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INVOLVEMENT OF 5-HYDROXYTRYPTAMINE IN THE ANALGESIC ACTION OF PETHIDINE AND MORPHINE IN MICE

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1 Groups of mice were pretreated with the 5-hydroxytryptamine (5-HT) depletors, fenfluramine or *p*-chlorophenylalanine (PCPA), followed by pethidine or morphine.

2 Fenfluramine alone produced a short lasting analgesia but PCPA was without any effect.

3 Pethidine and morphine both increased hot plate reaction times measured after 30 min.

4 Pretreatment with PCPA attenuated morphine analgesia but did not affect pethidine analgesia. Fenfluramine did not alter the response to either analgesic.

5 PCPA produced a significant depletion of brain 5-HT levels which was not reversed by the analgesics. The fenfluramine-induced decrease in 5-HT was reversed by morphine but not by pethidine.

6 The results support the involvement of 5-HT in the antinociceptive action of morphine in the mouse.

Introduction

It is well established that 5-hydroxytryptamine (5-HT) is involved in the antinociceptive action of morphine in rats (Sparkes & Spencer, 1971; Samanin, Ghezzi, Mauron & Valzelli, 1973; Vogt, 1974; Chance, Krynock & Rosencrans, 1978). After a single injection of morphine there is an increase in 5-HT turnover as shown by an elevated concentration of the metabolite, 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the brain (Haubrich & Blake, 1969; 1973; Goodlet & Sugrue, 1974; Miranda, Invernizzi & Samanin, 1979; Vasko & Vogt, 1981). Drug treatments that tend to increase brain 5-HT concentrations can potentiate morphine-induced analgesia. For example, pretreatment with the 5-HT reuptake inhibitors, fluoxetine or zimelidine (Sugrue & McIndewar, 1976; Sugrue, 1979; Gebhart & Lorens, 1980), or intracerebroventricular (i.c.v.) injection of 5-HT (Sparkes & Spencer, 1971) increase morphine analgesia.

In mice however, it has been shown that increased brain 5-HT concentrations are associated with an inhibition of morphine analgesia. Following a single injection of the monoamine oxidase inhibitor, phenelzine (Botting, Bower, Eason, Hutson & Wells, 1978), or 5-hydroxytryptophan (Botting, 1980), there was a significant decrease in morphine analgesia. This inhibition was reversed by pretreatment with the 5-HT antagonist, methysergide. In contrast, the antinociceptive action of pethidine was potentiated by phenelzine or 5-hydroxytryptophan (Botting *et al.*, 1978; Botting, 1980). However, this opposite effect of phenelzine on morphine and pethidine-induced analgesia was not related to any qualitative difference in brain 5-HT concentrations or uptake (Botting, 1980).

In the present study we have extended this work on the possible role of 5-HT in analgesia, by using the 5-HT depletors, fenfluramine (Reuter, 1975) and *p*-chlorophenylalanine (Koe & Weissman, 1966).

Methods

Analgesia and body temperature

Male albino CPLF mice (18–28 g) bred at NESCOT were used. Experiments were carried out between 09 h 00 min and 11 h 00 min and at an ambient temperature of 19–20°C. Analgesia was assessed by measuring the reaction time (to the nearest 0.5 s) of animals when placed on a hot plate maintained at 56°C (Janssen & Jageneau, 1957). Body temperatures were recorded by means of an electrical thermometer (Light Laboratories, Brighton) with a probe inserted 10 mm into the rectum.

The mice were divided into two main groups; one of which was given fenfluramine (7.5 mg/kg i.p.) and the other 0.9% w/v NaCl solution (saline, 10 ml/kg i.p.), 30 min before measurement of analgesia and temperature. Thirty minutes later sub-groups of 6 subjects were given subcutaneous injections of saline, pethidine (10 mg/kg) or pethidine (20 mg/kg), and analgesia and temperature measured after a further 30 min. When *p*-chlorophenylalanine

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(PCPA) was used, mice were injected with 300 mg/kg (i.p.) 12 h before the first analgesia and temperature assessment. Sub-groups received subcutaneous injections of saline or pethidine (10 or 20 mg/kg) and the analgesia and temperature testing repeated 30 min later, that is 13 h after the PCPA injections.

