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Potential role of peripheral benzodiazepine receptors in inflammatory responses

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Abstract

This study investigates the anti-inflammatory effects of 1-(2-chlorophenyl)-*N*-methyl-*N*(1-methylpropyl)-3-isoquinoline carboxamide and 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2-*H*-1,4-benzodiazepin-2-one in paw oedema induced by carrageenan in mice. Pretreatment (24 h) with both ligands inhibited oedema formation in at different doses (0.00001–10 mg/kg, i.p.) with range of inhibition of 25% to 70%, in animals with or without adrenal glands. These results demonstrate, for the first time, an in vivo anti-inflammatory property of peripheral benzodiazepine receptor ligands.

Keywords: Benzodiazepine receptor, Peripheral; Inflammation

In addition to binding of $GABA_A$ receptors in the central nervous system, benzodiazepines bind to other sites in peripheral tissues. This second type of recognition sites is termed peripheral type benzodiazepine receptor (Braestrup and Squires, 1977).

Although several studies have shown that peripheral benzodiazepine receptor ligands, 1-(2-chlorophenyl)-*N*-methyl-*N*(1-methylpropyl)-3-isoquinoline carboxamide (PK11195) and 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2-*H*-1,4-benzodiazepin-2-one (Ro5-4864), affect various cellular functions, of which the best characterized is steroid biosynthesis, a line of evidence suggests that peripheral benzodiazepine receptor plays a major role in the regulation of immune functions. In fact, when peripheral benzodiazepine receptor was identified in various types of human blood cells, the rank order of cell expression was: monocytes═neutrophils B, natural killer cells, limphocyte CD4 and CD8 cells ≫ platelets > erythrocytes (Canat et al., 1993). In this context peripheral benzodiazepine receptor ligands have been found to modulate monocyte functions such as chemotaxis and lymphoid cell proliferation (Ruff et al., 1985; Laird et al., 1989). PK11195 and Ro5-4864 inhibit interleukin-3-like activity secretion in human peripheral blood mononuclear cells, while interleukin-2 production is inhibited by Ro5-4864 (Bessler et al., 1992). Treatment of mice with by Ro5-4864 markedly reduces the capacity of macrophages to produce key mediators of inflammation such as reactive oxygen intermediates, interleukin-1, tumor necrosis factor and interleukin-6 (Zavala et al., 1990).

The purpose of the present study is to investigate the role of peripheral benzodiazepine receptor in inflammatory processes in the experimental model of carrageenan-induced paw oedema in mice by using PK11195 and Ro5-4864.

Male Swiss mice (20–25 g; $n = 5–11$) received an intraperitonial (i.p.) injection of 0.1 mg/kg of either PK11195 or Ro5-4864 at different times (0.5–48 h). In other experiments different doses, (0.00001–10 mg/kg) of ligands were administered 24 h before oedema induction. Control animals received a similar volume (0.1 ml, i.p.) of phosphate buffered saline (PBS). Following treatments with either saline or peripheral benzodiazepine receptor ligands, animals were anaesthetized with ether and received a subcutaneous injection of carrageenan (300 μg/paw) in the right hindpaw. The contralateral hindpaw received the same volume of PBS immediately after the

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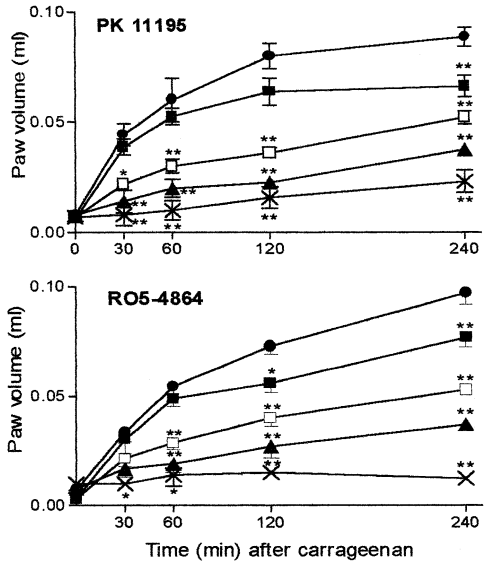

Fig. 1. Inhibition of carrageenan-induced paw oedema (●), by PK 11195 and Ro5-4864 at 0.00001 (■), 0.001 (□), 0.1 (▲), 10 (X) mg/kg when given 24 h before injection phlogistic agent. Results are expressed as the increase in paw volume (ml). Each point is the mean ± S.E.M. for $n = 6$ for 11 animals ($^{*}P < 0.05$; $^{**}P < 0.01$).

injection of carrageenan and served as control. The paw volume was measured with a plethysmometer. Subsequent readings of the same paw were carried out at several time-points (10, 20, 30, 60 and 120 min) after carrageenan injection or PBS. Oedema was expressed in ml as the difference between the test and the control paws.

In order to investigate the possible participation of adrenal glucocorticoids in the oedema inhibition caused by peripheral benzodiazepine receptor ligands, both adrenal glands were removed as described by Flower et al. (1986). After 7 days of surgery, animals received either PK11195 or Ro5-4864 (0.1 mg/kg, i.p.) or PBS (0.1 ml/100 g) 24 h before the oedema induction by carrageenan.

Data are reported as mean ± S.E.M. Differences between groups were determined by analysis of variance (ANOVA) followed by Dunnett's test or by Student's unpaired *t*-test when indicated. *P* values less than 0.05 were considered significant.

In relation to the effects induced by both peripheral benzodiazepine receptor ligands, one strong inhibition (50%), was observed 24 h after at the dose of (0.1 mg/kg, i.p.) in animals with or without adrenal glands. At this time, all tested doses (0.00001–10 mg/kg, i.p.) were significantly effective in inhibiting the studied inflammatory process (range of inhibition = 25% to 70%) (Fig. 1). No significant effect in both ligants were observed when the animals were treated 48 h after. Besides this novel anti-inflammatory properties presented by peripheral benzodiazepine receptor ligands, this effect is also long-lasting since an inhibitory effect was detected up to 24 h of pre-treatment. No sedative effects were observed along the experimental period with peripheral benzodiazepine receptor ligands.

Our data show in vivo, treatment with very small doses of peripheral benzodiazepine receptor ligands exerts a significant inhibitory effect upon carrageenan-induced paw oedema formation in the mouse paw injected with carrageenan in both groups.

These results suggest that peripheral benzodiazepine receptor ligands might be of pharmacological interest as potential anti-inflammatory agents. Further studies are being carried out to better understand the mechanism of the anti-inflammatory action of peripheral benzodiazepine receptor ligands.

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