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Research Report

Vasopressin-induced translocation and proteolysis of protein kinase Cα in an amphibian brain: Modulation by corticosterone

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ABSTRACT

In urodele amphibians, the hypothalamic neuropeptide arginine vasotocin and the adrenal steroid corticosterone interact to regulate reproductive behavior by actions in the brain. The present study investigated signal transduction pathways underlying acute effects of vasotocin and corticosterone, presumably mediated via "non-genomic" steroid action, in an amphibian brain. We used Western blot to examine the effects of corticosterone and the vasotocin receptor agonist arginine vasopressin, alone and in combination, on the subcellular localization and proteolytic processing of protein kinase C-alpha (PKCα) in tiger salamander brain tissue. Treatment of whole brain minces with vasopressin or vasotocin led to increases in PKCα in membrane fractions and concurrent decreases in PKCα in cytosolic fractions. Vasopressin or vasotocin treatment also induced the appearance in membrane and cytosolic fractions of a PKCα-immunoreactive band that corresponds to PKMα, the proteolytically generated, free catalytic subunit of PKCα. Treatment with corticosterone alone had no consistent effect on either PKCα or PKMα in either fraction. However, pretreatment with corticosterone reliably blocked vasopressin-induced increases in cytosolic PKMα. These data provide new information about the cellular mechanisms of action of vasopressin and corticosterone in the vertebrate brain and suggest a cellular mechanism by which the two hormones interact to regulate neuronal physiology and behavior.

1. Introduction

The neuropeptide hormone arginine vasotocin, the nonmammalian homologue of vasopressin, and the adrenal steroid hormone corticosterone play important roles in the acute regulation of vertebrate behavior (Rose and Moore, 2002; Goodson and Bass, 2001; Orchinik et al., 2002). In the urodele amphibian *Taricha granulosa*, the two hormones interact to regulate male reproductive behavior via actions in the brain (Coddington and Moore, 2003; Rose et al., 1995). Vasotocin increases the sensitivity of specific hindbrain neurons to reproductive stimuli, leading to enhanced courtship clasping of female *Taricha* by males (Moore and Miller, 1983; Rose et al., 1995). Corticosterone decreases the sensitivity of hindbrain

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Abbreviations: CORT, corticosterone; IOD, integrated optical density; IP_3, inositol 1,4,5 trisphosphate; MTr, mesotocin receptor; VTr, vasotocin receptor

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neurons to clasp-eliciting stimuli and rapidly inhibits clasping, an action mediated via a neuronal membrane-associated corticosteroid receptor (Orchinik et al., 1991). These actions probably underlie the ability of corticosterone to block the effects of vasotocin on behavior and neuronal function (Orchinik et al., 1991; Rose et al., 1995; Rose and Moore, 1999). The present study examines signal transduction pathways activated by vasotocin and corticosterone in the amphibian brain in an attempt to better understand cellular mechanisms underlying interactions between the two hormones in the acute regulation of behavior.

In amphibians, vasotocin and vasopressin bind to receptors similar to mammalian V_1 and/or V_2 type vasopressin receptors (Boyd and Moore, 1990; Larcher et al., 1992), as well as to the mesotocin receptor (MTr) (Acharjee et al., 2004). Behavioral effects of vasotocin appear to be mediated by a neuronal V_1-like receptor (Boyd and Moore, 1990). In mammals, V_1 and V_2 receptors preferentially activate phospholipase C (PLC) and adenylate cyclase (AC), respectively (Sabatier et al., 1998; Sabatier et al., 2004). Acharjee et al. (2004) demonstrated that amphibian vasotocin receptors (VTr) can activate both AC and PLC signaling pathways, while MTr preferentially activates PLC signaling (Acharjee et al., 2004). Vasotocin and vasopressin activate PLC and protein kinase C (PKC) in amphibian kidney cells (Larcher et al., 1992; Kohno et al., 2003; Ali et al., 1998), but little is known about signal transduction pathways activated by VTr in the amphibian brain.

In amphibian brain tissue, corticosterone binds to both cytosolic and membrane-associated receptors. The cytosolic receptor is similar to the mammalian glucocorticoid receptor, GR (Orchinik et al., 2000). The neuronal membrane receptor, which has been described in brain tissue from the tiger salamander (*Ambystoma tigrinum*) as well as the newt, is pharmacologically distinct from the cytosolic receptor. Specifically, dexamethasone, a potent GR agonist, and RU486, a potent GR antagonist, bind the amphibian cytosolic receptor, but not to the membrane-associated receptor (Orchinik et al., 2000; Orchinik et al., 1991). Rapid, "non-genomic," effects of corticosterone on *Taricha* neurons and behavior are mediated by the membrane-associated receptor (Orchinik et al., 1991), but the signal transduction pathways activated by this receptor are unknown.

