ELSEVIER

European Journal of Pharmacology 364 (1999) 147–150

ejp

Short communication

Citalopram elicits a discriminative stimulus in rats at a dose selectively increasing extracellular levels of serotonin vs. dopamine and noradrenaline

Mark J. Millan *, Alain Gobert, Sylvie Girardon, Anne Dekeyne

Department of Psychopharmacology, Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Seine, Paris, France

Received 13 August 1998; revised 27 October 1998; accepted 30 October 1998

Abstract

Citalopram (2.5 mg/kg, i.p.) increased (+145–+180%) extracellular levels of serotonin (5-hydroxytryptamine, 5-HT) in the frontal cortex, nucleus accumbens and striatum of freely-moving rats, whereas dopamine and noradrenaline were unaffected. At this dose, employing a two-lever, food-reinforced, drug discrimination procedure, citalopram generated reliable recognition and fully (>80%) generalized to itself with an Effective $Dose_{50}$ (ED_{50}) of 0.1 mg/kg, s.c. Two further selective 5-HT reuptake inhibitors, sertraline and paroxetine, fully generalized with ED_{50}s of 0.01 and 0.04 mg/kg, s.c., respectively. In contrast, the anxiolytic, diazepam (0.63), and the antipsychotic, clozapine (2.5), did not (≤20%) generalize. In conclusion, the selective 5-HT reuptake inhibitor, citalopram, elicits a pharmacologically-specific discriminative stimulus in rats at a dose *selectively* elevating extracellular concentrations of 5-HT.

Keywords: Drug discrimination; Microdialysis; 5-HT (5-hydroxytryptamine, serotonin) reuptake; Depression; Antidepressant; Selective 5-HT reuptake inhibitor

1. Introduction

Drug discrimination procedures have been proven of considerable importance in the characterization of anxiolytics, stimulants, drugs of abuse and other classes of psychotic agent (Stolerman et al., 1989). However, little information is available concerning antidepressants. While the 'atypical' antidepressant, mianserin, elicits a discriminative stimulus (Kelley et al., 1995), it does not affect monoamine uptake and its discriminative stimulus properties reflect antagonism of muscarinic receptors, an action underlying its side-effects rather than therapeutic actions. Further, although imipramine generates a discriminative stimulus in pigeons (Zhang and Barrett, 1991), the toxic actions of tricyclic agents has precluded pharmacological examination of their potential discriminative stimulus properties in rodents and primates (Jones et al., 1980; Schechter, 1983). Most notably, the discriminative stimulus properties of selective 5-hydroxytryptamine (5-HT) reuptake inhibitors have not been described, despite their key importance in the clinical treatment of depression (Frazer, 1997).

So that a selective 5-HT reuptake inhibitor might successfully generate a discriminative stimulus, its ability to increase synaptic 5-HT levels should be maintained upon repeated administration. Further, this increase should be transient: 24 h following administration, at the time of vehicle administration, extracellular 5-HT levels must be normal. Citalopram, which possesses a short half-life, fulfills these conditions (Arborelius et al., 1996; Gobert, A., unpubl. obs.) and was selected for the present work. However, certain selective 5-HT reuptake inhibitors also increase extracellular levels of dopamine and/or noradrenaline in vivo (Tanda et al., 1994; Gobert et al., 1997). Thus, in a parallel study, we determined whether citalopram *selectively* enhances extracellular levels of 5-HT, as compared to those of dopamine and noradrenaline simultaneously quantified in single dialysate samples of the frontal cortex, nucleus accumbens and striatum.

* Corresponding author. Tel.: +33-1-41-18-24-41; Fax: +33-1-41-18-24-70

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00802-4

2. Methods

Male Wistar rats (200–220 g; Iffa Credo, L'Arbresle, France) were implanted with a cannula in the frontal cortex, or in both nucleus accumbens and the contralateral striatum (Millan et al., 1997). Five days later, a concentric dialysis probe was introduced and, 2 h later, dialysis commenced. Three basal samples (20 μl) were collected before drug administration. Samples were taken for 3 h. Serotonin, dopamine and noradrenaline were simultaneously quantified by high performance liquid chromatography/coulometric detection (Millan et al., 1997; Gobert et al., 1998). Separate rats were trained to discriminate citalopram (2.5 mg/kg, i.p.) from saline with a standard two-lever, fixed-ratio 10, food-reinforced procedure (Schreiber et al., 1995). Each 15-min daily session commenced 15 min after injection. Drug or saline sessions alternated randomly. The discrimination criterion was 10 consecutive sessions with correct responding (less than 13 responses on both levers before first reinforcement). Thereafter, substitution tests were conducted twice weekly: test drugs were administered s.c. and the selected lever was that for which 10 responses were first recorded. All animal use procedures conformed to international European ethical standards (86/609-CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

