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Depletion of Caffeine-Sensitive Calcium Store Results in Diminution of ATP-Induced Metabotropic Calcium Responses in Rat Neocortical Neurons

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ATP receptor-mediated changes in the Ca^{2+} concentration were recorded from neurons of the sensorimotor cortex in brain slices from 3-week-old rats. To measure the cytoplasmic concentration of Ca^{2+}, slices were incubated with Fura-2/AM, and a microfluorimetry system was focused on an individual cell. Possible glutamatergic signals resulting from ATP-evoked glutamate release were excluded. After elimination of calcium from the extracellular solution, the first ATP-induced $[Ca^{2+}]_i$ transient decreased to 62 ± 9% of a similar response in the normal solution, suggesting the participation of metabotropic purinoreceptor-triggered Ca release in transient generation. Depletion of the caffeine-sensitive calcium store results in diminution of ATP-induced $[Ca^{2+}]_i$ transient in the Ca^{2+}-free solution by 31.4 ± 7.0% ($P < 0.01$). This may indicate that in pyramidal neurons of the sensorimotor cortex $InsP_3$- and Ca-induced Ca-releases demonstrate noticeable functional interaction. Nevertheless, there is no single compartment in the endoplasmic reticulum bearing both IICR and CICR channels.

INTRODUCTION

Two main types of Ca-releasing channels are described in excitable cells: the channels activated by elevation of cytosolic Ca^{2+} (Ca^{2+}-induced Ca^{2+} release, CICR) and those activated by an intracellular messenger, inositol-1,4,5-triphosphate ($InsP_3$-induced Ca^{2+} release, IICR) [1]. Neurons with functioning intracellular Ca^{2+} stores show significant differences in the capability of the latter to accumulate and hold Ca ions [2, 3]. Some cells (small-size DRG neurons transmitting predominantly nociceptive signals) do not reveal at all $InsP_3$-triggered Ca^{2+} release [4]; these cells are also lacking Ca^{2+}-induced Ca^{2+} mobilization through ryanodine/caffeine-sensitive release channels [5].

One of the important questions in the studies of the intracellular calcium release mechanism is whether the endoplasmic reticulum forms a single compartment endowed with both $InsP_3$-receptors and CICR channels, or whether CICR and IICR are realized from separate Ca^{2+} pools. The respective data are very limited and contradictory. For cultured hippocampal neurons, it was found that discharging the Ca^{2+}-sensitive pool does not affect IICR activated by quisqualate [6], which indicates to a separate nature of Ca^{2+} pools. Yet, later experiments performed on the same neurons demonstrated that IICR never can be activated after caffeine application [7]. In cultured cerebellar granule neurons, the data favoring the existence of single Ca^{2+} store involved in both CICR and IICR were obtained [8]. Thus, the existence of functionally segregated or co-localized release mechanisms seems to considerably vary between different neuronal types. In our experiments, we tried to solve the question about possible interactions between caffeine- and $InsP_3$-sensitive Ca^{2+} pools in neocortical neurons.

METHODS

Brain Slice Preparations and Solutions. Experiments were performed on brain slices freshly isolated from Wistar rats (postnatal day 21). Briefly, after anesthesia rats were decapitated, and the brain was removed and placed in cold (4°C) physiological saline for 1-2 min; then the hemispheres were separated, and one hemisphere was cut in the sagittal plane using a vibroslicer. The 250-300 μm thick slices were trans-

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ferred into physiological saline saturated with 95% O_2 + 5% CO_2. The physiological salt solution contained (mM): NaCl, 135; KCl, 2.5; $CaCl_2$, 2.5; $MgCl_2$, 1; $NaHCO_3$, 25; KH_2PO_4, 1.6; glucose, 10; pH 7.4, when continuously gassed with 5% CO_2 + 95% O_2. To obtain calcium-free solution, $CaCl_2$ was omitted; $MgCl_2$ was increased to 3 mM, and 1 mM EGTA was added. For preparation of high-potassium extracellular solutions, $[K^+]_o$ was altered by isoosmotic replacement of Na^+ with K^+. To block voltage-gated sodium channels, in all experiments 0.5 μM tetrodotoxin (TTX) was added to the physiological saline.

