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Research Report

Sustained depolarization-induced propagation of $[Ca^{2+}]_i$ oscillations in cultured DRG neurons: The involvement of extracellular ATP and P2Y receptor activation

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ABSTRACT

Recently emerging evidence implicates a number of neuroactive substances and their receptors in mediating complex cell-to-cell communications in the ganglia. In the present study, we characterized the nonsynaptic chemical coupling mediated by extracellular ATP in dorsal root ganglia (DRG) neuron cultures by using the real time imaging of ATP, whole-cell patch clamping, in conjunction with confocal calcium imaging. Sustained depolarization by electrical stimulation evoked intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) oscillations in individual DRG neurons, and subsequent ATP-dependent propagation $[Ca^{2+}]_i$ oscillations to surrounding non-stimulated neighbors. $[Ca^{2+}]_i$ oscillations were suppressed by inositol-1,4,5-trisphosphate (IP_3) receptor antagonist 2-APB, but not ryanodine. The propagation of $[Ca^{2+}]_i$ oscillations was prevented by the presence of the ATP-degrading enzyme, apyrase, and completely abolished by the blockase of G protein-coupled purinergic receptors-PLC-IP_3 pathway with suramin, U73122 or 2-APB. In parallel, sustained depolarization elicited robust ATP release and diffusion from the stimulation site. Moreover, exogenous application of ATP to DRG cultures in large concentration elicits the $[Ca^{2+}]_i$ oscillations in most neurons. Taken together, this data demonstrates that sustained membrane depolarization elicited ATP release, acting through a highly sensitive P2Y receptors/IP_3-mediated signaling pathway to mediate the propagation of intercellular Ca^{2+} signaling, which suggest a novel signaling pathway for neuronal communication in DRG.

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Abbreviations: DRGdorsal root ganglia; ATPadenosine 5′-triphosphate disodium salt; EGTAethyleneglycol bis(aminoethylether) tetraacetate; DMSOdimethyl sulfoxide; $[Ca^{2+}]_i$intracellular Ca^{2+} concentration; IP_3Rsinositol-1,4,5-trisphosphate receptors; RyRsryanodine receptors; PLCphospholipase C; ERthe endoplasmic reticulum; TGthapsigargin; BSSbalanced salt solution; DMEMDulbecco's modified Eagle's medium; HEPESN-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; BAPTA-AM1, 2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid; PPADSPyridoxal-5-phosphate-6-azophenyl-2′4′-disulfonic acid

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1. Introduction

Spike activity was elicited by an intracellular voltage step in primary afferent neurons in dorsal root ganglia (DRG) *in vivo* (Devor and Wall 1990) and *in vitro* (Utzschneider et al. 1992) that excited adjacent passive neurons that share the same DRG. It appears that such cell-to-cell interactions within DRG result from neither synaptic nor electrical junctions, because adjacent neurons are isolated in individual satellite cell sheaths (Lieberman 1976). It has been inferred that chemically mediated cross-depolarization and the net increase in the input resistance of neurons contributed to the phenomenon (Amir and Devor 1996, 2000), but exact mechanisms remain elusive. During the last decade, the general assumption has been that the cell body (soma) of a neuron, which contains the nucleus, is mainly responsible for synthesis of macromolecules and has a limited role in cell-to-cell communication. Huang and Neher (1996) first reported that depolarization in small DRG neurons (15–25 μm in diameter, C-type) could trigger capacitance increase, indicating somatic exocytosis. Among others, some groups have found quantal release of preloaded transmitters or dyes, and pain-related peptides such as CGRP and substance P from the DRG neuron somata in response to action potentials (Zhang et al. 1995; Bao et al. 2003; Zhang et al. 2004; Ouyang et al. 2005). The somatic secretion most likely provides a way for an excited DRG neuron in an autocrine or paracrine fashion via extrasynaptic mechanisms to modulate the activity of adjacent ganglion neurons (including itself), and thereby affect the transmission of sensing and nociceptive inputs to the spinal cord and higher central nervous system (CNS). As for reception of the chemical signals, DRG neurons are known to contain a wealth of receptors for various neurotransmitters such as purinergic

