The Mechanism of Cyclic AMP Stimulation of Secretion in the Dogfish Rectal Gland

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Summary. 1. Cyclic AMP induces pronounced increases in ouabain binding and ouabain-sensitive oxygen consumption in rectal gland slices but does so in the absence of any change in the Na-K-ATPase activity of tissue homogenates. It is suggested that this apparent paradox results from the presence of inactive or concealed ‘latent sites’ in the membrane of the unstimulated gland which become exposed or activated in the presence of cAMP.

2. Evidence suggests that, in the absence of cAMP, the exposure and activation of these latent sites is largely determined by the rate of sodium entry into the cells. It appears that sodium can enter the cells either by a simple passive diffusion down the electrochemical gradient or via a furosemide-sensitive, chloride-coupled pathway.

3. Experiments have shown that the cAMP effect on ouabain binding and oxygen consumption is absent in conditions designed to eliminate the gradient for sodium entry into the cells. Similarly, no cAMP effect on these parameters is observed in the absence of external chloride or in the presence of furosemide. Furthermore, cAMP has been shown to produce a marked increase in sodium entry into the rectal gland cell and this enhanced entry is entirely furosemide-sensitive.

4. It appears therefore that cAMP has no direct effect on the recruitment of latent sites per se but instead has a specific, stimulatory action on the furosemide-sensitive entry of sodium which, in turn, leads to an increase in functional sodium pump sites and an enhanced sodium efflux. Chloride efflux appears to be increased in a passive manner as a result of the increased gradient on this ion subsequent to an enhanced entry via the cAMP-stimulated, furosemide-sensitive pathway.

Introduction

The elasmobranch rectal gland is metabolically a highly active structure producing a secretion rich in sodium and chloride ions, and as such is intimately involved in the maintenance of ionic balance in the animal (Burger 1962, 1965). The studies of Stoff et al. (1977) and Silva et al. (1977) have demonstrated the potent stimulatory effect of the cyclic nucleotide dibutyryl cyclic AMP (cAMP) on secretory activity in the gland. cAMP has also been shown to produce marked increases in ouabain binding (Shuttleworth and Thompson 1978, 1979b) and ouabain-sensitive oxygen consumption (Shuttleworth and Thompson 1980). As such, cAMP and the cyclic nucleotide phosphodiesterase antagonist theophylline have been routinely used in most studies investigating the process of secretion by the gland and have provided evidence for the development of a model for ion transport in this tissue (Silva et al. 1977). However, apart from some preliminary suggestions involving modifications of chloride uptake and/or efflux by Stoff et al. (1979), no analysis of the actual mechanism of stimulation has been described. Clearly such studies are of some importance, as elucidation of the mechanism of cAMP-mediated stimulation of secretion may well provide further information on the processes of ion transport in the gland, as well as describing possible control mechanisms.

Materials and Methods

Fish

Dogfish (Scyliorhinus canicula), weighing between 700 and 1,000 g, were obtained from the Plymouth Laboratory of the Marine Biological Association of the United Kingdom. They were kept in
large fibre-glass tanks supplied with re-circulating sea water at a temperature of 11 °C.

Slice Preparation and Oxygen Consumption

The technique for the preparation of thin (200–300 μm) slices of rectal gland tissue and the use of these slices to determine oxygen consumption was identical to that described previously (Shuttleworth and Thompson 1980).

Ouabain Binding

The binding of ouabain to slices of rectal gland was determined using a similar method to that previously described (Shuttleworth and Thompson 1978), involving the incubation of tissue slices in saline, using a similar method to that previously described (Shuttleworth and Thompson 1980). The binding of ouabain to slices of rectal gland was determined otherwise stated, 2.2 x 10^(-6) M unlabelled ouabain. Incubation was carried out for two hours in a shaking water bath at 11 °C. After incubation, the tissue was rinsed with three five-minute washes of ice-cold ouabain-free saline, blotted dry and placed on pre-weighed fragments of coverslip. Following overnight drying at 105 °C and weighing to determine dry weight, the tissue was transferred to scintillation vials, moistened with 50 μl of distilled water and treated with 0.2 ml of tissue solubilizer (Soluene, Packard).

5 ml of scintillation fluid (Instagel, Packard) was then mixed with 2.45 ml of incubation medium containing 1 mmol I−1 magnesium chloride, 5 mmol L−1 potassium chloride, 48 mmol L−1 sodium chloride, 30 mmol L−1 bistidine (pH 7.6). The homogenates were then frozen by plunging into liquid nitrogen and stored at −20 °C until assayed. The tissue to be assayed was weighed and then homogenised in 1.8 M H2SO4 (Atkinson et al. 1973) and the colour allowed to develop at room temperature for 10 min. After centrifugation, the inorganic phosphate (Pi) in the supernatant was determined using the technique of Lowry et al. (1951). Results are expressed as μmol of ouabain bound per mg of dry tissue.

