Results and discussion. The comparative effectiveness of PC, SAT and EHDP and pyrophosphate to block heterogeneously induced crystal growth is shown in figure 1. Both PC and EHDP at 0.5 μM concentration were equally potent as inhibitors; SAT and pyrophosphate required much higher concentrations to exert similar effects. Despite the reduced response for SAT in this physicochemical system, the compound could still be a useful controller of events in the initiation of calcification.

In studies with calcium uptake by MV, purified fractions were used in preference to heterogeneous MV enriched microsomes because the characteristics of calcium uptake differ. It is known that microsomes accumulate calcium actively whereas MV are believed to use a non-energy dependent process[12,15]. The data (fig. 2) reveal that PC was the most effective of the inhibitors, reducing accumulation by 85%. This effect could not be attributed to possible cleavage products as in separate experiments, Pi and citrate at concentrations up to 10 μM had no effect. This is interesting, that despite a high alkaline phosphatase activity associated with the MV, PC was obviously still active. Inhibition (63%) was also seen with 1 μM EHDP but 100 μM SAT was required to inhibit to the same degree.

The effect of different concentrations of inhibitors on calcium accumulation by MV is shown in figure 3. PC exhibited a maximal response at 1 μM which remained unaffected with increased concentrations up to 100 μM. By contrast, EHDP which also exhibited maximal response at 1 μM showed declining effectiveness at concentrations in the range 10-100 μM. A different pattern was seen with SAT, where a linear increase in inhibition up to 100 μM was observed. Thereafter (not shown here) no change in effectiveness was apparent up to 200 μM.

The differences noted may be in response to their various mechanisms of action because many factors interplay to determine the inhibitor potency of a compound[16]. The fact that SAT is less effective than PC in both test systems is not surprising considering their structural differences. Another area yet to be investigated is the ability of PC und SAT to bind to target cell membranes and cross the plasma membrane of cells. The present studies in highlighting the potential of SAT as a calcification inhibitor do suggest that if the compound proves to be non-toxic during long term administration, it could be useful as a therapeutic agent in some disorders of calcium metabolism.

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Identification of calcium antagonist receptor binding sites using (^{3}H)nitrendipine in bovine tracheal smooth muscle membranes

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Summary. (^{3}H)Nitrendipine binding to the bovine tracheal muscle membrane at 25 °C was rapid, saturable (B_{max} = 14.8 ± 3.9 fmol/mg protein) and of high affinity (K_d = 0.15 ± 0.04 nM). The rank order of Ca^{2+} antagonists competing for airway (^{3}H)nitrendipine binding was nitrendipine ≒ nisoldipine ≒ nifedipine ≫ verapamil. Cromolyn, however, neither inhibited nor increased the binding.

Calcium antagonists such as nifedipine, verapamil and cromolyn have recently been shown to be effective in preventing exercise- or deep inspiration-induced bronchospasm in asthmatics[2-5]. In addition to their stabilizing effect on the mast cell membrane against degranulation of the cell[6-8], the antagonists, except for cromolyn, an antiasthmatic agent used prophylactically, have a direct influence on the tone of the airway muscle. Thus nifedipine produces a potent relaxation of isolated tracheal muscle either with intrinsic tone present[9,10] or precontracted by a bronchocon-

strictor[10]; and nifedipine[10] and verapamil[11] at a relatively high concentration inhibited contractions of the muscle strip due to chemical mediators. The potent in vitro effect of nifedipine on KCl- or $CaCl_2$-induced contractions[10] and on muscle relaxation implies a direct interaction at muscle membrane Ca^{2+}-channel receptor sites. Recently, several investigators[12-17] have used (^{3}H)nitrendipine (NTD), an analogue of nifedipine, to identify successfully Ca^{2+}-channel receptor binding sites in homogenates of many tissues including brain, heart and intestinal smooth muscle. To date, however, there is no report directly dealing with (^{3}H)NTD binding to an airway smooth muscle preparation. In view of the potent effect of nifedipine on the tone of tracheal muscle, we attempted to use this radioligand to identify and quantitate its binding to the crude membrane of bovine tracheal muscle and to assess interactions of Ca^{2+} antagonists with (^{3}H)NTD binding in this preparation.

