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Effects of Na_2 EDTA and Alpha-chymotrypsin on Aqueous Humor Outflow Conductance in Monkey Eyes

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ABSTRACT

In glaucoma there is partial clogging of the outflow routes for aqueous humor between the anterior chamber and the canal of Schlemm. In monkeys chelating agents perfused from the anterior chamber markedly reduce the outflow resistance, but the effect is short-lasting. In the present study an attempt was made to prolong the effect of Na_2EDTA with alpha-chymotrypsin and the effect of this agent alone was also tested. After 30 min of perfusion with 50 U/ml alpha-chymotrypsin there was a marked rise in outflow conductance, which was well maintained during subsequent perfusion without the enzyme. Two hrs after enzyme perfusion the rise in outflow conductance was 1.25 ± 0.20 $\mu l \cdot min^{-1} \cdot cm\ H_2O^{-1}$ from a starting level of 0.33 ± 0.08 $\mu l \cdot min^{-1} \cdot cm\ H_2O^{-1}$. In eyes perfused with alpha-chymotrypsin and 0.5 mmol/l Na_2EDTA in $^{++}$Na- and $^{++}$Mg-free perfusate for 30 min the rise in outflow conductance observed 2 hrs later was 1.72 ± 0.20 $\mu l \cdot min^{-1} \cdot cm\ H_2O^{-1}$. No adverse effects were observed in the eyes during the experiments and the next few weeks. The results indicate that perfusion with alpha-chymotrypsin produces relatively wide routes for aqueous drainage into the canal of Schlemm, which remain patent at least for several hours. In addition the enzyme seems to prolong the effect of Na_2EDTA

INTRODUCTION

In primates the outflow of aqueous humor takes place in the angle of the anterior chamber (2). Part of the flow goes via the trabecular and endothelial meshwork into the canal of Schlemm and from there via collector channels into intra- and episcleral veins. Another part passes via the ciliary muscle into the supraciliary and supra-choroidal spaces. After modification - both with respect to composition and volume - the fluid is then drained through the scleral substance and via perivascular spaces into the episcleral tissue. Infusion of fluid into the anterior chamber increases the flow via Schlemm's canal but the flow via the ciliary muscle is very little affected.

The intraocular pressure, IOP, is dependent on the flow via the canal of Schlemm, F_s, the episcleral venous pressure, P_v, and the conductance in the outflow routes, C:

$$IOP = P_v + \frac{F_s}{C}$$

In open angle glaucoma the conductance in the outflow routes is usually lower than normal, which results in a high IOP. The precise mechanism underlying the rise in resistance is not known but the result seems to be clogging of the outflow routes with material of unknown origin. The traditional medical treatment of the disease includes attempts at reducing the rate of aqueous formation and increasing the outflow conductance. None of the conventional agents used is likely to affect the clogging of the outflow routes.

Recent studies have indicated that both cytochalsin B (6) and the chelating agents Na_2EDTA and EGTA (3) perfused through the anterior chamber angle cause marked increments in the outflow conductance. Both cytochalasin B and Na_2EDTA produced marked effects in the outflow routes: rounding of endothelial cells in the trabecular and endothelial meshwork, detachment of cells and wash-out of ground substance. There were also ruptures in the inner wall of Schlemm's canal (8, 3).

One could speculate then that these agents might be of interest in the treatment of glaucoma by permitting wash-out of the material clogging the meshwork (3). But with all three agents there was rapid restitution of the outflow resistance with falling concentrations.

Attempts at increasing the outflow conductance with enzymes in enucleated human eyes, perfused at room temperature have not given promising results (4). One of the agents tested was alpha-chymotrypsin. In vitro experiments have shown, however, that effects of trypsin may be masked at low temperatures (7). And it has been observed also that a combination of EDTA and trypsin may have a marked effect in dissociating cultured cells not easily dissociated by either agent alone (9). The purpose of the experiments reported here was to try and prolong the effect of Na_2EDTA with alpha-chymotrypsin and also to investigate the effect of this agent alone in the normal monkey eye.

