approximately four times higher than those for 5-ASA. These levels are similar to those obtained from patients taking up to 3 g of sulphasalazine daily (Fischer & Klotz 1979). In the urine, the acetyl form predominated (98%).

These low levels would suggest that the action of 5-ASA may be largely topical rather than systemic and it is probable that 5-ASA rather than the acetyl 5-ASA is the active compound (Binder et al 1981), although both have anti-inflammatory activity.

Renal damage due to 5-ASA has been reported in rats (Calder et al 1972) after intravenous administration of 1·4–5·7 mM kg which might be expected to give higher serum levels than those obtained by our enemas or sulphasalazine administration. As the levels of 5-ASA and acetyl 5-ASA documented after 5-ASA enema administration in our patients (most of whom had active inflammation and might be expected to absorb more of the drug) were low, this should be a safe and non-toxic preparation in man.

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p-Chlorophenylalanine antagonism of the analgesia and increase in brain noradrenaline metabolism produced by morphine

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Although the potential roles of noradrenaline and 5-hydroxytryptamine (5-HT) in opiate analgesia have been extensively studied (Messing & Lytle 1977; Iwamoto & Way 1979), information concerning their interaction in the expression of opiate effects is relatively lacking (Sewell & Spencer 1976). We here report an attempt to establish such a relationship by evaluating the effect of *p*-chlorophenylalanine (PCPA), an inhibitor of 5-HT synthesis (Koe & Weissman 1966), on the analgesia and increase in brain 3-methoxy-4-hydroxyphenylethylene glycol sulphate (MOPEG-SO_4), the major noradrenaline metabolite in rat brain (Schanberg et al 1968), produced by morphine.

Methods

Male Sprague-Dawley rats (Holtzman, Madison, WI) 200–300g were housed in pairs in automatic watering cages with food freely available. *p*-Chlorophenylalanine methyl ester HCl (Sigma, St. Louis, MO) (PCPA), dissolved in distilled water with the pH adjusted to 6·0 with 5 M NaOH, was administered at a dose of 300 mg kg^{-1} base i.p. 72 h before i.p. injection of 10 mg kg^{-1} morphine sulphate (Mallinckrodt, St. Louis, MO) or an equivalent volume of its saline (0·9% NaCl) vehicle (2 ml kg^{-1}). Analgesia was measured by a modification (Bass & Vander Brook 1952) of the tail flick procedure of D'Amour & Smith (1941). Baseline (BL) latency (3–4s) was determined immediately before the injection of morphine or saline and analgesia tested (T) 60 min thereafter. A 12s cut off was employed in the absence of a response and the degree of analgesia (DA) was calculated according to the following formula (Mayer & Hayes 1975): $DA = 100\ (T-BL)/(12-BL)$. The mean and standard errors of these ratios were calculated through the use of an arcsine transformation (Sokal & Rohlf 1969). Animals were decapitated following analgesic testing and whole brain noradrenaline and dopamine were measured in the same samples by the fluorometric procedures of Anton & Sayre (1962) and Carlsson & Waldeck (1958). Separate groups were employed for the fluorometric determination of brain 5-HT (Maickel et al 1968) and MOPEG-SO_4 (Meek & Neff 1972). Statistical comparisons among treatment groups were made with a one way analysis of variance and/or Student's *t*-test ($\alpha = 0{\cdot}05$).

To verify the selectivity of the PCPA treatment and to obtain an indication of the degree of 5-HT depletion, whole brain concentrations of dopamine, noradrenaline, 5-HT and MOPEG-SO_4 were measured in rats given PCPA (300 mg kg^{-1} i.p.) 73 h and saline 1 h before death. PCPA produced a 75% decrease in brain 5-HT ($55 \pm 3{\cdot}9$ vs $215 \pm 12{\cdot}4$ ng g^{-1} in untreated controls; $P < 0{\cdot}01$) but did not significantly alter the concentration of dopamine ($547 \pm 35{\cdot}7$ vs $560 \pm 12{\cdot}3$ ng g^{-1}), noradrenaline ($260 \pm 24{\cdot}4$ vs $265 \pm 7{\cdot}8$ ng g^{-1}) or MOPEG-SO_4 ($423 \pm 18{\cdot}6$ vs $430 \pm 13{\cdot}5$ p mol g^{-1}). This treatment also did not significantly alter baseline tail flick latency in the analgesic test.

The effect of PCPA on the analgesia and increase in

* Correspondence.

Table 1. Effect of PCPA on the degree of analgesia and increase in brain MOPEG-SO_4 produced by morphine. PCPA (300 mg kg^{-1} i.p.) or saline (2 ml kg^{-1} i.p.) was administered 72 h before the injection of morphine sulphate (10 mg kg^{-1} i.p.) or saline and animals were killed immediately following analgesic testing 1 h after the second injection. † Means ± s.e.m. followed by number of animals in parentheses. ** $P < 0.01$ compared with PCPA + saline. ‡ $P < 0.01$ compared with saline + morphine and PCPA + saline. *** $P < 0.01$ compared with saline + morphine.

Treatment	Degree of analgesia (%)	MOPEG-SO_4 (p mol g^{-1})
PCPA + saline	−0·7 ± 0·1 (8)†	422·6 ± 18·6 (7)
Saline + morphine	93·6 ± 1·2 (10)**	473·8 ± 7·6 (8)**
PCPA + morphine	20·2 ± 0·5 (9)‡	400·0 ± 4·9 (8)***

brain MOPEG-SO_4 produced by morphine (10 mg kg^{-1} i.p.) is illustrated in Table 1. In agreement with LoPachin & Reigle (1978), this dose of morphine produced a significant increase in brain MOPEG-SO_4 and a nearly maximal analgesic effect. However, PCPA significantly reduced the analgesic effect of morphine and prevented the opiate-induced increase in brain MOPEG-SO_4.

These findings strongly suggest that the ability of morphine to increase brain noradrenaline metabolism is dependent on mediation by a 5-HT system. PCPA alone did not appear to alter the metabolism of brain noradrenaline and, in agreement with previous findings (Koe & Weissman 1966), produced a selective depletion of brain 5-HT. This was associated with a previously demonstrated (Tenen 1968) reduction in morphine analgesia and an antagonism of the ability of morphine to increase brain MOPEG-SO_4.

According to the brain area investigated and the methodology employed, evidence for 5-HT enhancement and inhibition of noradrenergic transmission has been advanced (Watabe & Satoh 1979; Wang et al 1979; Ferron et al 1982) and either mechanism may contribute to the action of morphine. However, increases in brain MOPEG-SO_4 have been directly related to increased noradrenergic neuronal activity (Korf et al 1973) and morphine does not appear to increase the intraneuronal metabolism of noradrenaline, as would be expected if neuronal activity were reduced (Huff & Reigle 1980). In contrast, several studies have suggested that increased noradrenergic activity antagonizes morphine analgesia (Price & Fibiger 1975; Paalzow & Paalzow 1975) and, although contradictory evidence also exists (Sasa et al 1977), it is possible that morphine induces neuronal activity which attenuates its analgesic effect. It is also of interest that PCPA significantly reduced but did not completely prevent the analgesic action of morphine (Table 1). This may reflect residual 5-HT activity but could also indicate the participation of an additional transmitter such as dopamine (Iwamoto & Way 1979).

It is evident that the involvement of brain monoamines in opiate analgesia is highly complex, with the present study indicating that the participation of noradrenaline requires an initial interaction of morphine with brain 5-HT systems.

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