Short Communication

Arg-vasopressin Facilitates Calcium Channel Currents in Osteoblasts

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Abstract

The hypothalamic nonapeptide and neurohypophyseal hormone arg-vasopressin (AVP), also known as antidiuretic hormone, is best known for its effects on water reabsorption in kidney. Osteoblasts play a major role in bone formation, employing intracellular Ca^{2+} as a second messenger to modulate hormonal responses and as a cofactor for mineralization. Voltage-dependent Ca^{2+} channels (VDCCs) mediate the influx of Ca^{2+} in response to membrane depolarization. The purpose of this study was to investigate the effects of AVP on VDCC currents in osteoblasts using a patch-clamp recording method. An application of $1\ \mu\text{M}$ AVP facilitated VDCC currents in osteoblasts. To our knowledge, the data presented here demonstrate for the first time that AVP facilitates VDCCs in osteoblasts.

Key words: Arg-vasopressin — Osteoblast — Voltage-dependent Ca^{2+} channels — Patch-clamp experiment

Introduction

Intracellular Ca^{2+} signals coordinate cell behavior, which translates into systemic con-

trol of Ca^{2+} homeostasis and maintenance of normal bone mineral density. The activities of bone cells have been reported to be regulated by various systemic and auto/paracrine

factors.

Arg-vasopressin (AVP) is a nonapeptide, mainly produced in two hypothalamic nuclei, the supraoptical and the paraventricular. It has been demonstrated that AVP stimulates proliferation of human osteoblast-like (hOB) cells⁷.

Voltage-dependent Ca^{2+} channels (VDCCs) serve as crucial mediators of membrane excitability⁹ and many Ca^{2+} -dependent functions such as growth of bone³, regulation of proliferation⁸, enzyme activity¹⁴ and gene expression¹⁰. The purpose of this study was to investigate the effects of AVP on VDCC currents (I_{Ca}) in osteoblastic MC3T3-E1 cells.

Materials and Methods

Murine osteoblastic MC3T3-E1 cells were purchased from Summit Pharmaceuticals International Corporation (Tokyo, Japan). The cells were cultured at 37°C in a 5% CO_2 atmosphere with α -modified minimal essential medium (α -MEM; Gibco BRL, Grand Island, NY, U.S.A.). Unless otherwise specified, the medium contained 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Cell culture medium was changed every 2–3 days. For patch-clamp experiments, cells were harvested using a 0.05% trypsin/0.02% EDTA solution after reaching confluence. Cells were plated at very low density in 35-mm tissue culture dishes. Prior to recordings, the cells were washed at least 3 times with Krebs solution of the following composition (in mM): 136 NaCl, 5 KCl, 2.5 CaCl_2 , 0.5 MgCl_2 , 10.9 glucose; 11.9 NaHCO_3 and 1.1 NaH_2PO_4 . The pH was 7.3–7.4. Cell culture reagents were purchased from Sigma (Tokyo, Japan).

Voltage-clamp recordings were obtained using whole-cell configuration patch-clamp technique⁶. Fabricated recording pipettes (2–3 M Ω) were filled with internal solution of the following composition (in mM): 150 CsCl, 5 EGTA, 10 glucose, and 10 HEPES. The pH was adjusted to 7.3 with CsOH. After the formation of a giga seal, in order to record

I_{Ca} carried by Ba^{2+} (I_{Ba}), the extracellular solution was replaced with Krebs solution to a solution containing the following (in mM): 115 BaCl_2 and 20 HEPES. The pH was adjusted to 7.4 with tetraethylammonium hydroxide. Command voltage protocols were generated with the computer software pCLAMP version 10 (Axon Instruments, Union City, CA, U.S.A.) and transformed to an analogue signal using the DigiData1440A interface (Axon Instruments). The command pulses were applied to the cells through an L/M-EPC7 amplifier (HEKA Elektronik, Lambrecht, Germany). The currents were recorded with an amplifier and the computer software pCLAMP10 acquisition system. Access resistance (<15 M Ω) was determined by transient responses to voltage commands. Arg-vasopressin was purchased from Sigma. The AVP was dissolved in distilled water and diluted to the desired final concentration in the external solution just before use.

Results

The properties of VDCCs in osteoblasts have been demonstrated previously using current-voltage relationships¹⁵. Osteoblasts have VDCCs, which are fully activated at a test potential (= 10 mV) from a holding potential (= –80 mV). Therefore, in this study, full activation of I_{Ba} was obtained by applying a test pulse from a holding potential of –80 mV in depolarizing voltage steps of 10 mV evoked every 5 sec.

Superimposed I_{Ba} traces in the absence or presence of 1 μM AVP are shown in Fig. 1. As shown in Fig. 1, application of 1 μM AVP rapidly and reversibly facilitated I_{Ba} from –242 to –338 pA in this cell ($n = 1$).

Discussion

The results of this study showed that AVP facilitated VDCCs in osteoblasts.

