CALCIUM AND MAGNESIUM SECRETION IN THE RECTAL GLAND OF THE SHARK (SQUALUS ACANTHIAS)

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There exists remarkable similarity between the mechanisms of Cl^- secretion in the shark rectal gland (RG) and those of Cl^- absorption in the thick ascending limb of Henle's loop (TALH) in mammalian nephron. While the transport of divalent ions in the TALH has been fairly well characterized (Agus and Goldfarb, The Kidney: Physiology and Pathophysiology, ed. Seldin and Giebisch, 1985), similar information for the RG is lacking. In the TALH, cAMP enhances net Ca^{2+} and Mg^{2+} absorption while furosemide, in high doses, inhibits this absorption. This study is an initial attempt to examine the secretion rate of Ca^{2+} and Mg^{2+} in the RG and the response to cAMP and furosemide.

METHODS

The isolated perfused shark rectal gland was used as previously described (Silva et.al., Am. J. Physiol. 233 (2), F298-F306, 1977). The shark Ringer solution contained Ca^{2+}: 2.5mM and Mg^{2+}: 3.0 mM. The experimental protocol involved three 10-min control collections and six 10-min experimental collections. Ca^{2+} and Mg^{2+} were measured by atomic absorption spectrophotometry (Perkin Elmer, 170).

RESULTS

In the basal state, excretion rates of Ca^{2+}, Mg^{2+}, and Cl^- diminished progressively in parallel to the fall in duct flow rate, reaching 30% of initial rates at 30 min and 15% at 60 min. With time, the concentrations of Ca^{2+} and Mg^{2+} in the duct fluid were unchanged. The average Ca^{2+} concentration was 1.0 ± 0.2 mM (n=9) and Mg^{2+} concentration was 0.93 ± 0.25 mM (n=7). These values in the duct fluid are much lower than arterial perfusate concentrations, a situation opposite to that of monovalent ions such as Na^+ and Cl^-.

Table 1 illustrates the effects on Ca^{2+} and Mg^{2+} secretion of 8-p-chlorophenylthio-cAMP (8-CPT-cAMP), a potent long-acting cAMP analogue which also possesses phosphodiesterase inhibitory activity. Within 20 minutes of 8-CPT-cAMP stiumlation, Ca^{2+} secretion rate doubles and Mg^{2+} secretion rate increases by about 50%. Despite the variability in secretion rates among different glands, 8-CPT-cAMP increased these rates in each and every gland. This modest increase owes to the gradual fall in the concentrations of Ca^{2+} and Mg^{2+} in the duct fluid while duct flow is increased several fold. Only in the first 10 minutes of 8-CPT-cAMP administration does the Ca^{2+} concentration transiently increase, reaching 1.2 ± 0.2 mM. Mg^{2+} concentration remains virtually unchanged in this period (0.89 ± 0.26 mM). By 40 minutes of 8-CPT-cAMP stimulation, the concentration of Ca^{2+} and Mg^{2+} fall to 0.14 ± 0.03 and 0.12 ± 0.02 mM, respectively. Beyond 20 minutes of 8-CPT-cAMP stimulation, the secretion rates of Ca^{2+} and Mg^{2+} are maintained at pre-stimulation values, while duct flow and chloride secretion continue to increase.

TABLE 1. Effects of 10^{-4}M 8-p-CPT-cAMP(cAMP) in RG

Period	DUCT FLOW (µl/min/gWW)	Cl^- SECRETION (µEq/min/gWW)	Ca^{2+} SECRETION (nEq/min/gWW)	Mg^{2+} SECRETION (nEq/min/gWW)
Control (10'-30')	7.2 ± 1.6	3.4 ± 0.7	21.5 ± 7.0	16.5 ± 7.4
cAMP (30'-50')	41.7 ± 8.1*	19.4 ± 4.9*	41.9 ± 7.0*	25.5 ± 6.6*
cAMP (50'-70')	64.3 ± 9.3*	28.2 ± 5.2*	23.5 ± 7.5	18.1 ± 2.5

Values are means ± SE. Each period is 20 min. At 30' of perfusion 8-CPT-cAMP was added to the arterial perfusate.
Values are expressed per min per gram of wet weight (WW) of gland. *$p < 0.05$ as compared with the control period by paired t-test. Number of glands = 9. Mg^{2+} was measured in 7 glands.

