Turning behaviour as an index of the action of amphetamines and ephedrines on central dopamine-containing neurones

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Summary

1. Rats lesioned unilaterally in the substantia nigra show no obvious abnormalities after recovery from the operation but rotate towards the lesioned side after administration of drugs of the amphetamine and ephedrine groups.

2. (+)-Amphetamine and (−)-amphetamine are equally potent in producing turning behaviour but (+)-methylamphetamine is considerably more effective.

3. (−)-Ephedrine (with a β-OH group on the ethylamine side chain) induces turning but only in doses approximately 20 times greater than (+)-methylamphetamine.

4. (+)-Norpseudoephedrine was the most effective of the ephedrine isomers tested followed by (−)-ephedrine and (+)-pseudoephedrine.

5. Turning produced by (−)-ephedrine and (+)-methylamphetamine is not reduced by FLA63 (50 mg/kg) 4 h previously, but is almost completely inhibited by α-methyl-p-tyrosine (150 mg/kg) 12 h previously.

6. Reserpine pretreatment potentiates turning produced by (−)-ephedrine.

7. Chlorpromazine (5 mg/kg) completely blocks turning induced by (+)-methylamphetamine although it concurrently increases exploratory activity. The level of exploratory activity after the (+)-methylamphetamine-chlorpromazine combination is more than 3 times that attained after saline alone.

Introduction

The distribution of dopamine in the mammalian central nervous system differs from that of noradrenaline (Bertler & Rosengren, 1959). A large proportion of this amine is concentrated in the corpus striatum. Lesions in the ipsilateral ventral mesencephalon drastically reduce the dopamine content of the corpus striatum and lead to retrograde cell degeneration in the pars compacta of the substantia nigra (Sourkes & Poirier, 1965 ; Poirier, Singh, Sourkes & Boucher, 1967). These experiments suggest the existence of an uncrossed dopamine-containing pathway connecting the substantia nigra with the corpus striatum and this conclusion is strongly supported by studies with the Falck-Hillarp histochemical technique (Andén, Carlsson, Dahlström, Fuxe, Hillarp & Larsson, 1964 ; Andén, Dahlström, Fuxe, Larsson, 1966a ; Hökfelt & Ungerstedt, 1969). Such studies show that fluorescence attributable to the presence of catecholamines is concentrated in varicosities in the nerve terminals within the corpus striatum. Experiments suggesting release

of amine from the nerve terminals after stimulation in the region of the nigro-neostriatal pathway support the hypothesis that dopamine acts as a neurohumour (Portig & Vogt, 1969 ; Arbuthnott, Crow, Fuxe, Olson & Ungerstedt, 1970).

Andén (1966) suggested a method for studying the action of drugs on central dopamine neurones. Rats with unilateral lesions of the nigro-neostriatal pathway appear relatively normal after recovery from the lesioning procedure but show a marked rotation towards the side of the lesion when treated with combinations of certain drugs, for example with nialamide and DOPA, or with reserpine and nialamide (Andén, Dahlström, Fuxe & Larsson, 1966b). Turning also results from administration of moderate doses of amphetamines (Andén, Rubenson, Fuxe & Hökfelt, 1967 ; Crow & Gillbe, 1970 ; Ungerstedt & Arbuthnott, 1970 ; Arbuthnott & Crow, 1971). These effects therefore may represent an index of the action of drugs on central dopamine neurones. In that case, turning can be used to compare drug potencies and to investigate the interaction of drugs on central dopamine mechanisms.

