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Rapid communication

The molecular mechanism of action of peripheral morphine analgesia: stimulation of the cGMP system via nitric oxide release

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In addition to the central and spinal sites of action of morphine both our laboratory and others have demonstrated that opiates can also cause analgesia through a peripheral mechanism (Ferreira, 1983). However, the molecular basis of the mechanism of the analgesic actions of opiates remains unknown. In vitro studies show that morphine is able to inhibit activation of adenylate cyclase (Collier and Roy, 1974) as well as to stimulate formation of cGMP (Minneman and Iversen, 1976).

Recently, using the rat paw hyperalgesia test, we showed that in the periphery, acetylcholine-induced analgesia was mediated via the activation of the nitric oxide/cGMP pathway (Duarte et al., 1990). This conclusion was based upon the fact that intraplantar injection of sodium nitroprusside, a substance which non-enzymatically releases nitric oxide (NO), caused analgesia. Furthermore, the analgesic effects of acetylcholine (ACh) and sodium nitroprusside were enhanced by intraplantar injection of an inhibitor of cyclic GMP phosphodiesterase, My5445. In addition, methylene blue (MB), an inhibitor of guanylate cyclase, blocked the analgesia induced by acetylcholine and sodium nitroprusside. On the other hand, the analgesia induced by acetylcholine, but not by sodium nitroprusside, was blocked by N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of the formation of NO from L-arginine.

Due to the similarity of the local action of ACh and opiates we investigated whether agents which affect the arginine/NO-cGMP pathway also interfere with morphine-induced peripheral analgesia as tested with our modification of the Randall–Selitto rat paw pressure test (see Duarte et al., 1990). In this test a constant pressure of 20 mmHg is applied to the hind paw of rats (male Wistar rats, 130–180 g) and discontinued when the animals present a typical freezing reaction (reaction time). Hyperalgesia was induced by intraplantar injection of prostaglandin E_2 (PGE_2, 100 ng). The intensity of hyperalgesia was quantified as the change in the reaction time (delta reaction time, measured in s) between zero and 3 h after administration of PGE_2.

Morphine (Mph) or 3-morpholino-sydnonimine (SIN-1), a stimulator of NO generation, was injected into the paws 2 h after PGE_2. Morphine and SIN-1 caused dose-dependent analgesia (fig. 1, A and D). The highest intraplantar dose of morphine used is devoid of systemic effects since it did not affect the hyperalgesia induced by prostaglandin E_2 in the contralateral paws (results not shown). The effect of the molsidomine metabolite, SIN-1, was similar to that of another NO generator, sodium nitroprusside (Duarte et al., 1990), thus confirming that substances which release NO cause peripheral analgesia. Methylene blue (MB, 500 μg/paw), My5445 (50 μg/paw) or N-iminoethyl-L-ornithine (L-NIO, 100 μg/paw) injected 1 h before the analgesics, showed a different pattern of effects upon morphine or SIN-1. The inhibitor of activation of soluble guanylate cyclase, MB, in a dose (500 μg/paw) which did not affect prostaglandin-induced hyperalgesia abolished the morphine and SIN-1 analgesic effects (fig. 1, A and D). Although the specificity of MB as a guanylate cyclase inhibitor is open to question, the involvement of the cGMP system in the peripheral analgesic effect of morphine is further supported by the fact that the analgesia is potentiated by a specific inhibitor of guanylate cyclase, phosphodiesterase, My5445 (fig. 1, B). The effect of MY5445 was peripheral since the dose used did not interfere with the hyperalgesia of contralateral paws injected with PGE_2. Finally, administration of an inhibitor of NO synthesis, L-NIO, did not affect SIN-1 analgesia but antagonized morphine peripheral analgesia dose dependently (fig. 1, C and D). These results were confirmed using another argininase inhibitor, L-NMMA,

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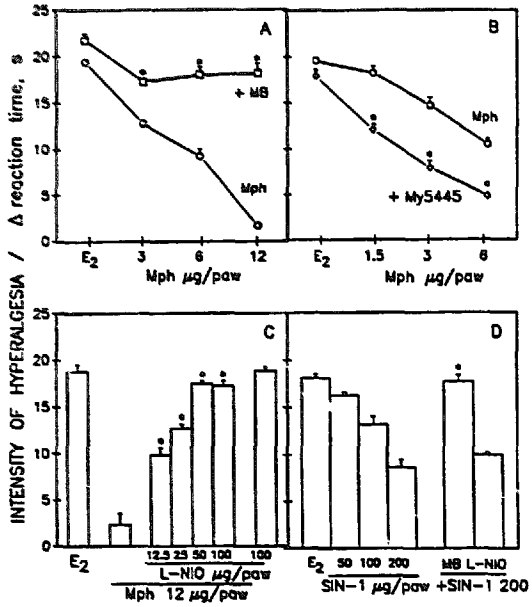

Fig. 1. Contribution of the NO/cGMP system to morphine-induced peripheral analgesia. (A) Blockade by methylene blue (MB, 500 μg/paw) of analgesia induced by intraplantar administration of morphine (Mph) on PGE_2-induced hyperalgesia. (B) Potentiation by My5445 (50 μg/paw) of analgesia induced by peripheral administration of morphine. (C) Dose–response effect of N-iminoethyl-L-ornithine (L-NIO) on the analgesia induced by intraplantar injection of morphine (12 μg/paw). (D) Peripheral analgesic effect of SIN-1 on PGE_2-induced paw hyperalgesia and its inhibition (200 μg/paw) by MB (500 μg/paw) but not by L-NIO (100 μg/paw). Statistical comparison was made by Dunnett's t-test. The stars indicate statistically significant differences ($P < 0.05$) between Mph or SIN-1 and the same group treated with MB, MY5445 or L-NIO. Each bar or symbol represents the mean ± S.E.M. for 54 animals.

which reproduced the effects of L-NIO. The intensity of hyperalgesia for the various groups was: PGE_2 = 19.3 s, Mph 12 μg/paw = 2 s, L-NMMA 50 μg/paw + Mph = 18.3 s. D-NMMA (50 μg/paw), had no effect on the intensity of the antinociceptive effect of an intraplantar injection of Mph. Thus, inhibitors of arginase do not block the direct peripheral analgesic effect of NO generators, SIN-1 and sodium nitroprusside (Duarte et al., 1990), but abolish the morphine analgesia.

It is interesting that enkephalin and opiate narcotics are known to increase cGMP in neuronal tissues and that NO synthetase is present in discrete areas of the CNS and is abundant in the endothelium of small blood vessels with which sensory neurones are closely associated (Snyder and Bredt, 1991).

In conclusion, our results support the involvement of the arginine/NO-cGMP pathway in peripheral morphine analgesia since it was inhibited by L-NIO, L-NMMA or methylene blue and potentiated by My5445. Finally, we may speculate that the anti-anginal effect of nitrates is not only dependent on their vasodilator effect but consequent to a true analgesic effect via stimulation of the cGMP system.

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