INHIBITION OF CHLORIDE SECRETION BY NUCLEOTIDES IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

Patricio Silva,[1] Richard Solomon,[1] Heather Brignull,[2] Elizabeth Franco,[3] Christina Pathwick-Paszyc,[4] Hadley Solomon,[5] Katherine Spokes,[6] Melissa Taylor,[1] and Franklin H. Epstein.[6]
[1,6]Department of Medicine, Harvard Medical School, Boston, MA 02115
[1]New England Deaconess Hospital and Joslin Diabetes Center, Boston, MA 02215
[2]Dartmouth College, Hanover, NH 03755
[3]Colby College, Waterville, ME 04901
[4]Ellsworth High School, Ellsworth, ME 04605
[5]Barnard College, New York, NY10025
[6]Beth Israel hospital, Boston, MA 02215

The transport of chloride in many epithelia is regulated by humoral substances including a variety of peptide hormones, prostaglandins and autacoids and also by neural mechanisms. Over the past few years, nucleotides have been shown to have a stimulatory effect on the secretion of chloride by respiratory and other epithelia (Wong, P.Y., Br. J. Pharmacol. 95:1315,1988; Mason, S.J., et al., Br. J. Pharmacol. 103:1649,1991; Knowles, M.R., et al. N. Engl. J. Med. 325:533,1991; Brown, H.A., et al. Mol. Pharmacol. 40:648,1991; Dho, S., et al. Am. J. Physiol. 262:C67,1992; Clarke, L.L. and Boucher, R.C., Am. J. Physiol. 263:C348,1992; Stutts, M.J., et al. Proc. Natl. Acad. Sci. U. S. A. 89:1621,1992). Since the mechanism of chloride transport in all these epithelia is essentially the same as that of the rectal gland of S. acanthias, we investigated the effect of nucleotides on chloride secretion by the rectal gland.

Shark rectal glands were perfused in vitro as previously described (Silva, P., et al. Methods Enzymol. 192:754,1990).

In order to avoid any confusion that might arise from the stimulation of adenosine receptors by nucleotides or their hydrolysis products, initial experiments were carried out on glands stimulated with 2.5 x 10^{-4}M theophylline, an adenosine receptor antagonist. Both ATP, 10^{-4}M, and UTP, 10^{-4}M, inhibited chloride secretion in a reversible manner (Figures 1 and 2). In unstimulated glands, UTP had no discernible effect, while ATP, 10^{-4}M, induced marked

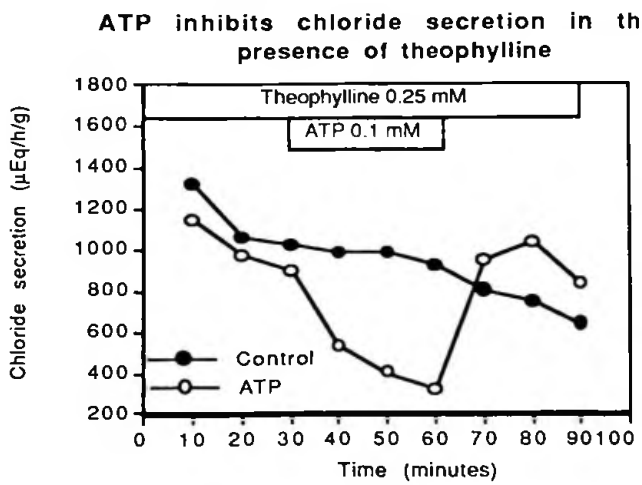

Figure 1. ATP inhibits chloride secretion in the presence of theophylline. ATP inhibits chloride secretion in isolated perfused rectal glands stimulated to secrete chloride with theophylline. Shown are two representative experiments.

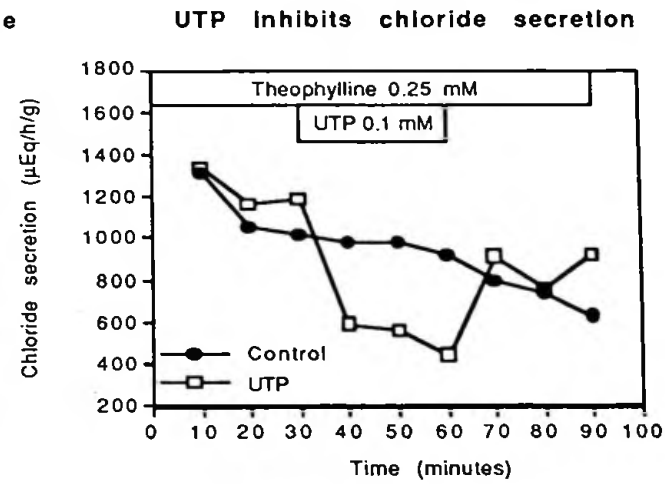

Figure 2. UTP inhibits chloride secretion in the presence of theophylline. UTP inhibits chloride secretion in isolated perfused rectal glands stimulated to secrete chloride with theophylline. Shown are two representative experiments.

stimulation, presumably because it was hydrolyzed to adenosine by ecto-5'-nucleotidases. When UTP was added to the perfusate of glands stimulated with VIP, it inhibited the secretion of chloride by 78±7 %.

