Ca\textsuperscript{2+}-DEPENDENT PHOSPHATASE AND Ca\textsuperscript{2+}-DEPENDENT ATPase ACTIVITIES IN PLASMA MEMBRANES OF EEL GILL EPITHELIUM—III. STIMULATION OF BRANCHIAL HIGH-AFFINITY Ca\textsuperscript{2+}-ATPase ACTIVITY DURING PROLACTIN-INDUCED HYPERCALCEMIA IN AMERICAN EELS

GERT FLIK and SJOERD E. WENDELAAR BONGA
Department of Zoology, University of Nijmegen, Toernooiveld 25, 6525 ED Nijmegen, The Netherlands
(Tel: (80)55-88-33)
and
JAMES C. FENWICK
Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5
(Received 5 March 1984)

Abstract—1. Infusions of ovine prolactin for 10 days induced hypercalcemia in unfed American eels, Anguilla rostrata LeSueur, that tentatively was related to stimulation of branchial Ca\textsuperscript{2+}-uptake mechanisms.
2. Analysis of ATPase activities in the plasma membranes of the branchial epithelium in prolactin treated eels showed a specific stimulation of high-affinity Ca\textsuperscript{2+}-ATPase.
3. The results of this study form further evidence that the high-affinity Ca\textsuperscript{2+}-ATPase activity represents the Ca\textsuperscript{2+}-pump of the branchial epithelium.

INTRODUCTION

Fish can regulate their serum calcium levels with great precision (Bailey and Fenwick, 1975; Copp and Ma, 1978) and this ability must be attributed, at least in part, to the capacity of their gills to absorb calcium directly from their aquatic environment (Berg, 1968; Simmons, 1971; Simkiss, 1974; So and Fenwick, 1977; Payan et al., 1981). Unfortunately, there is a paucity of information concerning the mechanisms of this branchial calcium uptake.

Recently we reported the simultaneous occurrence of non-specific phosphatase activity and high-affinity Ca\textsuperscript{2+}-ATPase activity in American eel gill plasma membranes (Flik et al., 1983, 1984). Further, we equated the heterogeneous non-specific phosphatase activity with the Ca\textsuperscript{2+}-activated ATPase activity which has been reported present in the gills of many teleostean species and which has been described as related to calcium transport (Ma et al., 1974; Fenwick, 1976; Moon, 1977; Fenwick, 1979; Ho and Chan, 1980; Doneen, 1981). However, the characteristics of this activity more closely resemble those of an alkaline phosphatase rather than a transport Ca\textsuperscript{2+}-ATPase (Ghysen et al., 1980). On the other hand, we identified the branchial high-affinity Ca\textsuperscript{2+}-ATPase as a calmodulin-sensitive ATPase that was stimulated by intracellular Ca\textsuperscript{2+}-concentrations in the presence of excess Mg\textsuperscript{2+} (Flik et al., 1984). These characteristics suggested that it was this latter activity which is associated with the branchial Ca\textsuperscript{2+}-pump in fish.

The present study was directed towards testing the effect of ovine prolactin on both the Ca\textsuperscript{2+}-dependent phosphatase and the high-affinity Ca\textsuperscript{2+}-ATPase in American eel gill plasma membranes to determine which, if either, of the activities respond to prolactin treatment.

Prolactin is known to induce hypercalcemia in several species of fish (Pang et al., 1978; Wendelaar Bonga and Flik, 1982), including American eel (Copp et al., 1982). It was further reported that ovine prolactin enhanced calcium influx in perfused American eel gills (Ma and Copp, 1981). The rationale behind our present study was that if prolactin stimulates gill calcium absorption it should stimulate enzymic activities associated with active calcium transport.

MATERIALS AND METHODS

Adult female yellow eels, Anguilla rostrata LeSueur, with an average body weight of 1.7 kg were obtained from a commercial dealer in Quebec City, Quebec, Canada. The eels were held in running dechlorinated Ottawa tapwater (0.45 mM Ca\textsuperscript{2+}, 12 °C) with 16 hr of light alternating with 8 hr of darkness. The animals were not fed.

