Effect of depletion of rat brain 5-hydroxytryptamine on morphine-induced antinociception

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Central 5-hydroxytryptaminergic pathways are believed to be implicated in the antinociceptive action of morphine (Messing & Lytle 1977). Fenfluramine and *p*-chloroamphetamine are structurally similar halogenated phenethylamines and both elicit a long term reduction in rat brain 5-hydroxytryptamine (5-HT) content which is thought to be due to a neurotoxic action following their uptake into central 5-HT-ergic neurons (Harvey et al 1977). The objective of this study was to investigate the antinociceptive effect of morphine in rats pretreated with either fenfluramine or *p*-chloroamphetamine. Rats pretreated with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Koe & Weissman 1966) or the neurotoxin 5,7-dihydroxytryptamine (Baumgarten et al 1973) were also included in the study.

Male Wistar rats (ALA/CFHB strain), 180–250 g were used. Antinociceptive activity was quantified using the hot plate (55 °C) test. The dose of morphine used (2 mg kg^{-1}, s.c.) was one which approximately doubled the reaction time of rats injected with vehicle (saline, 0·9% NaCl). The time between injection and placement on the hot plate was 30 min, the time of peak antinociceptive activity. Using this protocol it has been demonstrated that increasing 5-HT availability at its central receptors markedly potentiates the antinociceptive action of morphine in rats of the same strain and weight as those used in this study (Sugrue 1979). Reaction time was the time between placement on the hot plate and the licking or flicking of hind paws and was timed to the nearest second. Fenfluramine hydrochloride (15 mg kg^{-1}), *p*-chloroamphetamine hydrochloride (10 mg kg^{-1}) or *p*-chlorophenylalanine methylester hydrochloride (PCPA) (300 mg kg^{-1}) was dissolved in saline and injected i.p. 4 days before antinociceptive testing. Rats were pretreated with desipramine hydrochloride (25 mg kg^{-1}, i.p.) 30 min before 5,7-dihydroxytryptamine infusion in order to prevent the uptake of the neurotoxin into noradrenergic neurons and a reduction in brain noradrenaline (NA) content (Björklund et al 1975). In addition, the rats also received pentobarbitone sodium (40 mg kg^{-1}, i.p.) 10 min before 5,7-dihydroxytryptamine to eliminate post infusion convulsions (Baumgarten et al 1973). Rats were anaesthetized with halothane/nitrous oxide and 200 μg of 5,7-dihydroxytryptamine creatinine sulphate, dissolved in saline containing 1 mg ml^{-1} ascorbic acid and kept on ice, was infused in a volume of 20 μl over a 1 min interval into the right lateral ventricle (Noble et al 1967). Antinociceptive testing was 7 days later. All doses refer to the free base. Each result is the mean of 6–8 observations and statistical significance was determined by means of Student's *t*-test (two tailed). Other groups of 5–6 rats were subjected to identical experimental procedures except that instead of antinociceptive testing, they were killed and brain concentrations of 5-HT (Snyder et al 1965), NA and dopamine (DA) (Laverty & Taylor 1968) determined spectrophotofluorometrically. Results were expressed as μg g^{-1} wet tissue and were corrected to 100% recovery. In all cases controls were treated in a similar manner except that drug vehicle was injected.

None of the 5-HT-depleting procedures changed hot plate reaction times (Table 1). The ability of morphine to significantly increase reaction times was not antagonized by pretreatment with 5,7-dihydroxytryptamine or PCPA. In contrast, fenfluramine or *p*-chloroamphetamine pretreatment markedly attenuated the ability of morphine to increase hot plate reaction times. All four procedures decreased rat brain 5-HT content, the magnitude of the fall ranging from approx. 40 to 80%. Brain concentrations of NA and DA were unaltered (Table 2).

