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European Journal of Pharmacology 454 (2002) 19–23

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The κ-opioid agonist (±)-bremazocine elicits peripheral antinociception by activation of the L-arginine/nitric oxide/cyclic GMP pathway

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Received 10 April 2002; received in revised form 8 August 2002; accepted 13 August 2002

Abstract

In view of the scarce information about the analgesic mechanism of κ-opioid receptor agonists, the objective of the present study was to determine whether nitric oxide (NO) is involved in the peripheral antinociception of bremazocine, a κ-opioid receptor agonist. Three drugs all interfering with the L-arginine/NO/cyclic GMP pathway were tested using the rat paw model of carrageenan-induced (250 μg) hyperalgesia: (a) N^G-nitro-L-arginine (a nonselective NO-synthase inhibitor), (b) methylene blue (a guanylate cyclase inhibitor), and (c) zaprinast (a cyclic GMP phosphodiesterase inhibitor).

Intraplantar administration of bremazocine (20, 40 and 50 μg) caused a dose-dependent peripheral antihyperalgesia against carrageenan-induced hyperalgesia. The possibility of the higher dose of bremazocine (50 μg) having central or systemic effect was excluded since administration of the drug into the left paw did not elicit antinociception in the contralateral paw. However, when the dose of bremazocine was increased to 100 μg, a significant increase in the nociceptive threshold was observed, as measured in the hyperalgesic contralateral paw.

Peripheral antihyperalgesia induced by bremazocine (50 μg) was significantly reduced in a dose-dependent manner when N^G-nitro-L-arginine (6, 9, 12 and 25 μg) or methylene blue (250, 375 and 500 μg) was injected before. Previous treatment with 50 μg of zaprinast (which had no effect when administered alone) potentiated the antihyperalgesic effect of bremazocine (20 μg).

Our data suggest that bremazocine elicits peripheral antinociception by activation of the L-arginine/NO/cyclic GMP pathway and that nitric oxide is an intermediary in this mechanism, forming cyclic GMP.

Keywords: Bremazocine; κ-Opioid receptor; Peripheral antinociception; Nitric oxide; Cyclic GMP; Carrageenan

1. Introduction

There are several evidences for the involvement of the second messenger system formed by G-protein activation, in the peripheral antinociceptive effect of opioid agonists (Sharma et al., 1975; Ferreira and Nakamura, 1979; Levine and Taiwo, 1989). On the other hand, nitric oxide (NO) has an important role in central (Duarte and Ferreira, 1992; Song et al., 1998) and peripheral analgesia (Duarte et al., 1990). Ferreira et al. (1991) showed that the peripheral antinociceptive effect of low doses of morphine (a μ-opioid receptor agonist), injected into the hind paw of hyperalgesics rats was inhibited by the enzymatic NO biosynthesis blockers L-N^5-(1-iminoethyl)ornithine (L-NIO) and N^G-monomethyl-L-arginine (L-NMMA) and by methylene blue, a guanylate cyclase inhibitor, whereas this antinociception was potentiated by 1-(3-chlorophenylamino)-4-phenylphthalazine (MY5445), a specific cyclic GMP phosphodiesterase inhibitor. These results led us to suggest the involvement of the L-arginine/NO/cyclic GMP pathway in the peripheral antinociception induced by μ-opioid receptor agonists.

Rodrigues and Duarte (2000) showed that the ability of morphine to induce peripheral antinociception is dependent on the activation of ATP-sensitive K^+ channels. Recently, Soares et al. (2000) demonstrated that sulfonylureas, selective ATP-sensitive K^+ channel blockers (Edwards and Weston, 1993), reduced the peripheral antinociception elicited by the NO-donor sodium nitroprusside.

