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Presynaptic release of ATP from superior cervical ganglion of rats modulated by various receptors

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KEY WORDS adenosine triphosphate; superior cervical ganglion; oxotremorine; serotonin; atropine; yohimbine; sulpiride; histamine

ABSTRACT

AIM: To determine whether adenosine 5'-triphosphate (ATP) is released from the superior cervical ganglion (SCG) of rats and whether the release is regulated by presynaptic mechanism. **METHODS**: Using the luciferin-luciferase technique. **RESULTS**: Electric stimulation evoked the release of ATP from the rat SCG. Adenosine (100 $\mu mol \cdot L^{-1}$), P_1 (A_1) purinoceptor agonist N_6-cyclopentyladenosine (0.1 $\mu mol \cdot L^{-1}$), the muscarinic agonist oxotremorine (1 $\mu mol \cdot L^{-1}$), and 5-hydroxytryptamine (100 $\mu mol \cdot L^{-1}$) decreased the evoked release of ATP from the rat SCG. On the contrary, P_1 (A_1) purinoceptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (10 $nmol \cdot L^{-1}$), P_2 purinoceptor antagonist pyridoxal-5-phosphate-6-azophenyl-2',4'-disulphonic acid (10 $\mu mol \cdot L^{-1}$), muscarinic antagonist atropine (1 $\mu mol \cdot L^{-1}$), α_2 adrenoceptor antagonist yohimbine (3 $\mu mol \cdot L^{-1}$), D_2 dopamine receptor antagonist sulpiride (20 $\mu mol \cdot L^{-1}$), and histamine (100 $\mu mol \cdot L^{-1}$) increased the evoked release of ATP from the rat SCG. **CONCLUSION**: ATP is released from the rat SCG and the release of ATP can be presynaptically modulated by P_1 (A_1), P_2, muscarinic, α_2 adrenergic, D_2, 5-HT, and H_1 receptor agonists and antagonists.

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INTRODUCTION

Neurotransmitter release may be regulated by presynaptic receptors on nerve terminals[1,2]. Presynaptic receptors include autoreceptors and heteroreceptors[2]. Autoreceptors are located at nerve terminals, through which neuron's own transmitter may modulate its own release. Heteroreceptors are located on the axon terminal and stimulated by transmitters released from different types of neurons. There were presynaptic muscarinic receptor[3], α- and β-adrenoceptors[4] in the superior cervical ganglion (SCG). Immunohistochemical investigations showed that small intensely fluoresent (SIF) cells of the SCG contained dopamine[5], histamine, and 5-hydroxytryptamine (5-HT)[6]. It appears that synaptic transmission in the SCG can be affected by different transmitters or modulators.

Strong evidence has been provided that ATP can act as a transmitter in the nervous system[7]. In addition, ATP has been suggested to mediate fast synaptic transmission in sympathetic ganglia[8]. The rat SCG contains P_1 purinoceptors that hyperpolarize the ganglion and P_2 purinoceptors that depolarize the SCG[9]. Studies on ATP release in various nervous tissues will provide some clues to explore the role of ATP in the neurotransmission. The aim of this work was to determine whether ATP was released from the rat SCG and whether the release of ATP was regulated by presynaptic mechanism in the rat SCG.

MATERIALS AND METHODS

Chemicals ATP assay mix, luciferin-luciferase, adenosine, oxotremorine sesquifumarate, atropine, sulpiride, histamine, 5-HT were purchased from Sigma. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX), yohimbine, N_6-cyclopentyladenosine

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(CPA) were purchased from Research Biochemical Inc (Natick MA, USA). Pyridoxal 5-phosphate-6-azophenyl-2',4'-disulphonic acid 4-sodium salt (PPADS) was obtained from Tocris-Cookson (Langford, Bristol, UK).

Experimental procedure Sprague-Dawley rats (180 – 220 g) of either sex provided by the Animal Centre of our Institute were anesthetized with urethane (1.5 $g \cdot kg^{-1}$ ip). The SCG together with pre- and postganglionic fibers was excised. The tissues were transferred to an organ bath (2 mL) with a pair of platinum wire electrodes and were perfused with Krebs' solution: NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 11.5 $mmol \cdot L^{-1}$ at a rate of 0.6 $mL \cdot min^{-1}$ at 37 ℃ and gassed with 95 % O_2 + 5 % CO_2. Samples were collected by an automatic fraction collector for 5 min after a 60-min equilibrium and assayed for ATP content. During superfusion two stimulation periods (S_1, S_2) were performed at 30-min intervals and drugs were added to the perfusion solution between S_1 and S_2. Square wave pulses of a 2.5-ms duration were delivered at supramaximal voltage (> 1 $kV \cdot m^{-1}$). The stimulation at 10 Hz for 0.5 min (total of 300 shocks) was administered by an Eltron (Hungary) stimulator. One ganglion of each pair, left or right, served as control.

Assay of ATP The ATP released from the ganglia was assayed using the luciferin-luciferase technique[10]. A standard calibration curve was established using ATP (10 $pmol \cdot L^{-1}$ to 1 $nmol \cdot L^{-1}$). Sample (100 μL) was added to 50 μL of ATP assay mixture containing luciferin-luciferase. The luminescence was measured in an LKB 1250 luminometer for 10 s. The Krebs' solution was used as a blank.

