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# NEUROPEPTIDE Y MODULATES EXCITATORY SYNAPTIC TRANSMISSION IN THE OLFACTORY BULB

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**Abstract—Although the olfactory bulb contains one of the highest concentrations of neuropeptide Y in the CNS, its function in the bulb remains unclear. In this study, we used whole-cell electrophysiological, molecular, and primary culture techniques to investigate neuropeptide Y gene expression and neuromodulatory actions of neuropeptide Y on rat olfactory bulb neurons. Northern analysis showed that neuropeptide Y mRNA increases with animal age or time in culture, in a parallel manner. In electrophysiology experiments, agonists that activate neuropeptide Y receptors (whole neuropeptide Y) and the Y2 receptor subtype (neuropeptide Y 13-36) reduced spontaneous excitatory activity in bulb interneurons. In investigating potential presynaptic effects, both agonists reduced the amplitude of calcium channel currents in the presynaptic (mitral/tufted) cell. Also consistent with a presynaptic effect, both agonists reduced the frequency but not the amplitude of miniature excitatory postsynaptic currents (or "minis") in interneurons. In examining potential postsynaptic effects, both agonists slightly increased membrane resistance but had no effect on currents evoked by glutamate. Together, these data suggest that neuropeptide Y inhibits excitatory neurotransmission between olfactory bulb neurons via a presynaptic effect on transmitter (glutamate) release. © 2005 Published by Elsevier Ltd on behalf of IBRO.**

**Key words: NPY, presynaptic, glutamate, gene expression, olfaction.**

Neuropeptide Y (NPY) is one of the most abundant and widely distributed neuropeptides in the peripheral nervous system and CNS (Tatemoto et al., 1982; Adrian et al., 1983; Chronwall et al., 1985). It plays several key physiological roles, including regulation of food and water intake (Clark et al., 1984; Stanley and Leibowitz, 1984), hormone secretion (Kalra and Crowley, 1984, 1992), neural excitability (Colmers et al., 1985; Colmers and Bleakman, 1994), and memory processing (Flood et al., 1987; Howell et al., 2003). NPY also has been implicated in a variety of disease states including depression, anxiety, pain, seizures, intestinal dysfunction, and cardiovascular disease (see Gehlert, 1998; Balasubramaniam, 2002; Redrobe et al., 2002b; Thorsell and Heilig, 2002).

NPY gene and peptide expression has been investigated in a variety of species including humans and rats. Typically, the NPY gene is expressed in neurons in which NPY is located and NPY transcript and peptide levels are highly correlated (Caberlotto et al., 1999; Thorsell and Heilig, 2002). Of potential significance to olfaction, the rat olfactory bulb (OB) contains more NPY mRNA than most other brain regions (Larhammar et al., 1987; Rutkoski et al., 1999). In addition, NPY-like immunoreactivity has been observed in several layers and cell types in the OB (Gall et al., 1986; Scott et al., 1987). Therefore, one objective of this study was to use Northern analysis to quantify levels of NPY mRNA in the rat OB. As others have reported age-related changes in the number of neuropeptide Y-immunoreactive (NPY-IR) neurons in the rat main OB (Won et al., 2000), we also investigated the developmental regulation of OB NPY mRNA.

In addition to examining NPY gene expression, some investigators have examined potential sources of NPY in the OB. One potential extrinsic source of NPY is from the locus ceruleus. Bouna et al. (1994) used a combination of autoradiography of [$^3$H]NA retrograde labeling with immunodetection of NPY to demonstrate that some scattered locus ceruleus noradrenergic neurons (about 26%) projecting to the OB contain NPY. In regard to intrinsic sources, several immunocytochemical studies have examined NPY expression by OB neuron subtypes. In one study, two types of short axons (SA) cells that express NPY-like immunoreactivity were identified. The first was a deep SA cell that lies deep in the granule cell layer (GCL), with dendrites lying parallel to that layer (Scott et al., 1987). The second was a superficial SA cell with dendrites that lie within the periglomerular region and in the superficial external plexiform layer (EPL) of the OB, generally lying parallel to the glomerular layer (Scott et al., 1987). Recent evidence suggests that SA cells send interglomerular axons over long distances to form excitatory synapses with inhibitory periglomerular (PG) cells as far as 20–30 glomeruli away (Aungst et al., 2003).

