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Changes of μ-opioid receptors and GABA in visual cortex of chronic morphine treated rats

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Abstract

Electrophysiological and biochemical studies have indicated that GABAergic modulation is involved in the opioid-induced altering of response properties of visual cortical cells and impairing of short-term synaptic plasticity in the geniculo-cortical visual pathway. The aim of the current study was to examine whether there were changes in the localization and density of μ-opioid receptor subtype (MOR1) and GABA in the visual cortex of morphine-dependent and abstinent rats. Immunofluorescence histochemical method was applied to display MOR1 and GABA distribution. We found that MOR1-like immunoreactive neurons were significantly lowered in layer I–VI of visual cortex of rats sacrificed immediately after the last injection (defined as morphine-dependent (MD)) than saline-control group. In rats sacrificed just before the last injection (defined as morphine-abstinent (MA)), the density of μ-opioid receptor was higher than that in dependent group in layer I–V neurons of visual cortex, but remained lower than those in control group. Three hours after the last morphine injection (defined as 3 h after morphine-abstinent (3 h)), MOR1-like immunoreactive neurons in layer I and layer IV of visual cortex were still significantly lower than control. As to GABA-like immunoreactive neurons, they were significantly decreased in abstinent group compared to dependent group. These results provide morphological evidence that opioid-induced altering of response properties of visual cortical cells might be due to the change of visual cortical GABAergic system induced by opioid via μ-opioid receptor, which result in disinhibition of visual cortex projection neurons and their abnormal firing.

Keywords: Immunofluorescence histochemistry; μ-Opioid receptor; GABAergic neuron; Visual cortex; Rat

Changes of endogenous opioid peptides system have been considered to constitute an important mechanism of opioid dependence. Repeated use of addictive drugs leads to multiple adaptive neuronal responses. Increasingly, morphine-like drugs decreased visual sensitivity in humans, impacted visual discrimination performance in rats and affected cortical potentials evoked from optic chiasm stimulation in cats [5]. Recent work in our lab also showed that chronic morphine exposure influences the response properties of cortical neurons in cats and impaired short-term synaptic plasticity in the geniculo-cortical visual pathway of rats [20]. It has been proposed that the modulation of morphine on GABAergic system could play a key role in their results.

Neuroanatomy study indicated that the pyramidal cells of morphine treated rats showed a significant decrease in the total dendritic length and the dendritic spine density of dendritic arborization of layer III in the primary visual cortex in rats [12]. It may contribute to synaptic plasticity and modify synaptic efficacy by altering fast synaptic neurotransmission [9] or the local chemical environment [18]. Our previous neurochemical research showed that GABA content and the activity of its synthetase-glutamic acid decarboxylase in the visual cortex of morphine-abstinent rats were significantly decreased. Other researches also shown that GABAergic synaptic transmission is influenced by opiates [4,6,11]. It has been suggested that the visual system is subject to opiate modulation. Although opioid receptors express extensively in the visual system of rats, it is unknown whether and how chronic opioid expo-

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sure affects response modulation in visual cortical neurons of rats.

Our previous work on rats and cats indicated the chronic morphine exposure could impair the inhibition on visual cortical cells [7]. However, this phenomenon still lacks morphological evidence. Therefore, it is critical to investigate the effects of chronic morphine exposure on the distribution pattern of μ-opioid receptor and GABA in visual cortex. The current study was undertaken to examine the expression of μ-opioid receptor and GABA in the visual cortex of morphine-dependent and morphine-abstinent rats at different time intervals by using immunofluorescence histochemical staining for μ-opioid receptor and GABA.

Thirty Male Sprague Dawley (200–230 g) rats were obtained from the Laboratory Animal Center, An'hui Medical University (Hefei, China). Rats were housed in groups and maintained with food and water ad libitum on a 12 h light/dark cycle. Fifteen rats were injected with morphine (10 mg/kg) (SC injection) twice per day at 12 h intervals for 10 days as described previously [17,19]. Fifteen rats were treated similarly with the normal saline (NS) instead of morphine. Of the 15 morphine-treated rats, 5 were sacrificed immediately after the last injection, 5 sacrificed just before the last injection and the last 5 rats were sacrificed 3 h after the last injection. Of the 15 saline-control rats, they were sacrificed timely as above. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

