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IMMUNOHISTOCHEMICAL LOCALIZATION OF OXYTOCIN RECEPTORS IN HUMAN BRAIN

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Abstract—The neuropeptide oxytocin (OT) regulates rodent, primate and human social behaviors and stress responses. OT binding studies employing ^{125}I-d(CH$_2$)$_5$-[Tyr(Me)$_2$,Thr$_4$,-Tyr-NH$_2^9$] ornithine vasotocin (^{125}I-OTA), has been used to locate and quantify OT receptors (OTRs) in numerous areas of the rat brain. This ligand has also been applied to locating OTRs in the human brain. The results of the latter studies, however, have been brought into question because of subsequent evidence that ^{125}I-OTA is much less selective for OTR vs. vasopressin receptors in the primate brain. Previously we used a monoclonal antibody directed toward a region of the human OTR to demonstrate selective immunostaining of cell bodies and fibers in the preoptic-anterior hypothalamic area and ventral septum of a cynomolgus monkey (Boccia et al., 2001). The present study employed the same monoclonal antibody to study the location of OTRs in tissue blocks containing cortical, limbic and brainstem areas dissected from fixed adult, human female brains. OTRs were visualized in discrete cell bodies and/or fibers in the central and basolateral regions of the amygdala, medial preoptic area (MPOA), anterior and ventromedial hypothalamus, olfactory nucleus, vertical limb of the diagonal band, ventrolateral septum, anterior cingulate and hypoglossal and solitary nuclei. OTR staining was not observed in the hippocampus (including CA2 and CA3), parietal cortex, raphe nucleus, nucleus ambiguus or pons. These results suggest that there are some similarities, but also important differences, in the locations of OTRs in human and rodent brains. Immunohistochemistry (IHC) utilizing a monoclonal antibody provides specific localization of OTRs in the human brain and thereby provides opportunity to further study OTR in human development and psychiatric conditions. © 2013 Published by Elsevier Ltd. on behalf of IBRO.

Key words: oxytocin receptor, immunohistochemistry, human brain, limbic system, hypothalamus, OT.

INTRODUCTION

Oxytocin (OT) plays a significant role in a broad range of mammalian social behaviors, emotions and stress responses (Insel, 1992; Carter, 1998; Gimpl and Fahrenholz, 2001; Kendrick, 2004; Bartz and Hollander, 2006; Lee et al., 2009). Autoradiographic studies have documented that distribution of OT receptors (OTRs) in the brain varies greatly among rats, mice, guinea pigs, hamsters, voles, marmosets, and rabbits (Dubois-Dauphin et al., 1992; Elands et al., 1988a; Insel et al., 1993; Tribollet et al., 1992). These studies have employed autoradiography using the radioligand, ^{125}I-labeled d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH$_2$(^{9})] ornithine vasotocin (^{125}I-OTA), which has high affinity and selectivity for the OTR in rat brain tissue (Elands et al., 1987). The distribution of central OT binding differs between monogamous and promiscuous species of voles (Insel and Shapiro, 1992). Although OT binding was not compared, differences in OT concentrations in the cerebrospinal fluid were also related to contrasting sociability in two species of macaques (Rosenblum et al., 2002). In sheep, OTR mRNA was identified in many but not all of the same brain areas in which OT binding is found in rats (Broad et al., 1999).

OT immunostaining or immunoreactive content has been found in the brains of all primate species studied to date including humans, rhesus monkeys, squirrel monkeys, marmosets, and tree shrews (Sofroniew et al., 1981; Jenkins et al., 1984; Caffe et al., 1989; Wang et al., 1997). In these species, OT projections have been found in many of the same brain areas as in the rat (e.g., the amygdala, brainstem nuclei) but not all areas (e.g., hippocampus).

Loup et al. (1989, 1991) conducted autoradiographic studies of OT binding on human brain sections using ^{125}I-OTA and ^{3}H-OT. Binding was visualized in the ventrolateral septum, and throughout the preoptic and hypothalamic area which is somewhat similar to findings in rats although less discretely localized. Unlike observations in rats, they found no binding in the amygdala or the hippocampus. However, the validity of

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Abbreviations: IHC, immunohistochemistry; MPOA, medial preoptic area; OT, oxytocin; OTRs, OT receptors; VMN, ventromedial nucleus of the hypothalamus.

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the Loup et al. (1989, 1991) studies has been brought into question by evidence that ^{125}I-OTA binds V1a as potently as OTRs in the rhesus monkey brain (Toloczko et al., 1997).

Immunohistochemistry (IHC) has not yet been employed to locate OTRs in the human brain. The monoclonal antibody, 2F8, and other selective antibodies have been used successfully to demonstrate the presence of OTRs in human reproductive or tissues (Takemura et al., 1994; Einspanier et al., 1998; Frayne and Nicholson, 1998; Kimura et al., 1998; Lee et al., 1998; Cassoni et al., 2004; Vignozzi et al., 2004, 2005; Wakasa et al., 2009). We previously employed 2F8 to visualize OTRs in cynomolgous monkey brain (Boccia et al., 2001). In the current study, we used this antibody to examine the location of OTRs in numerous areas of the human brain.

