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Regulation of somatodendritic dopamine release by corticotropin-releasing factor via the inhibition of voltage-operated Ca^{2+} channels

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ABSTRACT

Dopamine (DA) neurons in the substantia nigra pars compacta release DA from their somata and dendrites, which regulate motor activity and muscle tone. Previously, we reported that Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOCCs) contributes to spontaneous somatodendritic DA release. Since corticotropin-releasing factor (CRF) regulates VOCC, we sought to determine whether urocortin affects somatodendritic DA release in the isolated DA neurons using amperometry method. The application of urocortin reversibly inhibited both VOCC and the frequency of DA release events via the activation of type-1 CRF receptor. The blockers for L- and T-type Ca^{2+} channels effectively abolished the effects of urocortin both on the frequency of DA release events and on Ca^{2+} current. These results indicate that CRF inhibits somatodendritic DA release by inhibiting L- and T-type Ca^{2+} channels. Thus, the inhibition of somatodendritic DA release by stress hormone may be one of the molecular mechanisms underlying the effect of stress on motor function.

The midbrain dopamine (DA) neurons play an important role in reward and motivational behavior, cognitive function, and motor control [30,6]. In addition to the classical axon-terminal release of DA at the striatum, DA neurons in the substantia nigra pars compacta (SNc) also release DA from their somata and dendrites [3,11,7]. DA released from the somata and dendrites activates D_2 autoreceptors, and thereby regulates spontaneous firing and prevents further release of DA [26] or modulates non-DA cells [22,25]. These feedback mechanisms of somatodendritic DA release might regulate motor activity and muscle tone [10,29,2,1]. Stress triggers a cascade of events leading to the activation of the hypothalamic-pituitary-adrenal axis, influencing cognition and emotion [27,28]. Moreover, stress adversely affects motor performance directly via hormonal change and indirectly via changes in emotion [19,21]. Furthermore, both stress and stress hormone treatments reduce the accuracy of skilled movements in reaching and walking in rats [20]. The stress hormone, corticotropin-releasing factor (CRF), acts by binding two receptor subtypes, CRFR1 and CRFR2 [23,18]. Although both CRF receptors are expressed in DA neurons, the functional role and the mechanism of CRF action on DA neurons are not clear.

Influx of Ca^{2+} through voltage-operated Ca^{2+} channels (VOCCs) may be involved in the release of DA from the somata of DA neurons [14]. We have shown that DA neurons spontaneously release DA, and that Ca^{2+} influx through VOCCs regulates DA release from the somata of DA neurons [16]. We have also reported that CRF inhibits T-type Ca^{2+} channels in dopaminergic MN9D cells, a model of DA neuron [17]. Since native DA neurons express all types of VOCCs [17,15,5], and inhibition of VOCCs by CRF may affect DA release from the somata of DA neurons, we sought to determine if urocortin affects somatodendritic DA release from SNc DA neurons.

All chemicals and reagents used in this study were purchased from Sigma (St. Louis, MO) except for nifedipine, ω-conotoxin GVIA, and ω-agatoxin IVA, which were purchased from Tocris (Ellisville, MO).

Animal handling was in accordance with the guidelines of the Animal Welfare Committee of Sungkyunkwan University. DA neurons were freshly isolated from Sprague–Dawley rats at postnatal days 9–14 as described previously [16]. Briefly, whole brains were quickly removed and placed in ice-chilled oxygenated HEPES-buffered saline. The brain was cut into a midbrain block containing the SNc, and coronal slices of 300-μm thickness were obtained with a vibratome (TPI, St. Louis, MO). Subsequently, the dark regions of slices were dissected out, and digested with fully oxygenated papain (4–10 U/ml) for 20–30 min at 34 °C. The tissue segments were rinsed with enzyme-free saline and then gently triturated with a graded series of fire-polished Pasteur pipettes. The isolated cells were plated on poly-D-lysine-coated glass coverslips, and used within 3 h after preparation.