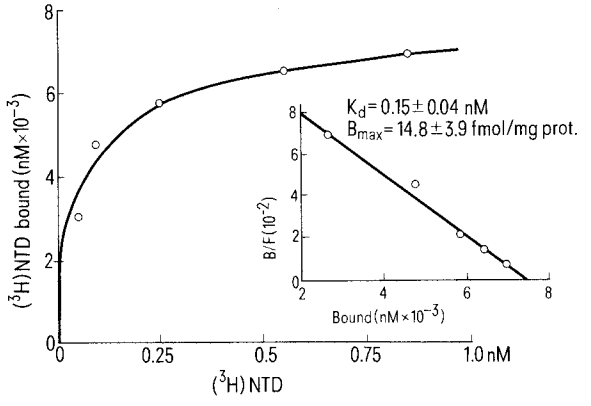

Figure 1. Specific (^{3}H)NTD binding as a function of (^{3}H)NTD. The tracheal muscle preparation (0.5 mg/ml) was incubated with increasing concentrations of (^{3}H)NTD (0.04-0.7 nM) in the absence and presence of 1 µM nitrendipine. Points are the means of triplicate determinations from a representative experiment. (^{3}H)NTD binding was linear with tissue concentrations from 0.25 to 1.25 mg/ml. Inset: Scatchard analysis of the binding curve ($r^2 = 0.976$, determined by linear regression). The dissociation constant (K_d) and the concentration (B_{max}) of (^{3}H)NTD binding sites are determined from 5 experiments (mean ± SE).

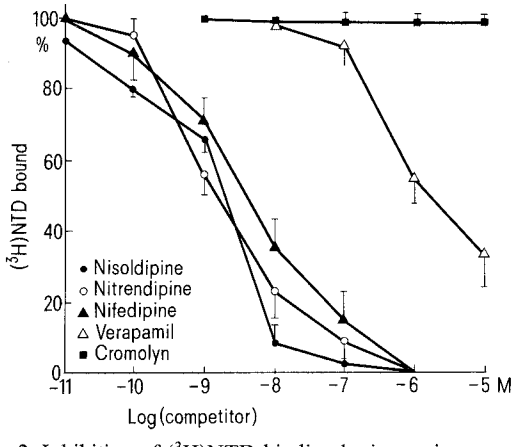

Figure 2. Inhibition of (^{3}H)NTD binding by increasing concentrations of competitors in the bovine tracheal muscle preparation. The muscle preparation (1.0 mg/ml) was incubated with 0.5-1.0 nM (^{3}H)NTD in the absence and presence of 1.0 µM nitrendipine and the listed competitor. Percent specific binding is binding in the absence of competitor divided by binding at the indicated concentration of the competitor. Each point represents the mean ± SE of 3-5 experiments, each in triplicate.

Methods. We prepared the crude membranes of bovine tracheal muscle, performed a (^{3}H)NTD binding assay and analyzed binding data according to the methods reported previously[18]. The routine assay included 200 µl of the membrane protein (0.5-1.0 mg/ml), 25 µl (^{3}H)NTD and 25 µl of 50 mM Tris · HCl buffer (pH 7.4) with or without a competitor. We used a Whatman GF/C glass fiber filter to separate free and bound (^{3}H)NTD, with 4×4 ml buffer washes. The assay was performed in triplicate for each experiment. Specific binding, defined was binding in the absence of nitrendipine minus binding in the presence of 1 µM nitrendipine, was 30-50% of total binding in the tracheal muscle preparation. Protein concentrations were determined by the method of Lowry with bovine serum albumin as standard. Nifedipine, nitrendipine and nisoldipine were gifts from Bayer AG, Wuppertal, FRG. Verapamil was obtained from Hoffmann-La Roche AG, Basel, Switzerland. Disodium cromolyn was from Fisons Corp., Mass., USA. (^{3}H)NTD (87.0 Ci/mmole) was purchased from New England Nuclear Corp., Boston, USA. In this report, we express values as the mean ± SE.

Results and discussion. (^{3}H)NTD bound rapidly to the bovine tracheal muscle preparation. Equilibrium was reached within 5 min at 25 °C. As shown in figure 1, (^{3}H)NTD binding to the muscle preparation was saturable and of high affinity when the radioligand concentration was increased from 0.04 to 0.7 nM. Scatchard analyses of the equilibrium experiments resulted in a straight line (Hill slope = 1.01), indicating the presence of a single population of (^{3}H)NTD binding sites within the concentration range. The dissociation constant (Kd) of the binding sites in the tracheal muscle preparation was about 0.15 nM, a value which is close to the Kd demonstrated in other tissues[12-17]. However, the concentration of (^{3})NTD binding sites in this preparation is apparently lower than the concentration found in other tissues such as the heart[12-17]. In a similar bovine tracheal muscle preparation, the number of muscarinic and beta-adrenergic receptor sites is about 190- and 13-fold, respectively[18], greater than that of (^{3}H)NTD binding sites when the numbers are expressed per mg protein.

Figure 2 shows that the ability of selected Ca^{2+} antagonists to compete with (^{3}H)NTD binding is of great diversity. Nitrendipine ($K_i = 0.28 \pm 0.05$ nM), nisoldipine (0.35 ± 0.04 nM) and nifedipine (0.70 ± 0.14 nM), all 1,4-dihydropyridine Ca^{2+} antagonists, had the highest affinity for the binding sites, whereas verapamil (0.29 ± 0.08 µM) was about 1000-fold less potent (n = 3-5 for each competitor). Cromolyn, a 2-carboxychromone derivative structurally different from nifedipine and verapamil, had no inhibition on airway (^{3}H)NTD binding (n = 5). No effect of cromolyn at the binding sites is compatible with its inability to produce a direct muscle effect[7,10].

