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Dopamine-induced graded intracellular Ca^{2+} elevation via the Na^+—Ca^{2+} exchanger operating in the Ca^{2+}-entry mode in cockroach salivary ducts

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Abstract

Stimulation with the neurotransmitter dopamine causes an amplitude-modulated increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in epithelial cells of the ducts of cockroach salivary glands. This is completely attributable to a Ca^{2+} influx from the extracellular space. Additionally, dopamine induces a massive $[Na^+]_i$ elevation via the Na^+—K^+—$2Cl^-$ cotransporter (NKCC). We have reasoned that Ca^{2+}-entry is mediated by the Na^+—Ca^{2+} exchanger (NCE) operating in the Ca^{2+}-entry mode. To test this hypothesis, $[Ca^{2+}]_i$ and $[Na^+]_i$ were measured by using the fluorescent dyes Fura-2, Fluo-3, and SBFI. Inhibition of Na^+-entry from the extracellular space by removal of extracellular Na^+ or inhibition of the NKCC by 10 μM bumetanide did not influence resting $[Ca^{2+}]_i$ but completely abolished the dopamine-induced $[Ca^{2+}]_i$ elevation. Simultaneous recordings of $[Ca^{2+}]_i$ and $[Na^+]_i$ revealed that the dopamine-induced $[Na^+]_i$ elevation preceded the $[Ca^{2+}]_i$ elevation. During dopamine stimulation, the generation of an outward Na^+ concentration gradient by removal of extracellular Na^+ boosted the $[Ca^{2+}]_i$ elevation. Furthermore, prolonging the dopamine-induced $[Na^+]_i$ rise by blocking the Na^+/K^+-ATPase reduced the recovery from $[Ca^{2+}]_i$ elevation. These results indicate that dopamine induces a massive NKCC-mediated elevation in $[Na^+]_i$, which reverses the NCE activity into the reverse mode causing a graded $[Ca^{2+}]_i$ elevation in the duct cells.

Keywords: Ca^{2+}-signaling; Amplitude modulation; Na^+—Ca^{2+} exchanger; Na^+—K^+—$2Cl^-$ cotransporter; Salivary glands; Dopamine; Biogenic amines; Cockroach; Insect

1. Introduction

Ca^{2+} acts as a universal intracellular messenger and regulates a plethora of physiological functions. The specific and coordinated activation of many cellular processes by stimulus-induced changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) requires appropriate temporal and spatial coding patterns [1–3]. In electrically non-excitable cells, the primary mechanism of Ca^{2+} mobilization involves the activation of the inositol 1,4,5-trisphosphate ($InsP_3$) second messenger cascade. As a result, low agonist concentrations cause Ca^{2+} oscillations or repetitive Ca^{2+} spikes arising from cyclical Ca^{2+} release from, and Ca^{2+} reuptake into, the endoplasmic reticulum [4,5], whereby agonist concentration and the frequency of Ca^{2+} oscillations are correlated [6–8]. Thus, the Ca^{2+} signal is frequency-modulated. However, Ca^{2+} signals can also be amplitude-modulated. Such graded Ca^{2+} signals activate Ca^{2+}-sensitive processes that differ in their Ca^{2+} affinity [1,9]. The ubiquitous $InsP_3$-mediated Ca^{2+} release from the endoplasmic reticulum, although inherently regenerative because of its Ca^{2+} sensitivity [10–12], can produce such amplitude-modulated Ca^{2+} signals by recruiting a variable number of elementary events resulting from the opening of either individual or small groups of Ca^{2+} channels [2,9]. Nevertheless, amplitude-modulated Ca^{2+} signals certainly also require Ca^{2+} influx from the extracellular space, such as a store-operated Ca^{2+} influx [13,14]. Amplitude modulation of agonist-induced Ca^{2+} signals has been observed in a few cell types. Among these are mice B lymphocytes [15], human endothelial cells [16], human mast cells [17], and invertebrate microvillar photoreceptors [18,19].

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The salivary glands in the cockroach, *Periplaneta americana*, are an excellent system for studying amplitude-modulated Ca^{2+} signals, since the epithelial cells of their ducts exhibit agonist-induced graded $[Ca^{2+}]_i$ elevation. The acinar salivary glands are innervated by dopaminergic and serotonergic fibers [20] and secrete a NaCl-rich primary saliva upon stimulation with dopamine or serotonin [21]. The ducts of the glands are also stimulated by dopamine and modify the primary saliva by Na^+ reabsorption and K^+ secretion [22,23]. Although the Ca^{2+}-signaling pathway is believed to play an important role in regulating saliva modification, the mechanisms that contribute to the regulation of $[Ca^{2+}]_i$ have not been identified. Dopamine causes a slow dose-dependent tonic increase in $[Ca^{2+}]_i$ in duct epithelial cells. This increase spreads over the duct epithelium as a Ca^{2+}-tide and is completely attributable to a Ca^{2+} influx from the extracellular space [24].

