THE EFFECT OF ORGANOTIN ON THE ISOLATED PERFUSED RECTAL GLAND OF SQUALUS ACANTHIAS PRELIMINARY STUDIES, R. Solomon, L.Dick, M. Taylor, G. Solomon, P. Silva, New York Medical College and Harvard Medical School.

INTRODUCTION: Organotins are widely used as PVC plastic stabilizers, catalysts for silicone rubber, biocides for the control of a variety of organisms from fungi to rodents, and preservatives of marine wood and metals (Piver, WT, Environ Health Perspect 4:61-80, 1973). Organotins find their way into man as a result of diffusion from medical PVC products and absorption from the marine environment into the food chain. The potential medical hazards of these compounds is reflected in the deaths of nearly 100 people in France in 1954 following the use of Stalinon, an anti acne preparation, containing mono, di, and triethyltin iodides. In animal studies, the trialkyltins have been the most toxic. The size of the organic ligand appears to be important in determining the extent and site of toxicity. The trimethyltin and triethyltin compounds produce severe neurotoxicity, the triphenyltin and tributyltin appear less neurotoxic but more immunotoxic. The higher substituted trialkyltins appear to have minimal toxicity (Snoeij NJ, et al, Toxicol Appl Pharmacol 81:274-86, 1985).

We report herein preliminary data on the toxicity of bis (tributyltin) oxide, TBTO, the major compound used in marine paints. The antifoulant properties of this paint result from the gradual leaching of the organotin into the surrounding marine environment. This compound thus enters the food chain and poses a potential hazard to biologic processes. For example, TBTO is known to produce alterations in the eye of the rabbit. Following injection into the corneal sac, erythema and edema appeared within 3 hours followed by hemmorrhage and opacity of the cornea (Pelikan Z. Brit J. Ind. Med 26: 165, 1969). These effects may be interpreted as evidence of membrane damage and alteration in the permeability to proteins.

METHODS: Isolated rectal glands were perfused in vitro with shark Ringer's solution via standard techniques. Rectal glands were first perfused with theophylline 2.5×10^{-4}M and dibutyryl cAMP 5×10^{-5}M to stimulate chloride secretion. Following the control collections during which chloride secretion was stable, TBTO was added in varying concentrations. After stable secretory rates were again observed, the perfusate was changed to the original solution without TBTO. Following these preliminary dose finding studies, rectal glands were perfused under basal conditions, i.e., without theophylline and db CAMP. Three ten minute collections were obtained after which 5 ug rat atriopeptin II (sANP) (Peninsula Labs) in 1 ml of shark Ringer's was given as a bolus into the arterial line. Following an additional 3 ten minute collection periods, 0.5 ug VIP in 1 ml of shark Ringer's was given as bolus into the arterial line. In separate glands, bis (tributyltin) oxide, TBTO, 10^{-10} to 10^{-9} M was added to the perfusate either prior to the start of the basal collections or at the same time that secretagogues were given to increase chloride secretory rate. TBTO was obtained from M and T Chemicals as a 95% solution, dissolved in ethanol and diluted to final concentrations in shark's Ringers solution.

Chloride concentration was determined on a Buchler chloridometer. Chloride secretory rate was calculated from the product of chloride concentration and duct flow rate, corrected for gland weight, and reported as uEq/10 min/gram wet weight. The data was analyzed by paired and unpaired students "t" test.

RESULTS: Table I indicates that concentrations of TBTO between 10^{-7} and 10^{-6}M had little effect on the chloride secretory rate while higher concentrations produced an irreversible inhibition of the chloride secretory rate. Therefore in the subsequent experiments, a TBTO concentration of 10^{-10} to 10^{-9}M was used.

Table 1. **The effect of various concentrations of TBTO on chloride secretory rate (uEQ/10/min/gww) in rectal glands stimulated with theophylline and dibutyryl cAMP.**

CONCENTRATION	BASAL	TBTO	RECOVERY
TBTO 10^{-5}M	1475	320	211
TBTO 5 x 10^{-6}M	1235	639	448
TBTO 10^{-6}M	1423	1673	1683
TBTO 10^{-7}M	1247	1308	1312
TBTO vehicle only	1586	1308	1272

Data are the means of at least 2 perfusions at each concentration.

Both sANP and VIP produced significant increases in the chloride secretory rate in the rectal gland perfused in the absence of TBTO (Table 2). The VIP stimulatory effect was significantly greater than that for sANP. This observation contrasts with the equimolar potency described by us previously (Solomon, R., et al, Amer. J. Physiol. 249:R348-354, 1985). All the experiments reported here were conducted using the same batch of sANP. We subsequently employed other batches of sANP from the same supplier and noticed enhanced efficacy of the peptide.