Hormone treatment

Ovine prolactin was kindly supplied by the Hormone Distribution Agency of the National Institutes of Health, Bethesda, MD and was administered continuously for 10 days by means of Alzet osmotic minipumps implanted intraperitoneally. The dosage was 0.1 U/g fish/day, dissolved in 0.05 N HCl. Controls received equivalent amounts of solvent. Ovine prolactin, at the doses used in this study,
Table 1. Effects of ovine prolactin on blood plasma mineral composition. Plasma mineral content is expressed in mM. Mean values ± SD are given, with the number of animals in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Controls (5)</th>
<th>Experiments (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>141.5 ± 14.1</td>
<td>153.8 ± 6.9</td>
</tr>
<tr>
<td>K</td>
<td>1.89 ± 0.06</td>
<td>1.84 ± 0.19</td>
</tr>
<tr>
<td>Mg</td>
<td>0.91 ± 0.05</td>
<td>0.50 ± 0.12*</td>
</tr>
<tr>
<td>Ca</td>
<td>2.84 ± 0.17</td>
<td>3.40 ± 0.20*</td>
</tr>
<tr>
<td>P</td>
<td>1.35 ± 0.13</td>
<td>1.12 ± 0.20</td>
</tr>
</tbody>
</table>

*P < 0.01.

---

DISCUSSION

Prolactin and blood plasma mineral composition

Ovine prolactin induced hypercalcemia in fresh water yellow eels and this result agrees with earlier findings from killifish (Pang et al., 1978), sticklebacks (Wendelaar Bonga and Greven, 1978), the tilapia Sarotherodon mossambicus (Wendelaar Bonga and Flik, 1982; Wendelaar Bonga et al., 1983) and American eels (Ma and Copp, 1981). In addition, prolactin induced hypomagnesemia, a phenomenon also reported earlier for tilapia (Wendelaar Bonga et al., 1983). In mammals such a hypomagnesemia is considered to be a direct effect of the concomitant hypercalcemia (Ebel and Guenther, 1980). Prolactin did not affect plasma Na, K or P levels, which indicates the specific nature of the hypercalcemic action of prolactin under these conditions. Ma and Copp (1981) have shown for American eels, the species used in this study, that this effect can be ascribed to stimulation by prolactin of Ca2+-uptake in the gills.

Prolactin and branchial ATPase activities

Prolactin enhanced high-affinity Ca2+-ATPase activities in the plasma membranes of eel branchial epithelium, which suggests that prolactin may influence Ca-metabolism by activating a Ca2+-pump in the gills. Prolactin did not stimulate the non-specific Ca ~ ATP-phosphatase activity. We take these observations as evidence that it is the high-affinity Ca2+-ATPase and not the non-specific phosphatase which functions as a Ca2+-transporting enzyme. Further, the recovery of branchial total Na+/K+-ATPase, total Ca ~ ATP-phosphatase and total protein were similar in prolactin treated and control eels. The absence of an effect on either the Ca ~ ATP-phosphatase or the Na+K+-ATPase activities indicates that the action of prolactin on the gills is not of a general trophic character. This is also supported by the fact that the ratios of total Na+/K+-ATPase to high-affinity Ca2+-ATPase activities differed between prolactin treated and control eels. The decrease of the ratio from 11.7 in the controls to 8.1 in prolactin treated eels suggested a specific induction of high-affinity Ca2+-ATPase in branchial plasma membranes.

Interestingly, our results show some close simi-
Prolactin stimulates high-affinity Ca\(^{2+}\)-ATPase

<table>
<thead>
<tr>
<th>Controls (4)</th>
<th>Experiments (4)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-affinity Ca(^{2+})-ATPase</td>
<td>2.56 ± 0.50</td>
<td>4.94 ± 0.58</td>
</tr>
<tr>
<td>Na(^{+})/K(^{-})-ATPase</td>
<td>75.7 ± 20.0</td>
<td>74.5 ± 15.1</td>
</tr>
<tr>
<td>Ca (~) ATP-phosphatase</td>
<td>81.2 ± 10.0</td>
<td>80.3 ± 7.4</td>
</tr>
</tbody>
</table>

Acknowledgements—The authors are greatly indebted to Mrs Lise Belanger and to Mr Frans Wilms for their excellent assistance during the experiments. Experiments were carried out in the department of Biology, University of Ottawa and were supported by a NSERC of Canada operating grant (No. A6246) to Dr J. C. Fenwick.

REFERENCES


So Y. P. and Fenwick J. C. (1977) Relationship between net 45Ca influx across a perfused isolated eel gill and the