3. Results

Citalopram (2.5 mg/kg, i.p.) markedly increased extracellular levels of 5-HT in frontal cortex, nucleus accumbens and striatum of freely-moving rats, whereas there was no alteration in levels of dopamine or noradrenaline (Fig. 1). At this dose, the mean ± S.E.M. number of training sessions to citalopram discrimination criterion was 42 ± 6 (Fig. 2). Citalopram, paroxetine and sertraline, administered s.c., dose-dependently and fully (> 80%) generalized (Fig. 2). In distinction, diazepam (0.63 mg/kg, s.c.) did not generalize (17%), though response rates were signifi-

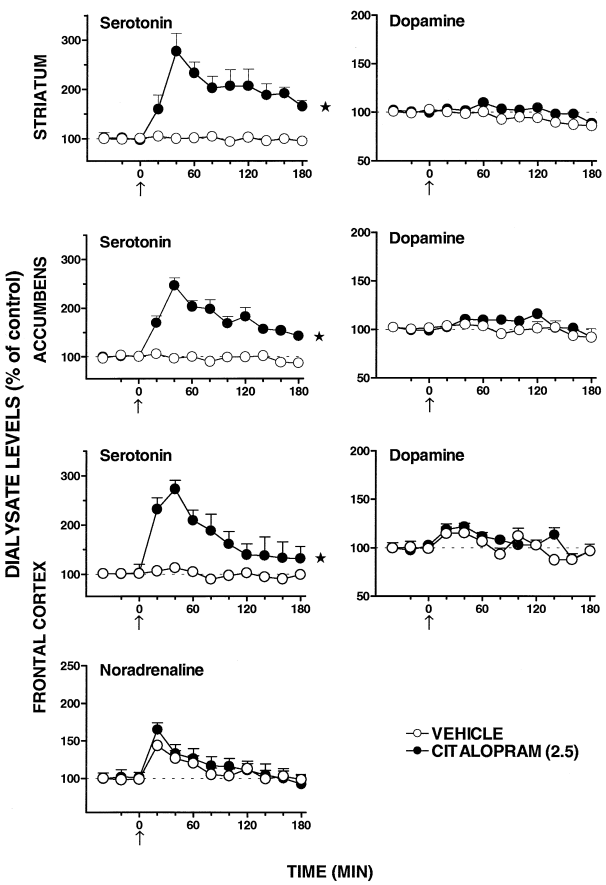

Fig. 1. Influence of citalopram upon dialysate levels of serotonin, dopamine and noradrenaline in the frontal cortex, striatum and nucleus accumbens of freely-moving rats. Data are means ± S.E.M. of serotonin, dopamine and noradrenaline levels expressed as a percentage of pre-injection values (= 100%). $N \geq 5$ per value. Absolute levels: frontal cortex, 1.08 ± 0.11, 1.01 ± 0.05 and 1.74 ± 0.10 for serotonin, dopamine and noradrenaline, respectively; striatum, 0.52 ± 0.06 and 9.2 ± 1.3 for serotonin and dopamine, respectively and accumbens, 1.03 ± 0.20 and 8.2 ± 1.5 pg 20 μl^{-1} dialysate for serotonin and dopamine, respectively. For comparison of individual values with vehicle-treated group, ANOVA (20–180 min) as follows. (Frontal cortex) serotonin: $F(1,12) = 12.0$, $P < 0.01$; dopamine: $F(1,12) = 1.0$, $P > 0.05$ and noradrenaline: $F(1,12) = 0.6$, $P > 0.05$. (Striatum) serotonin: $F(1,13) = 41.2$, $P < 0.01$ and dopamine: $F(1,13) = 2.1$, $P > 0.05$. (Accumbens) serotonin: $F(1,13) = 86.1$, $P < 0.01$ and dopamine: $F(1,12) = 1.0$, $P > 0.05$. $* P < 0.05$ for drug vs. vehicle.

