Effects of agmatine on the electrical activity of subfornical organ neurons

WANG Ze-Min, JI Shu-Mei, ZHANG Hao, SUN Guang-Qi, WANG Zhi-An, HE Rui-Rong*
Department of Physiology, Institute of Basic Medicine, Hebei Medical University, Shijiazhuang, Hebei 050017, China

Abstract: The aim of this study was to investigate the effects of agmatine (Agm) on the electrical activity of neurons in subfornical organ (SFO) slices using extracellular recording technique. The results are as follows. (1) In response to the application of Agm (1.0 µmol/L) into the superfusate for 2 min, the discharge rate of 24/28 (85.7%) subfornical neurons was decreased significantly, while the discharge rate of 4/28 (14.3%) neurons were not affected. (2) Pretreatment with L-glutamate (0.3 mmol/L) led to a marked increase in the discharge rate of 19/24 (79.2%) subfornical neurons in an epileptiform pattern and the activity of the remaining 5/24 (20.8%) neurons was unaffected. By application of Agm (1.0 µmol/L) into the superfusate for 2 min, the epileptiform discharge of 15/19 (78.9%) neurons was suppressed significantly, while that of the other 4 (21.1%) neurons was not inhibited. (3) In 12 neurons, perfusion of the selective L-type calcium channel agonist, Bay K-8644 (0.1 µmol/L), induced a significant increase in the discharge rate of 10/12 (83.3%) neurons, while the other 2 (16.7%) neurons showed no change. The increased discharge of 8/10 (80%) neurons was reduced by application of Agm (1.0 µmol/L) into the superfusate and that of 2/10 (20%) neurons was not affected. (4) Application of nitric oxide synthase (NOS) inhibitor N^o-nitro-L-arginine methyl ester (L-NAME, 50 µmol/L) into the superfusate also significantly increased the discharge rate of 6/9 (66.7%) neurons, and that of 3/9 (33.3%) neurons had no response. Agm (1.0 µmol/L) applied into the superfusate reduced the increased discharge of all 6/6 (100%) neurons. These results suggest that Agm can inhibit the spontaneous discharge, and L-glutamate, Bay K-8644 or L-NAME-induced discharge of neurons in SFO. These inhibitory effects of Agm may be related to the blockade of NMDA receptors and reduction in calcium influx in SFO neurons.

Key words: subfornical organ; agmatine; L-glutamate; Bay K-8644; N^o-nitro-L-arginine methyl ester

脇丁胺对大鼠穹隆下器神经元电活动的影响

王泽民，季淑梅，张 浩，孙光启，王志安，何瑞荣*
河北医科大学基础医学研究所生理室，石家庄 050017

摘要 应用细胞外记录单个单位电位技术，在73个大鼠穹隆下器组织片中观察了脙丁胺 (agmatine, Agm) 对神经元电活动的影响。实验结果如下：(1) 在28个穹隆下器组织片中接触Agm (1.0 µmol/L) 2 min，有24个单位(85.7%)自发放频率明显降低，4个单位(14.3%)无明显变化；(2) 预先用L-谷氨酸 (0.3 mmol/L) 浸润，24个放电单位中有19个单位 (79.2%) 放电频率明显增加，表现为癫痫样放电，5个单位(20.8%)的变化不明显。在此基础上接触Agm (1.0 µmol/L) 2 min，有15个单位 (78.9%) 的癫痫样放电被抑制，另外4个单位 (21.1%) 无明显变化；(3) 浸润 L-型Ca^{2+}通道激动剂Bay K-8644 (0.1 µmol/L)，在12个神经元放电单位中有10个单位 (83.3%) 的放电频率明显增加，另外2个单位 (16.7%) 变化不明显。然后接触Agm (1.0 µmol/L) 2 min，有8个单位 (80%) 的放电频率被抑制，其余无明显变化；(4) 9个单位在浸润一氧化氮合酶 (NOS) 抑制剂N^o-nitro-L-arginine methyl ester (L-NAME, 50 µmol/L) 后，其中6个单位 (66.7%) 放电频率明显增加，另外3个单位 (33.3%) 放电频率变化不明显。在此基础上再给予Agm (1.0 µmol) 2 min，增加的放电频率被抑制。上述结果提示：脙丁胺可抑制大鼠穹隆下器神经元自发放电以及由L-谷氨酸，Bay K-8644 和 L-NAME 诱发的放电，这可能与脙丁胺阻断了神经元的 NMDA 受体，从而减少了钙离子内流有关。

