EFFECTS OF CALCIUM CHANNEL BLOCKERS VERAPAMIL AND D600 ON ADENOSINE-STIMULATED CHLORIDE SECRETION IN THE ISOLATED PERFUSED RECTAL GLAND OF SQUALUS ACANTHIAS

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The rectal gland of the dogfish shark Squalus acanthias secretes a hypertonic NaCl solution by an active chloride transport mechanism responsive to cyclic AMP (Stoff et al., J Exp. Zool. 199: 443-448, 1977; Silva et al., Am. J. Physiol., 233(4): F298-F306, 1977). Because calcium has been implicated as a second intracellular mediator of secretion in a number of epithelia, we have used several approaches to investigate the possible role of calcium in the secretion of chloride by the rectal gland. The calcium ionophore A23187 enhances calcium exchange in epithelia and has been shown to increase chloride secretion in the rabbit ileum and colon, suggesting that chloride secretion in these tissues is calcium dependent (Bolton and Field, J. Memb. Biol., 35: 159-173, 1977). Recent studies with A23157 (Forrest et al., Bull. MDIBL 18: 10-13, 1979) showed that the ionophore caused a modest but significant increase in secretion above baseline, although the response was less dramatic than in the above-mentioned tissues. To further define the role of calcium in rectal gland secretion, in the present studies we observed the effect of calcium channel blockers on chloride secretion in the isolated perfused gland. These compounds, verapamil and D600 (a methoxy derivative) inhibit cellular calcium uptake by blocking membrane calcium channels, thereby reducing the availability of external calcium to the cell. We studied the effect of these two compounds on maximal adenosine-stimulated chloride secretion in rectal glands perfused either with regular shark Ringer's solution (2.5 mM Ca) or low Ca Ringers (0.5 mM). Stimulation with adenosine was chosen since in other tissues and presumably the rectal gland (see Forrest et al., this bulletin) this substance activates secretion proximal to cyclic AMP by binding to an external receptor that activates adenylate cyclase.

Dogfish of either sex weighing 3 to 6 kg were taken by hook and line from Frenchman Bay, Maine, and kept in marine livecars for up to 3 days until killed by spinal cord transection. The rectal gland was excised and its artery, vein and duct cannulated with PE 90 tubing. The gland was then placed in a plexiglass chamber at 15°C and perfused by gravity flow from an oxygenated reservoir at an approximate flow rate of 4 to 9 ml/min. The shark ringers perfusate, as described by Silva et al., Am. J. Phys., 233(4): F298-F306, 1977, contained Na, 280 mM; K, 5.5 mM; Cl, 270 mM; HCO_3, 8 mM; Mg, 1.2 mM; phosphate, 1 mM; sulfate, 0.5 mM; urea, 350 mM; glucose, 5 mM and Ca, either 2.5 or 0.5 mM. Adenosine and verapamil or D600 were added directly to the perfusate.

In all experiments, glands were first perfused for 30 minutes with Ringers alone to achieve low basal secretory rates. Adenosine 10^{-4}M was then added for the next 30-minute interval, followed by 30 minutes of adenosine with the calcium channel blocker (10^{-4}M). Experiments were concluded with a final 30 minutes of perfusion with adenosine alone. This sequence was followed with both verapamil and D600 at both calcium concentrations. Control glands were perfused for the same time period with adenosine alone. In addition, a d isomer of D600 which has no effect on Ca channels was tested in 3 glands.

Rectal gland fluid and venous effluent were collected at 10-minute invervals throughout the experiments. Chloride concentration of samples was determined with a Buchler-Cotlove chloridometer. Chloride secretion was calculated as μEq Cl/hr/g wet weight. Mean values are provided for the final basal period (30 min.), final period of initial adenosine stimulation (60 min.), first and last periods of adenosine and blocker (70 and 90 min.), and last period of adenosine recovery (120 min.). (Table 1.)

TABLE I. Effects of verapamil and D600 (10^{-4}M) on adenosine-stimulated (10^{-4}M) chloride secretion in the isolated rectal gland perfused with regular Ca (2.5 mM) and low Ca (0.5 mM) Ringers solution.

