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Potentiation of morphine analgesia by caffeine

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Significant potentiation of morphine (5 mg kg^{-1} s.c. or 1 mg kg^{-1} i.v.) analgesia (tail-withdrawal reflex at 55°C) was observed in caffeine-treated (100 mg kg^{-1} i.p.) rats as compared to the control group and lower doses of caffeine (2mg kg^{-1} i.p.) did not show this effect. Potentiated analgesia was reversed by naloxone. Pharmacokinetic or dispositional factors appear to be involved in part in this potentiation.

Introduction Caffeine has been added to a large number of over-the-counter mild analgesic, antipyretic and anti-rheumatic products for many years either to counter the sedative and depressant effects of other ingredients or to potentiate their action. Apart from recent work (Laska *et al.*, 1984) which has provided evidence for caffeine as an analgesic adjuvant, little evidence and insufficient data existed for many years to demonstrate any additive contribution of caffeine to the action of these analgesic ingredients. This study was undertaken to determine the effect of caffeine on morphine analgesia assessed in tail-withdrawal reflex in rats.

Methods

Injection solutions Caffeine injection solution, (2.57×10^{-1} M) was prepared in warm 40% aq. propylene glycol. Morphine injection solutions were prepared in 0.9% w/v NaCl (saline); sp. act of [6-^{3}H (n)]-morphine injection solution was 10 μCi mg^{-1}.

Nociceptive testing The terminal 5 cm of the tail of male Wistar rats (120–160 g) was immersed in a water bath (55°C) and the time elapsing between immersion and flicking of tail was recorded as the reaction time (cut-off time 15 s). Average control tail-withdrawal latencies were approx. 1.5–2.0 s. Two separate groups of rats were pretreated intraperitoneally with either the vehicle or 100 mg kg^{-1} dose of caffeine and 30 min later injected subcutaneously with either 5 mg kg^{-1} or 1 mg kg^{-1} morphine intravenously. The response latencies were measured at selected times after morphine injection between 10 h 00 min and 15 h 00 min. The effect of a 25 mg kg^{-1} (i.p.) dose of caffeine on morphine analgesia was also determined.

Estimation of morphine concentrations Male Wistar rats (120–160 g) were injected intraperitoneally with either the vehicle (control) or with a 100 mg kg^{-1} dose of caffeine. A 1 mg kg^{-1} intravenous bolus dose of [6-^{3}H (n)]-morphine was injected 30 min after the injections ($n = 5$) and 20 min later, the animals were killed to obtain plasma and tissues for the analyses of morphine. Aliquots (2 ml) of plasma (diluted 1:5 with distilled water) or tissue homogenates (10% w/v in 0.5 M HCl) containing 1 ml non-radioactive morphine hydrochloride as carrier (500 μg ml^{-1} as free base) were adjusted to pH 9–9.5 with 1 M NaOH and the solution buffered with 2 ml 40% w/v K_2HPO_4 solution and extracted with 15 ml ethylene dichloride-*n*-amyl alcohol (7:3 v/v) as described previously (Misra *et al.*, 1971). Corrections for quenching of radioactivity in extracted tissue samples were made using [^{3}H]-toluene as internal standard.

Statistical analyses The statistical significance of data in the control vehicle and caffeine-treated groups was evaluated by Student's *t* test.

Results

The response latencies of rats given a 100 mg kg^{-1} (i.p.) dose of caffeine alone were not significantly different from those receiving an injection of the control vehicle over a 3 h testing period. The latencies of caffeine-treated animals at 1.0, 1.5 and 3.0 h after a 5 mg kg^{-1} (s.c.) morphine injection were significantly higher than those in control vehicle group (Figure 1a). The area under the analgesia-time curve of caffeine-treated group after morphine injection (11.70 ± 1.37 s-h) was significantly higher ($P < 0.05$) than that in control vehicle group (8.17 ± 0.53 s-h). The response latencies of caffeine-treated animals at 0.5, 2.0 h after a 1 mg kg^{-1} (i.v.) morphine injection were significantly higher than those in vehicle-treated group (Figure 1b) and the area under the analgesia-time curve of caffeine-treated group (8.03 ± 0.68 s-h) was also significantly higher ($P < 0.05$) than the vehicle-treated group (6.30 ± 0.18 s-h). The % increases in analgesia in caffeine-treated animals after subcutaneous and intravenous injections of morphine were 43.2 and 27.5 respectively. Animals pretreated with a 25 mg kg^{-1}

(i.p.) dose of caffeine and injected with a 5 mg kg^{-1} (s.c.) dose of morphine 30 min later, showed a greater stimulant and startle response than the vehicle controls and although the response latencies in these animals at 0.75 and 1 h and the area under the analgesia-time curve were somewhat higher than the control group, the differences were not statistically significant.

Comparative concentrations of morphine in plasma and tissues of contrl vehicle and caffeine-treated rats. The concentrations of morphine (mean ± s.e.mean, ng g^{-1} tissue or ml^{-1} fluid) in plasma and brain respectively of caffeine-treated rats 20 min after a 1 mg kg^{-1} (i.v.) injection of [6−^{3}H (n)]-morphine (148 ± 13; 65 ± 4) were significantly higher ($P < 0.05$) than those in the control group (102 ± 8; 53 ± 2). The concentrations in liver, heart and lung respectively (696 ± 91; 407 ± 35; 804 ± 37) were not significantly different from the corresponding values in the control group (588 ± 92, 341 ± 27, 673 ± 53), nor were the concentrations of morphine metabolites in plasma and liver or the ratio of concentration of morphine metabolites to unchanged drug in liver.