关键词：穹隆下器 脙丁胺 L-谷氨酸，Bay K-8644；N^o-硝基-L-精氨酸甲酯

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*Corresponding author. Tel/Fax: +86-311-6062490; E-mail: syho@hebmu.edu.cn
Agmatine (Agm) is a polyamine cation that can be synthesized from decarboxylation of L-arginine by arginine decarboxylase and is hydrolysed to putrescine, the precursor of spermine, by agmatine uryl hydrolase (agmatinase). It has long been known to be a constituent of bacteria, plants and many invertebrates. Until 1994, it has been proved to exist in the mammalian brain as an endogenous clonidine-displacing substance[1]. It has been well established that Agm is an endogenous agonist at imidazoline receptors (IR) and noncatecholamine ligand at alpha-2 adrenergic receptors (α2-AR)[1]. In the brain, Agm has extensive but regional distribution that has been mapped immunocytochemically using a specific polyclonal anti-agmatine antibody. Most of the neurons that exhibit agmatine-like immunoreactivity are present in hypothalamus, periventricular area, and rostral midbrain[2]. Accumulating evidence indicates that Agm may serve as a novel neurotransmitter or neuromodulator[3], and can block NMDA receptor channels and other ligand-gated cationic channels[4]. In the central nervous system (CNS), Agm possesses many physiological and pharmacological functions, such as releasing luteinizing hormone releasing hormone (LHRH) from the hypothalamus, antagonizing some hyperalgesic states without altering pain thresholds, enhancing morphine analgesia, attenuating morphine abstinence syndrome and conferring neuroprotection[4].

The subfornical organ (SFO) is one of the circumventricular organs (CVO), located in the dorsorostral extension of the third ventricular wall. SFO lacks a blood-brain barrier, and its neurons are consequently accessible to circulating messenger molecules. SFO integrates the systemic and central components to control fluid balance and vascular resistance, through direct neuronal connections to CNS regions regulating the release of neurohypophysial hormones and sympathetic outflow[5]. Our previous study demonstrated that superfusion with Agm decreased the discharge of the neurons in CA1 area of rat hippocampal slices[6]. However, the effect of Agm on the neurons of rat SFO has not been reported. Therefore, this study was designed to examine the effect of Agm on the electrical activity of the neurons in slice preparation of rat SFO using extracellular recording technique.

1 MATERIALS AND METHODS
1.1 Slice preparation. Male or female Sprague-Dawley rats (21 ± 3 d) were decapitated. The skull was opened. The brain was removed quickly and placed into a dish with ice-cold oxygenated artificial cerebrospinal fluid (ACSF).

A tissue block (4 mm × 4 mm × 2 mm) containing SFO and its surrounding tissue was dissected. The SFO could easily be indentified by its protrusion into the third ventricle and the lateral blood vessels lining the organ on both sides. Then, the SFO was cut into 350–500 µm coronal slices using a razor blade. Figure 1 is a schematic diagram of SFO slice (Fig. 1).

Fig. 1. Schematic diagram showing the coronal brain slice of subfornical organ. CC, corpus callosum; TS, triangular septal nucleus; LV, lateral ventricle; VHC, ventral hippocampal commissure; SFO, subfornical organ; 3V, third ventricle.

The slices were preincubated in ACSF aerated with 95% O2 and 5% CO2 for 60–90 min at room temperature before recording started. The slice was then transferred to a chamber perfused with prewarmed ACSF at 2 ml/min. The temperature was held at 34–35ºC. The ACSF contained (in mmol/L): NaCl 124, KCl 3.5, KH2PO4 1.24, MgSO4 1.3, CaCl2 1.5, NaHCO3 18 and glucose 20; pH 7.40–7.50.

