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Short communication

Catalepsy induced by the 5-HT_{1A} receptor antagonist WAY 100635 in rats pretreated with the selective serotonin reuptake inhibitor citalopram

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Abstract

Neither a high dose of the selective serotonin reuptake inhibitor citalopram (100 μmol kg^{-1} s.c.), nor the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide 3HCl (WAY 100635) (0.1–0.4 μmol kg^{-1} s.c.) produced any evidence of catalepsy in adult male rats. When combined with citalopram, however, WAY 100635 produced a dose-dependent, and statistically significant, catalepsy in the inclined grid test.

Keywords: 5-HT_{1A} receptors; 5-HT (5-hydroxytrypytamine, serotonin) reuptake inhibitor, selective; Catalepsy; (Rat)

1. Introduction

Selective serotonin reuptake inhibitors may precipitate parkinsonism in susceptible individuals, in all probability due to increased availability of synaptic serotonin, since it is an effect shared by a number of structurally different selective serotonin reuptake inhibitors (see, e.g., Gerber and Lynd, 1998). On the other hand, laboratory studies have shown that stimulation or blockade of brain 5-HT_{1A} receptors may modulate dopamine $D_{2/3}$ receptor-induced catalepsy in rats. Thus, raclopride-induced catalepsy can be antagonized by treatment with the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Wadenberg and Ahlenius, 1991; Prinssen et al., 1999), and is enhanced by treatment with the 5-HT_{1A} receptor antagonist WAY 100635 (Prinssen et al., 2000). The fact that 8-OH-DPAT also stimulates locomotor activity in reserpine-treated rats (Ahlenius and Salmi, 1995) provides further support for a role of brain 5-HT_{1A} receptors in extrapyramidal motor functions. Thus, it is possible that stimulation of brain 5-HT_{1A} receptors protects against an inherent potential for parkinsonism of a selective serotonin reuptake inhibitor. This working hypothesis was in the present study approached by examining the effects of the selective serotonin reuptake inhibitor citalopram on catalepsy, as observed on an inclined grid, in the presence of the 5-HT_{1A} receptor antagonist WAY 100635.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (B&K Universal, Sollentuna, Sweden), approximately 300g body weight, were used. The animals, housed three to four per cage (Makrolon® IV), were acclimatized for at least 10 days to controlled conditions of temperature (21.0 ± 0.4°C) and relative humidity (55–65%). Food (R34, Ewos, Södertälje, Sweden) and tap water were available ad libitum. The rat is a nocturnal animal, and thus, the rats were kept on a reversed light–dark cycle (12:12 h, lights off at 06:00 h) (see Hillegaart and Ahlenius, 1994). The rats were transferred from the animal quarters to the laboratory, 1 h before the experiments started, and were housed in a ventilated cabinet between the catalepsy measurements. All experiments were performed between 10:00 and 16:00 h.

The studies were approved by the *Stockholm North Local Ethical Committee on Animal Experiments*.

2.2. Drugs

The drugs used were *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxam-

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ide 3 HCl (WAY 100635), molecular weight 550.0 (Wyeth-Ayerst, Princeton, NJ), and citalopram HBr, molecular weight 405.3 (Lundbeck, Copenhagen, Denmark). Both drugs, or the saline vehicle, were administered subcutaneously in a volume of 2 ml kg^{-1}.

2.3. Catalepsy measurements

Catalepsy was observed by placing the animals on an inclined grid (60°) for a maximum of 2.25 min, in a dimly lit room. The animals were allowed 30 s of adaptation on the grid before observations started. The catalepsy was expressed as a score from 0 to 5, according to the time (square root transformation) the rat remained immobile (min): *0* = 0.00–0.08; *1* = 0.09–0.35; *2* = 0.36–0.80; *3* = 0.81–1.42; *4* = 1.43–2.24; *5* = > 2.25 min, i.e., if the rat remained immobile for 0.08 min, it was scored as 0, etc. (see Ahlenius and Hillegaart, 1986). The animal was considered immobile as long as it did not let go from the grid with any of its four paws.

