Brain Research, 279 (1983) 105–110
Elsevier

Analgesia Induced by Brief Footshock: Blockade by Fenfluramine and 5-Methoxy-N,N-Dimethyltryptamine and Prevention of Blockade by 5-HT Antagonists

P. H. HUTSON, M. D. TRICKLEBANK* and G. CURZON**

Department of Neurochemistry, Institute of Neurology, Queen Square, London WC1N 3BG (U.K.)

(Accepted March 22nd, 1983)

Key words: 5-hydroxytryptamine — analgesia — footshock — memory — learning

Analgesia induced by footshock (2 mA, 30 s) is decreased by the 5-HT releaser, fenfluramine, and the rapidly acting 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT). These decreases are blocked by the 5-HT antagonists, cyproheptadine and methiothepin. However, the antagonists when given alone do not influence shock-induced analgesia. Therefore, analgesia induced by brief footshock in the absence of drugs may not involve 5-HT-dependent mechanisms even though it may be influenced by pharmacologically provoked changes of 5-HT release or by 5-MeODMT. This drug was also able to attenuate the analgesia after its induction, possibly reflecting a disruption of memory processes rather than of nociceptive mechanisms per se.

INTRODUCTION

Exposing rats to acute footshock induces a transient analgesia as indicated by increased latency to lick the paws or flick the tail when noxious heat is subsequently applied[7,13]. Various drug experiments have shown that the analgesic effect of 30 s footshock is inversely related to the availability of 5-hydroxytryptamine (5-HT) in the CNS as analgesia is attenuated by the 5-HT releasers, *p*-chloroamphetamine and fenfluramine, by the 5-HT re-uptake inhibitor, fluoxetine, and by the indolic 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), while it is enhanced by depleting 5-HT with *p*-chlorophenylalanine (pCPA)[17], or by lesioning spinal 5-hydroxytryptaminergic tracts with 5,7-dihydroxytryptamine[8].

However, (apart from its attenuation by 5-MeODMT), the analgesia is markedly insensitive to drugs acting on 5-HT receptors, i.e. the agonists trifluoromethylphenylpiperazine and quipazine and the antagonists methysergide, metergoline, cyproheptadine, methiothepin and mianserin[17].

Results thus reveal two interesting characteristics of the analgesia induced by brief footshock. Firstly, there is an inverse relationship with 5-HT availability which contrasts with other nociceptive mechanisms which occur in the absence of prior stressful stimulation as these exhibit a positive relationship between 5-hydroxytryptaminergic activity and nociceptive thresholds[10,16,21]. Secondly, the effects on the analgesic response of procedures altering the availability of 5-HT contrast strikingly with the lack of effect of a range of 5-HT agonists and antagonists. These findings were investigated further in a series of experiments on the response of the analgesia induced by brief footshock to the 5-HT releaser, fenfluramine, and the indolic 5-HT agonist, 5-MeODMT.

Results obtained show that the blockade of the analgesia by these drugs was prevented by 5-HT antagonists which were confirmed to be without direct effect on the analgesic response in the absence of fenfluramine or 5-MeODMT. Also, it was found that the latter drug opposed the analgesia even when given shortly after shock. Thus it cannot simply act by reducing the perceived intensity of shock.

* Present address: Centre de Recherche Merrell International, 16 Rue d'Ankara, 67084 Strasbourg Cedex, France.
** To whom correspondence should be addressed at: Institute of Neurology, 33 John's Mews, London, WC1N 2NS, U.K.

0006-8993/83/$03.00 © 1983 Elsevier Science Publishers B.V.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (weight range 200–250 g; Charles River U.K., Margate), were fed ALGH standard rodent diet and housed singly for 8–9 days under a 12 h light–dark (white-red) cycle, with white light commencing at 06.00 h.

Drugs

5-MeODMT was purchased from Sigma Chemicals, Poole, U.K. The following drugs were donated by their respective manufacturers: cyproheptadine (Merck, Sharpe and Dohme), fenfluramine (Servier), methiothepin (Roche). All drugs were dissolved or suspended in 0.9% NaCl and were injected i.p. in a volume of 1 ml/kg body wt.