Statistics Data were expressed as $\bar{x} \pm s$ and compared by paired t-test.

RESULTS

Electric stimulation-evoked release of ATP from SCG The release of ATP at rest was (0.033 ± 0.013) $nmol \cdot min^{-1} \cdot g^{-1}$ (tissue) ($n = 32$) in the superfusate. ATP releases evoked by S_1 and S_2 were (3.3 ± 0.4) and (1.92 ± 0.24) $nmol \cdot min^{-1} \cdot g^{-1}$ (tissue), respectively ($n = 32$), the ratio of ATP release at S_2/S_1 was 0.57 ± 0.08.

Effects of purinoceptor agonists and antagonists on stimulation-evoked release of ATP from SCG Adenosine and P_1 (A_1) purinoceptor agonist CPA decreased the evoked release of ATP from the SCG. P_1 (A_1) purinoceptor antagonist DPCPX and P_2 purinoceptor antagonist PPADS increased the evoked release of ATP from the SCG (Tab 1).

Tab 1. Effects of different receptor agonists and antagonists on the evoked release of ATP from rat SCG. $n = 4$ **ganglia from 4 rats.** $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **control.**

Drug/μmol·L^{-1}	Ratio of ATP release (S_2/S_1)	
	Control	Drug present
Adenosine/100	0.54 ± 0.03	0.29 ± 0.04^c
CPA/0.1	0.494 ± 0.018	0.273 ± 0.017^b
DPCPX/0.01	0.663 ± 0.103	0.824 ± 0.024^b
PPADS/10	0.56 ± 0.03	0.84 ± 0.09^b
Oxotremorine/1	0.514 ± 0.015	0.232 ± 0.013^b
Atropine/1	0.61 ± 0.08	0.80 ± 0.03^b
Yohimbine/3	0.471 ± 0.023	0.68 ± 0.04^b
Sulpiride/20	0.492 ± 0.016	0.714 ± 0.019^b
Histamine/100	0.50 ± 0.03	0.61 ± 0.03^b
5-HT/100	0.513 ± 0.014	0.371 ± 0.022^b

Effects of heteroreceptor agonists and antagonists on stimulation-evoked release of ATP from SCG The muscarinic agonist oxotremorine and 5-HT reduced the evoked release of ATP from the SCG. The muscarinic antagonist atropine, α_2 adrenoceptor antagonist yohimbine, D_2 dopamine receptor antagonist sulpiride, and histamine enhanced the evoked release of ATP from the SCG (Tab 1).

DISCUSSION

The study indicates that there is the release of ATP from the rat SCG. The results of this paper provide evidence that the evoked release of ATP can be modulated when presynaptic autoreceptors and heteroreceptors were stimulated.

The purine receptors can be classified into P_1 and P_2 purinoceptors[11]. P_1 purinoceptor is activated by the degradation product adenosine of ATP. P_2 purinoceptor is activated mainly by ATP. P_1 purinoceptor is further classified into A_1 and A_2 subtypes. Adenosine and P_1 (A_1) purinoceptor agonist

CPA decreased the evoked release of ATP. $P_1(A_1)$ purinoceptor antagonist DPCPX and P_2 purinoceptor antagonist PPADS increased the evoked release of ATP. These results showed that the release of ATP from the SCG was regulated by preganglionic $P_1(A_1)$ and P_2 purinoceptors.

Synaptic transmission *via* presynaptic mechanism can be affected by oxotremorine[3], noradrenaline[12], dopamine[13], histamine[14], and 5-HT[15]. Similar findings have been obtained in our works. It is clear that transmitter and modulator state in the SCG is considerably complex and the release of ATP in the SCG can be regulated by different endogenous ligands through different receptors. The experiments support the opinion that the preganglionic nerve terminals of rat SCG are equipped with $P_1(A_1)$, P_2, M, α_2, D_2, H_1, and 5-HT receptors.

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大鼠颈上神经节突触前释放腺苷三磷酸的多种受体调制

R971.9
R965.2

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关键词 腺苷三磷酸; 颈上神经节; 氧化震颤素; 血清素; 阿托品; 育亨宾; 舒必利; 组胺

目的: 研究大鼠颈上神经节 ATP 的释放及其突触前调制. **方法**: 荧光素-荧光素酶测定技术. **结果**: 电刺激大鼠颈上神经节引起 ATP 释放. 腺苷, $P_1(A_1)$嘌呤受体激动剂环戊腺苷, M 胆碱能受体激动剂氧化震颤素, 5-羟色胺减少神经节 ATP 的释放. $P_1(A_1)$嘌呤受体拮抗剂 8-环戊基-1,3-二丙基黄嘌呤, P_2 嘌呤受体拮抗剂吡多醛-5-磷酸-6-偶氮苯基-2′,4′-二磺酸, M 胆碱能受体拮抗剂阿托品, α_2 肾上腺素受体拮抗剂育亨宾, D_2 多巴胺受体拮抗剂舒必利和组胺增加颈上神经节 ATP 的释放. **结论**: 大鼠颈上神经节释放 ATP. $P_1(A_1)$嘌呤受体, P_2 嘌呤受体, M 胆碱能受体, α_2 肾上腺素受体, D_2 多巴胺受体, 5-羟色胺受体及 H_1 组胺受体激动剂或拮抗剂可通过突触前机制调节 ATP 释放.

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