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Immobility test: effects of 5-hydroxytryptaminergic drugs and role of catecholamines in the activity of some antidepressants

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Fenfluramine and *m*-chlorophenylpiperazine, two drugs purported to enhance central 5-hydroxytryptaminergic transmission, and metergoline, a 5-HT antagonist, did not modify the duration of immobility induced in rats made to swim in a restricted space. Nomifensine, desipramine and amineptine, three antidepressants known to block neuronal catecholamine uptake, significantly reduced the duration of immobility. Penfluridol, a dopamine antagonist at central receptors, counteracted the effect of nomifensine and amineptine but not that of desipramine. Propranolol and phenoxybenzamine respectively reduced the effects of desipramine and nomifensine but did not modify amineptine's effect. Metergoline pretreatment did not counteract the effect of any drug. The results indicate that various antidepressants can reduce the duration of immobility in rats by activating dopaminergic and/or noradrenergic mechanisms in the brain. Either α- or β- noradrenergic receptors could contribute to the anti-immobility effects, depending on the drug used. The immobility test appears to be insensitive to drugs activating or reducing 5-HT-ergic mechanisms in the brain.

Rats made to swim in a restricted space adopt a typical immobile posture after an initial period of vigorous attempts to escape (Porsolt et al 1977). Since most drugs known to be active in the treatment of human depression reduce the duration of immobility in this test, it was suggested that this may serve as a useful model for the study of antidepressants (Porsolt et al 1978). According to Porsolt et al (1979), drugs acting on 5-hydroxytryptamine (5-HT) are not particularly active in this test, whereas compounds known to modify central catecholaminergic transmission cause significant changes in the duration of immobility.

We have further investigated 5-HT-ergic involvement in this test using metergoline as a central 5-HT receptor blocker (Mawson & Whittington 1970) and *m*-chlorophenylpiperazine as a specific agonist at central 5-HT receptors (Samanin et al 1979). In addition we attempted to identify which of the two catecholamines is the more important in shortening the immobility induced by amineptine, desipramine (DMI) and nomifensine, three antidepressants known to affect differently catecholamine mechanisms in the brain (Hunt et al 1974; Schacht & Heptner 1974; Samanin et al 1975, 1977; Koe 1976; Dankova et al 1977). To this end we investigated how (±)-propranolol, phenoxybenzamine and penfluridol, blockers respectively of central β-adrenergic, α-adrenergic and dopamine receptors (Barrett & Cullum 1968; Bylund & Snyder 1976; Nose & Takemoto 1975; Andén & Strombom 1974) modified the action of these drugs. The specificity of catecholamine involvement was also assessed by studying their effect on duration of immobility in rats pretreated with metergoline.

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MATERIALS AND METHODS

Animals

Male CD-COBS rats (Charles River, Italy), 180–200 g, were housed at constant room temperature (21 ± 1 °C) and relative humidity (50%). Each experimental group consisted of 7 rats.

Measurement of immobility

Rats were individually placed in plexiglass cylinders (height 40 cm; diameter 18 cm) containing 15 cm of water maintained at 25 °C. After 15 min they were removed to a 30 °C drying room for 30 min. The next day, after drug administration, the animals were placed in the cylinders again for a 5 min test. A rat was judged to be immobile whenever 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 observers who did not know which treatments rats had received.

Drug treatment

The following drugs were given after the drying period by three i.p. injections 24, 5, and 1 h before the 5 min test: amineptine 10, 20, 40 mg kg^{-1}; metergoline maleate 1·25, 2·5, 5 mg kg^{-1}; *m*-chlorophenylpiperazine hydrochloride (mCPP) 1·25, 2·5, 5, 10 mg kg^{-1}; (±)-fenfluramine hydrochloride 2·5, 5, 10 mg kg^{-1}. When pretreatment was used, we selected 20 mg kg^{-1} DMI, 20 mg kg^{-1} amineptine and 5 mg kg^{-1} nomifensine since these doses had approximately the same effect in decreasing the duration of immobility. The pretreatment drugs were injected with the following schedule: (±)-propranolol hydrochloride (5 mg kg^{-1} i.p.), 25, 6 and 2 h before the test, metergoline maleate (1 mg kg^{-1} i.p.) and phenoxybenzamine hydrochloride (10 mg kg^{-1} i.p.) 26 and 6 h before the test, penfluridol (2·5 mg kg^{-1} oral) 26 h before the test. Penfluridol was suspended in carboxymethylcellulose 0·5%. Phenoxybenzamine was dissolved in a vehicle containing 1·7% ethanol (100%), 2·8% propylene glycol, 1·1% M HCl and distilled water to volume. Other drugs were dissolved in 0·9% NaCl (saline). Control animals were injected with vehicle alone.

