ELSEVIER

European Journal of Pharmacology 285 (1995) 95–98

ejp

Short communication

Morphine-induced changes in cerebral and cerebellar nitric oxide synthase activity

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Received 30 March 1995; revised 27 July 1995; accepted 1 August 1995

Abstract

The effect of acute and chronic morphine treatment on nitric oxide (NO) synthase activity (determined by the rate of conversion of [^{14}C]arginine into [^{14}C]citrulline) on mouse brain was studied. Acute morphine treatment induced an increase in Ca^{2+}-dependent NO synthase in cerebellum. This effect was blocked by coadministration with naloxone. Chronic morphine treatment (by s.c. pellet) also produced an increase in cerebellar NO synthase, with a maximum on the second day of implantation. No significant changes were found in frontal cortex and forebrain during acute or chronic morphine treatment. The relationship between opiate effects and the L-arginine:NO pathway is discussed.

Keywords: Morphine; Nitric oxide (NO); Nitric oxide (NO) synthase; Brain

1. Introduction

The elucidation of the role of nitric oxide (NO) in brain is one of the most interesting fields in neuroscience. NO is formed from the amino acid L-arginine by the enzyme NO synthase, identified in the brain by Knowles et al. (1989). Three classes of NO synthases have been defined: two constitutive and Ca^{2+}-dependent enzymes (neuronal and endothelial), and one inducible and Ca^{2+}-independent enzyme (Knowles and Moncada, 1994).

NO is the mediator responsible for the increases in cyclic GMP that follow the stimulation of glutamate receptors, mainly the NMDA (*N*-methyl-D-aspartate) type (Garthwaite et al., 1988; Bredt and Snyder, 1989). Glutamate and other excitatory amino acids have been implicated in opiate effects, as well as in the development of tolerance and dependence (Trujillo and Akil, 1991; Kolesnikov et al., 1993).

The L-arginine:NO-cyclic GMP pathway has been involved in opiate and opioid peptide effects, and there is some pharmacological evidence for a modulating role of NO in opiate analgesia (Duarte and Ferreira, 1992; Brignola et al., 1994) and in the development of dependence (Cappendijk et al., 1993). Furthermore, Askew and Charalampous (1976) have demonstrated that injection of analgesic doses of morphine increases brain cyclic GMP in a naloxone-reversible manner.

In this study we try to find biochemical evidence for these relationships between opiate effects and the L-arginine:NO-cyclic GMP pathway by analyzing the effect of acute and chronic morphine treatment on nitric oxide synthase activity in mouse brain.

2. Materials and methods

2.1. Animals

Male CD1 mice (Charles-River), aged 5–6 weeks and weighing 25–30 g were used and were allowed free access to food and water.

2.2. Experimental groups

(a) Acute morphine treatment: mice were s.c. injected with morphine HCl (1, 5, 10 and 30 mg/kg) and the brains were removed after 30 min. The time-course (15, 30, 60, 90 and 120 min) of the effects was studied. Coadministration of the opiate antagonist, naloxone (1 mg/kg) plus morphine (5 mg/kg), was carried out. To

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Elsevier Science B.V.
SSDI 0014-2999(95)00474-2

investigate the effects of naloxone (1 mg/kg) in naive mice, the brains were removed 15 and 30 min after injection. (b) Chronic morphine treatment: mice were implanted with a 75-mg morphine pellet and were decapitated on days 1, 2, 3 and 4 post-implantation. A placebo pellet (containing the same amount of saccharose) was also studied.

The brains were divided into sections, cerebellum, frontal cortex and forebrain. The forebrain (from one hemisphere) was cut coronally and the central portion was taken. All tissues were freeze-clamped in liquid nitrogen and stored at −80°C until studied.

2.3. Nitric oxide synthase enzyme activity

NO synthase activity was determined by measuring the conversion of L-[U-^{14}C]arginine into L-[U-^{14}C]citrulline (Salter et al., 1991). The Ca^{2+}-dependent NO synthase activity was calculated by subtracting the activity in samples containing 1 mM EGTA from total activity (control samples). Measurements were expressed as pmol/min per mg wet tissue.

