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Short communication

Effect of caffeine coadministration and of nitric oxide synthesis inhibition on the antinociceptive action of ketorolac

Francisco J. López-Muñoz, Gilberto Castañeda-Hernández, Francisco J. Flores-Murrieta, Vinicio Granados-Soto *

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología del Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado Postal 22026, 14000 México, D.F., Mexico

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Abstract

The effects of caffeine and nitric oxide synthesis inhibition on the antinociceptive action of ketorolac were assessed using the pain-induced functional impairment model in the rat. Nociception was induced by the intra-articular injection of uric acid. Ketorolac, but not caffeine, produced an antinociceptive effect which was reduced by N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis. Caffeine coadministration potentiated the ketorolac effect. L-NAME induced a dose-dependent reduction of this potentiation. The results suggest the participation of the L-arginine-nitric oxide-cyclic GMP pathway in the caffeine potentiation of ketorolac-induced antinociception.

Keywords: Ketorolac; Caffeine; Nitric oxide (NO); N^G-Nitro-L-arginine methyl ester (L-NAME); Potentiation; Antinociception

1. Introduction

It is generally accepted that non-steroidal antiinflammatory drugs (NSAIDs) produce their antinociceptive effect by prostaglandin synthesis inhibition (Ferreira, 1972). Nevertheless, it has been recently reported that, at least in certain cases, nitric oxide (NO) is also involved (Granados-Soto et al., 1995; Tonussi and Ferreira, 1994). It has been proposed that activation of soluble guanylate cyclase by NO increases intracellular cyclic GMP levels, which in turn modulate pain and analgesia (Tonussi and Ferreira, 1994). On the other hand, caffeine is able to increase NSAID-induced analgesia (Castañeda-Hernández et al., 1994). It has been suggested that such potentiation is due to caffeine-induced phosphodiesterase inhibition (Sawynok and Yaksh, 1993) as caffeine increases intracellular cyclic GMP in the periphery (Hatano et al., 1995). Therefore, we decided to study the interaction between ketorolac and caffeine in presence and absence of NO synthesis inhibition.

2. Materials and methods

Antinociception was assessed by the pain-induced functional impairment model in the rat as described previously (López-Muñoz et al., 1993). All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and the recommendations of the Canadian Council on Animal Care (1989). The study was approved by the local Animal Care Committee.

Male Wistar rats (180–220 g) were used. Nociception was induced by the intra-articular injection of 0.05 ml of a 30% uric acid suspension in mineral oil in the right hind knee. The animals were then forced to walk in a rotating cylinder for 2-min periods every 30 min. As a result of uric acid injection, the rats developed a progressive dysfunction of the injured limb, reducing the time of contact between the right hind limb and the cylinder. The data are expressed as the functionality index, i.e. the time of contact of the injected limb divided by the time of contact of the control left limb, multiplied by 100.

Once the functionality index was zero, the rats received an intra-articular injection of 800, 1600 or 3200 μg N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of

* Corresponding author. Tel.: +(525) 675 9075; fax: +(525) 675 9168.

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NO synthesis (Rees et al., 1990), dissolved in 0.05 ml of isotonic saline or of vehicle. One hour later, the animals received ketorolac tromethamine (1.8 mg/kg) and/or caffeine (32 mg/kg) by gavage. The functionality index was determined every 30 min in the subsequent 4 h. Additional groups were pretreated with intra-articular L-NAME or saline, but those who did not receive any analgesic agent served as controls. Recovery of the functionality index was considered as the expression of the antinociceptive effect.

It should be noted that, after uric acid injection, we did not observe any behavioral sign of severe discomfort, such as licking, elevating, biting, shaking, or vocalization, as occurs in other experimental pain models (Tjölsen et al., 1992).

2.1. Data analysis and statistics

Functionality index against time curves were constructed and the area under the curve (AUC_E) was considered as an expression of the overall antinociceptive activity during the 4-h observation period (López-Muñoz et al., 1993). AUC_E values were compared by means of Student's *t*-test for unpaired data.

