THE EFFECT OF ANP ON ISOLATED AND CULTURED TUBULES OF THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously shown that ANP stimulated Cl secretion by the isolated perfused rectal gland (RG) of the Squalus acanthias (P. Silva et al., Am J Physiol 252:F99-103, 1987) by inducing the release of VIP from nerves within the RG. The stimulatory effect of ANP can be abrogated by procaine, calcium-channel blockers or perfusion with high Mg^{++} and low Ca^{++}; all of these maneuvers inhibit neurotransmitter release from nerve terminals. Isolated cells and single perfused tubules could not be stimulated by ANP or shark cardiac extracts, presumably because neural elements were absent or hormone receptors were damaged or removed by the isolation procedures.

In order to explore the mechanism of ANP stimulation in the rectal gland, we examined two independent parameters of ANP-mediated stimulation in freshly isolated tubules and in tubules in primary culture. First, we measured oxygen consumption (QO_2), since the ouabain-sensitive moiety of QO_2 is an index of transport-related metabolism; and, second, the cGMP production in response to ANP, since ANP exerts its effect via guanylate cyclase in mammalian kidney and other tissues.

Tubules were isolated from the RG of the shark, according to previously described methods (P. Silva, Min Electr Metab 12:286-92, 1986), with minor modifications. Preparation and maintenance of the RG tubules in culture have also been described in previous reports (J. Valentich, Bull MDIBL 26:91-94, 1986; S. Lear, Bull MDIBL 28:70-71, 1989).

QO_2 was measured as previously described for isolated cells (P. Silva, Am J Physiol, op. cit.). For QO_2 studies of cultured cells, the cells were grown on collagen-coated plastic dishes (35 mm) with a circle of Nytex mesh (18 mm in diameter) embedded therein. After 10-14 days of the initial plating, the mesh with adherent collagen and cells was excised and placed in the same chamber that was used for the isolated cells. QO_2 of the cultured tubules was then measured in the same manner as the isolated cells.

The total and ouabain-sensitive QO_2 of freshly isolated and cultured tubules are listed in Table I. Whereas 51% of basal QO_2 was ouabain-sensitive in the fresh tubules, very little of the basal QO_2 in the cultured cells was ouabain-sensitive. Both cell preparations showed highly significant stimulatory responses to VIP. Most of the VIP-stimulated QO_2 was ouabain-sensitive, that is, related to active transport. ANP did not significantly increase QO_2 in the fresh cells, but significantly stimulated total and ouabain-sensitive QO_2 in the cultured cells. The degree of stimulation achieved by VIP invariably exceeded that seen with ANP treatment.

We measured intracellular cGMP in freshly isolated tubules, using ^{125}I radioimmunoassay (NEN). Tubules were incubated with vehicle (control), ANP (1 μm), IBMX (1 mM), or both ANP and IBMX for 10 minutes at 25°C, and then extracted with hydrochloric acid (1 N). Since experiments by Karnaky et al. (published in this Bulletin and cited with the author's permission) had already demonstrated an ANP-mediated increase in cGMP in cultured cells, we performed limited, but confirmatory, experiments in cultured cells. The cGMP production by freshly isolated tubules is depicted in Figure 1. There was a highly significant increase in cGMP in the freshly isolated tubules after the addition of both ANP and IBMX together ($p < 0.005$). In analogous studies, there was no significant change in cAMP production by the freshly isolated tubules (data not shown).

In summary, we found that cultured cells showed a significant increase in ouabain-sensitive (i.e., transport-related) QO_2 in response to ANP. Although ANP treatment did not significantly increase the QO_2 of freshly isolated tubules, ANP (in the presence of IBMX) did significantly increase tubule production of cGMP, but not cAMP. The activation

of guanylate cyclase as a consequence of ANP binding has been demonstrated both in freshly isolated kidney cells, e.g., rabbit collecting duct (M. Gunning, Am J Physiol 256:F766-75, 1989), and in kidney epithelial cell lines, e.g. LLC-PK1 (D.C. Leitman, Endocrinology 122:1478-85, 1988). In rectal gland epithelium, it seems likely that binding to the cell surface receptor for ANP stimulates the intracellular production of cGMP as a second messenger, and that ANP stimulates active Cl transport in this tissue.

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TABLE I The Effect of ANP on the QO_2 of RG Tubules

Freshly Isolated Tubules

Total QO_2				Ouabain-sensitive QO_2		
Basal	ANP	VIP	Ouabain	Basal	ANP	VIP
67.2±11.7 (11)	88.8±16.2 (11)	214.1±54.6** (9)	33.1±5.5^{++} (11)	34.1±7.4 (11)	55.7±13.3 (11)	184.9±48.7^{+} (9)

Tubules in Primary Culture

Total QO_2				Ouabain-sensitive QO_2		
Basal	ANP	VIP	Ouabain	Basal	ANP	VIP
56.5±5.3	80.9±6.6* (7)	90.7±9.4^{+} (7)	53.2±5.7** (7)	3.3±5.0 (9)	27.7±6.8** (9)	40.6±4.4^{++} (9)

QO_2 is expressed in μM O_2/mg (wet wt)/h. For statistical analysis by Student's t-test: QO_2 values after treatment with ANP and VIP are compared to basal QO_2; QO_2 after ouabain is compared to QO_2 after VIP, which is the maximal QO_2 achieved in these studies. *$p < 0.025$; **$p < 0.01$; $^{+}p < 0.005$; $^{++}p < 0.001$.

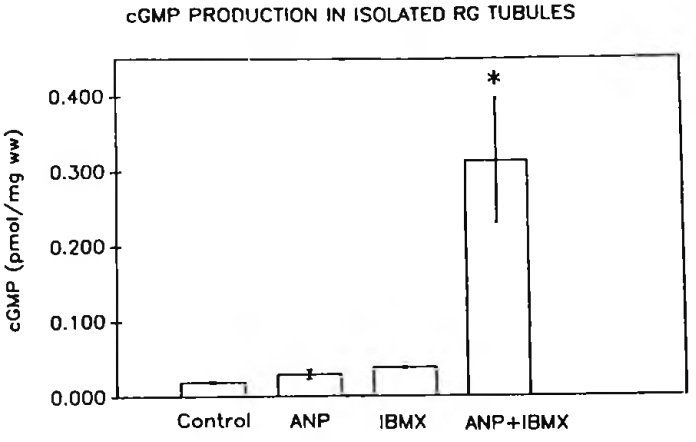

FIGURE 1. Production of cGMP by freshly isolated tubules. *$p<0.005$ vs. control. ANP vs. control —NS; IBMX vs. control - $p<0.025$.