Neuroscience 228 (2013) 139–162

SYNAPTIC INNERVATION TO RAT HIPPOCAMPUS BY VASOPRESSIN-IMMUNO-POSITIVE FIBRES FROM THE HYPOTHALAMIC SUPRAOPTIC AND PARAVENTRICULAR NUCLEI

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Abstract—The neuropeptide arginine vasopressin (AVP) exerts a modulatory role on hippocampal excitability through vasopressin V_{1A} and V_{1B} receptors. However, the origin and mode of termination of the AVP innervation of the hippocampus remain unknown. We have used light and electron microscopy to trace the origin, distribution and synaptic relationships of AVP-immuno-positive fibres and nerve terminals in the rat hippocampus. Immuno-positive fibres were present in all areas (CA1–3, dentate gyrus) of the whole septo-temporal extent of the hippocampus; they had the highest density in the CA2 region, strongly increasing in density towards the ventral hippocampus. Two types of fibres were identified, both establishing synaptic junctions. Type A had large varicosities packed with immuno-positive large-granulated peptidergic vesicles and few small clear vesicles forming type I synaptic junctions with pyramidal neuron dendrites, dendritic spines and with axonal spines. Type B had smaller varicosities containing mostly small clear vesicles and only a few large-granulated vesicles and established type II synaptic junctions mainly with interneuron dendrites. The AVP-positive axons in stratum oriens appeared to follow and contact metabotropic glutamate receptor 1α (mGluR1α)-immuno-positive interneuron dendrites. Fluoro-Gold injection into the hippocampus revealed retrogradely labelled AVP-positive somata in hypothalamic supraoptic and paraventricular nuclei. Hypothalamo-hippocampal AVP-positive axons entered the hippocampus mostly through a ventral route, also innervating the amygdala and to a lesser extent through the dorsal fimbria fornix, in continuation of the septal AVP innervation. Thus, it appears the AVP-containing neurons of the magnocellular hypothalamic nuclei serve as important sources for hippocampal AVP innervation, although the AVP-expressing neurons located in amygdala and bed nucleus of the stria terminalis reported previously may also contribute.

Key words: arginine vasopressin (AVP), Fluoro-Gold, electron microscopy, Neurolucida, anatomical tracing.

INTRODUCTION

Vasopressin (VP), also referred to as antidiuretic hormone (ADH), is a neuropeptide originated in several brain regions but preferentially in the supraoptic (SON) and paraventricular (PVN) nuclei (Buijs, 1978; Hou-Yu et al., 1986). As a hormone, VP regulates water–electrolyte metabolism, hepatic glucose metabolism, and cardiovascular function (Hatton, 1990). In the central nervous system (CNS), VP exerts influences on behaviours and cognitive function (Goodson, 2008). For instance, arginine vasopressin (AVP) plays a prominent role in the regulation of aggression, affiliation and certain aspects of pair bonding, as well as social recognition (for a review, see Caldwell et al., 2008). AVP system abnormalities have also been reported to be linked to stress over-responsiveness, anxiety, and depressive states (Wigger et al., 2004; Zhang et al., 2010). In peripheral cells, VP binds to three distinct receptors: (i) vasopressin V_{1A} receptors that trigger phospholipase-Cβ (PLCβ) activation and calcium mobilization, and are present in smooth muscle, liver and platelets; (ii) vasopressin V_{1B} receptors that are also coupled to PLCβ and are found in the anterior pituitary; (iii) vasopressin V_2 receptors, that are coupled to adenylyl cyclase, and are present in the kidney (Barberis et al., 1998; Schoneberg et al., 1998; Thibonnier et al., 1998; Birnbaumer, 2000). In the CNS, V_{1A} is the prevalent receptor with a wide distribution whereas V_{1B} receptors are expressed in only a few brain regions (Lolait et al., 1995; Hernando et al., 2001; Stemmelin et al., 2005).

