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Effect of coadministration of caffeine and either adenosine agonists or cyclic nucleotides on ketorolac analgesia

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Abstract

Caffeine potentiation of ketorolac-induced antinociception in the pain-induced functional impairment model in rats was assessed. Caffeine alone was ineffective, but increased the effect of ketorolac without affecting its pharmacokinetics. Intra-articular administration of adenosine and N^6-cyclohexyladenosine (CHA, an adenosine A_1 receptor agonist), but not 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride (CGS-21680, an adenosine A_{2A} receptor agonist), significantly increased ketorolac antinociception. This effect was not local, as contralateral administration was also effective. Ipsilateral and contralateral administration of adenosine and CHA also increased antinociception by ketorolac–caffeine. Intra-articular 8-Bromo-adenosine cyclic 3′,5′-hydrogen phosphate sodium or 8-Bromo-guanosine-3′,5′-cyclophosphate sodium (cGMP) given ipsilaterally or contralaterally did not affect ketorolac-induced antinociception. Nevertheless, ipsilateral, but not contralateral, administration of 8-Br-cGMP significantly increased antinociception by ketorolac–caffeine, suggesting a local effect. The results suggest that caffeine potentiation of ketorolac antinociception is mediated, at least partially, by a local increase in cGMP and rule out the participation of adenosine receptor blockade.

Keywords: Ketorolac; Caffeine; Potentiation; Adenosine A_1 receptor; cGMP

1. Introduction

There are a number of reports that caffeine can potentiate the antinociceptive effects of non-steroidal anti-inflammatory drugs (NSAIDs) in animal models (Siegers, 1973; Vinegar et al., 1976; Seegers et al., 1980; Granados-Soto et al., 1993). In humans, caffeine has been extensively used as an analgesic adjuvant (Laska et al., 1984; Forbes et al., 1991; reviewed Sawynok and Yaksh, 1993). However, the ability of caffeine to increase the analgesic effect of NSAIDs has been questioned, since there are reports that caffeine is ineffective to increase the antinociceptive effect of NSAIDs (Cass and Frederik, 1962; Moertel et al., 1974). In contrast, in the last decade an increasing number of controlled clinical trials have produced evidence that caffeine can indeed improve analgesic efficacy, but only in certain pain states and dose ratios (Laska et al., 1984; Forbes et al., 1991; Sawynok and Yaksh, 1993). Despite the fact that potentiation of the antinociceptive effect of NSAIDs by caffeine has been attributed to the inhibition of adenosine receptors (Sawynok and Yaksh, 1993), the mechanism of such potentiation is not well understood. Therefore, we decided to determine whether the mechanism of this synergy is pharmacokinetic or pharmacodynamic. In addition, we studied the potentiation by caffeine of the antinociceptive activity of ketorolac in the presence and absence of cyclic nucleotides (8-Bromo-adenosine cyclic 3′,5′-hydrogen phosphate monosodium, 8-Br-cAMP and 8-Bromo-guanosine-3′,5′-cyclophosphate sodium, 8-Br-cGMP) and adenosine agonists (adenosine, N^6-cyclohexyladenosine (CHA) and 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamido-adenosine hydrochloride (CGS-21680)) (Fredholm et al., 1996) by way of assessing the participation of peripheral

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cyclic nucleotides and adenosine receptors activation, respectively, in this action.

2. Material and methods

2.1. Animals

Female Wistar rats (weight range, 180–220 g) from our own breeding facilities [Crl:(WI)BR] were used in this study. Twelve hours before the initiation of experiments, food was withheld, but the animals had free access to drinking water. Rats to be used in the pharmacokinetic study were lightly anaesthetized with ethyl ether. Then, polyethylene catheters (a combination of a PE-10 and PE-50 was used; i.d. 0.28 mm, o.d. 0.61 mm and i.d 0.58 mm, o.d. 0.96 mm, respectively, Clay Adams, Parsippany, NJ, USA) were surgically implanted into the caudal artery for the collection of blood samples. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the local Animal Care Committee.

