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COMMUNICATIONS

Mesulergine antagonism towards the fluoxetine anti-immobility effect in the forced swimming test in mice

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Abstract—The anti-immobility effect of fluoxetine (40 mg kg^{-1}) in the forced swimming test in mice was antagonized by the 5-$HT_{1c/2}$ antagonist mesulergine (7·5 mg kg^{-1}) and the dopamine D_2 antagonist (±)-sulpiride (12.5 mg kg^{-1}) but not by the 5-$HT_{2/1C}$ antagonist ritanserine (2 mg kg^{-1}), the 5-$HT_{1A/1B}$ antagonist (−)-propranolol (20 mg kg^{-1}) or the 5-HT_3 antagonist DAU 6215 (0·1 mg kg^{-1}). All compounds were administered intraperitoneally (i.p.) 6 min before fluoxetine, given i.p. 30 min before testing. The anti-immobility effect of fluoxetine was also prevented by pretreatment with *p*-chlorophenylalanine (300 mg kg^{-1} twice daily for 3 days) which produced an 80% reduction of 5-HT in brain. The results suggest that fluoxetine reduces immobility time in mice forced to swim, by acting indirectly through a mesulergine-sensitive site, probably the 5-HT_{1C} receptor.

The problem of which subtype of 5-hydroxytryptamine (5-HT) receptor is mainly involved in the activity of antidepressants in behavioural animal models of depression is still unresolved. In a recent paper (Borsini et al 1991), we have suggested a contribution of 5-HT_{1C} receptors in the mechanism of the imipramine effect in the forced swimming test in mice. This finding was in agreement with studies by Broekkamp & Berendsen (1992) which showed that the effects of the 5-HT-uptake inhibitors in their behavioural animal models, could be mimicked by 5-HT_{1C}-receptor agonists. In addition, the 5-HT_{1C} agonist *m*-chlorophenylpiperazine (mCPP), a metabolite of the antidepressant trazodone, has recently been shown to exert antidepressant actions in man (Mellow et al 1990). The aim of the present work was to investigate further the role of 5-HT_{1C} receptor stimulation in the antidepressant-induced reduction of the immobility time in this test. For this purpose we have utilized the antidepressant fluoxetine, which affects the 5-HT-ergic system by blocking the synaptic uptake of 5-HT more selectively than imipramine (Hall et al 1984).

Materials and methods

Animals. Male Crl:CD-1 Swiss mice (Charles River, Calco, Italy), 22–28 g, were housed 10 to a cage, at constant room temperature (21 ± 1°C) and relative humidity (55 ± 5%), with water and food freely available, and a 12 h light–dark cycle (light on: 0700 h). The light intensity was 350 lux, 1 m from the floor. Each experimental group was chosen according to a completely randomized schedule (Borsini 1985). Experiments were carried out between 1000 and 1300 h.

Forced swimming test. The test was performed according to Porsolt et al (1977). A 1000 mL Pyrex beaker (14 cm height, 12 cm internal diam.) was filled with water (20–22°C) up to a level of 7·5 cm from the bottom. The animal was placed in the cylinder and left for 6 min. Duration of immobility was assessed throughout the last 4 min period by an observer unaware of the treatment. A mouse was judged to be immobile when it remained floating, making only the movements necessary to keep its head above water. Groups of 10 mice each were used.

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Drug treatments. Fluoxetine was administered 30 min before the forced swimming test. The antagonists were administered 6 min before fluoxetine. The dose of each compound, with the exception of *p*-chlorophenylalanine (pCPA) refers to the base. All compounds were administered intraperitoneally in a volume of 20 mL kg^{-1}.

The doses of antagonists were chosen on the basis of published data regarding mice and non-interference with the swimming of mice: ritanserine was used at 2 mg kg^{-1}, 10-fold the dose blocking to some extent 5-HT_2-mediated behaviour (Goodwin & Green 1985); (−)-propranolol was used at a dose of 20 mg kg^{-1} which has been reported as possibly antagonizing 5-HT_1-mediated behaviour (Goodwin & Green 1985); DAU 6215 was used at a dose of 0·1 mg kg^{-1} which is 10 times greater than the dose shown to exert pharmacological effects through blockade of 5-HT_3 receptors (Borsini et al 1990).

Brain 5-HT was depleted by pCPA administration. The pCPA schedule of treatment (300 mg kg^{-1}, i.p., twice daily for 3 days) was chosen on the basis of biochemical assays (Table 1) in order to obtain a marked reduction of brain 5-HT content. The pCPA-treated animals were tested 18 h after the last pCPA administration.