Statistical analysis

Dunnett's test (two-tailed) was used for statistical analysis in the dose-response studies. The other data were analysed by ANOVA (2 × 2) factorial analysis followed by Tukey's test (Linton & Gallo 1975).

Drugs

Amineptine (Servier, Paris, France), nomifensine (Hoechst, Frankfurt, Germany), desipramine hydrochloride (Ciba-Geigy, Milan, Italy), metergoline maleate (Farmitalia, Milan, Italy), *m*-chlorophenylpiperazine (Angelini, Rome, Italy), (±)-fenfluramine hydrochloride (Servier, Paris, France), phenoxybenzamine hydrochloride (SKF, Philadelphia, Pa., U.S.A.), (±)-propranolol hydrochloride (Icpharma, Milan, Italy), penfluridol (Janssen, Beerse, Belgium).

RESULTS

As shown in Fig. 1, neither metergoline, mCPP nor fenfluramine, modified the total duration of immobility in rats, whereas amineptine, nomifensine and desipramine all reduced significantly the duration of immobility to different extents depending on doses. Pretreatment with penfluridol, which on its own prolonged immobility by about 25%, significantly counteracted the effects of amineptine and nomifensine but not of DMI (Fig. 2).

Fig. 3 shows the effect of propranolol on the reduction of immobility induced by amineptine, nomifensine and DMI. This pretreatment reduced the effect of DMI but not that of nomifensine or amineptine. The effect of nomifensine was partially prevented by phenoxybenzamine pretreatment (Fig. 4) while that of DMI and amineptine was unaffected. Propranolol and phenoxybenzamine when given alone did not significantly change the duration of

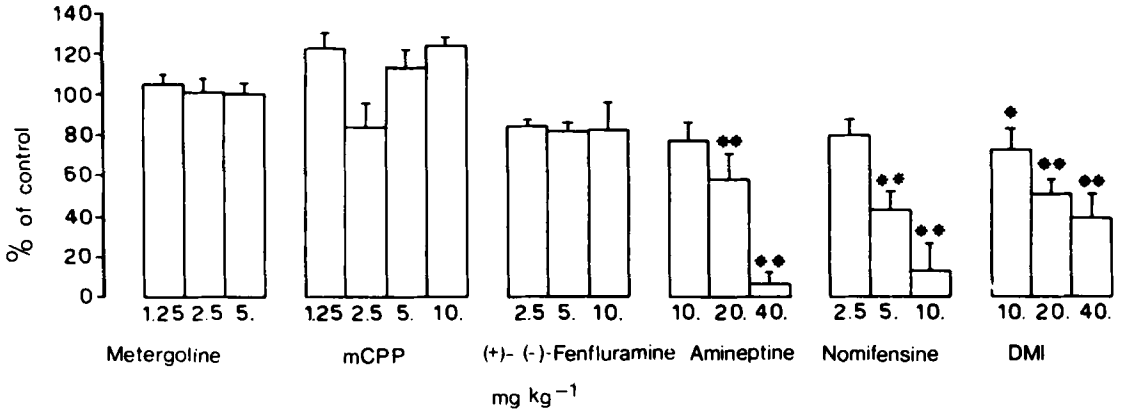

FIG. 1. Effects of metergoline, mCPP, (±)-fenfluramine, amineptine, nomifensine and DMI on total duration of immobility during a 5 min test. Each column represents the mean ± s.e. of 8 animals. Mean duration of immobilities (± s.e.) in control: 225 ± 15. The data were analysed by Dunnett's test (two-tails). $*P < 0.05$, $**P < 0.01$ compared with vehicle-treated rats.