A similar protocol was followed for morphine, except that the doses used were half those of pethidine.

Biochemical determinations

Mice were killed by cervical dislocation 60 min after pethidine or morphine. The whole brain (minus cerebellum and cortex) was immediately placed in preweighed 10 ml polypropylene tubes standing in an ice bath. After weighing, the tissue was homogenized in 1 ml 0.4 M $HClO_4$ by sonication for 20 s at 4°C (amplitude = 20 μM). Homogenates were centrifuged for 30 min at 4500 rev/min (4°C) in an MSE Mistral 2L. The supernatants were decanted onto 8 × 40 mm columns of Sephadex G-10 (Bio-Rad Laboratories, Watford), and tryptophan, 5-HT, and 5-HIAA were separated according to the method of Earley (1981). Recovery of the indoles from the columns was estimated by carrying mixtures of authentic standards in 1 ml 0.2 M $HClO_4$ through the entire procedure. The concentration of tryptophan (Hess & Udenfriend, 1959), 5-HT (Vanable, 1963), and 5-HIAA (Curzon & Green, 1970) in each sample was measured using a Perkin-Elmer 204 fluorescence spectrophotometer.

Statistics

Two-way analysis of variance (ANOVA) was used to evaluate the effects of pretreatment with 5-HT depletors on the analgesia, temperature and biochemical changes following treatment with pethidine or morphine. Significant interactions between the two main effects were further assessed using Tukey's test for unconfounded means. An α-value of 0.05 was deemed to be statistically significant. The strength of association (w^2) between the independent and dependent variables was calculated for each significant F-ratio (Vaughan & Corballis, 1969).

Drugs

Fenfluramine hydrochloride (LLS), *p*-chlorophenylalanine methyl ester hydrochloride (Sigma), pethidine hydrochloride (Roche) and morphine sulphate (MacFarlane Smith) were dissolved in saline. L-Tryptophan (BDH), 5-hydroxytryptamine creatinine sulphate complex (Sigma) and 5-hydroxyindol-3-yl-acetic acid (Sigma) were dissolved in 0.01 M HCl. All doses and concentrations used are expressed as the free base.

Results

Analgesia and body temperature

Fenfluramine treatment Fenfluramine alone showed significant analgesic properties in the hot plate test ($F = 15.64$, d.f. = 1,44, $w^2 = 0.17$), and there was also a significant drug × time interaction ($F = 21.85$, d.f. = 1,44, $w^2 = 0.25$; Table 1). This interaction was due to an increase in reaction time after fenfluramine at 30 min but not 90 min (Tukey test). Fenfluramine also produced hyperthermia ($F = 6.66$, d.f. = 1,44, $w^2 = 0.10$), even though there was a decrease in body temperature with time ($F = 6.55$, d.f. = 1,44, $w^2 = 0.09$; Table 1).

Both pethidine ($F = 6.43$, d.f. = 2,30, $w^2 = 0.22$) and morphine ($F = 13.02$, d.f. = 2,30 $w^2 = 0.39$) caused analgesia (Figure 1), although only morphine produced significant hypothermia ($F = 6.48$, d.f. = 2,30, $w^2 = 0.24$; Figure 2). Pretreatment with fenfluramine had no effect on morphine or pethidine-induced analgesia (Figure 1); nor on morphine-induced hypothermia (Figure 2).

p-*Chlorophenylalanine treatment* PCPA was without any significant analgesic or hypothermic action (Table 2), although body temperature fell over the 60 min between tests ($F = 10.25$, d.f. = 1,44, $w^2 = 0.16$).

Table 1 Effect of fenfluramine on reaction time and body temperature

	Reaction time (s)		*Temperature* (°C)	
	30 min	90 min	30 min	90 min
Saline	5.1 ± 0.7	8.8 ± 1.2	38.0 ± 0.3	37.4 ± 1.4
Fenfluramine	15.1 ± 1.7	7.9 ± 0.7	38.8 ± 0.1	38.0 ± 0.1

Analgesia and rectal temperature were measured 30 and 90 min after fenfluramine (7.5 mg/kg) or saline. Each value is the mean reaction time or body temperature ± s.e.mean of 12 animals.
Statistical significance was assessed with a 2 × 2 ANOVA followed by Tukey's test for unconfounded means (details in text).