2.2. Drugs

Racemic ketorolac tromethamine was obtained from Roche-Syntex (Mexico City). Caffeine, adenosine, 8-Br-cAMP, 8-Br-cGMP and uric acid were purchased from Sigma (St. Louis, MO, USA). CHA and CGS-21680 (adenosine A_1 and A_{2A}-selective receptor agonists, respectively) (Fredholm et al., 1996) were purchased from Research Biochemical International (Natick, MA, USA). Acetonitrile was chromatographic grade (Merck, Darmstadt, Germany). Deionized water was obtained through a Milli-Q system (Continental Water Systems, El Paso, TX, USA). Other reagents used in this study were of analytical grade.

2.3. Measurement of antinociceptive activity

Antinociception was assessed with the pain-induced functional impairment model in the rat, as described previously (López-Muñoz et al., 1993). Nociception was induced by the intra-articular injection of 50 μl of 30% uric acid suspension in mineral oil into the right hind knee and an electrode was attached to each hind limb behind the plantar pads. At selected times, the rats were required to walk on a cylinder of 30-cm diameter rotating at 4 rpm for 2-min periods. The variable measured in this model was the time of contact of each electrode with the cylinder floor. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. The animals were allowed to rest between recording periods. During resting periods the rats did not show any sign of discomfort, such as licking, biting, shaking, elevating, vocalization.

As a result of uric acid injection, the rats developed a progressive dysfunction of the injured limb. This was recorded as a diminished time of contact between the right hind limb and the cylinder. 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. After 2 h, the injected limb made no contact with the cylinder, the rats received the drugs and recordings were carried out during the next 4 h. Recovery of the functionality index was considered as the expression of the antinociceptive effect.

2.4. Ketorolac determination

Blood concentrations of ketorolac were determined by a high performance liquid chromatographic (HPLC) method previously described (Flores-Murrieta et al., 1994).

2.5. Study design

In the first experimental series, six groups of at least six rats each were used to test the potentiation by caffeine of the antinociceptive effect of ketorolac. Once the functionality index was zero, the animals received vehicle (carboxymethyl cellulose), 1.8 mg/kg of ketorolac tromethamine, 32 mg/kg of caffeine or the combination of

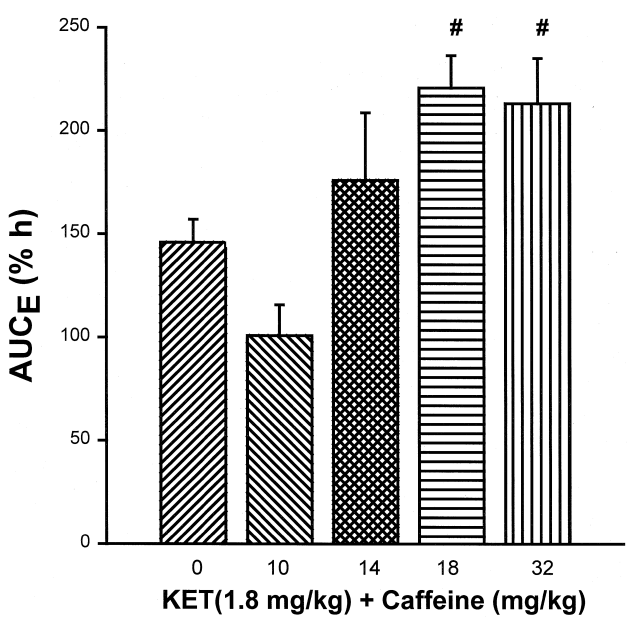

Fig. 1. Area under functionality index against time curve (AUC_E), which represents a global antinociceptive effect, observed in rats with pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. Rats received ketorolac alone (1.8 mg/kg) or combined with 10, 14, 18 or 32 mg/kg caffeine. Data are expressed as means ± S.E.M. of six determinations. # Significantly different from the ketorolac–caffeine combination alone ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