Drugs and sources. The following drugs were used: fluoxetine hydrochloride (Menarini), mesulergine (Sandoz), (±)-sulpiride as Dobren (Ravizza), ritanserine (R.B.I.), (−)-propranolol hydrochloride (Sigma), (±)-4-chlorophenylalanine methyl ester hydrochloride (Aldrich). DAU 6215 (endo-2,3-dihydro-*N*-(8-methyl-8-azabicyclo [3.2.1.]oct-3-yl)-2-oxo-1H-benzimidazole-1-carboxamide) hydrochloride was synthesized in the Chemical Department of Boehringer Ingelheim, Italy. All drugs were dissolved in 0·9% NaCl (saline). Ritanserine was solubilized by means of a few drops of 0·1 M HCl.

5-HT assay. Groups of five mice were treated with different doses of pCPA or saline as reported in Table 1. Twenty-four or eighteen (treated once or twice daily, respectively) hours after the last pCPA injection, animals were killed by cervical dislocation. The brain was removed quickly, the cerebellum was dissected out and the remaining tissues were frozen by dry ice and stored at −80°C until assay. The amount of 5-HT in brain tissue was measured by HPLC with electrochemical detection (Invernizzi et al 1991). Samples were homogenized using an ultraturax with 0·4 M $HClO_4$ (1:10 w/v) and centrifuged at 17000 *g* for 10 min. Supernatant (50 μL) was injected onto a reverse-phase column (Chrompack, 200 × 3 mm, packed with Nucleosil) using the following mobile phase: 0·15 M chloroacetic acid, 37 mg L^{-1} Na_2EDTA and 15% methanol adjusted to pH 3 with 10 M NaOH. 5-HT was oxidized at +0·45 V with a graphite paste electrode (Coulochem detector, model 5100A with analytical cell model 5011 ESA, USA).

Table 1. Effects of *p*-chlorophenylalanine (pCPA) on 5-HT in mouse-brain (excluding cerebellum).

Dose of pCPA (mg kg^{-1}, i.p.)	Treatment for 3 days	Brain 5-HT (ng (g tissue)$^{-1}$)	% Variation vs control
0	Once daily	538±40	—
300	Once daily	209±39*	−61
0	Once daily	597±116	—
400	Once daily	248±48*	−58
150	Twice daily	196±60*	−67
0	Twice daily	665±133	—
300	Twice daily	134±42*	−80
450	Twice daily	103±11*	−85

Brains were removed 24 h (once daily treated mice) or 18 h (twice daily treated mice) after the last pCPA injection. Values are the mean±s.e. of five mice. *$P<0.01$ compared with corresponding control.

Statistics. Brain 5-HT was assessed by Student's *t*-test for unpaired data. The results relative to the dose-response of fluoxetine in the forced swimming test were analysed by Dunnett's test (two-tailed). For the evaluation of the antagonism, data were analysed by factorial analysis of variance and Tukey's test was used for the post-hoc comparisons.

Results

In the forced swimming test, the 5-HT uptake blocker fluoxetine dose-dependently reduced mice immobility time (Table 2). Mesulergine (7.5 mg kg^{-1}) and (±)-sulpiride (12·5 mg kg^{-1}) antagonized the anti-immobility effect of fluoxetine (40 mg kg^{-1}) whereas 3·75 mg kg^{-1} mesulergine, 6·25 mg kg^{-1} (±)-sulpiride, 20 mg kg^{-1} (−)-propranolol, 2 mg kg^{-1} ritanserine and 0·1 mg kg^{-1} DAU 6215 did not exert any antagonism (Table 3). None of the antagonists modified the level of immobility. The pCPA treatment, which produced an 80% reduction of 5-HT in the whole brain (Table 1), had no effect on immobility time when compared with saline treatment, but antagonized the anti-immobility activity of 40 mg kg^{-1} fluoxetine (Table 4).

