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μ和δ阿片样受体在自发高血压大鼠和正常血压WKY大鼠中枢神经系统中的分布

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关键词 μ阿片样受体；δ阿片样受体；高血压；放射自显影法；近交SHR大鼠；近交WKY大鼠

(6) 中枢神经 大鼠
目的：比较自发高血压大鼠(SHR)和对照组WKY大鼠中枢神经系统中阿片受体亚型的分布. **方法**：用放射自显影法，选用^{3}H-OMF，^{3}H-U69593分别标记μ和κ受体，用遮盖法以^{3}H-etorphine标记δ受体. **结果**：δ受体密度在SHR下丘脑、中央灰质高于WKY，μ受体密度在SHR杏仁基底外侧核、僵核、孤束核低于WKY，κ受体密度没能检测出. **结论**：阿片受体亚型不同分布与SHR的血压有关，并且δ受体对高血压的维持作用大于μ受体.

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Modulatory effects of gonadorelin on GABA-induced depolarization and GABA-activated current in rat spinal ganglion neurons[1]

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KEY WORDS gonadorelin; GABA; spinal ganglia; microelectrodes; electrophysiology

AIM: To explore the modulatory effects of gonadorelin on GABA-induced depolarization and GABA-activated current in membrane of rat primary sensory neurons. **METHODS**: Intracellular recordings and whole-cell patch clamp techniques were performed on neurons in rat spinal ganglia (SG) preparation and neurons freshly isolated from rat SG, respectively. Drugs were applied by superfusion and/or by bath application. **RESULTS**: In the majority of neurons GABA (10 $\mu mol \cdot L^{-1}$ — 1 $mmol \cdot L^{-1}$) induced a depolarization, which was blocked by bicucullin (100 $\mu mol \cdot L^{-1}$). Pretreatment with gonadorelin (50 $\mu mol \cdot L^{-1}$) decreased the GABA-induced depolarization by 79 ± 22 % ($n = 29$), while gonadorelin elicited no effect or slight depolarization alone. In 6 of 11 cells, GABA-activated currents were also inhibited by pretreatment with gonadorelin (50 $\mu mol \cdot L^{-1}$), while in 5 of 11 cells, there was no change or a slight potentiation. **CONCLUSION**: Gonadorelin exerts an inhibitory effect on GABA-induced depolarization and GABA-activated current in the primary sensory neurons.

GABA is the major neurotransmitter involved in the formation of primary afferent depolarization (PAD) and thus related to the generation of presynaptic inhibition[1,2]. Our previous study revealed that peptide gonadorelin modu-

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lated the responses of muscarinic receptors and α-adrenoceptors in the somatic membrane of toad spinal ganglion (SG) neuron[4]. Using the SG neuron as a model for studying the properties of primary afferent terminals of sensory neuron, the present work was to investigate the modulatory effects of gonadorelin on the responses mediated by $GABA_A$ receptors in rat SG neurons by intracellular recordings and whole cell patch-clamp techniques.

MATERIALS AND METHODS

Intracellular recordings Intracellular recordings[4] were made on SG *in vitro* from 2-3-wk SD rats of either sex. The rats were anesthetized with either. Laminectomy was done at L_4 or L_5. The SG with dorsal root and spinal nerves attached was dissected out and transferred into oxygenated balanced salt solution (BSS) containing NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, Tris HCl 5 mmol·L^{-1} (pH 7.4, 25±3 ℃) and then pinned onto a silicone gum block. The sciatic nerve was placed on a pair of platinum stimulating electrods in the neighboring compartment. The flow rate was 3—5 mL·min^{-1}.

The glass microelectrodes for intracellular recordings were filled with potassium acetate 4 mol·L^{-1}, tip resistance being 25 — 60 MΩ. Membrane potentials were amplified by microelectrode amplifier (MEZ 8201, Nihon Kohden) and recorded with a pen recorder (LMS-2B, Chengdu).

