

SCA 125

VASOPRESSIN MEDIATED POTENTIATION OF ADRENERGIC VASOCONSTRICTION IN THE RAT MESENTERY.

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Background: Vasopressin (VP), a potent vasoconstrictor peptide released by the posterior pituitary, mediates vasoconstriction by activating G-protein coupled VP receptors (V1a), Phospholipase C (PLC) resulting in intracellular Ca²⁺ release. Moreover, VP also potentiates the vasoconstrictor effects of catecholamines at concentrations of the former that have no intrinsic pressor effects. This effect of VP has important therapeutic implications in pathophysiologic conditions associated with vasodilatory shock and catecholamine refractoriness as occurs in septic shock, cardiopulmonary bypass, and following ventricular assist device placement.

Introduction: The cellular mechanisms responsible for the potentiating effects of VP on adrenergic signaling remain unclear. Recent studies investigating the role of K⁺ channels have yielded potentially conflicting results. Electrophysiological studies indicate that K_{ATP} channels are important while data from cultured smooth muscle cells suggest that K⁺ rectifier channels (K_v) are also important. In our study we hypothesize that VP inhibits K_{ATP} channels and/or K_v channels. K⁺ channels are modulated following activation of VP receptors by signaling pathways that involve G protein coupling to PLD and distally include protein kinase C (PKC), the src family of kinases, PYK2 and tyrosine kinase (TK) as highlighted in figure 1. We used a vascular ring ex-vivo bioassay to investigate the mechanisms and signaling pathways underlying this VP effect.

Methods: Ring Physiology Experiments:

Wistar rats, 2-4 months of age, were anesthetized. The superior mesenteric artery was excised and immediately placed in cold Krebs solution and the surrounding connective tissue was cleaned. Rings were prepared for isometric recording. Changes in tension were recorded online using MacLab system and analyzed using Dose Response (D-R) Software (AD Instruments, MA).

Each artery ring was placed in a 5ml bath filled with Krebs, and the solution was continuously bubbled with 95% O₂ and 5% CO₂, to provide a physiological pH of approximately 7.4. Temperature was maintained at 37C. The rings were first stretched in 100mg increments to 600 mg (resting tension for maximal force development), for an hour and were allowed to equilibrate. 60Mm KCl was added to the bath to determine maximal contraction of smooth muscle tissue. D-R curves were constructed for VP. The threshold response

concentration was determined to be 10⁻⁹M. The rings were washed and then D-R curves were constructed, for NE in the presence and absence of VP (10⁻⁹M) and again repeated following Glybenclamide (GB) (a K_{ATP} channel inhibitor) (10⁻⁶M) administration. The role of Protein Kinase C (PKC), PYK2, and K_{ATP} channels on VP-induced augmentation of NE responses was studied by testing the effects of appropriate antagonists on these responses.

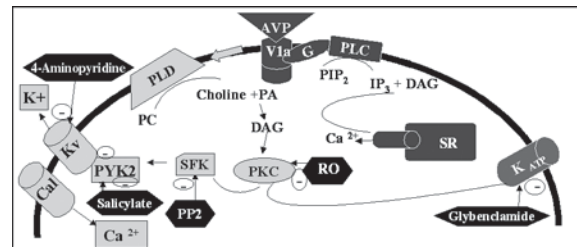


Fig 1: Schematic diagram showing the two hypothetical pathways for Vasopressin stimulated contractions in vascular smooth muscles. The first whereby binding of physiological concentrations of Vasopressin to vasopressin receptors activates a cascade of events including activation of PLC, PKC, an Src family kinase, and PYK2. PYK2 activation leads to tyrosine phosphorylation and the inhibition of current through the delayed rectifier K⁺ channels (K_v). This results in membrane depolarization and firing of action potentials involving Ca²⁺ influx via L-type voltage-sensitive Ca²⁺ channels (Cal). It has also been hypothesized that vasopressin modulates the activity of the ATP-sensitive K⁺ channels causing inhibition of the K_{ATP} channels through a mechanism that involves Protein Kinase C. Low concentrations of Vasopressin activate PLC and release of calcium from the sarcoplasmic reticulum (SR).

Results and Discussion: VP (10⁻⁹M – 10⁻⁴M) caused concentration-dependent contractions in rat mesenteric rings. VP potentiated NE induced contractions (The contraction induced by NE 10⁻⁵M was increased by 75% by VP). GB simulated the potentiating effect of VP on the NE dose-response relationship. Both Ro-31-8220 (RO) (PKC inhibitor) and Genistein(GN) (TK inhibitor) attenuated the NE response and the augmented NE response observed in the presence of VP (The contraction induced by NE 10⁻⁵M were attenuated 100% and 100%, and the VP-induced augmentation was attenuated by 95% and 90% by RO and GN respectively). Salicylate (PYK2 inhibitor) inhibited only the VP augmented contractions (by 75%) and did not effect the NE response. These results indicate: 1) that VP augments NE contractions by mechanisms that involve both PKC and TK; 2) PYK2 is important in mediating the effect of VP; 3) K_{ATP} sensitive channels may also play a role in the VP response.