Discussion

Fluoxetine is a potent and selective 5-HT-uptake blocker (Richelson & Pfenning 1984) which induces an anti-immobility effect in mice forced to swim (De Graaf et al 1985). Pretreatment with *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor which lowered brain 5-HT by blocking 5-HT synthesis, antagonized this fluoxetine effect. This antagonism suggests that presynaptic rather than post-synaptic 5-HT mechanisms are involved in the fluoxetine-induced anti-immobility action. As observed for imipramine (Borsini et al 1991), the anti-immobility activity of fluoxetine was blocked by mesulergine, which preferentially binds to 5-HT$_{1C}$ rather than to 5-HT$_2$ receptors (Van Wijngaarden et al 1990). The failure of ritanserine, which preferentially binds to 5-HT$_2$ rather than 5-HT$_{1C}$ receptors (Van Wijngaarden et al 1990), to antagonize fluoxetine activity suggests that 5-HT$_{1C}$ receptors, instead of 5-HT$_2$ receptors, are mainly involved in the mechanism of action of fluoxetine. It must be pointed out, however, that the blockade of 5-HT$_{1C/2}$ receptors by mesulergine may also be observed at doses lower than that used herein. In fact, mesulergine is able to antagonize head-twitches induced by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane at a dose of 0.2 mg kg^{-1} intraperitoneally (Rizzi et al 1991). Furthermore, it should be mentioned that mesulergine has an affinity for dopamine D$_2$ receptors which is only one-tenth its affinity for 5-HT$_{1C}$ receptors (Van Wijngaarden et al 1990). Thus we cannot exclude that an interference of mesulergine with dopamine D$_2$ can play a role in this antagonistic activity. Mesulergine has been reported to exert dopamine mimetic activity (Fluckinger et al 1979; Lamberts et al 1984) and an activation of D$_2$ receptors leads to an anti-immobility effect in the forced swimming test (Duterte-Boucher et al 1988). From this point of view, addition or potentiation, rather than antagonism towards fluoxetine-induced anti-immobility effects should be expected after mesulergine administration. The lack of affinity of fluoxetine for dopamine D$_2$ receptors (Hall et al 1984) seems to rule out a direct interaction between mesulergine and fluoxetine on these receptors. However, it must be pointed out that a dopaminergic antagonistic activity has also been reported for mesulergine (Stahle & Ungerstedt 1987) and that an active metabolite can contribute to the pharmacological effects of

Table 2. Effect of fluoxetine in the forced swimming test in mice.

	Dose (mg kg^{-1}, i.p.)	Immobility time (s)	% Variation vs control
Vehicle	—	219±6	—
Fluoxetine	15·0	201±7	−8
	30·0	190±10*	−13
	40·0	180±8**	−18

The compound was administered 30 min before testing. Values are the mean±s.e. of 10 mice. Two-tailed Dunnett's test: *$P<0.05$, **$P<0.01$ compared with value for vehicle alone.

Table 3. Effects of some antagonists on the reduction of immobility induced by fluoxetine in the forced swimming test in mice.

Pretreatment	Dose (mg kg^{-1})	Immobility (*s*) Saline	Immobility (*s*) Fluoxetine	Analysis of variance 2×2 F_{int} (df)
Vehicle	—	235±3	183±7*	7·0 (1/36)
Mesulergine	7·5	226±5	210±10#	$P<0.05$
Vehicle	—	226±5	183±9*	1·9 (1/36)
Mesulergine	3·75	223±5	198±7	NS
Vehicle	—	230±5	133±23*	7·0 (1/36)
Sulpiride	12·5	215±9	196±15#	$P<0.05$
Vehicle	—	214±6	147±17*	0·2 (1/36)
Sulpiride	6·25	217±5	139±18	NS
Vehicle	—	228±5	176±13*	1·1 (1/36)
Ritanserine	2·0	217±5	186±13	NS
Vehicle	—	232±5	160±14*	0·2 (1/36)
(−)-Propranolol	20	224±4	162±18	NS
Vehicle	—	226±8	165±12*	0·1 (1/36)
DAU 6215	0·1	218±6	161±8	NS

Antagonists were given 6 min before fluoxetine (40 mg kg^{-1}, i.p.) administered 30 min before testing. The values represent the mean±s.e. from 10 mice. Tukey's test: *$P<0.01$ vs control group; #$P<0.01$ vs respective vehicle group; NS = not significant.

Table 4. Effect of pCPA pretreatment on the reduction of immobility induced by fluoxetine in the forced swimming test in mice.

Pretreatment	Immobility time (s) Saline	Immobility time (s) Fluoxetine	Analysis of variance 2×2 F_{int} (df)
Vehicle	213±7	159±6*	5·2 (1/36)
pCPA	206±8	187±10#	$P<0.05$

pCPA was given at 300 mg kg^{-1}, i.p. twice daily for 3 days, the last injection 18 h before the test. Fluoxetine (40 mg kg^{-1}, i.p.) was administered 30 min before testing. The values represent the mean±s.e. from 10 mice. Tukey's test: *$P<0.01$ vs control group; #$P<0.05$ vs vehicle group.

mesulergine (Enz 1981). The anti-immobility activity of fluoxetine is antagonized by (±)-sulpiride, a dopamine D_2 blocker which does not show affinity for 5-$HT_{2/1C}$ receptors (Canton et al 1990). Therefore, a possible antidopaminergic action of mesulergine in blocking fluoxetine effects cannot be excluded. Neither 5-HT_{1A}-receptor blockade by (−)-propranolol (Middlemiss et al 1977; Goodwin & Green 1985) nor 5-HT_3-receptor blockade by DAU 6215 (Turconi et al 1990) modified the fluoxetine-induced anti-immobility effects. Therefore, if mesulergine should counteract fluoxetine effects through dopamine blockade, and not through 5-HT_{1C} blockade, one should consider which type of 5-HT receptor mediates the fluoxetine action. The failure of the 5-HT_{1A} antagonist (−)-propranolol to antagonize the activity of fluoxetine in the forced swimming test in mice does not appear so surprising because a similar finding was also reported for imipramine (Borsini et al 1991). However, how much (−)-propranolol is appropriate to block 5-HT_{1A} receptors in the brain regions important for the swimming test remains open. The present results suggest that, like imipramine, fluoxetine reduces immobility time in mice forced to swim by acting through mesulergine-sensitive sites, probably the 5-HT_{1C} receptors.

