Optical Recording of Neuronal Excitability in the Rat Dorsolateral Septal Nucleus

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Key words lateral septum, rat, voltage-sensitive dye, optical recording, pseudo-color image

The septal area participates in a variety of physiological and behavioral processes related to learning, memory, emotions (fear and aggression), as well as autonomic regulation. The lateral septal nuclei, the largest nuclear mass in the septum, receive the massive hippocampo-septal projection, which contains the main axons of the CA1 and CA3 pyramidal neurons [1,2]. Intracellular recordings demonstrated that stimulation of the fimbria/fornix pathway evoked an excitatory postsynaptic potential (EPSP) mediated by an excitatory amino acid in the dorsolateral septal nucleus (DLSN) [3,4]. The principal neurons in the lateral septum which contain γ -aminobutyric acid (GABA) project primarily to the hypothalamic and amygdaloid areas. In addition to the extraseptal projection, GABAergic neurons participate in local inhibitory circuits within the lateral septum which mediate the inhibitory postsynaptic potential (IPSP), via local axon collaterals [2,5]. In this study, the propagation of neuronal activity from the hippocampal CA3 to the septal nuclei was examined with optical recording techniques [6-11].

Parasagittal slices of the rat brain containing both the hippocampal CA3 and the lateral septum were obtained in a manner described previously [12]. Briefly, male Wistar rats (80-150 g) were killed by decapitation. Slices (500 μ m in thickness) were cut with a Vibroslice (Campden Instruments) and superfused with ACSF pre-bubbled with 95% O₂-5% CO₂. The composition of the ACSF was as follows: 117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM

MgCl₂, 25 mM NaHCO₃, 1.2 mM NaHPO₄, and 11 mM d-glucose (pH 7.4 and 295-305 mOsm). Optical images were acquired using a 128 $\times$ 128 photodiode array (HR Deltaron 1700, Fuji Photo Film Co., LTD.). With the 10 $\times$ objective, each photodiode corresponded to a tissue area of 11.5 $\times$ 11.5 μ m, and the whole array corresponded to an area of 1.48 $\times$ 1.48 mm (Fig. 1). Brain slices were stained with a voltage-sensitive dye, RH-482 (0.1 mg/ml) (Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan) for 20 min. The stained slices were mounted on an upright microscope (Optiphot-2, Nikon) that was equipped with a tungsten-halogen lamp (100 W), interference filters (700 nm), a mechanical shutter, and an objective (Plan $\times$ 10). Optical responses were recorded as absorption signals of RH-482. Image data files and analysis data files were stored on an IBM PC/AT computer and used for later analysis. Drugs were purchased from the following sources. CNQX and bicuculline methiodide were obtained from Sigma (St Louis, MO). Tetrodotoxin (TTX) was obtained from Wako Pure Chemical Industries, Ltd. All experiments were carried out at 22-24 $^{\circ}$ C.

Electrical stimulation of the fimbria/fornix pathway induced optical responses near the stimulus electrode and these responses initially spread toward the medial septum by the fimbrial pathway. The optical images then propagated to the dorsolateral portion of the lateral septum (Fig. 2). The apparent velocity of propagation was 0.1-0.2 m/s. TTX (1 μ M) blocked the spreading of the optical responses in the slice preparation (Fig. 2A). The responses which had

Fig. 1. A schematic diagram of the optical response recording system. The microscope is an Optiphot-2 (Nikon) with a tungsten-halogen lamp (100 W). The HR Deltaron 1700 has a camera head which contains the MOS image sensor with a 128×128 photodiode array. PC: IBM PC/AT computer

Optical response in septum

Fig. 2. Optical images in the lateral septal nuclei evoked by stimulation of the fimbria/fornix pathway. A: Effect of TTX ($1 \mu\text{M}$) on the optical signals which propagate to the lateral septum. B: Effect of a low Ca^{2+} concentration on the optical signals. C: Effect of CNQX, an antagonist for non-NMDA receptors, on the optical signals. Note that these agents block the excitatory optical images in the lateral septum.

spread to the lateral septum were blocked by a low Ca^{2+} solution (Fig. 2B). Application of CNQX (10 μM) to the Krebs solution also blocked the propagation of the optical images at the DLSN, where excitation signals only occurred near the stimulus electrode (Fig. 2C). In contrast, the application of bicuculline (50 μM), a GABA_A receptor antagonist, markedly enhanced the optical signals.

Electrical stimulation of the fimbria/fornix pathway which contains axons of the hippocampal CA1 and CA3 pyramidal neurons, induces an EPSP followed by IPSPs in DLSN neurons [5]. The EPSP was blocked by CNQX (10 μM) and AP5 (50 μM). The IPSPs were blocked by bicuculline and CGP55845A, GABA_A and GABA_B receptor antagonists, respectively. In this study, neuronal activity and synaptic transmission between the lateral septum and the hippocampal CA3 area of the rat were examined using optical recording techniques. Excitatory optical responses evoked by stimulation of the fimbrial pathway spread toward the lateral portion of the septum. The optical signals in the lateral septum involve excitatory synaptic transmission mediated by an excitatory amino acid [4]. The principal neurons in the lateral septum which contain γ -aminobutyric acid (GABA) participate in local inhibitory circuits within the lateral septum, via their local axon collaterals [2]. It has been shown that GABA mediates the IPSPs in the lateral septal neurons [5]. The inhibition of the IPSPs by bicuculline and CGP55845A may result in the disinhibition of inhibitory synaptic transmission in the lateral septum. These results indicate that the propagation of optical signals involve excitatory synaptic transmission in the hippocampo-septal pathway.

ACKNOWLEDGMENTS: This work was supported by a Grant-in-Aid for Scientific Research (B) from the Ministry of

Education, Science, Sports and Culture of Japan (08457017).

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