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Short communication

Effect of a 5-lipoxygenase inhibitor on nerve growth factor-induced thermal hyperalgesia in the rat

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Abstract

Intraplantar injection of mouse β (2.5S) nerve growth factor (NGF) caused thermal hyperalgesia and stimulated release of immunoreactive leukotriene B_4 from the rat paw skin. Both effects of NGF were prevented by the 5-lipoxygenase inhibitor, (*R*)-2-[4-quinolin-2-yl-methoxy)phenyl]-2-cyclopentyl acetic acid (BAY X1005). BAY X1005 did not affect bradykinin-induced thermal hyperalgesia. These results suggest the participation of 5-lipoxygenase products of arachidonate in NGF-induced local thermal hyperalgesia.

Keywords: Leukotriene; NGF (nerve growth factor); Thermal hyperalgesia

1. Introduction

Nerve growth factor (NGF) administration produces hyperalgesia in laboratory animals (Lewin et al., 1994) as well as in man (Petty et al., 1994). Studies of NGF effects in rats have suggested that systemic treatment with high doses of NGF (1 mg/kg i.p.) induces initial generalized thermal hyperalgesia via serotonin release from mast cells followed by thermal and mechanical hyperalgesia probably maintained by central mechanisms (Lewin et al., 1994). Injection of lower doses of NGF in rats does not produce detectable general hyperalgesia, but lowers the thermal nociceptive threshold at the injected site (Woolf et al., 1994). We have previously observed (Amann et al., 1995) that intraplantar injection of NGF in rats caused indomethacin-resistant thermal hyperalgesia of the injected paw. Pretreatment of rats with compound 48/80 in order to degranulate mast cells was only partially effective to prevent hyperalgesia, suggesting that degranulation of mast cells is less important in this model than after i.p. administration of NGF.

Metabolites of the 5-lipoxygenase pathway of arachidonate metabolism could be mediators of NGF-induced acute thermal hyperalgesia. Thus, it is known that NGF can influence the synthesis of leukotrienes (Bischoff and Dahinden, 1992), among which leukotriene B_4 has been shown to sensitize C-polymodal afferent neurons (Martin et al., 1987). In order to investigate the involvement of leukotrienes in NGF-induced thermal hyperalgesia, we determined if intraplantar NGF stimulates the production of immunoreactive leukotriene B_4 in the paw skin, and if BAY X1005, a 5-lipoxygenase inhibitor (Müller-Peddinghaus et al., 1993), inhibits the NGF-induced decrease in thermal nociceptive threshold.

2. Materials and methods

2.1. Determination of thermal nociceptive threshold

Male Sprague-Dawley rats (300–350 g) received unilateral intraplantar injections (50 μl) of mouse β (2.5S) NGF (4 μg; Chemicon, Temecula, USA) or bradykinin (0.5 μg, together with captopril, bestatin and thiorphan, 50 pmol each; all obtained from Sigma). The thermal nociceptive threshold was determined in the Plantar Test Apparatus (Ugo Basile) as described previously (Amann et al., 1995). BAY X1005 ((*R*)-2-[4-quinolin-2-yl-methoxy)phenyl]-2-cyclopentyl acetic acid; provided by Bayer) was administered s.c. 75 min before NGF. Control groups received the vehicle (dimethylsulfoxide, DMSO; 0.5 ml/kg s.c.) injections.

