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ATTENUATION OF PETHIDINE-INDUCED ANTINOCICEPTION BY ZIMELIDINE, AN INHIBITOR OF 5-HYDROXYTRYPTAMINE REUPTAKE

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1 The effect of selective inhibition of 5-hydroxytryptamine (5-HT) re-uptake by fluoxetine and zimelidine on morphine- and pethidine-induced antinociception was studied in rats. The hot plate (55°C) and tail flick test procedures for measurement of analgesia were employed to assess antinociception.

2 Pretreatment with fluoxetine and zimelidine potentiated the antinociceptive effect of morphine (4.5 mg/kg, as base); zimelidine was without effect on a lesser dose of morphine (3.0 mg/kg, as base).

3 Pretreatment with zimelidine but not fluoxetine, significantly attenuated pethidine-induced antinociception (24 mg/kg, as base) and prevented the expression of pethidine-induced antinociception at a lesser 10 mg/kg (as base) dose of pethidine.

4 These and other results support (a) a role for 5-HT in the expression of morphine-induced antinociception, and (b) a different mode of antinociceptive action of morphine and pethidine. The role of 5-HT in pethidine-induced antinociception remains unclear.

Introduction

Evidence has steadily accumulated suggesting that 5-hydroxytryptamine (5-HT) is involved in mediating morphine-induced antinociception (see Messing & Lytle, 1977). For example, electrolytic destruction of the midbrain raphe nuclei (Samanin, Gumulka & Valzelli, 1970; Yaksh, Plant & Rudy, 1977) and nucleus raphe magnus (Proudfit & Anderson, 1975), as well as *p*-chlorophenylalanine administration (Tenen, 1968), have been shown to attenuate morphine-induced antinociception. Further, it has been reported that the selective inhibition of 5-HT reuptake by fluoxetine produces hypoalgesia and potentiates morphine-induced antinociception (Sugrue & McIndewar, 1976; Messing, Phebus, Fisher & Lytle, 1975). Surgue (1979) recently found that several specific inhibitors of 5-HT reuptake, fluoxetine and zimelidine (Ross & Renyi, 1977) among them, potentiated morphine- but not pethidine-induced antinociception in rats. As detailed in this paper, our data suggest an attenuation of pethidine-induced antinociception by zimelidine.

Methods

Male Albino Sprague-Dawley derived rats (Biolab, St. Paul, MN), weighing 250 to 350 g at the time of testing, were used. The animals were housed 5/cage in a temperature (22°C) and light (12:12 h dark-light cycle; lights on 07 h 00 min) controlled room. Food and water were available *ad libitum*. Standard hot plate and tail flick tests for measurement of analgesia were employed. The tail flick apparatus consisted of a photocell-activated timer capable of recording tail flick latencies to the nearest 0.01 s. The lamp intensity was adjusted to yield average tail flick latencies of 2.5 to 3.5 s (approximately 60°C); a trial was terminated if animals failed to respond within 15 s. Hot plate tests were conducted on an aluminum hot plate thermostatically maintained at 55 ± 0.5°C. The animals were contained in a clear plexiglas cylinder (20 cm diameter, 30 cm high) during testing; a trial was terminated if animals failed to respond within 120 s. Reaction latency on the hot plate was the time between placement on the heated surface and licking of any of the four paws. Reaction times were recorded to the nearest 0.1 s.

Animals received 0.9% w/v NaCl solution (saline, 1.0 ml/kg), fluoxetine (10.0 mg/kg, as the salt), or zimelidine (10.0 mg/kg, as the salt) intraperitoneally followed 1.5 h later by saline (1.0 ml/kg), morphine sulphate (3.0 or 4.5 mg/kg, as the base), or pethidine hydrochloride (10.0 or 24.0 mg/kg, as the base) subcutaneously. Post-drug testing started 30 min after the second injection (i.e., 2 h after fluoxetine or zimelidine administration). All testing was conducted between 13 h 00 min and 17 h 00 min.

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Results

The paw lick reaction time on the hot plate was unaltered by either fluoxetine or zimelidine 2 h after administration. As expected, both morphine and pethidine produced dose-related significant increases in the latency to respond on the hot plate (Table 1). Results of the tail flick test were comparable (Table 2).

The effect of pretreatment with the inhibitors of 5-HT reuptake on the antinociception induced by morphine and pethidine are presented in Table 3. At the 3 mg/kg dose of morphine, inhibition of 5-HT reuptake by zimelidine did not enhance morphine-induced antinociception in either the hot plate or tail flick tests. At 4.5 mg/kg morphine, both fluoxetine and zimelidine significantly enhanced morphine-induced antinociception on the hot plate. However, the effect of zimelidine pretreatment on pethidine-induced antinociception revealed a significant attenuation of antinociception by 24 mg/kg pethidine in the hot plate test and a suggestion that the same might occur at the single 10 mg/kg dose of pethidine in the tail flick test. Fluoxetine did not enhance pethidine-induced antinociception in the hot plate test, confirming other reports.