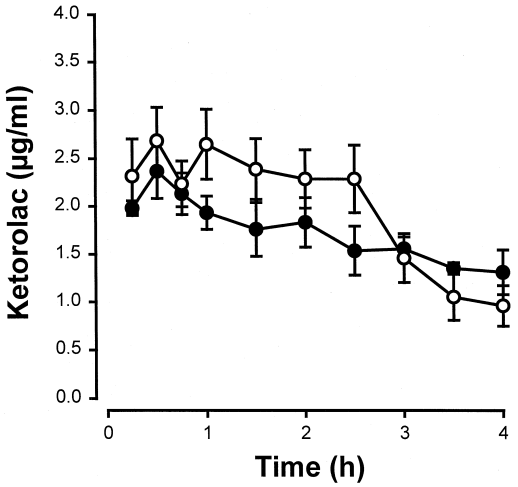

Fig. 2. Time course of the whole blood ketorolac levels observed in rats with pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind limb. Animals received 1.8 mg/kg of ketorolac tromethamine (clear circles) alone or combined with 32 mg/kg of caffeine (dark circles). Data are expressed as means ± S.E.M. of six determinations.

ketorolac tromethamine (1.8 mg/kg) with 10, 14, 18 or 32 mg/kg of caffeine dissolved in 0.5% carboxymethyl cellulose by gavage. Functionality index was evaluated at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h after drug administration. Ketorolac and caffeine doses were selected based on previous studies (Granados-Soto et al., 1993, 1995).

In the second experimental series, we examined whether the potentiation of ketorolac-induced antinociception by caffeine had a pharmacokinetic mechanism. For this purpose, 1.8 mg/kg of ketorolac tromethamine alone or combined with 32 mg/kg of caffeine was given. Whole blood samples (100 µl) were obtained, through the cannula inserted into the caudal artery, at 0, 15, 30 and 45 min and at 1, 1.5, 2, 2.5, 3, 3.5 and 4 h after drug administration. Then, blood samples were frozen and stored at −70°C until analyzed. In the third experimental series, once functionality index had reached zero, the animals received either ipsilaterally or contralaterally an intra-articular injection of increasing doses of saline, adenosine, 8-Br-cAMP, 8-Br-cGMP, CHA or CGS-21680 and simultaneously a dose of ketorolac tromethamine (1.8 mg/kg) or the combination of ketorolac–caffeine (1.8–14 mg/kg p.o.).

Table 1
Values for pharmacokinetic and pharmacodynamic parameters of ketorolac obtained after oral administration of ketorolac tromethamine alone (1.8 mg/kg) or combined with 32 mg/kg of caffeine. Data are expressed as means ± S.E.M. of six determinations

Parameter	Ketorolac	Ketorolac–caffeine
C_{max} (µg/ml)	2.38 ± 0.26	2.72 ± 0.28
t_{max} (h)	1.25 ± 0.43	0.75 ± 0.43
AUC (µg h/ml)	6.70 ± 0.58	7.50 ± 0.81
E^{obs}_{max} (%)	66.0 ± 6.72	85.4 ± 4.86[a]
t_{Emax} (h)	1.58 ± 0.20	1.16 ± 0.10
AUC_E (% h)	145.9 ± 11.1	213.1 ± 21.9[a]

[a] Significantly different from ketorolac alone ($P < 0.001$), as determined by Student's *t*-test.