Nozacki-Taguchi and Yamamoto (1998) demonstrated that the NO-releasing agent (±)-(*E*)-ethyl-2-[(*E*)-hydroxyiminol]-5-nitro-3-hexeneamide (FK409) enhanced the analgesic effect of peripheral injection of (−)trans 1,S,2S

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PII: S0014-2999(02)02275-6

U50,488H hydrochloride and [D-Pen^{2,5}] enkephalin (DPDPE) which are κ- and δ-opioid receptor agonists, respectively, in the formalin test. However, little is known about the role of NO in the peripheral antinociception induced by these opioid receptor agonists. Thus, the objective of the present study was to obtain data that would support our suggestion that the L-arginine/NO/cyclic GMP pathway is involved in the peripheral antihyperalgesic effect of bremazocine, a potent κ-opioid receptor agonist (Römer et al., 1980; Ko et al., 1999). For this purpose, we tested the following drugs that interfere with the L-arginine/NO/cyclic GMP pathway in the hyperalgesic rat paw model: (a) N^G-nitro-L-arginine (a NO-synthase inhibitor), (b) methylene blue (a guanylate cyclase inhibitor), and (c) zaprinast (a cyclic GMP phosphodiesterase inhibitor).

2. Materials and methods

2.1. Animals

Male Wistar rats weighting 180–250 g from The Animal House of the Institute of Biological Sciences, Federal University of Minas Gerais, Brazil, were used for the experiments. The animals were housed in a temperature-controlled room (23 ± 1 °C) on an automatic 12-h light/dark cycle (6:00 AM to 6:00 PM). All tests were conducted during the light phase (8:00 AM to 2:00 PM). Food and water were freely available until the beginning of the experiments. Naive animals were used throughout.

2.2. Measurement of hyperalgesia

Subcutaneous administration of a carrageenan suspension (250 μg) into the hind paw elicited hyperalgesia which was measured by the pressure test described by Randall and Selitto (1957). We used an analgesimeter (Ugo-Basile, Italy) with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the plantar surface of the paw. The weight in grams (g) required to elicit nociceptive responses such as paw flexion or struggling was defined as the nociceptive threshold. A cut-off value of 300 g was used to prevent damage to the paws. The nociceptive threshold was always measured in the right hind paw and determined by the average of three consecutive trials recorded before (zero time) and 3 h after carrageenan injection. The results were calculated by the difference between these two averages (Δ of nociceptive threshold) and were expressed in grams.

2.3. Experimental protocol

All drugs were administered subcutaneously into the right hind paw and the nociceptive threshold was measured in this same paw, except in the protocol used to determine whether bremazocine was acting at central sites. In this protocol, carrageenan was injected into both hind paws, bremazocine was administered into the left or right paw and nociceptive threshold was measured in the right hind paw. Bremazocine was always administered 15 min before measurements, and the other drugs (N^G-nitro-L-arginine, methylene blue and zaprinast) were administered 1 h before bremazocine.

2.4. Drugs

Carrageenan (Sigma, St. Louis, MO, USA), (±)-bremazocine hydrochloride (RBI, Natick, MA, USA), N^G-nitro-L-arginine (RBI), Nor-Binaltorphimine (Sigma) and methylene blue (RBI) were dissolved in physiological saline. Zaprinast (Sigma) was dissolved in dimethyl sulfoxide (DMSO) at 10%. Carrageenan, bremazocine and vehicles were injected in a volume of 100 μl per paw and the other drugs in a volume of 50 μl per paw. For acidic or alkaline solutions, the pH was adjusted to approximately 7.4.

2.5. Statistical analysis

Statistical analysis was carried out by one-way Analysis of Variance (ANOVA) followed by Bonferroni's test for multiple comparisons. Probabilities smaller than 5% ($P < 0.05$) were considered to be statistically significant.