The aim of the present study was to investigate the molecular mechanisms responsible for the generation of this amplitude-modulated Ca^{2+} signal. We discovered that the reverse Ca^{2+}-entry mode of the Na^+–Ca^{2+} exchanger alone caused the graded, thus amplitude-modulated, $[Ca^{2+}]_i$ elevation in the duct epithelial cells during stimulation with the neurotransmitter dopamine. This Ca^{2+} elevation was initiated by a massive elevation in $[Na^+]_i$, caused by the activity of the Na^+–K^+–$2Cl^-$ cotransporter.

2. Materials and methods

2.1. Animals and preparation

A colony of *P. americana* (L.) (Blattodea, Blattidae) was reared at 27 °C under a light/dark cycle of 12 h:12 h. The animals had free access to food and water. Only male adults aged between 4 and 6 weeks were used for experiments. The salivary glands were dissected in physiological saline (PS) as described previously [21]. Small pieces of the glands consisting of one lobe with its acini and ducts were examined.

2.2. $[Ca^{2+}]_i$ and $[Na^+]_i$ measurements

For $[Ca^{2+}]_i$ measurements, isolated lobes of the salivary glands were loaded with the acetoxymethyl ester of Fura-2 at room temperature during a 30 min incubation in 5 μM Fura-2/AM (Biotrend Chemikalien, Cologne, Germany) in PS. The lobes were then mounted in a rhomboid coverslip-bottomed chamber and continuously superfused with PS. The coverslip was coated with the tissue adhesive Vectabond Reagent (Axxora, Grünberg, Germany) and changed after every experiment. The chamber was mounted on a Zeiss Axiovert 135TV inverted microscope equipped with epifluorescence optics and a Zeiss Fluar 20/0.75 objective. For fluorescence excitation, a 75 W xenon arc lamp monochromator unit (VisiChrome, Visitron, Puchheim, Germany) was connected to the microscope by a quartz fiber-optic light guide. The epifluorescence filter-block in the microscope contained a 485 nm dichroic mirror and a 515–565 nm bandpass emission filter. Pairs of fluorescence images were acquired and digitized with a cooled image transfer CCD camera (CoolSnap-HQ, Roper Scientific Inc., Tucson, USA) at excitation wavelengths of 340 nm and 380 nm at rates of 0.1–0.2 s^{-1} at 12-bit resolution. Monochromator control, image acquisition, and processing were carried out by using the imaging software MetaFluor 6.1 (Universal Imaging Corp., Downingtown, USA). Calcium signals were expressed as fluorescence ratios (F_{340}/F_{380}) calculated after the subtraction of background fluorescence and cell autofluorescence determined at the end of each experiment by quenching Fura-2 fluorescence with 20 mM $MnCl_2$.

For simultaneous $[Na^+]_i$ and $[Ca^{2+}]_i$ measurements isolated lobes of the salivary glands were loaded with the acetoxymethyl esters of Fluo-3 and sodium-binding benzofuran isophthalate (SBFI) at room temperature during a 150–180 min incubation in 15 μM SBFI/AM and 3.75 μM Fluo-3/AM in PS in the presence of 0.125% Pluronic F-127 (Invitrogen, Karlsruhe, Germany). Fluorescence images were acquired at excitation wavelengths of 340 nm, 360 nm, and 480 nm. Sodium signals were expressed as fluorescence ratios (F_{340}/F_{360}), and calcium signals were expressed as Fluo-3 fluorescence (F_{480}).

2.3. Solutions and chemicals

The cockroach PS contained 160 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM glucose, and 10 mM Tris. The pH was adjusted to 7.4 with HCl. In Na^+-free saline, equimolar amounts of choline chloride were substituted for NaCl. Dopamine, bumetanide, and ouabain (all from Sigma, Deisenhofen, Germany) were stored as 10 mM stock solutions in small aliquots at −20 °C and diluted in PS immediately before an experiment. Dimethyl sulfoxide (DMSO) from the bumetanide stock solution did not affect $[Ca^{2+}]_i$ ($n = 3$, data not shown).

2.4. Statistical analysis

Statistical comparisons were calculated by either Student's paired *t*-test or repeated-measures ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant. All analyses were performed by using GraphPad Prism 4.01 (GraphPad Software, San Diego, USA). Results are given as mean ± S.E. Figures displaying original recordings are representative of at least five independent experiments.

3. Results

3.1. Dopamine induces graded elevations in $[Ca^{2+}]_i$

Stimulation of isolated lobes by a brief application of 1 μM dopamine induced a reversible $[Ca^{2+}]_i$ elevation in