In the presence of TBTO, inhibition of the sANP stimulatory effect on chloride secretion was observed although the VIP effect remained unchanged. Because the absolute magnitude of the stimulatory effect of sANP was attenuated already (vide supra), caution must be exercised in interpeting this finding despite its statistical significance.

Table 2. **The effects of sANP and VIP on the chloride secretory rate (uEq/10 min/gww) of the perfused rectal gland.**

Condition	Basal	ANP	P value	VIP	P Value
TBTO- (n=8)	118±48	337±81	<.005	1221±212	<.01
TBTO+ (n=10) (10^{-9} - 10^{10}M)	134±23	248±87	NS	1333±101	<.005

Values are mean ± SEM, P values by paired "t" test compared to basal period.

A biologic effect, however, is suggested by the additional observation that chloride concentration in the duct fluid falls progressively in the absence of stimulation. As can be seen in Figure 1, both sANP and VIP led to an increase in chloride concentration in the absence of TBTO. This presumably reflects membrane effects of these drugs to enhance chloride transport into the duct lumen. In the presence of TBTO, the initial chloride concentration in duct fluid is significantly lower than in the absence of TBTO. This alone suggests that TBTO has some effect on the membrane of the chloride secreting cells. Furthermore, in the presence of

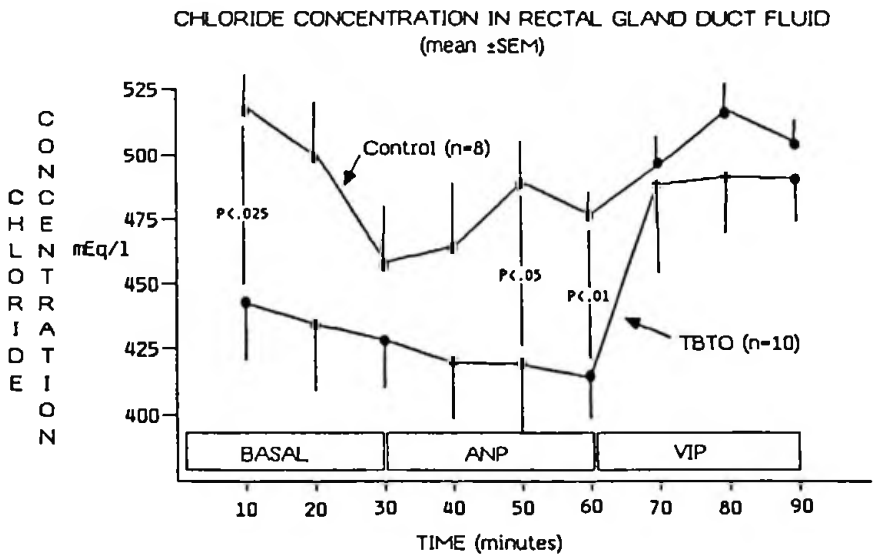

Figure 1. The effect of perfusion with TBTO (10^{-10} - 10^{-9}M) on the chloride concentration of the duct fluid of the in vitro rectal gland perfused under basal conditions and stimulated with sANP and VIP.

TBTO, sANP fails to increase duct chloride concentration although the ability of VIP to raise chloride concentration is unimpaired. The similarity of the VIP effects in the presence and absence of TBTO suggest that the TBTO effect is not a non-specific toxic effect on the cells.

DISCUSSION: Organotins are known to interfere with a number of membrane related metabolic processes including mitochondrial oxidative phosphorylation (Aldrige, W.N., Biochem J., 61:406-418, 1955), PGE stimulated cAMP production in thymus cells (Snoeij, N.J., Toxicology 39:71-83, 1986), and pancreatic islet cell insulin release (Manabe S. Diabetes 30:1013-1021, 1981). We chose to look at the effect of TBTO on another membrane dependent process, the active transport of chloride, in a model in which the transport process could be manipulated acutely by a number of secretagogues. The doses of TBTO chosen for study were based upon the reported levels of organotin in the marine environment of 10^{-10} to 10^{-9}M (U'ren S., Marine Pollution Bull 14:303-306, 1983) and the lack of toxicity on secretion in glands stimulated with db CAMP. Our results suggest that TBTO, in these nanomolar concentrations, interferes with the stimulation of transport following sANP but not VIP. This result is particularly interesting because the mechanisms of action of sANP and VIP appear to be different. VIP acts directly on the membrane of the chloride secreting cell and stimulates chloride secretion by receptor mediated activation of adenylate cyclase. In contrast, the available evidence suggests that sANP has an indirect effect to release VIP from neuronal stores within the gland. Thus sANP does not have effects on the membranes of the chloride secreting cells themselves but rather on the membranes of the neuronal cells within the gland. Thus the inhibitory effects of TBTO observed in this study are consistent with the hypothesis that TBTO has predominantly a neurotoxic effect.