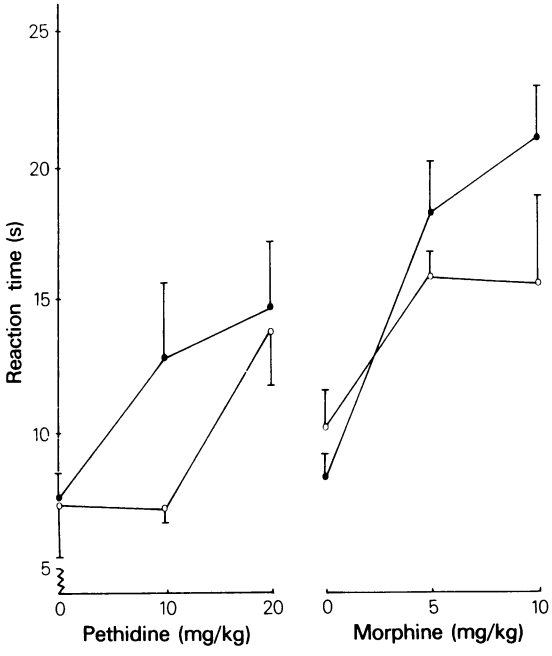

Figure 1 Effect of fenfluramine (7.5 mg/kg) on pethidine and morphine-induced analgesia. Fenfluramine (●) or saline (○) were given 1 h before the analgesics, and reaction times were measured 30 min later. Each point represents the mean for 6 mice; vertical bars show s.e.mean.

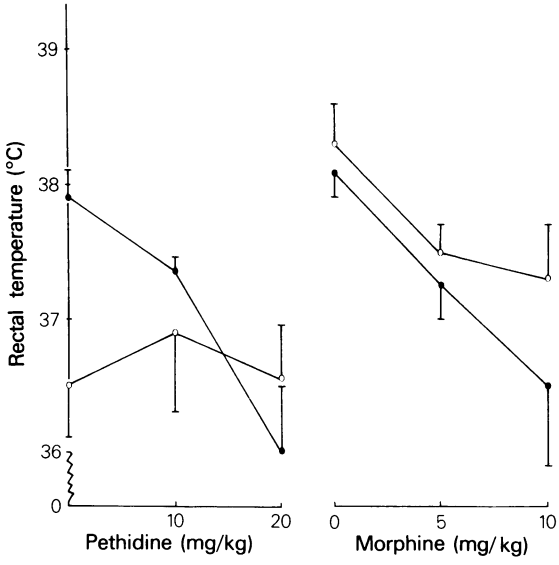

Figure 2 Effect of fenfluramine (7.5 mg/kg) on pethidine- and morphine-induced hypothermia. Fenfluramine (●) or saline (○) pretreatment were as described in the legend to Figure 1. Each point is the mean value ($n = 6$); vertical bars show s.e.mean.

PCPA significantly attenuated morphine- ($F = 5.59$, d.f. = 1,30, $w^2 = 0.06$), but not pethidine-induced analgesia (Figure 3). A significant hypothermic effect of morphine ($F = 4.35$, d.f. = 2,30, $w^2 = 0.16$) was again apparent, but this was unaltered by pretreatment with PCPA.

Biochemistry

The recoveries (mean ± s.e.mean) of tryptophan (0.5–2.0 μg/ml), 5-HT(50–200 ng/ml) and 5-HIAA (50–200 ng/ml) applied to the Sephadex microcolumns were 77 ± 3% ($n = 22$), 94 ± 2% ($n = 32$) and 83 ± 3% ($n = 8$) respectively. The values presented in Tables 3–6 inclusive are uncorrected for % loss.