Isolated SG neurons Immature SD rats were decapitated under ether. The vertebrate column in thoracic and lumbar segments was dissected out and longitudinally divided into two halves along the median lines on both dorsal and ventral sides. The SG together with dorsal and ventral roots and spinal nerves attached were transferred into Dulbecco's modified Eagle's medium (DMEM, pH 7.4) immediately. After removal of attached nerves and surrounding connective tissues the SG were minced with iridectomy scissors and incubated with the enzymes including trypsin (type Ⅱ, Sigma) 0.55 g·L^{-1}, collagenase (type 1 A, Sigma) 1.1 g·L^{-1} and DNase (type 1 A, Sigma) 0.11 g·L^{-1} in DMEM at 35 ℃ for 40 min. To stop the enzymatic digestion soya bean trypsin inhibitor (Type Ⅰ-S, Sigma, 1.25 g·L^{-1}) was added. The isolated neurons were transferred into a culture dish and kept still at least for 30 min.

Patch-clamp recordings Whole cell patch-clamp recordings were carried out by PC-1 patch-clamp amplifier (Huazhong University of Science and Technology). The patch electrode was filled with internal solution KCl 140, $MgCl_2$ 2, HEPES 10, egtazic acid 11 mmol·L^{-1}. The external solution was composed of: NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2, HEPES 10, d-glucose 10 mmol·L^{-1}. The tip resistances of patch-clamp electrode were 2 — 4 MΩ. A small membrane patch underneath the pipette tip was aspirated to form a tight seal and then a more negative pressure was applied to rupture it. Compensation for capacitance and series resistence were done before the start of experiment. Membrane currents were filtered at —10 Hz (—3 dB). Data were recorded and analyzed on an Envision/386 computer using a Labmaster data acquisition system (Huazhong University of Science and Technology) or recorded by a pen recorder (Nihon Kohden).

Drugs GABA, bicuculline, muscimol, isoguvacine, diazepam, pentobarbital and gonadorelin were prepared by dissolving in BSS (pH 7.4). The drug solutions were delivered by gravity flow from a linear barrel array consisting of fused silica tubes (OD 0.5 mm), which were connected to a series of independent reservoirs. The distance from tube mouth to the cell was arround 100 μm (80—120 μm). This rapid solution exchange system was manipulated by shifting the pipette horizontally with a micromanipulator. The inhibitory rate of gonadorelin on the responses mediated by $GABA_A$ receptor = [(control of GABA response — GABA response after application of gonadorelin)/(control of GABA response)]×100 %.

RESULTS

Membrane responses induced by GABA Experiments were performed on 127 cells from 40 SG. The resting potential (RP) of the cells was —63±10 mV (n=127), membrane resistance (R_m)=38±8 MΩ (n=18). The conduction velocities were 1.25—15 m·s^{-1} (n=55). In majority of neurons (106/127) examined the depolarization in membrane potential was induced by GABA (10 μmol·L^{-1}—1 mmol·L^{-1}). The responses were concentration-dependent and sensitive to bicuculline (100 μmol·L^{-1}). In 4/127 spontaneous discharge was seen. During depolarization the membrane conductance increased simultaneously (106/127). In a small part of neurons biphasic response appeared in response to GABA, ie, depolarization followed by a more lasting hyperpolarization (5/127). There appeared hyperpolarization response only in one cell (1/127) during application of GABA.

Inhibitory effect of gonadorelin on GABA-induced depolarization Superfusing SG with gonadorelin (50 μmol·L^{-1})-contained BSS for 5 —20 min, neither the RP nor the amplitude and duration of action potential (AP) evoked by stimulation of sciatic nerve had any change in comparison with the control.

Gonadorelin (50 μmol·L^{-1}) had no effect or induced a slight depolarization in membrane potential. However, pretreatment of SG with gonadorelin (10 — 50 μmol·L^{-1}) 30 s prior to the application of GABA (0.1 — 1 mmol·L^{-1}), the GABA-induced depolarization was attenuated markedly. The decrease in amplitude of GABA-induced depolarization was 79 ± 22 % (n = 29), which recovered after washing for 10 — 30 min (n = 21).

Inhibitory effect of gonadorelin on GABA-induced response started from 30 s to 10 min following gonadorelin. The course of recovery varied with the cells: in majority of cells examined the removal of inhibitory effect of gonadorelin was fairly slow, in 24/29 cells the restoration remained incomplete even over 1 h (Fig 1), while in 5 of 29 cells the recovery was rather quick and complete after washing.

