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Reversal of neuroleptic-induced catalepsy by novel aryl-piperazine anxiolytic drugs

BRIAN A. MCMILLEN, SARA M. SCOTT, ELIZABETH A. DAVANZO, *Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858, USA*

Abstract—The novel anxiolytic drug, buspirone, reverses catalepsy induced by haloperidol. A series of aryl-piperazine analogues of buspirone and other 5-hydroxytryptaminergic agonists were tested for their ability to reverse haloperidol induced catalepsy. Those drugs with strong affinity for 5-hydroxytryptamine$_{1a}$ receptors were able to reverse catalepsy. Drugs with affinity for other 5-HT receptors or weak affinity were ineffective. However, inhibition of postsynaptic 5-HT receptors neither inhibited nor potentiated reversal of catalepsy and leaves open the question as to the site or mechanism for this effect.

Buspirone, an *N*-alkyl-substituted arylpiperazine drug, reduces clinical symptoms of anxiety without sedating side-effects or interactions with sedating drugs (Rickels et al 1982; Moskowitz & Smiley 1982). The drug does not interact with the benzodiazepine-GABA chloride channel ionophore complex (Riblet et al 1982; McMillen et al 1983) and its behavioural effects are not blocked by benzodiazepine antagonists (Weissman et al 1984). Instead, buspirone and several chemical analogues stimulate 5-hydroxytryptamine (5-HT) receptors (5-HT$_{1a}$ subtype) both at postsynaptic sites (Glaser & Traber 1983; Yocca & Maayani 1985) and at presynaptic sites (VanderMaelen et al 1986; McMillen et al 1987). Because buspirone behaves as a partial agonist at post-synaptic 5-HT$_{1A}$ receptors and a full agonist at presynaptic receptors on the raphe neurons, it was postulated that a decrease in 5-HT activity may mediate the antianxiety effect of buspirone and related drugs (McMillen et al 1987). This hypothesis is in harmony with the observation that the anti-aggressive effect of gepirone, a buspirone analogue, is potentiated by co-administration of methysergide or methiothepin, postsynaptic 5-HT receptor antagonists (McMillen et al 1987).

Buspirone has another behavioural effect, apparently unrelated to its anxiolytic effects, that is not well understood. Buspirone and gepirone potently reverse the immobility (catalepsy) induced by either blocking dopamine receptors or depleting dopamine (McMillen & Mattiace 1983; McMillen & McDonald 1983) and it was concluded that these drugs must be acting at a site efferent from the postsynaptic dopamine receptors. The 5-HT-like action of these drugs may have a role in this phenomena. Carter & Pycock (1978) demonstrated that reducing 5-HT activity can decrease the ability of a dopamine receptor inhibitor to induce catalepsy. Inhibitors of 5-HT re-uptake potentiate haloperidol-induced catalepsy and increased dopamine metabolism (Waldmeier & Delini-Stula 1979). If buspirone and its analogues are acting through reduced 5-HT activity, then other drugs with similar activity should also decrease catalepsy scores. 5-HT-like agonists that do not decrease raphe neuronal impulse flow would be expected to be inactive. In the following report we examine the ability of various drugs to reverse haloperidol-induced catalepsy and compare the results with the affinities of these drugs for the 5-HT$_{1A}$ binding site in hippocampus published previously.

Methods and materials

Catalepsy was scored according to the method of Shore & Dorris (1975). At 30 min intervals after injection of 1·0 mg kg^{-1} s.c. haloperidol, each rat was scored 0·5 point for each forepaw, placed one at a time on a 3·0 cm high peg, held in place 10 s. Then the rat was scored 1·0 point for each forepaw held for 10 s on a 10 cm peg. The rat was scored an additional 1·0 point if the hind limb could be placed and held over the homolateral fore limb for 10 s. In this manner each rat was scored from 0 for no catalepsy to 4·0 for full catalepsy. Scores around 1·0 indicate akinesia, while scores of 3 to 4 indicate the rigid posturing associated with catalepsy.

Data for ability of these drugs to displace 1·0 nM [^{3}H]5-HT binding from hippocampal membranes has been published elsewhere (McMillen et al 1988). Binding assay conditions are described in McMillen et al (1987). The IC50 values were calculated from semi-log plots often using the maximum displacement by the test drug as the blank value for calculating specific binding since several of these drugs do not displace [^{3}H]5-HT from the 5-HT$_{1B}$ site which comprises about 25% of the hippocampal 5-HT$_1$ receptor population (Pazos & Palacios 1985; McMillen et al 1987).

