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Repeated treatment with imipramine or amitriptyline increases the locomotor response of rats to (+)-amphetamine given into the nucleus accumbens

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Effects of bilateral injections of (+)-amphetamine (5 μg/0·5 μl) into the nucleus accumbens were investigated in rats treated repeatedly with imipramine or amitriptyline. Repeated but not acute administration of either drug enhanced the locomotor hyperactivity induced by (+)-amphetamine given 2 or 72 h after the last dose of antidepressant. The results indicate that the increased responsiveness of the mesolimbic dopaminergic system may be involved in the action of antidepressant drugs.

We have recently demonstrated that prolonged, but not acute, treatment with various antidepressant drugs potentiates the locomotor hyperactivity induced by (+)-amphetamine given systemically to mice and rats (Maj 1984b; Maj et al 1984a). Similar effects in rats have been reported for some antidepressant drugs by other authors (Spyraki & Fibiger 1981; Martin-Iverson et al 1983; Arnt et al 1984). Apomorphine-induced behaviour, evaluated in the open field test in rats treated repeatedly with various antidepressants, was also enhanced (Maj et al 1984b), as has been reported for desipramine (Spyraki & Fibiger 1981). Since apomorphine- or (+)-amphetamine-induced stereotypy is not intensified after repeated administration of antidepressants (Delini-Stula & Vassout 1979; Maj et al 1979; Spyraki & Fibiger 1981), it may be supposed that the mesolimbic rather than the nigrostriatal dopamine (DA) system is the system modified when antidepressants are given repeatedly. We have therefore injected (+)-amphetamine locally into the nucleus accumbens, a structure of the mesolimbic DA system, of rats treated repeatedly with imipramine and amitriptyline, and measured the locomotor activity since in the rat, (+)-amphetamine injected into the nucleus accumbens induces locomotor hyperactivity mediated by DA (Pijnenburg et al 1975, 1976).

Method

Male Wistar rats, 150–220 g, were treated in groups of 6–8 with imipramine or amitriptyline (hydrochlorides) 10 mg kg^{-1} in 0·9% NaCl orally, acutely or repeatedly (twice a day for 14 days), controls receiving saline according to the same schedule, after surgical implantation of cannulae in the nucleus accumbens.

At the time of the surgery the rats were fixed in the David Kopff stereotaxic frame under pentobarbitone anaesthesia (Vetbutal, Biovet, 30 mg kg^{-1} i.p.). Steel cannulae (o.d. 0·4 mm) were then implanted bilaterally into the upper border of the nucleus accumbens (König & Klippel (1963) coordinates: A, 9·5; L, 1·9; H, 6·1 from the surface of the skull).

To avoid the ventricular system, an angle of 10° with a midsagittal plane was used. The cannulae were fixed to the skull with an acrylic dental cement and fastened with steel screws. The rats were left for 7 days before treatment with the antidepressants or saline.

(+)-Amphetamine sulphate (in distilled water), spiperone (in 0·1 M tartaric acid), or vehicle were injected into the nucleus accumbens bilaterally with internal cannulae (o.d. 0·3 mm) protruding from the guide cannula by 0·5 mm and attached to a Hamilton syringe with a Teflon tube. After injection, the internal cannula was left in-situ for 30 s. The volume of injections was always 0·5 μl.

Locomotor activity was measured in photoresistor actometers (two light beams) in which rats were placed individually and habituated for 30 min before intracerebral drug administration. (+)-Amphetamine was given in a dose of 5 μg/per side 2 or 72 h after the single dose or after the last dose (repeated treatment) of antidepressant or saline. Spiperone (0·5 μg per side) was injected 30 min before (+)-amphetamine. Locomotor activity was measured for 60 min, starting immediately after the injection of (+)-amphetamine.

At the end of experiments the rats were killed by an overdosage of pentobarbitone and the injection sites identified histologically. Data from animals having cerebral infections or in which the sites were not clearly within the nucleus accumbens were not used. Statistical significance was calculated according to Student's *t*-test.

