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COMMUNICATIONS

Evidence that *m*-chlorophenylpiperazine inhibits some nociceptive responses of rats by activating 5-hydroxytryptamine mechanisms

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Several studies suggest that 5-hydroxytryptamine (5-HT) in the central nervous system (c.n.s.) acts by inhibiting animal responses to painful stimuli. The evidence is based mainly on experiments using lesions or stimulation of 5-HT-containing neurons or direct administration of 5-HT into various areas of the c.n.s. (Samanin et al 1970; Samanin & Valzelli 1971; Oliveras et al 1975; Messing & Lytle 1977; Sewell & Spencer 1977; Yaksh & Wilson 1979).

There is some controversy about whether drugs increasing 5-HT transmission have antinociceptive effects when administered systemically. Quipazine, a 5-HT agonist (Jacoby et al 1976) was found by Samanin et al (1976) to inhibit nociceptive responses of rats in the hot plate and tail compression tests. However, other authors failed to find any effect of quipazine in the hot plate test (Malec & Langwinski 1980). More recently, quipazine was found to reduce rat responses in the hot plate but not in the tail flick test (Minnema et al 1980). There are also some differences about the ability of 5-HT uptake inhibitors to cause antinociception in rats (Messing et al 1975; Larson & Takemori 1977).

There is no clear explanation for the different results, although differences in the antinociceptive tests may play a major role (Ogren & Holm 1980; Dennis & Melzack 1980).

m-Chlorophenylpiperazine (mCPP), which displaces [^{3}H]5-HT bound to brain membranes (Samanin et al 1979; Fuller et al 1981) and has behavioural and biochemical effects compatible with a stimulatory action on 5-HT receptors (Samanin et al 1979), has been examined for its effects on various nociceptive responses of rats and to assess whether these depend on its effect on 5-HT.

Materials and methods

Male CD-COBS rats (Charles River, Italy) 200–225 g were kept in groups of 5 per cage at constant room temperature (21 ± 1 °C) and relative humidity (60%). The animals were tested for their responses to three noxious stimuli.

Tail immersion. The method described by Janssen et al (1963) was used. The animals were placed in a specially constructed rat holder with the tail hanging freely. The tail was dipped in a beaker containing water kept at 55 °C and the time elapsed until its sudden withdrawal was measured to the nearest 0.1 s. The animals were allowed to adapt to the cages for 30 min before testing and were kept in the cages for the period of test. If an animal failed to respond within 30 s, a score of 30 was recorded.

Hot plate, licking response. Rats were placed on a hot plate with the temperature thermostatically held at 55 °C. A Plexiglass cylinder (26 cm high, 20 cm in diameter), open at the top, confined the rat to a defined area of the plate. The time (in 0·1 s) between contact with the plate and licking of paws was recorded. If an animal failed to reach the end point of 30 s, it was removed from the plate and given a score of 30.

Hot plate, jumping response. The method was similar to that described by Jacob & Blozovski (1961). The day before the experiments the animals were placed 3 times (at intervals of 30 min) on the hot plate (55 °C) as described above. Animals responded with a jump adjusted on the border of the cylinder at the third exposure and maintained this response 24 h later when drugs were tested. The time between placing the animal on the plate and the adjusted jump was measured to the nearest 0·1 s. If an animal failed to jump after 30 s it was removed from the plate and assigned a score of 30.

Treatment. In one experiment the effects of 0·3, 1, 3 and 10 mg kg^{-1} of mCPP hydrochloride (Aldrich-Europe, Belgium) injected subcutaneously were assessed in the various tests. In another experiment, doses of mCPP with nearly maximal effects on jumping (3 mg kg^{-1}) and licking (10 mg kg^{-1}) in the hot plate test were administered subcutaneously to rats which had received a subcutaneous injection of metergoline (5 mg kg^{-1}) 3 h earlier. mCPP and metergoline were dissolved respectively in 0·9% NaCl (saline) and ascorbic acid solution (1%). A group of saline-treated rats was used as controls in the various experiments. In each test the animal's response was recorded before and 30, 60, 120 and 180 min after drug injection.

Results

As shown in Table 1, mCPP significantly decreased the latency for tail withdrawal at 60 min after the injection of

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Table 1. Effects of mCPP on various nociceptive responses of rats.