3. Results

Fig. 1 shows the time course of the antinociceptive effect, expressed as functionality index recovery, of ketorolac and/or caffeine in saline- and L-NAME-pretreated rats. Ketorolac was able to induce an increase of the functionality index which was greater in saline-pretreated animals. Caffeine coadministration resulted in a potentiation of the antinociceptive effect of ketorolac. Caffeine alone, however, was ineffective in this model.

Fig. 2 shows the overall antinociceptive effect, expressed as AUC_E, of ketorolac alone or combined with caffeine in rats pretreated with several doses of L-NAME. In both cases, L-NAME induced a dose-dependent reduction of the antinociceptive effect, although a complete inhibition of the response was not achieved. The effect of the ketorolac/caffeine combination was significantly greater in the presence of 0, 800 and 1600 μg of L-NAME. However, at the highest dose of the NO synthesis inhibitor, i.e. 3200 μg, the effects of ketorolac and ketorolac/caffeine were similar.

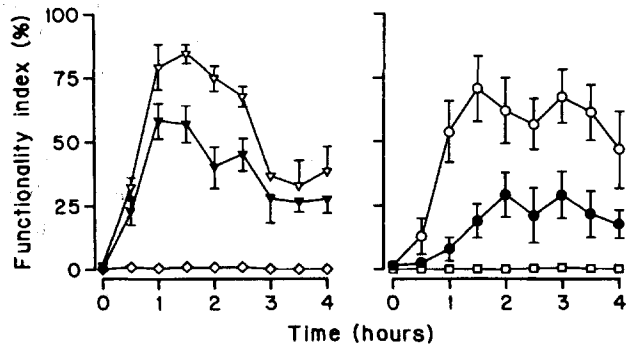

Fig. 1. Time course of the antinociceptive effect, measured as functionality index recovery in rats. Left: animals pretreated with an intra-articular injection of saline then given 1.8 mg/kg ketorolac (▼), 32 mg/kg caffeine (◇), or the ketorolac/caffeine combination (▽). Right: animals pretreated with 800 μg N^G-nitro-L-arginine methyl ester (L-NAME) then given vehicle (□), ketorolac (●) or the ketorolac/caffeine combination (○). Data are presented as means ± S.E.M., $n = 6$.

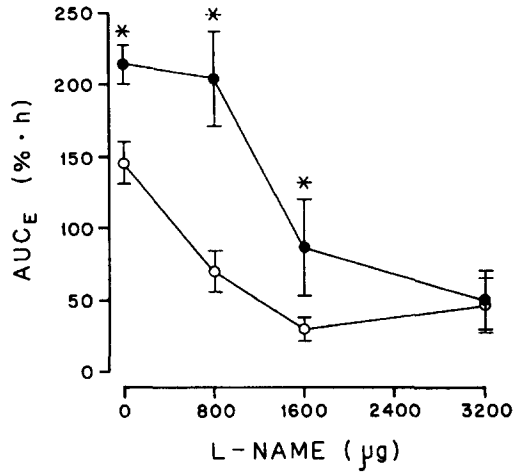

Fig. 2. Overall antinociceptive effect, measured as the functionality index against time curve (AUC_E), of ketorolac in presence (●) and absence (○) of caffeine in rats pretreated with several doses of N^G-nitro-L-arginine methyl ester (L-NAME). Data are presented as means ± S.E.M., $n = 6$. * Significantly different from ketorolac alone ($P < 0.05$) as determined by means of Student's *t*-test.

4. Discussion

It has been demonstrated that caffeine coadministration is able to increase NSAID-induced antinociception (Castañeda-Hernández et al., 1994). The mechanism of action of such potentiation, however, remains unclear. It has been suggested that caffeine may enhance NSAID effects by a pharmacokinetic mechanism (Sawynok and Yaksh, 1993). This, however, seems unlikely, as there is experimental evidence that caffeine does not significantly alter NSAID concentrations (Castañeda-Hernández et al., 1994; Granados-Soto et al., 1993). It then appears that the caffeine action as an antinociceptive adjuvant is exerted through a pharmacodynamic mechanism.