Experiments on the inhibition of tritiated amine uptake into cortical and striatal brain slices suggest that the uptake process into dopamine-containing neurones differs from that into noradrenaline-containing neurones (Ross & Renyi, 1967). The relative potencies of $(+)$- and $(-)$-amphetamine in blocking uptake into these two types of central catecholamine neurone may also differ (Taylor & Snyder, 1970). Ephedrines act on peripheral sympathetic postganglionic fibres, blocking noradrenaline uptake (McNeil, Muschek & Commarato, 1970), and causing increased noradrenaline release (Valette, Cohen & Bralet, 1966) as has been suggested for amphetamines (Burn & Rand, 1958 ; Axelrod & Glowinski, 1965), but differ from the amphetamines by the presence of a $-OH$ group on the carbon atom of the ethylamine side chain, that is in the manner in which noradrenaline differs from dopamine. In these experiments we have investigated the activity of amphetamines and ephedrines in eliciting turning behaviour and the antagonism of such turning by amine synthesis inhibitors and neuroleptic drugs. The introduction of the dopamine-β-oxidase inhibitor FLA63 (bis-(4-methyl-1-homopiperazinylthiocarbonyl)-disulphide (for structural formula see Svensson & Waldeck, 1969) has provided a means of distinguishing between drugs which act on central noradrenaline and those which act on central dopamine stores. FLA63 (40 mg/kg) produces a greater depletion of brain noradrenaline at 2–5 h than does α-methyl-p-tyrosine (200 mg/kg), without decreasing brain dopamine concentrations as does the latter drug (Persson & Waldeck, 1970), and the action of α-methyl-p-tyrosine is maximal at 12 hours (Nagatsu, Levitt & Udenfriend, 1964). In these experiments we have used α-methyl-p-tyrosine (150 mg/kg) 12 h previously to deplete brain noradrenaline and dopamine stores, and FLA63 (50 mg/kg) 4 h previously to deplete brain noradrenaline alone.

Methods

Male hooded Lister rats, weighing 200 ± 20 g, were anaesthetized with an intraperitoneal injections of sodium pentobarbitone (1 ml/kg body weight). Unilateral lesions in the substantia nigra were made by passing a charge of 40 mC through a varnished steel electrode (anode) implanted stereotaxically (co-ordinates according to the atlas of Fifkova & Marsala (1967): P4·5 L1·0V8·5), the circuit being completed by an anal cathode.

At least 1 week after the operation, the rats were tested for turning towards the lesioned side after an intraperitoneal injection of (+)-methylamphetamine (5 mg/kg). A group of ten rats which turned well under (+)-methylamphetamine were selected for further drug trials.

The test drugs were administered to the same group of rats, at least 1 week being allowed between each drug trial. The number of turns in 1 min towards the side of the lesion was recorded for each rat at 15 min intervals.

Exploratory activity was assessed in an open field apparatus (diameter 120 cm). The number of entries into squares (40 cm × 40 cm) marked on the base of the apparatus were counted in 1 min periods of observation for each rat.

