SIGNAL TRANSDUCTION OF INHIBITORY RECEPTORS IN THE SHARK RECTAL GLAND: CONTRASTING EFFECTS OF ADENOSINE AND SOMATOSTATIN ON CYCLIC AMP ACCUMULATION DURING MAXIMAL INHIBITION OF TRANSPORT.

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Adenosine and somatostatin (SRIF) are known to inhibit hormone stimulated chloride transport in the shark rectal gland. Adenosine inhibits forskolin and vasoactive intestinal peptide (VIP) through an A_1 inhibitory receptor by a mechanism that is both cAMP dependent and cAMP independent (Kelley et al. Bull. MDIBL 26:177-179, 1986). SRIF also inhibits forskolin and VIP stimulated secretion. The mechanism of this inhibition is less clear. Silva et al have shown that SRIF inhibits secretion stimulated by both VIP and by dibutyryl cyclic AMP/theophylline, suggesting that the inhibition is both cAMP dependent and independent (Am. J. Physiol. 249:R329-R334, 1985). In the present experiments we compared the effects of maximal inhibitory concentrations of 2 chloro adenosine (2Clado) and SRIF on forskolin stimulated cyclic AMP accumulation and chloride secretion in the perfused gland.

Rectal glands were isolated from male dogfish and perfused as previously described. Secretion was measured at 10 minute intervals and expressed as uEq of chloride secreted per hour per gram wet weight (uEq/h/g). Samples for tissue cAMP determination were taken at 50 and 80 minutes of the perfusion. At 50 minutes, the tip of the gland proximal to the artery was transected and frozen in liquid nitrogen and a ligature was placed around the severed end of the gland to prevent leakage of fluid. At 80 minutes a second slice was transected from the middle of the gland. Tissue cAMP content was measured by radioimmunoassay as previously described (Kelley et al. MDIBL Bull 26:177-179, 1986) and expressed as pmol/mg protein.

Under basal conditions secretion was 109 ± 9 uEq/h/g (average of 50-80 minute periods) and tissue cyclic AMP was 6 ± 0.8 pmol/mg protein at 50 minutes and 9.1 ± 0.4 at 80 minutes. When 1 uM forskolin was added to the perfusate after 30 minutes of basal perfusion, secretion increased 6.6 fold and cAMP increased 45 fold at 50 minutes and 58 fold at 80 minutes. The addition of 0.1 uM 2Clado completely inhibited forskolin stimulated secretion to basal levels. Tissue cAMP content, however, was inhibited by only 40 percent at 50 minutes ($p<0.01$) and by 38 percent at 80 minutes ($p<0.05$). Tissue cAMP levels, remained 27 fold greater than basal at 50 minutes ($p<0.01$) and 38 fold greater than basal at 80 minutes ($p<0.001$). Furthermore, the cAMP content at 80 minutes in the presence of 2Clado was greater than the cAMP content at 50 minutes in the forskolin controls that did not contain 2Clado. These results indicate that 2Clado, in addition to a modest inhibition of cAMP accumulation, inhibits forskolin-stimulated secretion by a mechanism that is cAMP independent.

Perfusion with 0.1 uM SRIF also completely inhibited forskolin-stimulated secretions to basal levels. Unlike 2Clado, however, SRIF inhibited cAMP accumulation by 86 percent at 50 minutes ($p<0.01$) and by 88 percent at 80 minutes ($p<0.001$). Cyclic AMP content, however, was still 7 fold greater than basal at 80 minutes ($p<0.05$).

In summary, these experiments demonstrate that 2Clado and SRIF completely inhibit forskolin stimulated secretion in perfused glands. Although

both inhibitors inhibit cAMP accumulation, SRIF was much more potent (88% vs 38%) than 2 chloroadenosine. We conclude that both 2Clado and SRIF inhibit cyclic AMP accumulation in the rectal gland. However, a major portion of the inhibition by 2Clado is by a cyclic AMP independent mechanism.

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