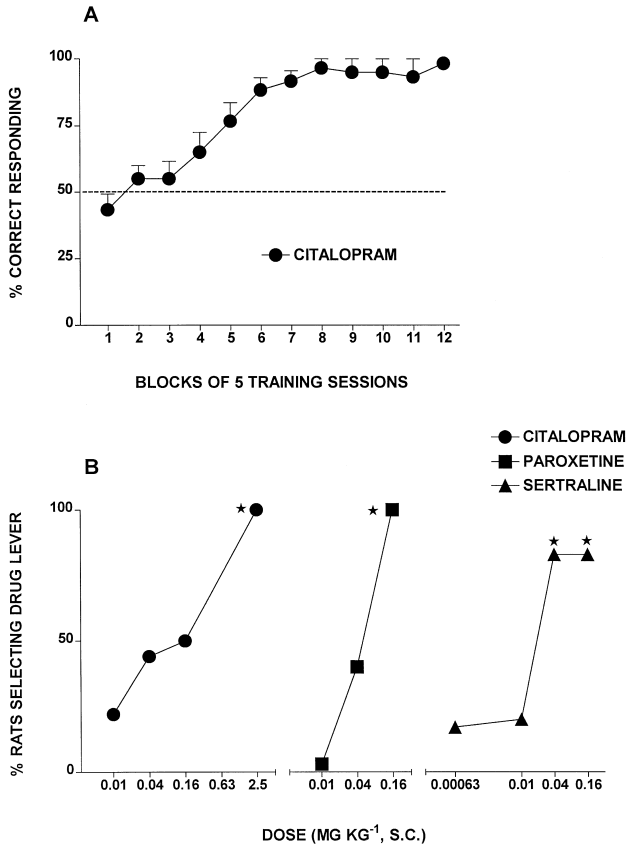

Fig. 2. (Panel A) Percent correct responding during acquisition of the discriminative stimulus effects of citalopram (2.5 mg/kg, i.p.). (Panel B) Generalization to the discriminative stimulus properties of citalopram. $N \geq 5$ per value. Citalopram, paroxetine and sertraline dose-dependently generalized with ED_{50}s (95% confidence limits) of 0.1 (0.02–0.12), 0.04 (0.02–0.07) and 0.01 (0.003–0.06) mg/kg, s.c., respectively. Asterisks indicate significance of differences ($* P < 0.05$; Fisher Exact Probability test) as compared to control value (0% drug lever selection). Response rates were not lower than 89% of values of the preceding saline training session.

cantly suppressed by $32 \pm 9\%$. A higher dose (2.5) abolished responding (not shown). Similarly, clozapine (2.5) did not generalize (20%), though response rates were significantly decreased by $68 \pm 17\%$.

4. Discussion

Previous studies have shown that citalopram increases extracellular 5-HT levels in the frontal cortex of freely-moving rats (Arborelius et al., 1996) and, herein, we extend these data in showing that citalopram increases 5-HT levels in the nucleus accumbens and striatum, as well as the frontal cortex, in the absence of an influence upon dopamine or noradrenaline levels, simultaneously determined in the same dialysate samples. These observations are of significance inasmuch as the selective 5-HT reuptake inhibitor, fluoxetine, increases levels of dopamine and noradrenaline as well as 5-HT in frontal cortex (Tanda et al., 1994; Gobert et al., 1997). This difference may reflect the lesser selectivity of fluoxetine for 5-HT vs. dopamine and noradrenaline uptake sites (Frazer, 1997). Thus, although an enhancement in frontocortical dopaminergic transmission may be involved in the actions of certain selective 5-HT reuptake inhibitors and other antidepressants (Tanda et al., 1994), at the dose utilized herein, citalopram selectively enhances serotonergic vs. dopaminergic and noradrenergic transmission.

Citalopram rapidly generated a stable discriminative stimulus, to which both citalopram and two further potent selective 5-HT reuptake inhibitors, paroxetine and sertraline (Frazer, 1997), fully generalized. In distinction, indicative of the specificity of the citalopram discriminative stimulus, the anxiolytic diazepam, which decreases corticolimbic release of 5-HT (Millan et al., 1997), did not generalize. Similarly, the antipsychotic clozapine, which likewise inhibits serotonergic transmission (Brunello et al., 1995), failed to generalize to citalopram. Notably, antidepressants do not generalize to clozapine (Hoenicke et al., 1992). These observations are of interest in the light of ongoing discussion concerning the relationship between depressive states and the deficit symptoms of schizophrenia (Brunello et al., 1995; Frazer, 1997). The present data, thus, provide evidence that the discriminative stimulus properties of clozapine *differ* to those of selective 5-HT reuptake inhibitors in rats.