2.4. Drugs

Morphine base (for pellets) and morphine HCl (for injections) were from the Centro Nacional de Estupefacientes y Psicotropos; L-[U-^{14}C]arginine was from Amersham, and all the other chemicals were from Sigma. The drugs were dissolved in distilled water in a volume of 0.5 ml/mouse.

2.5. Statistical analysis

The results are presented as means ± S.E.M. for a minimum of 6 animals per group. Statistical comparisons were made using the one-way Student's *t*-test and differences with $P < 0.05$ or less were considered significant.

3. Results

3.1. Acute morphine treatment

Acute morphine treatment by s.c. injection of morphine (1, 5, 10, 30 mg/kg) caused a significant increase in cerebellar NO synthase activity (Fig. 1A), without changes in forebrain (data not shown) and a slight decrease in frontal cortex (control activity: 4157 ± 605; 30 min after morphine 5 mg/kg: 3048 ± 507, expressed as pmol citrulline per min per mg tissue). Based on these findings, the dose of 5 mg/kg was selected to study the time course. A bell-shaped time-response curve, with a maximum at 30 min, was found in cerebellum (+16% at 15 min; +51% at 30 min, $P < 0.01$; +36% at 60 min, $P < 0.05$; +42% at 90 min, $P < 0.05$; −17% at 120 min, not significant, all vs. control). Naloxone (1 mg/kg) blocked this effect of morphine (5 mg/kg) when it was coadministrated (Fig. 1B). Naloxone itself (1 mg/kg) did not modify the NO synthase activity in naive animals at either of the times studied (15 or 30 min).

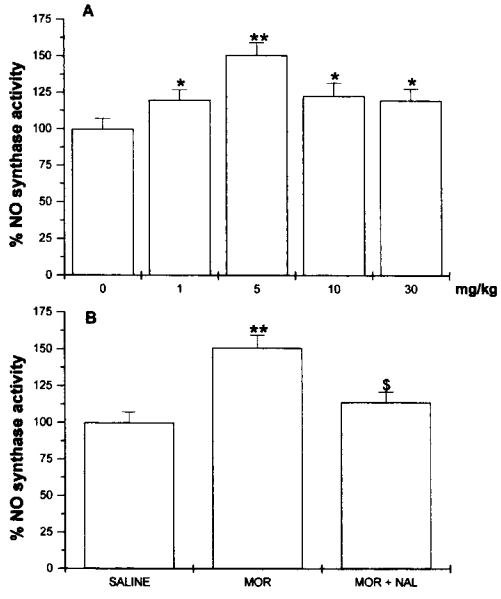

Fig. 1. (A) Effect of a single dose of morphine (1, 5, 10 and 30 mg/kg s.c.), and (B) coadministration of naloxone (NAL, 1 mg/kg) plus morphine (MOR, 5 mg/kg) on Ca^{2+}-dependent NO synthase activity in cerebellum, shown as percent of untreated values (mean ± S.E.M.). Brains were removed 30 min after treatments. The control activity was (pmol citrulline per min per mg tissue): 3236 ± 242. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control group; $^{\$}P < 0.05$ vs. morphine 5 mg/kg-treated group (*t*-test).

This stimulation of NO synthase in cerebellum by morphine was not a result of direct stimulation of the

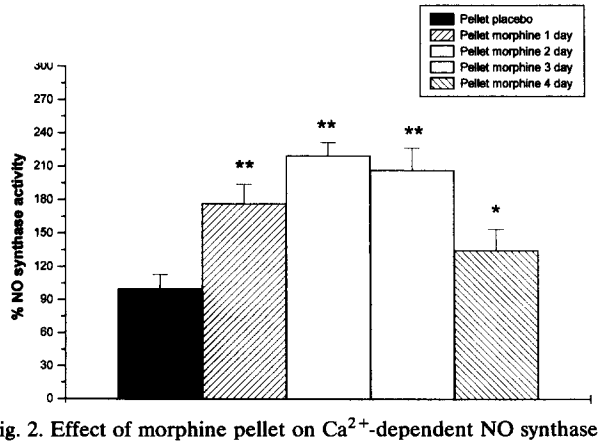

Fig. 2. Effect of morphine pellet on Ca^{2+}-dependent NO synthase in cerebellum, shown as percent of placebo pellet effect (mean ± S.E.M.). Brains were removed on days 1, 2, 3 and 4 post-implantation. The placebo pellet activity on the 4th day of implantation was (pmol citrulline per min per mg tissue): 3009 ± 268. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. placebo-implanted group (*t*-test).

