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# Chronic treatment with iprindole reduces immobility of rats in the behavioural 'despair' test by activating dopaminergic mechanisms in the brain

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Iprindole, 10 mg $kg^{-1}$ i.p., once daily for 21 days, enhanced the metabolism of dopamine in the frontal cortex and striatum of rats with no effect in the nucleus accumbens 1 h after the last injection. Noradrenaline metabolism in the brainstem and telecephalon was also increased in these conditions. No effect on dopamine or noradrenaline metabolism was seen 24 h after the last injection. The same repeated treatment schedule with iprindole markedly reduced the immobility of rats in the behavioural 'despair' test 1 h after the last injection and the effect was prevented by 0·5 mg $kg^{-1}$ i.p. haloperidol and 100 mg $kg^{-1}$ i.p. sulpiride but not by 3 mg $kg^{-1}$ s.c. prazosin or 5 mg $kg^{-1}$ i.p. (±)-propranolol. The data show that enhanced metabolism of brain dopamine and noradrenaline is associated with the presence of iprindole during repeated treatment and the effect on dopamine mechanism is important in iprindole's ability to reduce rats' immobility in the behavioural 'despair' test.

Iprindole is a clinically effective antidepressant drug (Fann et al 1972; Rosloff & Davis 1978) which, unlike other tricyclic compounds, has little or no effect on monoamine uptake, release or receptors in in-vitro preparations or intact animals after a single dose (Randrup & Braestrup 1977; Hall & Ögren 1981). Because repeated treatment with iprindole enhanced amphetamine-induced hyper-locomotion in mice and rats (Spyraki & Fibiger 1981; Maj et al 1984) it was suggested that the activity of the mesolimbic dopamine (DA) system was increased in these animals. Chronic iprindole treatment has been found to cause subsensitivity of DA autoreceptors (Chiodo & Antelman 1980), variable effects on DA metabolism and no changes of DA receptor binding in the nigro-striatal system (Holcomb et al 1982; Diggory & Buckett 1984; Peroutka & Snyder 1980). However, no information is available on the effects of iprindole on DA-containing neurons in the nucleus accumbens and frontal cortex, areas known to influence amphetamine-induced hypermotility (Glowinski et al 1984).

In the present study DA metabolism was examined in these brain areas of rats which had received 10 mg $kg^{-1}$ iprindole once daily for 21 days. Since biochemical, behavioural and electrophysiological studies suggest that chronic iprindole affects central noradrenergic activity (Menkes & Aghajanian 1981; Sulser 1982; Maj et al 1982), the metabolism of brain noradrenaline (NA) was also measured in these animals. Recent studies have shown that DA is specifically involved in the ability of repeated treatment with desipramine to reduce immobility of rats in the behavioural 'despair' test (Borsini et al 1984) while the effect of amitriptyline seemed to involve both DA and NA mechanisms (Borsini et al 1985). We have, therefore, examined the ability of drugs which block DA and NA receptors to modify the anti-immobility effect of a 21-day iprindole treatment in rats. Three injections of 40 mg $kg^{-1}$ iprindole were previously reported to reduce significantly the immobility of rats in the behavioural 'despair' test (Porsolt et al 1978).

*Materials and methods*

Male CD-COBS rats (Charles River, Italy), 175–200 g at the beginning of the treatment, were housed 5 to a cage at constant room temperature (21 ± 1 °C) and relative humidity (60%) with free access to food and water. For the biochemical experiments, the rats received intraperitoneally 10 mg $kg^{-1}$ iprindole hydrochloride or distilled water once daily for 21 days and were decapitated 1 or 24 h after the last injection; the brain was quickly removed and dissected into striatum, nucleus accumbens, brainstem and remaining telencephalon according to Glowinski & Iversen (1966). The frontal cortex was separated from the remaining telencephalon by a perpendicular cut at the A 10·500 level of the König & Klippel atlas (1963) excluding tissue below the rhinal sulcus. Homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were measured in the striatum, nucleus accumbens and frontal cortex according to Wightman et al (1977) with minor modifications (Invernizzi & Samanin 1981). 3-Methoxy-4-hydroxyphenylethylene glycol sulphate (MHPG-$SO_4$) was measured in the brainstem and telencephalon according to Kohno et al (1979).