The following drugs were used: (+)-methylamphetamine hydrochloride (Burroughs Wellcome); (+)-amphetamine sulphate (Smith, Kline & French); (—)-amphetamine sulphate (Smith, Kline & French); (—)-ephedrine hydrochloride (Evans Medical); (+)-norpseudoephedrine hydrochloride (Pierce); (+)-pseudo-ephedrine hydrochloride (Burroughs Wellcome); α-methyl-*p*-tyrosine (Axel Kistner AB); FLA63 (AB Biotec); reserpine (B.D.H.); chlorpromazine hydrochloride (May & Baker); cocaine hydrochloride (Macfarlan Smith). All drugs were dissolved in 0·9% NaCl solution with the exceptions of reserpine, which was dissolved in the minimum amount of glacial acetic acid and diluted with distilled water, and FLA63 which, together with an equal weight of (—)-ascorbic acid, was dissolved in distilled water. The standard formulae of the amphetamine and ephedrine derivatives used are shown in Fig. 1.
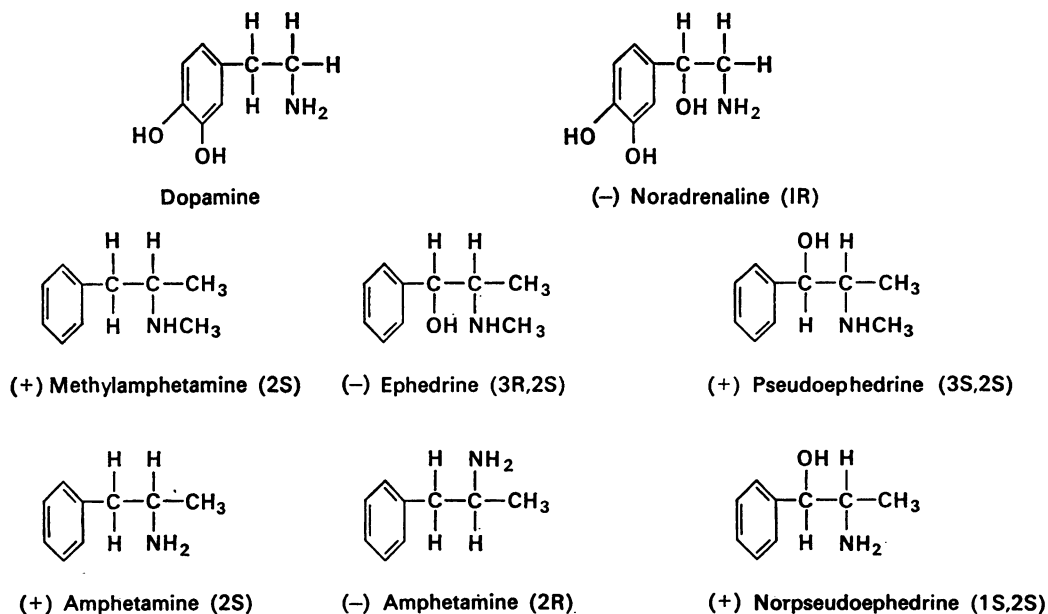

FIG. 1. Structural formulae of the amphetamine and ephedrine isomers used. R and S are notations of absolute configuration according to the sequence rule.

Results

Amphetamines

The effects of intraperitoneal injections of (+)-methylamphetamine, (+)-amphetamine and (−)-amphetamine, each in a dose of 5 mg/kg are recorded in Fig. 2. (+)-Methylamphetamine was significantly the most potent with a mean maximum turning rate of over twenty turns/minute. (+)-Amphetamine and (−)-amphetamine were approximately equipotent.
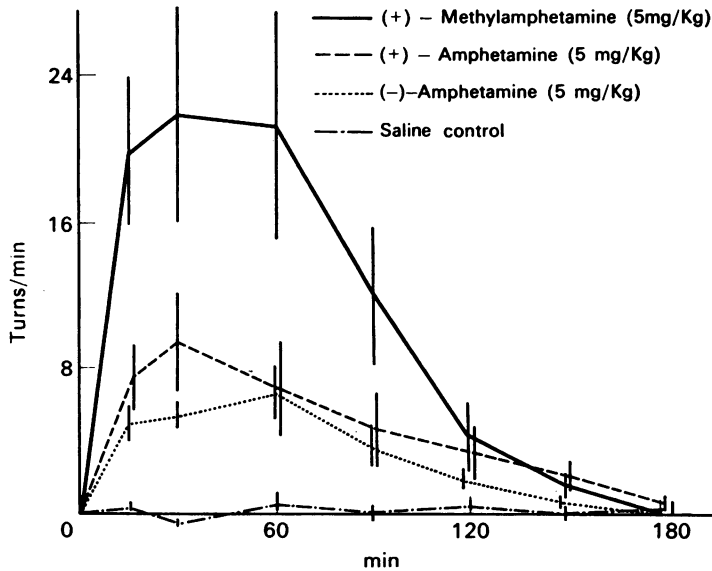
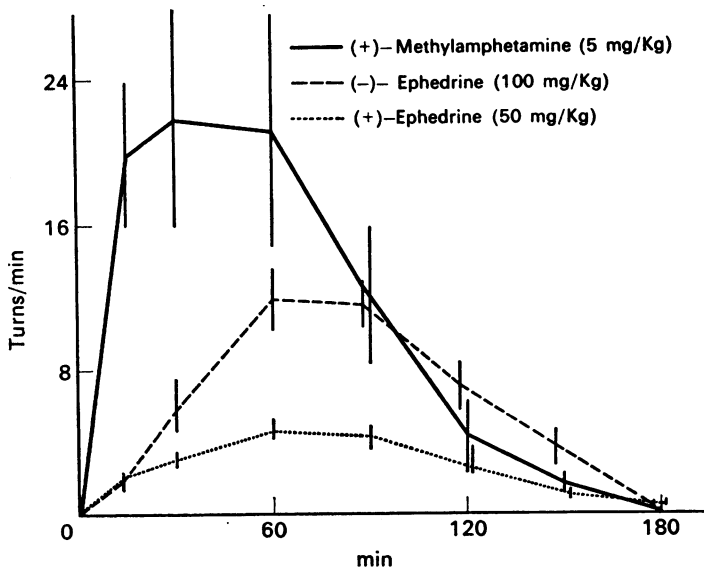FIG. 2. Turning rates/min after administration of amphetamines and saline control. The vertical bars represent ±S.E.M.