Fluorimetric $[Ca^{2+}]_i$ Measurements. Fluorimetric $[Ca^{2+}]_i$ measurements were performed in neurons loaded with acetoxymethyl ester of fura-2 (fura-2/AM, Molecular Probes, USA). For loading, brain slices were incubated in a solution supplemented with fura-2/AM (10 μM, diluted in DMSO) and pluronic F-127 detergent (0.02%) for 20 min at 35°C. Later on, slices were incubated in physiological solution for an additional 40 min to ensure fura-2/AM deesterification. For fura-2 excitation, the cells were alternately illuminated at the wavelength of 360 ± 5 and 390 ± 5 nm. Excitation filters were mounted in a filter wheel set at five revolutions per second. The emitted light was collected at 530 ± 10 nm by a photomultiplier. The filter wheel and photomultiplier outputs were controlled by a Fura-2 system (Luigs and Neumann, Germany). Signals corresponding to both excitation wavelengths were fed to an IBM-compatible PC via a TIDA interface (Batelle, Germany). Dye-loaded neocortical neurons were positioned in such a way that the fluorescent signal was collected from their somata. The actual $[Ca^{2+}]_i$ was calculated from the ratio (R) of fluorescence recorded at 360 and 390 nm excitation wavelengths using the equation of Grynkiewicz et al. [9]. The system was calibrated *in vitro*. The calibration parameters K_{dB}, R_{min}, and R_{max} characterizing the system were 1512 nM, 0.6, and 4, respectively. After incubation for 1 h at 32°C and 1 h at room temperature (about 22°C), the selected slice was transferred to an experimental chamber, mounted on the stage of an upright microscope (Axioscope, Zeiss, Germany) and continuously superfused (10-20 ml/min) with a Tyrode salt solution. The cells were visualized using a long-distance water-immersion objective (40×, NA 0.75). All experiments were carried out at 32°C.

RESULTS AND DISCUSSION

Ten-sec-long application of ATP in the concentration of 100 μM produced a transient increase in $[Ca^{2+}]_i$ in the majority of pyramidal neurons from the sensorimotor cortex (18 of 24 units). The mean amplitude of ATP-induced $[Ca^{2+}]_i$ transients reached 86.3 ± 8 nM (n = 18). This effect of ATP could be mediated by post-synaptic iono- and metabotropic purinergic receptors or ATP-induced release of other neurotransmitter from pre-synaptic terminals [10]. Since glutamate is the main excitatory neurotransmitter in the brain [11], we recorded ATP-induced $[Ca^{2+}]_i$ transients in the presence of glutamate receptor antagonists (20 μM 6-cyano-7-nitroquinoxaline-2,3-dione, CNQX, and 25 μM 2-amino-5-phosphovaleric acid, d-APV) in the bath solution. The amplitude and time course of intracellular calcium transient was not altered (n = 9) under such conditions (Fig. 1). In this way we avoided glutamatergic signals resulting from ATP-evoked glutamate release and made it certain that the observed Ca^{2+} rise was triggered by postsynaptic purinergic receptor activation.

Applications of 50 mM KCl (5-sec-long) were per-

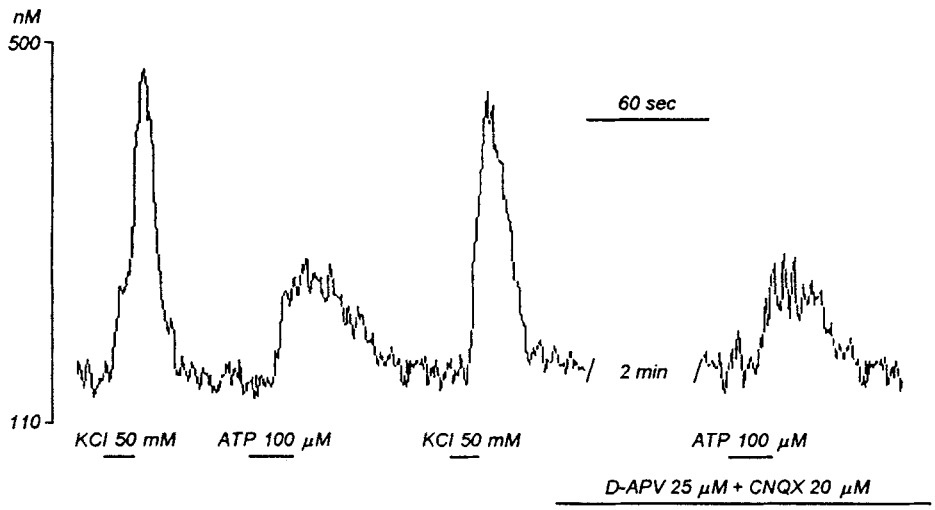

Fig. 1. Glutamate receptor antagonists exert no effect on ATP-induced $[Ca^{2+}]_i$ transients in neurons of the sensorimotor cortex from 3-week-old rats. Examples of Ca^{2+} transients evoked by 100 μM ATP bath application without and with glutamate antagonists (2-amino-5-phosphovaleric acid, d-APV, 25 μM, and 6-cyano-7-nitroquinoxaline-2,3-dione, CNQX, 20 μM).