It would be of interest to extend the present findings in several ways. First, does the citalopram discriminative stimulus generalize to all classes of antidepressant, irrespective of their mechanisms of action, or is an increase in extracellular 5-HT levels necessary and sufficient for generalization? Second, do drugs which enhance the influence of selective 5-HT reuptake inhibitors upon extracellular 5-HT levels and, possibly, their therapeutic efficacy, such as 5-HT_{1A} autoreceptor antagonists (Artigas et al., 1996; Gobert et al., 1997), similarly enhance their discriminative stimulus properties? Third, which type(s) of 5-HT receptor intervene in the discriminative stimulus elicited by citalopram? This is a fundamental issue in the light of continuing uncertainty as to which 5-HT receptor type(s) mediate the therapeutic action of selective 5-HT reuptake inhibitors (Frazer, 1997).

In conclusion, at a dose which selectively increases extracellular levels of 5-HT vs. dopamine and noradrenaline in the frontal cortex, citalopram generates a robust and pharmacologically-specific discriminative stimulus in rats. A selective increase in synaptic concentrations of 5-HT can, thus, produce reliable stimulus control. To our knowledge, this is the first occasion on which a discriminative stimulus induced by a selective 5-HT reuptake inhibitor has been described. The present model provides a novel and powerful tool for an evaluation of the interoceptive properties and mechanisms of action of antidepressants.

References

Arborelius, L., Nomikos, G.G., Hertel, P., Salmi, P., Grillner, P., Höök, B.B., Hacksell, U., Svensson, T.H., 1996. The 5-HT_{1A} receptor antagonist (*S*)-UH-301 augments the increase in extracellular concentration of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 630–640.

Artigas, F., Romero, L., De Montigny, C., Blier, P., 1996. Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. TINS 19, 378–383.

Brunello, N., Masotto, C., Steardo, L., Markstein, R., Racagni, G., 1995. New insights into the biology of schizophrenia through the mechanism of action of clozapine. Neuropsychopharmacology 13, 177–213.

Frazer, A., 1997. Antidepressants. J. Clin. Psychiatry 58, 9–25.

Gobert, A., Rivet, J.-M., Cistarelli, L., Millan, M.J., 1997. Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely-moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY 100,635 and GR 127,935. J. Neurochem. 68, 1159–1163.

Gobert, A., Rivet, J.-M., Audinot, V., Newman-Tancredi, A., Cistarelli, L., Millan, M.J., 1998. Simultaneous quantification of serotonin, dopamine and noradrenaline levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. Neuroscience 84, 413–429.

Hoenicke, E.M., Vanecek, S.A., Woods, J.H., 1992. The discriminative stimulus effects of clozapine in pigeons: involvement of 5-hydroxytryptamine$_{1C}$ and 5-hydroxytryptamine$_2$ receptors. J. Pharmacol. Exp. Ther. 263, 276–284.

Jones, C.N., Howard, J.L., McBennett, S.T., 1980. Stimulus properties of antidepressants in the rat. Psychopharmacology 67, 111–118.

Kelley, B.M., Porter, J.H., Varvel, S.A., 1995. Mianserin as a discriminative stimulus in rats: asymmetrical cross-generalization with scopolamine. Psychopharmacology 120, 491–493.

Millan, M.J., Hjorth, S., Samanin, R., Schreiber, R., Jaffard, R., De Ladonchamps, B., Veiga, S., Goument, B., Peglion, J.-L., Spedding, M., Brocco, M., 1997. S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)$_{1A}$ receptors: II. Modulation of hippocampal serotonin release in relation to potential anxiolytic properties. J. Pharmacol. Exp. Ther. 282, 148–161.

Schechter, M.D., 1983. Discriminative stimulus control with imipramine: transfer to other antidepressants. Pharmacol. Biochem. Behav. 19, 751–754.

Schreiber, R., Brocco, M., Lefèbvre De Ladonchamps, B., Monneyron, S., Millan, M.J., 1995. A drug discrimination analysis of novel serotonin$_{1A}$ receptor ligands in the rat using the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin. J. Pharmacol. Exp. Ther. 275, 822–831.

Stolerman, O.P., Rasul, F., Shine, P.J., 1989. Trends in drug discrimination research analysed with a cross-indexed bibliography, 1984–1987. Psychopharmacology 98, 1–19.

Tanda, G., Carboni, E., Frau, R., Di Chiara, G., 1994. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential?. Psychopharmacology 115, 285–288.

Zhang, L., Barrett, J.E., 1991. Imipramine as a discriminative stimulus. J. Pharmacol. Exp. Ther. 259, 1088–1093.