3. Results

3.1. Peripheral antihyperalgesic effect of bremazocine

Fig. 1 shows a dose-dependent antagonism of bremazocine (20, 40, 50 μg) against hyperalgesia induced by prior local injection of carrageenan (250 μg). The effect of

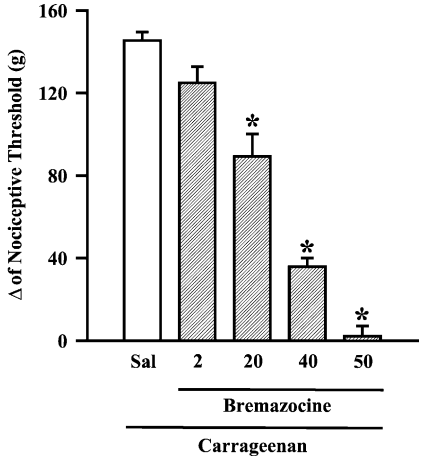

Fig. 1. Effect of bremazocine on the nociceptive threshold in carrageenan-induced hyperalgesia in rats. Bremazocine (μg) was administered intraplantarly 2 h and 45 min after local administration of 100 μl of a carrageenan suspension (250 μg). Each column represents the mean ± S.E.M. ($n = 5$). *Indicates significant difference from the carrageenan + saline (Sal) control group ($P < 0.05$, ANOVA + Bonferroni's test).

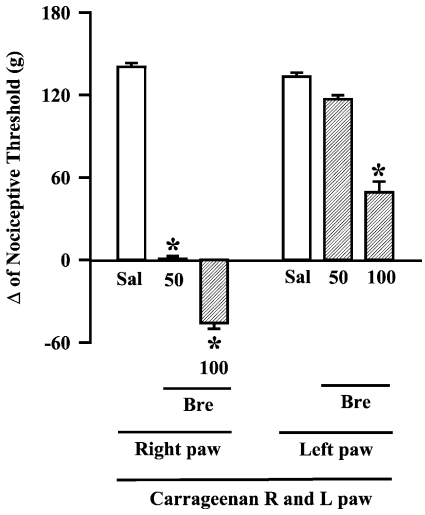

Fig. 2. Exclusion of the central or systemic antihyperalgesic effect of bremazocine (Bre). Carrageenan (250 μg) was injected into both hind paws while Bre (50 or 100 μg) was administered subcutaneously into the right (R) or left (L) hind paw. The nociceptive threshold was measured in the right hind paw, as described in Section 2. Each column represents the mean ± S.E.M. ($n = 5$). *Indicates a significant difference from the carrageenan + saline (Sal) control group ($P < 0.05$, ANOVA + Bonferroni's test).

bremazocine (50 μg/paw) was reversed by a selective κ-opioid antagonist (Song and Takemori, 1990), Nor-binaltorphimine (Nor-BNI, 200 μg/paw, results not shown). When administered into the left paw, bremazocine (50 μg) did not elicit antihyperalgesia in the right paw. However, when a dose of 100 μg of bremazocine was injected into the left paw, a potent antihyperalgesic effect in the contralateral paw was induced (Fig. 2).

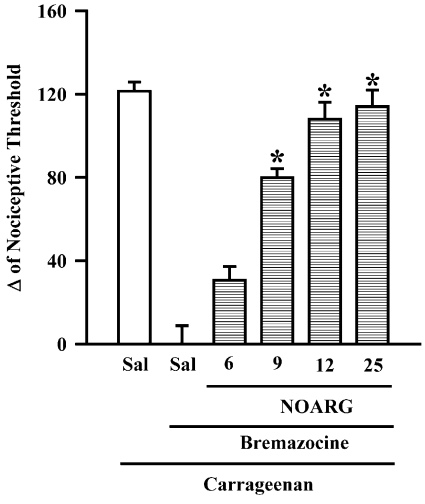

Fig. 3. Antagonism induced by intraplantar administration of N^G-nitro-L-arginine (NOARG) against peripheral antihyperalgesia produced by bremazocine (50 μg) in the hyperalgesic paw. NOARG (μg) was administered 1 h before bremazocine (50 μg). Each column represents the mean ± S.E.M. ($n = 5$). *Indicates a significant difference from the carrageenan + bremazocine + saline (Sal) group ($P < 0.05$, ANOVA + Bonferroni's test).

