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Peripheral anti-hyperalgesia by oxcarbazepine: involvement of adenosine A_1 receptors

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In this study we determined whether oxcarbazepine (OXC) could produce local peripheral antinociceptive effects in a rat model of inflammatory hyperalgesia, and whether adenosine receptors were involved. When coadministered with the pro-inflammatory compound concanavalin A, OXC (1000–3000 nmol/paw) caused a significant dose- and time-dependent anti-hyperalgesia. Caffeine (1000–1500 nmol/paw), a nonselective adenosine receptor antagonist, as well as 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (10–30 nmol/paw), a selective A_1 receptor antagonist, coadministered with OXC, significantly depressed its anti-hyperalgesic effect. Drugs injected into the contralateral hind paw did not produce significant effects. These results indicate that OXC produces local peripheral anti-hyperalgesic effects, which is mediated *via* peripheral A_1 receptors.

Oxcarbazepine (OXC), a relatively novel anticonvulsant drug, has been used in neuropathic pain treatment (Carrazana and Mikoshiba 2003). Recently, it has been shown that it has anti-hyperalgesic activity in animal models of inflammatory pain (Kiguchi et al. 2004; Tomić et al. 2004). However, the sites and mechanisms of analgesic actions of OXC are not fully understood. Beside a blockade of ion currents (Kiguchi et al. 2001; Ambrosio et al. 2002), there is an evidence indicating that some receptors are also involved in analgesic action of OXC. We have previously shown that systemic OXC reversed the mechanical hyperalgesia of an inflamed rat paw, and that this effect is mediated *via* A_1 receptors (Tomić et al. 2004). It is well known that activation of both central and peripheral A_1 receptors inhibits pain in rodents (Sawynok 1998). The interaction of OXC with central adenosine receptors has been demonstrated in receptor binding studies (Marangos et al. 1983; Fujiwara et al. 1986). However, the ability of OXC to produce a local peripheral anti-hyperalgesic effect has not been evaluated before. Moreover, it remained unknown whether peripheral adenosine receptors are involved in the OXC-induced antinociception. In this study, we determined (1) the effects of locally administered OXC on Con A-induced inflammatory hyperalgesia in the rat and (2) the effects of caffeine, a nonselective A_1

and A_2 receptor antagonist, and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), a selective adenosine A_1 receptor antagonist, on OXC-induced antinociception.

The coadministration of OXC (1000–3000 nmol/paw; i.pl.) with Con A produced a significant dose- and time-dependent reduction in differences in forces exerted by inflamed and non-inflamed rat hind paw (Fig. A). The ED_{50} (95% confidence limits) obtained at 150 min is 3094 (1990–4810) nmol/paw. Coadministration of 1000 nmol/paw and 1500 nmol/paw of caffeine (i.pl.) with OXC (2000 nmol/paw; i.pl.) dose- and time-dependently depressed the anti-hyperalgesic effects of OXC (Fig. B), with maximal inhibitory effect (%I) of 40.96 and 88.52%, respectively (not shown). DPCPX (10 and 30 nmol/paw), coadministered with OXC also decreased the local anti-hyperalgesic effects of anticonvulsive in a dose- and time-dependent manner (Fig. C). Maximal %I of 10 and 30 nmol/paw of DPCPX (i.pl.) on the effect of OXC were 47.78 and 70.13%, respectively (not shown). The effects of OXC, caffeine and DPCPX were due to local effects, since they were not observed after injection of these drugs into the contralateral hindpaw (Fig.). The highest doses used of caffeine and DPCPX did not affect the Con A-induced hyperalgesia ($P > 0.05$, Student's t-test, data not shown).

The present experiments demonstrated a local peripheral antinociceptive effect of OXC in the rat model of inflammatory hyperalgesia. The local nature of this action was verified by the lack of effect when OXC was injected into the contralateral i.e. non-inflamed hind paw. This is the first evidence on the local peripheral anti-hyperalgesic effect of OXC.

The reversal of local periheral anti-hyperalgesic action of OXC by caffeine revealed in this study could mean that both A_1 and A_2 receptors are involved in this action. This is in agreement with previous findings of Kiguchi et al. (2002) that theophylline, another nonselective adenosine receptor antagonist, reversed antinociception of systemic OXC in a tail flick test in diabetic mice. Our further experiments using a selective A_1 receptor antagonist revealed that OXC-induced antinociception is most probably due to interaction with A_1 receptors.

