Pharmacology Biochemistry & Behavior, Vol. 32, pp. 657–660. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Apparent Antinociceptive Properties of Piperazine-Type Serotonin Agonists: Trifluoromethylphenylpiperazine, Chlorophenylpiperazine, and MK-212

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Received 27 June 1988

McKEARNEY, J. W. *Apparent antinociceptive properties of piperazine-type serotonin agonists: Trifluoromethylphenylpiperazine, chlorophenylpiperazine, and MK-212.* PHARMACOL BIOCHEM BEHAV **32**(3) 657–660, 1989.—Squirrel monkeys were studied under a titration procedure in which responding adjusted the intensity of an electrical stimulus delivered to the tail (0.1–3.3 mA range in 15 steps). The 5-HT agonists trifluoromethylphenylpiperazine (TFMPP), chlorophenylpiperazine (mCPP), and 6-chloro-2(1-piperazinyl)pyrazine (MK-212) increased the intensity at which shock was maintained. The order of potency was: MK-212 > mCPP > TFMPP. Reductions in absolute rates of responding were small, and not related systematically to increases in shock intensity. Pretreatment with the nonselective 5-HT antagonist methysergide (0.1–1.0 mg/kg) resulted in a 3- to 10-fold shift to the right of the dose-effect curves for the 5-HT agonists. In contrast, the selective 5-HT_2 antagonists ketanserin (0.3–1.7 mg/kg) and pirenperone (0.001–0.1 mg/kg) did not alter the effects of these agonists. This suggests that the apparent antinociceptive actions of these 5-HT agonists are probably mediated by effects at the 5-HT_1 receptor subtype.

Serotonin (5-HT) Trifluoromethylphenylpiperazine (TFMPP) Chlorophenylpiperazine (mCPP) 6-Chloro-2(1-piperazinyl)pyrazine (MK-212) Methysergide Serotonin antagonists Antinociception Pain Squirrel monkeys

THERE is considerable evidence that serotonin neurotransmission is involved in mediation of reactions to noxious stimuli such as electric shock. For example, manipulations that result in enhanced 5-HT function (e.g., administration of uptake inhibitors or direct agonists) have antinociceptive effects, whereas presumed reductions in 5-HT activity (e.g., synthesis inhibition, neurotoxin lesions, pharmacological antagonists) lead instead to heightened reactivity to noxious stimuli [for reviews see (5, 11, 13)].

Although a number of experiments have made use of pharmacological manipulations of 5-HT neurotransmission (e.g., uptake inhibition, inhibition of synthesis), few have studied the possible antinociceptive actions of direct 5-HT agonists. 5-HT itself, given intrathecally, does have antinociceptive effects (16), as does the indole agonist compound 5-methoxy-N,N-dimethyltryptamine (2). Results with quipazine, which has both pre- and postsynaptic effects, have not been consistent. Samanin (14) and Malec and Langwiska (10) reported that rather large doses of quipazine (10–30 mg/kg) increased latencies in the hot-plate procedure. Other investigators (12) reported that quipazine (7.5–30 mg/kg) was without activity in the tail-flick test. Doses of 15–30 mg/kg quipazine did increase response latencies in the hot-plate test, but these investigators report that the behavior of animals treated with quipazine was qualitatively different from that of untreated animals or animals treated with morphine. Untreated and morphine-treated animals licked their paws, but quipazine-treated animals attempted to jump from the hot surface. Interpretation of the results with quipazine is complicated further by the fact that doses this large produce marked alterations in a variety of motor movements (the so-called "serotonin syndrome").

To our knowledge, there have been no published studies of the possible antinociceptive effects of 5-HT agonists on behavior under the fractional-escape or shock-titration procedure originally used by Weiss and Laties (15). Under this procedure, the subject is able to adjust the intensity of electrical stimulation by engaging in a simple and easily performed motor response (e.g., lever pressing). Changes in the intensity at which shock is maintained without appreciable changes in the rate at which the motor response is executed can be interpreted as reflecting antinociceptive drug effects. In the present experiments, the shock-titration procedure was employed to assess the possible antinociceptive actions of three 5-HT agonists of the substituted-piperazine type: trifluoromethylphenylpiperazine (TFMPP), chlorophenylpiperazine (mCPP), and MK-212.

METHOD

Subjects and Apparatus

A total of six adult male squirrel monkeys were used. All had been exposed previously to drug administration and to a variety of

behavioral procedures. Subjects were housed individually with unlimited access to food and water.

Experiments were conducted with monkeys seated in primate chairs. Electric current was delivered through brass electrodes resting on a shaved portion of the restrained tail. A response key (BRS/LVE, No. 121-05 or Coulbourn No. E21-03) requiring about 15 g force for operation was mounted on a clear panel facing the monkey. Three pairs of 7-W colored lights were also mounted behind this panel. Chairs were housed in ventilated, sound-attenuating chambers in a room distant from programing and recording equipment.