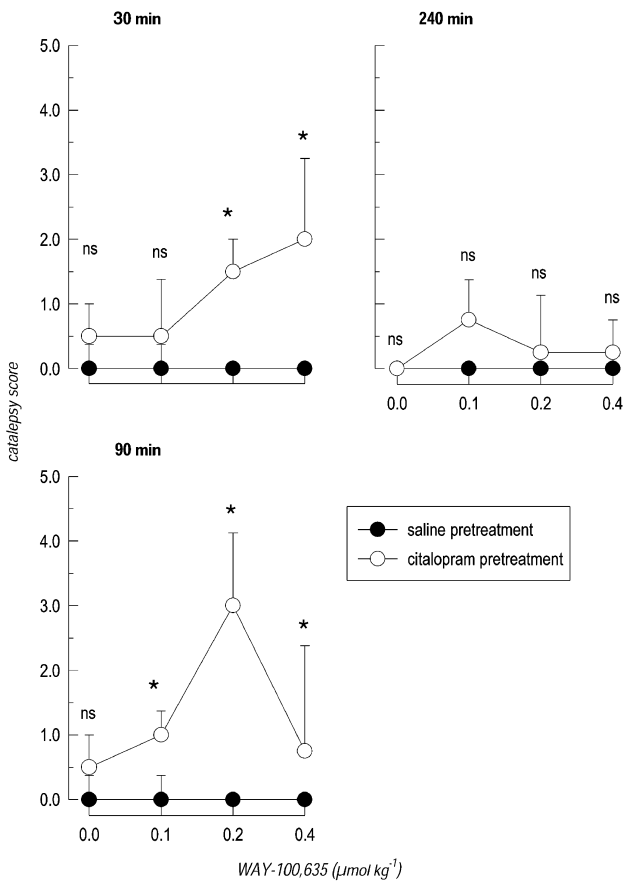

Fig. 1. Effects of WAY 100635 on catalepsy in rats pretreated with citalopram. The animals were observed at various time intervals after the administration of citalopram (100 μmol kg^{-1} s.c.), or the saline vehicle, as indicated in the figure. WAY 100635 (0.0–0.4 μmol kg^{-1} s.c.) was given 20 min after the citalopram injection. The results are presented as medians ± semi-interquartile range, based on repeated observations of four animals per group. Statistical evaluation was performed by means of the Mann–Whitney *U*-test for comparisons between saline and citalopram pretreated animals at the different doses of WAY 100635 (see Hollander and Wolfe, 1999), as shown in the figure. $^{ns}P > 0.05$; $^{*}P < 0.05$.

2.4. Experimental design and statistics

Separate groups of four animals each were used for the two pretreatment conditions, saline or citalopram. Repeated observations at 2–3 days intervals were made for the different doses of WAY 100635 (0.0, 0.1, 0.2 and 0.4 μmol kg^{-1}) and the respective pretreatment condition. Citalopram was administered 40, 90 and 240 min before the catalepsy test, and WAY 100635 was given 20 min after the citalopram injection. The animals served as their own controls in a change-over design (see Li, 1964). Nonparametric procedures were used for statistical description and analysis, as detailed in the figure legend.

3. Results

As shown in Fig. 1, neither citalopram (100 μmol kg^{-1}) nor WAY 100635, by themselves, produced any catalepsy at any time interval. However, when combined, there was a marked, and statistically significant, increase in catalepsy. The effect was observed at the 40–90-min time intervals, and the effect was gone by 240 min after the citalopram injection. The maximal catalepsy obtained by the WAY 100635/citalopram combination appears to be less than the catalepsy obtained in experiments on the dopamine $D_{2/3}$ receptor antagonists haloperidol or raclopride, which readily score 5.0 under present conditions (Ahlenius and Hillegaart, 1986; Wadenberg and Ahlenius, 1991).

