NEUROPEPTIDE Y INHIBITS CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS.

A.R. Grasso[1], S. Aller[1], C. Aller[1], J. Landsberg[2], M. Silva[2], F.H. Epstein[2], J.N. Forrest[1] and P. Silva[2].
Departments of Medicine, [1]Yale University School of Medicine, New Haven, CT 06510, and [2]Harvard Medical School and Beth Israel Hospital, Boston, MA 02215.

Neuropeptide Y is a 36 amino acid polypeptide that is widely distributed in the central nervous system and peripheral nerves throughout the body. It is found in association with catechola- mines in the adrenal medulla (Peptides 9:393, 1988) and in some nerves (Peptides 8:145, 1987), and with a number of other neurotransmitters, among them vasoactive intestinal peptide (VIP), in other nerves (Reg Peptides 10:47, 1984). Because of the co-distribution with vasoactive intestinal peptide in nerves in the intestine we examined the effect of neuropeptide on the secretion of chloride by the rectal gland.

Rectal glands were perfused *in vitro* by gravity, with shark Ringer's, at a pressure of 40 mm Hg and a temperature of 15°C. Two different protocols were used. In the first, the rectal glands were allowed to reach a basal rate of chloride secretion prior to the addition to the perfusate of neuropeptide Y or other agents that modify chloride secretion. In the second protocol, the secretion of chloride was stimulated with either VIP or cyclic AMP from the beginning of the perfusion. Collections of rectal gland secretion were made at ten minute intervals and the volumes measured. Chloride in the secretion was measured by amperometric titration.

Neuropeptide Y had no effect on basal, unstimulated, chloride secretion by the rectal gland. However, neuropeptide Y had a dose dependent inhibitory effect on the secretion of chloride stimulated with VIP. Figure 1 shows a representative experiment in which neuropeptide Y at a concentration of 10^{-7} M evoked a 50% decrease in the secretion of chloride in a gland stimulated to secrete chloride with 10^{-9} M VIP. The inhibitory effect was completely reversible. Examination of the dose response curve for neuropeptide Y inhibition in such experiments showed maximal inhibition at 10^{-7} M, and half-maximal at 3×10^{-9} M.

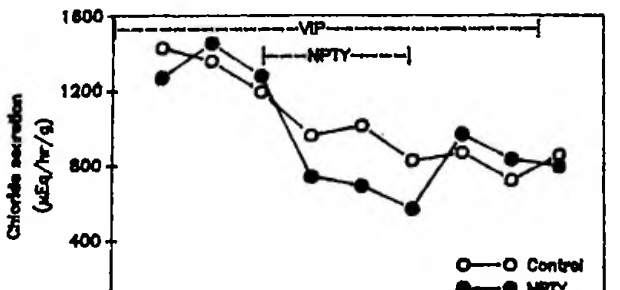

Figure 1. Representative experiments, out of 6 control and 4 experimental, showing the effect of neuropeptide Y on chloride secretion stimulated with VIP in isolated perfused rectal glands. Both glands were perfused with VIP throughout the experiment. Neuropeptide Y was added to the perfusate starting at thirty minutes for a total of thirty minutes. Neuropeptide Y induced a marked but reversible inhibition in chloride secretion.

Because the stimulation of chloride secretion by VIP is mediated by adenylate cyclase and neuropeptide Y has been shown to inhibit adenylate cyclase in the mammalian intestine (Endocrinology 124:692, 1989), we tested for the effect of neuropeptide Y on chloride secretion stimulated by

forskolin, which directly activates the catalytic subunit of the cyclase. Figure 2 shows a representative experiment in which neuropeptide Y, at a concentration of 5×10^{-8} M, prevented the stimulatory effect of forskolin 10^{-6} M. The inhibitory effect was immediately reversible upon removal of neuropeptide Y from the perfusate.

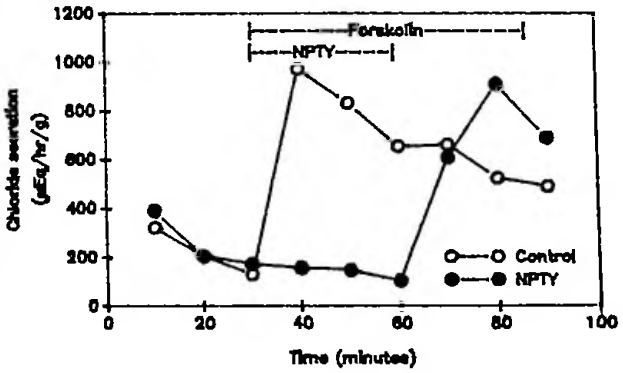

Figure 2. Representative experiments, out of 6 control and 6 experimental, showing the effect of neuropeptide Y on the stimulation of chloride secretion by forskolin in isoleted perfused rectal glands. After a control period of 30 minutes both glands received forskolin 10^{-6} M that was continued for the duration of the experiment. One gland, closed circles, received the simultaneous infusion of neuropeptide Y 5×10^{-8} M that was continued for thirty minutes. Neuropeptide Y completely prevented the normal stimulatory effect of forskolin. The effect of neuropeptide Y was completely reversible.