enzyme because 10^{-3}–10^{-7} M morphine failed to cause significant stimulation of the activity of the extracted enzyme (control, 4002 ± 316; morphine 10^{-3}, 10^{-5} and 10^{-7} M, 3608 ± 299, 4111 ± 302, 4201 ± 250 pmol/min/mg tissue respectively, $n = 4$).

3.2. Chronic administration

Chronic administration of morphine caused an enhancement in cerebellar NO synthase activity that reached a maximum on the 2nd day of addiction, decreasing on days 3 and 4 (Fig. 2), when compared with that in placebo-implanted mice. Chronic morphine treatment slightly diminished NO synthase activity in forebrain and frontal cortex, but without statistical significance (data not shown). The placebo pellet did not modify NO synthase activity after 4 days of implantation.

Inducible, Ca^{2+}-independent, NO synthase activity was observed in all tissues, but it averaged < 10% of the total activity and was not altered by any treatment.

4. Discussion

Previous studies have demonstrated that the L-arginine: NO pathway is implicated in the effects of opiates. A modulating role for NO in opiate analgesia has been described by Duarte and Ferreira (1992), and also one in processes induced by chronic administration of opiates, such as tolerance and dependence. Kolesnikov et al. (1993) demonstrated that NO synthase inhibitors block morphine tolerance, and Cappendijk et al. (1993) found that NO synthase inhibitors administered prior to abstinence may reduce some of the symptoms and signs of abstinence. Unpublished data from our laboratory indicate that NO synthase activity is enhanced in cerebellum during abstinence and that some antiwithdrawal agents act by inhibiting this NO increase.

The findings presented here demonstrated that acute and chronic administration of morphine to mice increases Ca^{2+}-dependent NO synthase activity in cerebellum. Furthermore, the changes observed in cerebellum are opiate-specific, since naloxone, a pure opiate antagonist, blocks the NO synthase activity increase when it is coadministered with morphine. These biochemical findings are consistent with the pharmacological findings of other authors mentioned above. For example, Askew and Charalampous (1976) demonstrated an increase in cerebellar cyclic GMP after acute administration of morphine to mice. The lack of effects on forebrain could be due to the fact that it is not a simple structure but a complex system in which the effect of a drug on one cell type could be masked by the effect on another type.

We have found that acute administration of morphine produces a bell-shaped dose-response curve for Ca^{2+}-dependent NO synthase activity. Interestingly, Minneman and Iversen (1976) found that morphine (10^{-7}–10^{-5} M) increased the accumulation of cyclic GMP in rat striatal slices and the dose-response curve was also bell-shaped, with higher concentrations (10^{-4}–10^{-3} M) causing no significant changes. Similar results have been described by Gullis et al. (1975) who used neuroblastoma × glioma hybrid cells. Several possibilities might explain this result: (a) it is known that low doses of morphine stimulate μ receptors while high doses stimulate other receptors (δ, κ) that could mask the μ-mediated effects (Benoliel et al., 1994); (b) morphine could exert a partial μ agonistic action; and (c) alternatively, there could be one cell system affected in which morphine stimulates the activation of NO synthase and parallel stimulation of another cell system in which morphine inhibits it by negative feedback which cannot be excluded.

Chronic effects of morphine on NO synthase may be related with tolerance processes in which glutamate and other excitatory amino acids have been involved. Kolesnikov et al. (1993) suggest that morphine tolerance involves the activation of NMDA receptors followed by the subsequent release of nitric oxide, since NMDA antagonists and NO synthase inhibitors block the development of morphine tolerance. The increase in NO synthase activity that we have found in cerebellum could explain these findings, since the tolerance secondary to chronic administration of morphine is correlated with an increase and a posterior decrease in cerebellar NO synthase.