To increase the frequency of spontaneous exocytotic events from the somata, the isolated DA neurons were preincubated with 100 μM 3,4-dihydroxy-L-phenylalanine (L-DOPA) for 30 min prior to recordings [16]. Recordings were performed at 32–35 °C in L-DOPA-free HEPES-buffered saline with continuous perfusion. High K^+ saline solution contained 60 mM KCl, which substituted NaCl. A

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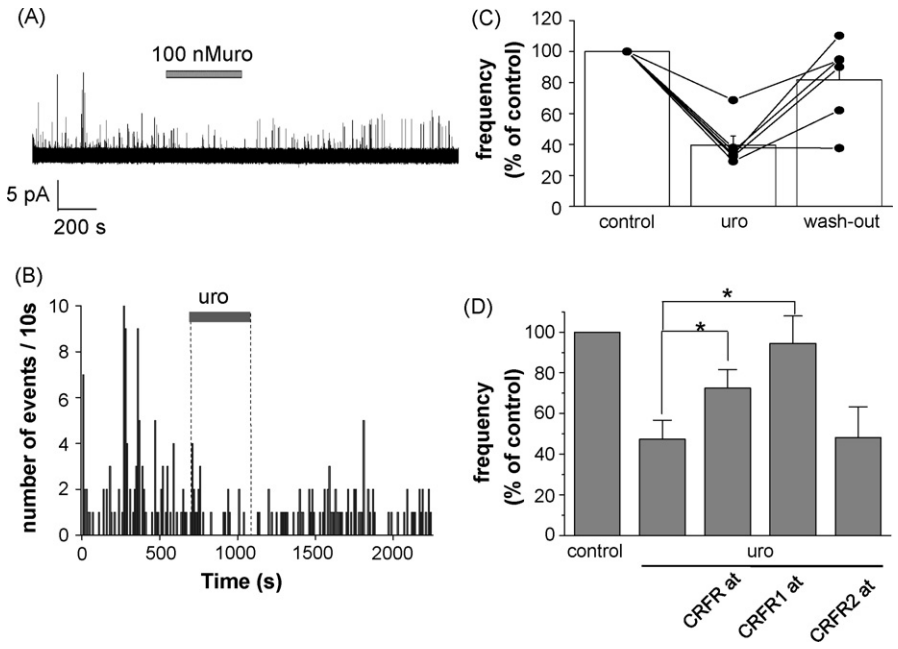

Fig. 1. Urocortin inhibits somatodendritic DA release from acutely isolated DA neurons. (A) Amperometry was used to measure DA release from isolated single SNc DA cells. Urocortin reversibly decreased the frequency of DA release events. (B) The number of amperometric events during each 10 s bin from (A) is plotted. (C) Results from five different DA neurons are shown as closed symbols, and the averaged result is represented as bars. (D) Somatodendritic DA release is inhibited by CRFR1 activation. The presence of the CRF receptor antagonist, astressin (CRFR at, $n = 6$), or CRFR1 antagonist, antalarmin (CRFR1 at, $n = 11$), prevented the inhibitory effect of urocortin on the frequency of DA release, while CRFR2 antagonist, astressin 2B (CRFR2 at, $n = 6$), was not able to prevent the effect of urocortin. * represents $p < 0.05$ from paired t-tests.

5 μm diameter carbon fiber electrode (Dagan Company, Minneapolis, MN) was held at +600 mV. Sampling was at 5 kHz with low-pass filtering at 100 Hz.

Ca^{2+} currents were recorded using whole-cell mode at 32–35 °C. Currents were filtered at 5 kHz, and recorded using an Axonpatch-200B amplifier (Axon Instruments, Foster City, CA). The bath solution contained 15 mM $BaCl_2$, 106 mM TEA–Cl, 10 mM HEPES, 1 mM $MgCl_2$, 5 mM KCl, and 19 mM glucose (pH 7.2). The pipette solution contained 140 mM CsCl, 2 mM $MgCl_2$, 10 mM HEPES, 2 mM Na-ATP, and 1 mM Na-GTP (pH 7.4).