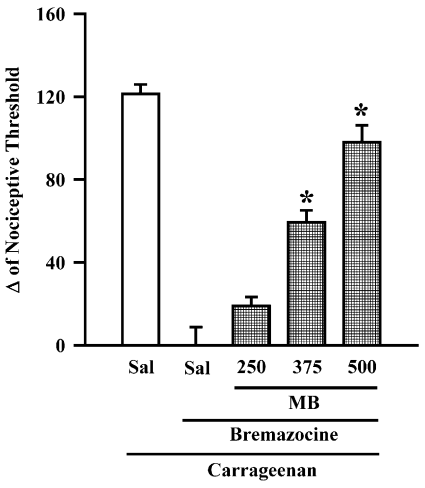

Fig. 4. Antagonism induced by intraplantar administration of methylene blue (MB) against peripheral antihyperalgesia produced by bremazocine in the hyperalgesic paw. MB (μg) was administered 1 h before bremazocine (50 μg). Each column represents the mean ± S.E.M. ($n = 5$). *Indicates a significant difference from the carrageenan + bremazocine + saline (Sal) group ($P < 0.05$, ANOVA + Bonferroni's test).

3.2. Antagonism of bremazocine-induced antihyperalgesia by N^G-nitro-L-arginine or methylene blue

The intraplantar injection of N^G-nitro-L-arginine (9, 12 and 25 μg) or methylene blue (375 and 500 μg) significantly reduced the peripheral antihyperalgesia induced by 50 μg bremazocine, in a dose-dependent manner (Figs. 3 and 4). N^G-nitro-L-arginine (25 μg) or methylene blue (500 μg)

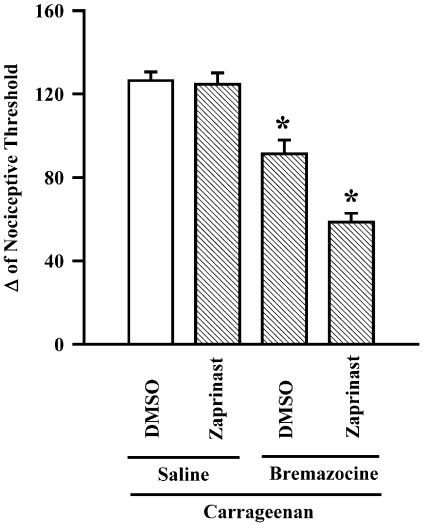

Fig. 5. Effect of intraplantar administration of the zaprinast–bremazocine combination against hyperalgesia induced by local administration of 100 μl of a carrageenan suspension (250 μg). Zaprinast (50 μg) was injected 1 h before administration of the low dose (20 μg) of bremazocine or isotonic saline. Each column represents the mean ± S.E.M. ($n = 5$). *Indicates a significant difference from the carrageenan + saline + dimethyl sulfoxide (DMSO) group ($P < 0.05$, ANOVA + Bonferroni's test).

when given alone did not modify significantly the nociceptive threshold in control animals and did not induce any overt behavioural effect at the doses used (not shown).

3.3. Effect of the zaprinast–bremazocine combination against carrageenan-induced hyperalgesia

Fig. 5 shows that intraplantar injection of zaprinast (50 μg) alone did not affect carrageenan-induced hyperalgesia. Nevertheless, the combination of a low dose of bremazocine (20 μg) with the same dose of zaprinast (50 μg) produced a moderate but statistically significant ($P < 0.05$) potentiation of bremazocine action by zaprinast.

4. Discussion

The role of NO in the pathogenesis or modulation of pain seems to be ambiguous. Although some studies have reported that NO plays a role in the development of central and peripheral pain (Machelska et al., 1997; Levy et al., 1999; Chen and Levine, 1999; Levy et al., 2000), our results are consistent with the idea that NO release elicits peripheral analgesia, at least as a result of κ-opioid receptor activation.