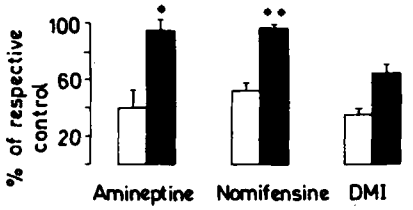

FIG. 2. Effect of penfluridol on the reduction of immobility induced by amineptine, nomifensine and DMI in rats. Each column represents the mean ± s.e. of 7 animals. Animals were injected i.p. with 20 mg kg^{-1} of amineptine, 5 mg kg^{-1} of nomifensine, 20 mg kg^{-1} of DMI. Penfluridol was administered orally, 2·5 mg kg^{-1}. Mean duration of immobility (s ± s.e.): in vehicle-treated rats: 202 ± 16, in penfluridol-treated rats: 257 ± 4. $*P < 0.05$, $**P < 0.01$ with ANOVA (2 × 2) test. Open columns: vehicle pretreated, closed columns: penfluridol pretreated rats.

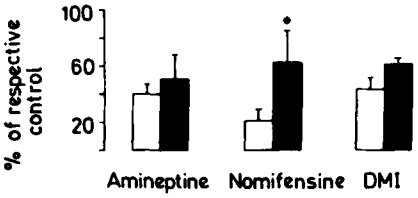

FIG. 4. Effect of phenoxybenzamine on the reduction of immobility induced by amineptine, nomifensine and DMI in rats. Each column represents the mean ± s.e. of 7 animals. Animals were injected i.p. with 20 mg kg^{-1} of amineptine, 5 mg kg^{-1} of nomifensine, 20 mg kg^{-1} of DMI. Phenoxybenzamine was injected i.p., 10 mg kg^{-1}. Mean duration of immobility (s ± s.e.) in vehicle-treated rats 249 ± 7, in phenoxybenzamine-treated rats: 180 ± 25. $*P < 0.05$ with ANOVA (2 × 2) test. Open columns: vehicle pretreated, solid columns: phenoxybenzamine pretreated rats.

immobility. As shown in Fig. 5, metergoline pretreatment did not modify the effect of nomifensine or DMI and significantly potentiated that of amineptine.

DISCUSSION

A major problem in the experimental study of depression is the lack of reliable models to predict the activity of antidepressant drugs. Recently Porsolt et al proposed 'the immobility test', which revealed not only the activity of classical drugs of the imipramine type but also that of non-typical antidepressants such as mianserine and iprindole, which are not detectable in classical screening tests (Porsolt et al 1978).

Since catecholamines and 5-HT in the brain have been involved in the etiology of depression (Van Praag & Korf 1971; Post et al 1973; Garelis et al 1974; Åsberg et al 1976; Garattini 1978), we examined whether drugs with known effects on these brain monoamines had an anti-immobility effect in this test. In agreement with Porsolt et al (1978), DMI and nomifensine, blockers respectively of neural noradrenaline and catecholamine uptake, significantly reduced the duration of immobility. In addition, amineptine, a new antidepressant (Roster 1979; Poignant 1979) with a more specific effect on dopamine uptake (Samanin et al 1977; Dankova et al 1977) also shortened immobility. These data indicate that either noradrenergic or dopaminergic mechanisms can be involved in the effect of the drugs examined.

A differential involvement of these mechanisms in the drug effects was shown by the ability of propranolol, a β-receptor blocker (Barrett & Cullum 1968; Bylund & Snyder 1976) to reduce the effect of DMI but not that of nomifensine, while penfluridol, a central dopamine antagonist (Nose & Takemoto 1975) significantly reduced the effect of nomifensine

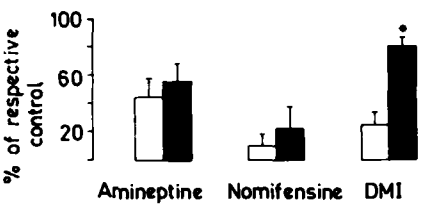

FIG. 3. Effect of (±)-propranolol on the reduction of immobility induced by amineptine, nomifensine and DMI in rats. Each column represents the mean ± s.e. of 7 animals. Animals were injected i.p. with 20 mg kg^{-1} of amineptine, 5 mg kg^{-1} of nomifensine, 20 mg kg^{-1} of DMI. (±)-Propranolol was injected i.p., 5 mg kg^{-1}. Mean duration of immobility (s ± s.e.): in vehicle-treated rats 222 ± 14, in propranolol-treated rats 238 ± 14. $*P < 0.05$ with ANOVA (2 × 2) test. Open columns: vehicle pretreated, solid columns: propranolol pretreated rats.

