ELSEVIER

European Journal of Pharmacology 340 (1997) 177–180

ejp

Short communication

Evidence for the involvement of the nitric oxide–cGMP pathway in the antinociception of morphine in the formalin test

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Received 11 August 1997; revised 6 October 1997; accepted 7 October 1997

Abstract

The effect of inhibition of nitric oxide synthesis and guanylate cyclase on the peripheral antinociceptive effect of morphine was assessed by using the formalin test in the rat. Saline, N^G-monomethyl-L-arginine, a nitric oxide synthesis inhibitor (50 μg) and methylene blue, a guanylate cyclase inhibitor (500 μg), did not exhibit any antinociceptive activity. However, morphine (10 μg) produced a significant antinociceptive effect in phases 2a and 2b, which was reduced by pretreatment with either N^G-monomethyl-L-arginine or methylene blue. These results suggest that the local administration of morphine induces antinociception by the activation of the L-arginine–nitric oxide–cGMP pathway. © 1997 Elsevier Science B.V.

Keywords: Morphine; Methylene blue; N^G-Monomethyl-L-arginine; Formalin

1. Introduction

In addition to the supraspinal and spinal sites of action of morphine, it has been found that opiates cause peripheral antinociception (Ferreira and Nakamura, 1979b; Ferreira, 1983). This observation was further confirmed by several authors (Stein, 1993). The molecular mechanism underlying the peripheral antinociceptive action of opiates, however, is still a matter of debate. Because inflammatory hyperalgesia was thought to be due to an increased neuronal content of Ca^{2+}/cAMP (Ferreira and Nakamura, 1979a) and this notion was supported by the in vitro studies of Collier and Roy (1974), it was suggested that the peripheral antinociceptive effect of opiates was associated with inhibition of activation of adenylate cyclase (Ferreira and Nakamura, 1979b). These results have been extended by several observations of Levine and Taiwo (1989) and Taiwo and Levine (1992). Recently, however, we proposed that peripheral opiate antinociception was due to the activation of the L-arginine–nitric oxide–cGMP pathway. This proposal was based on the observation that local administration of L-arginine produces antinociception in rats with carrageenin-induced hyperalgesia, the effect being blocked by nitric oxide synthesis inhibitors and methylene blue (Duarte et al., 1990). In prostaglandin- and carrageenin-induced hyperalgesia the local administration of opiates or non-enzymatic nitric oxide donors also produces antinociception. While pretreatment of the paws with methylene blue inhibit the action of morphine and of the nitric oxide donor, the nitric oxide synthase inhibitor only inhibited opiate analgesia (Duarte et al., 1990; Ferreira et al., 1991). The effect of morphine was potentiated by specific inhibitors of cGMP phosphodiesterase (MY5445), and intraplantar injections of dibutyryl–cGMP caused antinociception (Ferreira and Nakamura, 1979a). The finding that morphine stimulates cGMP formation (Minneman and Iversen, 1976) is in line with our suggestion.

There are, however, several observations indicating that the arginine–nitric oxide–cGMP pathway plays a hyperalgesic rather than a peripheral antinociceptive effect. In fact, it has been reported that either intraplantar (Haley et al., 1992) or systemic administration of N^G-nitro-L-arginine methyl ester (L-NAME), but not D-NAME, produces dose-dependent antinociception in the second phase of the formalin test. The nociceptive or inflammatory role

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of the arginine–nitric oxide pathway has been described for bradykinin, substance P, carrageenin and dextran (Kawabata et al., 1994). The simplest explanation for these apparent contradictions may lie in the fact that the arginine–nitric oxide–cGMP pathway may have different roles depending on the different groups of nociceptive stimuli involved in the participation of different types of primary sensory neurons. Moreover, some agents that are assumed to be specific inhibitors of nitric oxide synthase may have additional effects. Intraplantar L-NAME, for example, causes antinociception in both formalin (Haley et al., 1992; Malmberg and Yaksh, 1993) and paw mechanical hyperalgesia tests. L-NAME, however, is a rather peculiar agent since it seems to block, and even to induce if given chronically, nitric oxide synthase and guanylate cyclase in neural tissue (Miller et al., 1996). In the present study, we reinvestigated, using the behavioral nociceptive response induced by formalin in the rat, the peripheral effects of morphine, N^G-monomethyl-L-arginine (L-NMMA, a nitric oxide synthesis inhibitor), and methylene blue (an inhibitor of the soluble guanylate cyclase) and of their joint administration.

2. Material and methods

2.1. Animals

Male Wistar rats weighing 130–180 g with free access to food and water were used. All experiments were carried out according to the IASP guidelines on the use of animals in pain research.