After incubating DA neurons with 2–5 μM fura2-AM at room temperature for 20 min, single cell fluorescence intensity was measured using an Olympus IX70 inverted microscope (Center Valley, PA), attached with a charge coupled device (CCD) image intensifier camera (Quantix, Roper Scientific Inc., Tucson, AZ) and Metafluor software (Molecular Devices, Sunnyvale, CA). We used 340/380 dual excitations with a 400 nm diachronic mirror, and emitted light was collected with a 450-nm long pass filter. The ratio (F_{340}/F_{380}) of fluorescence intensities was measured at the cell bodies. EGTA (1 mM), instead of $CaCl_2$, was included for extracellular Ca^{2+}-free solution.

Data analysis was performed using pClamfit 7.0 (Axon Instruments). The peak Ca^{2+} currents at different voltages were analyzed using pClamfit 7.0. Data are presented as means ± standard error of the mean (S.E.M.). Both paired and unpaired Student's t-tests were used.

We used isolated large multipolar cells from the SNc, most of which were known as immunopositive to tyrosine hydroxylase antibody as described previously [8]. Most DA neurons exhibited spontaneous exocytotic events with a variety of amperometric peaks, as shown in Fig. 1A, representing the basic unit of synaptic transmission [24]. The application of 100 nM urocortin reversibly decreased the frequency of exocytotic events as shown in a typical example in Fig. 1A. The number of exocytotic events during each 10 s bin is plotted in Fig. 1B. In five different DA neurons, urocortin inhibited the frequency of exocytotic events to 39.0 ± 6.0% of the control level, and the effect was recovered to 81.6 ± 10.9% of the control level by washout (Fig. 1C). We also analyzed other parameters from exocytotic events from 5 different cells. Spike amplitudes (I_{max}) for control and for urocortin treated cells were 7.2 ± 0.5 pA and 7.5 ± 1.1 pA, respectively; the charge integrals, 2.85 ± 0.25 fC and 2.63 ± 0.36 fC; the median cube roots, 0.13 ± 0.01 $pC^{(1/3)}$ and 0.14 ± 0.01 $pC^{(1/3)}$; the average molecules of quantal events, 8880 ± 770 and 8190 ± 1140; the average $t_{1/2}$, 0.40 ± 0.04 ms and 0.38 ± 0.02 ms. Thus, urocortin decreased only the frequency of exocytotic events without any effect on the other parameters.

Since double staining results showed that DA neurons expressed both CRFR1 and CRFR2 (data not shown), we tested the effects of various ligands for CRF. As shown in Fig. 1D, a CRF receptor antagonist (CRFR at), 100 nM astressin, prevented the effect of urocortin (72.5 ± 9.2% of control level, $n = 6$). A specific CRFR1 antagonist (CRFR1 at), 100 nM antalarmin, also prevented the effects of urocortin (94.6 ± 13.4% of control level, $n = 11$). In contrast, a CRFR2 antagonist (CRFR2 at), 100 nM astressin 2B, failed to prevent the urocortin effect (48.1 ± 15.1% of control level, $n = 6$), indicating that the inhibitory effect of urocortin on the frequency of somatodendritic DA release occurs via the activation of CRFR1.

We have previously reported that Ca^{2+} influx through VOCCs regulates spontaneous somatodendritic DA release from DA neurons, and that a cocktail of VOCC blockers decreased the frequency of amperometric events without having any effect on other parameters [16]. Thus, the effects of urocortin were very similar to those of VOCC blockers, which may imply that urocortin inhibits somatodendritic DA release via the inhibition of VOCC. To test this possibility, we first applied a cocktail of VOCC blockers, which included 10 μM nifedipine (L-), 10 μM mibefradil (T-), 200 nM ω-conotoxin GVIA (N-), and 200 nM ω-agatoxin IVA (P/Q-type). As shown in Fig. 2A, the frequency of amperometric events decreased to 43% of the control level by these blockers (3.1 ± 0.7/10 s for control, 1.2 ± 0.3 events/10 s for VOCC blockers, $n = 5$). In the presence of these blockers, the application of urocortin did not inhibit the frequency of events further (1.0 ± 0.2 events/10 s; VOCC block-