Although it is well known that prostaglandins are involved in bone resorption, AVP

Fig. 1 Typical superimposed I_{Ba} traces in absence or presence of $1 \mu\text{M}$ AVP

I_{Ba} was evoked from holding potential of -80 mV in 100-msec voltage steps to 10 mV at 5-sec intervals.

stimulates the release of prostaglandins, and it has been suggested that AVP may stimulate bone formation through prostaglandin E_2 ^{2,5}. Arg-vasopressin acts through two types of receptor, V1 and V2, with the V1-receptor being subdivided into V1a and V1b. Arg-vasopressin is mainly associated with its vasoconstrictor effect mediated through V1a-receptors in the blood vessels and its antidiuretic effect mediated through V2-receptors located within the kidney. Petersson *et al.* demonstrated that the effects of AVP were mediated through the V1-receptor which is G-protein coupled to phospholipase C and the diacylglycerol/protein kinase C pathway in hOB cells¹².

Arg-vasopressin has been shown to trigger intracellular Ca^{2+} mobilization and Ca^{2+} oscillations in the inner medullary collecting duct^{13,16}. It also induced Ca^{2+} oscillation in mouse thick ascending limb, which was associated with AVP-stimulated secretion of nucleotides¹¹. Galizzi *et al.* reported that AVP inhibited L-type VDCCs measured as $^{45}\text{Ca}^{2+}$ uptake under depolarizing conditions in aortic muscle cells⁴. Bonev and Isenberg demonstrated the AVP-induced facilitation of VDCCs in guinea-pig ventricular myocytes¹.

In this study, we used $1 \mu\text{M}$ AVP to modulate I_{Ba} . The concentration of AVP applied was quite high. It has been demonstrated that AVP affects osteoblast proliferation in concentrations as low as 10 pmol/liter , which is close to that in normal physiological plasma

(5 pmol/liter)⁷. Therefore, we are now in the process of pharmacologically characterizing the effect of AVP on osteoblastic cells at our department.

As mentioned above, VDCCs are essential in the regulation of many cellular processes, including cell growth and proliferation. Therefore, further study is needed to elucidate the precise mechanism underlying the facilitation of VDCCs by AVP in osteoblasts.

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References

- 1) Bonev A, Isenberg G (1992) Arginine-vasopressin induces mode-2 gating in L-type Ca^{2+} channels (smooth muscle cells of the urinary bladder of the guinea-pig). *Pflügers Arch* 420:219–222.
- 2) Cooper CL, Malik U (1985) Mechanism of action of vasoactive hormones on prostaglandin synthesis in the kidney. *Adv Prostaglandin Thromboxane Leukot Res* 15:437–439.
- 3) Duriez J, Flautre B, Blary MC, Hardouin P (1993) Effects of the calcium channel blocker nifedipine on epiphyseal growth plate and bone turnover: a study in rabbit. *Calcif Tissue Int* 52:120–124.
- 4) Galizzi JP, Qar J, Fosset M, Van Renterghem C, Lazdunski M (1987) Regulation of calcium channels in aortic muscle cells by protein kinase C activators (diacylglycerol and phorbol esters) and by peptides (vasopressin and bombesin) that stimulate phosphoinositide breakdown. *J Biol Chem* 262:6947–6950.
- 5) Golde B (1992) New clues into the etiology of osteoporosis: the effects of prostaglandins (E_2 and F_{2a}) on bone. *Med Hypotheses* 38: 125–131.
- 6) Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 391:85–100.
- 7) Lagumdzija A, Bucht E, Stark A, Hulting A-L, Petersson M (2004) Arg-vasopressin increases proliferation of human osteoblast-like cells and decreases production of interleukin-6 and

- macrophage colony-stimulating factor. *Regul Pept* 121:41–48.
- 8) Loza J, Stephan E, Dolce C, Dziak R, Simasko S (1994) Calcium currents in osteoblastic cells: dependence upon cellular growth stage. *Calcif Tissue Int* 55:128–133.
 - 9) Miller RJ (1987) Multiple calcium channels and neuronal function. *Science* 235:46–52.
 - 10) Murphy TH, Worley PH, Baraban JM (1991) L-type voltage-sensitive calcium channels mediate synaptic activation of immediate early genes. *Neuron* 7:625–635.
 - 11) Odgaard E, Praetorius HA, Leipziger J (2009) AVP-stimulated nucleotide secretion in perfused mouse medullary thick ascending limb and cortical collecting duct. *Am J Physiol Renal Physiol* 297:F341–F349.
 - 12) Petersson M, Bucht E, Granbergz B, Stark A (2006) Effects of arginine-vasopressin and parathyroid hormone-related protein (1-34) on cell proliferation and production of YKL-40 in cultured chondrocytes from patients with rheumatoid arthritis and osteoarthritis. *Osteoarthritis Cartilage* 14:652–659.
 - 13) Pisitkun T, Jacob V, Schleicher SM, Chou CL, Yu MJ, Knepper MA (2008) Akt and ERK1/2 pathways are components of the vasopressin signaling network in rat native IMCD. *Am J Physiol Renal Physiol* 295:F1030–F1043.
 - 14) Reuter H (1983) Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* 301:569–574.
 - 15) Uchida Y, Endoh T, Tazaki M, Sueishi K (2012) Chronic bradykinin treatment alters $1\alpha,25$ -dihydroxyvitamin D₃-induced calcium current modulation in pre-osteoblasts. *Cell Calcium* 51:383–392.
 - 16) Yip KP (2002) Coupling of vasopressin-induced intracellular Ca²⁺ mobilization and apical exocytosis in perfused rat kidney collecting duct. *J Physiol* 538:891–899.

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