To determine whether the inhibitory effect of neuropeptide Y was exerted at a site distal to the generation of cyclic AMP we determined the effect of neuropeptide Y on chloride secretion stimulated by 8-chlorophenylthio cyclic AMP. Figure 3 shows that neuropeptide Y completely prevents the stimulatory effect of 8-CPTcAMP. The inhibitory effect of neuropeptide Y on 8-CPTcAMP stimulated chloride secretion was only partially reversible. After neuropeptide Y was removed from the perfusate, chloride secretion rose significantly but did not attain the levels normally seen with 8-CPTcAMP in

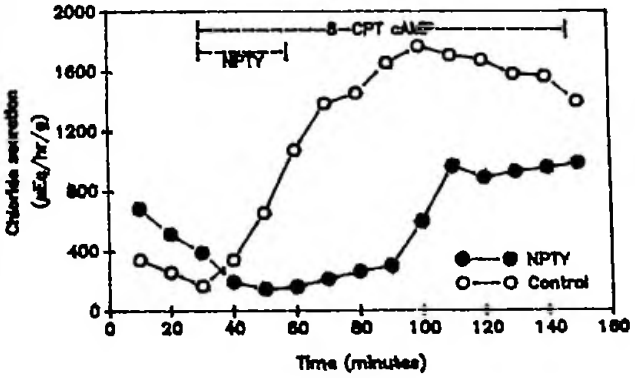

Figure 3. Representative experiments, out of 11 control and 6 experimental, showing the effect of neuropeptide Y on the stimulation of chloride secretion by 8-chlorophenylthio cyclic AMP in perfused rectal glands. After a control period of 30 minutes both glands received 8-CPTcAMP 5×10^{-5} M that was continued for the duration of the experiment. One gland, closed circles, received the simultaneous infusion of neuropeptide Y 5×10^{-8} M that was continued for thirty minutes. Neuropeptide Y completely prevented the normal stimulatory effect of 8-CPTcAMP. The effect was reversible.

the absence of the inhibitor. In additional experiments we found that neuropeptide Y reversibly inhibited the secretion of chloride by 50% when added to the perfusate after chloride secretion had been stimulated with dibutyryl cAMP and theophylline.

Neuropeptide Y appears to exert its inhibitory action directly on rectal gland cells, rather than secondarily via neural release of other

neurotransmitters. Procaine, 10^{-2}M, which blocks neurotransmission, did not prevent the inhibitory action of the peptide in perfused glands. Figure 4 illustrates the reduction (-45%), in a representative experiment, in ouabain-sensitive (transport dependent) O_2 uptake by isolated rectal gland tubules produced when neuropeptide Y was added subsequent to VIP. When added prior to VIP, neuropeptide Y reduced the stimulation in QO_2 normally evoked by VIP (not shown). In the basal, unstimulated state, neuropeptide Y did not significantly reduce further the low level of respiration.

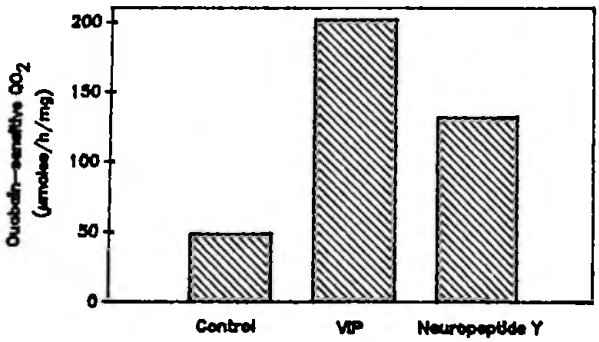

Figure 4. Representative experiment, out of 6, showing the effect of Neuropeptide Y on VIP-stimulated ouabain-sensitive oxygen consumption in isolated rectal gland tubules. VIP caused a four-fold increase in ouabain-sensitive oxygen consumption. Neuropeptide Y, added after VIP, reduced the ouabain-sensitive oxygen consumption by 45%.

The effect of neuropeptide Y on small intestinal secretion is prevented by nifedipine. We perfused rectal glands with nifedipine to determine whether it had a similar effect of on neuropeptide Y inhibition in the rectal gland. Nifedipine completely prevented the effect of neuropeptide Y on chloride secretion stimulated by VIP. Nifedipine alone had no effect on VIP stimulated chloride secretion.

These experiments demonstrate that neuropeptide Y, a widely distributed neurotransmitter, reversibly inhibits stimulated chloride secretion by the rectal gland of the shark. Neuropeptide Y directly inhibited VIP-stimulated transport-dependent oxygen consumption in isolated dispersed rectal gland tubules. Furthermore, the effect of neuropeptide Y in intact perfused glands was not inhibited by procaine, which blocks neurotransmitter release, suggesting that its effect is not mediated by the release of additional neurotransmitters such as somatostatin from nerves within the gland. The site of action of neuropeptide Y within the cell appears to be distal to the generation of cyclic AMP inasmuch it prevents and inhibits the stimulatory effect of exogenous cyclic AMP and forskolin.

This work was supported by grants provided by the USPHS NIHDK18078 and the American Heart Association: Maine Affiliate.