The finding that caffeine and DPCPX inhibit OXC-induced antinociception could mean that OXC acts directly on adenosine receptors, or indirectly, by increasing adenosine tissue levels. There is no available data on the enhancement of adenosine tissue levels by OXC. Contrary to this, several studies showed that OXC binds to brain adenosine receptors (Marangos et al. 1983; Fujiwara et al. 1986; Ambrosio et al. 2002). Also, caffeine- and DPCPX-induced depressions are dose- and time-dependent and almost complete (up to 90%) at higher doses, speaking in favour of direct adenosine receptor-mediated actions. Alzheimer et al. (1991) have shown that peripheral tissues possess A_1 receptors similar to those of the central nervous system. Furthermore, the adenosine A_1 receptor agonist N6-cyclopentyladenosine (CPA), when injected locally into the rat hindpaw, dose-dependently inhibited prostaglandin E_2-induced mechanical hyperalgesia in the paw pressure test (Aley et al. 1995; Aley and Levine 1997). It was also demonstrated that prostaglandins act as mediators in concanavalin A induced inflammation (Cottney and Lewis 1975).

Based on all these findings, our result that selective A_1 receptor antagonist suppressed the antinociceptive effect of OXC, means that the local peripheral anti-hyperalgesic effect of OXC is most probably mediated by direct activation of peripheral A_1 receptors. In conclusion, OXC produces local peripheral anti-hyperalgesia against inflammatory pain and peripheral A_1 receptors are involved in this action.

Experimental

1. Model of inflammatory hyperalgesia

Groups of 6–8 male Wistar rats (180–220 g) were used. Induction of inflammation and the paw pressure test was performed as previously described in