Table 1. Effect of pretreatment with fenfluramine, *p*-chloroamphetamine, *p*-chlorophenylalanine or 5,7-dihydroxytryptamine on morphine induced antinociception. Morphine (2 mg kg^{-1}) was injected s.c. 30 min before rats being placed on hot plate (55 °C). Reaction times are the mean ±s.e.m. of 6–8 observations. Fenfluramine (15 mg kg^{-1}), *p*-chloroamphetamine (10 mg kg^{-1}) or *p*-chlorophenylalanine (300 mg kg^{-1}) was injected i.p. 4 days before antinociceptive testing. 5,7-Dihydroxytryptamine (200 μg) was injected i.v.t. 7 days before antinociceptive testing.

Pretreatment	Reaction time (s)	
	Saline-treated	Morphine-treated
Vehicle	7·0 ± 0·6	14·7 ± 1·6**
Fenfluramine	6·3 ± 0·6	8·9 ± 1·0
p-Chloroamphetamine	7·4 ± 0·4	9·1 ± 1·2
p-Chlorophenylalanine	7·2 ± 0·6	12·8 ± 1·6*
5,7-Dihydroxytryptamine	7·7 ± 0·8	15·3 ± 1·0**

* Differs from saline treated, $P<0·05$.
** Differs from saline treated, $P<0·001$.

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Table 2. Effect of 5-HT-depleting procedures on rat brain concentrations of noradrenaline (NA), dopamine (DA) and 5-HT. Results are expressed as % of appropriately treated controls and are the mean ±s.e.m. of 5–6 observations. Control brain concentrations were: NA, 0·482 ± 0·017 μg g^{-1} (n = 17); DA, 1·057 ± 0·067 μg g^{-1} (n = 17) and 5-HT, 0·486 ± 0·015 μg g^{-1} (n = 18). For further information see legend to Table 1.

Pretreatment	Endogenous amine content as % of control (Mean ± s.e.m.) NA	DA	5-HT
Fenfluramine	106·9 ± 1·6	96·3 ± 3·8	61·8 ± 1·4*
p-Chloroamphetamine	100·7 ± 2·8	105·2 ± 4·2	32·2 ± 2·6*
p-Chlorophenylalanine	100·2 ± 2·3	106·6 ± 3·3	22·3 ± 1·2*
5,7-Dihydroxytryptamine	98·0 ± 3·6	101·0 ± 3·0	42·4 ± 4·2*

* Differs from control, $P<0·001$.

Hence of the four procedures used to selectively lower brain 5-HT concentrations, only pretreatment with fenfluramine or *p*-chloroamphetamine altered the antinociceptive action of morphine. The observed inability of PCPA to modify morphine-induced antinociception in the rat agrees with the findings of some (Buxbaum et al 1973; Harvey & Yunger 1973; Reinhold et al 1973) but not others (Tenen 1968; Görlitz & Frey 1972; Vogt 1974). Fenfluramine (Duncan & Spencer 1973) and *p*-chloroamphetamine (Tulunay et al 1976) antagonism of morphine induced antinociception has been reported for mice but not for rats. The observed antagonism of the antinociceptive effect of morphine cannot be correlated with the degree of 5-HT depletion since the increase in hot plate reaction times elicited by morphine is attenuated by fenfluramine (38·2% fall) but not by PCPA (77·7% fall). The mechanism of 5-HT depletion elicited by PCPA, i.e. tryptophan hydroxylase inhibition, differs from that of the other three procedures which achieve their effect via a neurotoxic action. However, antagonism is not a property of all 5-HT neurotoxins as indicated by the lack of effect of 5,7-dihydroxytryptamine. If, as has been suggested (Messing & Lytle 1977; Yaksh et al 1977; Deakin & Dostrovsky 1978), the antinociceptive effect of morphine is dependent upon intact 5-HT-ergic systems it would appear that both fenfluramine and *p*-chloroamphetamine selectively destroy 5-HT-ergic nerve tracts which are critical for the antinociceptive action of the drug. Following the administration of PCPA, 5,7-dihydroxytryptamine or *p*-chloroamphetamine to rats there are marked regional variations in the degree to which 5-HT is decreased. Following PCPA treatment 5-HT concentrations in various brain regions are generally uniformly decreased (Sanders-Bush & Massari 1977). In contrast, 5,7-dihydroxytryptamine and *p*-chloroamphetamine exhibit regional, albeit different, selectivities. For example, the former exerts a profound effect on spinal 5-HT content whereas the latter does not (Baumgarten et al 1973; Sanders-Bush & Massari 1977). Both spinal and supraspinal 5-HT-ergic systems have been implicated in morphine induced antinociception (Yaksh et al 1977). If morphine-induced antinociception was dependent solely upon spinal 5-HT-ergic neurons it would be anticipated that the antinociceptive effect of the drug would be unaltered by *p*-chloroamphetamine pretreatment since this agent does not destroy spinal 5-HT-ergic nerve tracts as evidenced by its lack of effect on spinal 5-HT concentrations. Conversely, it would be expected that 5,7-dihydroxytryptamine pretreatment would modify the drug-induced antinociception. This was not so and the finding that pretreating rats with fenfluramine or *p*-chloroamphetamine attenuates the ability of morphine to increase hot plate reaction times suggests the importance of supraspinal 5-HT-ergic structures in the antinociceptive effect of the drug.