Drugs and sources. Drugs generously donated were buspirone HCl, gepirone HCl, BMY 14802, BMY 20661 and *m*-chlorophenylpiperazine (mCPP, Bristol-Myers Co., Wallingford, CT), ipsapirone-HCl (TVX Q 7821, Miles Laboratories, Inc., West Haven, CT), fluprazine-HCl (Duphar B.V., Weesp), haloperidol (McNeil Pharmaceuticals, Spring House, PA), methysergide maleate and pindolol (Sandoz Pharmaceuticals, E. Hanover, NJ). Drugs purchased were (±)-8-hydroxydipropylaminotetralin HBr (DPAT, Research Biochemicals Inc., Wayland, MA), α,α,α-trifluoro-*m*-tolylpiperazine (TFMPP, Aldrich Chemical Co., Milwaukee, WI) and morphine SO_4 (Elkins-Sinn, Inc., Cherry Hill, NJ). All doses refer to the free base form of the drugs.

Results and discussion

Those drugs noted as having high affinity for 5-HT$_{1A}$ binding

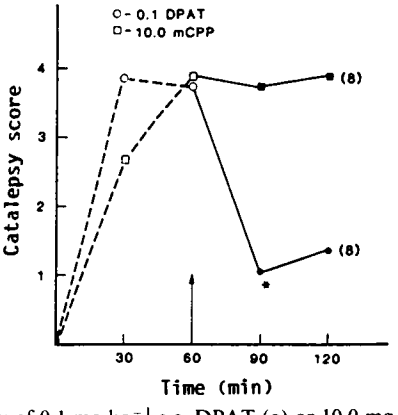

FIG. 1. Ability of 0·1 mg kg^{-1} s.c. DPAT (o) or 10·0 mg kg^{-1} s.c. mCPP (□) to reverse catalepsy induced by haloperidol 1·0 mg kg^{-1}. The test drugs were injected 60 min after injection of haloperidol (arrow). A significant difference from the 60 min haloperidol-only score is noted by an * where $P < 0.05$ (Wilcoxon signed ranks test). Number of rats in each group is shown in parentheses.

Correspondence to: B. A. McMillen Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858, USA.

sites reversed catalepsy induced by haloperidol. Fig. 1 compares data for DPAT with mCPP, a 5-HT_{1B}-preferring drug (Sills et al 1984), and shows that mCPP did not reverse catalepsy, but DPAT was potent for reversal of catalepsy. Indeed, those drugs noted as having a strong preference for the 5-HT_{1A} binding site all reversed haloperidol-induced catalepsy. Drugs that are 5-HT_{1B} preferring, mCPP and TFMPP, or weak displacers of [^{3}H]5-HT binding, fluprazine, failed to reverse catalepsy. Fig. 2

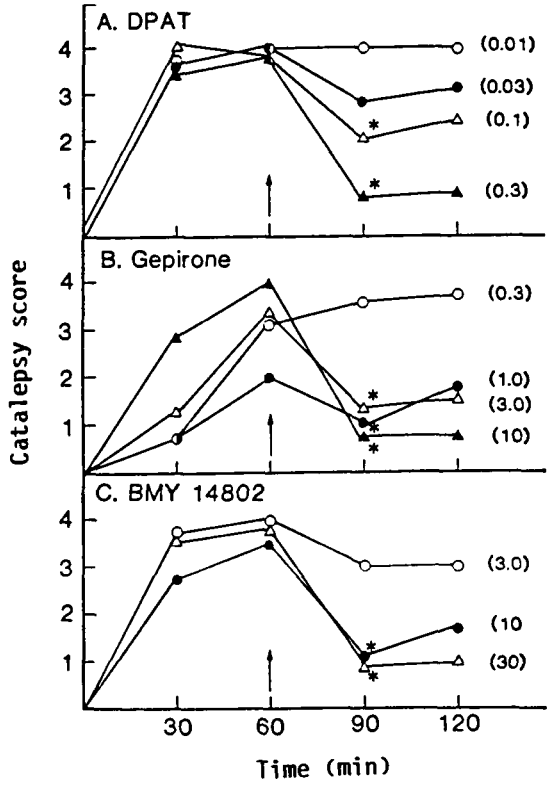

FIG. 2. Effects of various doses of A. 8-hydroxy-dipropylaminotetralin (DPAT); B. gepirone or C. BMY 14802 on catalepsy induced by haloperidol 1·0 mg kg^{-1}. The test drugs were injected 60 min after haloperidol (arrow). Significant differences from the 60 min haloperidol-only score are noted by an * where $P < 0.05$ (Wilcoxon signed ranks test) for groups of 8 animals per treatment.