Fenfluramine treatment Pethidine increased brain tryptophan ($F = 5.40$, d.f. = 2,30, $w^2 = 0.21$) and 5-HT concentrations ($F = 4.24$, d.f. = 2,30, $w^2 = 0.11$; Table 3). Fenfluramine decreased 5-HT ($F = 16.63$, d.f. = 1,30, $w^2 = 0.26$) and 5-HIAA concentrations ($F = 9.81$, d.f. = 1,30, $w^2 = 0.20$) over the dose range of pethidine studied (Table 3). Table 4 shows that fenfluramine decreased tryptophan ($F = 7.07$, d.f. = 1,30, $w^2 = 0.13$), 5-HT ($F = 13.97$, d.f. = 1,30, $w^2 = 0.25$) and 5-HIAA ($F = 34.96$, d.f. = 1,30, $w^2 = 0.45$) concentrations in mice given morphine. There was also a significant interaction for tryptophan ($F = 3.33$, d.f. = 2,30, $w^2 = 0.10$) and 5-HT ($F = 3.34$, d.f. = 2,30, $w^2 = 0.09$) due to a significant difference between the saline and fenfluramine controls (Tukey test).

p-*Chlorophenylalanine treatment* PCPA produced a decrease in brain 5-HT ($F = 76.71$, d.f. = 1,30, $w^2 = 0.67$) and 5-HIAA ($F = 77.14$, d.f. = 1,30,

Table 2 Effect of *p*-chorophenylalanine (PCPA) on reaction time and body temperature

	Reaction time (s)		*Temperature* (°C)	
	12 h	13 h	12 h	13 h
Saline	7.5 ± 0.7	7.4 ± 1.1	39.0 ± 0.2	38.4 ± 0.2
PCPA	7.8 ± 0.8	7.3 ± 0.6	39.1 ± 0.1	37.9 ± 0.1

Analgesia and rectal temperature were measured 12 and 13 h after PCPA (300 mg/kg) or saline.
Each value is the mean reaction time or temperature ± s.e.mean of 12 animals.
Statistical significance was assessed with a 2 × 2 ANOVA (details in text).

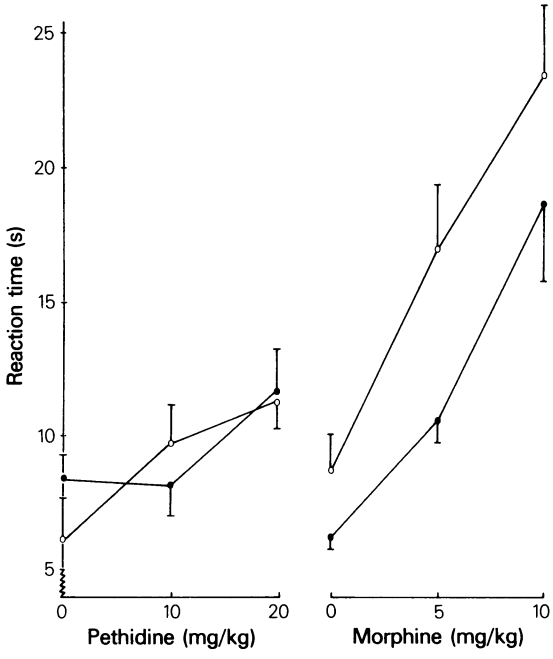

Figure 3 Effect of *p*-chlorophenylalanine (PCPA) (300 mg/kg) on pethidine- and morphine-induced analgesia. PCPA (●) or saline (○) was given 12.5 h before the analgesics and reaction times were measured 30 min later. Each point represents the mean for 6 mice; vertical bars show s.e.mean.

$w^2 = 0.68$) which was independent of the dose of pethidine (Table 5). In addition PCPA pretreatment prevented the pethidine-induced increase in tryptophan (cf. Table 3). A similar pattern was seen with morphine (Table 6); PCPA pretreatment resulting in a significant fall in tryptophan ($F = 4.67$, d.f. = 1,30, $w^2 = 0.10$), 5-HT ($F = 99.25$, d.f. = 1,30, $w^2 = 0.74$) and 5-HIAA concentrations ($F = 98.85$, d.f. = 1,30, $w^2 = 0.92$).