Results

Acute or repeated treatment with imipramine did not change the locomotor activity compared with saline controls (Table 1). When given at a single dose of 10 mg kg^{-1} it did not affect the locomotor hyperactivity induced by (+)-amphetamine (5 μg/0·5 μl) (Table 1).

Bilateral injection of (+)-amphetamine (5 μg/0·5 μl) into the nucleus accumbens of rats treated repeatedly with saline induced an increase in the locomotor activity (Table 1). A similar injection (2 h after the last dose of imipramine) into rats treated repeatedly with imipramine induced a much stronger effect (about 370%). An increase in hyperactivity was also observed when

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(+)-amphetamine was injected 72 h after the last dose of imipramine (about 311%).

Table 1. The effect of imipramine (IMI) given acutely or repeatedly on the locomotor hyperactivity induced in rats by (+)-amphetamine (AMPH) injected into the nucleus accumbens.

Group	Treatment Orally	Into the n. accumbens	Locomotor activity counts (mean ± s.e.m.)	P (t-test)
I	Vehicle	Vehicle (2 h)	51·2 ± 13·3	
II	Saline	AMPH (2 h)	351·0 ± 57·0	II/I<0·001
III	IMI 1 ×	Vehicle (2 h)	46·8 ± 13·5	III/I n.s.
IV	IMI 1 ×	AMPH (2 h)	304·0 ± 62·0	IV/II n.s.
V	Saline 14 × 2	Vehicle (2 h)	46·9 ± 13·5	
VI	Saline 14 × 2	AMPH (2 h)	395·0 ± 121·0	VI/V<0·005
VII	IMI 14 × 2	Vehicle (2 h)	60·7 ± 8·6	VII/V n.s.
VIII	IMI 14 × 2	AMPH (2 h)	1481·0 ± 269·0	VIII/VII<0·001
IX	Saline 14 × 2	Vehicle (72 h)	51·1 ± 13·3	
X	Saline 14 × 2	AMPH (72 h)	235·0 ± 28·0	X/IX<0·005
XI	IMI 14 × 2	Vehicle (72 h)	69·1 ± 7·7	XI/IX n.s.
XII	IMI 14 × 2	AMPH (72 h)	732·0 ± 18·5	XII/X<0·001

Imipramine was given at a dose 10 mg kg^{-1} orally acutely (1 ×) or repeatedly twice a day for 14 days (14 × 2). (+)-Amphetamine was injected into the nucleus accumbens bilaterally (5 µg/0·5 µl/per side) 2 or 72 h after the single dose of imipramine or saline or after the last dose of drug (repeated treatment). (n = 6–8.) For other explanations see the text.

Amitriptyline given in a single dose of 10 mg kg^{-1} did not change the locomotor activity in normal rats or in rats stimulated by (+)-amphetamine injected into the nucleus accumbens (Table 2).

Repeated administration of amitriptyline did not affect the locomotor activity in normal rats but increased the locomotor hyperactivity induced by (+)-amphetamine (Table 2). Such a potentiation was observed at 2 h (about 289%) as well as 72 h (about 311%) after the last dose of amitriptyline (Table 2).

Spiperone injected bilaterally into the nucleus accumbens (0·5 µg/0·5 µl) decreased the locomotor activity in normal rats (12·0 ± 6·2) as well as the hyperactivity induced by (+)-amphetamine (given into the nucleus accumbens) in rats treated acutely with imipramine (177·4 ± 79·9) and repeatedly with saline (161·0 ± 69·5) or imipramine (303·5 ± 67·0).

Discussion

The results indicate that repeated, but not single, administration of imipramine or amitriptyline to rats enhances the locomotor hyperactivity induced by (+)-amphetamine injected into the nucleus accumbens. The effect was antagonized by spiperone similarly administered, and is thus mediated by DA receptors. Therefore, it may be concluded that repeated treatment with the antidepressants imipramine and amitriptyline, enhances the responsiveness of the DA mesolimbic system (nucleus accumbens) (Wędzony & Maj 1983).

The increased effect of (+)-amphetamine occurs at 2 and also at 72 h after the last dose of antidepressant. These times were chosen because at 2 h the brain concentrations of imipramine and its metabolite desipramine, and that of amitriptyline are high, whereas at 72 h the drugs and their metabolites are not detectable (Daniel et al 1982).