Test	Treatment mg kg^{-1}	Times after injection (min) 0	30′	60′	120′	180′
Tail immersion	Controls	2·04 ± 0·197	2·95 ± 0·28	2·86 ± 0·22	3·09 ± 0·25	2·82 ± 0·21
	mCPP 0·3	2·32 ± 0·18	2·68 ± 0·37	2·34 ± 0·26	2·89 ± 0·34	2·73 ± 0·60
	mCPP 1	2·28 ± 0.16	2·13 ± 0·19	2·05 ± 0·13	2·18 ± 0·10	2·24 ± 0·20
	mCPP 3	2·21 ± 0·14	2·08 ± 0·14	1·84 ± 0·18**	2·01 ± 0·45	2·57 ± 0·82
	mCPP 10	2·60 ± 0·23	1·88 ± 0·11**	1·85 ± 0·17**	1·32 ± 0·15**	1·65 ± 0·19**
Hot plate: licking	Controls	5·02 ± 0·45	7·18 ± 0·97	5·54 ± 0·59	5·22 ± 0·31	5·08 ± 0·30
	mCPP 0·3	5.37 ± 0·37	6·16 ± 0·85	5·87 ± 0·42	6·34 ± 0·83	6·96 ± 1·06
	mCPP 1	4·69 ± 0·22	9·97 ± 2·36	10·18 ± 2·57	6·30 ± 0·95	6·68 ± 0·68
	mCPP 3	5·14 ± 0·20	12·42 ± 2·38	12·28 ± 1·63*	8·06 ± 0·79	6·70 ± 1·19
	mCPP 10	4·99 ± 0·40	19·75 ± 3·23**	20·72 ± 2·75**	18·80 ± 3·14	14·74 ± 4·89**
Hot plate: jumping	Controls	6·02 ± 0·68	7·64 ± 1·79	7·14 ± 1·75	7·85 ± 1·85	9·07 ± 2·67
	mCPP 0·3	4·50 ± 1·06	11.0 ± 3·57	7·11 ± 1·17	4·88 ± 0·86	5·81 ± 1·32
	mCPP 1	5·64 ± 0·85	14·39 ± 3·08	20·07 ± 3·02*	5·13 ± 1·12	4·02 ± 0·72
	mCPP 3	5·52 ± 0·85	23·27 ± 2·90**	23·70 ± 2·78**	14·95 ± 2·98	10·09 ± 2·60
	mCPP 10	4·49 ± 0·53	26·83 ± 1·79**	26·88 ± 2·19**	20·16 ± 3·3**	18·81 ± 3·55*

The figures are the means ± s.e. (in seconds) of 10 animals.
* $P < 0.05$
** $P < 0.01$ compared with controls (Dunnett's test, two tails).

3 mg kg^{-1} and at each time after 10 mg kg^{-1}. Jumping and paw licking on the hot plate were inhibited by mCPP, although to different extents. Jumping was significantly inhibited 60 min after the administration of 1 mg kg^{-1} of mCPP whereas 3 mg kg^{-1} was necessary to significantly affect paw licking. 10 mg kg^{-1} of mCPP caused long-lasting inhibition of both responses on the hot plate. Table 2 reports the findings in experiments with metergoline. Only the results at peak effect of mCPP (60 min after injection) are shown. Metergoline pretreatment completely prevented the effect of mCPP on jumping but only partially reduced that on paw licking.

Table 2. Effects of mCPP on animals pretreated with metergoline.

Pre-treatment	Treatment	mg kg^{-1}	Licking	mg kg^{-1}	Jumping
Saline	Saline	(—, —)	4·72 ± 0·30	(—, —)	5·07 ± 0·80
Saline	mCPP	(—, 10)	25·85 ± 2·06**	(—, 3)	19·12 ± 2·70**
Metergoline	Saline	(5, —)	5·93 ± 0·98	(5, —)	3·82 ±0·62
Metergoline	mCPP	(5, 10)	17·60 ± 2·43††	(5, 3)	4·86 ± 0·59††

Responses of animals at mCPP peak effect (60 mins after injection) are reported.
Each figure is the mean ± s.e. (in seconds) of 10 animals.
**$P < 0.01$ compared with controls Student's t-test.
††F int $P < 0.01$ ANOVA split-plot design 2 × 2 for unrepeated measures.

Discussion

The present findings confirm previous suggestions (Dennis & Melzack 1980; Minnema et al 1980; Ogren & Holm 1980) that agents activating 5-HT mechanisms inhibit some but not all responses of rats to painful stimuli.

mCPP blocked jumping and paw licking of rats on the hot plate but had no effects on tail withdrawal in the tail immersion test. mCPP preferentially inhibited jumping and the effect was prevented by pretreatment with metergoline, a 5-HT antagonist (Fuxe et al 1975), indicating that 5-HT is involved.