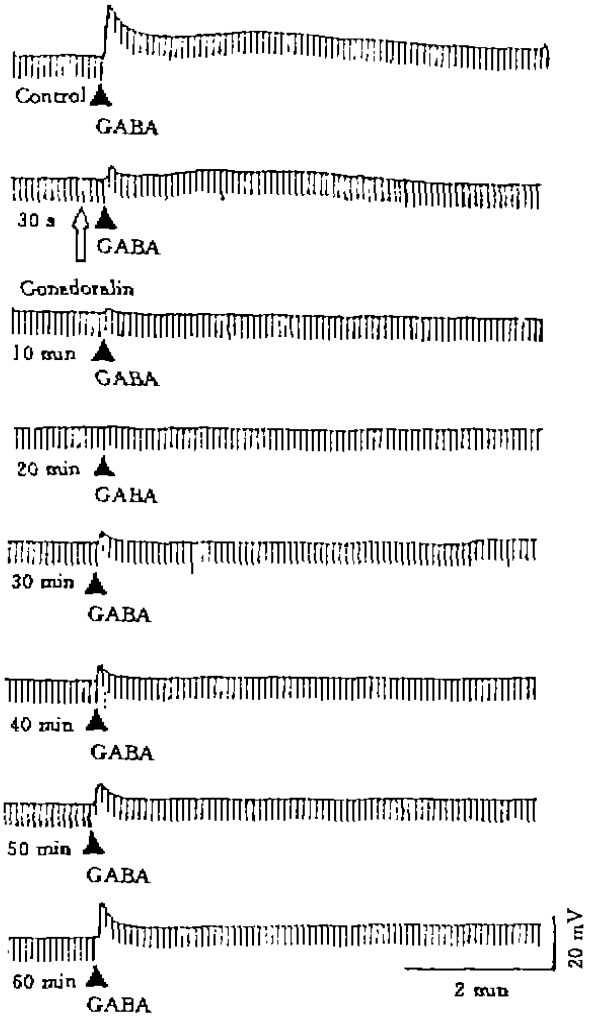

Fig 1. Inhibitory effect of gonadorelin on GABA-indoced response of rat SG neuron.

GABA receptor activated inward current All experiments were done with the holding potential at −60 mV. Most of the SG cells (67/74) responded to GABA (10 μmol·L^{-1} — 1 mmol·L^{-1}) with a concentration-dependent inward current, which was blocked completely by $GABA_A$ receptor antagonist bicuculline (10 μmol·L^{-1}). This kind of GABA-activated inward currents was mimicked by $GABA_A$ receptor agonist muscimol (20 μmol·L^{-1}) and isoguvation (20 μmol·L^{-1}). GABA-activated currents were potentiated greatly by application of GABA allosteric modulatory agents: diazepam (10 μmol·L^{-1}) or pentobarbital (10 μmol·L^{-1}) (Fig 2).

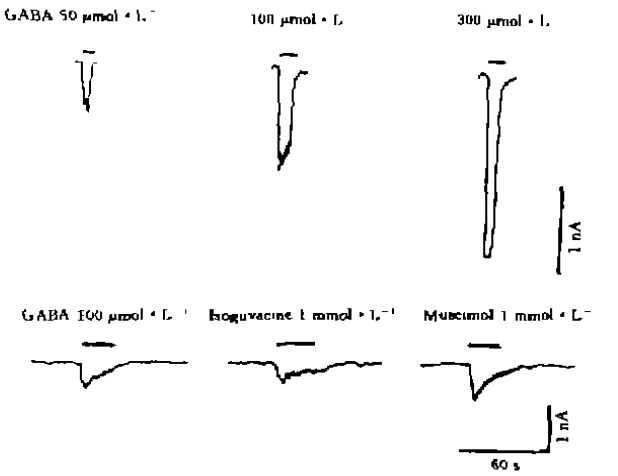

Fig 2. GABA and $GABA_A$ receptor agonists (isoguvacine, muscimol) activated currents.

Modulatory effect of gonadorelin on GABA-activated inward current There was no change in membrane current or just slight outward current (< 0.6 nA) observed with applying gonadorelin (20 μmol·L^{-1}) to the SG cells. However, when gonadorelin was preapplied, inhibition of GABA-activated current reached 72 ± 18 % in 6/11 neurons (Fig 3); no change or slight potentiated in 5/11 cells. All the changes were reversible but very slow.

