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Repeated treatment with amitriptyline reduces immobility in the behavioural 'despair' test in rats by activating dopaminergic and β-adrenergic mechanisms

F. Borsini, E. Nowakowska†, L. Pulvirenti, R. Samanin*, *Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milan, Italy*

Seven days of treatment with amitriptyline 10 mg kg^{-1} day^{-1}, reduced the immobility time in the behavioural 'despair' test in rats. 0·5, but not 0·25 mg kg^{-1} haloperidol significantly counteracted the reduction of immobility caused by amitriptyline. Its anti-immobility effect was reduced by 50 and 100 mg kg^{-1} sulpiride, another blocker of dopamine receptors, and 5 mg kg^{-1} (±)-propranolol, a β-adrenolytic drug. Prazosin, 3 mg kg^{-1}, an antagonist of post-synaptic α-adrenoceptors, had no effect. It is suggested that dopaminergic and β-adrenoceptors mediate the anti-immobility effect of repeated amitriptyline treatment in rats.

Rats repeatedly treated with desipramine show less immobility in the behavioural 'despair' test (Kitada et al 1981). This effect is reduced by atypical neuroleptics such as sulpiride, metoclopramide and clozapine but not by haloperidol or antiadrenergic agents (Borsini et al 1984). Repeated administration of amitriptyline also reduces rats' immobility in the behavioural 'despair' test by a mechanism which seems to involve an effect on presynaptic α-adrenoceptors (Zebrowska-Lupina 1980).

The present study examines whether blockade of dopamine transmission by sulpiride and haloperidol modifies the anti-immobility effect of amitriptyline. Furthermore, since repeated treatment increases noradrenergic transmission (Maj et al 1979a; Miyauchi et al 1981), the effect of propranolol, a β-adrenolytic drug (Wolfe et al 1978) or prazosin, a post-synaptic α-adrenoceptor antagonist (Fuller et al 1978), was also assessed.

Materials and methods

Animals. Male CD-COBS rats (Charles River, Italy), 210–250 g, were housed 5 to a cage, at constant room temperature (21 ± 1 °C) and relative humidity (60%), with free access to water and food. Each group consisted of 6–8 rats.

Measurement of immobility. Rats were placed individually in Plexiglas cylinders (height 40 cm, diameter 18 cm) containing 17 cm of water at 25 °C, and 15 min later they were removed to a 30 °C drying room for 30 min. For drug testing, animals were again placed in the cylinders and immobility was measured for 5 min. A rat was judged to be immobile when it remained floating in the water, in an upright position, making only very small movements necessary to keep its head above water.

The total duration of immobility during 5 min was recorded by an observer who did not know which treatments rats had received.

Drug treatment. Rats were treated intraperitoneally with amitriptyline hydrochloride (10 mg kg^{-1}) or with vehicle once daily for 7 consecutive days. All injections were made in the morning. The first dose was injected immediately after the 30 min drying period, the last dose 1 h before the 5 min test. The other drugs were given at doses, routes and pretreatment times reported to have a significant effect on adrenergic or dopaminergic mechanisms (the appropriate references for each compound are given in parentheses): (±)-propranolol hydrochloride 5 mg kg^{-1} i.p., 120 min (Borsini et al 1981); prazosin hydrochloride 3 mg kg^{-1} s.c., 90 min (Clineschmidt et al 1979); haloperidol 0·5 and 0·25 mg kg^{-1} i.p., 90 min (Ljungberg & Ungerstedt 1978); sulpiride (Dobren) 100 and 50 mg kg^{-1} i.p., 90 min (Ljungberg & Ungerstedt 1978).

Drugs. Amitriptyline hydrochloride (Lepetit, Milan, Italy) and (±)-propranolol hydrochloride (Icpharma, Milan, Italy) were dissolved in distilled water. Haloperidol (Lusofarmaco, Milan, Italy) was dissolved in distilled water with a few drops in 1 M HCl. Prazosin hydrochloride (Pfizer, Latina, Italy) was suspended in 1% carboxymethylcellulose. Sulpiride was administered as Dobren (Ravizza, Milan, Italy).

Statistics. Data were analysed by ANOVA factorial analysis followed by Tukey's test for unconfounded means.