FIG. 3. Turning elicited by (−)-ephedrine compared with (+)-methylamphetamine.

Ephedrines

(−)-Ephedrine produced turning at the rather high dosages of 50 mg/kg and 100 mg/kg (Fig. 3). The onset of turning was slower than with (+)-methyl-amphetamine but the duration of the response is similar. (+)-Norpseudoephedrine was significantly the most potent of the isomers tested, followed by (−)-ephedrine and (+)-pseudoephedrine (Fig. 4). All were administered in doses of 50 mg/kg.
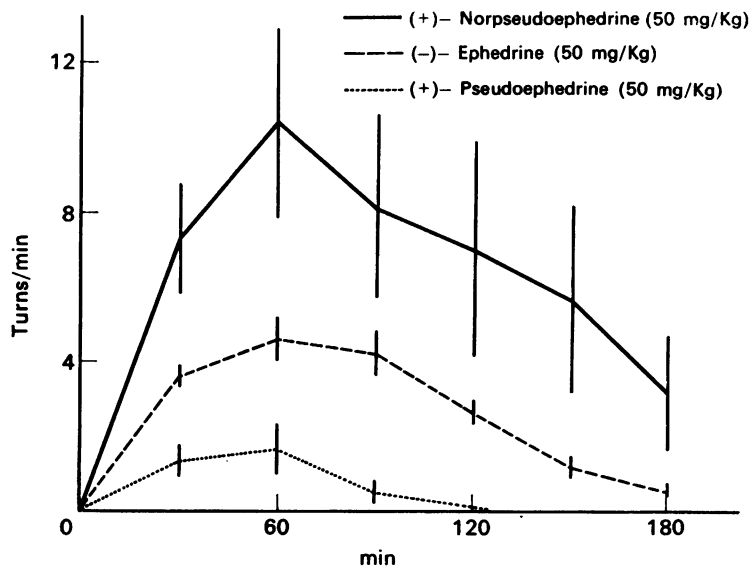

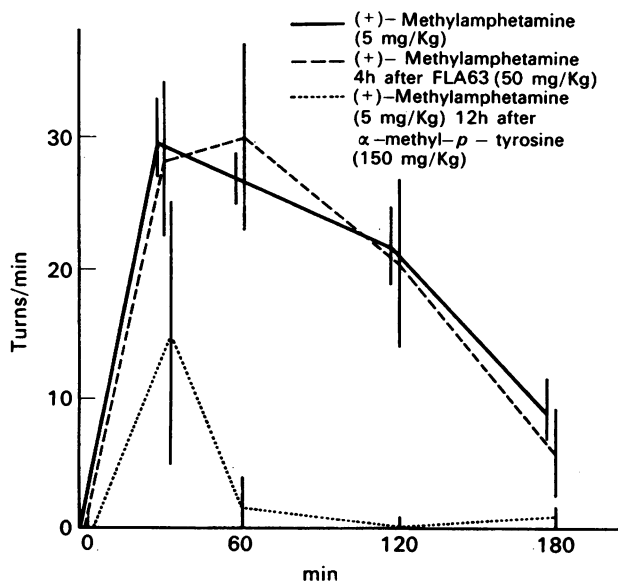FIG. 4. Comparison of the potency of ephedrine isomers in producing turning behaviour.