Only the highest dose of mCPP (10 mg kg^{-1}) caused a clear-cut inhibition of paw licking and the effect was partially prevented by metergoline. Since mCPP, at 10 mg kg^{-1}, increases the metabolism of brain noradrenaline, (Invernizzi et al 1981) which has also been involved in antinociception (Mayer & Price 1976; Zemlan et al 1980) it is conceivable that noradrenaline contributes to the inhibition of paw licking by mCPP.

Various authors have found that activation of 5-HT transmission in the spinal cord inhibits tail flick of rats (Yaksh & Wilson 1979; Wang 1977). The failure of mCPP to exert this effect in the present studies may depend on its inability to activate 5-HT mechanisms in this brain region. It has been found, however, that other drugs supposed to increase 5-HT transmission such as quipazine and fluoxetine fail to inhibit tail withdrawal of rats (Minnema et al 1980; Larson & Takemori 1977). This may therefore be a characteristic of these agents. It has been shown recently that increasing 5-HT transmission in the spinal cord or brain leads to opposite effects on some behavioural responses (Davis et al 1980). It may be therefore that the diffuse activation of 5-HT mechanisms in the c.n.s. caused by mCPP produces effects which mask its action on spinal 5-HT fibres involved in the inhibition of pain transmission. These effects may also have contributed to the decreased latency for tail withdrawal in animals treated with mCPP.

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Interindividual variation in apparent volumes of distribution of antipyrine in the rat

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Antipyrine pharmacokinetics have frequently been employed as a measure of in vivo rate of hepatic drug metabolism both in man and animals (Vesell & Page 1968; Bakke et al 1974; Stevenson 1977; Aarbakke 1978; Vesell 1979; Gadeholt et al 1980; Høyem-Johansen et al 1980). In these studies various kinetic variables have been used as indices of the hepatic metabolic capacity. Recently a thorough discussion of the relationship between antipyrine clearance, half-life and apparent volume of distribution in man was presented (Sultatos et al 1980). Analysis of pooled data from several studies of antipyrine in man revealed that over the range of commonly observed clearance values in healthy volunteers, the relationship between antipyrine clearance and half-life approximated linearity. It was further stated that changes in β or $t_{1/2}(\beta)$ reflect altered antipyrine elimination, provided that the apparent volume of distribution (V_β) is unchanged. The latter statement must, however, be qualified, because unchanged V_β is a prerequisite for the use of β or $t_{1/2}(\beta)$ both in experiments designed for interindividual and intraindividual comparison. In man, insignificant variation of the apparent volume of distribution of antipyrine has been reported in both types of studies (Roberts et al 1976; Sultatos et al 1980).

Recently, significant intraindividual variation of both V_c and V_β upon repeated testing of antipyrine kinetics in cannulated, but otherwise untreated rats, were demonstrated (Johannessen et al 1981). Thus, the validity of β and $t_{1/2}(\beta)$ as single measures of changes in antipyrine elimination in studies using rats as their own controls might be questioned. In this communication we present data on the interindividual variation of antipyrine apparent volumes of distribution, V_c, V_β and $V_{d(ss)}$, in a population of cannulated, but otherwise untreated rats.

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Materials and methods

Eighteen Male Wistar rats, 200–300 g, were studied. In fluanison/fentanyl anaesthesia (0·6/0·13 mg kg^{-1}), an inguinal artery and vein were cannulated with PE 50 tubing previously filled with heparinized 0·9% NaCl, the indwelling part of the tubing having been stretched to reduce its diameter and lubricated with silicone oil to facilitate insertion. The cannula was secured and the tubing was transferred dorsally through a subcutaneous tunnel and made accessible through a skin perforation in the lumbar region. After the surgery, 3 ml sterile 0·9% NaCl (saline) was injected s.c. in the rat to compensate for fluid losses. The rats were placed in restraining cages overnight and allowed free access to food and water. Pharmacokinetic experiments were conducted the next morning, 12–15 h after the cannulation. Antipyrine-*N*-methyl[^{14}C] (New England Nuclear) 15 mg kg^{-1}, 1–2 μCi/animal, was infused via the cannula in the inguinal vein and dissolved in a volume of 0·6–0·9 ml saline. The infusion, lasting for 30 s, was immediately followed by flushing of the cannula with 0·5 ml saline. Blood samples (0·1 ml) were drawn from the cannula in the inguinal artery 3, 6, 9, 15, 20, 30, 40, 60, 90, 120, 150 and 180 mm after dosing.

Concentrations of antipyrine in whole blood was determined essentially by the extraction method of Bakke et al (1974).

The data were analysed according to a two-compartment