Table 2 Effect of zimelidine, morphine and pethidine on reaction time of rats in the tail flick test

Agents (dose, mg/kg)	*n*	*Reaction time (s)* *Control*	*Change*[b]
Saline-saline[a]	6	3.3 ± 0.6	0.9 ± 0.7
Zimelidine (10)-saline	7	2.8 ± 0.5	0.4 ± 0.5
Saline-morphine (3)	8	2.7 ± 0.3	8.1 ± 2.2*
Saline-pethidine (10)	7	3.3 ± 0.5	8.1 ± 1.8*

[a,b,*] See legend, Table 1.

Discussion

These results support previous reports that inhibition of 5-HT reuptake by either fluoxetine or zimelidine can enhance morphine-induced antinociception in rats (Sugrue & Indewar, 1976; Sugrue, 1979). However, while fluoxetine and zimelidine potentiate morphine-induced antinociception, inhibition of 5-HT reuptake by these agents affect pethidine-induced antinociception differently. In this and other papers (Sugrue & McIndewar, 1976; Sugrue, 1979), pethidine-induced antinociception was unaffected by fluoxetine pretreatment. Zimelidine pretreatment, on the other hand, significantly attenuated pethidine-induced antinociception at the 24 mg/kg dose and prevented the expression of its antinociceptive effect at the 10 mg/kg dose on the hot plate. The indication of a similar trend was apparent in the tail-flick test. In this study, both the dose of pethidine at which zimelidine showed significant attenuation of antinociception and the time of pretreatment with zimelidine differ from the reports of Sugrue (Sugrue & McIndewar, 1976; Sugrue, 1979). At the 10 mg/kg dose of pethidine (as the salt), Sugrue (1979) observed no affect of zimelidine pretreatment on pethidine-induced antinociception whereas the 10 mg/kg dose of pethidine in this study (equivalent to approximately 12.5 mg/kg as the salt) failed to produce a significant antinociception in zimelidine-pretreated (2 h) animals on the hot plate. This dose of pethidine was clearly antinociceptive in the absence of pretreatment (Table 1).

Table 1 Effect of fluoxetine, zimelidine, morphine and pethidine on reaction time of rats on the hot plate

Agents (dose, mg/kg)	*n*	*Reaction time (s)* *Control*	*Change*[b]
Saline-saline[a]	14	7.6 ± 1.1	2.6 ± 1.3
Fluoxetine (10)-saline	15	7.7 ± 0.8	2.9 ± 1.1
Zimelidine (10)-saline	12	8.8 ± 0.9	2.2 ± 1.3
Saline-morphine (3)	17	7.8 ± 0.5	8.5 ± 3.4*
Saline-morphine (4.5)	9	8.4 ± 0.8	20.5 ± 11.7*
Saline-pethidine (10)	7	8.5 ± 1.0	17.9 ± 8.9*
Saline-pethidine (24)	14	7.7 ± 0.8	62.0 ± 10.9*

[a] Animals received saline (1.0 ml/kg), fluoxetine (10 mg/kg) or zimelidine (10 mg/kg) intraperitoneally followed 1.5 h later by saline (1.0 ml/kg), morphine (3.0 and 4.5 mg/kg, as base) or pethidine (10.0 and 24.0 mg/kg, as base) subcutaneously. Testing began 0.5 h after the last injection. Values reported as mean ± s.e. mean.

[b] Change = post-drug latency − pre-drug control latency.

* Differs from respective pre-drug saline control ($P \leq 0.05$, Student's t, paired, one tail) and from saline-saline group ($P \leq 0.05$, Student's *t*, grouped, one tail).