For the behavioural experiments, the 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. Immediately after the 30 min drying period, the animals received intraperitoneally 10 mg $kg^{-1}$ iprindole HCl or distilled water. This dose was administered once daily for 21 days. One hour after the last

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injection the 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. The other drugs were given at doses, routes and pretreatment times reported to have a significant effect on adrenergic or dopaminergic mechanism (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 mg $kg^{-1}$ i.p. 90 min (Ljungberg & Ungerstedt 1978); sulpiride 100 mg $kg^{-1}$ i.p. 90 min (Ljungberg & Ungerstedt 1978).

*Drugs.* Iprindole HCl (Wyeth, Taplow, UK) and (±)-propranolol HCl (Icipharma, Milan, Italy) were dissolved in distilled water. Haloperidol (Lusafarmaco, Milan, Italy) was dissolved in distilled water with a few drops of 1 M HCl. Prazosin HCl (Pfizer, Latina, Italy) was suspended in 1% carboxymethylcellulose. Sulpiride was administered as Dobren (Ravizza, Milan, Italy).

*Statistics.* Statistical analysis of the biochemical data was by Student's *t*-test. Behavioural data were analysed by ANOVA factorial analysis followed by Tukey's test for unconfounded means.

*Results*

As shown in Table 1, 1 h after the last of the repeated doses of 10 mg $kg^{-1}$ iprindole, DOPAC levels in the striatum and frontal cortex were significantly raised but no effect was found in the nucleus accumbens. HVA concentrations showed a similar pattern, although statistical significance ($P < 0·05$) was reached only in the striatum. No modifications of DA metabolites were observed in any brain area when the rats were killed 24 h after the last drug injection. MHPG-$SO_4$ concentrations in the brainstem and telencephalon were significantly increased 1 h, but not 24 h, after the last injection.

As shown in Table 2, none of the pretreatments significantly modified the immobility time of untreated rats. 0·5 mg $kg^{-1}$ haloperidol and 100 mg $kg^{-1}$ sulpiride significantly counteracted the reduction of immobility caused by iprindole (haloperidol $P < 0·01$ F = 35·4 df = 3/28; sulpiride $P < 0·01$ F = 51·5 df = 3/27) whereas no significant interaction was found between 3 mg $kg^{-1}$ prazosin or 5 mg $kg^{-1}$ propranolol and iprindole (prazosin $P > 0·05$ F = 0·9 df = 3/26; propranolol $P > 0·05$ F = 2·8 df = 3/36).

Table 2. Effect of catecholamine antagonists on the reduction of immobility caused by 21 days' treatment with 10 mg $kg^{-1}$ $day^{-1}$ iprindole HCl.

| Treatment | Dose mg $kg^{-1}$ | Immobility time (s) Controls | Iprindole |
|---|---|---|---|
| Vehicle | — | 239 ± 9 | 137 ± 17* |
| Haloperidol | 0·5 | 270 ± 4 | 282 ± 2† |
| Sulpiride | 100 | 214 ± 9 | 270 ± 6† |
| Vehicle | — | 237 ± 9 | 137 ± 20* |
| Prazosin | 3 | 234 ± 11 | 160 ± 10* |
| (±)-Propranolol | 5 | 236 ± 12 | 191 ± 20 |

Values are means ± s.e. of 8–9 rats. The last dose of iprindole was given 60 min before the test. Injection times before the test were 90 min for haloperidol, sulpiride and prazosin, 120 min for (±)-propranolol.
* $P < 0·01$ vs controls (Tukey's test)
† $P < 0·01$ (F interaction)

*Discussion*

Repeated treatment with iprindole enhanced DA metabolism in the striatum and frontal cortex with no effect in the nucleus accumbens. The changes in DA metabolism were seen 1 h, but not 24 h, after the last injection indicating that the presence of the drug is a prerequisite. Although a single dose of 10 mg $kg^{-1}$ iprindole had no effect, 40 mg $kg^{-1}$ produced effects on DA metabolites similar to those found after 21 days treatment with 10 mg $kg^{-1}$ (unpublished results). It is possible therefore that iprindole accumulated in the rat brain during repeated treatment as recently found in human plasma (Caillé et al 1982).