EXPERIMENTAL PROCEDURES

Tissue collection and treatment

Blocks of tissue were dissected from the brains of two deceased human females (44 and 28 years old) at autopsy. Standard protocol for brain autopsy in the hospital autopsy service is as follows: Brains are fixed in 10% formalin for 1 wk, then washed in tap water for 2 days before autopsy. Following neuropathological examination, samples were collected for this study. Immediately following collection, blocks were fixed in 4% paraformaldehyde for 1 wk, washed for 24 h in phosphate-buffered saline (PBS) and then embedded in paraffin.

Blocks of tissue collected include samples from sites which have been found in rodents to contain OTRs as well as other areas involved in social behavior. The areas collected included the olfactory nucleus, anterior hippocampus, anterior cingulate cortex, hypothalamus, preoptic area, amygdala, nucleus accumbens, and the brainstem. Several sites thought not to contain OTRs were also collected as negative controls, including blocks containing the parietal cortex and pons. Several blocks contained bilateral samples, including medulla, anterior cingulate, septum/caudate and hypothalamus. The remaining blocks were sampled from the left hemisphere.

Coronal sections (10 μm) were cut and mounted directly onto Plus^7 slides. The slides were placed in an incubator (37 °C) for 24 h in order to prevent detachment of the sections from the glass, and subsequently were stored at room temperature until processed.

Control tissue was included in the assays which were comprised of normal human endometrium, generously provided by Dr. Bruce A. Lessey, Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC. Tissues were fixed in 10% formalin by immersion. Samples were selected from women in their late secretory phase (day 14 of the luteal phase, when there is evidence that rising estrogen levels may increase OTR expression in some brain areas (Bale et al., 1995, 2001; de Kloet et al., 1986; Johnson et al., 1989; Insel et al., 1997).

Collection of all human tissues was approved by the Committee for the Protection of Human Subjects, UNC-CH.

Immunohistochemistry (IHC)

A monoclonal antibody suitable for IHC directed toward human uterine OTR was obtained from Rohto Pharmaceutical Co., Ltd. (Osaka, Japan). This antibody, designated 2F8, is directed against a 21 amino acid sequence, comprising the NH_2-terminal region of the human OTR (Takemura et al., 1994). It comprises the 20th through 40th amino acids of the OTR, and is sequenced PPGAEGNRTAGPPRRNEALAR.

Slides were deparaffinized and hydrated and incubated in methanol with 0.17% H_2O_2 to block endogenous peroxidase activity. Slides were washed with PBS and treated with 0.05% trypsin to reduce crosslinking of proteins. Slides were then incubated in normal goat serum (NGS, 2%) for 10 min and incubated in the primary antibody (2F8, 0.1 μg/m in 1% PBS/NGS/NaN_3) for 48 h at 4 °C. [The optimal dilution of the primary antibody was determined from preliminary staining at three different concentrations (10.0, 1.0 and 0.1 μg/ml) of the antibody]. After incubation in primary antibody, slides were washed in PBS, followed by incubation in 2% NGS. Subsequently, biotinylated anti-mouse IgM (1:200, Vector Laboratories, Burlingame, CA, USA) was applied for 1 h. After washing in PBS, the sections were treated with Avidin Biotin Complex (1:200, ABC, VectaLabs) reagent for 1 h. Slides were then immersed in 0.05% DAB (3,3′-diaminobenzidine tetrahydrochloride) in Tris with 0.002% H_2O_2, washed in Tris, and PBS. Finally, sections were exposed to vapors from 2% Osmium for 10 min to stabilize and darken the DAB reaction product. Sections were counterstained with 0.05% Toluidine Blue, dehydrated and coverslipped. A Nikon Eclipse E600 microscope equipped with a digital camera system (Spot camera and software, Diagnostic Instruments Inc.) was used to examine the location of OTRs in the tissue. Sites were identified and localized by reference to Mai et al. (2004) and Paxinos and Huang (1995).

Blocking studies were conducted, in which the OTR peptide, against which 2F8 was designed, and the analogous peptide sequence of the V1a receptor were used. IHC method was repeated as described above. One hour prior to incubation of the samples with the antibody, however, an equal volume of blocking peptide was added to the antibody solution for a final blocking peptide concentration of 0.5 mg/ml.

RESULTS

OTR staining was found predominantly in limbic and hypothalamic sites, although other areas, such as the hypoglossal and solitary nuclei, also exhibited positive staining. OTR staining was visible on the cell membrane as well as in the cytoplasm. The possibility of OTR staining within the nucleus of cells within the piriform

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