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Morphine inhibits the carrageenan-induced oedema and the chemoluminescence of leucocytes stimulated by zymosan

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Morphine inhibited the oedema formation induced by carrageenan. The anti-inflammatory activity developed 120 min after carrageenan injection, suggesting that inhibition of the kinin phase might be partly responsible. This assumption is supported by the findings that morphine inhibited bradykinin oedema but did not influence oedema formation induced by histamine, 5-HT or PGE_2. The anti-inflammatory activity of morphine was partially inhibited by naloxone (0·5–1 mg kg^{-1}) in the carrageenan oedema test. Zymosan-stimulated chemoluminescence of neutrophils of the rat was inhibited both by morphine (0·1–10 μM) and naloxone (1–100 μM). When morphine and naloxone were administered simultaneously (10 μM) their inhibitory effects were additive. Naloxone also failed to antagonize the inhibitory action of morphine in lower dose (0·1 μM). These results suggest that the effect of morphine in inflammation might be mediated either by one of the opiate receptor subtypes insensitive to naloxone or a non-opiate mechanism might be involved.

Narcotic analgesics produce a variety of actions such as analgesia, respiratory depression, sedation, catalepsy, which are thought to be mediated by receptors in the central nervous system. However, a peripheral component of morphine analgesia has been described by Ferreira & Nakamura (1979), Ferreira (1981), Ferreira et al (1982) who reported that hyperalgesia induced by PGE_2 injected to the paw of the rat was inhibited by a small dose of morphine injected to the same site. Bentley et al (1981) found morphine when given intraperitoneally, to be a potent antinociceptive agent in the acetic acid writhing test in mice. Morphine is more effective in inhibiting inflammatory than non-inflammatory pain (Gyires & Knoll 1979; Gyires & Torma 1984). These findings prompted us to study how morphine influences the inflammatory process itself. For this purpose we used two different types of test: an in-vivo model, the rat paw oedema test, and an in-vitro method, the measurement of chemoluminescence (CL) of neutrophils stimulated by zymosan.

METHODS

Oedema tests

Sprague-Dawley CFY rats of either sex, 120–140 g were randomly divided in groups of 10. The oedema was produced by intraplantar injection of 0·1 ml of carrageenan (10 mg ml^{-1}), histamine (10 mg ml^{-1}), 5-hydroxytryptamine (5-HT) (0·05 mg ml^{-1}), bradykinin (0·05 mg ml^{-1}) or PGE_2 (0·005 mg ml^{-1}). The volume of oedema was determined by means of a plethysmograph 3 h after carrageenan, 60 min after 5-HT, 30 min after histamine and bradykinin and 15 min after PGE_2 injection. In some experiments the volume of oedema was determined 30, 60, 90, 120 and 180 min after the injection of carrageenan. The test compounds were administered to rats either orally (p.o.) 16 h after withdrawal of food or subcutaneously (s.c.) 30 min before intraplantar injection of the phlogistic agents in a volume of 10 and 5 ml kg^{-1}, respectively. The activity of drugs was expressed as % inhibition of oedema. As we found that an antioedematous effect no greater than 60–70% could be reached, we determined the ED30 value—the dose which inhibits the oedema formation by 30%.

Chemoluminescence (CL) produced by rat leucocytes stimulated by zymosan

Leucocytes were isolated from the peritoneal fluid of Sprague Dawley CFY rats of either sex, 150–200 g, that were given 10 ml of 6% dextran solution intraperitoneally (i.p.) and 3 h later had peritoneal fluid withdrawn. This was centrifuged (150*g* 15 min) and the sedimented fraction was washed and centrifuged again three times. The centrifuged cells were diluted with Dulbecco's buffer to a final concentration of 1×10^6 leucocytes ml^{-1}. The method was according to Van Dyke et al (1979). CL was

* Correspondence.

measured, in triplicate, with a liquid scintillation counter (Model Intertechnique LM-40). The activity of the drugs was expressed in %; the maximal light production (in the 6–7th min) in control and treated groups was compared. Light production of the control groups was taken as 100%.

