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Arthropod D_2 receptors positively couple with cAMP through the Gi/o protein family

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Abstract

The pyloric network is an important model system for understanding neuromodulation of rhythmic motor behaviors like breathing or walking. Dopamine (DA) differentially modulates neurons within the pyloric network. However, while the electrophysiological actions of DA have been well characterized, nothing is known about the signaling events that mediate its effects. We have begun a molecular characterization of DA receptors (DARs) in this invertebrate system. Here, we describe the cloning and characterization of the lobster D_2 receptor, $D_{2\alpha Pan}$. We found that when expressed in HEK cells, the $D_{2\alpha Pan}$ receptor is activated by DA, but not other monoamines endogenous to the lobster nervous system. This receptor positively couples with cAMP through multiple Gi/o proteins via two discrete pathways: 1) a Gα mediated inhibition of adenylyl cyclase (AC), leading to a decrease in cAMP and 2) a Gβγ-mediated activation of phospholipase Cβ (PLCβ), leading to an increase in cAMP. Alternate splicing alters the potency and efficacy of the receptor, but does not affect monoamine specificity. Finally, we show that arthropod D_2 receptor coupling with cAMP varies with the cellular milieu.

Keywords: Central pattern generator; Crustacean; G protein coupled receptor; Heterologous expression; Signal transduction; Stomatogastric

1. Introduction

The crustacean stomatogastric ganglion is extensively used as a model to understand neuromodulatory effects on motor pattern generation (Nusbaum and Beenhakker, 2002; Harris-Warrick and Marder, 1992). A wealth of information exists on the monoaminergic modulation of ion currents and neuronal firing properties (Flamm and Harris-Warrick, 1986; Harris-Warrick et al., 1995b,a, 1998; Kloppenburg et al., 1999; Johnson et al., 2003), but nothing is known about the transduction cascades mediating these effects. To extend the usefulness of this model system and gain insight into how component neurons integrate biochemical and electrical processes, we have begun a molecular characterization of DARs in this central pattern generator (CPG).

In the traditional view, DARs are classified as type-1 or type-2: type-1 DARs couple to Gs proteins, leading to a Gα mediated increase in [cAMP]i and protein kinase A (PKA) activity, while type-2 DARs couple to Gi/o proteins to decrease [cAMP]i and PKA activity (Missale et al., 1998; Neve et al., 2004). It is now clear that this traditional view of DAR signaling is much too simple. First, DARs have been shown to couple with multiple G proteins in various heterologous and native systems (Kimura et al., 1995a,b; Sidhu et al., 1998; Zheng et al., 2003; O'Sullivan et al., 2004; Zhen et al., 2004). Moreover, GPCRs, including DARs, can switch G protein coupling over time in response to constant agonist application (Daaka et al., 1997; Baillie et al., 2003; Lezcano et al., 2000). Second, both the Gα and Gβγ subunits are known to mediate individual responses (Cabrera-Vera et al., 2003). Third, activated G protein subunits can directly interact with target proteins such as ion channels without altering second messenger levels (Dascal, 2001; Ivanina et al., 2004). Fourth, GPCRs are known to interact

Abbreviations: CPG, central pattern generator; GPCR, G protein coupled receptor; DA, dopamine; DAR, dopamine receptor; PKA, protein kinase A; PP2A, protein phosphatase 2A; PTX, pertussis toxin; Et-18-OCH_3, 1-*O*-Octadecyl-2-*O*-methyl-rac-glycero-3-phosphorylcholine; IBMX, 3-isobutyl-1-methylxanthine; FSK, forskolin; 5-HT, serotonin; OCT, octopamine; TYR, tyramine; HIS, histamine; AC, adenylyl cyclase; PLC, phospholipase C.

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directly with target proteins. For example, DARs can physically interact with, and activate ionotropic glutamate receptors (Zou et al., 2005; Lee and Liu, 2004; Pei et al., 2004; Liu et al., 2000). Fifth, GPCRs can activate additional cascades, like the mitogen activated protein kinase (MAPK) cascade via crosstalk (Werry et al., 2005). Finally, GPCRs can directly activate G protein independent cascades. One important mechanism involves recruitment of β-arrestin scaffolds to an activated receptor and subsequent stimulation of G protein independent cascades (Lefkowitz and Shenoy, 2005). In this regard, it was recently shown that the D_2 receptor modulates locomotor activity in mice via a β-arrestin 2-mediated signaling complex involving Akt and PP2A, as well as by traditional G protein cascades (Beaulieu et al., 2005).

There are three arthropod DARs: two type-1 receptors and one type-2 receptor (Gotzes et al., 1994; Feng et al., 1996; Han et al., 1996; Hearn et al., 2002; Blenau et al., 1998; Beggs et al., 2005; Mustard et al., 2003). A fourth arthropod receptor that responds to DA with an increase in cAMP has been cloned, but it is primarily activated by ecdysteroids and does not appear to belong to the DAR family (Srivastava et al., 2005). We have previously cloned and characterized the two type-1 DARs from the spiny lobster (Clark and Baro, 2006). Here we describe the cloning and characterization of the lobster D_2 receptor, $D_{2\alpha Pan}$.

2. Materials and methods

2.1. Cloning and expression in a heterologous system

The lobster $D_{2\alpha Pan}$ cDNA was cloned from nervous tissue of *Panulirus interruptus* using a degenerate PCR strategy with conventional library screening and RACE technology as previously described (Clark et al., 2004). The $D_{2\alpha.1Pan}$ sequence has been submitted to Genbank under accession number DQ900655 (Fig. 1). Full length constructs were created and inserted into a pIRESneo3 vector (B.D. Biosciences Clontech, Palo Alto, CA) using standard recombinant techniques. $D_{2\alpha Pan}$ and *Am*Dop3 constructs were stably expressed in HEK293 cells using methods previously described (Clark et al., 2004). *Am*-Dop3 was kindly provided by Dr. Allison Mercer, University of Otago. All tissue culture reagents were purchased from Invitrogen except the DMEM and the penicillin streptomycin solution (American Type Culture Collection), and the neomycin (Sigma).

In some experiments, the Gβγ scavengers, dexras1 (UMR cDNA resource center, University of Missouri-Rolla) or $\beta ARK_{495-689}$ (kindly provided by Dr. Robert Lefkowitz, Howard Hughes Medical Institute), were transiently expressed. In these cases, cells were maintained in DMEM supplemented

Fig. 1. The DAR family is conserved across arthropods. The *Panulirus* (lob), *Drosophila* (fly), *Apis* (bee), and human (hum) DAR proteins are aligned. Amino acids that are identical are highlighted. Black bars approximate the seven transmembrane regions. The points of alternate splicing on lobster DARs are indicated by black arrowheads. The accession numbers are as follows: lobster: DQ900655; fly: AAN15955; bee: NP_001014983; human: NP_000786.