2.6. Data analysis and statistics

Maximal blood concentrations (C_{max}) and time to reach the maximal blood concentration (t_{max}) were determined directly from the individual blood concentration against time curves. The area under the ketorolac blood concentra-

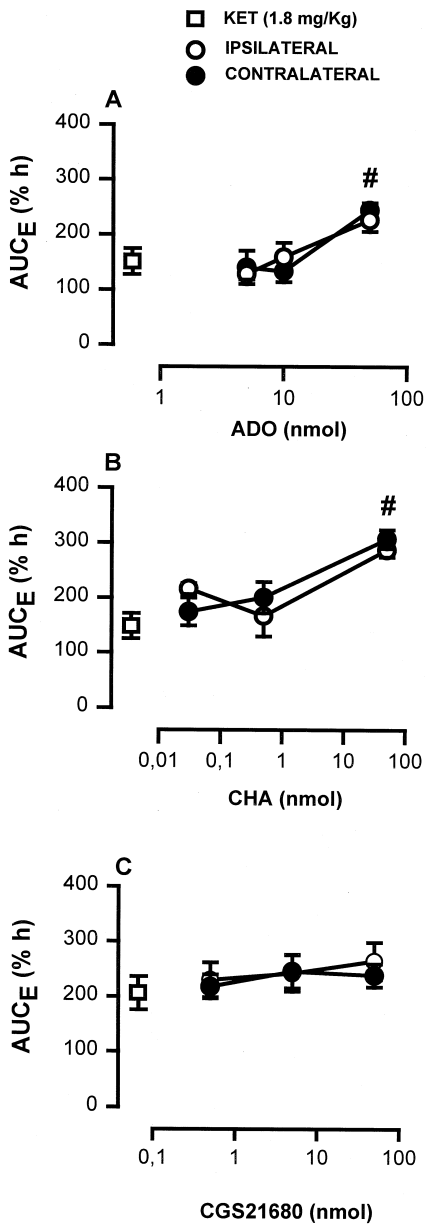

Fig. 3. Effect of ipsilateral or contralateral intra-articular administration of adenosine, ADO (A), CHA (adenosine A_1 receptor agonist) (B) and CGS-21680 (adenosine A_{2A} receptor agonist) (C) on ketorolac (1.8 mg/kg) antinociception. Data are expressed as the AUC_E for six animals ± S.E.M. # indicates a significant difference against ketorolac alone ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

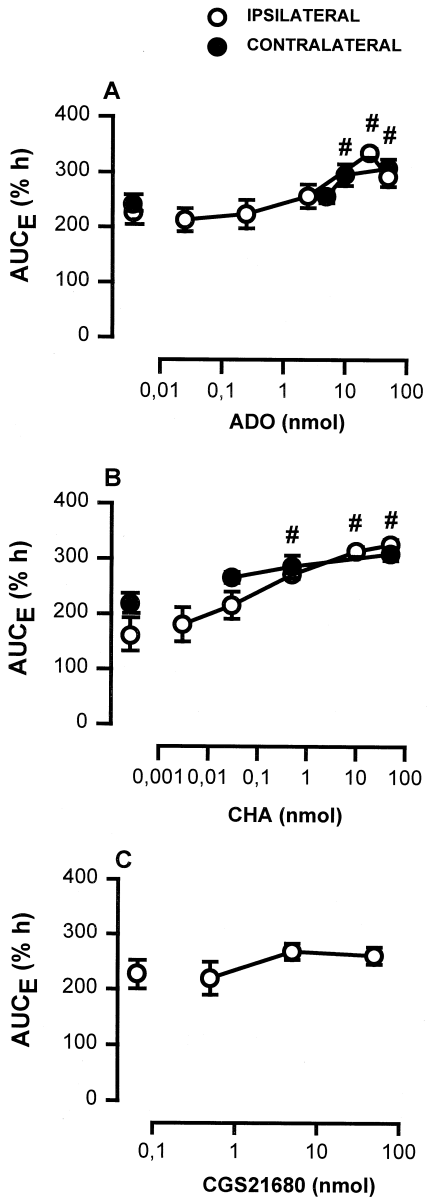

Fig. 4. Effect of ipsilateral or contralateral adenosine, ADO (A), CHA (adenosine A_1 receptor agonist) (B) and CGS-21680 (adenosine A_{2A} receptor agonist) (C) on ketorolac–caffeine (1.8–14 mg/kg) antinociception. Data are expressed as the AUC_E for six animals ± S.E.M. # indicates a significant difference against ketorolac–caffeine combination alone ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

tions against time curves (AUC) was calculated by the trapezoidal rule.