Materials. Carrageenan (Merck), PGE_2 (Upjohn), Naloxone HCl (Endo), histamine HCl (Serva), 5-HT creatinine sulphate (Sandoz), bradykinin (Sigma), morphine HCl (Alkaloida), indomethacin (Gedeon Richter), zymosan (Human), luminol (Reanal) were used.

Statistical evaluation. The confidence limits of ED30 values were calculated according to Litchfield & Wilcoxon (1949). The significance of differences between means was calculated using Student's unpaired test. Dose ratios (DR) were calculated as (ED30 of the drug in the presence of antagonist)/(ED30 control).

RESULTS

Paw oedema tests

Table 1 summarizes the effect of morphine, naloxone and indomethacin on oedema formation induced by carrageenan. Morphine inhibited the carrageenan oedema in a dose dependent manner (ED30: 1·6 mg kg^{-1} s.c.) more effectively than indomethacin (ED30: 2·7 mg kg^{-1} p.o.). While the anti-oedematous effect of indomethacin could be observed only 180 min after carrgeenan injection, the anti-inflammatory action of morphine appeared in 120 min. Neither drug influenced the oedema formation in the first 90 min (Fig. 1).

Table 1. The effect of morphine, naloxone and indomethacin on oedema formation induced by carrageenan.

Compound	Dose mg kg^{-1}	% Inhibition of oedema ± s.e.	ED30* and 95% confidence
Morphine (s.c.)	0·5	15·0 + 3·8	
	1·25	30·8 + 9·1	1·6
	2·5	34·0 + 5·92	(0·8–3·2)
	5·0	41·0 + 4·7	
Naloxone (s.c.)	0·5	15·0 + 2·9	
	1·0	17·0 + 3·8	—
	2·0	15·0 + 3·1	
Indomethacin (p.o.)	0·5	—	
	1·0	12·0 + 0·91	
	2·0	29·0 + 1·9	2·7
	5·0	40·0 + 4·6	(1·6–4·32)

* ED30: the dose which decreases the oedema by 30%.

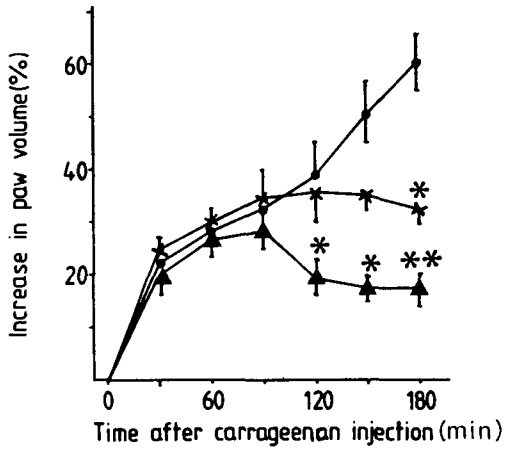

FIG. 1. Carrageenan-induced oedema of the rat paw as affected by indomethacin (2·5 mg kg^{-1}, p.o.) (—×—) and morphine (—▲—) (2·5 mg kg^{-1} s.c.). Control —●— Values indicate mean (n = 10) ± s.e. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.

Bradykinin oedema was inhibited by morphine to the same extent as the carrageenan-induced inflammation (ED30: 1·1 mg kg^{-1} s.c.). However, morphine did not influence the oedema formation produced by histamine, 5-HT, and PGE_2 significantly.

Naloxone alone failed to inhibit the oedema formation induced by carrageenan, however, the anti-inflammatory action of morphine decreased in the carrageenan oedema test in the presence of naloxone (0·5–1 mg kg^{-1} s.c.) (dose ratio: 2·27 and 6·8, respectively) (Table 2).

Effects of morphine, naloxone and indomethacin on the chemoluminescence (CL) of granulocytes stimulated by zymosan

As Table 3 and Fig. 2 demonstrate, both morphine and naloxone inhibited the zymosan-stimulated CL of the granulocytes. The dose dependency of this

Table 2. The inhibitory effect of naloxone on the anti-inflammatory action of morphine on rats (n = 10).