DISCUSSION

The changes in membrane potential induced by GABA mainly were depolarization[5, 6]. In the present study on the preparation of rat SG neurons there appeared biphasic response and hyperpolarization in response to GABA in addition to the depolarization. The literatures concerning the GABA-induced biphasic response up to now

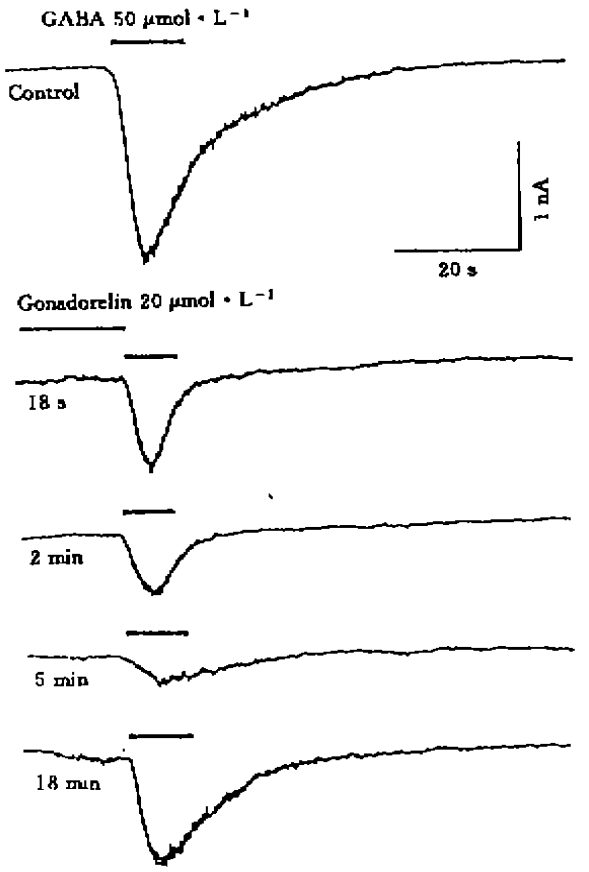

Fig 3. GABA-activated currents after preapplication of gonadorelin.

are very few[7,8]. The similar phenomena have not yet been reported in mammalian SG preparation. Moreover, the cause and ionic mechanism of GABA-induced hyperpolarization is not clear.

So far as we know there is no direct evidence for the existance of gonadorelin receptor in SG neurons. Our previous work demonstrated that gonadorelin potentiated depolarization induced by ACh and NA, while it attenuated the NA-induced hyperpolarization[3]. As all know GABA is the main neurotransmitter mediating presynaptic inhibition in primary afferent terminals. If the results on investigation of GABA receptor obtained from somatic membrane of SG neuron could be available to explain the status of GABA receptor existed in primary afferent terminals, it suggests from the observation in the present study that gonadorelin may act as a modulator taking part in the presynaptic inhibition in primary afferent terminals by modulating the sensitivity of GABA receptor.

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戈那瑞林对大鼠脊神经节细胞 GABA 引起的去极化及 GABA 激活电流的调制作用[1]

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关键词 戈那瑞林；γ-氨基丁酸；脊神经节；微电极；电生理学 大鼠 R338 R904

目的：探索戈那瑞林对大鼠初级感觉神经元膜 GABA 引起的去极化和 GABA 激活电流的调制作用. **方法**：应用细胞内记录和全细胞膜片钳技术分别在大鼠脊神经节(SG)标本和新鲜分离神经元进行实验. **结果**：GABA (10 μmol·L⁻¹—1 mmol·L⁻¹)在大多数神经元引起可为荷包牡丹碱(100 μmol·L⁻¹)所阻断的膜去极化. 预加戈那瑞林(50 μmol·L⁻¹)可减少 GABA 引起的去极化，抑制率为79±22 %（n=29），而戈那瑞林本身不产生膜反应或只引起轻微去极化. 在11个细胞中有6个细胞 GABA 激活电流也为戈那瑞林的预处理所抑制，另5个细胞无改变或反应稍有增加. **结论**：戈那瑞林对初级感觉神经元 GABA 介导的去极化和 GABA 激活电流具有抑制作用.