EFFECT OF INTRACAROTID ADMINISTRATION OF ADENOSINE ON THE ACTIVITY OF AREA POSTREMA NEURONS IN BARODENERVATED RATS

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ABSTRACT
To observe the effect of intracarotid administration of adenosine on the electrical activity of area postrema (AP) neurons, 76 spontaneous active units were recorded from 45 sinoaortic denervated Sprague-Dawley rats using extracellular recording technique. The results obtained are as follows. (1) Following intracarotid administration of adenosine (Ado, 25 μg/kg), the discharge rate of 29 out of 42 units decreased markedly from 6.126 ± 0.175 to 4.174 ± 0.176 spikes/s (P < 0.01), whereas that of 6 units increased from 4.113 ± 0.177 to 4.172 ± 0.183 spikes/s (P < 0.01), and the other 7 showed no response. Blood pressure (BP) and heart rate (HR) were unaltered throughout the experiment. (2) 8-phenyltheophylline (8PT, 15 μg/kg), a nonselective adenosine receptor antagonist, completely blocked the inhibitory effect of Ado in 10 units. (3) Selective A1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 50 μg/kg), blocked the effect of Ado in 12 units to a remarkable extent. (4) Glibenclamide (500 μg/kg), a blocker of ATP-sensitive potassium channel, abolished the effect of Ado in 12 units. The above results indicate that Ado can inhibit spontaneous electrical activity of AP neurons, which is mediated by adenosine A1 receptor with the involvement of ATP-sensitive potassium channels.

Key words: adenosine; area postrema; spontaneous single unit discharge; 8-phenyltheophylline; 8-cyclopentyl-1,3-dipropylxanthine; glibenclamide

Adenosine (Ado) may play a role as a central modulator in cardiovascular regulation[1]. Our previous study demonstrated that Ado does result in an enhanced neuronal expression of Fos (a marker of neuronal activation) in baroreflex pathway, including the nucleus of the solitary tract (NTS), area postrema (AP) and rostral ventrolateral medulla (RVLM)[2], specifically in the AP. Such a result suggests that Ado is able to affect the activity of some regions in CNS involved in the baroreflex function, especially in AP. Furthermore, we have showed that Ado exerts an inhibitory action on the spontaneous electrical activity of RVLM neurons[3], which receive projections from AP[4]. It is well known that the AP is a circumventricular organ located on the dorsal surface of the medulla, and it has a dense vascular supply and lacks the blood-brain barrier and thus even allows circulating peptides to gain direct access to specific central neural components within the AP. Substantial evidence documented...
that AP is an important site involved in cardiovascular regulation\textsuperscript{[5]}. Previous studies have shown that AP neurons might be excited by circulating arginine vasopressin (AVP)\textsuperscript{[6]}, endothelin\textsuperscript{[7,8]} and angiotensin II (ANG II)\textsuperscript{[9]}. In a study of our lab a strong case was built for the AP as the site where AVP acted to augment the baroreflex\textsuperscript{[10]}. So it is proposed that AP may participate in the Ado\textsuperscript{2} induced facilitation of baroreflex. However, owing to the superficial location of AP on the medulla oblongata and the small size of the neurons, it is difficult to keep the AP stable for long-lasting electrophysiological recording. Up to now, few electrophysiological studies have attempted to elucidate the direct effect of Ado on the electrical activity of AP neurons. The purpose of our study was to investigate the effect of Ado on the spontaneous electrical activity of AP neurons and to identify the involved subtype of Ado receptors.

1 MATERIALS AND METHODS

Forty-five male Sprague-Dawley rats weighing 280\textendash}350 g were anesthetized with urethane (110 g/kg) intraperitoneally. Supplemental doses of the anesthetics were given as necessary.

Surgical procedure

The animals breathed spontaneously through an intratracheal tube. The right femoral artery and vein were cannulated for measurement of blood pressure (BP) by a pressure transducer (MPU2015) and for saline infusion (015\textendash}017 ml/h). Heart rate (HR) was monitored by a heart rate counter (AT2601G, Nihon Kohden) triggered by the differential signal of the arterial pressure pulse. A polyethylene catheter inserted into common carotid artery was advanced to the bifurcation region for drug administration. \(pO_2\), \(PCO_2\) and \(pH\) of arterial blood monitored by a blood gas analyzer (ABL21, Radiometer) were 1314 ± 016 kPa, 419 ± 013 kPa and 7141 ± 0102, respectively.

