The possibility was explored that subsequent to the administration of myoglobin in circulation the hemoprotein undergoes biological transformations to render its heme moiety degradable by heme oxygenase. One mode of such a transformation could entail the introduction of a structural modification in the hemoprotein by serum factors, such as proteolytic enzymes, and the second could involve the transfer of the heme prosthetic moiety of myoglobin to serum albumin or other heme-binding proteins which would be degraded by tissue heme oxygenase subsequent to its cellular uptake. The heme–albumin complex is known to be an excellent substrate for heme oxygenase [1]. [^{14}C-heme]Myoglobin solutions (1 mM in physiological saline, pH 7.4) were preincubated with equal volumes of rat serum for various time intervals (0–2 hr) at 37°. Thereafter, the serum-treated myoglobin preparations were used as substrate for microsomal heme oxygenase employing the assay system and experimental procedures described in the legend for Table 2. No appreciable difference in the formation of [^{14}C]bilirubin was detected when the serum-treated and the untreated myoglobin preparations were used as substrates. Accordingly, it appears that serum factors do not convert myoglobin to a suitable substrate for heme oxygenase.

The findings reported in this communication suggest that, although myoglobin *in vivo* is a substrate for heme oxygenase, certain physicochemical changes *in vivo* in the structure of the hemoprotein are required prior to its conversion to a suitable substrate for the enzyme. Furthermore, these findings may be interpreted to suggest that such biotransformations of the hemoprotein take place at the cellular levels rather than at the plasma level. The latter suggestions are consistent with the suggested involvement of lysosomal enzymes in the initial process of the degradation of the globin moiety of hemoglobin [14]. Moreover, the present findings further suggest that the distinct possibility exists that the exposure of animals to metal ions not only promotes the induction of heme oxygenase activity but, in addition, stimulates the action of the biological processes which promote changes in the structural conformation of myoglobin to render the hemoprotein a suitable substrate for heme oxygenase.

On the basis of the present findings it would appear likely that, in the normal course of the degradation of musculature myoglobin, the hemoprotein is initially released into the circulation before it is transported to the ultimate cellular site(s) for degradation and bilirubin formation. This suggestion is consistent with the negligible levels of heme oxygenase activity detected in the musculature, and the appearance of myoglobin in the circulatory system and in the urine of patients suffering from certain skeletal and muscular disorders.

In summary the degradation of the heme moiety of myoglobin *in vivo* is a process which appears to involve the catalytic activity of heme oxygenase. This suggestion is based on the finding that [^{14}C]bilirubin was excreted in the bile of rats injected with [^{14}C-heme]myoglobin. Moreover, the treatment of rats with Sn^{2+} or Co^{2+}, potent inducers of heme oxygenase activity, enhanced the rate of the excretion of [^{14}C]bilirubin in the bile. Microsomal preparations obtained from the liver, kidney, spleen, heart and the muscles of control, Sn^{2+}- or Co^{2+}-treated rats failed to catalyze the formation of any detectable amount of [^{14}C] bilirubin from [^{14}C-heme]myoglobin. However, the formation of [^{14}C]bilirubin was detected when heme isolated from [^{14}C-heme]myoglobin or trypsin-, heat- or urea-treated [^{14}C-heme]myoglobin was used as substrate for the enzyme.

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The calcium antagonist nisoldipine stimulates the electrolyte transport of the isolated frog skin

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Calcium and sodium transport across epithelial cell membranes are thought to be linked by at least two mechanisms. First, a countertransport of sodium and calcium, energized by the sodium gradient, extrudes intracellular calcium. Second, sodium channels mediating passive influx of sodium are postulated to be modified by intracellular calcium [1]. The operation of such mechanisms, alongside Na/K-ATPase, should be able to regulate intracellular sodium

concentration and thus transepithelial sodium transport by a negative feedback mechanism [2, 3]. Considerable evidence has accumulated for the existence of a sodium–calcium countertransport in a variety of epithelial tissues [2, 3] including the frog skin [4], an epithelium that has been used extensively as a model for the study of pharmacological agents affecting sodium transport. Experimental procedures expected to increase intracellular calcium have been shown to decrease sodium transport, providing support for the negative feedback mechanism [2, 3]. However, the effect of decrease of intracellular calcium on sodium transport is less well documented and the application of the calcium channel blocker verapamil has led to conflicting results [3]. In this study, we have investigated the effect of a new calcium antagonistic compound, nisoldipine [5], on the short circuit current of the isolated frog skin. In this tissue, short circuit current is known to be predominantly due to sodium transport.

