TMAO has no effect on the secretion of chloride or its stimulation by VIP or CNP in *Squalus acanthias* rectal gland

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The body fluids of *Squalus acanthias* contain in addition to inorganic salts, organic compounds such as urea and trimethylaminoxide (TMAO). The normal concentration of TMAO in the body fluids of *Squalus acanthias* is approximately 70 mM. The effect of TMAO is to prevent the denaturing effect of high concentrations of urea on cellular proteins. The isolated rectal gland of *Squalus acanthias* is generally perfused *in vitro* with shark Ringer's containing inorganic salts and urea, but lacking TMAO. To ascertain whether TMAO is necessary for the normal function of the rectal gland *in vitro*, we compared the secretion of chloride by the isolated rectal gland perfused with and without TMAO under basal conditions and after stimulation with vasoactive intestinal peptide (VIP) or C-type natriuretic peptide (CNP).

Isolated rectal glands of *Squalus acanthias* were perfused with oxygenated shark Ringer's solution at pH 7.6, as previously described.[1] The glands were perfused with shark Ringer's with and without TMAO 70 mM. Duct fluid was collected at 10-minute intervals in small tared plastic centrifuge tubes, and the volume measured every 10 minutes by weighing. The concentration of chloride in the duct fluid was estimated by amperometric titration using a Buchler-Cotlove chloridrometer. An initial thirty minutes of control perfusion (three collection periods) allowed the gland to reach a stable basal state. At the end of this control period a 1 ml bolus of VIP, calculated to deliver a final concentration of 10^{-7}M to the gland, or of CNP calculated to give a final concentration of 5×10^{-7}M was injected directly into the arterial catheter over 1 min. In all experiments with CNP the perfusate contained procaine 10^{-2}M to prevent the release of VIP from the nerves within the gland. The results are expressed as mean ± SEM.

TMAO had no effect on the secretion of chloride by the isolated perfused rectal gland. The basal secretion of chloride was unaffected by its presence or absence (188 ± 36 μEq/h/g without TMAO, n = 17, and 167 ± 51 with TMAO, n = 8, $p > 0.05$). Stimulation of chloride secretion by either VIP or CNP was also not affected by TMAO. Peak stimulation with VIP was 2019 ± 236 without TMAO, n = 9, and 2563 ±402 with TMAO n = 4 ($p > 0.05$). Peak stimulation with CNP was 1518 ± 321 without TMAO, n = 7, and 795 ± 224 with TMAO, n = 4, $p > 0.05$. These experiments suggest that TMAO is not necessary for the functional integrity of the rectal gland *in vitro* during relatively short term experiments.

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1. Silva P, Solomon RJ, and Epstein FH. Shark rectal gland. In: *Methods Enzymol.* Vol 192, p. 754-766, 1990.