Discussion and Conclusion Recent studies (Laska *et al.*, 1984) on the combination of caffeine with mild analgesics have shown that to obtain the same amount of response from an analgesic without caffeine required a dose that was approx. 40% greater than one with caffeine. Our study shows that caffeine pretreatment (100 mg kg^{-1} i.p.) produced a significant potentiation of morphine analgesia. The potentiated analgesia in caffeine-treated rats was reversed by naloxone (2.5 mg kg^{-1} s.c.) administered 45 min after morphine injection. No antinociception was observed in rats with the 100 mg kg^{-1} (i.p.) dose of caffeine alone over a 3 h testing period. As in previous studies (Laska *et al.*, 1984), dosage of caffeine is an important factor in this potentiation and at lower doses (25 mg kg^{-1} i.p.) caffeine did not have effective analgesic adjuvancy. LD_{50} (oral) of caffeine in rats is 200–250 mg kg^{-1} and its plasma $t_{\frac{1}{2}}$ in human subjects about 4.0 h, with peak plasma levels occurring 1 h after oral ingestion.

The mechanism by which caffeine potentiates morphine analgesia is unclear. Significantly higher values of morphine in brain and plasma of caffeine-treated rats as compared to the controls suggested a reduced clearance of morphine in this group. Lack of significant changes in the ratio of morphine metabolites concentration to unchanged drug in the liver in the two groups suggested that caffeine pretreatment did not change morphine metabolism. Thus pharmacokinetic or dispositional factors are involved in part in the potentiation of morphine analgesia by caffeine. Reversal of potentiated analgesia by naloxone indicated the involvement of opiate receptor mechanisms. Caffeine

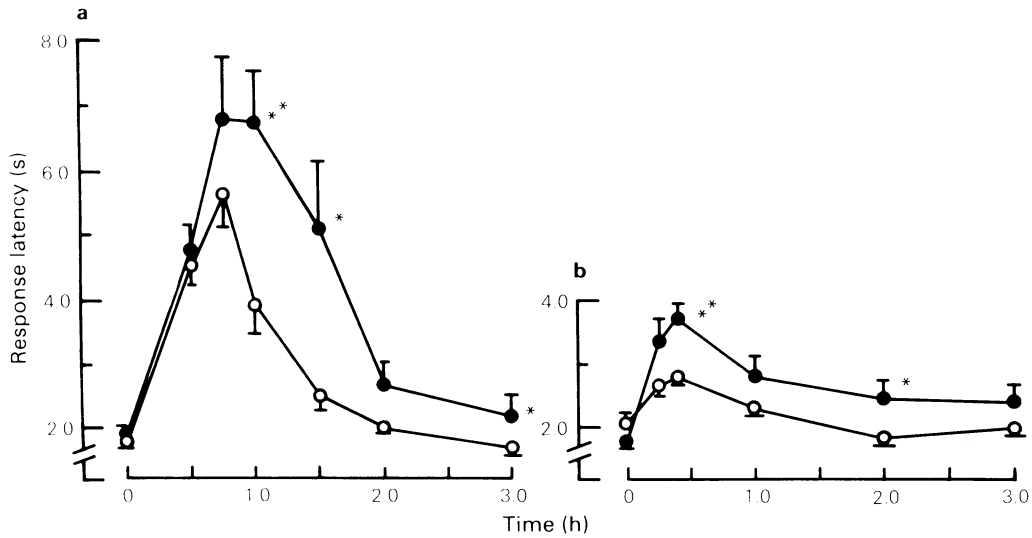

Figure 1 Tail-withdrawal response latencies (s) (mean with s.e.mean shown by vertical lines) of control vehicle (○) or caffeine-treated rats (●) ($n = 5$) after a 5 mg kg^{-1} s.c. or 1 mg kg^{-1} i.v. injection of morphine. The animals were injected i.p. either with the control vehicle or caffeine (100 mg kg^{-1}) and 30 min later injected with morphine. Response latencies were measured before and at selected times after morphine injection. Values significantly different from corresponding control values at $^{*}P < 0.05$ and $^{**}P < 0.01$ respectively.

has a very low binding to opiate receptors and has recently been shown to stimulate β-endorphin release in blood but not in CSF (Arnold *et al.*, 1982). Although medial thalamus and brain stem reticular formation have been suggested as important sites for the stimulant action of caffeine (Chou *et al.*, 1980), multiple sites in the CNS mediate the complex spectrum of pharmacological effects of caffeine. Such effects include inhibition of phosphodiesterases with consequent increases in cyclic AMP, potentiation of inhibitors of prostaglandin synthesis (Butcher & Sutherland, 1962), blockade of adenosine receptors (Daly *et al.*, 1981), increases in the rate of the turnover of noradrenaline and dopamine (Berkowitz *et al.*, 1970; Waldeck, 1971), increases in brain concentrations of 5-hydroxytryptamine (5-HT) and tryptophane without affecting the 5-HT turnover (Fernstrom *et al.*, 1984) and translocation of intercellular Ca^{2+}. However, the specific role of these various factors in the potentiation of morphine analgesia by caffeine remains to be determined.

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