Assessment of response to noxious thermal stimulation

Animals were tested between 14.00 h and 17.00 h. Latencies to withdraw the tail from a water bath at 51 °C were determined initially (L1), immediately after returning to the home cage for 30 s (L2), and then after exposure to 30 s inescapable footshock (2 mA, six 5 s pulses, less than 1 s between pulses) in a box with a lid which was too low to permit rearing.

RESULTS

Blockade of shock-induced analgesia by fenfluramine and 5-MeODMT and its prevention of 5-HT antagonists

The second latency value (L2) measured before shock was slightly greater than the first value (L1). This increase averaged 21%, was significant in 14/16 groups of rats and did not appear to be related to drug treatments (Tables I and II). After subsequent exposure to footshock there was a significant and much larger increase of latency which averaged 96%. This response was significantly attenuated in rats given the 5-HT releaser, fenfluramine (5 mg/kg i.p., 30 min before shock), but was unaffected by the 5-HT antagonists, cyproheptadine (10 mg/kg i.p., 90 min before shock), or methiothepin (2.5 mg/kg i.p., 60 min before shock) (Table I). These results agree with previous experiments in which analgesia was measured by a hot-plate method[17]. However, the above antagonists completely prevented the attenuation of analgesia due to fenfluramine.

Table II shows that the rapidly acting indolic 5-HT agonist, 5-MeODMT (1 mg/kg i.p., 1 min before

TABLE I

Effects of cyproheptadine and methiothepin on the inhibition of shock-induced analgesia by fenfluramine

Fenfluramine (5 mg/kg) was injected i.p. 30 min before shock (2 mA, 30 s). Cyproheptadine (10 mg/kg) was injected i.p. 90 min before shock. Methiothepin (2.5 mg/kg) was injected i.p. 60 min before shock. Analgesia was determined by tail flick as described under Materials and Methods, with the final latency value measured immediately after shock. Values are mean ± S.D., 10 or 9 rats/group.

Injected		*Tail flick latency (s)*				
		Pre-shock			*Post-shock*	
		L1	*L2*	*% change*	*L3*	*% change*
Experiment 1						
0.9% NaCl	0.9% NaCl	3.5 ± 0.6	4.3 ± 0.7**	23	9.1 ± 2.2***	111
Cyproheptadine	0.9% NaCl	3.6 ± 0.7	4.3 ± 0.7**	19	8.3 ± 2.9**	93
0.9% NaCl	Fenfluramine	3.5 ± 0.6	4.3 ± 0.4**	23	6.2 ± 1.1***,†	44
Cyproheptadine	Fenfluramine	3.3 ± 0.6	4.2 ± 0.6***	27	8.9 ± 2.7***,††	112
Experiment 2						
0.9% NaCl	0.9% NaCl	3.5 ± 0.5	4.0 ± 0.6**	14	7.7 ± 1.2***	93
Methiothepin	0.9% NaCl	3.5 ± 0.9	4.2 ± 1.2*	16	8.3 ± 2.4***	93
0.9% NaCl	Fenfluramine	3.6 ± 0.6	4.3 ± 0.4**	19	6.4 ± 0.7***,†	49
Methiothepin	Fenfluramine	3.5 ± 0.4	3.8 ± 0.6	9	8.4 ± 1.9***,††	121

Differences from preceding latency value for same group of rats; $^{*}P < 0.02$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t*-test, paired observations). Differences from L3 value for rats treated with 0.9% NaCl: $^{\dagger}P < 0.01$; differences from L3 values for rats treated with 0.9% NaCl and fenfluramine: $^{\dagger\dagger}P < 0.01$ (Student's *t*-test, unpaired observations). % changes are with respect to the preceding latency value.

TABLE II

Effects of cyproheptadine and methiothepin on the inhibition of shock-induced analgesia by 5-MeODMT

5-MeODMT (1 mg/kg) was injected i.p. 1 min before shock (2 mA, 30 s). Other drugs were injected 60 min before shock. All other conditions were as in Table I. Values are mean ± S.D., 10 rats/group.