References

Borsini, F. (1985) Randomization program for Apple IIe computer. Brain Res. Bull. 15: 279–281

Borsini, F., Templeton, D., Turconi, M., Nicola, M., Schiantarelli, P., Donetti, A. (1990) Anxiolytic effects of a new structural class of 5-HT_3 antagonists. Psychopharmacology 101: S7

Borsini, F., Cesana, R., Vidi, A., Mennini, T. (1991) Evidence that imipramine activates 5-HT_{1C} receptor function. Eur. J. Pharmacol. 203: 359–363

Broekkamp, C. L. E., Berendsen, H. H. G. (1992) Serotonin receptor subtypes for antidepressant effects. International symposium on the role of serotonin in psychiatric disorders. Castres, France, June 24–26, p. 14

Canton, H., Verrièle, L., Colpaert, F. C. (1990) Binding of typical and atypical antipsychotics to 5-HT1C and 5-HT2 sites: clozapine potently interacts with 5-HT1C sites. Eur. J. Pharmacol. 191: 93–96

De Graaf, J. S., van Riezen, H., Berendsen, H. H. G., van Delft, A. M. L. (1985) A set of behavioural tests predicting antidepressant activity. Drug Dev. Res. 5: 291–301

Duterte-Boucher, D., Leclère, J. F., Panissaud, C., Constentin, J. (1988) Acute effects of direct dopamine agonists in the mouse behavioural despair test. Eur. J. Pharmacol. 154: 185–190

Enz, A. (1981) Biphasic influence of a 8-alpha-amino-ergoline, CU 32-085, on striatal dopamine synthesis and turnover in vivo in the rat. Life Sci. 29: 2227–2234

Fluckinger, E., Briner, U., Burki, H. R., Marbach, P., Wagner, H. R., Doepfner, W. (1979) Two novel prolactin release-inhibiting 8-alpha-amino-ergolines. Experientia 35: 1677–1678

Goodwin, G. M., Green, R. (1985) A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists of 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmacol. 84: 743–753

Hall, H., Sallemark, M., Wedel, I. (1984) Acute effects of atypical antidepressants on various receptors in the rat brain. Acta Pharmacol. Toxicol. 54: 379–384

Invernizzi, R., Carli, M., Di Clemente, A., Samanin, R. (1991) Administration of 8-hydroxy-2-(*di-n-propylamino*) tetralin in raphe nuclei dorsalis and medianus reduces serotonin synthesis in the rat brain: differences in potency and regional sensitivity. J. Neurochem. 56: 243–247

Lamberts, S. W. J., Klyn, J. G. M., Oosterom, R. (1984) Mechanism of action and tolerance of mesulergine. Clin. Pharmacol. Ther. 36: 620–627

Mellow, A. M., Lawlor, B. A., Sunderland, T., Mueller, E. A., Molchan, S. E., Murphy, D. (1990) Effects of *m*-chlorophenylpiperazine in elderly depressed patients. Biol. Psychiatry 28: 588–594

Middlemiss, D. N., Blakeborough, L., Leather, S. R. (1977) Direct evidence for an interaction of beta-adrenergic blockers with the 5-HT receptors. Nature 267: 289–290

Porsolt, R. D., Bertin, A., Jalfre, M. (1977) Behavioural despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. 229: 327–336

Richelson, E., Pfenning, M. (1984) Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepinephrine uptake. Eur. J. Pharmacol. 104: 277–286

Rizzi, C. A., Prudentino, A., Borsini, F. (1991) Evaluation of drug-induced head twitches as a model to detect 5-$HT_{1C/2}$ receptor mediated functions. International meeting on Serotonin 1991, 14–17 July, Birmingham, p. 106

Stahle, L., Ungerstedt, U. (1987) One of the mode of action of six putative dopamine receptor agonists on suppression of exploratory behaviour in rats. Psychopharmacology 91: 139–146

Turconi, M., Nicola, M., Gil Quintero, M., Maiocchi, L., Micheletti, R., Giraldo, E., Donetti, A. (1990) Synthesis of a new class of 2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylic acid derivatives as highly potent 5-HT_3 receptor antagonists. J. Med. Chem. 33: 2101–2108

Van Wijngaarden, I., Tulp, M. T. M., Soudijn, W. (1990) The concept of selectivity in 5-HT receptor research. Eur. J. Pharmacol. 188: 301–312