1.2 Extracellular single-unit recording. Extracellular single-unit recordings were obtained from SFO neurons using glass microelectrodes with DC resistance of 5~25 MΩ, tip diameter less than 1 µm, and filled with 0.5 mol/L sodium acetate containing 2% Pontamine Sky Blue. The electrical signals were amplified by a microelectrode amplifier (MEZ-8201, Nihon Kohden), displayed on a dual-beam oscilloscope (VC-9, Nihon Kohden), and analyzed by a program for histogram (exploited by ourselves) on a personal computer.

1.3 Experimental protocols. After a stable spontaneous discharge of subfornical neurons had been obtained for at least 10 min and recorded for 3~5 min as control, a drug infusion was started. The neurons were classified as having been excited or inhibited if their firing rates were increased or decreased by more than 20% after application of drug. The latency of a response comprised the time from the arrival of the drug at the recording chamber to the start of the excitatory or inhibitory response. The pro-
protocols were divided into the following groups: (1) Agm (n=28): superfusion with Agm (1.0 µmol/L) into SFO slices for 2 min, and its effect on discharge rate (DR) was recorded; (2) L-glutamate+Agm (n=24): after pretreatment with L-glutamate (0.3 mmol/L), Agm (1.0 µmol/L) was administered for 2 min; (3) Bay K-8644+Agm (n=12): after pretreatment with the L-type calcium channel agonist, Bay K-8644 (0.1 mmol/L), Agm (1.0 µmol/L) was applied for 2 min; (4) L-NAME+Agm (n=9): after pretreatment with NOS inhibitor L-NAME (50 mmol/L), Agm (1.0 µmol/L) was administered for 2 min.

1.4 Drugs. Agmatine, Bay K-8644 and L-NAME were purchased from Sigma Co. L-glutamate was purchased from Institute of Shanghai Zheng-Xiang. For each experiment, all solutions were freshly prepared. Agmatine, L-glutamate and L-NAME were dissolved by ACSF. Bay K-8644 was first dissolved in 99% ethylalcohol and diluted in the ACSF, and the final concentration of ethylalcohol was less than 0.5%.

1.5 Data analysis. All values were presented as means ± SE. Statistical differences were evaluated by paired t test. Statistical significance was set at P<0.05.

2 RESULTS

Seventy-three spontaneously active single-units were recorded from 73 SFO slices. The patterns of discharge were irregular. The mean DR was 2.1 ± 0.07 Hz.

2.1 Effects of Agm on DR of neurons in SFO slices

In 28 single-units of SFO slice, after application of Agm (1.0 µmol/L) into the superfusate for 2 min, the DR of 24/28 (85.7%) neurons was decreased significantly from 1.97 ± 0.12 to 0.92 ± 0.12 Hz (P<0.001), with a latency of 58 ± 4 s; while that of 4/28 (14.3%) neurons was not affected (Fig. 2).

2.2 Effects of Agm on L-glutamate-induced discharges of neurons in SFO slices

Pretreatment with L-glutamate (0.3 mmol/L) led to a marked increase in the DR of 19/24 (79.2%) neurons in SFO slice, from 2.23 ± 0.14 to 4.46 ± 0.22 Hz (P<0.05), with a latency of 48 ± 3 s. However, the discharges of the other 5 (20.8%) neurons were not affected. After application of Agm (1.0 µmol/L) into the superfusate for 2 min, the DR of 15/19 (78.9%) neurons was suppressed significantly to 1.71 ± 0.18 Hz (P<0.01), with a latency of 82 ± 3 s. But, the discharges of 4/19 (21.1%) neurons showed no change (Fig. 3 and Table 1).

Table 1  Effects of Agm on L-glutamate-induced discharge rate of neurons in SFO slices

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Discharge rate ( Hz )</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>24</td>
<td>2.23 ± 0.14</td>
</tr>
<tr>
<td>L-glutamate</td>
<td>19</td>
<td>4.46 ± 0.22</td>
</tr>
<tr>
<td>L-glutamate + Agm</td>
<td>15</td>
<td>1.71 ± 0.18</td>
</tr>
</tbody>
</table>

*P<0.05 vs baseline; **P<0.01 vs L-glutamate.