Injected		*Tail flick latency (s)*				
		Pre-shock			*Post-shock*	
		L1	*L2*	*% change*	*L3*	*% change*
Experiment 1						
0.9% NaCl	0.9% NaCl	3.4 ± 0.4	4.1 ± 0.6**	20	7.8 ± 1.2***	90
Cyproheptadine	0.9% NaCl	3.2 ± 0.4	4.1 ± 0.6***	28	7.4 ± 1.2***	80
0.9% NaCl	5-MeODMT	3.6 ± 0.6	4.7 ± 0.5***	31	6.0 ± 0.8***,†	28
Cyproheptadine	5-MeODMT	3.4 ± 0.3	4.3 ± 0.6***	26	7.7 ± 1.8***,§§	79
Experiment 2						
0.9% NaCl	0.9% NaCl	3.7 ± 0.7	3.9 ± 0.9	5	7.4 ± 0.9***	90
Methiothepin	0.9% NaCl	3.3 ± 0.4	4.4 ± 0.6**	33	7.2 ± 1.0***	64
0.9% NaCl	5-MeODMT	3.9 ± 0.5	4.9 ± 0.9**	26	5.4 ± 0.8††	10
Methiothepin	5-MeODMT	3.7 ± 0.8	4.3 ± 1.1*	16	7.6 ± 3.2**,§	77

Differences from preceding latency value for same group of rats; $^{*}P < 0.02$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t*-test, paired observations). Differences from L3 value for rats treated with 0.9% NaCl; $^{\dagger}P < 0.01$, $^{\dagger\dagger}P < 0.001$: differences from L3 values for rats treated with 0.9% NaCl and 5-MeODMT; $^{\S}P < 0.05$, $^{\S\S}P < 0.02$ (Student's *t*-test, unpaired observations). % changes are with respect to the preceding latency values.

shock), significantly attenuated the development of analgesia. This agrees qualitatively with the previously found attenuation of analgesia as measured by hot-plate when 5-MeODMT (2 mg/kg i.p.) was given 20 min before shock[17]. In agreement with the fenfluramine results, the attenuation of analgesia by 5-MeODMT was prevented by cyproheptadine (10 mg/kg i.p., 60 min before shock) or by methiothepin (2.5 mg/kg i.p., 60 min before shock), even though (as in the experiments with fenfluramine) these drugs were without significant effect on shock-induced analgesia when given alone.

Effect of 5-MeODMT given before and after footshock on percentage analgesia score

To investigate whether 5-MeODMT (and hence by implication, 5-HT) affected the analgesia by means other than a reduction of the perceived intensity of shock, advantage was taken of its rapidity of action following intraperitoneal injection[4]. In the following experiments, the effect of giving the drug immediately after shock (when obviously it can no longer directly affect the perception of shock) was investigated.

Immediately after determining preshock latencies (L1 and L2), animals were injected i.p. with either 0.9% NaCl (Table III, Experiment 1, Groups 1 and 3) or 5-MeODMT (1 mg/kg) in 0.9% NaCl (Table III, Experiment 1, Group 2). After returning to the home cage for 1 min, animals were exposed to footshock and then immediately injected with either saline (Groups 1 and 2) or 5-MeODMT (Group 3) and returned to the home cage. Response latency was determined in all animals at 1 min and 2.5 min after termination of shock. In a separate experiment, the above procedure was repeated as described for Groups 1 and 3 except that animals were not shocked but instead remained in the home cage for an additional 30 s. The second latency value (L2) measured before shock was slightly greater than the first value (L1). This increase was significant in 3/5 groups of rats, averaged 15% and as in the previous experiments did not appear to be related to drug treatments.

Further changes did not occur on subsequent analgesia testing of saline-treated rats in the absence of footshock (Experiment 2, Table III, L3, L4). Similarly treated but shocked animals had marked and significant further increases of tail flick latencies 1 and 2.5 min after the termination of shock (Experiment 1, Table III, L3, L4). These changes no longer occurred in animals given 5-MeODMT 1 min before footshock. When 5-MeODMT was given immediately after shock, a significant increase in tail flick la-

TABLE III

Effect on analgesia of 5-MeODMT given before, after or without footshock (2 mA, 30 s)

Full details are given in the text. Each experiment was done twice and results combined. Values are means ± S.D. Numbers of rats given in brackets.

