EFFECT OF CADMIUM ON NEUROTRANSMITTER CONTROL OF SHARK RECTAL GLAND

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We have previously shown that cadmium chloride inhibits the secretion of chloride by the isolated perfused rectal gland of Squalus acanthias (Silva et al. CMTS 2nd annual report, 1987, pp 60-62). The inhibitory effect was found only at concentrations of 10^{-3}M. Recently, Grasso et al. (Bull MDIBL 29:57, 1990) made the observation that cadmium chloride, 2.5 x 10^{-4}M reverses the inhibitory effect of peptide YY (PYY) on forskolin stimulated chloride secretion. PYY is a peptide closely related to the neurotransmitter neuropeptide Y (NPY) that inhibits stimulated chloride secretion by the rectal gland (Silva et al Bull MDIBL 29:98-100, 1990). Because cadmium is known to inhibit neurotransmitter release (Cooper et al. Neurotoxicology 5:247-266, 1984) we explored its effect on neurotransmitters that inhibit or stimulate chloride secretion by the rectal gland either directly or through the release of other neurotransmitters.

Dogfish were pithed and the rectal glands removed via an abdominal incision. The rectal gland artery, vein and duct were catheterized with PE90. The glands were placed in an all glass perfusion chamber maintained at 15°C with running sea water. The glands were perfused by gravity at a pressure of 40 mm of Hg. The composition of the perfusate was (in mM) Na, 280; Cl, 280; K, 5; bicarbonate, 8; phosphate, 1; Ca, 2.5; Mg, 1; sulfate, 0.5; urea, 350; glucose, 5; pH 7.6 when gassed with 99% O_2/1% CO_2. Venous flow was collected over time to measure the rate of perfusate flow. Rectal gland secretion was collected in tared 1.5 ml centrifuge tubes over timed intervals, usually 10 minutes. The volume was measured by weight. When volume was 100 ul or less, collections were made directly into calibrated 100 ul glass pipettes. Chloride concentration in the rectal gland secretion was measured by amperometric titration using a Haake-Buchler chloridometer. The rate of chloride secretion was expressed as uEq/h/g.

In the initial series of experiments, we tested the effect of cadmium on the inhibitory effect of NPY in glands stimulated with vasoactive intestinal peptide (VIP). Figure 1 shows that cadmium, 2.5 x 10^{-4}M completely reverses the effect of NPY in glands stimulated with VIP.

We then examined the effect of cadmium on glands stimulated with forskolin, that activates adenylate cyclase at a site distal to that of VIP. The rectal glands were perfused for an initial control period of thirty minutes during which the rate of chloride secretion was allowed to stabilize at a basal rate. The perfusate was then changed to a solution containing forskolin 10^{-6}M, NPY 10^{-8}M and $CdCl_2$ at concentrations varying from 0 to 10^{-3}M. Figure 2 shows that cadmium reverses the inhibitory effect of NPY in a dose dependent fashion.

We then tested for the effect of cadmium on the inhibitory effect of somatostatin, a peptide that inhibits stimulated chloride secretion by the rectal gland. The chloride secretion was allowed to stabilize at a basal rate for a period of thirty minutes, after which the perfusate was changed to a solution containing forskolin 10^{-6}M, somatostatin 10^{-7}M and in half of the

Fig 1. Cadmium chloride reverses the effect of NPY in glands stimulated with VIP. Representative, 3 out of 28, experiments showing that NPY (closed circles) inhibits chloride secretion when compared with a perfusion with VIP alone (open circles). Cadmium chloride (open triangles) completely reverses the effect of NPY. Values are expressed as the percentage of the initial rate of chloride secretion for each experiment.