Sinoaortic denervation

Carotid sinus areas were fully exposed and sternohyoid muscles and superior laryngeal nerves were cut. The aortic depressor nerves and the carotid sinus nerves were sectioned under a dissecting microscope. The superior cervical sympathetic trunk and recurrent laryngeal nerves were also cut. Completeness of barodenervation was ensured by the absence of decrease in HR when hypertension was initiated by iv injection of phenylephrine (2\textendash}3\mu g).

Single unit recordings

The animals were placed in a stereotaxic frame (Model 1C, Jiangwan, China) with their head positioned vertically (nose down). A midline incision was made and the atlantooccipital membrane removed to expose the dorsal surface of the AP, and then immersed in warm liquid paraffin. Cerebrospinal fluid was constantly drained with a strip of twisted cotton. A pressure foot placed over this region was used to stabilize the exposed region. Extracellular single unit recordings were obtained using a glass micropipettes (<1 \(\mu m\) tip, DC resistance 10\textendash}20 M\(\Omega\)) filled with 2% Pontamine Sky Blue and 015 mol/L acetate sodium. The recording electrode was positioned to the middle surface of AP (stereotaxic coordinates: 00 013 mm lateral to midline, 414\textendash}418 mm posterior to interaural line, and 0\textendash}400\(\mu m\) from the dorsal surface of medulla oblongata) and inserted at a speed of 1\(\mu m/s\) by a micromanipulator (SM201, Narishige). The electrical activity was first amplified by a microelectrode amplifier (MEZ7200, Nihon Kohden) and then fed to a biophysical amplifier (AB2621G, Nihon Kohden). The amplified bioelectrical signals were recorded along with BP and HR.
on a polygraph system (RM26000, Nihon Kohden) with a thermal array recorder (WS2682G, Nihon Kohden; bandpass width: 0–218 kHz).

114 Histology At the end of the experiment, the dye filled in the glass micropipettes was diffused out with a negative current of 60μA/min. After the animals were deeply anesthetized, the brains were quickly removed and fixed in 10% formalin. After 7–10 d, frozen brain tissue was sectioned in the coronal plane (50μm). Histological verification was carried out with reference to the Bao and Shu’s stereotaxic Atlas of the rat brain[11]. Data from those electrode tips not in the desired area were excluded.

115 Protocols A period of 30 min was allowed for stabilization after operation before trying to record a spontaneous activity. After a stable recording was obtained for more than 5 min, the drugs were administered. Before application of the drugs, the vehicle (normal saline) was used as controls. The experimental animals were divided into the following groups. Groups 1: After a stable recording was obtained, intracarotid administration (ica) of adenosine (25μg/kg, Sigma) was carried out, and the changes of BP, HR and electroneurogram (ENG) of AP neurons were examined. Group 2: BP, HR and ENG were recorded following ica of adenosine before and after administration of a nonselective adenosine antagonist, 82phenyltheophylline (82PT, 15μg/kg, Sigma), by the same route. Group 3: BP, HR and ENG were examined following ica of adenosine before and after administration of a selective A1 adenosine receptor antagonist, 82cyclopentyl21,32dipropylxanthine, via the same route (DPCPX, 50μg/kg, Sigma). Group 4: BP, HR and ENG were examined following ica of adenosine before and after administration of a blocker of ATP-sensitive potassium channel, glibenclamide, via the same route (Gl, 500μg/kg, Sigma).

116 Statistics All data are expressed as means ±SE. The differences of the parameters between the vehicle and adenosine were compared by paired Student’s t test. Differences between groups were assessed using unpaired t test. Statistical significance was accepted when P < 0.05.

2 RESULTS

Seventy-six spontaneously active single units of AP within the desired area were recorded from 45 rats. The mean firing rate of the neurons was 5156 ±0193 spikes/s. Figure 1 is a composite picture of the locations where the spontaneously active single units were recorded.