In summary, the findings of this study reveal that antagonism of the antinociceptive effect of morphine, as assessed by the hot plate, is not a property common to all agents which selectively decrease rat brain 5-HT concentrations. The precise relationship between 5-HT depletion and antagonism of morphine antinociception is a complex one awaiting elucidation.

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REFERENCES

Baumgarten, H. G., Björklund, A., Lachenmayer, L., Nobin, A. (1973) Acta Physiol. Scand. Suppl. 391: 1–19

Björklund, A., Baumgarten, H. G., Rensch, A. (1975) J. Neurochem. 24: 833–836

Buxbaum, D. M., Yarbrough, G. G., Carter, M. E. (1973) J. Pharmacol. Exp. Ther. 185: 317–327

Deakin, J. F. W., Dostrovsky, J. O. (1978) Br. J. Pharmacol. 63: 159–165

Duncan, C., Spencer, P. S. J. (1973) J. Pharm. Pharmacol. 25: 124P–125P

Görlitz, B.-D., Frey, H. H. (1972) Eur. J. Pharmacol. 20: 171–180

Harvey, J. A., McMaster, S. E., Fuller, R. W. (1977) J. Pharmacol. Exp. Ther. 202: 581–589

Harvey, J. A., Yunger, L. M. (1973) in Barchas, J., Usdin, E. (eds): Serotonin and Behavior, Academic Press: London. pp. 179–189

Koe, K. B., Weissman, A. (1966) J. Pharmacol. Exp. Ther. 154: 499–516

Laverty, R., Taylor, K. M. (1968) Anal. Biochem. 22: 269–279

Messing, R. B., Lytle, L. D. (1977) Pain 4: 1–21

Noble, E. P., Wurtman, R. J., Axelrod, J. (1967) Life Sci. 6: 281–291

Reinhold, K., Bläsig, J., Herz, A. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 278: 69–80
Sanders-Bush, E., Massari, V. J. (1977) Fed. Proc. Fed. Am. Soc. Exp. Biol. 36: 2149–2153
Snyder, S. H., Axelrod, J., Zweig, M. (1965) Biochem. Pharmacol. 14: 831–835
Sugrue, M. F. (1979) Br. J. Pharmacol. in press.
Tenen, S. S. (1968) Psychopharmacologia 12: 278–285
Tulunay, F. C., Yano, I., Takemori, A. E. (1976) Eur. J. Pharmacol. 35: 285–292
Vogt, M. (1974). J. Physiol., 236: 483–498
Yaksh, T. L., Plant, R. L., Rudy, T. A. (1977) Eur. J. Pharmacol. 41: 399–408

Effect of drugs influencing central 5-hydroxytryptaminergic mechanisms on morphine-induced catalepsy in the rat

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High doses of morphine induce immobility and catalepsy in the rat (Fog 1970) either by blocking dopamine receptors (Lal et al 1975) or by decreasing the amounts of dopamine at post-synaptic dopamine receptor sites (Kuschinsky & Hornykiewicz 1972, 1974). However, morphine has been shown not to block dopamine receptors (Iwatsubo & Clouet 1975), and Costall & Naylor (1975), on the basis of lesion studies, have suggested an involvement of the 5-hydroxytryptaminergic raphé system in the mediation of morphine-induced behavioural states like catalepsy and stereotypy. Furthermore, the 5-HT antagonist methergoline antagonizes morphine-induced catalepsy (Scheel-Krüger et al 1977).