FIG. 5. Effect of pretreatment with FLA63 and α-methyl-*p*-tyrosine on turning induced by (+)-methylamphetamine.

Pretreatment with inhibitors of catecholamine synthesis

Turning produced by (+)-methylamphetamine (5 mg/kg) was very much reduced by administration of α-methyl-*p*-tyrosine (150 mg/kg) 12 h previously, but not by FLA63 (50 mg/kg) given 4 h previously (Fig. 5). Similarly, turning produced by (−)-ephedrine (100 mg/kg) was not reduced by FLA63 but greatly diminished by α-methyl-*p*-tyrosine administered in the same doses 4 and 12 h respectively before ephedrine (Fig. 6).
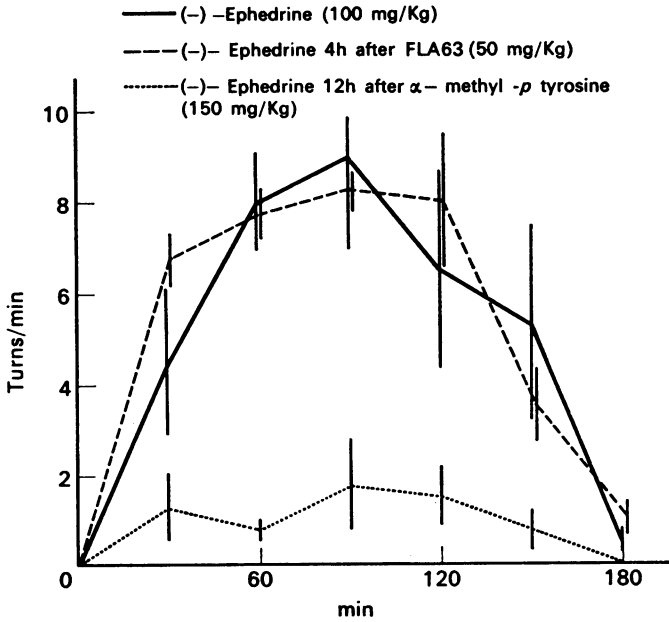

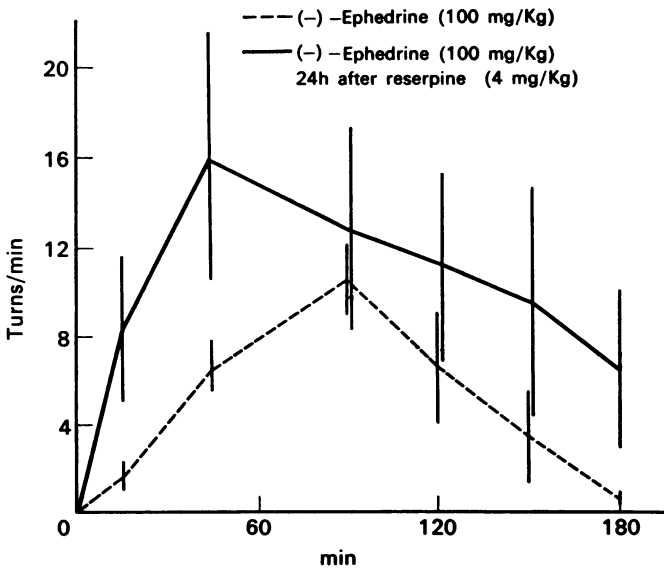FIG. 6. Effect of pretreatment with FLA63 and α-methyl-*p*-tyrosine on turning initiated by (−)-ephedrine.