Discussion

A single dose of fenfluramine resulted in a threefold increase in reaction time after 30 min. However, the analgesia was short acting, and by 90 min there was no significant difference between fenfluramine- and saline-treated mice. Fenfluramine also produced a small but significant hyperthermia, that outlasted the analgesic effect. Duncan & Spencer (1973) have reported a similar analgesic effect in mice, while Sulpizo, Fowler & Macko (1978) have found hyperthermia in rats housed at 26–28°C (but not 20°C). The hyperthermia is dependent on the uptake of fenfluramine into the neurone, as it is reduced by pretreatment with some 5-HT reuptake inhibitors (Pawłowski, 1981; Sugrue, 1981). This effect may be mediated via the release of 5-HT, as it is abolished by PCPA, methysergide or cyproheptadine (Sulpizo *et al.*, 1978). The finding that PCPA alone is devoid of analgesic properties, is in agreement with the results of Sethy, Pradhan, Mandrekar & Seth (1970), and Major & Pleuvry (1971) for mice; and Tenen (1968) for rats.

Pethidine and morphine both increased reaction time, with morphine being the more potent of the two. Only morphine produced hypothermia, although pethidine at higher doses does cause a fall in body temperature (Botting, 1980). Pretreatment with fenfluramine did not alter pethidine or morphine analgesia, but PCPA significantly decreased morphine analgesia. Fenfluramine has been shown to potentiate the action of morphine in the mouse tail immersion test (Duncan & Spencer, 1973), while PCPA causes an inhibition in both rats and mice (Tenen, 1968; Major & Pleuvry, 1971; Vogt, 1974).

Table 3 Effect of pethidine on fenfluramine-induced changes in brain tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA)

Treatment	*Dose* (mg/kg)	*Tryptophan*	*5-HT*	*5-HIAA*
Saline		4.68 ± 0.22	0.36 ± 0.03	0.45 ± 0.04
Fenfluramine	7.5	5.49 ± 0.30	0.30 ± 0.02	0.40 ± 0.03
Pethidine	10.0	5.23 ± 0.39	0.45 ± 0.01	0.49 ± 0.01
Pethidine	20.0	6.42 ± 0.60	0.41 ± 0.03	0.48 ± 0.03
Fenfluramine + pethidine	7.5 + 10.0	5.50 ± 0.56	0.30 ± 0.01	0.38 ± 0.03
Fenfluramine + pethidine	7.5 + 20.0	6.62 ± 0.64	0.38 ± 0.03	0.43 ± 0.03

Fenfluramine was injected (i.p.) 60 min before the analgesic (s.c.), and the biochemical assays were carried out 60 min after the second injection.
Each value is the mean (μg/g wet wt. of tissue) ± s.e.mean of 6 determinations.
The data were analysed by a 3 × 2 ANOVA and Tukey's test for unconfounded means (details in the text).

Table 4 Effect of morphine on fenfluramine-induced changes in brain tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA)

Treatment	*Dose* (mg/kg)	*Tryptophan*	*5-HT*	*5-HIAA*
Saline		4.60 ± 0.25	0.45 ± 0.02	0.52 ± 0.02
Fenfluramine	7.5	3.23 ± 0.27	0.31 ± 0.01	0.35 ± 0.02
Morphine	5.0	3.86 ± 0.29	0.38 ± 0.04	0.49 ± 0.02
Morphine	10.0	3.68 ± 0.46	0.38 ± 0.02	0.58 ± 0.05
Fenfluramine + morphine	7.5 + 5.0	3.10 ± 0.37	0.33 ± 0.02	0.41 ± 0.02
Fenfluramine + morphine	7.5 + 10.0	3.87 ± 0.21	0.35 ± 0.02	0.43 ± 0.02

Details are as in the footnotes to Table 3.

PCPA has been shown to reduce the effect of pethidine in the mouse tail compression and electric shock tests, but not on the hot plate (Sethy *et al.*, 1970).

Fenfluramine causes a significant depletion of rat brain 5-HT which is maximal 2 h after a single injection (Costa, Groppetti & Revuelta, 1971; Tagliamonte, Tagliamonte, Perez-Cruet, Stern & Gessa, 1971; Miranda *et al.*, 1979). In the mouse brain there was a consistent decrease in 5-HT and 5-HIAA concentrations, and in the morphine experiment there was a decrease in tryptophan. Fenfluramine-induced depletion of tryptophan and 5-HT was reversed by morphine while pethidine did not affect 5-HT levels. It is probable that pethidine reverses the depletion of tryptophan, as it was found to increase significantly the concentrations of the amino acid in both saline- and fenfluramine-pretreated animals. PCPA produced a greater depletion of 5-HT and 5-HIAA than fenfluramine. The decrease of 40–50% was in close agreement with that of Koe & Weissmann (1966) and Curzon, Kantamaneni & Tricklebank (1981). With larger doses of PCPA, depletion of 5-HT can be as much as 90% (Vogt, 1974; Curzon *et al.*, 1981).