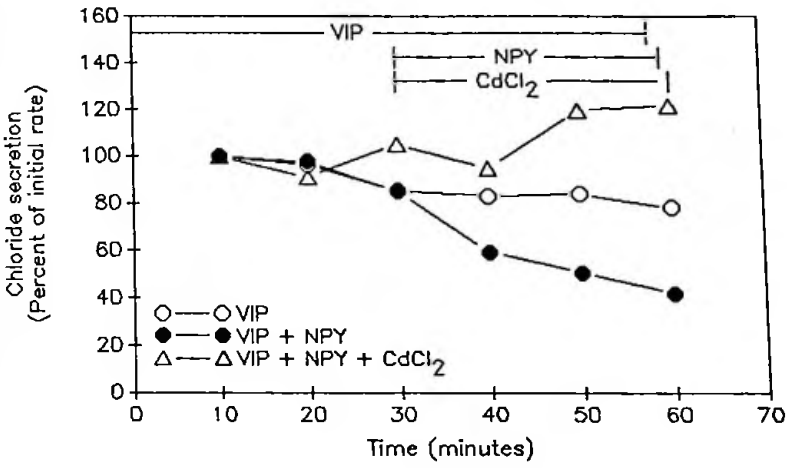


Fig 2. Cadmium chloride reverses the inhibitory effect of NPY in a dose dependent fashion. This graph shows a representative dose response curve, out of 21 experiments of the reduction in inhibitory effect of NPY as a function of the cadmium concentration in the perfusate expressed as a percentage of the inhibition induced by NPY in the absence of cadmium.

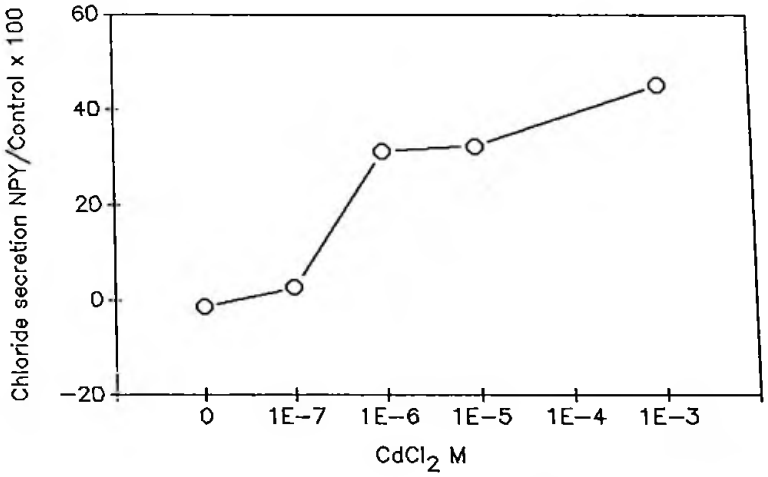
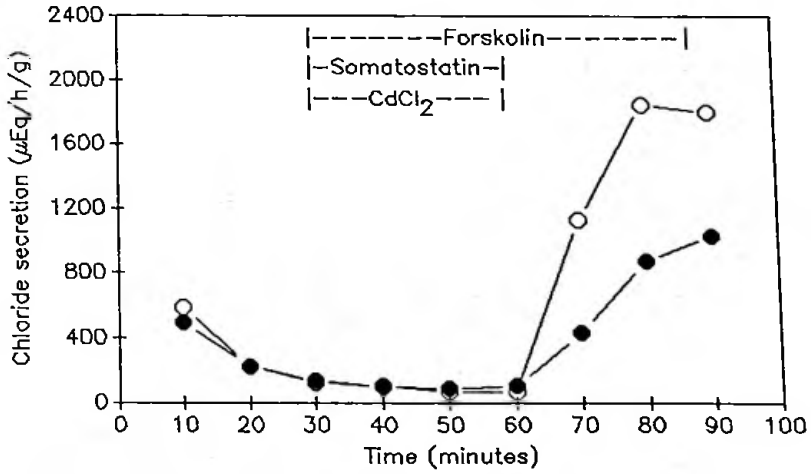


Fig. 3. Cadmium chloride does not prevent the inhibitory effect of somatostatin. Two out of eight experiments showing that somatostatin prevents the stimulatory effect of forskolin both in the presence and absence of cadmium chloride.



experiments cadmium chloride 2.5 x 10^{-4}M. Figure 3 shows that cadmium chloride does not alter the effect of somatostatin.

Another agent that inhibits chloride secretion by the rectal gland is adenosine. We examined the effect of cadmium chloride on the inhibitory effect of 2-chloro adenosine. In these experiments glands were perfused with forskolin throughout the experiment. After thirty minutes of control perfusion the perfusate was changed to one containing, in addition to forskolin, 2-chloroadenosine 10^{-6}M and cadmium chloride 10^{-4}M. Thirty minutes later the perfusate was changed to a solution without cadmium chloride. Figure 4 shows that cadmium chloride did not prevent the inhibitory effect of 2-chloro adenosine.