FIG. 7. Effect of reserpine pretreatment on turning produced by (−)-ephedrine.

Pretreatment with reserpine

Pretreatment with reserpine (4 mg/kg) 24 h before (—)-ephedrine (100 mg/kg) resulted in a potentiation of the ephedrine effect (Fig. 7). The reserpine syndrome (inactivity ; ptosis ; hunched-back posture) was maximal at this time but was effectively reversed by ephedrine or amphetamine administration.

Cocaine

Cocaine, which inhibits the uptake of dopamine into striatal slices in concentrations of 2×10^{-6}M (Ross & Renyi, 1967), did not produce turning in doses up to 20 mg/kg.

Effect of chlorpromazine

Chlorpromazine (5 mg/kg) was given 30 min after (+)-methylamphetamine (5 mg/kg). Turning ceased within 15 min (Fig. 8) although at this time the amphetamine response in animals not treated with chlorpromazine is still maximal. Exploratory activity, however, was markedly increased after chlorpromazine and this level of exploratory activity was significantly higher ($P<0.001$) than in a control experiment in which the same rats were given a saline injection alone.
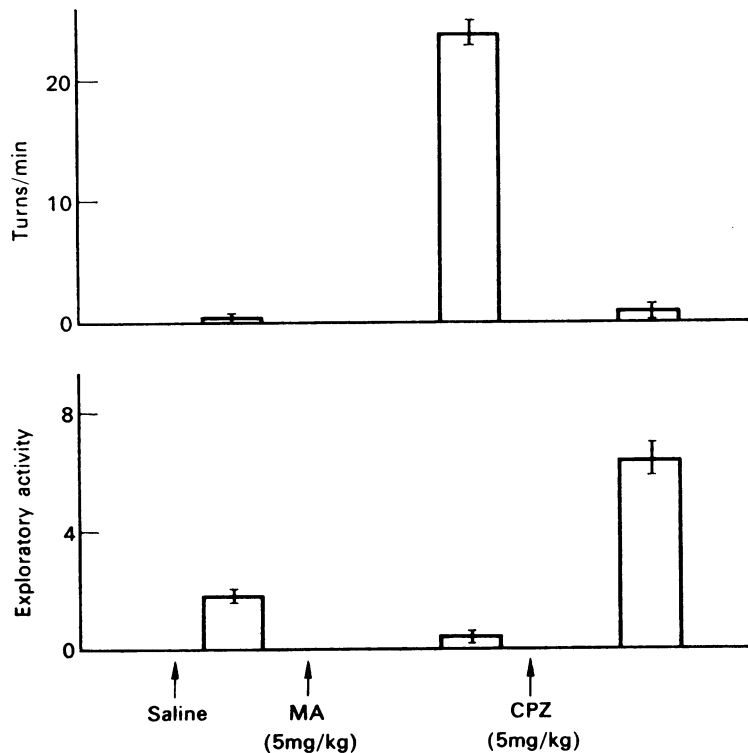

FIG. 8. Turns/min and exploratory activity recorded after a saline control injection ; 30 min after (+)-methylamphetamine (MA) (5 mg/kg) ; 15 min after chlorpromazine (CPZ) (5 mg/kg) (that is 45 min after the injection of (+)-methylamphetamine). Reduction of (+)-methyl-amphetamine induced turning after chlorpromazine $P<0.001$. Increase of exploratory activity after the (+)-methylamphetamine chlorpromazine combination from the saline control level $P<0.001$.

Discussion

Specific lesions in the region of the nigro-neostriatal tract alone cause amphet-amine-induced turning (Crow, 1971) and such lesions are associated with a depletion of dopamine in the ipsilateral striatum (Arbuthnott & Crow, 1971). Furthermore, unilateral stereotaxic injections of 6-hydroxydopamine which selectively destroy catecholamine-containing neurones, give results equivalent to those following electrolytic lesions (Ungerstedt, 1969; Ungerstedt & Arbuthnott, 1970). These findings are consistent with the hypothesis that turning results from excess dopamine release on one side of the brain, and somewhat similar turning results from elec-trical stimulation through electrodes implanted in the region of the substantia nigra (Arbuthnott & Crow, 1971). However, rats with unilateral nigro-neostriatal lesions do not turn unless treated with drugs. This may indicate either that the dopamine neurones are not normally active or that the animals can compensate for the absence of one nigro-neostriatal pathway.

