Effect of tempol on chloride secretion stimulated by vasoactive intestinal peptide in rectal gland of *Squalus acanthias*

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It has been suggested that small quantities of superoxide, liberated within the vascular smooth muscle cells by certain vasoconstrictors, act as a kind of intracellular messenger to enhance internal calcium release[1]. It seemed possible that superoxide might play a similar role in secretory epithelium. If that were the case, the destruction of superoxide by its dismutase, or by a chemical that mimicked the action of superoxide dismutase, might reduce the intensity of chloride secretion by a well-established agonist. We therefore examined the effect of tempol (4-OH-2,2,6,6-tetramethyl-l-piperidinyloxy, free radical) on chloride secretion stimulated by vasoactive intestinal peptide in the rectal gland of *Squalus acanthias*. Tempol, which enters the cells readily and destroys superoxide has been reported to decrease calcium transients and vasoconstriction produced by some agonists in vascular smooth muscle of sharks and mammals[1,2].

Shark rectal glands were perfused as previously described[3]. Duct fluid was collected at 10-minute intervals in tared plastic centrifuge tubes and the volume assessed by weight. The concentration of chloride in the duct fluid was measured by amperometric titration. All glands were perfused with shark Ringers containing 5 mM glucose and 100 mg/100 ml of bovine serum albumin. After three ten-minute periods to allow the gland to reach a steady state, a continuous infusion containing 10^{-9}M vasoactive intestinal peptide was started and continued for another 60 minutes. In six experiments, 1 mM tempol was added to the perfusate from the start of the experiment. The results were compared with those of six experiments in which tempol was omitted.

There was no significant difference between the chloride secretion induced by an infusion of 10^{-9}M VIP alone and that induced by the same infusion in a gland exposed to tempol, 1 mM (Figure 1). These results suggest that superoxide plays little if any role in amplifying the stimulated secretion of chloride by shark rectal gland.

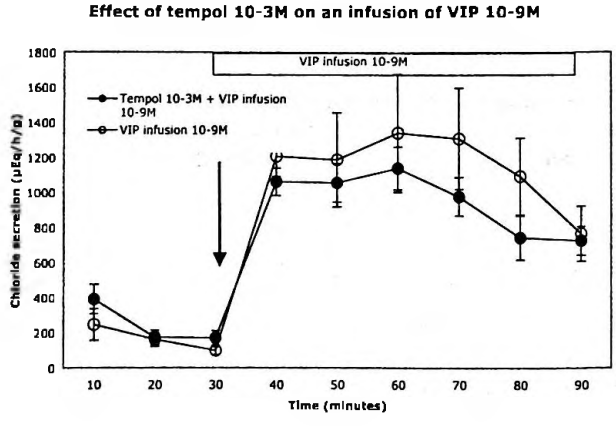

Figure 1. Effect of tempol on the secretion of chloride stimulated by VIP. Tempol, 10^{-3}M, was given from the beginning of the experiment. An infusion of VIP 10^{-}

^{9}M was started thirty minutes after the beginning of the experiment at the time indicated by the arrow. Tempol had no effect on the stimulation of the secretion of chloride induced by VIP. The symbols represent the mean±SEM, n= 6 for both series of experiments.

1. **Fellner S. K. and W. J. Arendshorst**. Angiotensin II, reactive oxygen species, and Ca2+ signaling in afferent arterioles. *Am J Physiol Renal Physiol.* 289:F1012-9, 2005.
2. **Fellner S. K. and L. Parker**. Endothelin-1, superoxide and adeninediphosphate ribose cyclase in shark vascular smooth muscle. *J Exp Biol.* 208:1045-52, 2005.
3. **Silva P., R. J. Solomon, and F. H. Epstein**. Shark rectal gland. *Methods Enzymol.* 192:754-66, 1990.