Curves were made for functionality index against time and the maximal antinociceptive effect (E_{max}^{obs}) and time to reach E_{max}^{obs} ($t_{E_{max}}$) were directly determined from these plots. The area under the functionality index against time curve (AUC_E), considered as an expression of the overall antinociceptive activity during the 4-h observation period, was estimated by the trapezoidal rule (López-Muñoz et al., 1993).

Analysis of variance followed by Dunnett's test was used to test differences in the potentiation studies. Comparison between pharmacokinetic parameter values observed with ketorolac alone and combined with caffeine were performed by the Student's *t*-test for unpaired data.

3. Results

3.1. Potentiation of ketorolac antinociception by caffeine

Oral administration of ketorolac produced a recovery of the functionality index (antinociceptive effect) which reached a peak of about 65% and then decayed gradually. Caffeine alone was not able to produce any significant antinociceptive action, but when it was coadministered with ketorolac, it induced a significant dose-dependent increase in the antinociceptive effect. The antinociceptive effect of the combination of either 10 or 14 mg/kg caffeine and 1.8 mg/kg of ketorolac was not different from that of ketorolac alone ($P > 0.05$). However, subsequent doses of caffeine (18 and 32 mg/kg) produced a clear potentiation of ketorolacs antinociceptive activity ($P < 0.05$) (Fig. 1).

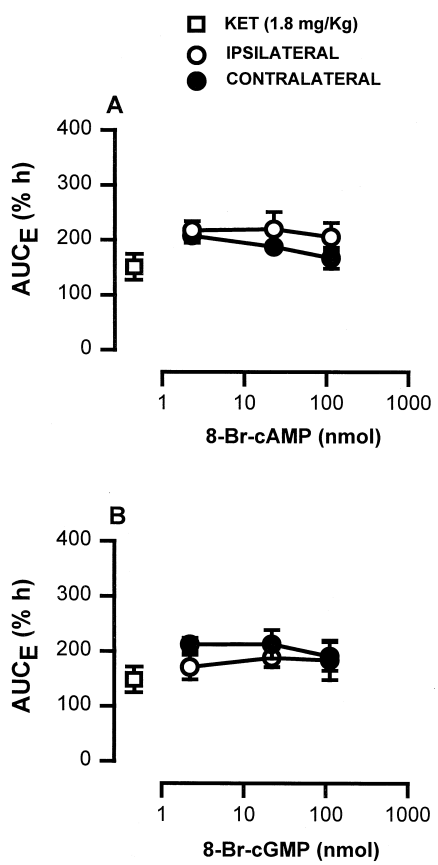

Fig. 5. Effect of ipsilateral or contralateral intra-articular administration of 8-Br-cAMP (A) and 8-Br-cGMP (B) on the ketorolac antinociception. Data are expressed as the AUC_E for six animals ± S.E.M.

3.2. Ketorolac–caffeine pharmacokinetic interaction

Since a clear potentiation was observed with 1.8–32 mg/kg ketorolac–caffeine, we decided to study this combination in comparison with 1.8 mg/kg ketorolac alone in order to study a possible pharmacokinetic interaction. Ketorolac blood concentrations were similar in the presence and absence of caffeine (Fig. 2), despite the fact that the association, ketorolac–caffeine, produced a higher antinociceptive effect than ketorolac alone (Fig. 1). Caffeine did not produce any significant modification of ketorolac pharmacokinetics, although the values of pharmacodynamic parameters E_{max}^{obs} and AUC_E were significantly ($P < 0.001$) increased (Table 1). Caffeine pharmacokinetics were not examined as this drug, by itself, failed to produce any significant antinociception.