Table 2. The effect of amitriptyline (AMI) given acutely or repeatedly on the locomotor hyperactivity induced in rats by (+)-amphetamine injected into the nucleus accumbens.

Group	Treatment Orally	Into the n. accumbens	Locomotor activity counts (mean ± s.e.m.)	P (t-test)
I	Saline	Vehicle (2 h)	46·5 ± 12·4	
II	Saline	AMPH (2 h)	283·0 ± 24·2	III/I<0·001
III	AMI 1×	Vehicle (2 h)	39·1 ± 11·5	III/I n.s.
IV	AMI 1×	AMPH (2 h)	236·0 ± 27·8	IV/II n.s.
V	Saline 14 × 2	Vehicle (2 h)	51·2 ± 19·2	
VI	Saline 14 × 2	AMPH (2 h)	290·0 ± 28·5	VI/V<0·05
VII	AMI 14 × 2	Vehicle (2 h)	59·0 ± 23·4	VII/V n.s.
VIII	AMI 14 × 2	AMPH (2 h)	830·0 ± 51·2	VIII/V<0·001
IX	Saline 14 × 2	Vehicle (72 h)	47P0 ± 12·3	
X	Saline 14 × 2	AMPH (72 h)	265·0 ± 28·5	IX/IX<0·05
XI	AMI 14 × 2	Vehicle (72 h)	59·0 ± 18·5	XI/IX n.s.
XII	AMI 14 × 2	AMPH (72 h)	436·0 ± 38·6	XII/IX<0·05

For explanations see Table 1.

The site of the changes induced by repeated treatment with the two antidepressants is unclear. According to Martin-Iverson et al (1983) there are no changes in the binding of [^{3}H]spiperone to DA receptors in the nucleus accumbens. Our other results indicate that imipramine, mianserin and citalopram, administered repeatedly, do not affect the levels of DA, 3,4-dihydroxyphenylacetic acid, homovanillic acid or 3-methoxytyramine in the nucleus accumbens (Maj unpublished).

In conclusion, repeated treatment with imipramine or amitriptyline induces increased responsiveness of the DA mesolimbic system. Increased responsiveness of the noradrenaline system after repeated treatment with antidepressants has also been found (see: Maj 1984). Both the increased responsiveness of the DA mesolimbic system and noradrenaline system result in a similar behavioural effect—an increase in the locomotor activity. This raises the question whether either or both these effects may be involved in the drugs' therapeutic actions.

REFERENCES

Arnt, J., Hyttel, J., Overø, K. F. (1984) Pol. J. Pharmacol. Pharm. 36: 221–230

Daniel, W., Adamus, A., Melzacka, M., Szymura, J. (1982) J. Pharm. Pharmacol. 34: 678–680

Delini-Stula, A., Vassout, A. (1979) European J. Pharmacol. 58: 443–451

König, J. R., Klippel, R. A. (1963) The Williams and Wilkins Company, Baltimore

Maj, J. (1984) Pol. J. Pharmacol. Pharm. 36: 87–99

Maj, J., Mogilnicka, E., Kordecka, A. (1979) Neurosci. Lett. 13: 337–341

Maj, J., Rogóż, Z., Skuza, G., Sowińska, H. (1984a) J. Pharm. Pharmacol. 36: 127–130

Maj, J., Rogóż, Z., Skuza, G., Sowińska, H. (1984b) J. Neural Transm. 60: 273–282

Martin-Iverson, M. T., Leclere, J.-F., Fibiger, H. C. (1983) European J. Pharmacol. 94: 193–201

Pijnenburg, A. J. J., Honig, W. M. M., Van Rossum, J. M. (1975) Psychopharmacologia (Berlin) 41: 175–180

Pijnenburg, A. J. J., Honig, W. M. M., Van Der Heyden, J. A. M., Van Rossum, J. M. (1976) European J. Pharmacol. 35: 45–58

Spyraki, C., Fibiger, H. C. (1981) Ibid. 74: 195–206

Wędzony, K., Maj, J. (1983) 8th Congress of Polish Pharmacological Society, Warsaw, September 26–28, 1983, Abstract p. 181

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Dopamine receptor agonistic activities of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts

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In-vitro and in-vivo studies were used to evaluate the presynaptic dopamine receptor stimulating activities of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts. Bioassay results show that the *R*-enantiomer is 100 times more potent than the *S*-enantiomer in both in-vitro and in-vivo preparations.