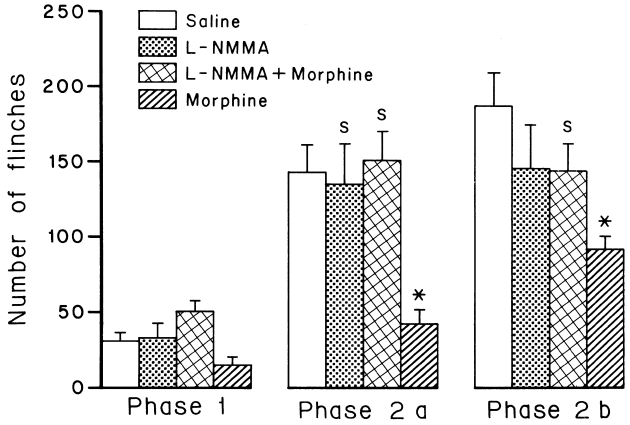

Fig. 1. Contribution of the nitric oxide–cGMP pathway to the peripheral antinociceptive effect of morphine. Blockade by N^G-monomethyl-L-arginine (L-NMMA, 50 μg/paw) of the antinociceptive effect of locally administered morphine on the 1% formalin-induced nociceptive responses (number of flinches). Each bar represents the mean number of flinches of 5–7 animals ± S.E.M. Statistical comparison was made by analysis of variance followed by the Bonferroni test ($P < 0.05$). The stars indicate significant differences between morphine and saline. S indicates significant differences of L-NMMA and L-NMMA + morphine groups from the morphine ($P < 0.05$) group.

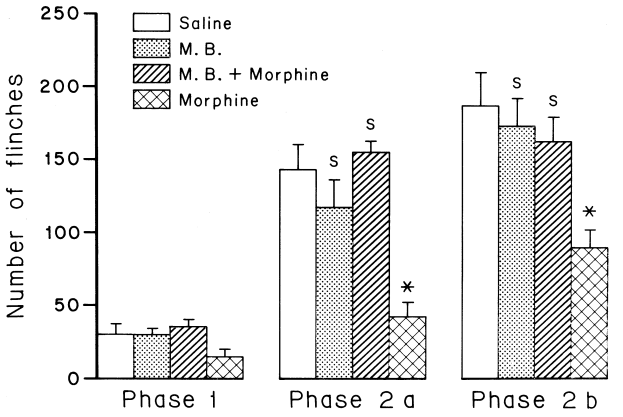

Fig. 2. Contribution of the nitric oxide–cGMP pathway to the peripheral antinociceptive effect of morphine. Blockade by methylene blue (MB, 500 μg/paw) of the antinociceptive effect of locally administered morphine on the 1% formalin-induced nociceptive responses (number of flinches). Each bar represents the mean number of flinches of 5–7 animals ± S.E.M. Statistical comparison was made by analysis of variance followed by the Bonferroni test ($P < 0.05$). The stars indicate significant differences between morphine and saline. S indicates significant differences of MB and MB + morphine groups from the morphine ($P < 0.05$) group.

2.2. Nociception test

Rats were placed in an open plexiglas observation chamber for 30 min to allow them to accommodate to their surroundings, then they were removed for formalin administration. The right hind paw of the rat was injected with 50 μl of dilute formalin (1%–5%), using a 30-gauge needle. The animal was then returned to the chamber for observation. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. The rats were observed for nociceptive behavior immediately after formalin injection in periods of 5 min until 60 min after injection. Nociceptive behavior was quantified as the number of flinches of the injected paw during the observation period. After the 1 h observation period, animals were immediately killed by cervical dislocation.

2.3. Drugs

The following drugs were used: Morphine sulfate (Merck), N^G-monomethyl-L-arginine (Wellcome), methylene blue (Reagen, Brazil).

2.4. Statistical analysis

All results are presented as means ± S.E.M. for 5–7 animals per group. Statistical comparisons were made by using an analysis of variance followed by the Bonferroni test. A $P < 0.05$ was considered significant.

3. Results

Local administration of 10 μg of morphine, given 20 min before formalin (1%), produced a significant reduction ($P < 0.05$) in the number of flinches in the second phase, 60 and 50% for phase 2a (5–40 min) and 2b (40–60 min), respectively, when compared to saline (Figs. 1 and 2). This effect was not observed in the first phase (0–5 min, Figs. 1 and 2) or when morphine was administered in the contralateral paw or when a higher concentration (5%) of formalin was injected (data not shown). Pretreatment of the paws with either 50 μg of N^G-monomethyl-L-arginine (Fig. 1) or 500 μg of methylene blue (Fig. 2), given 30 min before formalin, did not alter control responses but significantly attenuated the antinociceptive effect of morphine in phases 2a and 2b ($P < 0.05$).

4. Discussion

It is now becoming clear that the results obtained in the formalin test are highly susceptible to the concentration of formalin or agent tested. Low formalin concentrations elicit sub-maximal nociceptive behaviors. This facilitates the detection of the effects of weak analgesics. However, with injection of fixed concentration of formalin, intraplantar injections of L-arginine either enhance (low doses 0.1–1 μg per paw) or diminish (10 μg per paw) the frequency of the second-phase nociceptive response (Kawabata et al., 1994). In the present investigation we were able to detect peripheral morphine analgesia with 1% but not with 5% of formalin. Under these conditions, local pretreatment of the paw with N^G-monomethyl-L-arginine or methylene blue had no effect on the flinch frequency of the control groups but abolished morphine antinociception. Thus it is plausible that the molecular basis of peripheral analgesia induced by morphine in this test is similar to that of opiates and carbachol in other nociceptive tests (Ferreira et al., 1991; Duarte and Ferreira, 1992).