There is evidence suggesting that caffeine effects on nociception are due to the inhibition of phosphodiesterase activity (Sawynok and Yaksh, 1993), as caffeine is able to produce an intracellular accumulation of cyclic GMP (Hatano et al., 1995). It has also been proposed that, in addition to prostaglandin synthesis inhibition, ketorolac, dipyrone and diclofenac, stimulate NO production, which results in increased cyclic GMP levels and antinociception (Granados-Soto et al., 1995; Tonussi and Ferreira, 1994). The present results confirm the finding that inhibition of

NO synthesis by intra-articular L-NAME reduces the antinociceptive effect of ketorolac. Despite the fact that high L-NAME doses were used, ketorolac was still able to produce a significant effect. This is likely due to mechanisms independent of NO production, such as prostaglandin synthesis inhibition.

Caffeine was able to potentiate the antinociceptive effect of ketorolac. This potentiation, however, was abolished in a dose-dependent manner by L-NAME. These data can be interpreted as follows. Ketorolac promotes NO synthesis, which in turn results in an increase of cyclic GMP. Caffeine-induced phosphodiesterase inhibition prevents cyclic GMP degradation and, hence, the antinociceptive effect is potentiated. In the presence of a high L-NAME dose, the potentiation is not observed because, if cyclic GMP is not produced, phosphodiesterase inhibition is irrelevant. This also can explain why caffeine is ineffective in the absence of ketorolac.

The present results provide evidence that the L-arginine-NO-cyclic GMP pathway is involved in the caffeine potentiation of NSAID-induced antinociception. Further investigation, however, is required since the pharmacological effects of both caffeine and NO are complex. Therefore, the information available at present does not allow the possibility of involvement of other mechanisms of action to be discarded.

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References

Canadian Council on Animal Care, 1989, Ethics of Animal Investigation, Canadian Council on Animal Care, Ottawa, Ont.

Castañeda-Hernández, G., M.S. Castillo-Méndez, F.J. López-Muñoz, V. Granados-Soto and F.J. Flores-Murrieta, 1994, Potentiation by caffeine of the analgesic effect of aspirin in the pain-induced functional impairment model in the rat, Can. J. Physiol. Pharmacol. 72, 1127.

Ferreira, S.H., 1972, Prostaglandins, aspirin like drugs and analgesia, Nature 240, 200.

Granados-Soto, V., F.J. López-Muñoz, G. Castañeda-Hernández, L.A. Salazar, J.E. Villarreal and F.J. Flores-Murrieta, 1993, Characterization of the analgesic effects of paracetamol and caffeine combinations in the pain-induced functional impairment model in the rat, J. Pharm. Pharmacol. 45, 627.

Granados-Soto, V., F.J. Flores-Murrieta, G. Castañeda-Hernández and F.J. López-Muñoz, 1995, Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac in the rat, Eur. J. Pharmacol. 277, 281.

Hatano, Y., K. Mizumoto, T. Yoshiyama, M. Yamamoto and H Iranami, 1995, Endothelium-dependent and -independent vasodilation of isolated rat aorta induced by caffeine, Am. J. Physiol. 269, H1679.

López-Muñoz, F.J., L.A. Salazar, G. Castañeda-Hernández and J.E. Villarreal, 1993, A new model to assess analgesic activity: pain-induced functional impairment in the rat (PIFIR), Drug Dev. Res. 28, 169.

Rees, D.D., R.M.S. Palmer, R. Schulz, H.F. Hodson and S. Moncada, 1990, Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo, Br. J. Pharmacol. 101, 746.

Sawynok, J. and T.L. Yaksh, 1993, Caffeine as an anagesic adjuvant: a review of its pharmacology and mechanisms of action, Pharmacol. Rev. 45, 43.

Tjölsen, A., O.G. Berge, S. Hunskaar, J.H. Rosland and K. Hole, 1992, The formalin test: an evaluation of the method, Pain 51, 5.

Tonussi, C.R. and S.H. Ferreira, 1994, Mechanism of diclofenac antinociception: direct blockade of inflammatory sensitization, Eur. J. Pharmacol. 251, 173.

Zimmermann, M., 1983, Ethical guidelines for investigation on experimental pain in conscious animals, Pain 16, 109.