Hacksell et al (1981) reported that *RS*-4-hydroxy-2-di-n-propylaminoindan is slightly less active or equipotent to apomorphine in stimulating central dopamine receptors. Recently, Cannon et al (1984) synthesized *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan. Several reports using cat hearts have demonstrated that when presynaptic dopamine receptors (DA-2) are stimulated there is an inhibition of positive chronotropic responses produced by stimulating the right postganglionic cardioaccelerator nerves in both in-vivo and in-vitro experiments (Long et al 1975; Ilhan et al 1976a, b). We have evaluated the presynaptic dopamine receptor stimulating actions of *R*- and *S*-enantiomers of 4-hydroxy-2-di-n-propylaminoindan in cat hearts both in-vitro and in-vivo.

Method

In in-vivo experiments, cats were anaesthetized by i.p. injection of sodium pentobarbitone (30 mg kg^{-1}). The trachea was cannulated, the animal artificially respirated and the thorax opened by a midline incision. Right postganglionic cardioaccelerator nerves were placed on a bipolar electrode for stimulation. Arterial blood pressure was measured from the cannulated left femoral artery using a Statham pressure transducer (P23AA). Heart rate was monitored by a cardiotachometer which was triggered by the systolic pulse pressure. Solutions of experimental compounds were administered i.v. through a catheter placed in the right femoral vein and the arterial blood pressure and heart rate were recorded on a multichannel oscillograph (Beckman, Model R611). Cardioaccelerator nerves were stimulated for 30 s every 5 min with a Grass S48 stimulator at 20–30 V using a pulse width of 2 ms and a frequency of 2 Hz. After three consecutive reproducible responses to stimulation had been obtained, compounds were injected.

In in-vitro experiments, cats were anaesthetized as before. Following midline thoracotomy the heart was excised and the right atrium isolated and suspended between 2 platinum electrodes in a 100 ml organ bath containing Feigen solution (mM): NaCl 154·0; KCl 4·6; $CaCl_2$ 5·6; $NaHCO_3$ 23·8 and glucose 11·1. The atrium was transmurally stimulated at 2 Hz, 5 ms duration and 100 V for 10 s. Atrial rate was monitored with a Beckman Model 9857B cardiotachometer. Feigen solution was aerated with 95% O_2 and 5% CO_2 and maintained at 37 °C. A Statham force displacement transducer, Beckman recorder and a Grass S48 stimulator were used. IC50 values and 95% confidence intervals were determined by probit analysis as described by Finney (1952).

Results

In the in-vivo experiments, both compounds caused dose-dependent inhibition of the tachycardic response to cardioaccelerator nerve stimulation. The ID50 values (with 95% confidence intervals) of the *R*- and *S*-enantiomers are 0·011 (0·008–0·015) μmol kg^{-1} (n = 4) and 1·29 (0·62–15·8) μmol kg^{-1} (n = 4), respectively. Compound-induced inhibition of tachycardia during neuronal stimulation was completely reversed by haloperidol (0·13 μmol kg^{-1}) which also completely reversed the bradycardic and hypotensive response to the highest dose of compounds (data not shown). The *R*-enantiomer (0·03 μmol kg^{-1}) caused 37·0 ± 9·0% decrease of blood pressure and basal heart rate respectively (n = 4) while, the *S*-enantiomer (1 μmol kg^{-1}) lowered arterial blood pressure and basal heart rate by 30·1 ± 11·2% and 19·0 ± 8·1%, respectively (n = 4).

Both the *R*- and *S*-enantiomers produced inhibition of stimulation-induced heart rate increases in isolated cat atria and the IC50 values (with 95% confidence

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