METHODS

Cynomolgus monkeys of both sexes and weighing 2-3 kg were used.

The monkeys were anesthetized with methohexital (Brietal, Lilly) and anesthesia was maintained by injection of pentobarbital every 20 min. Each anterior chamber was cannulated with 3 needles, one connecting it to a pressure transducer, one to an infusion apparatus and the third connecting it to a reservoir, the weight of which was measured continuously.

The conductance in the outflow routes was determined by measureing the inflow from the reservoir at two different heights, conductance being defined as the change in inflow from the reservoir divided by the change in anterior chamber pressure. The infusion was then started at a rate of 20 µl/min. The reservoir was adjusted to such a level that most of the infused fluid passed through the anterior chamber into the reservoir. The height was increased after about 15 min to cause a pressure rise in the eye of about 7 cm H_2O. After another 10-15 min the reservoir was lowered, and such changes were repeated for the rest of the exeriments. The conductance was calculated by dividing the change in net inflow from the external circuit by the change in IOP. The composition of the perfusate was varied as shown under results.

The fluid used to perfuse the anterior chamber was the mock aqueous humor described by Bárány (1) or a modification of this fluid. Alpha-chymotrypsin (Zolyse, Alcon, Fort Worth, Texas, USA) was added to normal mock aqueous humor to give a concentration of 50 U/ml , or to ^{++}Ca- and ^{++}Mg-free mock aqueous humor containing 0.5 mmol/l Na_2EDTA. Preliminary experiments showed marked increments in conductance at this concentration. At 0.1 mmol/l the effects were variable.

RESULTS

Perfusion of the anterior chamber with alpha-chymotrypsin in mock aqueous humor for 30 min caused a marked increase in outflow conductance which lasted for the rest of the experiments, usually 2-3 hrs, Fig. 1. The conductance before the perfusion

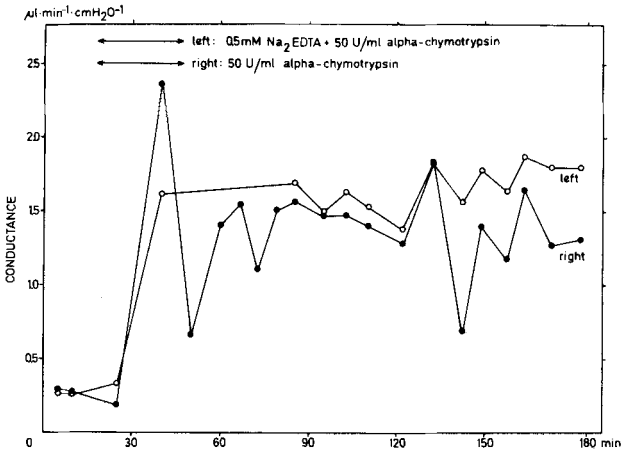

Fig. 1.
After determination of the normal outflow conductance the anterior chamber on one side was perfused for 30 min with mock aqueous humor containing 50 NF U/ml alpha-chymotrypsin, the other eye was perfused with $^{++}$Ca- and $^{++}$Mg-free aqueous humor containing 50 NF U/ml alpha-chymotrypsin and 0.5 ml/l Na_2EDTA. Both eyes were then perfused with mock aqueous humor containing $^{++}$Ca and $^{++}$Mg.

was started was 0.33 ± 0.08 µl·min^{-1}·cm H_2O^{-1} (n=6). 2 hrs after the perfusion with alpha-chymotrypsin the rise in conductance was 1.25 ± 0.20 µl·min^{-1}·cm H_2O^{-1}. In eyes perfused with 50 U/ml alpha-chymotrypsin and Na_2EDTA in $^{++}$Ca- and $^{++}$Mg-free mock aqueous humor for 30 min the rise in outflow conductance as observed 2 hrs later was 1.72 ± 0.20 µl·min^{-1} cm H_2O^{-1} (n=8). At the start of the experiments the conductance in this group was 0.34 ± 0.05 µl·min^{-1}·cm H_2O^{-1}. In 4 experiments with perfusion of Na_2EDTA plus alpha-chymotrypsin on one side and alpha-chymotrypsin only