		CHLORIDE SECRETION RATE µEq/Hr/G Wet Weight						
		REGULAR Ca (2.5 mM)			LOW Ca (0.5 mM)			
Period	Time (min)	Control n=17	D600 n=4	Verap n=3	Control n=6	D600 n=5	Verap. n=5	Na-D600†† n=3
Last Basal	30	154±17	23±10	17±17	40.2±11	30±11	25.9±10	12.6±5
Last Adenosine before Inhibitor	60	736±140	911±107	589±151	500±105	1630±209	1275±252	1193±234
First Adenosine with Inhibitor	70	717±358	492±115	372±108	692±133	692±215	512±135	823±52
	p value*	NS	<0.025	<0.025	NS	<0.01	<0.025	NS
Last Adenosine with Inhibitor	90	712±236	524±127	524±190	605±294	628±108	648±99	1299±284
	p†	NS	NS	NS	NS	<0.005	NS	NS
Last Adenosine Without Inhibitor	120	1106±377	781±109	375±129	630.3±174	1105±227	576±82	781±433
	p value†	NS	NS	NS	NS	<0.005	NS	NS

* Compared to last adenosine before inhibitor.

† Compared to last adenosine with inhibitor.

†† Experiments with an inactive d isomer of D600.

All values are mean ± SEM; n = no. of experiments per group.

Following 30 minutes of unstimulated perfusion, rectal gland chloride secretion consistently fell to between 12 and 150 µEq Cl/hr/gww. Adenosine was a potent and reliable stimulant of chloride secretion in the rectal gland and increased secretion approximately 10-15 fold in 30 minutes. There was considerable variability in adenosine response between glands. Note that the values at 60 minutes for both control groups (regular and low calcium) were lower than temporarilly identical values for the experimental groups destined to receive a calcium blocker. However, because adenosine stimulation of chloride secretion in both control groups persisted without decline for 120 minutes, it is reasonable to interpret significant declines in experimental groups as effects of the calcium antagonists.

In 17 experiments with both regular and low calcium perfusate, verapamil and D600 significantly decreased secretion in adenosine-stimulated glands during the first 10 minutes of perfusion with the blocker (all $p<0.025$, Table 1). The percent inhibition was greater in low calcium (58% for D600 and 60% for verapamil) than in regular calcium Ringers (46% for D600 and 37% for verapamil), and the inhibition was better maintained in low calcium Ringers suggesting that low calcium enhanced the inhibitory effect. Inhibition of secretion was demonstrated most clearly with D600 in low calcium media. The mean chloride secretion rate during 30 minutes of perfusion with adenosine and blocker (844±94) was significantly inhibited compared to adenosine alone (1346±145) ($p<0.01$) and recovery was observed following removal of D600 (1105±226) ($p<0.005$). In glands receiving verapamil or D600 in regular calcium media, chloride secretion rates often rose during the final 20 minutes of perfusion with blocker.

Adenosine stimulated recovery of inhibited glands was variable, but was clearly demonstrated following removal of D600 in low calcium experiments. Restimulation did not achieve the secretion rates observed before the blocker and did not occur in verapamil-treated glands.

Experiments with a d-isomer of D600 that is without effects on calcium transport served as a third control group. Adenosine-stimulated chloride secretion was not inhibited during any period of perfusion with this agent (Table 1).

The present experiments demonstrate a transient, but consistent and significant inhibition of chloride secretion in adenosine-stimulated glands following the calcium channel blockers, verapamil and D600. No similar change is seen in glands treated with adenosine alone or with an inactive isomer of D600. Inhibition was enhanced in low calcium media and its reversibility was clear only with low calcium media. Although direct measurement of intracellular calcium activity has not been performed in this tissue, it can be assumed that these blockers impair calcium uptake by rectal gland cells; recent work also suggests that the blockers affect the redistribution of intracellular calcium (Church and Zsoster, Can. J. Phys. and Pharm., 58: 254-264, 1980). These data support the hypothesis that adenosine-stimulated rectal gland fluid secretion is sensitive to acute changes in the intracellular concentration of calcium.