Compound	ED30 and confidence (mg kg^{-1} s.c.) (carrageenan rat paw oedema test)	Dose ratio
Morphine	1·6 (0·8–3·2)	1
Morphine + 0·5 mg kg^{-1} naloxone	2·5 (1·31–4·75)	1·56
Morphine + 1·0 mg kg^{-1} naloxone	7·5 (3·75–15)	4·6

Table 3. Effect of morphine, naloxone and indomethacin on the zymosan-stimulated chemoluminescence of granulocytes in the rat.

Compound	Concn (μM)	% Inhibition of CL ± s.e.	
Morphine	100	40·8 ± 5·5*	
	10	45·3 ± 6·8**	(n = 20)
	1	38·7 ± 11·7*	
	0·1	20·15 ± 5·2	
Naloxone	100	53·5 ± 10·6**	
	10	32·1 ± 7·7*	(n = 20)
	1	28·3 ± 6·1	
	0·1	Ineff.	
Morphine + naloxone	10 10	60·1 ± 11**	(n = 10)
Morphine + naloxone	10 0·1	43·0 ± 7·7**	(n = 10)
Indomethacin	10	47·3 ± 6·1**	(n = 10)

*$P < 0.05$; **$P < 0.01$.

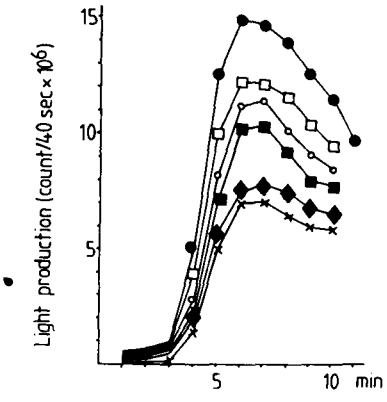

FIG. 2. Effects of indomethacin (—×—) (10 μM), morphine (1 μM —○—, 10 μM —◆—) and naloxone (1 μM —□—, 10 μM —■—) on luminol-dependent light production of rat leucocytes stimulated by zymosan. Control —●— (n = 15).

inhibitory action proved to be weak. The maximal inhibitory action of morphine could be observed at 10 μM, and no higher inhibitory action was obtained by increasing the dose. Naloxone was almost as potent as morphine in inhibiting the CL of leucocytes, 100 μM decreased the CL by 53·5% and at 10 μM naloxone also exerted significant inhibitory action.

When morphine and naloxone (10 μM) were administered simultaneously, the CL-inhibitory actions were additive. To check that the possible antagonistic effect of naloxone was not hidden by its agonistic-CL inhibitory action, we also combined a lower, ineffective dose of naloxone (0·1 μM) with morphine but this failed to influence morphine's inhibitory action.

DISCUSSION

Some indirect evidence in the literature referred to the ability of morphine to suppress acute inflammation (Randall & Selitto 1957; Winter & Flataker 1965). Arrigo-Reina & Ferri (1979) suggested that an opioid-peptidergic system may be involved in the control of inflammation since they found that inhibition of carrageenan oedema by a stress situation could be antagonized by naloxone. Their assumption is supported by the findings of Bläsig et al (1979) who found that endorphine plasma levels are concomitantly elevated after stress. Ferreira & Nakamura (1979) and Ferreira et al (1982) obtained an analgesic effect with intraplantar naloxone, when the hyperalgesia was induced by PGE_2. Low doses of naloxone were found to inhibit inflammatory pain induced either acutely by carrageenan (Rios & Jacob 1982) or chronically by Freud's adjuvant (Kaiser & Guilbaud 1981). However, the oedema formation was not influenced by naloxone (Kaiser & Guilbaud 1981). Bartho & Szolcsányi (1981) found that opiate agonists inhibit neurogenic plasma extravasation of the rat.

Our results would seem to support the idea that opiates may influence the inflammatory processes, since morphine was found to inhibit the formation of carrageenan oedema in a dose-dependent manner.

According to Di Rosa et al (1971), after the injection of carrageenan the first response is an increase in the output of histamine and 5-HT (90 min), this is followed by bradykinin formation (120 min) and prostaglandin (PG) release begins at about the third hour.