Initially, we tested the ability of bremazocine to induce peripheral antihyperalgesia. Our data showed that intraplantar administration of bremazocine caused a dose-dependent peripheral antihyperalgesia against carrageenan-induced hyperalgesia. This antinociception seems to be due κ-opioid receptor activation since Nor-BNI (Nor-binaltorphimine, 200 μg/paw) antagonized this effect (not shown). The possibility that bremazocine had a central or systemic effect at the dose of 50 μg per paw was excluded since its administration into the left paw did not elicit antihyperalgesia in the contralateral paw. However, when the dose of bremazocine was increased to 100 μg, a significant increase in the nociceptive threshold was observed, as measured in the hyperalgesic contralateral paw. This experiment showed that bremazocine had no central or systemic effects at the dose used in the present study (50 μg per paw).

Previous reports have shown that morphine stimulates the synthesis of cyclic guanylate cyclase in neuronal tissues (Minneman and Iversen, 1976) and causes peripheral analgesia by stimulation of the cyclic GMP system via nitric oxide release (Duarte et al., 1990; Ferreira et al., 1991). To confirm that the reported effect of bremazocine is dependent on L-arginine/NO/cyclic GMP pathway activation, we performed experiments based on NO biosynthesis. Since NO is formed by vascular endothelial cells from the terminal guanido nitrogen atoms of the amino acid L-arginine (Palmer et al., 1988), NO biosynthesis should be inhibited by N^G-nitro-L-arginine, which is a L-arginine analogue that competitively inhibits the enzyme NO-synthase, in a non-selective manner, blocking NO-generation (Wildhirt et al., 1997; Nelson and Eichinger, 2001).

A significant dose-dependent reduction in bremazocine-induced peripheral antihyperalgesia occurred after intraplantar administration of N^G-nitro-L-arginine. When applied alone, N^G-nitro-L-arginine (25 μg) did not antagonize the effect of carrageenan, showing that N^G-nitro-L-arginine itself has no hyperalgesic or antihyperalgesic effect, and that the effect of bremazocine is partially due to local NO-generation.

When methylene blue was applied 1 h before bremazocine administration, a significant dose-dependent reduction in the peripheral antihyperalgesia induced by bremazocine was observed. However, when applied alone, methylene blue had no effect on bremazocine antihyperalgesia. This protocol blocked the ability of guanylate cyclase to form cyclic GMP but did not interfere with NO-generation. This result led us to conclude that NO has an intermediary role in the peripheral antinociception because it was unable to induce antihyperalgesia in the hyperalgesic paw when guanylate cyclase was blocked by methylene blue. This experiment also showed that cyclic GMP is essential in the antihyperalgesic mechanism of bremazocine. In addition, in another experiment, it was demonstrated that zaprinast (50 μg per paw) potentiated the ability of a low dose of bremazocine (20 μg per paw) to cause antihyperalgesia against carrageenan-induced hyperalgesia. It is known that zaprinast is a specific inhibitor of cyclic GMP phosphodiesterase. Thus, the cyclic GMP formed after administration of bremazocine is not degraded and is accumulated in the inflamed paw, increasing the nociceptive threshold.

In conclusion, we suggest that κ-opioid receptor agonists like bremazocine elicit peripheral antinociception by activation of the L-arginine/nitric oxide/cyclic GMP pathway, although NO is only an intermediary in this mechanism. Even though it is known that this nitric oxide induces guanylate cyclase to form cyclic GMP, it is yet to be clarified how cyclic GMP elicits peripheral antinociception.

Acknowledgements

Conselho Nacional de Pesquisa (CNPq) supported this research and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

References

Chen, X., Levine, J.D., 1999. NOS inhibitor antagonism of PGE2-induced mechanical sensitization of cutaneous C-fiber nociceptors in the rat. J. Neurophysiol. 81, 963–966.

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.

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.

Edwards, G., Weston, A.H., 1993. The pharmacology of ATP-sensitive potassium channels. Annu. Rev. Pharmacol. Toxicol. 33, 597–637.