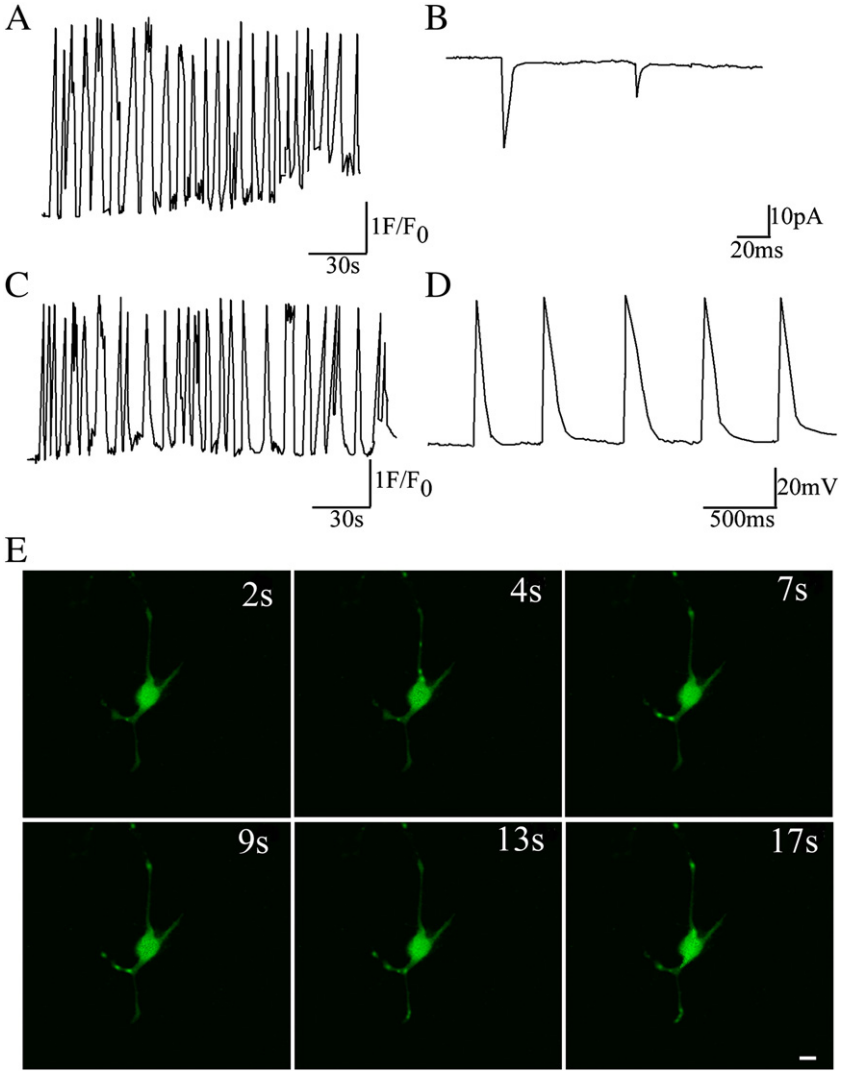

Fig. 1 – Sustained depolarization-evoked $[Ca^{2+}]_i$ oscillations in DRG neuron cultures. (A) a typical trace of $[Ca^{2+}]_i$ oscillations in response to 500 ms depolarization pulses in ROI of DRG cultures. (B) The fluorimetric Ca^{2+} signals are accompanied by the corresponding holding current recordings. (C) 500 ms, 1 Hz electrical currents induced a typical $[Ca^{2+}]_i$ oscillations in a DRG neuron under current clamp conditions. (D) corresponding action potentials evoked by current stimulus trains. (E) representative $[Ca^{2+}]_i$ wave propagation within a DRG neuron in response to sustained depolarizations. Signal initiation was observed first in the cell body, then the $[Ca^{2+}]_i$ wave propagated along the neuron axons (white arrows). Bar, 20 μm.