Treatment	*Tail flick latency (s)*						
	Pre-shock			*1 min post-shock*		*2.5 min post-shock*	
	L1	*L2*	*% change*	*L3*	*% change*	*L4*	*% change*
Experiment 1 (shock)							
Group 1: 0.9% NaCl before and after shock	4.4 ± 0.8 (20)	5.1 ± 1.4* (20)	16	8.0 ± 2.5*** (20)	57	7.6 ± 1.5*** (20)	49
Group 2: 5-MeODMT (1 mg/kg, i.p.) before shock; 0.9% NaCl after shock	5.2 ± 0.9 (9)	5.7 ± 1.3 (9)	10	5.3 ± 2.2^{+} (9)	—7	4.6 ± 3.0^{+} (9)	—19
Group 3: 0.9% NaCl before shock, 5-MeODMT (1 mg/kg i.p.) after shock	4.1 ± 0.9 (22)	5.0 ± 1.0*** (22)	22	8.1 ± 2.4*** (22)	62	5.6 ± 1.9^{++} (22)	12
Experiment 2 (no shock)							
0.9% NaCl injections as for Group 1	3.7 ± 1.0 (17)	3.9 ± 0.8 (17)	5	4.4 ± 1.0 (17)	13	4.5 ± 1.1 (17)	15
0.9% NaCl and 5-MeODMT injection as for Group 3	4.2 ± 1.1 (24)	4.6 ± 1.2** (24)	9	5.6 ± 1.6** (24)	22	5.4 ± 1.7 (24)	17

Differences from other values for same group of rats: L1 vs L2, L3 or L4 vs L2; *$P < 0.02$, **$P < 0.01$, ***$P < 0.001$ (Student's *t*-test, paired observations). Differences from value for rats treated with 0.9% NaCl: $^{+}P < 0.01$, $^{++}P < 0.001$ (Student's *t*-test, unpaired observations). % changes for L2 are with respect to L1. % changes for L3 and L4 are with respect to L2.

tency still occurred at 1 min (L3) which was essentially identical to that found with rats which were shocked but not given 5-MeODMT (probably because the drug had not had time to reach the brain). However, by 2.5 min the latency value was significantly lower than that of shocked animals not given 5-MeODMT and little greater than the pre-shock control values. 5-MeODMT given to non-shocked animals did not significantly affect the tail flick latencies over a similar period (Experiment 2, Table III, L3, L4). These results show that 5-MeODMT can reverse an established shock-induced analgesia although they do not exclude the possibility that it also decreases the perceived intensity of shock when given beforehand.

DISCUSSION

Results indicate that 30 s footshock caused considerable analgesia as measured by latency of tail flick response to noxious heat. These effects were markedly greater than the small differences between latencies obtained in the absence of shock (i.e. Tables I and II, L1 vs L2; Experiment 2, Table III, L1 vs L2 vs L3 vs L4).

Results also confirm our previous findings (using a hot-plate and paw lick method) that the analgesia was prevented by the 5-HT releaser, fenfluramine, and the indolic 5-HT agonist, 5-MeODMT. The action of the latter drug, even though various piperazine-containing 5-HT agonists were ineffective[17], is of interest in view of the finding that indole- but not piperazine-containing agonists induce 5-HT dependent myoclonus in the guinea pig[12]. These results suggest that the same type of 5-HT receptor may be involved in both the latter behaviour and the inhibition of shock-induced analgesia by fenfluramine.

We also showed that five 5-HT antagonists were without effect using the paw lick test. The lack of effect of two of these drugs (cyproheptadine and meth-

iothepin) was confirmed in the present work in which a tail flick test was used. However, these two drugs are now shown to oppose the attenuation of the analgesia by fenfluramine and 5-MeODMT. These two 5-HT antagonists also have anti-histaminergic and anti-dopaminergic properties, respectively[6,11,15]. Nevertheless, their actions at 5-HT receptors are probably involved in the present experiments as the drugs prevented the effects of a 5-HT releaser and a 5-HT agonist on shock-induced analgesia but did not alter the analgesic response when given alone. These results suggest that the analgesic effect of brief footshock may not normally involve 5-HT-dependent mechanisms, but that this transmitter, when released pharmacologically, acts on 5-HT receptors to prevent the analgesia. While gross depletion of 5-HT stores by the 5-HT synthesis inhibitor, *p*-chlorophenylalanine[17], or by lesioning spinal 5-HT neurons with 5,7-dihydroxytryptamine[8], increases the analgesic effect of shock, it should be noted that these experiments involved relatively chronic depletions of 5-HT, while the 5-HT antagonists which were found not to affect the analgesia were given acutely.