The time of pretreatment with fluoxetine and zimelidine was selected based on reports of maximum inhibition (*in vivo*) of 5-HT reuptake (Wong, Horng, Bymaster, Hauser & Molloy, 1974; Ross, Ogren & Renyi, 1976). Wong and co-workers (1974) reported that fluoxetine (10 mg/kg) produced a maximum inhibition of 5-HT reuptake into whole rat brain (synaptosomes) of approximately 50%, 2 to 4 h after administration. Zimelidine (10 mg/kg) produced a 70% inhibition of 5-HT uptake into rat brain hypothalamic slices 2 h after administration (Ross & Renyi, 1977), suggesting that at equivalent doses, zimelidine produces a greater degree of inhibition of 5-HT reuptake. Alternatively, different brain regions may be differentially sensitive to inhibition of 5-HT uptake by fluoxetine and zimelidine. Wong *et al.* (1974) found different fluoxetine sensitivity in striatum (19% inhibition of 5-HT uptake into synaptosomes), diencephalon (23%), brain stem (53%), cerebral cortex (70%) and cerebellum (2%). Ross & Renyi (1977) reported that the concentration of zimelidine producing 50% inhibition of 5-HT uptake differed in homogenates of rat hypothalamus (0.24 μM) and striatum (0.60 μM). Thus, regional differences in the degree of inhibition of 5-HT reuptake may also contribute to the different effects of fluoxitine and zimelidine on pethidine-induced antinociception.

Pethidine and morphine were administered at a time such that both they and fluoxetine and zimelidine would exert their peak effects simultaneously. Neither fluoxetine (R. Fuller, personal communication) nor zimelidine (S-O. Ögren and S. Agurell, personal communication) alter the metabolism of morphine or pethidine and it can be assumed that the agents in fact were at peak effect as administered in this study.

While there is considerable evidence suggesting that 5-HT plays a role in morphine-induced antinociception, the role 5-HT plays in pethidine-induced antinociception is unclear. Tenen (1968) observed no attenuation of the antinociceptive effect of pethidine (45 mg/kg) in a modified flinch-jump procedure following *p*-chlorophenylalanine pretreatment whereas the effect of morphine (10 mg/kg) was significantly attenuated. Goodlet & Sugrue (1974) reported that morphine, but not pethidine, increased the turnover of 5-HT in rat brain. However, the dose of morphine used (20 mg/kg) also produces catalepsy and is well in excess of that required to produce antinociception. Further, a 10 mg/kg dose of morphine was without effect on the turnover of 5-HT. Moreover, while pethidine (50 mg/kg) failed to increase the turnover of 5-HT, it was not without effect; pethidine significantly reduced the turnover of 5-HT. Sugrue (1979) reviews additional differences between morphine- and pethidine-induced antinociception.

While these results provide further support for a role for 5-HT in the expression of morphine-induced antinociception, the role played by 5-HT in pethidine-induced antinociception remains unclear. The significant attenuation of pethidine-induced antinociception by zimelidine implicates 5-HT as playing a critical role. However, since inhibition of 5-HT reuptake by fluoxetine failed to attenuate pethidine-induced anti-

Table 3 Effect of fluoxetine and zimelidine pretreatment on morphine- and pethidine-induced antinociception in rats

Agents (dose, mg/kg)	*n*	*Reaction time (s)*	
		Control	*Change*[b]
	Hot plate		
Saline-morphine (3)[a]	17	7.8 ± 0.5	8.5 ± 3.4
Zimelidine (10)-morphine (3)	11	9.0 ± 1.1	9.9 ± 5.1
Saline-morphine (4.5)	9	8.4 ± 0.8	20.5 ± 11.7
Fluoxetine (10)-morphine (4.5)	9	7.5 ± 1.1	56.3 ± 13.7*
Zimelidine (10)-morphine (4.5)	9	7.9 ± 0.7	86.6 ± 14.5*
Saline-pethidine (10)	7	8.5 ± 1.0	17.9 ± 8.9
Zimelidine (10)-pethidine (10)	7	8.5 ± 0.9	5.9 ± 2.6
Saline-pethidine (24)	14	7.7 ± 0.8	62.0 ± 10.9
Fluoxetine (10)-pethidine (24)	5	8.2 ± 0.4	68.6 ± 16.1
Zimelidine (10)-pethidine (24)	15	8.2 ± 1.1	36.9 ± 11.3*
	Tail flick		
Saline-morphine (3)	8	2.7 ± 0.3	8.1 ± 2.2
Zimelidine (10)-morphine (3)	7	2.7 ± 0.3	10.3 ± 1.3
Saline-pethidine (10)	7	3.3 ± 0.5	8.1 ± 1.8
Zimelidine (10)-pethidine (10)	7	2.7 ± 0.4	6.4 ± 1.5

[a,b] See legend, Table 1.

* Differs from appropriate saline-morphine or saline-pethidine group ($P \leq 0.02$, Mann Whitney U, two tail).

nociception (Table 3), a non-5-HT related mechanism may be involved in the interaction between zimelidine and pethidine e.g. blockade by zimelidine of pethidine entry into the CNS or of its access to opioid receptors. Clearly, the interactions between zimelidine and the two analgesics emphasize the difference between the mode of antinociceptive action of morphine and pethidine, but whether the zimelidine-pethidine interaction is due to an action on 5-HT alone requires further investigation.

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