Recently, Barjavel and Bhargava (1994) have reported that morphine, but not other μ or δ agonists, inhibits NO synthase activity in rat cerebral cortex homogenate at 10 mM. Our data show that acute and chronic morphine slightly diminishes cortical NO synthase activity in vivo, but this effect is not statistically significant. At this moment, we cannot explain these differences between in vitro and in vivo studies.

In summary, our data show an alternative biochemical mechanism of action of morphine and also of changes leading to tolerance. The increase in Ca^{2+}-dependent NO synthase activity induced by morphine suggests that the present classification of this family of enzymes needs to be revised (Knowles and Moncada, 1994). Further studies are needed to clarify the dual effect of morphine on cerebellar and cortical Ca^{2+}-dependent NO synthase activity in vitro and in vivo and the role of NO as neuromodulator in these structures.

Acknowledgements

This work was supported in part by grants from the Fondo de Investigaciones Sanitarias (F.I.S. 93/0166)

and Comisión Interministerial de Ciencia y Technología (C.I.C.Y.T. 94/0454). O.S.-M.-C. is a fellow from the Spanish Ministerio de Educación y Ciencia (AP92-722744).

References

Askew, W.E. and K.D. Charalampous, 1976, Effects of morphine administration on cerebellar guanosine 3′,5′-monophosphate, Experientia 32, 1454.

Barjavel, M.J. and H.N. Bhargava, 1994, Effect of opioid receptor agonists on nitric oxide synthase activity in rat cerebral cortex homogenate, Neurosci. Lett. 181, 27.

Benoliel, J.J., E. Collin, A. Mauborgne, S. Bourgoin, J.C. Legrand, M. Hamon and F. Cesselin, 1994, Mu and delta opioid receptors mediate opposite modulations by morphine of the spinal release of cholecystokinin-like material, Brain. Res. 653, 81.

Bredt, D.S. and S.H. Snyder, 1989, Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum, Proc. Natl. Acad. Sci. USA 86, 9030.

Brignola, G., A. Calignano and M. Di Rosa, 1994, Modulation of morphine antinociception in the mouse by endogenous nitric oxide, Br. J. Pharmacol. 113, 1372.

Cappendijk, S.L.T., R. De Vries and M.R. Dzolijc, 1993, Inhibitory effects of nitric oxide (NO) synthase inhibitors on naloxone-precipitated withdrawal syndrome in morphine-dependent mice, Neurosci. Lett. 162, 97.

Duarte, I.D.G. and S.H. Ferreira, 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.

Garthwaite, J., S.L. Charles and R. Chess-Williams, 1988, Endothelium-derived relaxing factor release on activation of NMDA receptor suggests a role as intracellular messenger in the brain, Nature 336, 385.

Gullis, R., J. Traber and B. Hamprecht, 1975, Morphine elevates levels of cyclic GMP in a neuroblastoma × glioma hybrid cell line, Nature 256, 57.

Knowles, R.G. and S. Moncada, 1994, Nitric oxide synthases in mammals, Biochem. J. 298, 249.

Knowles, R.G., M. Palacios, R.M.J. Palmer and S. Moncada, 1989, Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase, Proc. Natl. Acad. Sci. USA 86, 5159.

Kolesnikov, Y.A., C.G. Pick, G. Ciszewska and G.W. Pasternak, 1993, Blockade of tolerance to morphine but not to kappa opioids by a nitric oxide synthase inhibitor, Proc. Natl. Acad. Sci. USA 90, 5162.

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

Salter, M., R.G. Knowles and S. Moncada, 1991, Widespread tissue distribution, species distribution and changes in activity of Ca^{2+}-dependent and Ca^{2+}-independent nitric oxide synthases, FEBS Lett. 291, 145.

Trujillo, K.A. and H. Akil, 1991, Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801, Science 251, 85.