PHOSPHODIESTERASE ACTIVITY IN THE RECTAL GLAND OF *Squalus Acanthias*

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The shark rectal gland has both stimulatory (A_2) and inhibitory (A_1) adenosine receptors as shown by previous work in our laboratory (Forrest et al. Bull MDIBL 20, 1980; Kelley et al. Bull MDIBL 23, 1983; Osswald et al. Bull MDIBL 23, 1983).

Inhibitory (A_1) adenosine receptors may act by decreasing the concentration of the second messenger cAMP. This effect could result from inhibition of adenylate cyclase and/or from stimulation of cAMP phosphodiesterase (PDE) activity. Activation of A_1 receptors by adenosine and its analogs stimulates a low K_m (high affinity), membrane-bound, particulate PDE in rat brain (Mazancourt and Gindicelli. FEBS 167, 142, 1984) and rat adipocytes (Wong et al. Biochem J 227, 815, 1985). Elks and Manganiello (Endocrinology 155 1262, 1984) found that cilostamide, a selective inhibitor of particulate PDE, reverses the stimulatory effects of insulin and lipolytic hormones on PDE in 3T3-L1 adipocytes.

In other tissues, PDE activity is characterized by a non-linear Lineweaver-Burk plot over varying substrate (cAMP) concentrations. Pichard and Cheung (JBC 251, 18, 5726-5737) isolated interconvertible, multiple forms of PDE by fractionating homogenized tissue over a continuous sucrose density gradient. Thompson and Strada (Receptors and Hormone Action, Vol III, 1978) describe four distinct PDE entities: (1) a PDE with high affinity for cAMP, regulated by cAMP in a negatively cooperative manner, (2) a PDE with a greater affinity for cGMP than cAMP and regulated by Ca^{2+}/Calmodulin, (3) a low affinity cAMP PDE regulated by cAMP and (4) a membrane bound or particulate PDE with a high affinity for cAMP.

As a first step to exploring receptor mediated regulation of PDE activity in the rectal gland, we determined the kinetics of particulate and cytosolic PDE in this tissue and examined the effects of selective and non-selective PDE inhibitors.

Rectal glands from *Squalus acanthias* were added to 40 mM Tris buffer (pH 8.0) at a ratio of 1 gm:10 ml and homogenized 4 x (15 sec) with a Tekmar tissuemizer. The crude homogenate was filtered through gauze and part of this fraction was diluted to a final concentration of 0.5 mg protein/ml and used as the homogenate while the remainder was fractionated into particulate and cytosolic components by centrifuging at 100,000 x g for 60 min. The supernatant fraction was removed and diluted to a final concentration of 0.5 mg/ml with buffer and centrifuged at 12,000 x g for 30 min. This pellet was resuspended in buffer to a final concentration of 0.5 mg/ml.

The PDE assay used was the batch method modified from Thompson et al (Adv Cyc Nuc Res 1979, Vol 10). The assay system consisted of 100 ul of ^3H cAMP in 40 mM Tris buffer (pH 8.0) with 20 mM $MgCl_2$, 50 µl cAMP (various concentrations) in Tris buffer, 50 µl of 32 mM 2-mercaptoethanol (final concentration 4 mM), 100 ul of enzyme preparation and 100 ul of buffer with or without a test substance (e.g., a PDE inhibitor). Inhibitor assays required the use of 0.1 µM cAMP for the particulate and 10 µM cAMP for the cytosolic fraction with varying concentrations of the inhibitors. K_m and V_{max} values were determined by a computer program (Simplex) using an iterative, least squares fit of data to a Lineweaver-Burk plot.

The homogenate fraction, 100,000 x g particulate fraction and supernatant fraction each yielded non-linear Lineweaver-Burk plots with the best fit by iterative least squares revealing two K_m and V_{max} values for each fraction. As shown in Table I, the particulate fraction demonstrated kinetics mirroring the low K_m (K_{m1}) PDE in the homogenate fraction. The higher K_m (K_{m2}) properties of the homogenate were more clearly shown by the supernatant fraction.

TABLE 1: PDE Kinetics in the Shark Rectal Gland

Fraction	K_{m1}*	V_{m1}**	K_{m2}*	V_{m2}**
Homogenate	0.41±0.37	25.7±1.8	14.3±3.3	46.2±4.6
Particulate	0.45±0.20	20.9±3.5	27.8±3.5	35.4±4.4
Supernatant	1.28±0.3	50.8±11.4	16.0±2.2	78.7±4.7

*K_m in µM concentrations
**V_m in pm/min/mg protein

Figure 1 illustrates the selectivity of cilostamide in the rectal gland PDE fractions. Cilostamide (100 µM) was without significant effects on the cytosolic fraction but markedly inhibited particulate PDE activity with an IC_{50} of 0.1 µM (n=3).

We next examined three classes of adenosine receptor antagonists for their effects on PDE activity. Theophylline and IBMX are adenosine receptor antagonists that are potent PDE inhibitors in other systems. Both drugs inhibited PDE activity in the cytosolic and particulate fractions of the rectal gland. IBMX had an IC_{50} value of 125 µM in the cytosolic and 4 µM in the particulate assay. The IC_{50} for theophylline was 1.6 µM and 90 µM in the cytosolic and particulate fractions. 8-phenyl-theophylline (8PT) and 8-cyclo-pentyl-theophylline (8CPT) antagonize adenosine receptors at µM concentrations, but displayed IC_{50} values greater than 100 µM for 8CPT and greater than 10 µM for 8PT. Selective A_1 and A_2 antagonists CGS 15943, PD115,199 and PD116,948 did not significantly inhibit PDE activity at concentrations approaching their solubility limits.

Our results demonstrate that in the rectal gland, there are two distinguishable forms of PDE: a particulate form showing low K_m (high affinity) properties and a cytosolic fraction displaying high K_m (low affinity) properties. The selective effect of cilostamide on the particulate fraction is consistent with separate forms of PDE in the rectal gland.

Theophylline and IBMX markedly inhibit PDE activity at concentrations required to antagonize adenosine receptors. These nonselective agents cannot be used in the rectal gland when separation of PDE inhibition from adenosine receptor antagonism is required. In contrast, 8-PT and 8-CPT inhibit PDE activity only at concentrations much greater than required for adenosine receptor antagonism and can be used in the rectal gland to demostrate adenosine receptor antagonist effects. The selective A_1 and A_2 antagonists CGS 15943, PD 115,199 and PD 116,948 also have little PDE inhibition and are useful as selective adenosine receptor antagonists.

Figure 1. Dose dependent effects of cilostamide on phosphodiesterase activity in particulate and cytosolic fractions of homogenates of the shark rectal gland (n=3).