Materials and methods

The abdominal skin of *Rana esculenta* was mounted between lucite chambers [6] and bathed with Ringer solution (titrated to pH 7.6, bubbled with air) containing (in mM) 100 NaCl, 2.5 $KHCO_3$, 1 $CaCl_2$, 10 glucose and 15 HEPES. In calcium-free solution $CaCl_2$ was replaced by EGTA. After an equilibration period of 90 min control measurements (defined as 100%) were made. In a total of 60 experiments a mean (±S.E.M.) short circuit current of 28.9 ± 1.3 $\mu A/cm^2$ and a *trans*-epithelial potential difference of 45.8 ± 1.9 mV were recorded. Nisoldipine (Bay k 5552, Chemical name: 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid methyl 2-methylpropyl ester, generously provided by Dr. Kazda from Bayer AG, Wuppertal, F.R.G.) was dissolved in polyethylene glycol 400/ethanol (final concentration 1%) and diluted with Ringer solution. Because of the light sensitivity of the drug all experiments were performed in the dark.

Results and discussion

The results of 6 paired experiments are illustrated in Fig. 1. Under control conditions short circuit current slightly decreased 15 min after addition of the solvent and then remained constant. Addition of nisoldipine to both sides of the skin resulted in a marked increase in short circuit current with a maximum after 30 min. In most experiments changes of potential difference paralleled those of short circuit current and resistance was unchanged. When nisoldipine was added to either the apical or the basolateral bathing medium an identical effect of the drug on short circuit current was observed (N = 6).

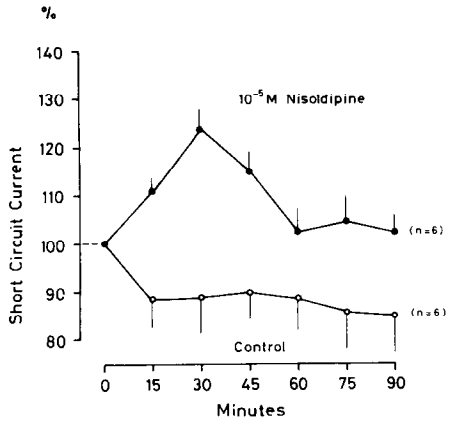

Fig. 1. Effect of control Ringer solution (1% polyethylene glycol/ethanol) and nisoldipine on short circuit current of the isolated frog skin.

The dose–response relationship between nisoldipine and short circuit current is depicted in Fig. 2. At the lowest concentration of nisoldipine tested (10^{-6} M) short circuit current of frog skins bathed in control Ringer (1 mM $CaCl_2$) increased to 113% ($P < 0.001$ as compared to 100%) 30 min after application of the drug. With higher concentrations of nisoldipine a greater stimulatory effect on short circuit current was observed, 129% at 10^{-5} M ($P < 0.001$) and 133% at 10^{-4} M ($P < 0.01$). In a second set of experiments frog skins were incubated in calcium-free Ringer solution for 90 min before nisoldipine was added. Incubation in calcium-free solution increased ($P < 0.001$ at 10^{-5} and 10^{-4} M) the sensitivity of short circuit current to nisoldipine.

The diuretic amiloride is a powerful inhibitor of sodium transport in frog skin and other epithelial membranes. Its mechanism of action can be ascribed to a blockade of sodium entry across the apical surface, thus depriving the transport mechanism of its supply [7]. Figure 3 shows the results of paired experiments from six different frogs. It can be seen (Fig. 3A) that amiloride almost completely inhibited short circuit current and thus sodium transport in tissues stimulated by nisoldipine. On the other hand, when amiloride was first applied (Fig. 3B) the effect of nisoldipine

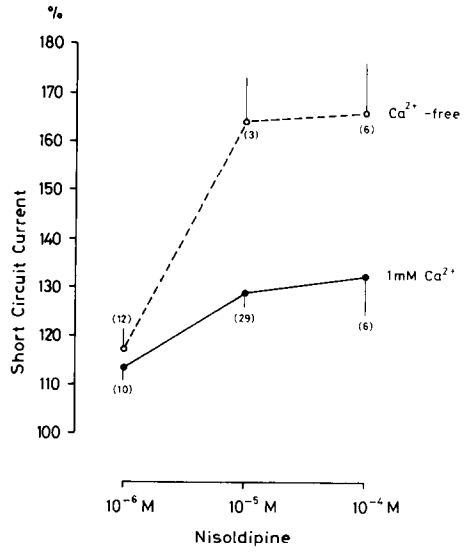

Fig. 2. Dose–response relationship between nisoldipine and short circuit current of the isolated frog skin incubated in either control Ringer solution (1 mM $CaCl_2$) or calcium-free solution (1 mM EGTA).

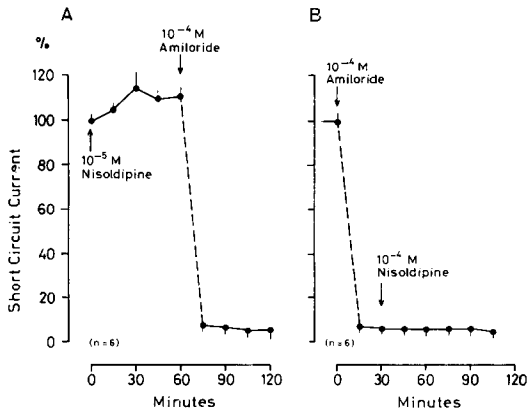

Fig. 3. Effect of amiloride and nisoldipine on short circuit current of the isolated frog skin.

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