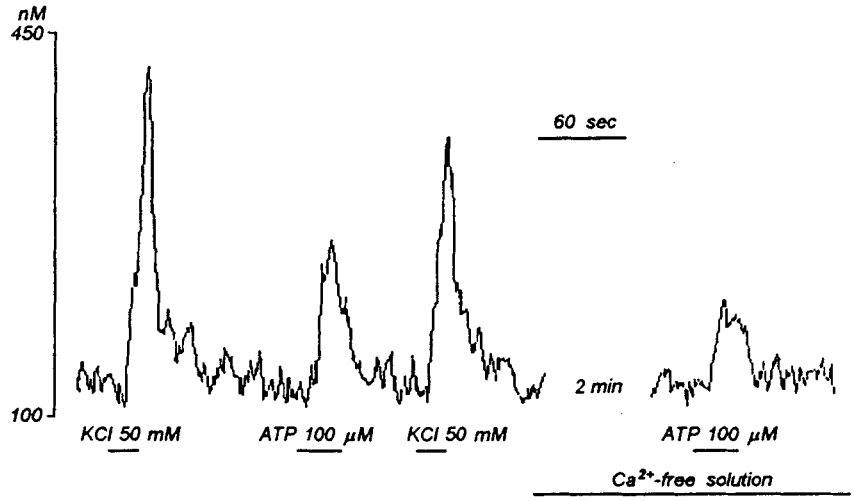

Fig. 2. ATP-induced Ca^{2+} mobilization and Ca^{2+} influx in pyramidal neurons of the sensorimotor cortex from 3-week-old rats. Examples of 100 μM ATP-evoked $[Ca^{2+}]_i$ signals measured under control conditions and after 2-min-long slice pre-incubation in the Ca^{2+}-free solution. Ca^{2+}-free solution started 2 min before ATP application in order to ensure Ca^{2+} removal from the slice.

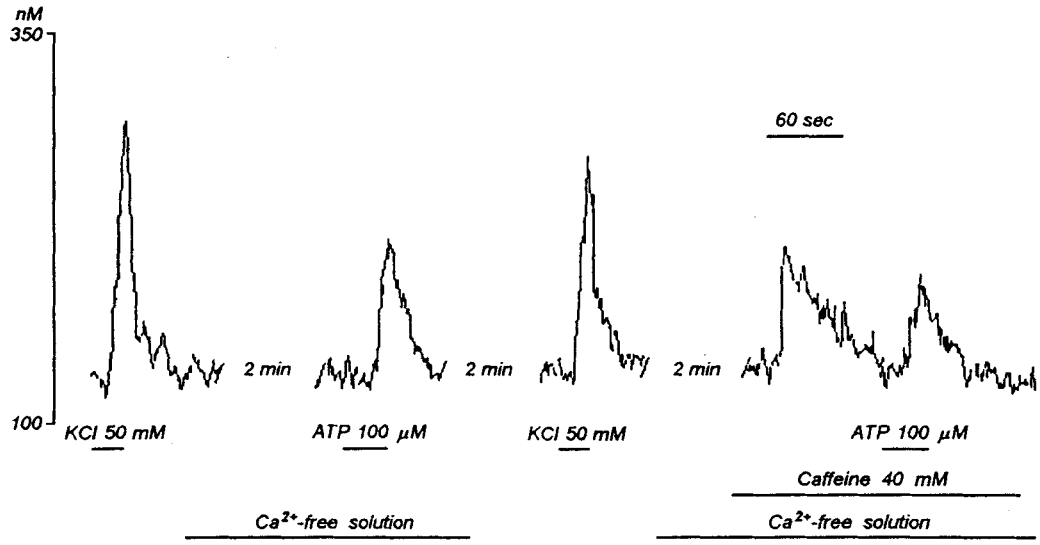

Fig. 3. Interaction between the $InsP_3$-sensitive and caffeine-sensitive Ca^{2+} pools. Long-term bath application of 40 mM caffeine reduced ATP-evoked $[Ca^{2+}]_i$ transient in Ca^{2+}-free solution in pyramidal neurons of the sensorimotor cortex from 3-week-old rats.

formed to ensure refilling of intracellular Ca^{2+} stores, taking into account the involvement of metabotropic purinergic receptors in generation of ATP-induced Ca^{2+} transients (Fig. 2). The presence of a considerable response in the absence of external Ca^{2+} indicates that cortical pyramidal neurons are endowed with metabotropic purinoreceptors. The first ATP-induced $[Ca^{2+}]_i$ elevation in the Ca^{2+}-free solution dropped to 62 ± 9% (n = 14) of a similar response in the normal solution. In this respect, the cortical neurons under study are similar to other brain neurons like hippocampal and thalamic cells [12], and cerebellar Purkinje cells [13], as well as DRG neurons [14]. In all studied structures, the metabotropic effect of ATP was mediated via the $InsP_3$ messenger system, as has been in detail analyzed by Hirano et al. [15].

Considering this finding, we examined the activity of another main intracellular signalling mechanism, which involves ryanodine/caffeine sensitive intracellular Ca^{2+} pools. In 16 cells of 22 investigated neurons, 10-sec-long application of 20-40 mM caffeine induced considerable $[Ca^{2+}]_i$ transients, which did not disappear in the Ca^{2+}-free solution. A longer caffeine application (over 30 sec) could result in complete depletion of the stores. Depletion of the caffeine-sensitive calcium store led to diminution of ATP-induced $[Ca^{2+}]_i$ transient in the Ca^{2+}-free solution by 31.47% (n = 15; Fig. 3); the difference was statistically significant ($P < 0.01$). This

fact may indicate that in pyramidal neurons of the sensorimotor cortex functional intraction between both main types of intracellulat Ca^{2+} stores does exist; however, this interaction is quite limited, indicating that there is no single compartment of endoplasmic reticulum bearing both $InsP_3$-receptors and CICR channels.

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