Pharmacological Reports
2013, 65, 152–163
ISSN 1734-1140

Mechanisms of $[Ca^{2+}]_i$ elevation following P2X receptor activation in the guinea-pig small mesenteric artery myocytes

Khrystyna Yu. Sukhanova[1,2], Maksym I. Harhun[4], Vitali A. Bouryi[1,2], Dmitri V. Gordienko[1,2,3]

[1]Laboratory of Molecular Pharmacology and Biophysics of Cell Signalling, A.A. Bogomoletz, Institute of Physiology, Bogomoletz 4, Kiev, 01024, Ukraine

[2]State Key Laboratory of Molecular and Cellular Biology, Bogomoletz 4, Kiev, 01024, Ukraine

[3]Inserm, U-1003, Université des Sciences et Technologies de Lille (USTL) Villeneuve d'Ascq, F-59655, France

[4]Division of Biomedical Sciences, St. George's, University of London, Cranmer Terrace, London SW17 0RE, UK

Correspondence: Khrystyna Yu. Sukhanova, e-mail: skhrist@biph.kiev.ua

Abstract:
Background: There is growing evidence suggesting involvement of L-type voltage-gated Ca^{2+} channels (VGCCs) in purinergic signaling mechanisms. However, detailed interplay between VGCCs and P2X receptors in intracellular Ca^{2+} mobilization is not well understood. This study examined relative contribution of the Ca^{2+} entry mechanisms and induced by this entry Ca^{2+} release from the intracellular stores engaged by activation of P2X receptors in smooth muscle cells (SMCs) from the guinea-pig small mesenteric arteries.
Methods: P2X receptors were stimulated by the brief local application of αβ-meATP and changes in $[Ca^{2+}]_i$ were monitored in fluo-3 loaded SMCs using fast x-y confocal Ca^{2+} imaging. The effects of the block of L-type VGCCs and/or depletion of the intracellular Ca^{2+} stores on αβ-meATP-induced $[Ca^{2+}]_i$ transients were analyzed.
Results: Our analysis revealed that Ca^{2+} entry *via* L-type VGCCs is augmented by the Ca^{2+}-induced Ca^{2+} release significantly more than Ca^{2+} entry *via* P2X receptors, even though net Ca^{2+} influxes provided by the two mechanisms are not significantly different.
Conclusions: Thus, arterial SMCs upon P2X receptor activation employ an effective mechanism of the Ca^{2+} signal amplification, the major component of which is the Ca^{2+} release from the SR activated by Ca^{2+} influx *via* L-type VGCCs. This signaling pathway is engaged by depolarization of the myocyte membrane resulting from activation of P2X receptors, which, being Ca^{2+} permeable, *per se* form less effective Ca^{2+} signaling pathway. This study, therefore, rescales potential targets for therapeutic intervention in purinergic control of vascular tone.

Key words:
confocal microscopy, Ca^{2+} signaling, vascular smooth muscle cells, P2X receptors, voltage-gated calcium channels, Ca^{2+}-induced Ca^{2+} release

Abbreviations: αβ-meATP - αβ-methylene-adenosine 5′-triphosphate, CICR – Ca^{2+}-induced Ca^{2+} release, CPA – cyclopiazonic acid, IP_3 – inositol 1,4,5-trisphosphate, IP_3R – inositol 1,4,5-trisphosphate receptor, $[Ca^{2+}]_i$ – intracellular concentration of ionized calcium, jSR – sub-plasmalemmal ("junctional") sarcoplasmic reticulum, RyR – ryanodine receptor,

SERCA – sarco-/endoplasmic reticulum Ca^{2+}-ATPase, SMC – smooth muscle cells, SPCU – sub-plasmalemmal $[Ca^{2+}]_i$ upstroke, SR – sarcoplasmic reticulum, VGCC – voltage-gated Ca^{2+} channel

Introduction

The control of total peripheral vascular resistance, blood flow and contraction of small arteries is mediated by sympathetic nervous system *via* activation of postjunctional receptors in smooth muscle cell (SMC) plasma membrane by neurotransmitters released from the nerve terminals [8, 27, 52]. One of the principal excitatory neurotransmitters – ATP, released from sympathetic nerves, acts on arterial myocytes *via* activation of P2X purinoceptors [1, 7]. The family of P2X purinoceptors comprises seven subunits (P2X1–P2X7), each encoded by distinct gene [34]. These subunits can be assembled in various configurations to form functional homo- or heteromeric cation channels [25, 49]. In the cardiovascular system, P2X receptors are expressed predominantly on smooth muscle cells [25, 52]. In rat mesenteric arteries, the predominant P2X receptor is homomeric P2X1 [30].

