on short circuit current was totally blocked. Consequently the present results suggest that an amiloride-sensitive sodium pathway is involved in the stimulation of electrolyte transport by nisoldipine.

In summary, nisoldipine, a new calcium antagonistic compound, stimulated short circuit current and transepithelial potential difference in the isolated frog skin. These results are compatible with the hypothesis [2, 3] that sodium entry across the apical cell membrane is modulated by intracellular calcium and that intracellular calcium is decreased by nisoldipine. The effect of nisoldipine could be blocked by amiloride, demonstrating that the effect of nisoldipine on short circuit current is indeed mediated by a transport of sodium. However, the effect of nisoldipine was enhanced by removing extracellular calcium. Thus, inhibition of calcium influx by nisoldipine can be excluded as the sole mechanism of this compound's effect on sodium transport. Nevertheless, a role of intracellular calcium cannot be ruled out even in calcium-free Ringer. It is tempting to speculate that intracellular calcium might be further decreased by nisoldipine via an inhibition of calcium release from intracellular stores. Interestingly, a marked increase in sensitivity of sodium transport to antidiuretic hormone (ADH) has been described in calcium-free Ringer [8]. Concerning the action of ADH on epithelial transport, intracellular calcium has been viewed either as a second messenger or as a coupling factor in relation to cyclic nucleotides. In the toad urinary bladder calcium antagonistic compounds have been shown to interfere with cAMP metabolism [9–11]. In our experiments the effect of supramaximal doses of ADH (1 I.U./ml) on short circuit current was tested. In preliminary experiments the stimulatory effect of ADH on short circuit current was not modified by nisoldipine in doses of 10^{-6}–10^{-4} M. However, from the data a possible action of nisoldipine on cAMP metabolism cannot be excluded. In addition to the discussed mechanism of action of nisoldipine on sodium transport other mechanisms of its action such as direct stimulatory effect on sodium entry or stimulation of Na/K-ATPase are also conceivable.

Calcium antagonists have been widely used in clinical medicine. Recently, it has been observed that verapamil [12] and nitrendipine [13] in addition to their peripheral vasodilating effect directly improve renal function and produce natriuresis. Our data obtained in a tissue with sodium transport properties similar to the ones observed in distal renal tubules are not compatible with the concept that natriuresis induced by calcium antagonistic compounds [12, 13] is directly caused by a change in sodium transport.

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Benoxaprofen does not inhibit formation of leukotriene B_4 in a model of acute inflammation

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Arachidonic acid is converted via the cyclo-oxygenase pathway to prostaglandins, prostacyclin and thromboxanes and via the 5′-lipoxygenase pathway to leukotrienes, hydroperoxy- and hydroxy-acids [1]. Non-steroidal anti-inflammatory drugs (NSAID, e.g. aspirin, indomethacin and flurbiprofen) provide symptomatic relief in inflammatory diseases by selectively inhibiting the cyclo-oxygenase [2]. These drugs, however, are not very active in reducing leukocyte-infiltration into inflamed tissues. The accumulation of leukocytes in chronic inflammatory reactions is associated with the pathogenesis of these diseases. Leukotriene B_4 (LTB_4), which is a product of arachidonate metabolism via the lipoxygenase pathway, is a potent leukotactic agent both *in vitro* and *in vivo* [3–6]. It has been hypothesised that LTB_4 may mediate cell infiltration and consequently inhibition of 5′-lipoxygenase could be the basis of improved anti-inflammatory therapy [7].

Benoxaprofen (2-[4-chlorophenyl]-α-methyl-5-benzoxazone acetic acid) is an effective anti-inflammatory agent in man; it reduces inflammatory symptoms in chronic joint inflammation [8] and psoriasis [9, 10]. Since the drug was reported to be a selective inhibitor of 5′-lipoxygenase when tested *in vitro* [11, 12], the observed clinical improvement could be due to reduced synthesis of LTB_4. Indeed, benoxaprofen has been used as a tool to establish whether the 5′-lipoxygenase pathway is involved in the pathophysiology of inflammation and hypersensitivity reactions [9, 10, 13, 14]. The effect of benoxaprofen on arachidonate metabolism has only been studied *in vitro*; these studies indicate that it inhibits 5′-lipoxygenase in polymorphonuclear leukocytes (PMN) stimulated with the calcium ionophore, A23187 [11, 12, 15–17]. However, the drug does not inhibit conversion of [^{14}C]-arachidonic acid by 12′-lipoxygenase in a cell-free system [12] or in thrombin-treated platelets [15]. In another study [18] benoxaprofen did not inhibit the synthesis of LTC_4 (also formed via the 5′-lipoxygenase) by resident mouse peritoneal macrophages exposed to zymosan although it did reduce the formation

of cyclo-oxygenase products. Recently, it was reported that benoxaprofen added to a cell-free system failed to inhibit 5'-lipoxygenase derived from human leukocytes [19]. Clearly, it is important to establish whether benoxaprofen inhibits LTB_4-formation *in vivo* before the effects observed in clinical situations can be attributed to inhibition of 5'-lipoxygenase.