NPY mediates its effects at five receptor subtypes, which have been designated as Y1, Y2, Y4, Y5 and y6 (Blomqvist and Herzog, 1997; Michel et al., 1998). In various brain regions (e.g. hippocampus, thalamus, hypothalamus), NPY has been shown to inhibit neural activity via presynaptic and/or postsynaptic effects (Colmers et al., 1985, 1991; Greber et al., 1994; McQuiston et al., 1996; van den Pol et al., 1996; Rhim et al., 1997; Silva et al.,

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*Abbreviations:* EPL, external plexiform layer; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GCL, granule cell layer; JG, juxtaglomerular; MEM, Minimal Essential Medium; mEPSCs, miniature excitatory postsynaptic currents ("minis"); M/T, mitral and tufted cells; NPY, neuropeptide Y; NPY-IR, neuropeptide Y immunoreactive; NPY 13-36, preferential agonist for neuropeptide Y2 receptor; OB, olfactory bulb; PG, periglomerular cells; SA, short axon; TTX, tetrodotoxin; Y1R-LI, Y1 receptor-like immunoreactivity.

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2001; Sun et al., 2001). These effects have been mediated by receptors including Y1 (McQuiston et al., 1996; Rhim et al., 1997; Sun et al., 2001) and Y2 (Bleakman et al., 1991; Colmers et al., 1991; Greber et al., 1994; Silva et al., 2001; Sun et al., 2001).

Various means have been used to examine the expression of NPY receptor subtypes in the OB. Autoradiography and immunocytochemical studies indicate that the rat OB expresses Y1 and Y2 (Dumont et al., 1996a,b; Migita et al., 2001; Kopp et al., 2002), Y5 (Dumont et al., 1998), and, potentially, Y4 (Dumont and Quirion, 2000). Y1 and Y2 have been localized to a number of bulb layers including the glomerular layer, EPL, internal plexiform layer, and GCL (Dumont et al., 1996a,b; Migita et al., 2001). Other evidence suggests that Y5 and potentially Y4 are expressed in the EPL (Dumont et al., 1998; Dumont and Quirion, 2000). Therefore, another objective of this study was to use whole-cell electrophysiology to investigate the effects of agonists and antagonists for NPY receptor subtypes on neurotransmission in the OB.

## EXPERIMENTAL PROCEDURES

### Tissue culture

The procedure for preparing primary cultures of OB neurons is described in detail elsewhere (Trombley and Blakemore, 1999). Briefly, OBs were harvested from P1–P5 Sprague–Dawley rat pups, cut into 1-mm cubes, and enzymatically treated in a calcium-buffered papain solution for 1 h at 37 °C. The tissue was then triturated with a fire-polished pipette until a single-cell suspension was achieved. The cells were plated in 35-mm culture dishes on a confluent monolayer of previously prepared OB astrocytes at a density of approximately 250,000 cells per dish.

The neuronal media consisted of 95% Minimal Essential Medium (MEM, Gibco, Carlsbad, CA, USA), 5% horse serum (Gibco), 6 g/l glucose, and a nutrient supplement (Serum Extender, Collaborative Research, Waltham, MA, USA). Astrocyte layers were obtained by plating a suspension of OB cells in a 75-$cm^2$ flask containing 90% MEM, 10% fetal calf serum, and 6 g/l glucose. Once confluent, the cells were treated with 0.125% trypsin, triturated to produce a single-cell suspension, and plated onto 35-mm dishes coated with poly-L-lysine (30,000–70,000 MW, 10 $\mu$g/ml, Sigma, St. Louis, MO, USA). Cytosine-$\beta$-D-arabinofuranoside ($10^{-5}$ M; Sigma) was added to the media 1 day after neuronal plating to prevent the overgrowth of astrocytes.

### Neuronal identity

Previously established morphological, physiological, and immunohistochemical criteria were used to identify and differentiate presumptive mitral and tufted cells (M/T) and interneurons (Trombley and Westbrook, 1990). Briefly, the OB cultures contained two morphologically distinct populations of neurons: a small number of large-diameter (20–40 $\mu$m soma), pyramidal-shaped neurons and a much larger population of small-diameter (5–10 $\mu$m soma) bipolar neurons. These characteristics correlate with M/T cells and granule/PG (OB interneurons), respectively.