These results are unexpected because in all chloride secreting epithelia where the effect of nucleotides has been studied, their effect is stimulatory. These results indicated that the secretion of chloride by the rectal gland was inhibited by nucleotides, and that the rectal gland had in addition to P1 purinoceptors (the adenosine receptors), P2 purinoreceptors.

We then proceeded to determine the order of potency of several different nucleotides in an effort to determine pharmacologically the type of P2 receptor present in the gland. Figure 3 shows the inhibitory dose response curves for a variety of nucleotides in glands stimulated with theophylline. The order of potency was ATP = ADP = ß, γ methylene ATP > UTP > uridine >> 2, methyl thio ATP. This order of potency is different from that of the reported sequence for P2x, P2y or the nucleotide receptors.

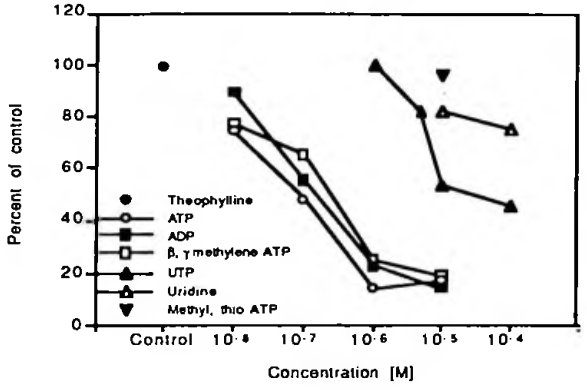

Figure 3. Dose response curves for inhibition of the secretion of chloride by different nucleotides. The inhibitory order of potency was ATP = ADP = ß, γ methylene ATP > UTP > uridine >> 2, methyl thio ATP. Representative experiments.

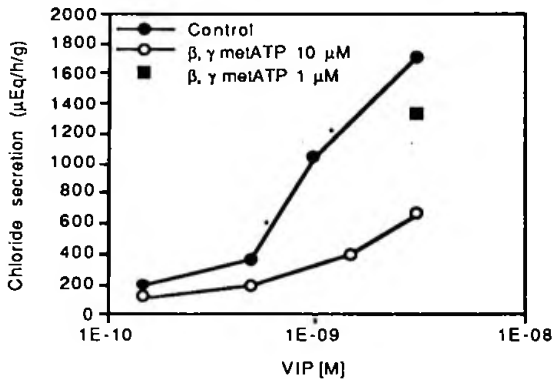

Figure 4. Effect of ß, γ methylene ATP ion isolated perfused rectal glands stimulated to secrete chloride with VIP. ß, γ methylene ATP inhibits the stimulatory effect of VIP. Representative experiments.

To assess the effect of the nucleotides on glands stimulated to secrete chloride with a stimulant other than theophylline we used ß, γ methylene ATP in glands stimulated with VIP. We used ß, γ methylene ATP because the methylene bond between the ß and γ phosphates of this compound render it resistant to 5' nucleotidases. Figure 4 shows that ß, γ methylene ATP inhibits the stimulatory effect of VIP.

The effect of the nucleotides on respiratory epithelia and rat epididymal cells is exerted on the apical side, whereas in T84 cells it is on the basolateral side. To test for the sidedness of the effect in the rectal gland, rectal gland cells were grown to confluence, in culture. ß, γ methylene ATP was then added to either the basolateral or apical surfaces of

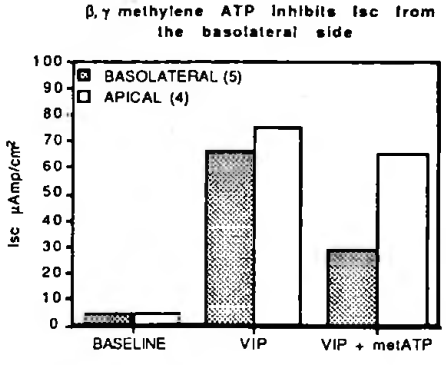

Figure 5. Effect of ß, γ methylene ATP on Isc of confluent monolayers of rectal gland cells mounted in an Ussing chamber. ß, γ methylene ATP inhibited Isc when added to the basolateral side and had only a minor effect when added to the apical side. Representative experiments.

confluent monolayers of rectal gland cells mounted in an Ussing chamber. The Isc of these cells was stimulated with VIP and theophylline. ß, γ methylene ATP inhibited Isc when added to the basolateral side and had only a minor effect when added to the apical side (Figure 5). Thus, in the rectal gland, the nucleotides exert their effect on the basolateral side.