We have developed a specific radioimmunoassay (RIA) which enabled the measurement of LTB_4 in the exudate derived from an animal model of acute inflammation [20, 21]. We previously reported the effect of several anti-inflammatory compounds on the concentrations of LTB_4 and cyclo-oxygenase products (prostaglandin E_2 [PGE_2] and thromboxane B_2 [TXB_2]) in the inflammatory exudate [22]. We now report data from a similar study showing that benoxaprofen is an inhibitor of cyclo-oxygenase *in vivo* but not of 5'-lipoxygenase.

Materials and methods

The methods employed were similar to those reported previously [22] for determining the effects of BW755C and other anti-inflammatory drugs on the concentrations of eicosanoids and numbers of leukocytes in exudate derived from an animal model of acute inflammation. Briefly, polyester sponges impregnated with carrageenin were implanted subcutaneously in male Wistar rats. After 6 hr the sponges were removed and the exudates squeezed into polypropylene tubes. An aliquot (40 μl) of the exudate was removed immediately for determination of the leukocyte count using a Model ZBI Coulter counter. The remainder of the exudate was centrifuged at 12,000 *g* for 1 min in a microfuge to precipitate cells and debris. The concentrations of LTB_4, PGE_2 and TXB_2 in the supernatant were determined by RIA after suitable dilution with buffer (1:5–1:20). The RIAs for LTB_4 [20], TXB_2 and PGE_2 [23] are highly specific. We have demonstrated [21] that direct analysis of these eicosanoids in the exudate was valid and reliable so that extraction and purification of the compounds prior to RIA was unnecessary.

Benoxaprofen (a gift from Dr. W. Dawson, Lilly Research Centre Ltd., Windlesham, Surrey, U.K.) was administered p.o. as a single bolus (5–200 mg/kg) immediately after sponge implantation. Each dose of drug was assessed in groups of 5 animals on at least 2 occasions.

For comparison, BW755C hydrochloride salt (synthesized by Dr. F. C. Copp, CRL, Wellcome Research Laboratories, Beckenham, U.K.) was administered p.o. to groups of rats and its effects on the concentrations of eicosanoids and cells in the exudate were determined as described above.

Results were expressed as the percentage of the vehicle-only (5% Tween 80 in water) control and the mean and standard error of the mean were calculated. The significance of difference between data was assessed using Student's unpaired *t*-test.

Results

Inflammatory exudate obtained 6 hr after sponge implantation from control groups of rats contained $16.37 \pm 0.82 \times 10^6$ leukocytes per ml (N = 25). The concentrations of PGE_2, TXB_2 and LTB_4 in the exudate were 16.72 ± 0.99, 21.76 ± 1.6 and 4.71 ± 0.44 ng/ml (N = 25) respectively. These data are similar to those previously reported [22].

The concentrations of all three eicosanoids in the inflammatory exudate were decreased in a dose related manner by BW755C and the ED_{50} against each of the products was approximately 20 mg/kg. This effect is similar to the data obtained previously after intraperitoneal injection of BW755C thus showing an efficient oral absorption of the compound. BW755C also reduced the accumulation of cells but, as noted previously, it was less active in this respect (ED_{50} approximately 70 mg/kg) and the dose–response curve was not parallel to that for the inhibition of eicosanoids.

Benoxaprofen reduced, dose-dependently, the concentration of both cyclo-oxygenase products (PGE_2 and TXB_2) with an ED_{50} of 18–22 mg/kg (Fig. 1). However, LTB_4 was not inhibited by benoxaprofen at doses up to 200 mg/kg; in fact the concentration of LTB_4 in the exudate was significantly increased after high doses of drugs (50–200 mg/kg). Leukocyte accumulation in the exudate was reduced but at doses higher than required to inhibit the cyclo-oxygenase products (approximate ED_{50} 200 mg/kg; see Fig. 1).

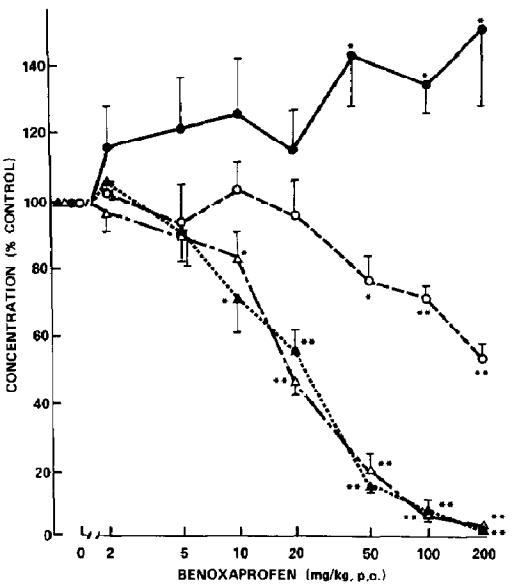

Fig. 1. Effect of benoxaprofen on leukocyte count and eicosanoid concentration in inflammatory exudate obtained from s.c. implantation in rats of sponges impregnated with carrageenan. – – ○ – –, Leukocyte number; —●—, LTB_4; — – △ — –, PGE_2; – – – ▲ – – –, TXB_2. Each point is the mean ± S.E. (N = 10–25). *P < 0.05; **P < 0.01.

