphetamines neither inhibit tryptophan-5-hydroxylase *in vitro* nor block the increase of cerebral 5-HT elicited by large doses of tryptophan (Pletscher, DaPrada, Burkard, Bartholini, Steiner, Bruderer & Bigler, 1966 and Pletscher, DaPrada, Burkard & Tranzer, 1968; Fuller *et al.*, 1965). Pletscher *et al.* (1964, 1968) and Fuller (1966) have suggested that *p*-chloramphetamine selectively releases brain 5-HT and inhibits monoamine oxidase. In rats receiving (±)-*p*-chloramphetamine the longlasting decrease of the tel-diencephalic 5-HT which begins after a latency of 1 h (Fig. 2), would be consistent with an indirect mechanism, for the concentration of *p*-chloramphetamine in brain decline significantly 1–2 h after intraperitoneal injections while the drug effect on the concentration of brain 5-HT has a latency time of 1 h (Nielsen & Dubnick, 1970). We are conducting experiments to investigate whether this discrepancy is compatible with the view that (±)-*p*-chloramphetamine decreases brain 5-HT because it inhibits 5-HT synthesis by a direct mechanism (Sanders-Bush & Sulser, 1970a, b).

Our results contribute evidence for the proposal (Strada, Sanders-Bush & Sulzer, 1970) that a lowering of brain 5-HT is independent of the increase in motor activity elicited by (±)-*p*-chloramphetamine since the dose injected in the experiment listed in Tables 2–4 increases the motor activity of rats without decreasing 5-HT concentrations in brain tissue. The effect of the three drugs on total motor activity estimated by direct inspection appeared to correlate well with the counts recorded by our meter. Perhaps the forty sensors placed on the floor of the cage partially eliminated the limitations described by Kršiak *et al.* (1970) for the conventional instruments provided with only two sensors placed 4 cm above the cage floor. Our experiments reveal that motor activity of rats can be increased by doses of (±)-*p*-chloramphetamine, aminorex and phenmetrazine which do not accelerate the turnover rate of NA in various brain parts we have analysed. Hence, minimal effective doses of these drugs do not seem to increase the motor activity of rats by acting on brain noradrenergic axons. We found that aminorex injected in doses which increase motor activity can decrease turnover time of striatum DM; therefore an action on dopaminergic axons should still be entertained as a possible indirect mechanism involved in the motor stimulation elicited by this drug. Our results suggest that

a similar conclusion applies to (±)-*p*-chloramphetamine and in this regard our studies modify the suggestion previously made by other workers (Strada *et al.*, 1970) that the stimulatory effect of this drug relates to its effect on brain NA metabolism. According to our studies, phenmetrazine fails to change the turnover time of brain catecholamines; therefore, this drug should be considered capable of stimulating motor activity without acting on brain adrenergic and dopaminergic axons.

Studies of Thomä & Wick (1954) had indicated that phenmetrazine is a direct acting sympathomimetic amine since its pressor effect and its effect on nictitating membrane were enhanced rather than antagonized by cocaine. A recent report on the action of phenmetrazine in man (Martin, Sloan, Sapira & Jasinski, 1971) tends to contraindicate that its central action is a consequence of the release of NA in the brain.

In conclusion, our study supports the view that aminorex, phenmetrazine and (±)-*p*-chloramphetamine, like (+)-amphetamine when given in pharmacological doses, elicit psychomotor stimulation either by acting directly on brain receptors or by releasing dopamine from nerve terminals. Therefore, our results support and extend to amphetamine congeners the proposal made by Van Rossum *et al.* (1962) and Smith (1963 and 1965) that the central action of minimal effective doses of (+)-amphetamine does not involve a release of brain NA.

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