shows the effects of increasing doses of DPAT, gepirone and BMY 14802. Those drugs all showed dose-response effects on catalepsy scores, but in different dose ranges. The percent decrease from 60 min to 120 min was used for log-probit determination of ED50 doses (Litchfield & Wilcoxon 1949) for those three drugs and were found to be 0·12, 3·2 and 9·5 mg kg^{-1} s.c. for DPAT, gepirone and BMY 14802, respectively. These differences in ED50 values compare favourably with the differences in IC50 concentrations for inhibition of [^{3}H]5-HT binding to hippocampal 5-HT_{1A} receptor sites shown in column two of Table 1.

Gepirone, in a dose of 1·0 mg kg^{-1} s.c. was the minimal dose to significantly decrease catalepsy scores. Fig. 3 shows that co-administration of methysergide or pindolol with haloperidol neither potentiated nor reduced the ability of gepirone 1·0 mg kg^{-1} to reduce catalepsy scores. The catalepsy scores at 90 or 120 min were not different from the haloperidol plus gepirone experiment (Mann-Whitney U-test). This is in marked contrast to data obtained from aggression testing which showed that methysergide greatly potentiated the anti-aggressive effects of gepirone and DPAT (McMillen et al 1987). The β-adrenoceptor blocking drug, pindolol, also blocks 5-HT_1 receptor sites

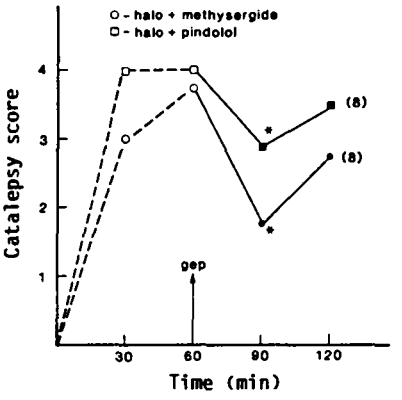

FIG. 3. Effects of 2·5 mg kg^{-1} methysergide or 5·0 mg kg^{-1} pindolol on gepirone's reversal of haloperidol-induced catalepsy. At 0 min, haloperidol, 1·0 mg kg^{-1} s.c., was injected and either methysergide or pindolol and catalepsy allowed to develop. Immediately after the 60 min catalepsy scoring, gepirone 1·0 mg kg^{-1} s.c., was injected (arrow) and the experiment continued. A significant difference from the 60 min catalepsy score is noted by an * where $P < 0.05$ (Wilcoxon signed ranks test). Number of rats in each group is shown in parentheses.

without much affinity for the 5-HT_2 receptor site (Glennon 1987). However, combining that drug with gepirone failed to modify, in either direction, the reversal of catalepsy.

To determine whether the reversal of catalepsy was a general phenomena, morphine was used to induce rigid posturing instead of haloperidol. The site and mechanism of morphine-induced catalepsy (or catatonia) is different from neuroleptics and is noted by a stiff body rigidity (lead pipe rigidity) such that it is impossible to get the rats to maintain the homolateral limbs on one side in a crossed position so that catalepsy scores rarely exceeded 3·0. Buspirone at 1·0 mg kg^{-1} did not reverse catalepsy induced by 20 mg kg^{-1} s.c. morphine (Fig. 4), but 5·0 mg kg^{-1} of

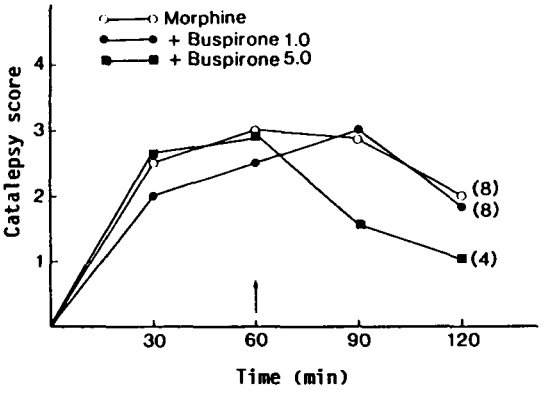

FIG. 4. Effect of buspirone on morphine induced rigidity. At 0 min, morphine, 20 mg kg^{-1} s.c., was injected and catalepsy scored every 30 min. A second group of 8 rats received buspirone, 1·0 mg kg^{-1} s.c., immediately after the 60 min scoring for catalepsy and a third group of 4 rats received buspirone, 5·0 mg kg^{-1} s.c. The score at 60 min after buspirone, 1·0 mg kg^{-1} (120 min after morphine) was without significant effect compared with the 60 min score (Wilcoxon signed ranks test). The combination of morphine, 20 mg kg^{-1}, and buspirone, 5·0 mg kg^{-1} caused a noticeable sedation in the rats.