211 Effects of ica of Ado on ENG, BP and HR (n = 42)

Ado injected into carotid artery resulted in remarkable decrease of the firing rate in 29 of the 42 AP units from 6126 ±0175 to 4174 ±0176 spikes/s (P < 0.01), while 6 units was increased from 4113 ±0177 to 4172 ±0183 spikes/s (P < 0.05), and the remaining 7 showed no response. BP and HR were unaltered throughout the experiment. Intracarotid injection of solvent caused no significant changes in the baseline values of these parameters (Table 1).
Fig 11 Histologically verified recording sites for microelectrode tips in AP
Each section represents 250 μm in rostro-caudal plane. X, dorsal motor nucleus of vagus; ts, tractus solitarius; XII, hypoglossal nucleus; C, central canal. NTS, nucleus of the solitary tract; AP, area postrema. Closed circle: units responsive to adenosine; triangle: units not in the AP.

Table 1 Effects of adenosine on MAP, HR and ENG AP neurons (n = 42)

<table>
<thead>
<tr>
<th></th>
<th>MAP/ kPa</th>
<th>HR/ bpm</th>
<th>ENG spikes/s ( \cdot ) ^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15136 ±0145</td>
<td>390 ±10</td>
<td>6126 ±0175</td>
</tr>
<tr>
<td>Adenosine</td>
<td>14178 ±0151</td>
<td>383 ±12</td>
<td>4174 ±0176 ^{33}</td>
</tr>
</tbody>
</table>

^{33} P < 0101 , compared with control.

212 Effect of 82PT on the actions of Ado (n = 10)

In 10 units, ica of Ado decreased the discharge rate from 5101 ±0194 to 3144 ±0183 spikes/ s (P < 0101). After ENG returned to the control level, ica of 82PT (15 μg/ kg) was conducted, but did not show any effect on ENG, BP nor HR, and further ica of Ado decreased the discharge rate of AP neurons only insignificantly (Table 2).

213 Effect of DPCPX on the actions of Ado (n = 12)

Ica of Ado markedly decreased ENG of 12 units. After administration of DPCPX at a dose of 50 μg/ kg, the inhibitory action of Ado on these units was blocked (Table 2), although DPCPX per se did not affect HR, BP or ENG.
Table 2  Blocking actions of 82PT, DPCPX and Gi on the effects of intracarotid injection of adenosine on MAP, HR and ENG of AP neurons

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MAP/ kPa</th>
<th>HR/ bpm</th>
<th>ENG spikes s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>14189 ±0143</td>
<td>386 ±8</td>
<td>5101 ±0194</td>
</tr>
<tr>
<td>Ado (25μg/ kg)</td>
<td>10</td>
<td>14176 ±0141</td>
<td>383 ±6</td>
<td>3144 ±0183</td>
</tr>
<tr>
<td>82PT (15μg/ kg) + Ado</td>
<td>10</td>
<td>15103 ±0136</td>
<td>385 ±10</td>
<td>4197 ±0186</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>15132 ±0153</td>
<td>393 ±9</td>
<td>5123 ±1118</td>
</tr>
<tr>
<td>Ado (25μg/ kg)</td>
<td>12</td>
<td>15113 ±0147</td>
<td>387 ±7</td>
<td>4115 ±1106</td>
</tr>
<tr>
<td>DPCPX(50μg/ kg) + Ado</td>
<td>12</td>
<td>15121 ±0142</td>
<td>391 ±8</td>
<td>5139 ±1113</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>15119 ±0131</td>
<td>389 ±5</td>
<td>5130 ±0181</td>
</tr>
<tr>
<td>Ado (25μg/ kg)</td>
<td>12</td>
<td>15107 ±0128</td>
<td>395 ±8</td>
<td>3168 ±0162</td>
</tr>
<tr>
<td>Gi (500μg/ kg) + Ado</td>
<td>12</td>
<td>15111 ±0132</td>
<td>392 ±7</td>
<td>5112 ±0179</td>
</tr>
</tbody>
</table>

33 P < 0101, compared with control.

214 Effect of glibenclamide on the action of Ado (n = 12)

Ica of Ado significantly decreased the ENG in other 12 units. Following administration of Gi at a dose of 500μg/ kg, the inhibitory action of Ado on ENG was abolished (Table 2), whereas Gi per se did not exert any action on HR, BP or ENG.

