BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1): 23—27

Dopamine-induced ionic currents in acutely dissociated rat neurons of CNS[1]

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KEY WORDS dopamine; potassium channels; hippocampus; pyramidal cells; patch-clamp techniques

AIM: To determine whether or not the dopamine (DA) can induce ionic current in single neuron acutely dissociated from different central areas including striatum, ventral tegmental area (VTA), substantia nigra pars compacta (SNC) and hippocampal CA_1 area. **METHODS**: Using a new patch-clamp whole-cell recording technic, namely nystatin-perforated whole-cell configuration under voltage-clamp mode. **RESULTS**: In 36 single neurons isolated from the striatum and VTA, DA 10—1000 $\mu mol \cdot L^{-1}$ was quite diverse to elicit ionic current response. In 19 SNC neurons, 5 neurons (26 %) response to 1 $mmol \cdot L^{-1}$ DA represented as a small outward current (11.3±2.4 pA) at a holding potential (V_H) of −20 mV. In 25 of 69 (36 %) examined hippocampal CA_1 pyramidal neurons, however, application of DA induced 3 types of current responses: outward current (8 neurons) accompanied with an increase of membrane conductance, slow inward current (5 neurons) with an decrease of membrane conductance and outward-following inward current (12 neurons) at a V_H of −20 mV. The concentration-response relationship of DA-induced currents showed the typical sigmoid shape with the threshold dose, being the maxium response dose are 3 $\mu mol \cdot L^{-1}$ and 1 $mmol \cdot L^{-1}$ respectively. The current-voltage (I-V) relationship of DA-induced responses did not show any voltage-dependent manner and the reversal potential (E_{DA}) was close to the equilibrium potential of potassium (E_K) calculated with the Nernst equation. TEA 5 $mol \cdot L^{-1}$ effectively inhibited the DA-induced response. **CONCLUSION**: These results suggest that DA-induced outward current is carried by K^+ in single hippocampal CA_1 pyramidal neuron.

In the central nervous system (CNS) many of the complex physiological actions of dopamine (DA) are mediated by its interaction with D_1 and D_2 receptors localized on the neurons of different brain areas. The corpus striatum is a major postsynaptic target of dopaminergic projections in mammalian brain. The loss of DA in the striatum as a result of the neuronal degeneration in the substantia nigra pars compacta SNC has been thought to be the main pathochemical correlate of the main symptoms of Parkinson's disease. The DA-containing neurons of the (SNC) project predominantly control the extrapyramidal movement. The release of DA by these neurons occurs not only in their striatal terminal field, but also in the SNC itself and this release appears to be self-regulating in that it can be inhibited by the evoked release of DA[1]. Although the hippocampus is not classically considered as a part of any dopaminergic system, there is both anatomical and biochemical evidence suggesting that it does receive a mesencephalic dopaminergic projection[2,3].

The electrophysiological experiments show that DA increases K^+ conductance mediated by a binding of D_2 receptors in rat SNC slice preparation[4]. DA opens the single K^+ channel in acutely dissociated rat corpus striatum neuron[5]. DA evokes the outward current in a main cell of VTA slice[6] and in hippocampal slice of CA_1 area. DA-induced hyperpolarization and membrane conductance increase are mediated by a Ca^{2+}-activated K^+ conductance[7]. However, whether or not DA is able to induce the whole-cell ionic currents in single acutely disociated CNS neurons still remains to examine. The present work is planning to determine if the DA in-

[1] Project supported by the National Natural Science Foundation of China, № 39270775.
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Received 1994-11-01 Accepted 1995-06-26

duces whole-cell current in single neurons acutely isolated from the striatum, VTA, SNC, and hippocampal CA_1 region using patch-clamp perforated whole-cell recording technic.