Pethidine increased brain tryptophan and 5-HT in fenfluramine and saline pretreated mice. Miranda *et al.* (1979) found that a higher dose of pethidine decreased 5-HIAA but not 5-HT concentrations in the brain stem of rats, although this may be non-specific, as it was not reversed by naloxone (Goodlet & Sugrue, 1974). Morphine increases 5-HIAA without affecting 5-HT concentrations in the rat (Haubrich & Blake, 1969; 1973; Goodlet & Sugrue, 1974; Miranda *et al.*, 1979; Vasko & Vogt, 1981). However, doses of at least 20 mg/kg were needed, except where the concentration was measured in discrete brain regions (Miranda *et al.*, 1979; Vasko & Vogt, 1981). A similar finding may have emerged in the present study, if separate brain regions had been analysed.

Our finding that morphine analgesia in the mouse is dependent on the integrity of brain tryptaminergic function is in agreement with results obtained from the rat. Thus, depletion of brain 5-HT by electrolytic or 5,6-dihydroxytryptamine lesions of the Raphe

Table 5 Effect of pethidine on *p*-chlorophenylalanine (PCPA)-induced changes in brain tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA)

Treatment	*Dose* (mg/kg)	*Tryptophan*	*5-HT*	*5-HIAA*
Saline		4.72 ± 0.44	0.43 ± 0.01	0.59 ± 0.03
PCPA	300	4.08 ± 0.51	0.26 ± 0.02	0.41 ± 0.02
Pethidine	10	4.46 ± 0.16	0.39 ± 0.01	0.64 ± 0.04
Pethidine	20	4.94 ± 0.31	0.37 ± 0.01	0.56 ± 0.02
PCPA + pethidine	300 + 10	4.25 ± 0.20	0.28 ± 0.02	0.38 ± 0.03
PCPA + pethidine	300 + 20	4.96 ± 0.37	0.27 ± 0.01	0.39 ± 0.01

PCPA was injected (i.p.) 12.5 h before the analgesic (s.c.), and the biochemical assays were carried out 60 min after the second injection.
Other details are as in the footnotes to Table 3.

Table 6 Effect of morphine on *p*-chlorophenylalanine (PCPA)-induced changes in brain tryptophan, 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid 5-HIAA

Treatment	*Dose* (mg/kg)	*Tryptophan*	*5-HT*	*5-HIAA*
Saline		5.01 ± 0.25	0.58 ± 0.03	0.33 ± 0.02
PCPA	300	4.67 ± 0.23	0.38 ± 0.03	0.21 ± 0.02
Morphine	5	5.38 ± 0.36	0.63 ± 0.04	0.38 ± 0.02
Morphine	10	4.87 ± 0.18	0.58 ± 0.01	0.34 ± 0.02
PCPA + morphine	300 + 5	4.72 ± 0.26	0.34 ± 0.02	0.20 ± 0.02
PCPA + morphine	300 + 10	4.52 ± 0.24	0.35 ± 0.03	0.19 ± 0.01

Details are as in the footnotes to Table 5.

nuclei (Samanin *et al.*, 1973; Vogt, 1974; Chance *et al.*, 1978; Miranda *et al.*, 1979), or systemic administration of PCPA (Tenen, 1968; Vogt, 1974) inhibit the antinociceptive action of morphine. Conversely, raising the 5-HT concentration with fluoxetine or zimelidine potentiated morphine analgesia but was without effect on pethidine analgesia (Sugrue & McIndewar, 1976; Sugrue, 1979; Gebhart & Lorens, 1980).

In conclusion, pretreatment with the 5-HT depletors, fenfluramine or PCPA did not alter the antinociceptive action of pethidine in mice. However, PCPA was associated with an attenuation of morphine-induced analgesia. The fenfluramine-induced depletion of 5-HT was reversed by morphine and this may reflect its lack of effect on morphine-induced analgesia.

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