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2.2. Determination of immunoreactive leukotriene B_4

In a different set of experiments, the rats were killed by an overdose of sodium pentobarbital 20 min after intraplantar injection of NGF, bradykinin or the respective vehicle. The plantar skin of the paw was removed, washed in ice-cold physiological salt solution (for composition see below), and cut into pieces of about 3 mm^3. The tissue samples were washed again, and then transferred to tubes containing 1.5 ml oxygenated (95% O_2, 5% CO_2) physiological salt solution (NaCl 118, KCl 4.6, $MgSO_4$ 1.17, $CaCl_2$ 2.5, NaH_2PO_4 1.17, $NaHCO_3$ 25, glucose 10 mM) at 37°C. After 30 min, the paw tissue was removed for determination of dry weight (30–70 mg), and the incubation medium was assayed for leukotriene B_4 using a rabbit leukotriene B_4 antiserum (gift of Dr. A.W. Ford-Hutchinson, Merck-Frost, Pointe Claire, Canada) which shows little cross-reactivity with other eicosanoids (Ford-Hutchinson et al., 1984), and [^{3}H]leukotriene B_4 (Amersham) as tracer. The detection limit of the assay (< 10% inhibition of binding) was 11 pg corresponding to 1.18–2.75 ng/g tissue). The identity of immunoreactive leukotriene B_4 was verified by high-pressure liquid chromatography (HPLC) according to Dreyling et al. (1986). Pooled incubation media (5 ml) were acidified with 1 M HCl to pH 3.0 and purified using SepPak C_{18} cartridges (Waters). After sequential washing with 0.1 M sodium phosphate buffer (pH 7.4), water and hexane, leukotriene B_4 was eluted with methyl formate. The solvent was evaporated, the residues were taken up in 200 μl methanol/water (30:70) and analysed by HPLC using a Nucleosil C_{18} column (Waters) and methanol/water/acetic acid (68:32:0.01), pH 5.5 (adjusted with NaOH) as mobile phase (flow rate 1 ml/min). The eluates were collected in 1-min fractions, lyophilized and analysed by radioimmunoassay. Synthetic leukotriene B_4 (Cayman Chemical Company, Ann Arbor, MI, USA) was used as reference.

2.3. Data analysis and statistics

The data were calculated as means ± S.E.M. Determination of immunoreactive leukotriene B_4 in all groups with the exception of one (NGF injection in vehicle-treated rats) yielded values close to or below the detection limit of the assay. For statistical analysis purposes, values which were below the detection limit of the assay were replaced with the lowest detectable value (10% inhibition of binding). Statistical comparisons were performed using the Kruskal-Wallis one-way analysis of variance followed by all pairwise multiple comparison procedures using Sigma Stat statistical software (Jandel Scientific, Erkrath, Germany).

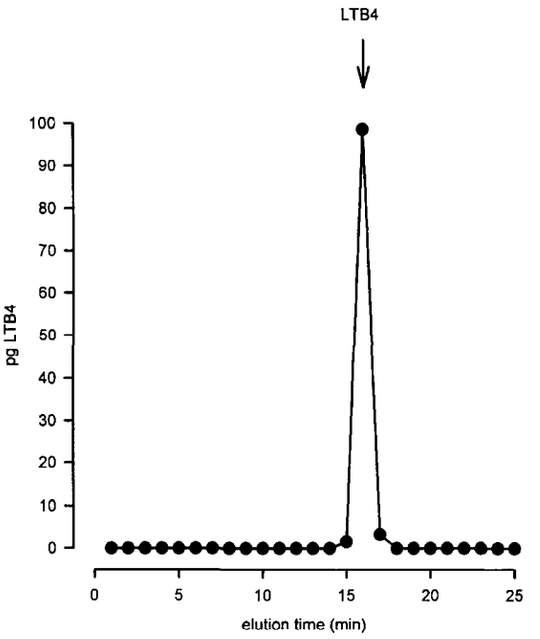

Fig. 1. HPLC elution profile of immunoreactive leukotriene B_4 (LTB_4) from incubation media of tissue samples taken from rats which had received intraplantar NGF. The arrow indicates elution position of synthetic leukotriene B_4.