Studies in other species suggest that PKCs play important roles in mediating rapid effects of corticosterone in the brain (ffrench-Mullen, 1995; Han et al., 2002), as well as the effects of corticosterone (He et al., 2003; Qiu et al., 2003; Orchinik et al., 2002) and aldosterone (Boldyreff and Wehling, 2004) in peripheral tissue. In addition, glucocorticoids rapidly modulate responses to neuropeptides and neurotransmitters that activate PKC signaling (Kasai and Yamashita, 1988; Ouyang and Wang, 2000; Shipston and Antoni, 1991). Activation of the classical PKC isoforms (PKCα, βI, βII, and γ) can occur by two related mechanisms. In the most-studied model of PKC activation, receptor-induced increases in concentrations of IP_3 and diacylglycerol lead to changes in the subcellular distribution of PKC, with inactive PKC residing mainly in the cytosol, and active PKC moving, at least transiently, to cellular membranes (Newton, 2003). However, an additional step associated with PKC activation can also occur. A protease-sensitive site separating the C-terminal catalytic from the N-terminal regulatory domain of PKC is exposed upon PKC activation and may be cleaved by the Ca^{2+}-dependent neutral protease, calpain (Pontremoli et al., 1990). Calpain-mediated cleavage at this site generates a free C-terminal catalytic domain, termed PKM. PKM is a cofactor-independent kinase which appears as a band of approximately 45 kDa molecular weight in Western blots using antibodies directed against the C-terminus of classical PKC isoforms (Shea, 2000). Recent reports suggest important roles for certain PKM isoforms in neuronal plasticity (Touyarot et al., 2002; Sutton et al., 2004; Muslimov et al., 2004; Serrano et al., 2005; Sajikumar et al., 2005; Ling et al., 2006).

Based on the interaction between corticosterone and vasotocin in the regulation of amphibian reproductive behavior, we tested the hypothesis that vasotocin receptors activate neuronal PKC signaling and that corticosterone acutely modulates vasotocin- or vasopressin-induced PKC activation in salamander brains. We examined the actions of the two hormones, alone and in combination, on the subcellular localization of PKCα, and the appearance of PKMα in acutely prepared tiger salamander (*A. tigrinum*) brain tissue.

2. Results

In preliminary studies, PKCα-, β-I-, β-II-, γ-, and μ-immunoreactive bands were all detected in Western blots of salamander brain tissue (data not shown). The reported studies focus on PKCα. Two main PKCα-like immunoreactive bands appeared in Western blots of salamander and rat (positive control) brain tissue (Fig. 1): full-length PKCα appeared as a band of approximately 80 kDa molecular weight, while putative PKMα appeared in both species as a PKCα-immunoreactive band of ~45 kDa. Neither band appeared in blots incubated in the absence of primary antibody or in blots incubated with primary antibody preadsorbed with a peptide

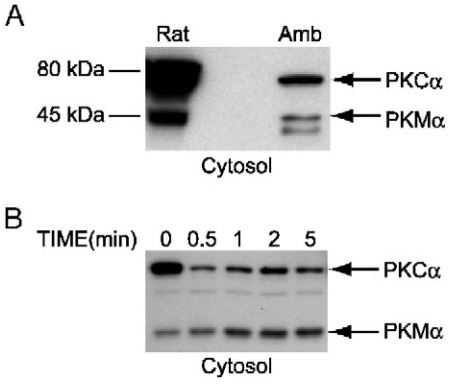

Fig. 1 – PKCα and PKMα immunoreactivity in rat and salamander brain tissue. (A) Western blotting revealed distinct PKCα-immunoreactive bands of approximately 80 kDa (PKCα) and 45 kDa (PKMα) in cytosol prepared from untreated rat and salamander (Amb) brain tissue. (B) Vasopressin alters PKCα and PKMα in amphibian brain cytosolic fractions: time course. Brain minces prepared from whole salamander brains ($n=5$) were pooled and treated with 1 μg/mL vasopressin for the indicated times.