Thus, we have quantitated (^{3}H)NTD binding sites and interactions of the Ca^{2+} antagonists with this binding in the tracheal muscle preparation. The high potency of the 1,4-dihydropyridine Ca^{2+} antagonists apparently reflects the specificity of the binding sites. The presence of identified airway (^{3}H)NTD binding sites permits us to investigate the correlation between the interaction of calcium channel blockers at the binding sites and their ability to relax airway muscle.

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Participation of microtubules and microfilaments in the transcellular biliary secretion of immunoglobulin A in primary cultures of rat hepatocytes[1]

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Summary. The biliary secretion of immunoglobulin A (IgA) in primary hepatocyte cultures was investigated by means of immunofluorescence. The characteristic accumulation of IgA in visible bile canaliculi was found to be strongly inhibited by vinblastine and colchicine or by cytochalasin B, but its surface binding was not.

Microtubules in conjunction with contractile microfilaments are known to play a role in phenomena of cellular and intracellular mobility, such as cellular organelle movements and endo- and exocytosis[2,3]. For instance, microtubular dysfunction in the liver caused by colchicine is associated with decreased secretion into the blood of albumin[4,5], fibrinogen[6], and and lipoproteins[7]. It is also established now that antimicrotubular and antimicrofilamentous drugs interfere with certain aspects of biliary secretion like transport of lipids[8], bile acids[9,10] or output of proteins into bile[11,12]. However, the mechanisms of this interference are only poorly understood and deserve further investigation in different experimental systems.

As pointed out in recent publications from this laboratory, primary monolayer cultures of adult rat hepatocytes form de novo a structural and functional biliary polarity[13,14] and provide a valuable model for examining the mechanisms of the transcellular and biliary transport of organic anions[15] and of immunoglobulin A[16] performed by the liver. The present report describes the influence of different drugs interacting with cytoskeletal elements on the biliary secretion of IgA in cultured hepatocytes.

Materials and methods. Male Sprague-Dawley rats (220–290 g) kept on a standardized diet of Alma[36] were used for isolating liver parenchymal cells as described[16]. Hepatocytes were maintained on collagen-coated cover slips in W/AB 77 medium[17] for up to 4 days. Details of cultivation are described[15,16]. Uptake and transcellular transport of IgA by the cultured cells was followed by direct immunofluorescence as described in a previous study[16]: on the 3rd day of cultivation cultures were exposed to human IgA (Biotest-Serum Institut, Frankfurt) at a concentration of 0.5 mg/ml for 2–5 h. Subsequently, cultures were fixed on ice with glutaraldehyde (2.5% for 3 min), methanol (5 min) and acetone (5 min) followed by a mixture of acetone and phosphate-buffered saline (PBS) pH 7.4 (1/1;v/v) (10 min) and were then rinsed extensively with PBS. The procedure for detection of IgA by direct immunofluorescence using fluoresceinated rabbit antihuman IgA (Behring Institute, Marburg) and the specificity of the immunologic reaction were described previously[16]. To define the influence of several drugs on uptake and secretion of IgA, hepatocyte cultures were incubated in the presence of these drugs either simultaneously with or prior to IgA as described in the legends to the figures. Individual experiments were repeated at least twice in order to evaluate the significance of the observations. Vinblastine sulfate and colchicine (Sigma, München) were used in a final concentration of 10 µg/ml and 50 µg/ml, respectively. Cytochalasin B (Sigma) dissolved in dimethylsulfoxide (10 mg/ml) was used in a final concentration of 100 µg/ml. Dimethylsulfoxide alone was without effect on the cells. Viability of the drug-exposed cells was routinely evaluated by several common viability tests[17] and on the basis of the ability of the cells to perform urea synthesis[18] and other metabolic functions[17].

Results and discussion. Cultured hepatocytes exposed to IgA have been shown to take up the immunoglobulin in a process mediated by its recepter, the so-called secretory component[16,19,20]. In a previous publication we were able to show that the internalized IgA is subject to a transcellular biliary secretion[16]. As illustrated in figure 1 (A and B) the final event of this transport visualized by immunofluorescence is the accumulation of the IgA within bile canaliculi[16] which are discernible by phase contrast microscopy as lucid enlargements of the intercellular space[14,15,18].

The accumulation of IgA could be considerably influenced by various agents known to interfere with cytoskeleton elements. The antimicrotubular drug vinblastine produced a complete inhibition of the transport (fig.3) including the intermediate 'vesicular' stage[16], whereas surface binding of