Inasmuch as ATP is considered a potential neurotransmitter, experiments were designed to examine its effect in the presence of procaine 10^{-2} M, a maneuver that blocks neurotransmission. Procaine did not alter the effect of UTP suggesting that the effect of the nucleotides is independent of the release of neurotransmitters.

Since the stimulatory effect of nucleotides on respiratory epithelia appears to be mediated by calcium, we used verapamil to determine whether extracellular calcium was required for their inhibitory effect. Glands were perfused with verapamil 10^{-4}M throughout the experiment. Verapamil did not prevent the inhibitory effect of ß, g methylene ATP (data not shown).

It is possible that the mode of action of these nucleotides involves the regulation of an ATP-gated potassium channel. To test for this possibility, experiments were performed in the presence of lemakalim, a potassium channel opener, and glyburide, a channel closer. Neither lemakalim nor glyburide inhibited the inhibitory effect of UTP, in the case of lemakalim, or ß, γ methylene ATP, in the experiments with glyburide (data not shown). Given these results it is unlikely that ATP-gated potassium channels mediate the effect of these nucleotides.

These experiments indicate that both purine and pyrimidine nucleotides inhibit the secretion of chloride by the rectal gland of the shark. Inhibition of the epithelial transport of chloride by these nucleotides is unexpected because in all other transporting epithelia where nucleotides have an effect this effect has been stimulatory (for more information see references cited in the opening paragraph of this report). To our knowledge this is the first such demonstration.

The order of potency of the effect of the nucleotides is different than that reported for the P2x, P2y and nucleotide receptors (Olsson, R.A. and J.D. Pearson, Physiol. Rev. 70:761,1990; El-Moatassim, C, et al., Biochem. Biophys. Acta, 1134:31,1992). Methylene substituted analogs such as ß, γ methylene ATP are considerably more potent than ATP at P2x, the excitatory purinoceptor, and equal or less potent at the P2y, or inhibitory purinoceptor. C-2 substituted analogs such as 2-methyl, thio ATP are more potent than ATP at P2y receptors and similar or less than ATP at P2x receptors. The order of potency found in the present studies, ATP = ADP = ß, γ methylene ATP > UTP > uridine >> 2, methyl thio ATP, differs from that of both the P2x and P2y receptors. It also differs from the nucleotide receptor where UTP is the same or greater than ATP. The order of potency observed in these experiments suggests that the rectal gland possesses a purinoceptor that is pharmacologically different from those previously described. Table I compares the order of potency of the rectal gland receptors with that of reported receptors.

Table I

Order of potency of the P2 and nucleotide receptors and rectal gland P2 receptor

P2x:	a, ß methylene ATP >> ATP = 2, methyl thio ATP > UTP
P2y:	2, methyl thio ATP > ATPγ S > ATP >> a, ß methylene ATP, UTP
Nucleotide:	UTP = ATP > ATPγ S > 2, methyl thio ATP > a, ß methylene ATP
Rectal Gland:	ATP = ADP = ß, γ methylene ATP > UTP > uridine >> 2, methyl thio ATP

The effect of the nucleotides to inhibit the transport of chloride is exerted on the basolateral side as judged by the experiments on confluent monolayers of rectal gland cells. The effect was prompt and marked when the nucleotides were applied to the basolateral side while it was delayed and of very small magnitude when applied to the apical side. These results also differ from those obtained in two other chloride transporting epithelia, the respiratory epithelia and rat epididymal cells. The sidedness of the effect is similar to that observed in T84 cells, that are a colon cancer cell line, and as such the like of the rectal gland that is an appendix of the alimentary tract. The effect of the nucleotides on the rectal gland differs from that on T84 cells in that it is inhibitory rather than stimulatory.

The present experiments do not provide a mechanism for the inhibitory effects of nucleotides on chloride transport. The effect of the nucleotides was not inhibited by procaine indicating that it is independent of neurotransmitter release. It was not inhibited by verapamil suggesting that it is not mediated by extracellular calcium. The failure of lemakalim and glyburide to prevent their effect implies that it is not mediated by an ATP gated potassium channel.

In summary, nucleotides applied to the basolateral side of the rectal gland inhibit the secretion of chloride, the first demonstration of an inhibitory effect on epithelial transport for these compounds. The receptor mediating the effect is pharmacologically different from the purinoceptors already described.

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