MATERIALS AND METHODS

Preparation The dissociated technic of rat central neurons is similar to previously described[8]. The Wistar rats, 2-week-old, were decapitated under ether anesthesia. The brain was sliced at a thickness of 400 μm with a microslicer (DSK, DTK-1000). The brain slices were preincubated in an incubation solution bubbled with 95 % O_2 + 5 % CO_2 gas at room temperature (22—24 ℃) for 60 min. Thereafter, slices were treated with pronase 167 mg·L^{-1} at 31 ℃ for 30 min. After the enzyme treatment, the slices were washed with incubation solution and incubated for 10 min. The examined central areas of the brain slices were micro-punched out from slices and transferred into a culture dish filled with well-oxygenated standard external solution. The single neurons under the phase-contrast microscope (Nikon, TMS-1). The cells usually adhered to the bottom of the culture dish within 30 min. In the present experiments, the isolated neurons maintained their original morphological features. For instance, the hippocampal CA_1 pyramidal neurons possess the pyramidal-like somata and dendritic processes (Fig 1, Plate 3).

Solution The incubation solution was composed of NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10 mmol·L^{-1}. The pH was adjusted to 7.4 gassed with 95 % O_2+5 % CO_2. The standard external solution contained: NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and HEPES 10 mmol·L^{-1}. The pH was adjusted to 7.4 with Tris-hydroxyethyl aminomethane (Tris-OH). The perforated patch-pipette solution was: KCl 150 and HEPES 10 mmol·L^{-1}. The pH was adjusted to 7.2 with Tris-OH. Nystatin was dissolved in acidified methanol and the stock solution was diluted with the internal (patch-pipette) solution to a final concentration of 20 mg·L^{-1}.

Nystatin perforated patch recording Electrical recordings were made on the dissociated neurons using the nystatin perforated patch recording configuration under voltage-clamp condition. The patch-pipettes were fabricated from glass tubes (Narishige, G-1.5) in 2 stages on a vertical pipette puller (Narishige, PB-7). The resistance between the patch pipette filled with the internal solution and the reference electrode in the standard external solution was 4—6 MΩ. The current and voltage were measured with a patch-clamp amplifier (Nihon Kohden, CEZ-2300), and monitored on both a storage oscilloscope (Tektronix, 5111A) and a thermal-head pen recorder (Nippondenki San-ei, RECTI-HORIZ-8K21), and stored on vidol tapes after digitization with a PCM processor (Nihon Kohden, PCM 501 ESN). All experiments were performed at room temperature (20—24 ℃).

Drugs and application The drugs used in the present experiments were pronase (Hoechst), nystatin, DA and caffeine (Sigma), tetraethylammonium (TEA, Tokyo, Kasei). DA usually was applied every five minutes, at which interval constant responses could be completely recovered from desensitization. Rapid application of the drugs was performed with the "Y-tube" method, as described elsewhere[9]. By this technic, the external solution surrounding a neuron could be exchanged within 10—20 ms.

Statistical analysis Experimental values represent the $\bar{x} \pm s$. For evaluation of half-maximal effective concentration (ED_{50}) and Hill coefficient of concentration response curve, data were fitted to the Michaelis-Menten equation using a least-squares fitting, $I = I_{max}(C^n + K^n)$

Where I is current, I_{max} is the maximal response, and C is the concentration of agonist.

RESULTS

Examining ionic current responses elicited by DA in isolated single neurons from the striatum, VTA and SN The caffeine was applied as a control response for monitoring the neuron condition in all of 55 examined neurons at a V_H of −20 mV. The application of DA failed to induce obvious ionic current responses in the striatum (11 neurons) or VTA (25 neurons) neurons even though the concentration of DA increased to 1 mmol·L^{-1} (Fig 2, left).

While in 5 of 19 SNC neurons (23 %), DA 1 mmol·L^{-1} induced a small outward current (I_{NS}), the averaged current amplitude of I_{SN} was 11.3 ± 2.4 pA (Fig 2). But the caffeine 10 mmol·L^{-1} induced the typical transient outward current at the same experimental conditions (Fig 2, right).