3.3. Adenosine agonists

Adenosine receptor agonists (adenosine, CHA and CGS-21680) were not able to produce any effect when administered to either normal or injured animals (data not shown). Either the ipsilateral or contralateral intra-articular administration of adenosine (50 nmol/knee) and the adenosine A_1 receptor agonist, CHA (50 nmol/knee), but not the adenosine A_{2A} receptor agonist, CGS-21680, simultaneously with the oral administration of ketorolac significantly increased antinociception ($P < 0.05$) (Fig. 3). Ipsilateral or contralateral adenosine, at the same dose level, was also able to significantly increase the antinociceptive effect of ketorolac–caffeine. Similar results were observed with CHA, although the dose of this adenosine A_1 agonist required to potentiate the effect of ketorolac–caffeine was 100 times lower than that needed to increase the effect of ketorolac alone (0.5 versus 50 nmol/knee) (Fig. 4).

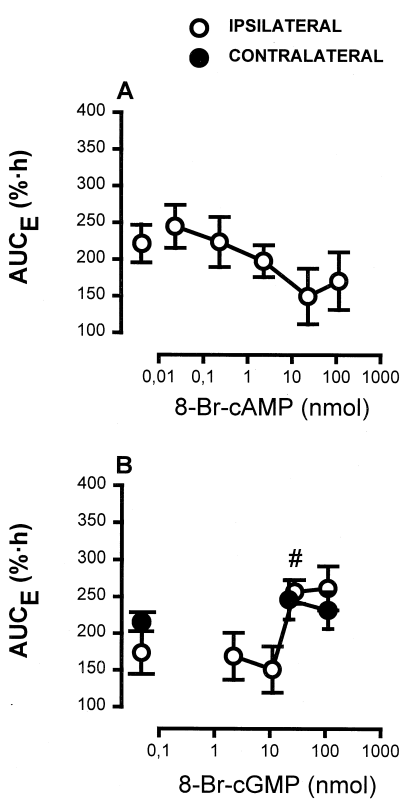

Fig. 6. Effect of ipsilateral intra-articular administration of 8-Br-cAMP (A) and ipsilateral or contralateral 8-Br-cGMP (B) on the ketorolac–caffeine (1.8–14 mg/kg) antinociception. Data are expressed as the AUC_E for six animals ± S.E.M. # indicates significant difference compared to the ketorolac–caffeine combination alone ($P < 0.05$), as determined by analysis of variance followed by Dunnett's test.

3.4. Cyclic nucleotides

8-Br-cAMP or 8-Br-cGMP were not able to produce any significant effect when given to either normal or injured rats (data not shown). Furthermore, the effect of ketorolac alone was not altered by intra-articular administration of increasing doses of 8-Br-cAMP or 8-Br-cGMP given either ipsilaterally or contralaterally simultaneously with oral ketorolac (Fig. 5). In contrast, ipsilateral, but not contralateral, administration of 8-Br-cGMP significantly increased ($P < 0.05$) the antinociceptive effect of the ketorolac–caffeine combination (Fig. 6). 8-Br-cAMP failed to increase the antinociceptive effect of ketorolac–caffeine.

4. Discussion

4.1. Potentiation of ketorolac antinociception by caffeine

Oral administration of caffeine did not produce antinociception in our model of hyperalgesia. However, it increased the antinociceptive effect of ketorolac in a dose-related manner. The caffeine dose that produced the best potentiation (18 or 32 mg/kg) in this study was similar to that used in combination with paracetamol (Granados-Soto et al., 1993) and tolmetin (Flores-Acevedo et al., 1995). These data confirm previous observations on the ability of caffeine to potentiate the effect of NSAIDs in experimental pain models (Vinegar et al., 1976; Seegers et al., 1980; Gayawali et al., 1991; Granados-Soto et al., 1993; Castañeda-Hernández et al., 1994; Flores-Acevedo et al., 1995) as well as in clinical situations (Laska et al., 1984).

