ADENOSINE RELEASE BY THE ISOLATED PERFUSED RECTAL GLAND OF SQUALUS ACANTHIAS

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INTRODUCTION

Adenosine is considered to be a factor in the metabolic regulation of cardiac function (Berne, Circ. Res. 47: 807-813, 1980). The idea is that adenosine as a metabolite from energy metabolism (mainly ATP) acts on the different structures of the organ (vasculature, myocardium, nerves), to adapt energy supply to the organ according to its different needs. Adenosine causes vasodilation in all organs except in the kidney where it causes vasoconstriction. The concept of metabolic control of renal function mediated by adenosine is recently reviewed by Osswald, in: Regulatory Function of Adenosine, edited by Berne, Rall, Rubio, Nijhoff Publishers Boston, 1983. The main question in that concept is: does the increased activity of the Na, K-ATPase lead to an enhanced release of adenosine by the transporting cells.

We chose the rectal gland of Squalus acanthias to test the hypothesis of an enhanced adenosine release during stimulation of active chloride transport because (1) in the rectal gland the Na, K-ATPase activity is the primary driving force for active chloride secretion (Epstein et al., Biochem. Nephrol., Huber, Bern, 1978), (2) the rectal gland has sufficient tissue to release adenosine in measurable amounts, (3) the rapid and several fold increase of chloride secretion rate above baseline following stimulation allows an easy experimental set up.

METHODS

Fresh isolated rectal glands of Squalus acanthias (2.2-3.7 g) were perfused in a standard fashion as previously described (Silva et al. Am. J. Physiol. 233, F298-F307, 1977). Secreted gland fluid and the venous effluent were collected during control periods and following stimulation by vasoactive intestinal polypeptide (VIP). The fluid samples were immediately frozen and stored in dry ice. After shipping the samples to Aachen the adenosine concentration was analysed in the Department of Pharmacology, RWTH Aachen, West Germany. On the day of arrival the dry ice box contained enough frozen $C0_2$ indicating that there was no thawing of the samples. The specimens were stored at -70°C until adenosine measurements were conducted. Measurements were completed within 12 weeks after collection at MDIBL. Before the adenosine measurements were carried out in a competitive binding assay (Sacher and Osswald, Arch. Pharmacol. 311, R49, 1980), the nucleoside in the diluted samples was adsorbed onto activated charcoal with isopropanol-water (50%, v/v, pH 9.5) at 22°C. The lyophilized eluate was spotted onto thin layer chromatographic plates and separated as previously described (Osswald et al. Pflüg. Arch. 371, 45-49, 1977). The adenosine spot was taken up in 500 µl assay buffer consisting of TRIS (100 mM) and dithiothreitol (1 mM) adjusted to pH 7.5. The recovery for the isolation procedure calculated by adding 1000 cpm ^{14}C-adenosine to each tube was 50-70%. The adenosine binding protein from beef heart (k_d=11 nM) was saturated with ^{3}H-adenosine for 4 hours at 0°C. The ^{3}H-adenosine-protein complex was separated from the incubation medium by a filtration technique. Known amounts of adenosine displaced the radioactive ligand as shown in Figure 1. Each sample was measured in duplicate and corrected for recovery.

RESULT AND DISCUSSION

Table 1 summarizes the results. VIP increased duct flow and chloride secretion rate from 5.2 ± 0.5 to 22.7 ± 2.1 µmol/min. Adenosine concentration in the duct fluid was 0.18 µM during unstimulated baseline. Since we can detect 5-10 pmoles of adenosine in one sample of 100 µl, the adenosine concentration in the duct fluid was well above detection level. The adenosine concentration in the venous effluent was one order of magnitude lower probably due to the high perfusion rate of 2.5 - 4.5 ml/min. Following VIP adenosine

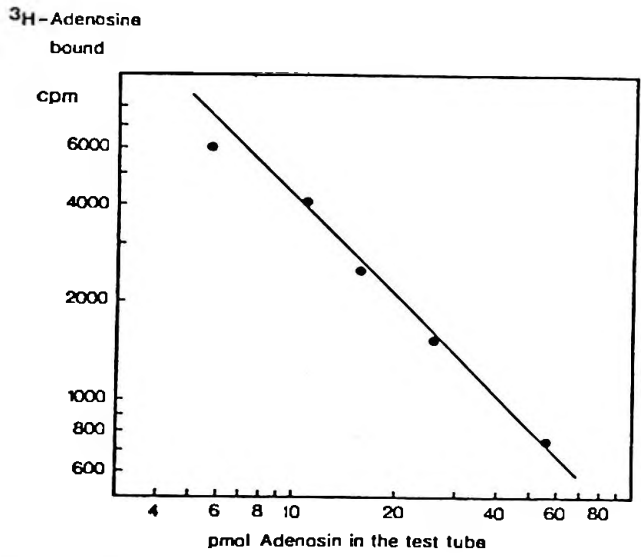

Figure 1.--Standard cruve of the adenosine assay. Ordinate: ^{3}H-adenosine recovered on cellulose filters due to binding to the adenosine-binding protein (Sacher and Osswald, 1980); Abszissa: added adenosine in the test tube, final volume 250 µl.

Table 1.--Adenosine release into the secreted duct fluid of the isolated perfused rectal gland during control conditions (2-3 periods) and following stimulation by VIP, 50 nM, (2-4 periods). Mean values ± SEM

	Control n = 5	VIP n = 7
Duct Flow Rate µl/min	12.5 ± 2.8	46.4 ±11.1
Adenosine Concentration nM	182 ± 36	423 ±207
Adenosine Release pmol/min	2.21 ± 0.21	13.5 ± 3.1

concentration in the duct fluid increased two-fold while chloride secretion increased fourfold. Adenosine release rate was enhanced from 2.2 to 13.5 pmol/min. This result clearly shows that adenosine can be released by an active NaCl transporting epithelium at an enhanced rate when the Na, K-ATPase is hydrolysing ATP at a higher rate. The data compare well with the finding of Rubio et al (MDIBL Bull. 20, 143-145, 1980) showing a fall of high energy phosphates in the gland tissue in response to stimulation of chloride secretion by saline loading the fish. Thus, active ion transport work in the rectal gland generates a metabolite which by itself at appropriate concentrations may regulate blood flow and chloride secretion. This work was supported by a Grant from the Deutsche Forschungsgemeinschaft, Os 42/6-7.

BINDING OF LIGANDS TO ADENOSINE RECEPTORS IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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Adenosine is released by the isolated perfused rectal gland into the venous effluent at an increased rate when