MHPG-$SO_4$ levels were significantly raised in the brainstem and telencephalon 1 h, but not 24 h, after the

Table 1. Effect of repeated treatment with iprindole on DA and NA metabolism in various brain regions of rats.

| Treatment | Time after last injection | Frontal cortex HVA | Frontal cortex DOPAC | Levels n. accumbens HVA | Levels n. accumbens DOPAC | ng $g^{-1}$ ± s.e. striatum HVA | striatum DOPAC | Brainstem telencephalon MHPG-$SO_4$ | |
|---|---|---|---|---|---|---|---|---|---|
| Vehicle | — | 16 ± 2 | 14 ± 1 | 432 ± 20 | 1292 ± 49 | 449 ± 27 | 781 ± 42 | 161 ± 11 | 103 ± 6 |
| Iprindole | 1 h | 22 ± 2 | 18 ± 1* | 448 ± 33 | 1318 ± 88 | 527 ± 22* | 988 ± 54* | 206 ± 5** | 134 ± 7** |
| Vehicle | — | 19 ± 1 | 14 ± 2 | 438 ± 13 | 1061 ± 33 | 443 ± 29 | 735 ± 60 | 180 ± 10 | 103 ± 11 |
| Iprindole | 24 h | 18 ± 2 | 10 ± 1 | 394 ± 21 | 991 ± 56 | 392 ± 29 | 711 ± 45 | 202 ± 13 | 119 ± 9 |

Animals received 10 mg $kg^{-1}$ iprindole HCl intraperitoneally once daily for 21 days. Each value is the mean of 7 determinations.
* $P < 0·05$ or ** $P < 0·01$ compared with vehicle (Student's *t*-test).

last injection of iprindole. A single dose of 10 mg $kg^{-1}$ iprindole had no effect on MHPG-$SO_4$ levels 1 h after injection (unpublished results).

A previous study showed no effect 12 h after the last of repeated doses of 10 mg $kg^{-1}$ iprindole twice daily for 10 days (Sugrue 1982). It seems therefore, as for DA, that the presence of the drug is necessary for the effect on NA metabolism.

One hour after the last injection, iprindole caused a marked reduction of rats' immobility in the behavioural 'despair' test and the effect was prevented by haloperidol and sulpiride but was not significantly modified by prazosin and propranolol. Thus it appears that brain dopamine, but not noradrenaline, is involved in the anti-immobility effect of iprindole. A similar suggestion has already been made for the anti-immobility effect of repeated treatment with desipramine in rats (Borsini et al 1984).

For the brain regions involved, the present data apparently exclude a role of presynaptic DA mechanisms in the nucleus accumbens. It has recently been shown that the amygdala is involved in the anti-immobility effect of desipramine and imipramine (Gorka et al 1979; Araki et al 1984), so correlations between effects in the behavioural 'despair' test and changes in dopamine function may perhaps be better in areas other than the nucleus accumbens. Considering the importance of the frontal cortex in the control of emotional behaviour (Glowinski et al 1984), it is of interest that DA metabolism in this area was increased in animals treated with iprindole.

Two recent studies showed an enhancement of amphetamine-induced hypermotility and stereotypy 3–8 days after withdrawal of repeated iprindole in rats (Spyraki & Fibiger 1981; Willner et al 1984). Amphetamine stereotypy was increased during iprindole administration as well but this effect was probably due to interference of the antidepressant with the metabolism of amphetamine (Freeman & Sulser 1972). Since no changes in DA metabolism are found in any brain region 24 h after the last iprindole injection, it is likely that the enhancement of amphetamine's effect after iprindole withdrawal depends on changes in DA pre- or post-synaptic receptor sensitivity. Subsensitivity of DA autoreceptors has been found after chronic treatment with iprindole (Chiodo & Antelman 1980), but no information is available on changes in post-synaptic DA receptors.

In conclusion, repeated treatment with iprindole increases the metabolism of brain catecholamines 1 h, but not 24 h, after the last injection and the effect on dopamine mechanisms is important for the ability of iprindole to reduce the immobility of rats in the behavioural 'despair' test.

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