Caffeine has been demonstrated to produce antinociception in threshold (Person et al., 1985; Malec and Michalska, 1988) and hyperalgesic tests (Siegers, 1973; Seegers et al., 1980; Sawynok et al., 1995). In contrast, there are a number of studies which have not detected antinociception with caffeine in both threshold and hyperalgesic studies (Fialip et al., 1989; Granados-Soto et al., 1993; Castañeda-Hernández et al., 1994; this study). Contradictory results of these studies are likely due to the use of different experimental models to detect antinociception.

4.2. Ketorolac–caffeine pharmacokinetic interaction

It has been suggested that the potentiation by caffeine could be due to an increase of NSAID bioavailability through an increase its absorption or impairment of its elimination (Sawynok and Yaksh, 1993). Our results, however, are not consistent with a pharmacokinetic interaction. When caffeine augmented the antinociceptive effect of ketorolac, the blood levels of the NSAID were similar to those observed in the absence of the xanthine, without any significant alteration of pharmacokinetic parameters. Although it has been reported that caffeine can modify gastric acidity, as well as gastric and hepatic blood flows (Debas et al., 1971; Beubler and Lambech, 1976; Onrot et al., 1986), these actions appear to have no relevance for the bioavailability of ketorolac. Our data are consistent with those previously reported for the combination of caffeine with paracetamol (Granados-Soto et al., 1993), aspirin (Vinegar et al., 1976; Collins et al., 1979; Castañeda-Hernández et al., 1994) and tolmetin (Flores-Acevedo et al., 1995), but disagree with those of Siegers (1973) and Seegers et al. (1980).

4.3. Adenosine agonists

Under our conditions, the adenosine agonists were not able to produce nociception or antinociception, but they increased the activity of ketorolac or the ketorolac–caffeine combination. The intra-articular administration of adenosine or the adenosine A_1 receptor agonist, CHA, but not the adenosine A_{2A} receptor agonist, CGS-21680, significantly increased antinociception by ketorolac. This effect was not due to the local effect of the agonists, as the contralateral administration of adenosine and CHA also significantly increased antinociception. These data suggest that adenosine is not producing a nociceptive effect, but facilitates the effect of ketorolac by a spinal or supraspinal action. Our results are supported by those studies of in vivo models of nociception which have suggested that the adenosine A1 receptor plays an important role in spinal antinociception by inhibiting sensory transmission related to nociceptive information at the spinal level (Reeve and Dickenson, 1995; Nakamura et al., 1997; Poon and Sawynok, 1998). In our model, we were not able to observe any antinociceptive effect with the adenosine receptor agonists. This discrepancy with previous studies could be due to the intensity of the nociceptive stimuli used. Recently, it has been reported that the antinociception produced by adenosine is intensity-dependent (Sawynok et al., 1998).

In addition to their effect on the antinociception produced by ketorolac, adenosine and CHA, but not CGS-21680, also increased the effect of the ketorolac–caffeine combination. Caffeine was not able to block the potentiation of the effect of adenosine agonists on ketorolac-induced antinociception. In fact, the addition of caffeine significantly increased the effect of ketorolac–adenosine and ketorolac–CHA, but not that produced by ketorolac-CGS-21680. Malmberg and Yaksh (1993) have reported an additive synergism of the combination of ketorolac and N^6-(L-2-phenyilisoptopyl)-adenosine (L-PIA), an adenosine A_1 receptor agonist. As in this study, the addition of caffeine, at a dose that antagonized L-PIA alone, failed to affect the combination ketorolac-L-PIA in the second phase of the formalin test. Therefore, our results are consistent with those of Malmberg and Yaksh (1993). However, our data are in contrast with other reports (Karlsten et al., 1992; Poon and Sawynok, 1998) in which theophylline or caffeine, non-specific antagonists of adenosine receptors, were able to block the antinociception produced by local or spinal administration of adenosine receptor agonists. Our results suggest that the effects of caffeine are not due to the blockade of the adenosine receptors, but that other effects of the xanthine are involved. However, it is also possible that there is a certain degree of adenosine antagonism, but that caffeine actions in other compartments predominate in such a way that the global result is a potentiation of the antinociceptive effect. It is not probable that caffeine produces antinociception or potentiation of NSAID-induced antinociception by a blockade of adenosine receptors, as it has been reported that spinal and supraspinal administration of adenosine analogs produces antinociception by activation of adenosine A_1 receptors (Post, 1984; Karlsten et al., 1990). Therefore, other effects of caffeine must be responsible for such antinociception or potentiation. The potentiation of the endogenous cholinergic activity at a central level (Ghelardini et al., 1997) and the activation of central noradrenergic pathways which regulate nociceptive thresholds has been suggested to play an important role in antinociception by caffeine in the several pain tests (Sawynok et al., 1995). Other effects, such as inhibition of phosphodiesterases (Choi et al., 1988), nitric oxide release (López-Muñoz et al., 1996) and stimulation of the central nervous system could also be contributing to the adjuvant effect of caffeine.