The hippocampus has been reported to be a highly sensitive site for the effects of VP on learning and memory. Behavioural studies show that VP injections into the ventral hippocampus improve memory when tested in a passive avoidance paradigm (Ibragimov,

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Abbreviations: ADH, antidiuretic hormone; AIS, axon initial segment; AVP, arginine vasopressin; BLA, baso-lateral amygdala; BNST, bed nucleus of stria terminalis; CNS, central nervous system; CoMeA, cortico-medial amygdala; DAB, 3,3′-diaminobenzidine; dcv, dense-core vesicle; dHi, dorsal hippocampus; EPSP, excitatory postsynaptic potential; FG, Fluoro-Gold; fi, fimbria; fx, fornix; HRP, horseradish peroxidase; ic, internal capsule; IR, immunoreaction; ir, immunoreactive; LTVP, long-term vasopressin potentiation; mGluR1α, metabotropic glutamate receptor 1α; NHS, normal horse serum; NSS, normal swine serum; PB, phosphate buffer 0.1 M; PLCβ, phospholipase-Cβ; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; STIA, stria terminalis, intra-amygdaloid division; TBS, Tris (0.05 M)-buffered saline; TBST, Tris (0.05 M)-buffered saline plus 0.3% of Triton X-100; vHi, ventral hippocampus; VP, vasopressin; VS, ventral subiculum; WD, water deprivation.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.10.010

1989). Ex-vivo electrophysiological studies showed that nanomolar concentrations of [Arg^8]-vasopressin (AVP) induced a prolonged increase in the amplitude and slope of the evoked population response in the presence of 1.5 mM calcium (Chen et al., 1993). This AVP-induced potentiation of the excitatory postsynaptic potential (EPSP) persisted following removal of AVP from the perfusion medium. The AVP-induced sustained increase of EPSP is known as long-term vasopressin potentiation (LTVP) (Chen et al., 1993). A pronounced effect of VP and its metabolite AVP (4–8) was found to elicit a long-lasting enhancement of hippocampal excitability, mostly in neurons within the ventral hippocampus (Chen et al., 1993; Urban, 1998; Chepkova et al., 2001; Dubrovsky et al., 2003). In the hippocampus V_{1A} receptors are present in the dentate gyrus as well as the CA1, CA2 and CA3 fields (Szot et al., 1994; Barberis and Tribollet, 1996; Raggenbass, 2001; Bielsky et al., 2005). In contrast, V_{1B} receptor expression is restricted to the CA2 field (Young et al., 2006).

In contrast to the detailed information of AVP on hippocampus excitability and its receptor distributions, much less is known about the neuronal sources of hippocampal VP innervation, its fibre-distribution across subfields and targets. Earlier anatomical studies suggested that both VP- and oxytocin-containing fibres originated from the hypothalamic PVN (Buijs, 1978, 1980). This view was indirectly supported by microdialysis studies (Landgraf et al., 1988; Landgraf and Neumann, 2004). However, due to the lack of evidence from anterograde- and retrograde-tracing studies performed in the following years, this hypothesis was gradually abandoned and replaced by the hypothesis that VP cells from the bed nucleus of stria terminalis (BNST) and medial amygdala served as the main sources of the hippocampal VP innervation in rats (De Vries and Buijs, 1983; Sofroniew, 1985; Caffe et al., 1987; van Wimersma-Greidanus et al., 2000).

In this study, we re-examined the sources of AVP innervation into the hippocampus by retrograde tract-tracing analysis and investigated the pattern of distribution of AVP fibres throughout different hippocampal subfields, types of synapses formed by AVP axons, and the nature of postsynaptic targets.

EXPERIMENTAL PROCEDURES

Chemicals

Chemicals were obtained from Sigma–Aldrich, St. Louis, MO, USA, if not indicated otherwise. Sources of primary antibodies and their dilutions are depicted in Table 1.

Animals

Experiments were carried out according to the principles set out in the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996. All animal procedures were approved by the local bioethical and research committees, with the approval ID 138-2009. All efforts were made to reduce the number of animals used.