Procedures

During experimental sessions, electric current (650 V AC, 60 Hz) was delivered continuously to the tail at varying intensities controlled by a two-way stepping switch and a series of variable resistances. The intensity increased by one step every 3 sec, but each depression of the response key decreased it by one step. Current was programmed to occur at 15 intensities ranging from 0.1 to 3.3 mA. The first six steps were separated by 0.1 mA, and the remainder by 0.3 mA. Experimental sessions were 60 min in duration. The time spent at each intensity was recorded separately, and this was used to calculate the proportion of the total session time spent at each shock intensity. A statistic was then calculated which represented the current intensity at or below which 50% of the total session time was spent. This will be referred to as the median shock intensity. Responding during the first 5 min of each session was excluded from the analysis of data.

Drugs

Compounds studied were: 1(*m*-trifluoromethylphenyl)piperazine (TFMPP, purchased from Aldrich Chemical), 1(*m*-chlorophenyl)piperazine diHCl (mCPP, purchased from Sigma Chemical), 6-chloro-2-(1-piperazinyl)pyrazine (MK-212, courtesy of Merck & Co.), methysergide maleate (courtesy of Sandoz), ketanserin tartrate, and pirenperone (both courtesy of Janssen). Where drug salts are specified, doses are expressed in these terms; otherwise they are expressed as the drug base. All were dissolved in sterile distilled water or 0.9% NaCl solution (except for the stock solution of pirenperone, which included a small amount of 1 N glacial acetic acid). Injections were usually 0.5 ml/kg, given in the thigh muscle. Agonists were given just before sessions, and antagonists were given 15 min before that. Experimental sessions were conducted 5 days weekly. Drugs were generally given on Tuesdays and Fridays, and performance on Thursdays was averaged to compute estimates of control responding.

RESULTS

In the absence of drugs, there was a steady moderate rate of responding (~0.3–0.5 responses/sec) and shock intensity was kept low (~0.1–0.2 mA).

Figure 1 shows the effects of TFMPP (open circles) in five monkeys. A dose of 1.0 mg/kg had little effect, but higher doses produced dose-related increases in median shock intensity. When given in combination with 0.3 mg/kg of the nonselective 5-HT antagonist methysergide (filled circles), there was a clear antagonism of the effects of TFMPP (about a 10-fold shift based on the full curves shown for S-565 and S-571). Selected doses of the 5-HT_2 antagonists ketanserin and pirenperone did not block the effects of TFMPP in the monkey tested (S-571—see figure legend).

Figure 2 shows similar effects of mCPP in three monkeys. Again, there were dose-related increases in median shock intensity (open circles), and these were blocked by pretreatment with 0.3 mg/kg of the nonselective 5-HT antagonist methysergide (filled circles). A dose of 0.003 mg/kg of the 5-HT_2 antagonist pirenperone, which was the highest dose devoid of effects of its own, did not change mCPP effects in the one monkey tested (×, S-572). The shift in the mCPP dose-effect curves by methysergide was similar to that seen with TFMPP.

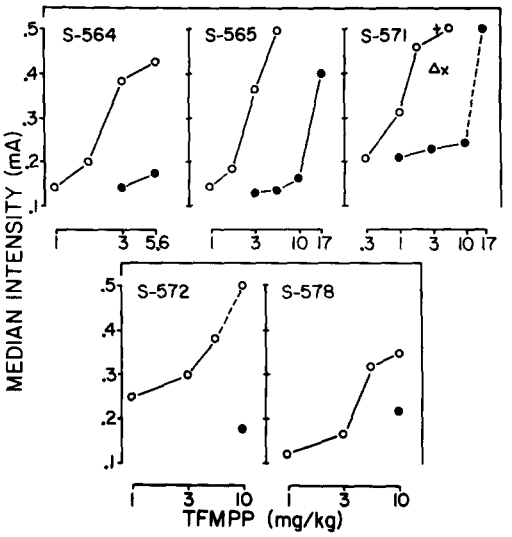

FIG. 1. Effects of TFMPP on median shock intensities. Open circles: TFMPP alone. Filled circles: in combination with 0.3 mg/kg methysergide. In this and subsequent figures, points are generally means of at least two determinations in each of the five monkeys. For monkey S-571, the point connected by the dashed line was higher than indicated but the figure was left at the same scale as for the other monkeys so as not to distort the effects of lower doses. Monkey S-571 was also tested with the following drugs in combination with TFMPP: 1.0 mg/kg ketanserin (triangle); 0.003 and 0.01 mg/kg pirenperone (× and +).

MK-212 likewise produced graded increases in median shock intensity (Fig. 3, open circles) which were blocked by 0.3 mg/kg methysergide (filled circles). The effects of MK-212 were not blocked by treatment with 0.3 (S-572) or 1.0 (S-585) mg/kg of the 5-HT_2 antagonist ketanserin.

