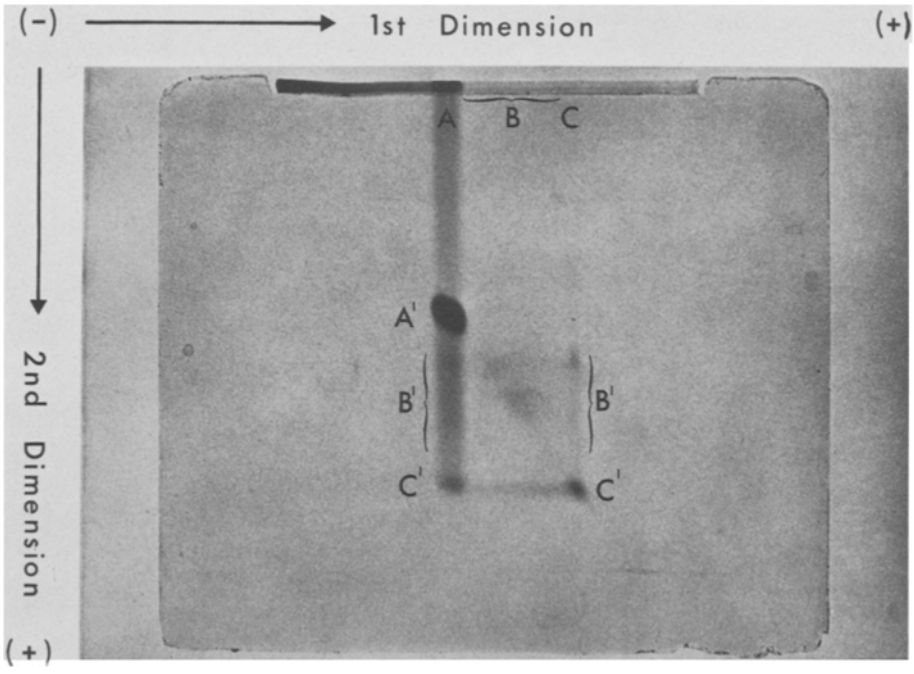

Fig. 2. 2 dimensional electrophoresis of purified *Triturus cristatus* toxin. Both dimensions: native toxin.

SDS in the 2nd dimension of polyacrylamide gel electrophoresis, the upper band A with the highest protein content splits up into the complete pattern of bands A′, B′, C′ obtained in the 1st dimension, band C forms B′ plus C′ and the smear B moves to 2 horizontal bands and a diagonal line, corresponding to the bands and the smear observed in the 1st dimension (figure 2). Apparently the components B and C are interconvertible and derive from the main component A, which contains the intact toxin. This is shown by incubation of the polyacrylamide slab on a blood agar slab. Hemolysis only occurs in an area corresponding to band A.

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V

Effect of theophylline on myocardial adenylate cyclase activity[1]

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Summary. Theophylline (0.01–10.0 mM) did not increase but rather decreased adenylate cyclase activity (AC) of guinea-pig auricles. Isoprenaline (1–100 μM) and sodium fluoride (0.3–10.0 mM) stimulated AC in a concentration-dependent manner.

We have recently shown[2] that the positive inotropic effect of theophylline in electrically driven left auricles from reserpine-pretreated guinea-pigs is consistently accompanied by an increase in cyclic AMP levels. The theophylline-induced increase in cyclic AMP, which was not impaired by propranolol, proceeded very rapidly: with 2 mM theophylline, cyclic AMP was elevated by about 25% within 5 sec. Under the same conditions, phosphodiesterase activity was inhibited by about 90%[3]. The conclusion from these data was that the theophylline-induced increase in cyclic AMP was due to the inhibition of the degradation of cyclic AMP and that the cyclic AMP turnover is a very fast process in the guinea-pig heart. However, the question arises whether the theophylline-induced increase in cyclic AMP proceeded so rapidly because theophylline also enhanced the formation of cyclic AMP via a stimulatory effect on myocardial adenylate cyclase. The present experiments were therefore designed to investigate whether theophylline increases adenylate cyclase activity of guinea-pig auricles. The effects of sodium fluoride and isoprenaline were studied for comparison.

Materials and methods. Guinea-pigs of either sex (220 to 300 g b.wt) were pretreated with reserpine (Serpasil® ampoules Ciba; 5 mg/kg i.p.; 18 h before sacrifice) to deplete cardiac catecholamine stores. Left auricles were isolated, suspended in aerated Tyrode solution (1.8 mM Ca^{++}; 5.4 mM K^{+}; 35 °C; pH 7.4) and stimulated electrically at 3 Hz as described previously[4]. After 30 min, the auricles were removed from the organ bath, blotted and weighed. Adenylate cyclase was prepared at 4 °C. 4 auricles were pooled, minced in a mortar for 2 min and suspended in 10 vol. (based on tissue weight) of a hypotonic medium consisting of 2.5 mM ATP, 2.5 mM $MgCl_2$, 1 mM $KHCO_3$, and 2 mM Tris-HCl at a final pH of 7.4. The resulting

