ATRIAL NATRIURETIC PEPTIDE STIMULATES cGMP ACCUMULATION IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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Silva et al (Am. J. Physiol. 252:F99, 1987) have shown that synthetic rat atrial natriuretic peptide (ANP II) and extracts of shark heart stimulate chloride secretion in the rectal gland of the dogfish shark. They conclude that the mechanism of this stimulation is through an ANP mediated release of VIP from VIP-containing nerves. Our present studies demonstrate that ANP increases cGMP accumulation in the *in vitro* perfused rectal gland and suggest that VIP release may not completely explain the effect of ANP on secretion.

Rates of chloride secretion, and tissue cAMP and cGMP content were measured simultaneously in rectal glands perfused, as previously described, with VIP or ANP. Rectal glands were perfused for 30 minutes of basal secretion and then VIP or ANP was added to the perfusate. Secretion was measured at 10 minute intervals. Twenty minutes after the addition of VIP or ANP, a portion of the rectal gland tissue was transected from the gland and tissue cAMP and cGMP content were determined by radioimmunoassay as previously described for cAMP.

Table 1 shows the effect of VIP and ANP on chloride secretion, and tissue cAMP and cGMP content. VIP (2 nM) and ANP (50 nM) both stimulated chloride secretion to 7.1 ($p<0.02$) and 7.5 ($p<0.001$) fold above basal respectively.

Both agents also increased tissue cAMP levels. From a basal value of 6.0 ± 0.8 pmol/mg protein, VIP increased tissue cAMP content 3.8 fold to 23 ± 1.6 ($p<0.001$) and ANP increased tissue cAMP 2.0 fold to 12.0 ± 1.4 ($p<0.01$). Since ANP does not stimulate adenylate cyclase activity in this tissue (data not shown) and since it has not been shown to increase cAMP accumulation in other tissues, the increase in cAMP content in glands perfused with ANP supports the hypothesis proposed by Silva et al. that the stimulatory effect of ANP on secretion is mediated by secretion of VIP from VIP-containing nerves in the gland. An increase in cAMP would therefore be expected because VIP stimulates secretion by increasing tissue cAMP as demonstrated above. For an equivalent stimulation of secretion, however, VIP increased cAMP content significantly more than ANP ($p<0.001$). This finding suggests that an ANP mediated release of VIP may not solely account for the stimulatory effects of ANP on secretion. If the action of ANP was solely mediated through VIP release, then cAMP levels would be expected to be the same for similar levels of secretion.

A possible candidate for mediating this additional effect is cGMP. As shown in Table 1, ANP increased tissue cGMP content from a basal of ≤ 0.01 to 4.3 ± 0.9 pmol/mg protein. Since the effects of ANP are thought to be mediated by cGMP in most other tissues, cGMP also may play an important role in this tissue, possibly by enhancing the response to released VIP.

TABLE 1 EFFECTS OF VIP AND ANF ON CHLORIDE SECRETION AND TISSUE CYCLIC NUCLEOTIDE CONTENT

	SECRETION (uEq/h/g)		cAMP (pmol/mg)		cGMP (pmol/mg)
BASAL (n=4)	127±28		6.0±0.8		≤0.01
VIP (2nM) (n=7)	907±182	@	23±1.6	+	Not Done
ANP (50nM) (n=5)	951±79	+	12.0±1.4	x	4.3±0.9

Data are mean ± SEM of indicated number of experiments.

@ $p < 0.02$ compared to basal.
+ $p < 0.001$ compared to basal.
x $p < 0.01$ compared to basal.
x $p < 0.001$ compared to VIP.

In summary, ANP stimulates both cAMP and cGMP production in the rectal gland. The increase in cAMP is likely secondary to released VIP as reported by Silva et al. However, for an equivalent secretory response, VIP stimulates AMP significantly greater than ANP suggesting that the secretory response to ANP is not solely dependent on VIP release but that an additional effector mechanism, possibly cyclic GMP, is involved in the stimulatory effect of ANP.

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