Results

As shown in Table 1, none of the pretreatments significantly modified the immobility time of rats. Haloperidol 0·5, but not 0·25 mg kg^{-1} significantly counteracted the reduction of immobility caused by amitriptyline (haloperidol 0·5 mg kg^{-1} $P < 0·01$ $F = 6·0$ $df = 1/25$; haloperidol 0·25 mg kg^{-1} $P > 0·05$ $F = 0·8$ df

* Correspondence.
† Visiting scientist from Dept of Pharmacology and Pharmacy, Medical Academy, Poznan, Poland.

Table 1. Effect of catecholamine antagonists on the reduction of immobility caused by 7-days' treatment with 10 mg kg^{-1} day^{-1} amitriptyline (AMI).

Treatment	Dose mg kg^{-1}	Immobility time (s) Saline	AMI
Vehicle	—	209 ± 11	121 ± 26**
Haloperidol	0·25	217 ± 14	150 ± 21^{ns}
Haloperidol	0·50	219 ± 9	208 ± 20††
Vehicle	—	268 ± 5	141 ± 13**
Sulpiride	50	266 ± 6	194 ± 17†
Sulpiride	100	275 ± 4	252 ± 8††
Vehicle	—	215 ± 17	155 ± 11**
(±)-Propranolol	5	233 ± 7	225 ± 12††
Vehicle	—	231 ± 9	183 ± 11*
Prazosin	3	219 ± 12	198 ± 13^{ns}

Values are mean ± s.e. of 8–9 rats. The last dose of AMI was given 60 min before the test. Injection times before the test were 90 min for haloperidol, sulpiride and prazosin, 120 min for (±)-propranolol.

**$P < 0.01$; *$P < 0.05$ vs respective control group (Tukey's test).

††$P < 0.01$; †$P < 0.05$; ns = not significant (F interaction).

= 1/27). Sulpiride 100 mg kg^{-1} completely counteracted the effect of amitriptyline ($P < 0.01$ F = 42·1 df = 1/26) whereas 50 mg kg^{-1} caused only partial, but significant, antagonism ($P < 0.05$ F = 5·5 df = 1/26). Propranolol, 5 mg kg^{-1} also reduced the effect of amitriptyline ($P < 0.05$ F = 4·5 df = 1/30) whereas prazosin had no significant effect ($P > 0.05$ F = 1·4 df = 1/33).

Discussion

The fact that haloperidol and sulpiride blocked the effect of amitriptyline indicates that this antidepressant drug reduces the immobility of rats in the behavioural 'despair' test by enhancing dopamine transmission. This agrees with previous findings that repeated treatment with amitriptyline reduces the sensitivity of presynaptic inhibitory dopamine receptors (Serra et al 1979). Other authors found that a 7-day treatment with amitriptyline reduced apomorphine-induced stereotypy (Delini-Stula & Vassout 1979), suggesting that the drug had induced hypofunction in the central dopamine system. The apparent discrepancy may depend on the different brain areas involved in stereotypy and reduction of immobility in the behavioural 'despair' test. Stereotypy caused by apomorphine is commonly attributed to the drug's ability to act as agonist at post-synaptic dopamine receptors in the striatum (Costall et al 1974), whereas it has been suggested that repeated treatment with antidepressants selectively increases dopamine transmission in limbic areas such as the nucleus accumbens (Borsini et al 1984; Spyraki & Fibiger 1981). Maj et al (1979b) found that 14-days treatment with amitriptyline did not affect the stereotypy induced by apomorphine but increased apomorphine-induced fighting supporting the suggestion that treatment with the antidepressant had increased dopamine function in extrastriatal areas.

In addition to dopamine, a central noradrenergic mechanism appears to play a role in the effect of amitriptyline since propranolol significantly reduced its effect. The fact that prazosin had no effect indicates that β- but not α-adrenergic mechanisms are involved. That increased noradrenergic transmission, probably consequent to a reduction in presynaptic α-adrenergic activity, is involved in the anti-immobility effect of long-term treatment with amitriptyline was suggested by Zebrowska-Lupina (1980).

In conclusion, as previously shown for desipramine (Borsini et al 1984), blockade of dopamine transmission prevents the effect of amitriptyline in the behavioural 'despair' test in the rat. Its effect was blocked by both sulpiride and haloperidol whereas only sulpiride prevented the effect of desipramine (Borsini et al 1981). This might indicate, in agreement with the findings of Spyraki & Fibiger (1981), a more selective effect of desipramine on particular dopaminergic mechanisms in the brain. The less selective action is also suggested by the fact that 7-days' treatment with amitriptyline, unlike 7-day treatment with desipramine, also used β-adrenergic mechanisms to reduce immobility of rats in the behavioural 'despair' test.

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