4.4. Cyclic nucleotides

We have previously reported that local administration of N^G-nitro-L-arginine methyl ester, a nitric oxide synthesis inhibitor, is able to block the potentiation by caffeine of ketorolac-induced antinociception (López-Muñoz et al., 1996). These data suggest a participation of the L-arginine-nitric oxide-cGMP pathway in antinocieption. In the present study we found that intra-articular administration of 8-Br-cAMP or 8-Br-cGMP, by themselves, did not produce any effect. Furthermore, these compounds were not able to produce any effect on the antinociception of oral ketorolac when given either ipsilaterally or contralaterally. In contrast, intra-articular administration of 8-Br-cGMP significantly increased ($P < 0.05$) the effect produced by the ketorolac–caffeine combination when injected ipsilaterally, but not contralaterally. These results suggest a local action of 8-Br-cGMP. Conversely, 8-Br-

cAMP did not produce any significant change of the ketorolac–caffeine effect. These results can be interpreted in the light of the results of Ferreira and Nakamura (1979) and Taiwo and Levine (1990), who reported an involvement of cGMP in the production of antinociception. The fact that 8-Br-cGMP, by itself, failed to induced any significant effect and also failed to potentiate the effect of ketorolac can be explained by a rapid degradation of the nucleotide. As caffeine is a nonspecific inhibitor of phosphodiesterases (Choi et al., 1988) it is likely that 8-Br-cGMP administration in the presence of caffeine was effective as its degradation was prevented. The available data suggest that caffeine could be producing the potentiation of NSAIDs by increasing cGMP or inhibiting its degradation at the site of inflammation. This suggestion is supported by the report of Hatano et al. (1995) that caffeine increases cGMP in two ways, by increasing nitric oxide and subsequently cGMP, or by inhibiting cGMP degradation by phosphodiesterase inhibition. In addition, it has been shown recently that nitric oxide can enhance antinociception in several models of hyperalgesia (Granados-Soto et al., 1997; Nozaki-Taguchi and Yamamoto, 1998).

In summary the data provided here indicate that potentiation by caffeine of the antinociceptive effect of ketorolac is not due to a pharmacokinetic interaction, but to a pharmacodynamic mechanism. The actions of caffeine appear to be complex, involving several mechanisms. Our results suggest that, in this model, adenosine does not have any pronociceptive or antinociceptive effect by itself, but is able to facilitate ketorolac-induced antinociception by activation of the adenosine A_1 receptor, probably at the central level. The potentiation of ketorolac by caffeine does not appear to result from the antagonism of pronociceptive adenosine actions. Our data suggest a peripheral effect of caffeine, involving local cGMP increase as a mechanism of potentiation of NSAID-induced antinociception. Additionally, there is evidence for a central antinociceptive action of caffeine which is also likely to be involved in the potentiation of NSAID effects by this xanthine (Sawynok et al., 1995; Ghelardini et al., 1997).

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