4. Discussion

The mechanism whereby WAY 100635 precipitates catalepsy in citalopram-pretreated rats is not clear. There are two obvious possibilities. Firstly, an effect whereby the increased synaptic availability of serotonin, produced by the selective serotonin reuptake inhibitor, indirectly affects neostriatal dopamine release, and thereby affects extrapyramidal motor functions. Secondly, an effect whereby serotonin directly affects extrapyramidal motor functions. There is experimental support for either possibility. Thus, it has been reported that neostriatal infusion of 5-HT may facilitate dopamine release in this brain area (Benloucif et al., 1993). It should be noted, however, that systemic administration of fluoxetine might cause a decreased release of dopamine in the neostriatum (Clark et al., 1996). These apparently conflicting observations could be explained by the fact that different 5-HT receptor subtypes affect dopamine release differently (Benloucif et al., 1993). Further complicating the picture, the net effect on dopamine

release must also take into account the fact that serotonergic neurotransmission will affect dopamine release differently depending on pre- vs. post-synaptic interactions in the nigrostriatal pathway (Cragg et al., 1997). In support of the second possibility, administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) enhances raclopride-induced catalepsy in the rat (Wadenberg and Ahlenius, 1995). It should be noted, however, that not only 8-OH-DPAT, but also the non-selective 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodo)-2-aminopropane (DOI), counteracts raclopride-induced catalepsy (see Wadenberg, 1996). Taken together, these observations suggest that the catalepsy provoked by WAY 100635 in citalopram pretreated rats should be due to stimulation of 5-HT receptors not being of the 5-HT_{1A} or 5-HT_2 subtypes.

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References

Ahlenius, S., Hillegaart, V., 1986. Involvement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: a comparison between the effects produced by pre- and post-synaptic inhibition of dopaminergic neurotransmission. Pharmacol. Biochem. Behav. 24, 1409–1415.

Ahlenius, S., Salmi, P., 1995. Antagonism of reserpine-induced suppression of spontaneous motor activity by stimulation of 5-HT_{1A} receptors in rats. Pharmacol. Toxicol. 76, 149–156.

Benloucif, S., Keegan, M.J., Galloway, M.P., 1993. Serotonin-facilitated dopamine release in vivo: pharmacological characterization. J. Pharmacol. Exp. Ther. 265, 373–377.

Clark, R.N., Ashby Jr., C.R., Dewey, S.L., Ramachandran, P.V., Strecker, R.E., 1996. Effect of acute and chronic fluoxetine on extracellular dopamine levels in the caudate/putamen and nucleus accumbens of rat. Synapse 23, 125–131.

Cragg, S., Rice, M.E., Greenfield, S.A., 1997. Heterogeneity of electrically evoked dopamine release and reuptake in substantia nigra, ventral tegmental area, and striatum. J. Neurophysiol. 77, 863–873.

Gerber, P.E., Lynd, L.D., 1998. Selective serotonin-reuptake inhibitor-induced movement disorders. Ann. Pharmacother. 32, 692–698.

Hillegaart, V., Ahlenius, S., 1994. Time course for synchronization of spontaneous locomotor activity in the rat following reversal of the daylight (12:12 h) cycle. Physiol. Behav. 55, 73–75.

Hollander, M., Wolfe, D.A., 1999. Nonparametric Statistical Methods. 2nd edn. Wiley, New York.

Li, C.C., 1964. Introduction to Experimental Statistics. McGraw-Hill, New York.

Prinssen, E.P.M., Kleven, M.S., Koek, W., 1999. Interactions between neuroleptics and 5-HT_{1A} ligands in preclinical behavioral models for antipsychotic and extrapyramidal effects. Psychopharmacology 144, 20–29.

Prinssen, E.P.M., Koek, W., Kleven, M.S., 2000. Effects of WAY 100635 on antipsychotic-induced catalepsy in 5-HT depleted animals: a role for tonic activation of 5-HT_{1A} receptors. Eur. J. Pharmacol. 395, 143–147.

Wadenberg, M.-L., 1996. Serotonergic mechanisms in neuroleptic-induced catalepsy in the rat. Neurosci. Biobehav. Rev. 20, 325–339.

Wadenberg, M.-L., Ahlenius, S., 1991. Antipsychotic-like profile of combined treatment with raclopride and 8-OH-DPAT in the rat: enhancement of antipsychotic-like effects without catalepsy. J. Neural Transm.: Gen. Sect. 83, 43–53.

Wadenberg, M.-L., Ahlenius, S., 1995. Antagonism by the 5-$HT_{2A/C}$ receptor agonist DOI of raclopride-induced catalepsy in the rat. Eur. J. Pharmacol. 294, 247–251.