Morphine did not influence the oedema formation in the first 90 min of inflammation. The anti-inflammatory activity developed 120 min after carrageenan injection, suggesting that the inhibition of the kinin phase of the oedema might be partly responsible for the anti-inflammatory activity of morphine in this test. The assumption is supported by the findings that morphine inhibited the bradykinin oedema but did not influence significantly the oedema induced by histamine and 5-HT.

Since the anti-inflammatory effect of morphine was practically the same (or somewhat higher) at 180 as at 120 min, the effect of morphine on the PG system must be also considered. Morphine does not inhibit, but increases the synthesis of PGs (Collier et al 1974), however, there are numerous reports on the antagonism existing between morphine and prosta-

glandins (Ehrenpreis et al 1973; Collier & Roy 1974; Ferri et al 1974; Beubler & Lembeck 1980; Warhurst et al 1983; Yamasaki et al 1983).

In our experiments morphine failed to antagonize the slight paw oedema-inducing effect of PGE_2. Therefore, it would seem that the anti-inflammatory action of morphine is not exerted by inhibition of the PG system.

Superoxides and other free radical production may play a basic role in inflammatory process (Fridovich 1975; Oyanagui 1976; Kuehl et al 1977). Therefore, we also studied the effect of morphine and naloxone on CL of leucocytes stimulated by zymosan, since CL is generally believed to come from the excited state of oxygen and free radicals.

We found that both morphine and naloxone inhibited the CL of leucocytes stimulated by zymosan. When morphine and naloxone (10 μM) were administered simultaneously, their CL-inhibitory effects were additive. A lower dose of naloxone (0·1 μM)—which had no effect on CL—failed to influence the CL-inhibitory action of morphine. Our results are in agreement with the findings of Ferreira et al (1979), who found that naloxone also exerted an analgesic (agonistic) effect on hyperalgesic paw similar to morphine, and gave an additive effect with that of morphine. However, lower, non-analgesic doses of naloxone antagonized the morphine-analgesia in contrast to our findings. On the other hand, according to Zucker-Franklin et al (1971), Stanley (1976), van Epps et al (1982) and Fischer & Falke (1984) opiates—in pharmacological concentrations— cause decrease of chemotactic response of PMNs and this effect could not be antagonized by naloxone.

The question thus raised is what can be the mechanism of CL-inhibitory action of morphine and naloxone? CL can be inhibited by drugs with different mechanisms of action, for example: compounds that interfere with PG synthesis (inhibitors of cyclooxygenase and lipoxygenase pathway, inhibitors of arachidic acid production, analogue of arachidonic acid), compounds that elevate the cyclic AMP level of neutrophils, and free radical scavengers (Van Dyke et al 1979; Cheung et al 1983).

Naloxone was recently reported to inhibit the formation of malondialdehyde in inflammatory fluid induced by the i.p. injection of acetic acid in mice (Fürst et al 1983). Inhibition of malondialdehyde formation can refer to the inhibition of PG synthesis and as it is known that inhibitors of PG synthesis are able to depress the CL of leucocytes stimulated by zymosan.

It is much more difficult to explain the CL-inhibitory action of morphine. Morphine neither inhibits the synthesis of PGs (Collier et al 1974) nor elevates the cAMP level, it even inhibits the activation of adenylate cyclase by PGs in different tissues (Ferri et al 1974; Collier & Roy 1974; Beubler & Lembeck 1980; Warhurst et al 1983; Yamasaki et al 1983). Though, we do not know whether morphine influences the cAMP-adenylate cyclase system in rat leucocytes, it is unlikely that morphine would have an opposite action just in these cells. The third possibility, scavenging of free radicals, cannot be yet excluded.

In any case, opiate receptors might not be involved in the anti-oedematous and CL-inhibitory action of morphine, since naloxone either failed to exert any antagonistic action (CL-test), or only slightly inhibited the effect of morphine (oedema test). Though the involvement of (other) opiate receptor subtypes insensitive to naloxone may be also supposed in the anti-oedematous and CL-inhibitory action of morphine.

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