THROMBIN ACTIVATES CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS.

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Thrombin is a serine protease that plays a an important role in the cascade of blood coagulation, transforming fibrinogen into fibrin. It has been conserved throughout the evolution of vertebrates, and thrombin or a closely related molecule is present in the plasma of elasmobranchs. Receptors for this protease are widely distributed among animal cells of all types, and are activated by peptides released by the hydrolytic action of the circulating enzyme on proteins in the plasma membrane. Exposure of cultured human duodenal crypt cells to thrombin stimulates the secretion of chloride (Buresi et al. Am J P 281:G323-32, 2001). In the present experiments we sought to determine if thrombin would stimulate the salt gland of the spiny dogfish shark, *Squalus acanthias*, to secrete chloride.

Shark rectal glands were perfused as described by Silva et al. (Methods in Enzymol. 192:754-66, 1990). Duct fluid was collected at 10 minute intervals in small tared plastic centrifuge tubes and the volume was estimated by weighing. The concentration of chloride was measured by amperometric titration using a Buchler-Cotlove chloridometer. An initial 30-40 minutes of control perfusion allowed the gland to reach a stable basal state. At the end of the control period a bolus of one ml of shark Ringer's solution containing bovine thrombin (Sigma T4648) was infused over one minute, in an amount calculated to deliver thrombin to the gland in a concentration of 5, 12.5, 25 ,or 50 units/ml. Three more 10 minute collection periods were obtained, after which, in some experiments, a one minute bolus of vasoactive intestinal peptide (VIP) 10^{-7}M was injected and collections were continued for another 20-30 minutes.

Thrombin stimulated chloride secretion in a dose dependent manner (Figure 1) with a maximum stimulation at 25 units/ml.

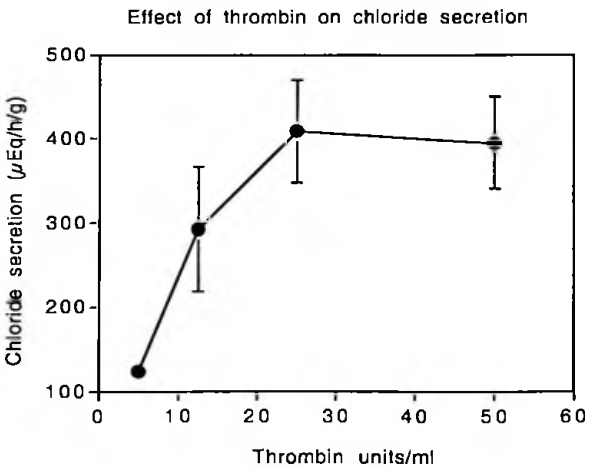

Figure 1. Thrombin stimulation of chloride secretion by the rectal gland. The values are peak chloride secretion after stimulation with thrombin. Each value represents mean±SEM, n= 3,6,16, and 6 for 5,12.5,25, and 50 units/ml, respectively.

Peak chloride excretion was was generally seen during the first 10 minutes after administration, averaging 409±61 μEq/h/g (mean±SEM, n=16) with 25 units thrombin/ml. The increment in chloride secretion stimulated by 25 units/ml averaged 37±7% of that which followed a bolus of 10^{-7}M VIP (n=8), in the same

gland.

In four experiments, the stimulatory effect of 25 units/ml of thrombin was completely prevented by the prior addition to the thrombin bolus of an equal number of units of bovine antithrombin III (Sigma A9141), activated with 500 units of heparin. A similar amount of heparin alone, without antithrombin III, did not prevent stimulation of rectal gland secretion by thrombin (n=6). In 5 experiments, bolus infusions of thrombin-receptor-associated-peptide (TRA, Sigma S-7152), Ser-Phe-Leu-Leu-Arg-Asn-Pro-Asn-Asp-Lys-Tyr-Glu-Pro-Pro, at concentrations varying from 1 to 5 x 10^{-5}M failed to stimulate the gland.

Cytochalasin D (10^{-6}M), when incorporated in the perfusing solution from the start of the experiments, produced virtually complete inhibition of the stimulatory effect of thrombin on the rectal gland secretion (1.4±1.9% of VIP stimulus, n=6).

These experiments suggest the presence of protease-activator-receptors (PARs) on the basolateral membranes of the shark rectal gland. When activated, they appear to stimulate chloride secretion through a pathway blocked by cytochalasin D, thus resembling the cascade initiated by CNP rather than VIP.

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