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Short Communication

EXTRACELLULAR ATP BOTH INHIBITS AND FACILITATES CALCIUM CHANNEL CURRENTS IN ACUTELY DISSOCIATED RAT NUCLEUS TRACTUS SOLITARIUS

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Abstract

The postsynaptic actions of exogenously applied adenosine 5′-triphosphate (ATP) were investigated in nucleus tractus solitarius (NTS) of the rat. Whole cell patch-clamp recordings were used to examine the regulation of voltage-dependent Ca^{2+} channels (VDCCs) currents (I_{Ca}) by ATP in freshly dissociated NTS. Application of ATP inhibited I_{Ca} from -905 pA to -741 pA. In addition to this inhibition, application of ATP facilitated I_{Ca} from -941 pA to $-1,094$ pA in other neurons. The data presented here demonstrate for the first time that ATP has both inhibitory and facilitative effects on I_{Ca} in NTS. It can be considered that ATP acts as a neurotransmitter in the NTS by having multiple regulatory effects on VDCCs.

Key words: Nucleus tractus solitarius—Adenosine 5′-triphosphate—Nucleotide receptor—Purinergic receptor—Voltage dependent calcium channel

INTRODUCTION

The nucleus tractus solitarius (NTS) plays a major role in the regulation of cardiovascular, respiratory, gustatory, hepatic and swallowing functions[4,17,19]. This nucleus receives primary afferent input from a wide variety of peripheral organs and tissues and is essential in the integration of autonomic nervous system functions. In accordance with the numerous integrative functions mediated by the NTS, over 30 neurotransmitter/neuromodulators have been found in both cell bodies and terminals within the NTS[19].

Extracellular adenosine 5′-triphosphate (ATP) is known to act as a neurotransmitter in the central and peripheral nervous systems[5,21,28]. It is also one of the many neurotransmitters thought to play a functionally important role in the NTS.

Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators of membrane excitability and Ca^{2+}-dependent functions such as neurotransmitter release, enzyme activity and

gene expression. The modulation of VDCCs is believed to be an important means of regulating Ca^{2+} entry and thus to have a direct influence on many Ca^{2+}-dependent processes.

Several electrophysiological studies have indicated that extracellular ATP inhibits VDCC currents (I_{Ca}) in chromaffin cells[14], in frog sympathetic ganglion[9], and in hamster submandibular ganglion[2]. In contrast, it has been demonstrated that extracellular ATP facilitates I_{Ca} in hippocampal neurons[7]. In NTS, however, the effect of extracellular ATP on neuronal VDCCs has not yet been clarified. Thus, the purpose of the present work was to examine the effects of ATP on I_{Ca} in NTS.

MATERIALS AND METHODS

Experiments were conducted according to international guidelines on the use of animals for experimentation. Young Wistar rats (7–18 days old) were decapitated, and their brains were quickly removed and submerged in ice-cold artificial cerebrospinal fluid (aCSF) saturated with 95% O_2 and 5% CO_2 of the following composition (in mM): NaCl 126, $NaHCO_3$ 26.2, NaH_2PO_4 1, KCl 3, $MgSO_4$ 1.5, $CaCl_2$ 1.5, and glucose 30; pH 7.4. Thin transverse slices from brainstems, $400\mu m$ in thickness, were prepared by a tissue slicer (DTK-1000; Dosaka EM Co., Ltd., Kyoto). After being sectioned, 3–5 slices obtained from a single brain were transferred to a holding chamber and stored in oxygenated aCSF at room temperature for at least 40 min before use. Then, slices were transferred to a conical tube containing gently bubbled aCSF at 36°C to which 1.8 U/ml dispase (grade I; 0.75 ml/slice) was added. After 60 min incubation, slices were rinsed with enzyme-free aCSF. Under a dissecting microscope, the NTS region was micropunched and placed on poly-l-lysine-coated coverslip (Fig. 1A). The cells were then dissociated by trituration using progressively smaller diameter pipettes and allowed to settle on a coverslip for 20 min.