2.3 Effects of Agm on Bay K-8644-induced discharges of neurons in SFO slices

In 12 units, during perfusion with the L-type calcium channel agonist, Bay K-8644 (0.1 µmol/L), the DR of 10/12 (83.3%) neurons was increased significantly, from 1.80 ± 0.25 to 4.05 ± 0.45 Hz (P<0.001), with a latency of 41 ± 4 s. But, the discharges of 2/12 (16.7%) neurons showed no change. The increased discharges of 8/10 (80%) neurons also were decreased by application of Agm (1.0 µmol/L) to 1.49 ± 0.17 Hz (P<0.001), with a latency of 68 ± 5 s, and others were not affected (Fig. 4 and Table 2).
2.4 Effects of Agm on L-NAME-induced discharges of neurons in SFO slices

Application of NOS inhibitor L-NAME (50 mmol/L) into the superfusate also significantly increased the DR of 6/9 (66.7%) neurons in SFO slice from 1.60 ± 0.20 to 3.81 ± 0.26 Hz (P<0.05), with a latency of 52 ± 3 s, and the other 3 neurons had no response to L-NAME. Application of Agm (1.0 µmol/L) inhibited the increased discharges of all 6/6 (100%) neurons from 3.81 ± 0.26 to 1.39 ± 0.15 Hz (P<0.001), with a latency of 88 ± 8 s (Fig.5 and Table 3).

3 DISCUSSION

The SFO, as one of CVO, plays an important role in central regulation of the autonomic nervous system and sends the efferent projections to various hypothalamic sites, including the paraventricular, supraoptic, and median preoptic nuclei[7]. The present study for the first time demonstrated that Agm inhibited spontaneous discharge and L-glutamate-, Bay K-8644- or L-NAME-induced discharges of neurons in SFO slice preparations. It was conformable with the effects of Agm on neurons in CA1 area of hippocampal slices in our previous study[6].

L-glutamate is a major excitatory amino acid in CNS and its receptors have NMDA and non-NMDA subtypes. L-glutamate has effects on both subtypes, but which one being predominant is unclear. Our previous experiment found that L-glutamate can induce the epileptiform discharge of neurons in hippocampal slices, an effect which was related to increased influx of calcium[6,8,9]. In cultured cerebellar granule cells, L-glutamate induced neurotoxicity[10], which results from the overstimulation of the NMDA receptors, leading to alteration in calcium homeostasis[11]. Agm can reverses these effects by blocking NMDA receptors[6,10]. L-glutamate also is a main excitatory neurotransmitter in the SFO and its action is at least partly mediated by non-NMDA receptors[12,13]. Liu et al.[14], using immunocytochemical staining technique demonstrated that L-glutamate AMPA receptors 2 and 3 subtypes distribution in SFO in abundance. Extracellular and patch-clamp recording from freshly dissociated rat SFO neurons showed that L-glutamate activates angiotensin II sensitive SFO neurons by opening ligand-gated cation channels[15]. It is well established that an activation of non-NMDA receptors can facilitate the voltage-dependent synaptic transmission, which results in an increase in the activity of NMDA receptors[14]. In the present study, Agm inhibited the L-glutamate- and Bay K-8644-induced discharges. It is suggested that Agm can block NMDA receptors, and thereby reduce calcium influx.
The presence of nNOS, the neuronal isoform of NOS, was demonstrated immunocytochemically in SFO slices\[16\]. Application of the NO donor sodium nitroprusside (SNP) led to an inhibition of the spontaneous electrical activity of the neurons in SFO\[17\]. NO also can protect SFO neurons from overexcitability by excitatory neurotransmitters\[18\]. In this experiment, application of NOS inhibitor L-NAME increased the DR, which was subsequently decreased by Agm. Agm might be a precursor for NO generation\[19\]. From this point of view, Agm would induce an increase in the production of NO, thereby lead to an increase in the levels of intracellular cGMP with a subsequent reduction in intracellular calcium. On the contrary, Galea et al.\[19\] believed that Agm was a competitive NOS inhibitor, but not a precursor for NO. So the mechanism that Agm decreases the effects of L-NAME remains further to be established.

In summary, Agm exerts inhibitory effects on spontaneous discharge and L-glutamate, Bay K-8644 and L-NAME-induced discharge of neurons in SFO, which may be related to the blockade of NMDA receptors and the reduction in calcium influx.

REFERENCES