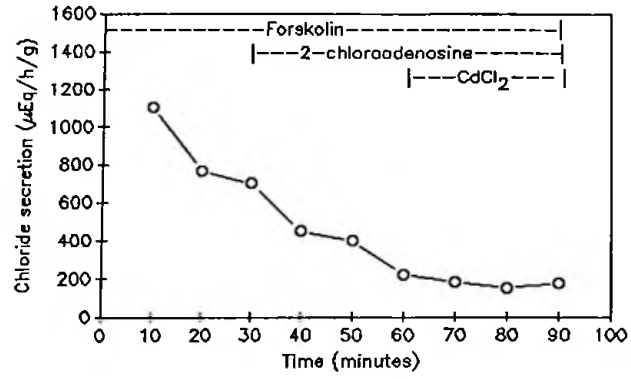


Fig. 4. Cadmium chloride does not reverse the inhibitory effect of 2-chloro adenosine. One representative experiment out of three showing that adenosine reduces the secretion of chloride in glands stimulated with forskolin. Cadmium chloride does not alter the effect of 2-chloro adenosine.

In all the above experiments the effect of cadmium was tested on <u>inhibitory</u> agents. We next tested the effect of cadmium on atrial natriuretic peptide (ANP), which <u>stimulates</u> chloride secretion by the gland. In these experiments chloride secretion was allowed to stabilize at a basal rate for 30 minutes. The perfusate was then changed to a solution containing 2.5×10^{-4}M cadmium chloride. Ten minutes later, 10 ug of ANP were administered directly into the arterial line over one minute. The secretion of chloride was monitored for thirty minutes and the solution containing cadmium chloride changed to one without cadmium and the injection of ANP repeated. A second series of experiments without cadmium was done as a parallel control. Figure 5 shows that cadmium chloride prevents the effect of ANP.

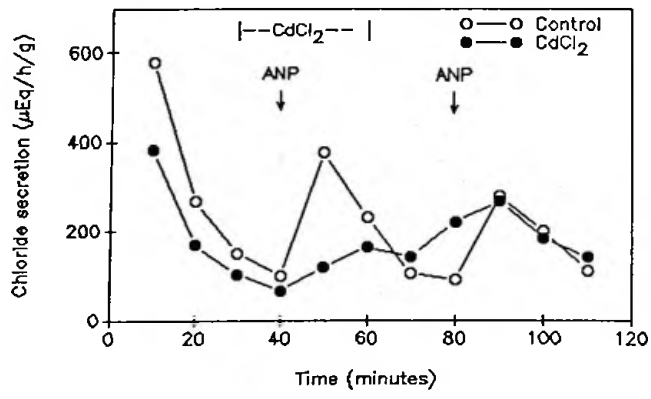


Fig. 5. Cadmium chloride prevents the stimulatory effect of ANP. Two representative experiments, out of fifteen, showing that boluses of ANP stimulate the secretion of chloride by the rectal gland (open circles). With $CdCl_2$ (closed circles) a bolus of ANP has no effect. Once $CdCl_2$ is removed, a similar bolus of ANP produces a normal response.

These experiments show that cadmium chloride prevents the effect of NPY, an inhibitory, and ANP, a stimulatory peptide. At the concentration used, cadmium chloride did not block the inhibitory effects of somatostatin, or that of 2-chloroadenosine. The observation that cadmium blocks the effect of ANP, which causes the release of VIP from nerves within the rectal gland, suggests that the effect of cadmium is to block neurotransmitter release in

the rectal gland as it does at the neuromuscular junction. Of great interest is the observation that cadmium reverses the effect of NPY. We have previously shown that NPY inhibits chloride secretion at a site distal to the generation of cyclic AMP, an effect reminiscent of that of somatostatin (Silva et al Bull MDIBL 29:98-100, 1990). On the other hand, cadmium did not prevent the effect of somatostatin, which exerts its inhibitory effect directly on the rectal gland cell at a site distal to the generation of cyclic AMP. The observation, that cadmium reverses the effect of NPY but does not prevent that of somatostatin suggest that the major inhibitory effect of NPY in these experiments is not mediated by a direct effect on the rectal gland cell but rather by another neurotransmitter such as somatostatin. The finding that cadmium did not inhibit the effect of 2-chloroadenosine, which has a direct inhibitory effect on chloride secretion, supports this hypothesis. In agreement with its known action to inhibit neurotransmitter release, cadmium probably prevents the effect of those inhibitory or stimulatory substances that act by releasing another neurotransmitter.

Supported by grants from the NIH, DK18078, NIEHS, 4 P30 ES03828, and the American Heart Association: Maine Affiliate.