Ferreira, S.H., Nakamura, M., 1979. Prostaglandin hyperalgesia, the peripheral analgesic activity of morphine, enkephalins, opioid antagonists. Prostaglandins 18, 191–200.

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

Ko, M., Butelman, E.R., Woods, J.H., 1999. Activation of peripheral κ-opioid receptors inhibits capsaicin-induced thermal nociception in rhesus monkeys. J. Pharmacol. Exp. Ther. 289, 378–385.

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

Levy, D., Höke, A., Zochodne, D.W., 1999. Local expression of inducible nitric oxide synthase in an animal model of neuropathic pain. Neurosci. Lett. 260, 207–209.

Levy, D., Tal, M., Höke, A., Zochodne, D.W., 2000. Transient action of the endothelial constitutive nitric oxide synthase (ecNOS) mediates the development of thermal hypersensitivity following peripheral nerve injury. Eur. J. Neurosci. 12, 2323–2332.

Machelska, H., Labuz, D., Przewlocki, R., Przewlocka, B., 1997. Inhibition of nitric oxide synthase enhances antinociception mediated by mu, delta and kappa opioid receptors in acute and prolonged pain in the rat spinal cord. J. Pharmacol. Exp. Ther. 282, 977–984.

Minneman, K.P., Iversen, L.L., 1976. Enkephalin and opiate narcotics increase cyclic GMP accumulation in slices of rat neostriatum. Nature 262, 313–314.

Nelson, R.S., Eichinger, M.R., 2001. Role of nitric oxide (NO) in pulmonary dysfunction associated with experimental cirrhosis. Respir. Physiol. 126, 65–74.

Nozacki-Taguchi, N., Yamamoto, T., 1998. Involvement of nitric oxide in peripheral antinociception mediated by κ- and δ-opioid receptors. Anesth. Analg. 87, 388–393.

Palmer, R.M., Ashton, D.S., Moncada, S., 1988. L-Arginine is the physiological precursor for the formation of nitric oxide in the endothelium-dependent relaxation. Biochem. Biophys. Res. Commun. 153, 1251–1256.

Randall, L.D., Selitto, J.J., 1957. A method for measurement of analgesic activity on inflamed tissues. Arch. Int. Pharmacodyn. 111, 209–219.

Rodrigues, A.R.A., Duarte, I.D.G., 2000. The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K^+ channels. Br. J. Pharmacol. 129, 110–114.

Römer, D., Büscher, H., Hill, R.C., Maurer, R., Petcher, T.J., Welle, H.B.A., Bakel, H.C.C.K., Akkerman, A.M., 1980. Bremazocine: a potent, long-acting opiate kappa-agonist. Life Sci. 27, 971–978.

Sharma, S.K., Niremberg, M., Klee, W., 1975. Morphine receptors as regulators of adenylate cyclase activity. Proc. Natl. Acad. Sci. U. S. A. 72, 590–594.

Soares, A.C., Leite, R., Tatsuo, M.A.K.F., Duarte, I.D.G., 2000. Activation of ATP-sensitive K^+ channels: mechanism of peripheral antinociceptive action of the nitric oxide donor, sodium nitropusside. Eur. J. Pharmacol. 400, 67–71.

Song, Z.H., Takemori, A.E., 1990. Involvement of spinal kappa opioid receptors in the antinociception induced by intrathecally administered corticotropin-releasing factor in mice. J. Pharmacol. Exp. Ther. 254, 363–368.

Song, H.K., Pan, H.L., Eisenach, J.C., 1998. Spinal nitric oxide mediates antinociception from intravenous morphine. Anaesthesiology 89, 215–221.

Wildhirt, S.M., Suzuki, H., Horstman, D., Weismuller, S., Dudek, R.R., Akiyama, K., Reichart, B., 1997. Selective modulation of inducible nitric oxide synthase isozyme in myocardial infarction. Circulation 96, 1616–1623.