Voltage-clamp recordings were conducted using the whole-cell configuration of the
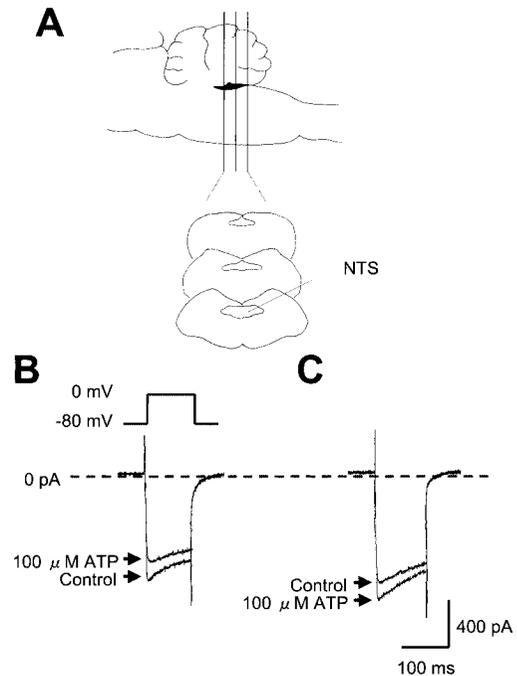

Fig. 1 (A) Location and morphology of acutely isolated nucleus tractus solitarius (NTS) neurons. NTS neurons were isolated from the area indicated. (B) Extracellular ATP-induced inhibition of I_{Ca}. Superimposed sample currents recorded from the indicated voltage protocol in the absence (Control) and presence of $100\mu M$ ATP. (C) Extracellular ATP-induced facilitation of I_{Ca}. Superimposed sample currents recorded from the indicated voltage protocol in the absence (Control) and presence of $100\mu M$ ATP. Current and time calibration bars represent 400 pA and 100 ms, respectively, for the current traces in both B and C.

patch-clamp technique[13]. Fabricated recording pipettes (2–3 MΩ) were filled with an internal solution of the following composition (in mM): 100 CsCl, 1 $MgCl_2$, 10 HEPES, 10 BAPTA, 3.6 MgATP, 14 $Tris_2$phosphocreatine (CP), 0.1 GTP, and 50 U/ml creatine phosphokinase (CPK). The pH was adjusted to 7.2 with CsOH. The inclusion of CP and CPK effectively reduced the "rundown" of I_{Ca}. After the formation of a giga seal, in order to record I_{Ca}, the external solution was replaced from Krebs solution to a solution containing the following (in mM): 67 choline-Cl, 100 tetraethylammonium chloride, 5.3 KCl, 5 $CaCl_2$, and 10 HEPES. The pH was adjusted to 7.4

with Tris base. Command voltage protocols were generated with a computer software pCLAMP version 8 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using a DigiData 1200 interface (Axon Instruments, Union City, CA, U.S.A.). The command pulses were applied to cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with the amplifier and a computer software pCLAMP 8 acquisition system.

RESULTS

The properties of VDCCs in NTS using current-voltage relationships have been demonstrated[24]. NTS has several types of VDCCs, which are full activated at a test potential = 0 mV from a holding potential = −80 mV. Therefore, in this study, it can be considered that full activation of I_{Ca} was obtained by applying a test pulse from a holding potential = −80 mV to a test potential = 0 mV (Fig. 1B and C). An example of extracellular ATP-induced inhibition of I_{Ca} is shown in Fig. 1B. Application of 100 μM ATP inhibited I_{Ca} from −905 pA to −741 pA (18.1% inhibition) in this neuron. In addition to inhibiting I_{Ca}, extracellular ATP-induced facilitation of I_{Ca} could be observed in a different neuron. An example of ATP-induced facilitation of I_{Ca} is shown in Fig. 1C. Application of 100 μM ATP facilitated I_{Ca} from −941 pA to −1,094 pA (16.2% facilitation) in this neuron.

The modulating manner, *i.e.*, inhibition or facilitation, did not depend on the experimental conditions. It was impossible to determine the modulating manner of the ionic current before agonist application.

DISCUSSION

In this study, it was demonstrated that extracellular ATP inhibited and facilitated I_{Ca} in NTS.

The actions of ATP are mediated by purinoceptors that are present on many neurons. These purinoceptors are classified into two major subtypes, P1 and P2, which are preferentially activated by adenosine and ATP, respectively. P2 purinoceptors have been categorized into two major groups, P2X and P2Y, based on their pharmacological properties as well as their molecular structure. P2X are ligand-gated ion channels, while P2Y are G-protein-coupled receptors[3,5]. The P2X purinoceptor family comprises seven cloned receptors: $P2X_{1-7}$[5]. The P2Y purinoceptor family comprises eight cloned receptors: $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$[1,3,6,22]. In addition, the localization and pharmacological profiles of the subtypes of the P2X and P2Y family have also been reported[5]. A study to clarify the pharmacological characterization of the purinoceptors on NTS neurons is now progress in our department.