preparation was homogenized in a cell homogenizer (Zell-Homogenisator, Colora-Messtechnik, Lorch; 10 min; 250 rpm) and was then centrifuged at 10,000 × g for 0.5 min. The precipitate was washed 4 times by resuspending in the hypotonic medium (original volume) and centrifuging as described above. Finally, the pellet was suspended in 1 mM $KHCO_3$ to give a protein concentration of about 2 mg per ml. The final cell suspension was used immediately for the adenylate cyclase assay. Adenylate cyclase activity was measured according to Schwabe et al.[5], using the protein-binding test of Gilman[6] to determine the cyclic AMP formed. Assay reaction constituents included 40 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.3 mM ATP, 0.01 mM EGTA, 7 µM 5'-guanylylimidodiphosphate, 0.3 mM papaverine-HCl, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase, 1 mg/ml bovine serum albumine, 200 µM $KHCO_3$ and 20 µl enzyme suspension (approximately 40 µg protein) in a final volume of 100 µl. The reaction mixture was preincubated for 5 min at 37 °C. The reaction was started with the enzyme preparation, carried out for 10 min at 37 °C and was terminated by the addition of 500 µl ice-cold sodium acetate buffer, 200 mM, pH 4.0. 50-µl samples were used for the cyclic AMP assay. Blanks (complete assay mixture inactivated at zero time) and recoveries (10 pmoles unlabelled cyclic AMP per 100 µl of assay mixture; average recovery 99.2±2.1%; N = 38), run with each experiment, were the same under all conditions. Adenylate cyclase activity was linear up to 30 min of incubation and up to 70 µg protein per 100 µl of assay mixture; activities are given as nmoles cAMP per mg protein per 10 min. Protein was determined by the method of Lowry et al.[7]. All assays were carried out in duplicate. The sources of the chemicals used were the same as described before[5]. Theophylline and (±)-isoprenaline-HCl were from Boehringer (Ingelheim); sodium fluoride was from Merck (Darmstadt). Statistical analysis was made with Student's t-test; $p < 0.05$ was considered significant.

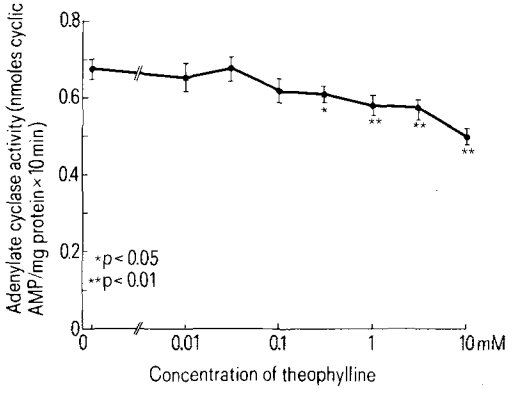

Fig. 1. Effect of theophylline (0.01–10.0 mM) on adenylate cyclase activity of guinea-pig left auricles. Adenylate cyclase activity is given as nmoles cyclic AMP/mg protein × 10 min. Means±SEM of 6 experiments. Significant differences from basal activity are marked with asterisks.

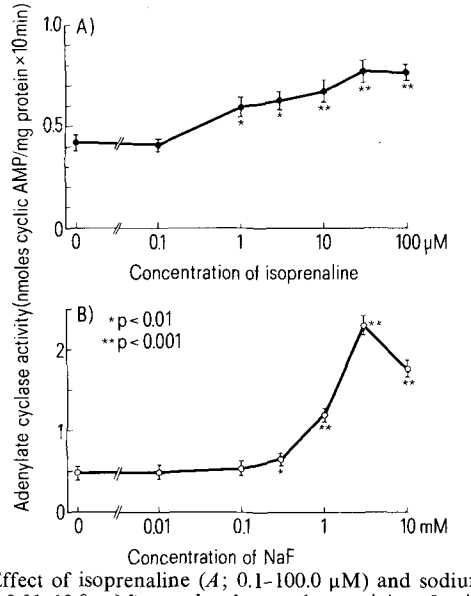

Fig. 2. Effect of isoprenaline (*A*; 0.1–100.0 µM) and sodium fluoride (*B*; 0.01–10.0 mM) on adenylate cyclase activity of guinea-pig left auricles. Adenylate cyclase activity is given as nmoles cyclic AMP/mg protein × 10 min. Means±SEM of 4 experiments each. Significant differences from basal activity are marked with asterisks.

Results and discussion. The basal adenylate cyclase activity of left auricles isolated from reserpine-pretreated guinea-pigs was 0.4–0.7 nmoles cyclic AMP per mg protein per 10 min. This value corresponds to those reported by others for guinea-pig ventricular tissue[8,9]. Figure 1 shows that adenylate cyclase activity was not increased by theophylline at all concentrations tested. Lower concentrations of the drug (0.01–0.1 mM) were ineffective, whereas higher concentrations (0.3–10.0 mM) significantly decreased adenylate cyclase activity by 9.7–26.3%. Similar results have been obtained in hepatic parenchymal cells[10], erythrocytes[11], kidneys[12], and fat cells[13,5] isolated from rats. Isoprenaline (figure 2, A) and sodium fluoride (figure 2, B) stimulated adenylate cyclase activity in a concentration-dependent manner. Maximal stimulation (1.9fold with isoprenaline and 4.8fold with NaF) occurred at 30 µM isoprenaline and 3 mM NaF, respectively.

The results obtained with isoprenaline and sodium fluoride demonstrate that the activity of the myocardial adenylate cyclase preparation used could be increased by stimulating agents, i.e. the preparation was actually responsive. The failure of theophylline to increase adenylate cyclase activity suggests that the formation of myocardial cyclic AMP is not enhanced by theophylline. This means that the theophylline-induced, very rapid increase in cyclic AMP levels in intact guinea-pig auricles[2] is mainly, if not entirely, due to an inhibition of phosphodiesterase activity with a subsequent inhibition of cyclic AMP breakdown. The results thus substantiate the view that the cyclic AMP turnover is a very fast process in the guinea-pig heart.

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