on the other side the effect on the conductance was greater in the former eyes; Fig. 1 shows the result of one of these experiments. In control eyes perfused with mock aqueous humor the starting conductance was 0.40 ± 0.05 $\mu l \cdot min^{-1} \cdot cm\ H_2O^{-1}$ (n=6). There was little rise in conductance in these experiments. The mean increase at the time of marked effects in the treated eyes was 0.23 ± 0.08 $\mu l \cdot min^{-1} \cdot H_2O^{-1}$.

There was no visible change in corneal transparency during the experiments. Pupil size was about 2 mm and was not appreciably affected by the perfusion with Na_2EDTA and/or alpha-chymotrypsin. Withdrawal of the needles in eyes treated with alpha-chymotrypsin or alpha-chymotrypsin plus Na_2EDTA resulted in loss of anterior chamber fluid and a bleeding from the chamber angle that most probably represented reflux of blood from the canal of Schlemm. In control eyes there was not such reflux.

DISCUSSION

The results reported here demonstrate that alpha-chymotrypsin perfused through the anterior chamber angle caused a marked increase in conductance in the outflow routes and that the effect lasted for several hours. The effect caused by perfusion for 30 min with a combination of Na_2EDTA and alpha-chymotrypsin tended to be larger and also lasted for several hours. The results suggest that treatment with alpha-chymotrypsin or alpha-chymotrypsin combined with Na_2EDTA produced wide routes for aqueous humor drainage into the canal of Schlemm. The bleeding into the anterior chamber from the reversal of the normal pressure gradient indicates that the routes created do not have the normal rectification property

of the normal outflow routes. Perfusion with mock aqueous humor only did not have these marked effects: in these eyes there was little change in conductance and no bleeding into the anterior chamber after withdrawal of the needles.

The perfusion with alpha-chymotrypsin or alpha-chymotrypsin plus Na_2EDTA had no obvious effect on the cornea or the iris. The chamber angle tissue thus seems to be more susceptible than the cornea and the structures of the iris.

In the experiments of Grant (4) alpha-chymotrypsin had no effect on the conductance of the outflow routes in enucleated eyes perfused at room temperature. The difference in result may be due to a species difference or the difference in temperature. That temperature may be an important factor is suggested by an observation of Rees, Lloyd and Thom (7). In their study trypsin was found to affect fibroblasts growing on glass even at low temperature, but the manifestation of the effect in terms of detachment required a normal fluidity of the membrane or normal metabolism.

In vitro experiments on different cells have suggested that cytochalsin B and chelating agents tend to disrupt the cytoskeleton of the cells. As a consequence of such disruption there is rounding of the cells and a tendency to detachment. With trypsin treatment there seems to be both such disruption and effects on some protein tending to maintain "sticky" surface proteins in appropriate configurations (7). Such additional effects of alpha-chymotrypsin may explain why the combination of this agent and Na_2EDTA has marked and long-lasting effects.

It is well known that when alpha-chymotrypsin is used in cataract surgery in order to produce lysis of the zonala fibres there is a tendency to increased intraocular pressure after the operation. Experiments in monkeys (3) have demonstrated that the pressure rise is most probably due to dissolution of the zonula fibres and subsequent blocking of the outflow routes with fragments rather than to direct effects in the anterior chamber. In other studies (5) when the anterior chamber in monkeys was perfused with alpha-chymotrypsin at high concentrations but in such a way that there was no access to the posterior chamber there was no pressure rise.

Much works remains to be done before anything can be concluded about the clinical usefulness of anterior chamber perfusion with the agents tested in this study. The experiments demonstrate, however, that the possibility exists that surgical treatment of some types of glaucoma may be replaced by the injection into the anterior chamber of agents cleaning the outflow routes.

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