3. Results

3.1. Effect of BAY X1005 on NGF-induced decrease of thermal nociceptive threshold

Intraplantar injection of NGF or bradykinin reduced the thermal nociceptive threshold of the injected paw. Treatment of rats with the 5-lipoxygenase inhibitor, BAY X1005 (5 mg/kg s.c.), prevented NGF-induced thermal hyperal-

Table 1
Effect of BAY X1005 (5 mg/kg s.c.) on thermal hyperalgesia produced by NGF (4 μg) or bradykinin (BK, 0.5 μg)

Systemic treatment	Intraplantar injection	Paw withdrawal latency (s), time after intraplantar injection			
		Before	10 min	20 min	30 min
Vehicle	NGF ($n = 18$)	7.94 ± 0.49	5.66 ± 0.56 [a]	4.57 ± 0.57 [a]	5.37 ± 0.76 [a]
BAY X1005	NGF ($n = 9$)	8.32 ± 0.81	8.38 ± 0.84	7.22 ± 0.69	7.30 ± 0.58
Vehicle	BK ($n = 8$)	9.19 ± 0.86	6.00 ± 0.91 [a]	9.29 ± 1.19	
BAY X1005	BK ($n = 12$)	9.98 ± 0.83	6.38 ± 0.71 [a]	7.88 ± 0.79 [a]	

Values are means ± S.E.M. [a] $P < 0.05$ as compared to corresponding pre-injection value.

gesia while not significantly reducing the effect of intraplantar bradykinin (Table 1).

3.2. Effect of BAY X1005 on NGF-induced increase of immunoreactive leukotriene B_4

In incubation media of tissue samples taken from solvent-treated rats which had received intraplantar NGF (4 μg, 20 min before, $n = 15$), the leukotriene B_4-like immunoreactivity was 13.2 ± 2.97 ng/g tissue. HPLC analysis showed co-elution of immunoreactive leukotriene B_4 and synthetic leukotriene B_4 (Fig. 1). In BAY X1005 (5 mg/kg s.c.)-treated rats ($n = 9$) which received intraplantar NGF, the value was 2.04 ± 0.2 ng leukotriene B_4-like immunoreactivity/g tissue, and not significantly different from that in rats which had received an intraplantar vehicle injection (3.61 ± 0.39 ng leukotriene B_4-like immunoreactivity/g tissue, $n = 15$). In contrast to NGF, intraplantar bradykinin (0.5 μg, $n = 7$) did not cause a detectable increase of immunoreactive leukotriene B_4 (data not shown).

4. Discussion

It is known that low concentrations (10–100 ng/ml) of mouse NGF are sufficient to augment the leukotriene release induced by other stimulants from human basophils in vitro, while NGF (up to 1 μg/l) alone has no appreciable effect (Bischoff and Dahinden, 1992). In the present experiments, NGF (4 μg) was injected in vivo, and resulted in increased synthesis of immunoreactive leukotriene B_4. It remains an open question, whether under the present in vivo conditions, the high local concentration of NGF was sufficient to trigger leukotriene synthesis on its own.

The NGF-induced increase of immunoreactive leukotriene B_4 seemed to be causally related to the development of thermal hyperalgesia since the 5-lipoxygenase inhibitor, BAY X1005, prevented the increase of immunoreactive leukotriene B_4 as well as thermal hyperalgesia. In contrast to NGF, bradykinin has been shown to produce indomethacin-sensitive thermal hyperalgesia (Schuligoi et al., 1994; Amann et al., 1995). In the present experiments, we observed no effect of bradykinin on immunoreactive leukotriene B_4, nor did we find inhibition of bradykinin-induced hyperalgesia by BAY X1005. This suggests that BAY X1005 had no general analgesic action, but selectively prevented hyperalgesia resulting from increased leukotriene synthesis.

In conclusion, the present results suggests that, in rats, inhibitors of the 5-lipoxygenase pathway of arachidonate metabolism can attenuate the local thermal hyperalgesia produced by intraplantar injection of mouse NGF. It remains to be investigated if inhibitors of 5-lipoxygenase are also effective to reduce NGF-induced hyperalgesia in humans.

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