DA induced three types of ionic current responses in the single hippocampal CA_1 pyramidal neurons Whole-cell recordings were made on 69 pyramidal neurons freshly dissociated from the hippocampal CA_1 region. When the cell membrane was patched with a pipette containing nystatin over 3—5 min, DA 1 mmol·L^{-1} induced 3 types of current responses, such as a transient outward current (8/69 neurons, 11.6 %) accompamied by an increase in membrane conductance (Fig 3A), a slow inward current (7/69 neurons, 10.2 %) with a decrease in membrane conductance (Fig 3B) and a combination of 2 types

responses in 10 (14.5 %) neurons (Fig 3C). Remainder 44 neurons (63.8 %) did not respond to DA.

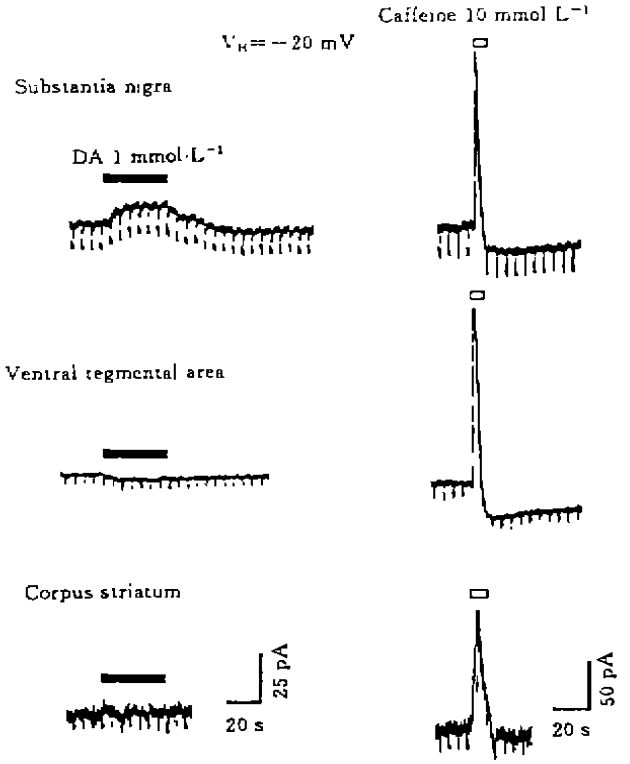

Fig 2. Current responses to DA (left) and caffeine (right) in single neurons.

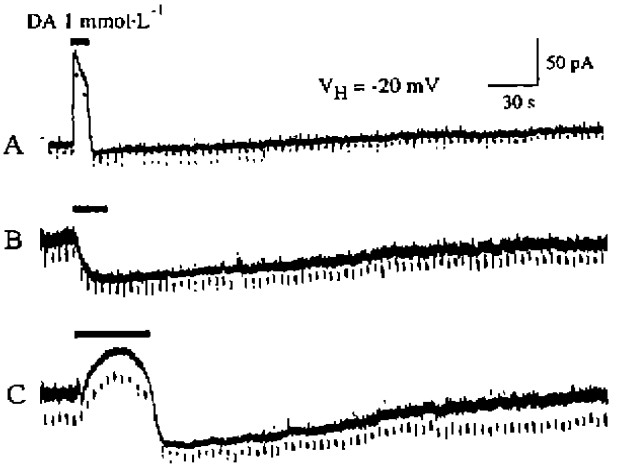

Fig 3. DA-induced 3 types of current responses in hippocampal CA_1 pyramidal neurons, typical from 25/69 neurons. A: a transient outward current; B: a slow inward current; and C: combination of 2 types of current. A hyperpolarizing pulse of 10 mV and 40 ms duration was applied every 5 s.