Additionally, ATP is rapidly hydrolysed by ecto-nucleotidases to ADP, AMP, and adenosine[26]. Adenosine, the final hydrolysed metabolite of ATP[12,18,23], is another strong neuromodulator that activate P1 purinoceptors[23]. Therefore, we can not rule out the possibility of the contribution of P1 purinoceptors in the ATP-induced modulation of I_{Ca}.

It has been reported that several different types of VDCCs exist in NTS[24]. From the functional point of view, VDCCs have been classed into "high" and "low" threshold on the basis of the voltage range at which they are activated. Low-threshold VDCCs have been also become known as T-type, which serve as prolong the duration of the Ca^{2+}-dependent spike burst and of the generation of rhythmic firing[15,16]. On the other hand, high-threshold VDCCs can be grouped into various classes according to their sensitivity to VDCCs blockers and the fact that they have different roles[10]. It is well established that these activities result from the expression of a family of related VDCC molecules that exist as multisubunit complexes. For example, neurotransmitter secretion seems to be preferentially coupled to one or more of these VDCC types under

different circumstances[20,25]. In our department, several studies suggest that many neurotransmitters modulate distinct types of VDCCs. For example, opioid inhibits L, N, and P/Q-type VDCCs. CGRP facilitates L- and N-type VDCCs. VIP both inhibits and facilitates L- and N-type VDCCs, respectively[10]. It will be important in future experiments to determine which types of VDCC contribute to the ATP-induced modulation in NTS neurons.

In conclusion, the activation of purinoceptors inhibits and facilitates VDCCs in NTS. These dual effects can be explained as follows: Reduction of the Ca^{2+} influx through VDCCs might inhibit the Ca^{2+}-activated K^+ currents responsible for post-spike hyperpolarization (AHPslow), thereby increasing neuronal excitability. In contrast, entry of Ca^{2+} through VDCCs opens a class of small-conductance Ca^{2+}-activated K^+ channels (SK channels); these induce a prolonged AHPslow and contribute to the accommodation of spike discharge frequency during sustained depolarization[8].

In combination with previous observations, the widespread distribution of P2Y and P2X purinoceptors in NTS[11,27] and the present demonstration of the purinoceptor's modulation of VDCCs, provide mounting evidence for the likely role of purinergic neurotransmission in NTS and central autonomic regulation. Therefore, the purinoceptors' pathway must be investigated in a further study.

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REFERENCES

1) Abbracchio, M.P., Boeynaems, J.M., Barnard, E.A., Boyer, J.L., Kennedy, C., Miras-Portugal, M.T., King, B.F., Gachet, C., Jacobson, K.A., Weisman, G.A. and Burnstock, G. (2003). Characterization of the UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family. *Trends Pharmacol Sci* **24**, 52–55.

2) Abe, M., Endoh, T. and Suzuki, T. (2003). Extracellular ATP-induced calcium channel inhibition mediated by P1/P2Y purinoceptors in hamster submandibular ganglion neurons. *Br J Pharmacol* **138**, 1535–1543.

3) Boarder, M.R. and Hourani, S.M.O. (1998). The regulation of vascular function by P2 receptors: Multiple sites and multiple receptors. *Trends Pharmacol Sci* **19**, 99–107.

4) Bradley, R.M., King, M.S., Wang, L. and Shu, X. (1996). Neurotransmitter and neuromodulator activity in the gustatory zone of the nucleus tractus solitarius. *Chem Senses* **21**, 377–385.

5) Burnstock, G. (1997). The past, present and future of purine nucleotides as signaling molecules. *Neuropharmacology* **36**, 1127–1139.

6) Communi, D., Gonzales, N.S., Detheux, M., Rezillon, S., Lannoy, V., Parmentier, M. and Boeynaems, J.M. (2001). Identification of a novel human ADP receptor coupled to G_i. *J Biol Chem* **276**, 41479–41485.

7) Dave, S. and Mogul, D.J. (1996). ATP receptor activation potentiates a voltage-dependent Ca channel in hippocampal neurons. *Brain Res* **715**, 208–216.

8) Davies, P.J., Ireland, R.J. and McLachln, E.M. (1996). Sources of Ca^{2+} for different Ca^{2+}-activated K^+ conductances in neurones of the rat superior cervical ganglion. *J Physiol* **495**, 353–366.