3 DISCUSSION

AP contains small neurons that project mainly to the NTS, the parabrachial nucleus and RVLM⁴,¹². There is much evidence indicating that the AP plays an important role in cardiovascular regulation, particularly when acting as a link between circulating hormones and central autonomic path¬
ways. However, there have been relatively few successful attempts to record neuronal activity within this region in vivo. The difficulty in recording from AP stems mainly from the instability of the medulla. In addition, AP neurons are small and have a low, or indeed may lack, spontaneous activity, thus making them difficult to locate and isolate electrophysiologically. The method developed in this paper enables us to keep a stable recording in order that the effect of Ado on AP neurons can be examined.

The present experiment indicated that Ado exhibited an inhibitory effect on the spontaneous electrical activity of AP neurons. This was caused by the direct action of Ado rather than a secondary effect. As sinoaortic denervated rats were used to examine the effects on AP neurons and the BP had no significant change, the indirect effects from baroreflex on AP neurons and the secondary response to the changes in cerebral blood flow were ruled out.

Ado exerts its actions via membranebound receptors which have been characterized using biochemical, electrophysiological and radioligand binding techniques. The remarkable feature of AP neurons is that although small in size, these cells have receptors for a great number of substances. In our experiments, the responses elicited by Ado were virtually abolished by 8PT, suggesting that these responses were mediated by Ado receptors in the AP neurons. Furthermore, since Krestew et al. had utilised radioligand binding assay to characterize the properties of Ado A1 receptors in the dorsal vagal complex (NTS, AP and dorsal motor nucleus of the vagus) of rats, a selective Ado A1 receptor antagonist, DPCPX, was used in our experiment, and it could completely block the action of Ado on ENG of AP neurons. It is likely that Ado displays an inhibitory effect on AP neurons through A1 Ado receptors.

Ado is considered to be an inhibitory neuromodulator in the mammalian brain, since it can inhibit the release of neurotransmitter or increase the channel conduction of the postsynaptic membranebound receptors. In vivo, parental administration of EHNA, an Ado deaminase inhibitor, profoundly decreases spontaneous motor activity in mice and rats. It has been demonstrated that Ado is capable of inhibiting the release of neurotransmitter, increasing K+ channel conductance of the postsynaptic membrane and reducing the activity of adenylate cyclase or phospholipase C. Such effects are operated by A1 receptor. It is well established that A1 receptor belongs to the G protein-coupled receptor superfamily, and Ado binding to A1 receptors coupled with G protein might activate ATP2-sensitive K+ channels. In our experiment, pretreatment with G1, a potent blocker of ATP2-sensitive K+ channels, could attenuate the inhibitory effect of Ado on AP neurons. This result is consistent with our previous study on RVLM neurons.

In summary, the results of the present study suggest that Ado may inhibit the spontaneous electrical activity of AP neurons in vivo, which is an effect mediated by A2 receptor with the involvement of ATP2-sensitive K+ channels.
REFERENCES


颈动脉内注射腺苷对去缓冲神经大鼠最后区神经元放电的影响

陈爽 何瑞荣

摘要 在45只切断双侧缓冲神经的Sprague-Dawley大鼠，应用细胞外记录方法，观察了颈动脉内注射腺苷对76个最后区（AP）神经元自发放电活动的影响。所得结果如下：（1）在记录到的42个自发放电单位中，颈动脉内注射腺苷（25μg/kg）引起其中29个单位的放电频率由6126±0175下降至4174±0176 spikes/s（P<0101），6个单位放电频率由4113±0177增加至4172±0183 spikes/s（P<0105），另外7个单位放电频率无明显变化，而血压和心率在实验中无变化；（2）在应用非选择性腺苷受体拮抗剂82 phenyltheophylline（15μg/kg）的10个单位，腺苷对放电的抑制效应可被完全阻断；（3）应用选择性腺苷A1受体拮抗剂82cyclopentyl1,3dipropylxanthine（50μg/kg）的12个单位，腺苷的上述效应亦可被消除。以上结果提示，腺苷对AP区神经元自发放电有抑制作用，而此作用与A1受体介导的ATP敏感性钾通道开放有关。

关键词：腺苷；最后区；自发放电单位；82 phenyltheophylline；82cyclopentyl1,3dipropylxanthine；格列苯脲

学科分类号：Q463；R33113