These effects of TFMPP, mCPP, and MK-212 on median shock intensities under the titration schedule were not secondary to changes in absolute response rate. Although there were moderate reductions in response rate of some monkeys at the higher doses, these were small by comparison with the changes in shock

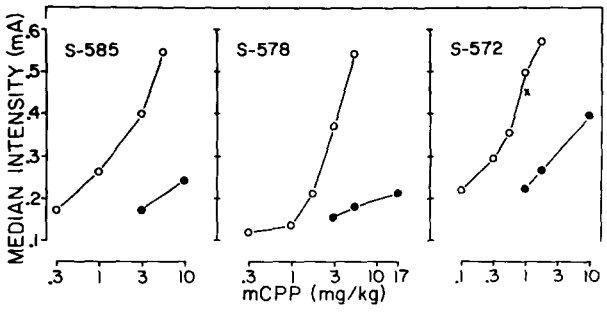

FIG. 2. Effects of mCPP on median shock intensities. Open circles: mCPP alone. Filled circles: in combination with 0.3 mg/kg methysergide. Monkey S-572 was also tested with 0.003 mg/kg pirenperone (×) in combination with mCPP.

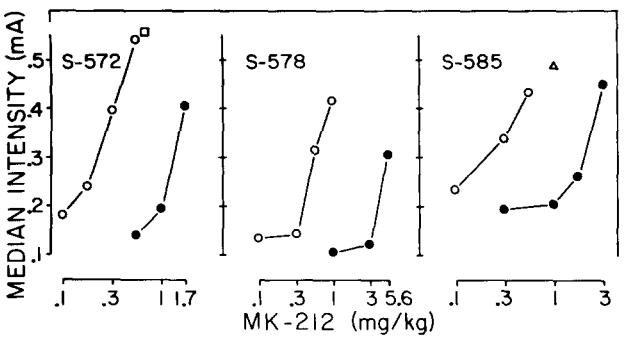

FIG. 3. Effects of MK-212 on median shock intensities. Open circles: MK-212 alone. Filled circles: in combination with 0.3 mg/kg methysergide. Monkey S-572 (square, 0.3 mg/kg) and S-585 (triangle, 1.0 mg/kg) were also tested with ketanserin in combination with MK-212.

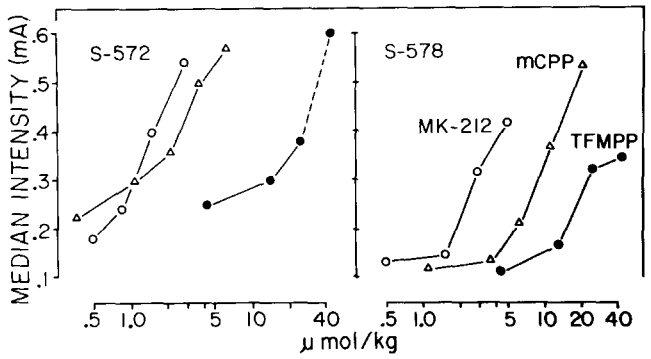

FIG. 4. Potency comparison of TFMPP, mCPP, and MK-212. Data are replotted from the other figures, except that doses are expressed in μmol/kg.

intensity and were accounted for primarily by drug-induced reductions in the frequency of ineffective responses (i.e., "extra" responses occurring when shock was already at the lowest intensity). In no case did these drugs cause response rate to fall below the level needed to maintain shock at a constant intensity (i.e., 1 response per 3 seconds).

For all monkeys studied, MK-212 was most potent and TFMPP least potent, with mCPP being intermediate. Potency comparisons for the drugs in the two monkeys studied with all three are shown in Fig. 4 (note that doses are expressed in μmol/kg). In order to estimate the relative potencies of the three drugs in all monkeys, we calculated by linear interpolation from dose-effect curves a dose of each drug which increased median shock intensity to 0.4 mA (which was about half way between control and the intensity observed at the higher drug doses). These doses expressed in μmol/kg (and their 95% confidence limits) are as follows: MK-212—2.2 (n = 3, 1.0–4.8); mCPP—5.2 (n = 3, 1.5–18.9); TFMPP—16.1 (n = 5, 6.1–42.6).

DISCUSSION

The 5-HT agonists MK-212, mCPP, and TFMPP produced dose-related increases in the intensity at which electric shock was maintained under a titration procedure, lending support to previous suggestions that 5-HT neurotransmission may be involved in mediation of the perception of and reaction to noxious stimuli. The order of potency, MK-212 > mCPP > TFMPP, was the same as that seen with these drugs studied under schedules of food presentation or stimulus-shock termination (1, 8, 9). Methysergide, an effective antagonist at both the 5-HT_1 and 5-HT_2 putative receptor subtypes, blocked the effects of each of these drugs to about the same extent. Limited tests with the selective 5-HT_2 antagonists ketanserin and pirenperone, however, did not reveal any antagonism of the effects of MK-212, mCPP, or TFMPP. This block of the behavioral effects of these agonists by a nonselective 5-HT antagonist but not by selective 5-HT_2 antagonists is in accord with results from experiments using locomotor activity [e.g., (6,7)], drug discrimination procedures [e.g., (3,4)], and other types of schedule-controlled behavior (8,9). Taken together, all of these results suggest that the behavioral effects of MK-212, mCPP, and TFMPP may be mediated predominantly by actions at 5-HT_1 sites.

ACKNOWLEDGEMENTS

This work supported primarily by grant DA-01015 and partially by grant MK-18421, both from the USPHS. I thank Janssen Pharmaceutica, Merck Sharp & Dohme, and Sandoz Pharmaceuticals for gratis samples of drugs.

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