The present pharmacological studies reinforce the case that turning behaviour is dependent upon an action on dopamine-containing neurones. Turning produced by methylamphetamine and (−)-ephedrine is substantially reduced by the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine but not by the dopamine-β-oxidase in-hibitor FLA63 at the time at which the depletion of noradrenaline produced by this drug is maximal.

There are several possible modes of action of the amphetamines: blockade of amine uptake at the nerve ending; monoamine oxidase inhibition and release of catecholamines from the nerve terminals (Carlsson, 1970; Fuxe & Ungerstedt, 1970). We have shown that turning is not produced by cocaine, which is the most effective agent inhibiting uptake of dopamine into striatal slices (Ross & Renyi, 1967), and that nialamide, a potent monoamine oxidase inhibitor, by itself produces only minimal turning (Andén, 1966). By exclusion therefore it seems likely that the actions of the amphetamines in eliciting turning reflect an active release of dopamine from the nerve terminals.

(+)-Amphetamine and (−)-amphetamine are equally effective in eliciting turning behaviour. Taylor & Snyder (1970) have shown that the relative potencies of these drugs on dopamine uptake are similar and these results suggest that (−)-amphet-amine may be used to influence central dopamine mechanisms with proportionately less effect, as Taylor & Snyder have demonstrated, on central noradrenaline stores.

The ephedrines initiate turning behaviour and this turning is not in any way reduced by FLA63, the dopamine-β-oxidase inhibitor, but is practically abolished by α-methyl-*p*-tyrosine. These results suggest that the ephedrines do not act directly but are dependent on the continuing synthesis of dopamine but not of nor-adrenaline. Ephedrines therefore probably resemble amphetamines in their action on central dopamine mechanisms.

The most potent of the ephedrines, (+)-norpseudoephedrine, was approximately 20 times less active than (+)-methylamphetamine. Presumably, the OH group, whether in the 1R position (as in (−)-ephedrine) or in the 1S position (as in (+)-norpseudoephedrine) reduces the action on central dopamine. (+)-Norpseudo-ephedrine, the most active in the series, is also the most active in increasing loco-motor activity (Fairchild & Alles, 1967). The interpretation of this result in structural terms is obscure. (+)-Pseudoephedrine differs from (+)-norpseudo-

ephedrine by the addition of a methyl group attached to the amine group on the αcarbon atom. This, however, is the difference between (+)-methylamphetamine and (+)-amphetamine, but in this instance (+)-methylamphetamine is the more active.

In view of the suggestion that the ephedrines release mainly granular stores (Rech & Stolk, 1970) the potentiation of the effects of ephedrines by reserpine is surprising. This action is comparable to the enhancement by reserpine of the actions of (+)-amphetamine on locomotor activity (Smith, 1963).

Turning produced by amphetamines is completely blocked by chlorpromazine (5 mg/kg). This appears to be a specific effect as the animals show no signs of sedation at this dose, and on the contrary exploratory activity is increased. It might be argued that the nature of the turning greatly limits exploratory activity but phenothiazine administration also increases exploratory activity in rats given similar doses of the amphetamines to induce stereotyped behaviour (Randrup & Munkvad, 1965 ; Del Rio & Fuentes, 1969). It is also pertinent that exploratory activity after the amphetamine-chlorpromazine combination is significantly higher than the control levels for the same rats given only a saline injection. The specificity of action of chlorpromazine against amphetamine-induced turning is compatible with the hypothesis that a major mode of action of the phenothiazines is to block dopamine receptors (Van Rossum, 1966 ; O'Keefe, Sharman & Vogt, 1970).

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