The general lack of effect of 5-HT agonists and antagonists on shock-induced analgesia is not paralleled by our findings using drugs acting on DA receptors as these were active. Thus, shock-induced analgesia was decreased by the specific D2 agonist, LY141865, and was increased by a non-cataleptogenic dose of the antagonist pimozide[3,19].

Although the inhibition by 5-MeODMT and fenfluramine of shock-induced analgesia appears to contradict numerous reports of positive relationships between serotonergic activity and nociceptive thresholds in the absence of stressful stimulation[10,16,21], a single mechanism could underly all the findings. For example, as analgesia varies directly with shock intensity[9], 5-HT might reduce the analgesia simply by reducing the perceived intensity of footshock. However, the rapidly acting[4] 5-HT agonist, 5-MeODMT, still prevented the analgesic effect of shock even when given after its termination. This cannot merely be explained by an action of 5-MeODMT on analgesic responses per se, as the drug did not decrease latencies to tail flick in the absence of shock. The finding is consistent with previous work[2] in which comparable doses were given intracerebroventricularly. The lack of effect of 5-MeODMT at 1 min probably merely reflects the time required for the drug to reach the brain.

How 5-MeODMT might decrease shock-induced analgesia is suggested by our finding that analgesia after brief footshock depends on exposing the animals to the pre-shock latency measurement. When this was omitted, latency after 30 s shock was significantly less than the corresponding values for rats concurrently put through the complete test procedure[18]. This phenomenon is reminiscent of 'superstition', a kind of learning in which the animal responds as if there was a causal relation between its behaviour (in this case, the motor response to the analgesia test) and a subsequent event (in this case, the shock) even though the relation is not causal but merely temporal[14]. Therefore, the attenuation of established analgesia by 5-MeODMT may be due to this drug and (by implication) 5-HT, disrupting memory processes. This suggestion does not exclude the possibility of other interactions between pharmacologically released 5-HT and mechanisms involved in the analgesia. However, it is relevant that intracranial injection of 5-HT into mice at pharmacological dosage[5] or systemic injection of the 5-HT releaser, *p*-chloroamphetamine[1], are reported to cause retrograde amnesia.

The finding that 5-MeODMT can attenuate analgesia *after* it has been established may be contrasted with the attenuation by naloxone of analgesia following 90 s shock to the front paws. This analgesia, unlike that studied here[17], is prevented by naloxone, but the drug must be given *before* analgesia is established[20]. If 5-HT decreases the analgesia induced by 30 s footshock on learning and memory rather than by directly altering nociception, then it is hardly surprising that its action does not accord with its antinociceptive properties. These are revealed if shock is prolonged to 30 min when the resultant analgesia no longer depends on exposure to pre-shock latency measurement and is increased if 5-HT synthesis is inhibited[18].

ACKNOWLEDGEMENTS

We thank the Medical Research Council and the Basil Samuel Trust for financial support.

REFERENCES

1 Archer, T., Ogren, S. O. and Johansson, C., The acute effect of *p*-chloroamphetamine on the retention of fear conditioning in the rat: evidence for a role of serotonin in memory consolidation, *Neurosci. Lett.*, 25 (1981) 75–81.

2 Berge, O. G., Hole, K. and Dahle, H., Nociception is enhanced after low doses and reduced after high doses of the serotonin receptor agonist 5-methoxy-N,N-dimethyltryptamine, *Neurosci. Lett.*, 19 (1980) 219–223.

3 Curzon, G., Hutson, P. H. and Tricklebank, M. D., Involvement of dopamine in the analgesic response induced by footshock, *Brit. J. Pharmacol.*, 74 (1981) 761P.