Cation channels formed by P2X subunits have similar permeability for Na^+ and K^+, and much greater permeability for Ca^{2+} [12, 25], e.g., relative Ca^{2+} over Na^+ permeability (P_{Ca}/P_{Na}) of 4.8 and 4.2 was reported for P2X1 and P2X4 receptors, respectively [11, 34]. An increase in cationic conductance upon P2X receptor activation results in depolarization of the SMC plasma membrane which, in turn, activates voltage-gated Ca^{2+} channels (VGCCs) [16, 21]. Ca^{2+} entering the cell *via* P2X receptors and VGCCs may potentially trigger Ca^{2+} release from intracellular calcium stores *via* Ca^{2+}-induced Ca^{2+} release (CICR) mechanism [13] engaging Ca^{2+} sensitive Ca^{2+}-release channels in the sarcoplasmic reticulum (SR) of SMC. However, recruitment of CICR mechanism in SMCs is still an area of debates and controversy [28, 51]. Indeed, relative contribution of the SR Ca^{2+}-release to intracellular $[Ca^{2+}]_i$ mobilization varies in different SMC types, and often depends on the strengths and mechanism of stimulation. Although activation of the SR Ca^{2+} release by Ca^{2+} entering the cell *via* VGCCs was demonstrated in voltage-clamp experiments performed on different types of visceral and vascular SMCs [4, 9, 24, 26, 44], there is a number of studies [e.g., 5, 6] demonstrating that complete depletion of the SR of Ca^{2+} does not reduce $[Ca^{2+}]_i$ transients induced by step-like depolarization of the cell membrane. The latter suggests that CICR is not recruited. An alternative explanation given by Bradley et al. [6] suggests that the SR and sarcolemma may form a passive physical barrier to Ca^{2+} influx ("Ca^{2+} trap"), which normally limits the $[Ca^{2+}]_i$ rise evoked by depolarization. The drugs, which open the SR Ca^{2+} release channels and facilitate the SR Ca^{2+} leak, diminish the influence of "Ca^{2+} trap" and may, thereby, increase amplitude of $[Ca^{2+}]_i$ transients resulting from Ca^{2+} entry *via* VGCCs even when the SR contains little or no Ca^{2+} [6].

Another important aspect of SMC Ca^{2+} signaling system is difference in the ability of various Ca^{2+} entry mechanisms to trigger Ca^{2+} release from the SR. This variability may arise from spatial organization and molecular composition of intracellular Ca^{2+}-release units [18, 19, 23, 32, 33]. Imaging microdomain Ca^{2+} in myocytes has reshaped our understanding of Ca^{2+} signaling and provided direct evidence validating the concept that a closed organelle system contains specialized biochemical functions ("local control concept"; [2]). Furthermore, an emerging and more revolutionary concept is that areas of the cell that are between organelles, as a consequence of their nanostructure, are also structurally specialized regions of distinct and important functions [37, 38]. We have recently demonstrated that in response to activation of P2X receptors in renal microvascular SMCs, Ca^{2+} entry *via* VGCCs is the major trigger of CICR, even though relative contribution of P2X receptors to Ca^{2+} entry under this conditions is greater than that of VGCCs [39]. This suggests co-localization of plasmalemmal VGCCs and the SR Ca^{2+}-release channels, and "local control" of Ca^{2+}-release mechanisms in these myocytes. The latter was also supported by the gradual dependence of $[Ca^{2+}]_i$ transients on P2X agonist concentration, despite the fact that a regenerative CICR mechanism was recruited.

Participation of P2X receptors in sympathetic control of vascular SMCs offers an attractive therapeutic target mediating substantial vasoconstrictor drive resistant to adrenoceptor antagonists [50]. As sympathetically driven splanchnic vasoconstriction is an important reflex responsible for stabilization of systemic blood pressure during exercise [29], understanding of the mechanisms linking P2X receptor activation to an increase of $[Ca^{2+}]_i$ in mesenteric ar-