EFFECTS OF GTP AND IONS ON THE KINETICS OF SPECIFIC BINDING OF 3H NECA TO A_1 ADENOSINE RECEPTORS IN THE SHARK RECTAL GLAND (SQUALUS ACANTHIAS)

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Previous work in our laboratory has provided evidence for two external adenosine receptors in the shark salt gland. These two receptors, A_1 and A_2, have been shown to inhibit (A_1) and stimulate (A_2) both adenylate cyclase and chloride secretion in the gland (Forrest et al. Bull MDIBL 20, 1980; Kelley et al. Bull MDIBL 23, 1983; Poeschla et al. Bull MDIBL 23, 1983; Osswald et al. Bull MDIBL 23, 1983).

Radioligand binding studies of these receptors have been performed using several adenosine analogues. In addition to confirming the existence of the two receptors, these studies showed binding at the high-affinity inhibitory receptor, A_1, to be inhibited by both GTP and chloride ion in the absence of Mg^{++} (Poeschla et al. Bull MDIBL 24, 1984). The binding studies described below examined the effects of both GTP and sodium chloride on the kinetics of binding of N-Ethylcarboxamido[8(n)-^{3}H]adenosine (^{3}H NECA) in rectal gland membranes. In addition the specificity of sodium chloride inhibition was further examined by testing other salts for inhibition.

Membranes were prepared in the manner described by Osswald et al (1983) except that magnesium was omitted from all buffers used in membrane preparation and binding assay. The procedure for binding was the same as that described by us previously except that assays were incubated for 45 minutes at 0°C. All studies utilized ^{3}H NECA as the radioactive ligand. ^{3}H NECA was obtained from Amersham at an activity of 22 Ci/mmol.

^{3}H NECA binding confirmed the existence of two binding sites. One site was found to have a Kd = 37±3 nM and Bmax = 3.8±0.3 pmole/mg consistent with a high-affinity inhibitory A_1 receptor. The second site had a Kd = 5±2 µM and Bmax = 20±5 pmole/mg (n=5) consistent with a low-affinity stimulatory A_2 receptor. These values agree well with those described by Poeschla (Clin. Res. 1985). Kinetic studies were also performed in the presence and absence of 100 µM GTP and 100 mM NaCl. GTP was found to inhibit binding by reducing the affinity of the receptor for the ligand, while Bmax remained essentially unchanged (Kd = 90±20 nM, Bmax = 4±1 pmole/mg, n=3). 100 mM NaCl was found to have profound effects on the kinetics of binding. This concentration reduced the affinity of the receptor for ^{3}H NECA over threefold while leaving the number of receptors unchanged (Kd = 130±40 nM, Bmax = 2.5±0.9 pmole/mg, n=2).

To examine the specificity of the inhibition of binding by sodium chloride, a number of salts were tested (Table 1). Although chloride was the most inhibitory of the anions examined, other anions, including gluconate and nitrate, were found to be nearly equal in potency to chloride. The cations employed also inhibited binding and showed an order of potency Na>K>Li. In a single dose-response experiment, potassium salts of chloride,

biphosphate, and aspartate achieved 50% of maximum inhibition at a concentration of 10 mM (maximum inhibition examined was 200 mM).

TABLE 1: Inhibition of ^3H NECA Binding by Various Salts

Na salt	% Inhibition	K salt	% Inhibition	Li salt	% Inhibition
NaCl	84	KCl	66	LiCl	43
Na gluconate	79	K gluconate	64	Li nitrate	42
Na nitrate	78	K nitrate	53		
Na biphosphate	73	K biphosphate	45		
Na sulfate	65	K sulfate	33		
		K aspartate	63		

Table 1: Effects of salts on ^3H NECA binding in the shark rectal gland. All salts were examined at a concentration of 100 mM. Total binding (0% inhibition) was 810 fmole/mg protein. ^3H NECA was bound at a concentration of 10 nM.

Binding of GTP to the inhibitory N_i protein causes dissociation of the subunits of N_i and a subsequent decrease in the affinity of the receptor for agonist. In the rectal gland, we observed that GTP inhibits specific binding of ^3H NECA by a decrease in receptor affinity. These results are similar to those reported for other inhibitory receptors and indicate that the affinity state of the A_1 receptor in the rectal gland is regulated by guanine nucleotide binding regulatory proteins. The effects of sodium to decrease binding of A_1 agonists in the present study were not observed in the fat cell (Ukena et al. 1984), but have been observed in brain (Goodman et al. 1982). The observation that both cations and anions decrease the specific binding of ^3H NECA in the rectal gland suggests that binding sites for both may be present on either the receptor or the Ni protein.