We have investigated on morphine-induced catalepsy in the rat, the effect of pretreatment with clomipramine, a selective blocker of neuronal reuptake of 5-HT (Ross & Renyi 1975), quipazine, a drug that stimulates 5-HT post-synaptic receptors (Rodriguez et al 1973) and also stimulates the release (Hamon et al 1976) and inhibits the reuptake of 5-HT (Medon et al 1973), L-tryptophan, a precursor of 5-HT (Aghajanian & Asher 1971) and methysergide, a 5-HT receptor antagonist.

Male albino rats, 150–200 g, with free access to a standard diet and tap water were used. They were individually housed in wire netting cages at 27–30° C in a noiseless room. All observations were made between 10 and 16 h.

Catalepsy was scored according to Costall & Naylor (1975). Animals were tested for the presence of catalepsy by placing both front limbs over a horizontal bar placed 10 cm above the bench surface. If the animal maintained the imposed posture for at least 10 s it was said to be cataleptic and scored one point. For each further 10 s it continued to maintain the cataleptic posture one point was given. The animals were tested at 30 min intervals beginning 30 min after morphine treatment (5–40 mg kg^{-1}, i.p.).

Clomipramine HCl (Ciba-Geigy), quipazine maleate (Miles Laboratories), methysergide hydrogen maleinate (Sandoz Products Ltd) were dissolved in distilled water while L-tryptophan (Sigma) was dissolved in a minimum quantity of HCl and made up to volume with distilled water. Morphine sulphate given by injection in the commercial preparation (Burroughs Wellcome) and was diluted to required strength with distilled water. All agents were injected intraperitoneally in a volume of 5 ml kg^{-1} weight. Doses refer to the salt except for L-tryptophan which refer to the base. For each dose 10 animals were used. Clomipramine, quipazine and methysergide were injected 30 min and L-tryptophan 60 min before morphine treatment. Control groups received vehicle (5 ml kg^{-1}, i.p.). Statistical differences were analysed by Student's *t*-test.

Morphine 5 mg kg^{-1}, induced no catalepsy (n = 10 rats) while 10 mg kg^{-1} induced catalepsy in about 70% of the animals tested (n = 30 rats). At higher doses (20, 30, 40 mg kg^{-1}) it induced a dose-dependent degree of catalepsy in 100% of the animals, which was maximum at 30 min. Thereafter it declined rapidly and, depending upon the dose, lasted for 1·5–2·5 h after injection. The rats showed exophthalmus and increased sensitivity to acoustic and tactile stimuli during the presence of catalepsy. During the testing the rats frequently jumped or showed a short-lasting but rapid motility before they again became immobile. The cataleptic phase was followed by the 'excitation phase', characterized by increased motility, episodical and non-stereotyped biting. All rats were silent 3–4 h after morphine.

Clomipramine (5, 10, 20 mg kg^{-1}) did not induce catalepsy. Higher doses were not tested as they tended to produce motor incoordination and ataxia. Clomipramine pretreatment potentiated the cataleptic effect of morphine (10, 20 mg kg^{-1}) dose-dependently (Fig. 1).

Quipazine (1, 2, 4 mg kg^{-1}) did not induce a cataleptic state. Shortly after its injection animals exhibited a behavioral syndrome, comprising of increased locomotor activity, slight tremor, intensive sniffing and rubbing of the nose, which lasted for 30–40 min. After about 40 min the rat behaviour was almost normal. With higher doses (10, 20 mg kg^{-1}) there was

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