4 Davis, M., Astrachan, D. I., Gendelman, P. M. and Gendelman, D. S., 5-Methoxy-N,N-dimethyltryptamine: spinal cord and brainstem mediation of excitatory effects on acoustic startle, *Psychopharmacology*, 70 (1980) 123–130.

5 Essman, W. B., Serotonin in learning and memory. In W. B. Essman (Ed.), *Serotonin in Health and Disease, Vol. 3, The Central Nervous System*, Spectrum, New York, 1978, pp. 69–143.

6 Green, A. R., Hall, J. E. and Rees, A. R., A behavioural and biochemical study in rats of 5-hydroxytryptamine receptor agonists and antagonists with observations on structure–activity requirements for the agonist, *Brit. J. Pharmacol.*, 73 (1981) 703–719.

7 Hayes, R. L., Bennett, G. J., Newlon, P. G. and Mayer, D. J., Behavioural and physiological studies of non-narcotic analgesia in the rat elicited by certain environmental stimuli, *Brain Research*, 155 (1978) 69–90.

8 Hutson, P. H., Tricklebank, M. D. and Curzon, G., Enhancement of footshock-induced analgesia by spinal 5,7-dihydroxytryptamine lesions, *Brain Research*, 237 (1982) 367–372.

9 Hutson, P. H., Tricklebank, M. D. and Curzon, G., Analgesia induced by brief footshock: characteristics and roles of 5-hydroxytryptamine and dopamine. In M. D. Tricklebank and G. Curzon (Eds.), *Stress Induced Analgesia*, J. Wiley, 1983, in press.

10 Lints, C. E. and Harvey, J. A., Drug induced reversal of brain damage in the rat, *Physiol. Behav.*, 4 (1969) 29–31.

11 Lloyd, K. G. and Bartholini, G., The effect of methiothepin on cerebral monoamine neurons, *Advanc. Biochem. Psychopharmacol.*, 10 (1974) 305–309.

12 Luscombe, G., Jenner, P. and Marsden, C. D., Pharmacological analysis of the myoclonus induced by 5-hydroxytryptophan in the guinea pig suggests the presence of multiple 5-hydroxytryptamine receptors in the brain, *Neuropharmacology*, 20 (1981) 819–831.

13 Madden, J., Akil, H., Patrick, R. L. and Barchas, J. D., Stress induced parallel changes in central opioid levels and pain responsiveness in the rat, *Nature (Lond.)*, 265 (1977) 358–360.

14 Skinner, B. F., 'Superstition' in the pigeon, *J. exp. Psychol.*, 38 (1948) 168–172.

15 Stone, C. A., Wenger, H. C., Ludden, C. T., Stavorski, J. M. and Ross, C. A., Antiserotonin-antihistaminic properties of cyproheptadine, *J. Pharmacol. exp. Ther.*, 131 (1961) 73–84.

16 Tenen, S. S., The effects of *p*-chlorophenylalanine, a serotonin depleter, on avoidance acquisition, pain sensitivity and behaviour in the rat, *Psychopharmacologia*, 10 (1967) 204–219.

17 Tricklebank, M. D., Hutson, P. H. and Curzon, G., Analgesia induced by brief footshock is inhibited by 5-hydroxytryptamine but unaffected by antagonists of 5-hydroxytryptamine or by naloxone, *Neuropharmacology*, 21 (1982) 51–56.

18 Tricklebank, M. D., Hutson, P. H. and Curzon, G., Analgesia induced by brief or more prolonged stress differs in its dependency on naloxone, 5-hydroxytryptamine and previous analgesia testing, *Neuropharmacology*, submitted.

19 Tricklebank, M. D., Hutson, P. H. and Curzon, G., Involvement of dopamine in the analgesic response to footshock, *Psychopharmacology*, in press.

20 Watkins, L. R. and Mayer, D. J., Involvement of spinal opioid systems in footshock-induced analgesia: antagonism by naloxone is possible only before induction of analgesia, *Brain Research*, 242 (1982) 309–316.

21 Yaksh, T. L. and Wilson, P. R., Spinal serotonin terminal system mediates antinociception, *J. Pharmacol. exp. Ther.*, 208 (1979) 446–453.