The results of the present experiments confirm published data showing the antinociceptive effect of the L-arginine–nitric oxide–cGMP pathway both in the periphery (Ferreira et al., 1991) and centrally (Duarte and Ferreira, 1992). The present results for the formalin test are similar to those observed for different pain models such as E_2 prostaglandin- and carrageenin-induced hind paw hyperalgesia and even the tail-flick test (Duarte and Ferreira, 1992). These results, however, are in contrast with the antinociceptive effect of nitric oxide synthesis inhibitors observed in the formalin test (Haley et al., 1992; Malmberg and Yaksh, 1993; Kawabata et al., 1994). As we pointed out earlier in the introduction, the simplest explanation for these apparent contradictions may lie in the fact that the L-arginine–nitric oxide–cGMP pathway may have different roles depending on the nociceptive stimuli and the type of primary sensory neurons involved. This suggestion may be particularly important for drugs injected into the central nervous system because of the complexity of the neuronal network involved in the relay and interpretation of nociception. In conclusion, our results show that the local administration of morphine induces antinociception as a result of the activation of the L-arginine–nitric oxide–cGMP pathway.

Acknowledgements

The authors gratefully acknowledge the technical assistance of I.R. dos Santos and S.R. Rosas. Dr. Vinicio Granados-Soto is a postdoctoral fellow from Third World Academy of Sciences South–South Fellowship Program and from the Department of Pharmacology of the Faculty of Medicine of Ribeirão Preto, USP, Brazil. Support from CNPq is gratefully acknowledged.

References

Collier, H.O.J., Roy, A.C., 1974. Hypothesis: Inhibition of E prostaglandin-sensitive adenyl cyclase as the mechanism of morphine analgesia. Prostaglandins 7, 361–370.

Duarte, I.D.G., Lorenzetti, B.B., Ferreira, S.H., 1990. Peripheral analgesia and activation of the nitric oxide–cyclic GMP pathway. Eur. J. Pharmacol. 186, 289–293.

Duarte, I.D.G., Ferreira, S.H., 1992. The molecular mechanism of central analgesia induced by morphine or carbachol and the L-arginine–nitric oxide–cGMP pathway. Eur. J. Pharmacol. 221, 171–174.

Ferreira, S.H., 1983. Prostaglandins: Peripheral and central analgesia. In: Bonica, J.J. (Ed.), Advances in Pain Research and Therapy, vol. 5. Raven Press, New York, pp. 627–630.

Ferreira, S.H., Duarte, I.D.G., Lorenzetti, B.B., 1991. The molecular mechanism of action of peripheral morphine analgesia: Stimulation of the cGMP system via nitric oxide release. Eur. J. Pharmacol. 201, 121–122.

Ferreira, S.H., Nakamura, M., 1979a. I-Prostaglandin hyperalgesia: A $cAMP/Ca^{2+}$ dependent process. Prostaglandins 18, 179–190.

Ferreira, S.H., Nakamura, M., 1979b. II-Prostaglandin hyperalgesia: The peripheral analgesic activity of morphine, enkephaline and opioid antagonists. Prostaglandins 18, 191–200.

Haley, J.E., Dickenson, A.H., Schachter, M., 1992. Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in the rat. Neuropharmacology 31, 251–258.

Kawabata, A., Manabe, S., Manabe, Y., Takagi, H., 1994. Effect of topical administration of L-arginine on formalin-induced nociception in the mouse: A dual role of peripherally formed NO in pain modulation. Br. J. Pharmacol. 112, 547–550.

Malmberg, A., Yaksh, T.L., 1993. Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 54, 291–300.

Miller, M.J., Thompson, J.H., Liu, X., Eloby-Childress, S., Sadowska-krowicka, H., Zhang, X.J., Clark, D.A., 1996. Failure of L-NAME to cause inhibition of nitric oxide synthesis: Role of inducible nitric oxide synthase. Inflamm. Res. 45, 272–276.

Minneman, K.P., Iversen, L.L., 1976. Enkephalin and opiate narcotics increase cyclic GMP accumulation in slices of rat neostriatum. Nature 262, 313–319.

Levine, J.D., Taiwo, Y.O., 1989. Involvement of the mu-opiate receptor in peripheral analgesia. Neuroscience 32 (2), 571–575.

Stein, C., 1993. Peripheral mechanisms of opioid analgesia. In: Herz, A. (Ed.), Opioids II. Springer-Verlag, London, Paris, pp. 91–103.

Taiwo, Y.O., Levine, J.D., 1992. Mediation of serotonin hyperalgesia by the cAMP second messenger system. Neuroscience 48 (2), 479–483.