PRELIMINARY DATA ON INTRACELLULAR SIGNALLING MECHANISMS IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: A PHARMACOLOGIC APPROACH

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The rectal gland of Squalus acanthias increases chloride secretion in response to a number of extracellular agonists, including vasoactive intestinal peptide (VIP), adenosine, and C-type natriuretic peptide (CNP). The intracellular events which mediate the stimulatory response are not fully known. A role for cyclic nucleotides, protein kinases, phospholipases, and calcium have all been hypothesized. We attempted to use a variety of inhibitors of these potential intracellular mediators to further characterize the events which follow stimulation with VIP and CNP. The role of intracellular calcium is described in a separate abstract.

Rectal gland tubules were freshly prepared as previously described (Silva et al., Am. J. Physiol. 265:R439, 1993). The tubules were kept on ice and used within 4 hours of preparation. Aliquots containing 5-10 µg wet weight of tubules were diluted into 2 ml of shark Ringer's buffered with HEPES and containing glucose (5 mM), pyruvate (10 mM) and acetate (10 mM) as metabolic substrates. Oxygen consumption (QO_2) was continuously monitored in a temperature controlled chamber (15 °C) using a Clark-type electrode. After basal QO_2 was established, the experimental inhibitor was added in a volume of 1-20 µl followed 5 minutes later by the secretory agonist, either VIP or CNP. After exposure to the agonist, ouabain (10^{-3}M) was added. Ouabain-inhibitable QO_2 was defined by subtraction of the QO_2 obtained in the presence of ouabain from the values obtained in the presence of the agonist ± the inhibitor.

A dose response curve for VIP and CNP was determined. We tested various inhibitors against both submaximal and maximal doses of either agonist. We determined QO2 simultaneously in 4 oxygen chambers one of which was a control experiment (i.e. agonist alone) to ensure the viability of the tubules.

A role for protein kinase A (PKA) in both the action of VIP and CNP is supported by the effects of H89 though KT5720 failed to have an effect. Likewise, a role for protein kinase C (PKC) is suggested by the effects of DHCP to inhibit maximal secretory responses to both VIP and CNP though staurosporin failed to produce any inhibition at 10^{-8}M. A possible role for protein kinase G (PKG) is also suggested by the effects of RpcGMP to inhibit the response to a submaximal dose of CNP.

This pharmacologic approach suffers from a lack of specificity of the inhibitors available for study. Additionally, K_i's reported in mammalian cells may not be applicable to the rectal gland of Squalus acanthias. For these reasons, the lack of an effect of an inhibitor in these studies is difficult to interpret. The inhibitor may have failed to adequately inhibit the target signalling mechanism or the mechanism may not play a crucial role in the secretory response. Thus, we are cautious in interpreting the above results.

Table 1. Effect of inhibitors of intracellular signalling mechanisms postulated to mediate an increase in chloride secretion in the rectal gland of Squalus acanthias. The increase in ouabain inhibitable oxygen consumption, μMO_2/min*mg (mean±sem) for different doses of either VIP or CNP and the effects of inhibitors are depicted (n=number of tubule preparations). * $p<.05$ by t test compared to agonist alone.

AGONISTS			$5x10^{-9}$	$1x10^{-8}$	$5x10^{-8}$	$1x10^{-7}$	$5x10^{-7}$	$1x10^{-5}$
VIP			124±36 (11)	177±21 (27)	203±18 (16)	270±32 (8)	209±33 (6)	
CNP			54±7 (18)	62±8 (39)	137±17 (16)	127±13 (30)		
INHIBITORS	Dose							
PKC Inhibitor								
Staurosporin	$1x10^{-8}$	VIP		287±38 (6)	145±31 (3)		260±21 (5)	
		CNP		71±15 (16)		101±46 (4)		
DHCP	$8x10^{-6}$	VIP			37±16 (6)*	20±9 (6)*		
		CNP			30±6 (6)*			
	$8x10^{-7}$	VIP		195±78 (5)				
		CNP		79±31 (6)				
PKA Inhibitor								
KT5720		VIP					232±44 (5)	
		CNP			76±3 (2)	113±13 (4)		
H89	$1x10^{-5}$	VIP			97±22 (8)*	68±33 (4)*	139±29 (6)	
		CNP				17±6 (7)*		
	$1x10^{-6}$	VIP		171±1 (2)				
		CNP			40±12 (5)*	61±32 (5)		
	$1x10^{-7}$	VIP		185±17 (3)	241±29 (3)			
PKG Inhibitor								
RpcGMP	$4x10^{-5}$	CNP		31±10 (9)*	133±22 (5)			

Supported by grants from Maine Heart Association, NIEHS, and EPSCoR.