Discussion

Benoxaprofen (600 mg daily) provides significant clinical improvement of inflammatory diseases [8–10]. The clinical benefit achieved with benoxaprofen was attributed to decreased cell influx and this, in turn, could be due to reduced formation of the leukotactic agent LTB_4. The data obtained in the present study demonstrate that benoxaprofen does not reduce the concentration of LTB_4 in exudate obtained in an animal model of acute inflammation although it does lower the concentration of both PGE_2 and TXB_2. Therefore, benoxaprofen is not a selective inhibitor of 5'-lipoxygenase in this *in vivo* model; in fact, benoxaprofen exhibits a similar profile of activities to those reported for the specific cyclo-oxygenase inhibitors indomethacin and flurbiprofen [22]. *In vivo* inhibition of PGE_2 by benoxaprofen was reported previously by Ford-Hutchinson *et al.* [24]. The mechanism by which benoxaprofen decreases cell-influx [24, 25, and confirmed in the present study] should now be reconsidered.

The explanation for the apparent difference between *in vitro* and *in vivo* data on the effect of benoxaprofen on 5'-lipoxygenase is unclear. Several investigators used A23187 to stimulate 5'-lipoxygenase in PMN and with this system we have confirmed that benoxaprofen inhibits the formation of LTB_4 (measured by RIA) although it was approximately 5 times less active than BW755C (data not shown). Benoxaprofen inhibited TXB_2-synthesis more effectively than LTB_4-formation in this *in vitro* system.

Humes *et al.* [18] reported that benoxaprofen did not reduce LTC_4 synthesis by zymosan-stimulated mouse macrophages. Since LTC_4 is also a product of 5′-lipoxygenase activity these data do not support the idea that benoxaprofen actually inhibits the enzyme. In preliminary experiments we have demonstrated that benoxaprofen is approximately 100 times less active than BW755C in inhibiting LTB_4-synthesis by human PMN stimulated with serum treated zymosan (unpublished data). The latter data is consistent with the failure of benoxaprofen to reduce LTB_4-synthesis *in vivo* observed in the present study.

In conclusion, benoxaprofen failed to reduce *in vivo* synthesis of LTB_4 in a model of acute inflammation. Therefore, this observation casts doubt on the hypothesis that the reported clinical benefit afforded by benoxaprofen is due to inhibition of 5′-lipoxygenase, although caution should be exercised in extrapolating from data derived in an animal model of acute inflammation to chronic clinical disease. Benoxaprofen inhibited the cyclo-oxygenase and inhibited cell accumulation by a mechanism which is unknown and these activities probably contribute to the drug's reported clinical success. The toxic effects of benoxaprofen, which necessitated its withdrawal from clinical use, probably cannot be attributed to inhibition of 5′-lipoxygenase and therefore inhibition of the latter enzyme remains an attractive approach to novel anti-inflammatory therapy.

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Effect of disulfiram on rat liver cholesterol 7α-hydroxylase

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The major pathway for cholesterol degradation is the biosynthesis of bile acids in the liver. The first and rate-limiting step is the 7α-hydroxylation of cholesterol [1]. This hydroxylation is catalyzed by a microsomal monooxygenase system involving a cytochrome P-450 and NADPH-cytochrome P-450 reductase [2]. It has been reported that treatment of alcoholics with disulfiram (Antabus^R) can result in increased serum cholesterol levels [3]. It should therefore be of interest to study possible correlations between the effect of disulfiram on serum cholesterol concentration and on the 7α-hydroxylation of cholesterol. The present communication reports such a study with a purified cholesterol 7α-hydroxylase from rat liver.

Materials and methods

[4-^{14}C]-Cholesterol (61 Ci/mol) was obtained from the Radiochemical Centre, Amersham, England. 5β[7β-^{3}H]-Cholestane-3α,7α-diol (500 Ci/mol) was prepared as described previously [4]. Disulfiram (Antabus^R) was obtained from Dumex Läkemedel AB, Helsingborg, Sweden and from Sigma. Nyco-test^R Kolesterol was obtained from Nyegaard & Co AB, Stockholm, Sweden.

Male rats of the Sprague–Dawley strain weighing about 250 g were used. Rats used for serum cholesterol determinations were given disulfiram orally, 40–400 mg/kg body weight daily, for 3 weeks. Rats used for cytochrome P-450 preparation were treated with cholestyramine 3% (w/w) in the diet for one week. In the preparation of enzyme fractions dithiothreitol was excluded from all buffers. Cytochrome P-450 was prepared from rat liver microsomes as described previously [4, 5] except that the microsomes were not subjected to the slow freezing and thawing procedure and that the CM-Sephadex chromatography was omitted. The final cytochrome P-450 fraction was treated on a