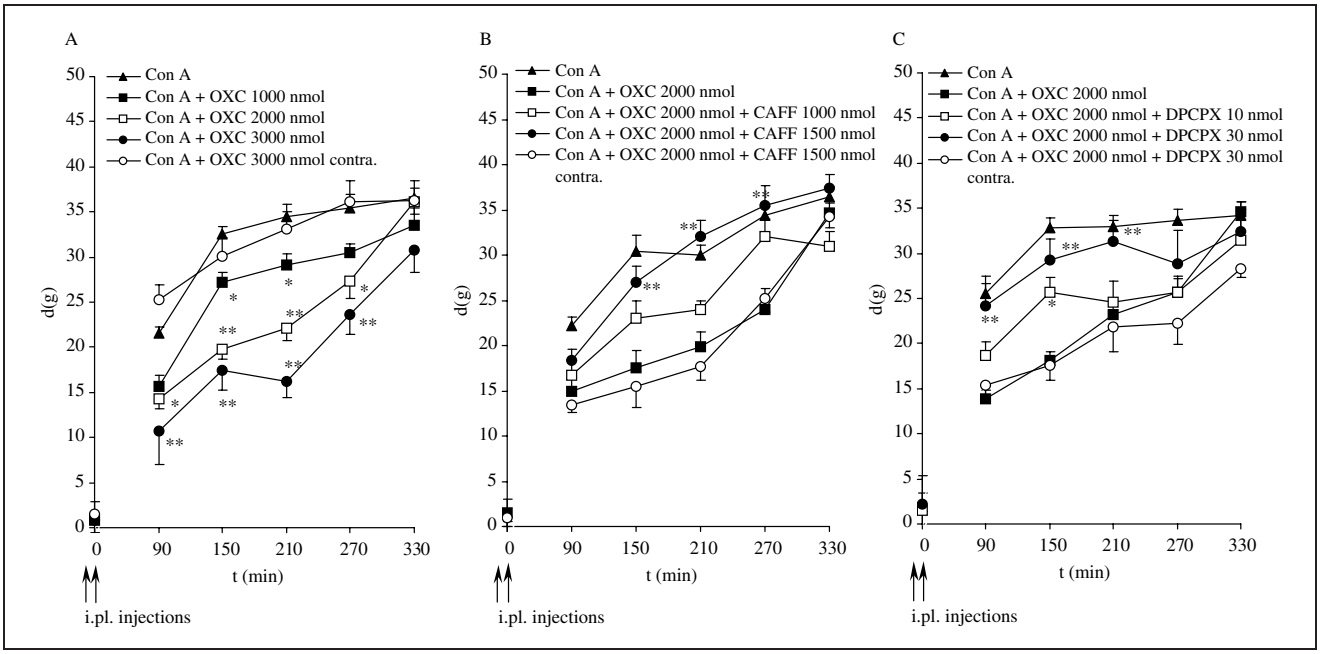

Fig.: Time course of the: local peripheral anti-hyperalgesic effects of oxcarbazepine (OXC) expressed as paw pressure differences in g (d) between non-inflamed and inflamed rat hind paw (**A**); inhibitory effect of local caffeine (CAFF) on local peripheral anti-hyperalgesic effects of OXC expressed as d (g) (**B**); inhibitory effect of local DPCPX on local peripheral anti-hyperalgesic effects of OXC expressed as d (g) (**C**). Predrug d (plotted at vertical axis) was obtained before intraplantar (i.pl.) injections (denoted by arrows). Each point represents the mean ± S.E.M. of d (g) obtained in 6–8 animals. Statistical significance (* $P < 0.05$, ** $P < 0.01$; One-Way ANOVA followed by Tukey's HSD test) was determined by comparison with the curve for Con A alone (**A**) or Con A + OXC 2000 nmol (**B, C**). Contra. = contralaterally.

detail (Tomić et al. 2004). Briefly, the rat was placed with its hind paws on two transducer platforms of the apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany) and pushed slowly and smoothly downwards, until one of the paws exceeds the trigger level set at 100 g. The difference (d) in pressures applied to non-inflamed (vehicle-injected) and inflamed (Con A-injected) rat hind paw is determined after each measurement.

2. Experimental protocol

In order to examine the peripheral effects of OXC, the drug and the Con A were coadministered intraplantarly (i.pl.), into the right hind paw. Control animals received the same volume of Con A. To exclude the possible systemic effect of the i.pl. injected drug, the highest dose of OXC used was given contralaterally. The influences of caffeine and DPCPX on the peripheral anti-hyperalgesic actions of OXC were examined after i.pl. coadministration of each antagonist with OXC and Con A. The comparative group of animals received Con A with OXC. To exclude the possible systemic effect of intraplantarly injected antagonists, the highest doses tested were given contralaterally. Finally, the effects of the highest doses of caffeine and DPCPX coadministered with Con A have been evaluated and compared with the effect of Con A alone.

3. Chemicals

OXC (Novartis Pharma AD), caffeine (Galenika) and DPCPX (Sigma) were dissolved or suspended in a vehicle containing 50% polyethylene glycol 400 and 50% saline, and sonicated for 15 min. Con A was used in a fixed dose of 0.8 mg/paw. All substances were injected i.pl. in a final volume of 0.1 ml/paw.

4. Statistics

Results are expressed as means ± S.E.M. Calculations for percent anti-hyperalgesic activity (%AA), percent inhibition (%I) of that activity, and ED_{50} values were done according to Tallarida and Murray (1986) and Tomić et al. (2004). Statistical difference was determined by Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey's HSD test. A value of $P < 0.05$ was considered significant.

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NO-Synthase inhibitors provide influence on protective effect of modified endotoxine diphosphoryl lipid A in a rat heart model of ischemic-reperfusion injury

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The present study was designed to assess whether a protective effect of the modified diphosphoryl lipid A (modLA) against myocardial ischemia-reperfusion injury (IRI) in rats can be related to the mechanism involving inducible nitric oxide synthase (iNOS). Pre-treatment with modLA significantly reduced the duration of both ventricular tachycardia ($p < 0.01$) and ventricular fibrillation ($p < 0.001$) compared to controls. Under these conditions the incidence of animal death was reduced ($p < 0.05$). The beneficial effect of modLA was markedly attenuated by the prior administration of selective iNOS inhibitor S-methylisothiourea (SMT). In this animal group, mortality was significantly increased ($p < 0.01$) partially in consequence of sustained ventricular arrhythmias. These results indicate that induction of iNOS can be responsible for cardioprotection of modLA.

Reperfusion of previously obstructed coronary arteries is known to produce paradoxically both morphological and functional damage of the myocardial tissue to a considerable greater extent than ischemia alone (Bolli 1990). Although numerous drugs have been shown to exert a protective effect against ischemic-reperfusion injury (IRI), new pharmacological strategies are being developed (Maxwell and Lip 1997).

Recently the modified lipids A obtained from bacterial lipopolysaccharides have been demonstrated to be a suitable means to decrease deleterious effects of IRI. Modified lipids A possess the immunomodulatory activity of parent lipids A (Salkowski 1997), but enjoy reduced toxicity. Monophosphoryl lipid A (MLA) has been the most widely studied substance, which represents a novel agent capable of enhancing myocardial tolerance to IRI, when pre-treated 24 h prior to ischemia. As with delayed ischemic preconditioning this cardioprotective activity of MLA manifests itself as a reduction in infarct size, polymorphonuclear leucocyte infiltration into the ischemic myocardium, myocardial stunning and arrhythmias in multiple animal species and various animal models of IRI, as