buspirone did reduce catalepsy scores. However, this latter dose of buspirone may have non-specific quieting effects on the rats (McMillen & McDonald 1983; Olivier et al 1984) and the rats appeared limp and immobile. These data further demonstrate the differences between opiate-induced and neuroleptic-induced rigidity and suggest that either the arylpiperazines do not

Table 1. Comparison of ability of various drugs to reverse catalepsy induced by haloperidol, 1.0 mg kg^{-1} s.c. with inhibition of 1·0 nM [^{3}H]5-HT binding to hippocampal 5-HT_1 binding sites. Catalepsy reversal values are drug dose times catalepsy score 120 min after injection of haloperidol (60 min after injection of test drugs). No score was calculated if scores at 120 min were not significantly different from 60 min haloperidol-only score (Wilcoxon signed ranks test). Each group consisted of 8 rats.

Drug mg kg^{-1} s.c.	Catalepsy reversal	IC50
DPAT, 0·1	0·14	1·0 nM
Buspirone, 1·0	0·87	10·0
BMY 20661, 1·0	1·75	31·0
Gepirone, 1·0	1·75	50·0
Ipsapirone, 3·0	4·89	20·0
BMY 14802, 10	16·90	170·0
Fluprazine, 10	NR	1,429
mCPP, 10	NR	100
TFMPP, 10	NR	177

NR = no reversal
BMY 14802—α-(4-fluoro-phenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-n-butanol
BMY 20661—4-[4-(4-(4-fluoro[3,2c]pyridinyl)-1-piperazinyl) butyl]-3,5-morpholinedione

interact with opiate-induced rigidity or are less potent as inhibitors of this behaviour.

Table 1 compares the ability of various drugs, mostly arylpiperazines, to reverse catalepsy with their IC50 values for displacement of 1·0 nM [^{3}H]5-HT from hippocampal 5-HT_1 receptors. The table shows a rough agreement between the two parameters except for the arylpiperazines lacking an *N*-alkyl substitution. Although those drugs can displace the [^{3}H]5-HT binding, they have mixed activity at 5-HT_{1A} and 5-HT_{1B} binding sites (Sills et al 1984; McMillen et al 1987) and do not inhibit impulse flow in 5-HT neurons of the dorsal raphe (McMillen et al 1987; Sprouse & Aghajanian 1987). Thus, the simple arylpiperazine drugs exhibit a different spectrum of behavioural effects than drugs with 5-HT_{1A} only activity (Lucki & Frazer 1985). These data demonstrate that reversal of haloperidol-induced catalepsy is a general phenomenon of *N*-alkyl-substituted arylpiperazines and DPAT provided that such drugs have a reasonable affinity for the 5-HT_{1A} binding site. The ability to reverse catalepsy is similar to each drug's ability to inhibit intraspecies aggression, except for fluprazine. Fluprazine is a striking exception in that it potently inhibits intraspecies aggression (Olivier et al 1984; McMillen et al 1988), but neither displace 5-HT_1 binding nor reverses catalepsy.

The major difference between the inhibition of aggression and reversal of catalepsy with the arylpiperazines is the failure of 5-HT antagonists to modify the reversal of catalepsy. That 5-HT antagonists of either the mixed type (methysergide or methiothepin) or 5-HT_1 preferring type (pindolol) can potentiate inhibition of aggression by gepirone or DPAT suggested a presynaptic action to reduce 5-HT impulse flow was critical for inhibition of aggression (McMillen et al 1987). Since reduced 5-HT function reduces the ability of fluphenazine to induce catalepsy (Carter & Pycock 1978), it was expected that small doses of 5-HT antagonists would potentiate catalepsy reversal. This result did not occur, which suggests that a mechanism different from that involved in the antiaggressive effect mediates the reversal of catalepsy. Since the only known common sites of action for these drugs are the pre- and postsynaptic 5-HT_{1A} binding sites, it is hard to envisage what this mechanism might be.

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