Adult male Wistar rats (N = 26, 250–310 g) were obtained from the local animal facility. Animals were housed 3 per cage under controlled temperature (22 °C) and illumination (12 h), with water and food *ad libitum*. After surgery, animals were kept warm until fully recovered from anaesthesia and then kept individually under the above-mentioned conditions for 1 week and then returned to the original housing conditions.

Fixation of animals by vascular perfusion and immunohistochemistry (IHC) for light microscopy AVP fibre density and pathways to hippocampus analysis

Ten rats were used in this study. Four hours before the perfusion, the rats received an i.p. 900 mM hypertonic saline injection. The rats were restrained from drinking water for the first 2 h and then they were allowed to drink *ad libitum*. It is worth mentioning that according to our observation, the nonapeptide AVP immunoreactivity is variable among individuals under the animal's basal conditions, which might be related to the individual oscillatory state and physiological demands at the time of the perfusion. The salt induction was intended to up-regulate the hypothalamic osmotic-sensitive magnocellular AVP systems (Verney, 1947; Robertson et al., 1976; Summy-Long et al., 1978), but not to stimulate its release by allowing the rat to drink *ad libitum* during the last 2 h before the perfusion-fixation. With this measure a more homogenous AVP immunoreaction (IR) was achieved among the subjects for the AVP-immuno-positive fibre-density analysis. It is worth mentioning that the osmotic stimuli increase Fos proteins mRNAs, which mediate the rapid transcriptional induction of the vasopressin gene (Yoshida, 2008).

Rats were deeply anaesthetized with an overdose of sodium pentobarbital (63 mg/kg, Sedalpharma, Mexico) and perfused transaortically with 0.9% saline followed by cold fixative containing 4% of paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4) plus 15% v/v saturated picric acid for 15 min. Brains were immediately removed, blocked, then thoroughly rinsed with PB. Brains were sectioned soon after perfusion using a Leica VT 1000S vibratome, at 70 µm thickness in the following four planes: sagittal (n = 3), coronal (n = 3), semihorizontal (30° to the horizontal plane, n = 2) and septo-temporal (between coronal and sagittal planes, 45° to both planes, n = 2). Freshly-cut freely-floating alternate sections from different cutting planes were blocked with 20% normal swine serum (NSS, for immunoperoxidase reaction) or 20% normal horse serum (NHS, for immunofluorescence reaction) in Tris-buffered (0.05 M, pH 7.4) saline (0.9%) plus 0.3% of Triton X-100 (TBST) for 1 h at room temperature and incubated with the following primary antibodies: rabbit anti-AVP antibodies, guinea pig anti-metabotropic glutamate receptor 1α (mGluR1α) and mouse anti-parvalbumin (Table 1) in TBST plus 1% NSS or 1% NHS (for corresponding reactions) over two nights at 4 °C with gentle shaking. For immunoperoxidase reaction, sections were rinsed and incubated with swine anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (P021702, 1:100, Dako, Denmark) in TBST + 1% NSS overnight at 4 °C. This IR was developed using 3,3′-diaminobenzidine (DAB, 0.05%, Electron Microscopy Sciences, Fort Washington, PA, USA) and hydrogen peroxide (H_2O_2, 0.01%) as the substrate. Sections were then mounted in gelatine solution (0.5 g gelatine, 0.05 g chromic potassium sulfate in 200 ml of dH_2O) and air-dried overnight. After passing briefly through 100% ethanol and xylene, the slides were coverslipped with permanent synthetic mounting medium Permount. For immunofluorescence reactions the following secondary antibodies were used: Cy3-donkey anti-rabbit IgG, Alexa Fluor 488 donkey anti-mouse IgG (1:1000, Molecular Probes Inc., Eugene, USA) and donkey anti-guinea pig IgG DyLight 649 (1:1000, Jackson ImmunoResearch Laboratories,