Our previous electrophysiological and immunocytochemical analyses of these neuronal populations further support this notion. In monosynaptically coupled pairs, intracellular stimulation of neurons with M/T cell-like morphology invariably evoked glutamate-mediated excitatory postsynaptic potentials (EPSPs), whereas intracellular stimulation of the small bipolar neurons evoked GABA-mediated inhibitory postsynaptic potentials (Trombley and Westbrook, 1990). In addition, the large pyramidal neurons (presumptive M/T cells) were immunoreactive for *N*-acetylaspartylglutamate, whereas the small bipolar neurons (presumptive interneurons) were immunoreactive for glutamic acid decarboxylase (Trombley and Westbrook, 1990).

### Northern analysis

The acid guanidinium thiocyanate–phenol–chloroform extraction method was used to isolate total cellular RNA from the following brain regions in an adult rats: OB, cortex, brain stem, hypothalamus, thalamus, and cerebellum. This method was also used to isolate total cellular RNA from OB cultures. The purity of the RNA was verified by the $A_{260}/A_{280}$ ratio. Equal amounts of total RNA (15 $\mu$g) were fractionated by size on a 1% (w/v) agarose gel containing 0.66 M formaldehyde. RNA was then transferred to a nylon membrane (GeneScreen, NEN, Boston, MA, USA) via capillary blotting. RNA transfer was confirmed by visualization of ethidium bromide-stained RNA under UV light. Blots were UV cross-linked and stored at 4 °C until hybridization with a random primed $^{32}$P-labeled pre-pro-NPY cDNA probe (RadPrime DNA Labeling System, Gibco BRL, Gaithersburg, MD, USA). Equal loading of lanes was confirmed by hybridization to a $^{32}$P-labeled 28S rRNA probe. Filters were exposed to Kodak X-OMAT AR film at −80 °C. Relative amounts of bound cDNA probe were determined by computer-evaluated densitometry (Quantity One quantification program; Protein and DNA Imaging PDI, Boston, MA, USA) and expressed as a function of 28S rRNA abundance.

### Electrophysiology

Electrophysiological recordings were obtained at room temperature from OB neurons after 7–25 days in culture. The acquisition software (AxoData and AxoGraph, Axon Instruments, Foster City, CA, USA) was run on a Macintosh G4 computer and used to control an AxoClamp 2B amplifier and an Axopatch 200B amplifier (Axon Instruments).

The 35-mm culture dishes functioned as the recording chambers and were perfused at 0.5–2.0 ml/min with a bath solution consisting of (mM): 162.5 NaCl, 2.5 KCl, 2 $CaCl_2$, 10 HEPES, 10 glucose, 1 $\mu$M glycine (pH 7.3, osmolarity 325 mOsm). Patch electrodes were pulled from borosilicate glass to a final tip resistance of 4–6 MOhm and filled with a solution containing (mM): 145 $KMeSO_4$ or CsCl, 1 $MgCl_2$, 10 HEPES, 5 Mg-ATP, 0.5 Mg-GTP, and 1.1 EGTA (pH 7.2, osmolarity 310 mOsm).

Drugs were diluted in bath solution and then applied via a gravity-fed flow-pipe perfusion system, consisting of a row of 600 $\mu$m-OD, square glass barrels. An electronic manipulator (Warner Instruments Corporation, Hamden, CT, USA) controlled the position of the flow pipes, and pinch clamps regulated drug delivery. The efficiency of the solution delivery produced complete drug exchange within 100 ms. In the text and figures, "control" data represent cells perfused with bath solution. The drugs used in these experiments were glutamate (Sigma), tetrodotoxin (TTX; Sigma), whole NPY (Tocris, Ellisville, MO, USA), NPY13-36 (Tocris), and BIIE 0246 (Tocris).

### Use of NPY agonists and antagonists

In whole-cell electrophysiology studies, we examined the effects of two NPY agonists: whole NPY and preferential agonist for Y2 receptor (NPY 13-36). Although whole NPY is a potent agonist for the Y1 receptor, its actions are nonspecific; whole NPY activates Y1, Y2, and Y5 receptors (Michel et al., 1998; Redrobe et al., 2002b). In contrast, NPY 13-36 is considered to be a selective agonist for Y2 receptors (Redrobe et al., 2002a,b). We also examined the effect of a potent and highly selective antagonist of Y2 receptors-BIIE 0246 (Dumont et al., 2000).

### Experimental procedures

Spontaneous synaptic activity was recorded from postsynaptic neurons (interneurons) in both current-clamp and voltage-clamp