9) Elmslie, K.S. (1992). Calcium current modulation in frog sympathetic neurones: Multiple neurotransmitters and G proteins. *J Physiol* **451**, 229–246.

10) Endoh, T. (2004). Modulation of voltage-dependent calcium channels by neurotransmitters and neuropeptides in parasympathetic submandibular ganglion neurons. *Arch Oral Biol.* (in press)

11) Fong, A.Y., Krstew, E.V., Barden, J. and Lawrence, A.J. (2002). Immunoreactive localisation of $P2Y_1$ receptors within the rat and human nodose ganglia and rat brainstem: comparison with [$\alpha^{33}P$] deoxyadenosine 5′-triphosphate autoradiography. *Neuroscience* **113**, 809–823.

12) Goldon, E.L., Pearson, J.D., Dickinson, E.S., Moreau, D. and Slakey, L.L. (1989). The hydrolysis of extracellular adenine nucleotides by arterial smooth muscle cells. Regulation of adenosine production at the cell surface. *J Biol Chem* **264**, 8986–8995.

13) Hamill, O.P., Marty, A., Neher, E., Sakmann, B.

and Sigworth, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch Eur J Physiol* **391**, 85–100.

14) Hernandez-Guijo, J.M., Carabelli, V., Gandia, L., Garcia, A.G. and Carbone, E. (1999). Voltage-independent autocrine modulation of L-type channels mediated by ATP, opioids and catecholamines in rat chromaffin cells. *Eur J Neurosci* **11**, 3574–3584.

15) Herrington, J. and Lingle, C. J. (1992). Kinetic and pharmacological properties of low voltage-activated Ca²⁺ current in rat clonal (GH₃) pituitary cells. *J Neurophysiol* **68**, 213–232.

16) Huguenard, J.R., Gutnick, M.J. and Prince, D.A. (1993). Transient Ca²⁺ current in neurons isolated from rat lateral habenula. *J Neurophysiol* **70**, 158–166.

17) Jean, A. (2001). Brain stem control of swallowing: Neuronal network and cellular mechanisms. *Physiol Rev* **81**, 929–969.

18) Kennedy, C., McLaren, G.J., Westfall, T.D. and Sneddon, P. (1996). ATP as a co-transmitter with noradrenaline in sympathetic nerves-function and fate. *Ciba Found Symp* **198**, 223–235.

19) Lawrence, A. and Jarrott, B. (1996). Neuro-chemical modulation of cardiovascular control in the nucleus tractus solitarius. *Prog Neurobiol* **48**, 21–53.

20) Luebke, J.I., Dunlap, K. and Turner, T.J. (1993). Multiple Ca²⁺ channel types control glutamatergic synaptic transmission in the hippocampus. *Neuron* **11**, 895–902.

21) Nieber, K., Polechen, W. and Illes, P. (1997). Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurones of the rat. *Br J Pharmacol* **122**, 423–430.

22) Ralevic, V. and Burnstock, G. (1998). Receptors for purines and pyrimidines. *Pharmacol Rev* **50**, 413–492.

23) Rathbone, M.P., Middlemiss, P.J., Gysberg, J.W., Andrew, C., Herman, M.A., Reed, J.K., Ciccarelli, R., Diiorio, P. and Caciagli, F. (1999). Trophic effects of purines in neurons and glial cells. *Prog Neurobiol* **59**, 663–690.

24) Rhim, H. and Miller, R.J. (1994). Opioid receptors modulate diverse types of calcium channels in the nucleus tractus solitarius of the rat. *J Neurosci* **14**, 7608–7615.

25) Takahashi, T. and Momiyama, A. (1993). Different types of calcium channels mediate central synaptic transmission. *Nature* **366**, 156–158.

26) Welfold, L.A., Cusack, N.J. and Hourani, S.M.O. (1986). ATP analogues and the guinea-pig taenia coil: A comparison of the structure-activity relationships of ectonucleotidases with those of the P₂-purinoceptor. *Eur J Pharmacol* **129**, 217–224.

27) Yao, S.T., Barden, J.A. and Lawrence, A.J. (2001). On the immunohistochemical distribution of ionotropic P2X receptors in the nucleus tractus solitarius of the rat. *Neuroscience* **108**, 673–685.

28) Zimmermann, H. (1994). Signaling *via* ATP in the nervous system. *Trends Neurosci* **17**, 420–426.

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