TABLE 2. Effects of 10^{-4}M Furosemide in RG

Period	DUCT FLOW (µl/min/gWW)	Cl^- SECRETION (µEq/min/gWW)	Ca^{2+} SECRETION (nEq/min/gWW)	Mg^{2+} SECRETION (nEq/min/gWW)
Furosemide (10'-30')	8.5 ± 2.4	3.6 ± 0.8	22.5 ± 10	23.9 ± 18.1
Furosemide + cAMP (30'-50')	11.4 ± 1.9*	5.2 ± 0.7	28.9 ± 7.5	18.1 ± 9.1
Furosemide + cAMP (50'-70')	18.7 ± 3.6*	8.4 ± 1.3*	37.4 ± 10*	27.2 ± 17.3

Values are means ± SE. Each period is 20 min. At 30' of perfusion 8-CPT-cAMP was added to the arterial perfusate.
*$p < 0.05$ as compared with the furosemide period by paired t-test. Number of glands = 6. Mg^{2+} was measured in 5 glands.

Table 2 shows the effect of furosemide on the 8-CPT-cAMP response. Here furosemide (10^{-4}M) was added to the arterial perfusate throughout the experiment and 8-CPT-cAMP (10^{-4}M) was added at 30 minutes. During the first 20 minutes of 8-CPT-cAMP administration, furosemide inhibited the increase in Ca^{2+} and Mg^{2+} secretion along with the expected inhibition of Cl^- and fluid secretion. In the following 20 minutes this inhibition was not complete, except for Mg^{2+} secretion.

We also examined the effects of indomethacin on Ca^{2+} and Mg^{2+} secretion. The results were qualitatively similar to those of 8-CPT-cAMP although they were quantitatively less pronounced. Indomethacin (10^{-5}M) resulted in a doubling of fluid as well as Cl^- secretion rates, and a transient increase by 50% in Ca^{2+} and Mg^{2+} secretion reaching a peak at 10-20 minutes (n=4). In additional experiments, furosemide (10^{-4}M) inhibited the increase in duct flow and in Cl^-, Ca^{2+} and Mg^{2+} secretion (n=4). It remains unclear whether the effects of indomethacin in these studies are due to cyclo-oxygenase inhibition or to modest inhibition in phosphodiesterase activity and subsequent increase in endogenous cAMP.

DISCUSSION

This study demonstrates that 8-CPT-cAMP augments the secretion rate of Ca^{2+} and Mg^{2+} in the isolated perfused RG and that furosemide attenuates this increase. The increase in divalent ion secretion could be accounted for, at least in part, by a favorable electrochemical gradient across the gland epithelium from blood side to lumen. The lumen negativity is increased several fold with cAMP addition (Silva, et.al., op. cit), and we showed a duct fluid concentration of Ca^{2+} and Mg^{2+} persistently lower than that of arterial perfusate. It appears, however, that there exists a permeability limitation to divalent ion secretion. Thus, beyond 20 minutes of 8-CPT-cAMP stiumlation, and despite the persistence of a rather steep and favorable electrochemical gradient, Ca^{2+} and Mg^{2+} secretion begin to fall and return to control values. Other cations such as Na^+, to which the gland is relatively more permeable, continue to be secreted at much higher rates for longer periods of time. In the presence of furosemide, the increase in secretion with 8-CPT-cAMP is delayed and attenuated but lasts longer, consistent with this interpretation.

Despite the modest increase in divalent ion secretion observed with 8-CPT-cAMP stiumlation, the magnitude of this increase makes it unlikely for the rectal gland to have a physiologically important role in Ca^{2+} and Mg^{2+} balance similar to its role in NaCl balance. The shark kidney is the predominant excretory organ for divalent ions.

This study illustrates yet another similarity between the shark rectal gland and the mammalian TALH: cAMP-stimulated Ca^{2+} and Mg^{2+} transport (secretion in RG and absorption in TALH).

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Key Words: cyclic AMP, furosemide, indomethacin