The animals were deeply anaesthetized with urethane and 0.9% NaCl was perfused through the ascending aorta till the liver became pale, followed immediately by 0.1 M phosphate-buffered saline (PBS; pH7.4) containing 4% (w/v) paraformaldehyde and 0.5% (w/v) glutaraldehyde. After perfusion, the skull was opened and the visual cortex was dissected out. After further fixation for 20–24 h in the perfusing fixative, the samples were washed in PBS, dehydrated in ethanol, transparentized with xylene and embedded in paraffin. Consecutive coronal sections 8 μm in thickness were cut and mounted for histological staining and immunostaining on poly-L-lysine-coated microscope slides. The sections were placed into three different dishes according to their numerical order while cutting (e.g., sections 1, 3, 5 to dish 1, sections 2, 4, 6 to dish 2, sections 3, 6, 9 to dish 3, respectively). All sections were washed carefully with 0.1 M PBS.

All of the sections were deparaffinized in xylene, hydrated through a graded series of ethanol and washed for 5 min in distilled water. Sections in the first and second dish were treated at room temperature for 10 min with 10% goat serum to suppress background staining. For μ-opioid receptors, sections in the first dish were incubated sequentially with: (1) guinea pig anti-μ-opioid receptor subtype (MOR1) polyclonal antibody (AB1774, 1:8000 dilution; Chemicon) for 48–72 h at 4 °C; (2) Cy3 conjugated secondary antibody (1:800 dilution, Chemicon) for 1 h at 37 °C. For GABA, sections in the second dish were incubated sequentially with: (1) rabbit anti-GABA serum (A2052, 1:400 dilution; Sigma, St. Louis, MO) for 48–72 h at 4 °C; (2) Goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC; F6005; 1:700 dilution; Sigma) for 1 h at 37 °C. The sections were rinsed at least three times in 0.1M PBS (pH7.4) after each incubation, lasting over 10 min every time. Then, the sections were air dried and cover-slipped with a mixture of 50% (v/v) glycerin and 0.1M PBS. Finally, the sections were observed with a fluorescence microscope (IX-70; Olympus, Tokyo, Japan) under appropriate filters for green-emitting FITC and for red-emitting, respectively. In the control experiments, the primary antibodies were omitted or replaced with PBS. No immunofluorescence histochemical staining for the omitted or replaced antibodies was detected. The sections in the third dish were processed for Nissl staining to identify the visual cortex according to Paxinos and Watson [16].

Quantitative measurement was done by a person blind to experimental groups. At 400×, according to the Nissl stained sections and the rat brain atlas, the numbers of GABA-like and MOR-like immunopositive neurons in visual cortex were counted in a calibrator (50 μm × 50 μm) and the density (cells/mm^2) was calculated. The criterion for acceptance as a neuron was the clear differentiation from background staining and a profile of soma.

All the data were expressed as mean ± S.E.M. The significance of the difference between the groups was evaluated by a two-way analysis of variance (ANOVA), and a P-value of <0.05 was considered significant.

MOR1-like immunoreactive non-pyramidal neurons were distributed diffusely throughout layers I–VI of rats' visual cortex. MOR1-like immunoreactive neuronal cell bodies were round, fusiform, ovoid or triangular in shape (Fig. 1). The diameters of MOR1-like immunoreactive neuronal cell bodies were about 10–20 μm. There was significant group and time differences in layer IV in the density of MOR1 between experiment and control groups ($F[1, 24] = 4.55$, $P < 0.05$; $F[2, 24] = 4.01$, $P < 0.05$). In morphine-dependent group, MOR1-like immunoreactive neurons significantly decreased in layer VI than that in saline-control (Table 1). Three hours after last morphine injection, MOR1-like immunoreactive neurons were counted predominantly more than morphine-dependent groups. In morphine-abstinent rats, MOR1-like immunoreactive neurons were notably more than morphine-dependent ones but still fewer then saline-control. In conclusion, morphine-dependence leads to decreased MOR1 expression while morphine-abstinence increases density of MOR1-like immunoreactive neurons.

GABA-like immunoreactive labeling was usually observed in the perikary of non-pyramidal neurons and their processes. Immunopositive labeling of GABA-like immunoreactive neurons was predominantly located underneath the cytomembrane of the perikarya and their processes. GABA-like immunoreactive neurons had similar morphological features to the MOR-like immunoreactive neurons, i.e., GABA-like immunoreactive neuronal cell bodies were also round, fusiform ovoid or triangular in shape. The diameters of GABA-like immunoreactive neuronal cell bodies were the same as the MOR-like immunoreactive neurons (about 10–20 μm) (Fig. 2).

GABA-like immunoreactive neuronal cell bodies were located scattered through all layers of visual cortex, but their