INHIBITION OF CHLORIDE SECRETION BY SOMATOSTATIN IN ISOLATED CHLORIDE-CELL RICH OPERCULAR EPITHELIA (FUNDULUS HETEROCLITUS)

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The adaptation to lower salinities in the sea water adapted fish consists of two responses at the level of the gill chloride cell: a rapid one with reduction in chloride secretion and increased membrane resistance and the second one, of longer duration with complete adaptation and absorption of salt. The second one is controlled mainly by prolactin while the mechanisms for the rapid response is not yet clear (see Fish Physiology, Vol. Xb, pp 129-176, Hoar and Randall, Ac.Press 1984 New-York).

Rapid hormonal effects, both inhibitory and stimulatory have been described on the chloride secretion of the opercular epithelium. The catecholamines, mainly adrenaline, produce a rapid and drastic reduction in the short circuit current and chloride secretion that spontaneously recovers to normal levels of current output. The inhibitory effect is mediated by the alpha receptors and has been shown to be blocked by phentolamine. A rapid stimulation is induced by beta agonists such as isoproterenol . In order to search further for rapidly acting, locally secreted hormones that could be involved in the rapid response to change in salinities the effects of the peptide somatostatin were tested on the isolated opercular membranes, and its interactions with the catecholamines examined.

Specimens of Fundulus heteroclitus were obtained from brackish waters in Mount Desert Island and kept in running sea water aquaria. The fish were fed commercial fish food once daily and used within 2-3 weeks of capture. Opercular epithelia were mounted in Ussing type chambers and electrical measurements performed as previously described (J. Physiol. 271, 155-191, 1977).

The addition of somatostatin (Sigma Chemical Co.) at concentrations higher that 10^{-9} M produces a rapid inhibitory effect on the chloride secretion. The effect lasts 15-20 minutes and the level of transport then returns to the original unstimulated level. The response is elicited only from the basolateral side of the preparation, shows typical dose response characteristics and can be elicited repeatedly in the same preparation.

In Table I the inhibitory effect of concentrations 10^{-8} M to 10^{-6} M are

TABLE 1 Per cent inhibition by Somatostatin (10^{-8} to 10^{-6}M) of the short circuit current of isolated operculi of Fundulus heteroclitus and the action of previous treatment with Forskolin (10^{-6}M).

	10^{-8}M	10^{-7}	10^{-6}
Somatostatin alone	17.0 ± 2.8 (6)*	65.0 ± 4.2 (11)	75.6 ± 4.3 (21)
Somatostatin after the effects of 10^{-6}M Forskolin	0 ± 0 (3)*	33.5 ± 6.4 (4)	45.0 ± 5.3 (11)

* Number of experiments.

Table values are mean percentages and standard errors.

presented. The percentage effect is close to maximal at 10^{-6} M with a range of inhibition of 42 to 100% of the initial chloride current.

The mechanism of the action of somatostatin on the adenylate cyclase and cyclic AMP cascade was tested, at least as far as possible interactions with forskolin, the activator of adenylate cyclase. There is a post-receptional interaction between somatostatin and the activation of adenylate cyclase. In Table 1 it is observed that the response to somatostatin is reduced in magnitude after stimulation with forskolin. The dose response curve is displaced, with a complete lack of inhibitory effect at 10^{-8}M and only partial inhibition at higher concentrations. These effects are in some way similar to the interaction between forskolin and somatostatin found in the chloride secretion of the isolated rectal gland (Amer.J. Physiol. 249, R329-R334, 1985).

Searching for a possible interaction with the activation of alpha receptors, the effect of somatostatin was tested after the preparation had been treated with the alpha receptor blocker phentolamine at a concentration of 10^{-5}M. The concentration used was 10^{-7} M of somatostatin and the full inhibitory effect of the peptide was found as indicated by the percentage inhibition of Table 2. Therefore it is not likely that the inhibitory effect of somatostatin occurs via alpha receptors. Interactions with the beta receptor stimulation with isoproterenol were also tested in a limited number of experiments, as seen in Table 2. After the full stimulation of isoproterenol, somatostatin had complete inhibitory effects. It reduced the current to levels compatible with the inhibitory effect found without the addition of isoproterenol.

TABLE 2 Interaction of Somatostatin with other agents on the short circuit current of isolated operculi of Fundulus heteroclitus.

	% Inhibition
Somatostatin (10^{-7}M)	58.0 ± 3.6 (8)
Somatostatin (10^{-7}M) after Phentolamine (10^{-5}M)	50.5 ± 4.53 (6)
Somatostatin (10^{-6}M) after Isoproterenol (10^{-7})	68.0 ± 5.3 (2)
Somatostatin (10^{-6}M) after Phorbol Ester 10^{-6}	82.8 ± 3.2 (6)

An interaction of somatostatin with the beta receptor itself is unlikely. However the stimulation by catecholamines through beta receptors and inhibition by somatostatin could play together a role in secretory control during the rapid adaptation to different salinities.

Also presented in Table 2 is the lack of interaction with phorbol ester, the tumor promoting agent. Phorbol ester had no effect of its own on this preparation, and there was no change in the action of somatostatin.

Other interactions tested indicated in a limited number of experiments that bombesin is not involved in the action of somatostatin in the opercular epithelium. A group of operculi and gill arches were analyzed for somatostatin by radioimmunoassay by Dr. Seymour Reichlin at the New England Medical Center, in Boston. The results indicate the presence of detectable amounts of somatostatin (between 0.04 and 0.62 pg/mg protein) in both opercular membranes and gill arches in Fundulus heteroclitus, indicating that this peptide could be a definite regulator of chloride secretion in the chloride cell epithelium.

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