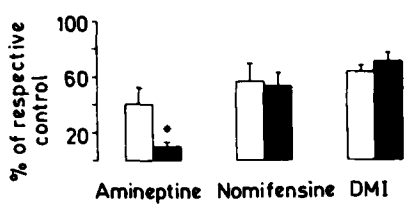

FIG. 5. Effect of metergoline on the reduction of immobility induced by amineptine, nomifensine and DMI in rats. Each column represents the mean ± s.e. of 7 rats. Animals were injected i.p. with 20 mg kg^{-1} of amineptine, 5 mg kg^{-1} of nomifensine, 20 mg kg^{-1} of DMI. Metergoline was injected i.p., 1 mg kg^{-1}. Mean duration of immobility (s ± s.e.): in vehicle-treated rats 229 ± 10, in metergoline treated rats 256 ± 11. $*P < 0.05$ with ANOVA (2 × 2) test. Open columns: vehicle pretreated, solid columns: metergoline pretreated rats.

and amineptine but not that of DMI. The anti-immobility effect of nomifensine was also reduced, although partially, by phenoxybenzamine, an α-adrenergic receptor blocker (Bylund & Snyder 1976; Andén & Strombon 1974), suggesting that besides the effect on dopamine, there may also be an α-adrenergic component in nomifensine reduction of rat immobility.

Porsolt et al (1979) assign to central α-adrenoceptors a primary role in mediating the effect of drugs acting on noradrenaline. The present data with propranolol, however, indicate that β-adrenoceptors might also be involved, at least in the action of DMI. That central β-receptors may have a more general role in antidepressant activity is indicated by the fact that salbutamol, a drug with β-adrenoceptor agonist properties, has been recently reported to have powerful antidepressant activity in man (Widlöcher et al 1977).

Central α- and β-adrenoceptors could mediate different aspects of antidepressant activity, which could be preferentially revealed by the particular experimental conditions used.

The specificity of the role of catecholamines in the anti-immobility effect of DMI, nomifensine and amineptine is shown by the fact that metergoline, a central 5-HT antagonist, did not counteract their effect. Metergoline actually potentiated the effect of amineptine. Several factors could contribute to this effect, including metergoline interfering with the metabolism and distribution of amineptine. However, only amineptine, which preferentially acts on dopaminergic mechanisms in the brain (Samanin et al 1977; Dankova et al 1977) was enhanced by metergoline.

Various studies suggest that changes in 5-HT-ergic function modify the activity of dopaminergic neurons in the brain and central 5-HT antagonists have been reported to enhance the effect of drugs believed to specifically activate dopaminergic function in the brain (Mabry & Campbell 1973; Cools 1974; Costall & Naylor 1978; Samanin & Garattini 1975; Tanner 1978; Giambalvo & Snodgrass 1978). Therefore, metergoline may have increased the effect of amineptine by releasing dopamine neurons from an inhibitory 5-HT-ergic influence.

In contrast to drugs acting on catecholamines, agents such as fenfluramine, mCPP and metergoline, at doses known to affect 5-HT-ergic mechanisms in the brain, did not significantly modify the duration of immobility in rats. It thus appears that the immobility test is not a suitable model for revealing antidepressant activities of drugs acting on 5-HT. The same conclusion was drawn recently by Porsolt et al (1979) and Gorka et al (1979), who found various 5-HT-ergic agents inactive in this test. The only exception, at variance with our data, was fenfluramine, a releaser of 5-HT and 5-HT uptake blocker (Kannengiesser et al 1976) which showed some anti-immobility effect in Porsolt test. Since fenfluramine can also act on brain catecholamines (Ziance & Rutledge 1972) it must be considered that this component could be revealed more or less depending on the different experimental conditions or drug doses used. The same holds true for mianserine and imipramine, which can act on both 5HT-ergic and noradrenergic mechanisms in the brain (Carlsson et al 1969a,b; Van Riezen 1972; Koe 1976; Raiteri et al 1976).

In conclusion, the present study shows that antidepressants such as desipramine, amineptine and nomifensine reduce the duration of immobility in rats by activating either noradrenergic or dopaminergic mechanisms or both. Independently of how useful it is in revealing antidepressant activity in man, the test may therefore serve for assessing drug ability to activate catecholaminergic mechanisms in the brain. The data show clearly that 5-HT agonists or antagonists are not active in this test. Obviously, this does not affect the possibility that drugs modifying central 5-HT mechanisms may be useful in certain forms of human depression.

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