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Research report

Fast neurotransmission in the rat medial preoptic nucleus

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Abstract

The functional properties of neurotransmission in the medial preoptic nucleus (MPN) were studied in a brain slice preparation from young male rats. The aims were to evaluate the thin slice preparation for studying evoked synaptic responses in MPN neurons, to characterize the fast responses triggered by activation of presynaptic nerve fibers in the MPN, and to identify the involved receptor types. Presynaptic stimulation within the MPN evoked postsynaptic voltage and current responses that were blocked by 200 μM Cd^{2+} or by 2.0 μM tetrodotoxin and were attributed to action potential-evoked transmitter release. The relation to stimulus strength and comparison with spontaneous synaptic currents suggested that in many cases only one presynaptic nerve fiber was excited by the stimulus. Furthermore, the transmission was probabilistic in nature, with frequent failures. Thus, response probability, most likely reflecting transmitter release probability, could be evaluated in the thin slice preparation. Evoked excitatory postsynaptic currents recorded under voltage-clamp conditions were, due to kinetics, *I–V* relation, and pharmacological properties, attributed to AMPA/kainate receptors and NMDA receptors, whereas inhibitory currents were attributed to $GABA_A$ receptors. No responses that could be attributed to glycine or other types of primary transmitters were detected. Although serotonin (5-HT) did not appear to function as a primary transmitter, glutamate- as well as GABA-mediated transmission was suppressed by 500 μM 5-HT, with a clear reduction in response probability observed. 5-HT also reduced the frequency, but not the amplitude, of spontaneous postsynaptic currents and was therefore ascribed a presynaptic site of action.

Theme: Excitable membranes and synaptic transmission
Topic: Ligand-gated ion channels

Keywords: Medial preoptic nucleus; Synaptic transmission; Glutamate; GABA; 5-HT

1. Introduction

The medial preoptic nucleus (MPN) is a major structure in the anterior hypothalamic region. The MPN plays an essential role in the regulation of several important physiological functions such as thermoregulation [2,17,31], arterial pressure [12], and sleep [17]. The MPN is also crucially involved in the control of sexual behavior [1,3,6,7,33,42] and male and female maternal behavior [22,40]. In general, MPN neurons have been suggested to integrate cognitive and sensory information with information on the behavioral state in order to regulate complex social behavior, including reproductive behavior [46,48].

Some aspects of synaptic transmission in the MPN have been studied. Earlier studies showed that inhibitory postsynaptic potentials in medial preoptic neurons were blocked by $GABA_A$ receptor blockers [13,26] and that spontaneous excitatory postsynaptic currents were blocked by an AMPA/kainate-receptor blocker [14]. We have later confirmed that isolated MPN neurons directly respond to GABA and glutamate with currents that could be attributed to $GABA_A$ receptors and AMPA/kainate receptors respectively [20,21]. However, medial preoptic neurons also

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generate currents attributed to glycine receptors [20,26] as well as to NMDA receptors [21], although it has not been established if these two receptor types are involved in synaptic transmission in the MPN.

Anatomical and histochemical studies have also revealed the presence of other possible transmitter substances in the MPN. Thus, besides a large number of peptides, input fibers in the MPN contain 5-hydroxytryptamine (5-HT, serotonin) [44], which may be important for the control of male sexual behavior [25,38] and brain temperature [16]. Although evidence for a clear role in synaptic transmission within the MPN has been lacking, it thus seems likely that 5-HT plays an important role in controlling the functions of the MPN.

From the evidence presented above, it seems possible that several different transmitters and receptor types mediate synaptic transmission within the MPN. Earlier studies of evoked synaptic responses within the MPN have used sharp microelectrodes and have, to our knowledge, not used voltage-clamp conditions. Furthermore, presynaptic stimulation has been confined to a few specific anatomical sites. Thus, evoked synaptic currents have not been characterized and several types of synaptic responses may have been missed.

The present study aimed at characterizing the rapid postsynaptic responses that can be evoked by activation of presynaptic fibers to MPN neurons and identifying the receptor types mediating these responses in brain slices from young male rats. This also implied a test of the thin hypothalamic slice preparation for studying evoked synaptic responses in MPN neurons. To increase the probability of detecting different types of responses, postsynaptic currents were measured under voltage-clamp conditions with the perforated-patch technique, and presynaptic stimulation was made within or in the close vicinity of the MPN. We show that the common types of postsynaptic responses were mediated by $GABA_A$ receptors, AMPA/kainate receptors, and NMDA receptors. We did not find any evidence for 5-HT receptors mediating the primary signal evoked by presynaptic stimulation. However, 5-HT, when applied externally, modulated GABA- as well as glutamate-mediated transmission. Although MPN neurons respond strongly to glycine, no evidence was found for glycine receptors being activated by the presynaptic stimulation.

2. Materials and methods

All experiments were carried out according to the local ethics committee for animal research.

2.1. Slice preparation

Thin brain slices (150 μm), containing the preoptic area, were obtained from young (3–4 weeks) Sprague–Dawley rats. After decapitation without anesthetics, the brain was isolated and a block of tissue including the preoptic area was cut out. Slices were prepared with a vibroslicer (752 M Vibroslice, Campden Instruments, Leicestershire, UK). During slicing, the chamber was filled with preoxygenated (100% O_2) ice-cold incubation solution (see below). Subsequently, the slices were incubated at 27 °C for at least 1 h in oxygenated incubation solution. Before recording, the slices were transferred to an experimental chamber perfused with extracellular solution (see below) saturated with a mixture of 95% O_2 and 5% CO_2. The anatomical location of electrical recordings was assessed with reference to the atlas by Swanson [47]. All parts of the MPN were used for electrical recording as well as for stimulation (see below). Neurons of the MPN were visually identified and prepared for recording by cleaning with a stream of the extracellular solution applied from a glass pipette [8].

2.2. Electrophysiological stimulation and recording

The electrophysiological recordings from postsynaptic neurons of the MPN were performed under voltage-clamp or current-clamp conditions using the tight-seal amphotericin-B-perforated-patch technique [35]. An extracellular stimulation pipette was placed 60–160 μm from the postsynaptic cell in the MPN. Postsynaptic potentials as well as postsynaptic currents were evoked by presynaptic stimulus pulses of 0.1–0.5 ms duration and 2–22 V amplitude. Spontaneous postsynaptic currents were also recorded. All recorded electrical signals were low-pass filtered at 2–5 kHz.

Stimulation pipettes as well as recording pipettes were prepared from borosilicate glass (GC150-10, Harvard Apparatus, Edenbridge, UK). The resistance of patch pipettes was 2.5–3.5 MΩ when they were filled with intracellular solution and immersed in incubation solution. Stimulation pipettes, filled with extracellular solution, had a resistance of 0.5–1.0 MΩ. A gravity-fed fast perfusion system, with the common outlet of a four-barrelled pipette positioned about 400–500 μm from the studied cell, was used for continuous application of standard extracellular solution or of test solutions. Solenoid valves controlled switching between solutions. Visually guided whole-cell patch-clamp experiments were made using a Zeiss Axioscope FS microscope equipped with water-immersion objective (×40) and differential interference contrast optics. Electrical recordings were made using an Axopatch 200B patch-clamp amplifier, a Digidata 1200B interface, and the pCLAMP software (version 8.0; all from Axon Instruments, Union City, CA, USA), controlled via a Pentium-processor based personal computer. Typically, recordings started 10–15 min after patch-penetration by amphotericin B. Series resistance was evaluated repeatedly throughout each experiment, although non-exponential decay of capacitative transients, likely due to dendritic