ATPase Activity

The tissue to be assayed was weighed and then homogenised in ice-cold medium consisting of 0.1% (w/v) sodium deoxycholate, 5 mmol L−1 disodium EDTA, 0.25 mmol L−1 sucrose and 30 mmol L−1 histidine (pH 7.6). The homogenates were then frozen by plunging into liquid nitrogen and stored at −20 °C until assayed. The assay was carried out at 37 °C in a shaking water bath. 2.45 ml of incubation medium containing 1 mmol L−1 magnesium chloride, 5 mmol L−1 potassium chloride, 48 mmol L−1 sodium chloride, 92 mmol L−1 Tris HCl buffer (pH 7.6) and 2 μmol L−1 ATP (disodium salt, Sigma) was thermostoequilibrated at 37 °C and the reaction started by the addition of 50 μl of the homogenate. After exactly 10 min the incubation was stopped by the addition of 4 ml of a mixture of 1% Lubrol (L.C.I.) and 1% ammonium molybdate in 1.8 M H2SO4 (Atkinson et al. 1973) and the colour allowed to develop at room temperature for 10 min. After centrifugation, the inorganic phosphate (Pi) in the supernatant was determined by reading at 390 nm on an SP80-100 spectrophotometer and comparing with standard curves. Assays were corrected for tissue phosphate and non-enzymatic hydrolysis of ATP using control flasks in which the homogenate was added after the incubation period, immediately prior to the addition of the Lubrol/ammonium molybdate mixture. Sodium-potassium-ATPase activity was assessed as the difference in inorganic phosphate liberated from ATP in the presence and in the absence of 10−4 M ouabain. Protein concentrations in the homogenates were measured using the technique of Lowry et al. (1951). Results are expressed as μmol P i liberated per mg protein per hour.

Intracellular Ions

Changes occurring in intracellular sodium and chloride in tissue slices were determined after incubation at 11 °C under various conditions (see text). The tissue was removed from the incubation flasks, blotted dry and weighed on pre-weighed fragments of coverslip. Tissue dry weight was determined by re-weighing after over-night drying at 105 °C and tissue water content calculated. The dry tissue was then digested in an appropriate volume of 0.1 M HNO3 for 48 h and the digest analysed for chloride (Corning-Eel chloride meter) and sodium (Eel flame photometer). Intracellular volume in rectal gland slices was measured under identical conditions in a separate group of fish. To determine this, slices were incubated under the appropriate conditions in saline containing 0.2 μCi ml−1 14C-polyethylene glycol (Radiochemical Centre, Amersham). Following determination of total tissue water content, as described above, the tissue was digested and radioactivity counted as described in the section on ouabain binding. Samples of the incubation media were taken and treated in an identical manner and hence extracellular space was determined. Preliminary experiments showed that equilibration of the polyethylene glycol in the incubation medium with the extracellular space of the tissue was complete within twenty minutes. Subtraction of the extracellular space from the total tissue water enabled intracellular volume to be determined and a mean value of this was calculated for each of the different treatments. Intracellular concentrations of sodium and chloride were calculated using the individual values of total water and total tissue ions and the mean value of the intracellular volume appropriate for the particular incubation treatment.

The composition of the normal dogfish saline used is given in Table 1. To obtain a ‘low-sodium saline’ (2.5 mmol Na+ 1−1), the sodium chloride and sodium sulphate were replaced with equimolar quantities of washed choline chloride. ‘Chloride-free saline’ was obtained by replacing the sodium chloride with equimolar sodium methyl sulphate, and the potassium chloride and calcium chloride with equimolar potassium nitrate and calcium nitrate respectively.

Drugs used were dibutyryl cyclic AMP (Sigma), theophylline (BDH), ouabain (BDH) and furosemide (Lasix, generous gift from Hoechst Pharmaceuticals).

Table 1. Composition of the normal dogfish saline (mmol I−1).

<table>
<thead>
<tr>
<th>Sodium chloride</th>
<th>257</th>
</tr>
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<tbody>
<tr>
<td>Sodium sulphate</td>
<td>7</td>
</tr>
<tr>
<td>Sodium hydrogen carbonate</td>
<td>2.5</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>4</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>3</td>
</tr>
<tr>
<td>Urea</td>
<td>400</td>
</tr>
<tr>
<td>Dsodium hydrogen phosphate</td>
<td>0.27</td>
</tr>
<tr>
<td>Sodium dihydrogen phosphate</td>
<td>0.01</td>
</tr>
<tr>
<td>Trimethylamine oxide</td>
<td>70</td>
</tr>
</tbody>
</table>

Results

ATPase activity was determined in tissue taken directly from the fish and in slices after incubation for two hours in normal saline either with or without 0.05 mmol I−1 cAMP and 0.25 mmol I−1 theophylline. The results are presented in Table 2 and clearly show that incubation of tissue slices produced no