Concentration-response relationships of DA-induced current in hippocampal pyramidal neurons Experiments were carried out at a V_H of −20 mV with nystatin perforated patch recording mode. The application of DA to the dissociated pyramidal neurons elicited ionic current responses in 21 examined neurons. The threshold concentration for the DA-induced current (I_{DA}) was approximately 3 $\mu mol \cdot L^{-1}$ (Fig 4A), leading to a sigmoid shaped concentration-response curve (Fig 4B). The maxium response dose of DA-induced response was 1 $mmol \cdot L^{-1}$. The averaged amplitude of DA (0.2 $mmol \cdot L^{-1}$)-induced response was 36 ± 5 pA (n = 21). The concentration-response relationship of I_{DA} peak current component was summarized in Fig 4. All responses were normalized to the peak current (outward current component) elicited by DA 0.1 $mmol \cdot L^{-1}$.

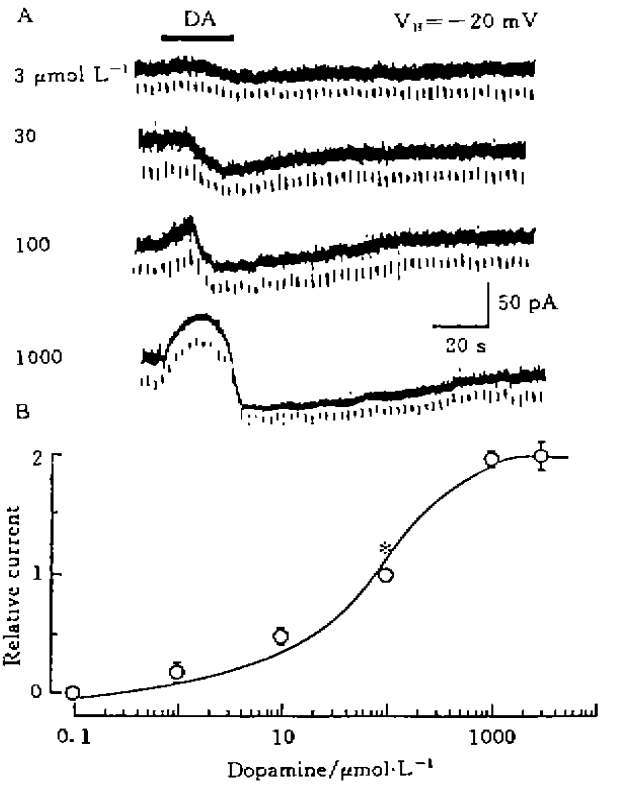

Fig 4. DA-induced ionic current responses in hippocampal CA_1 pyramidal neurons. A: the ionic currents induced by DA in various concentrations at a holding potential (V_H) of −20 mV. B: All responses were normalized to the current response induced by DA 0.1 $mmol \cdot L^{-1}$ (astcrisk, *). n=3−8 neurons ($\bar{x} \pm s$).

Current-Voltage (I-V) relationship of DA-induced current in hippocampal pyramidal neurons The dissociated neurons were perfused with the external and internal solutions contain-

ing K^+ ($[K^+]_o$ and $[K^+]_i$, 5 and 150 mmol·L^{-1}, respectively. The insides in figure 5A show the currents induced by DA 1 mmol·L^{-1} at various V_H. Detailed I-V relationship of the peak current component of I_{DA} is shown in Fig 5A. The I-V relationship for the transient peak current component showed linear-shaped. The reversal potential of DA-induced response (E_{DA}) value of peak current component estimated from intersects on voltage axis in the I-V curve was -81 ± 8 mV ($n=3$). This value was close to the K^+ equilibrium potintial (E_K) of -85.7 mV calculated from the Nernst equation which shows the extra- and intracellular K^+ concentrations. The K^+ channel blocker, TEA (5 mmol·L^{-1}) obviously inhibited the I_{DA} (Fig 5B). These results suggest that DA-induced outward current response is carried by K^+.

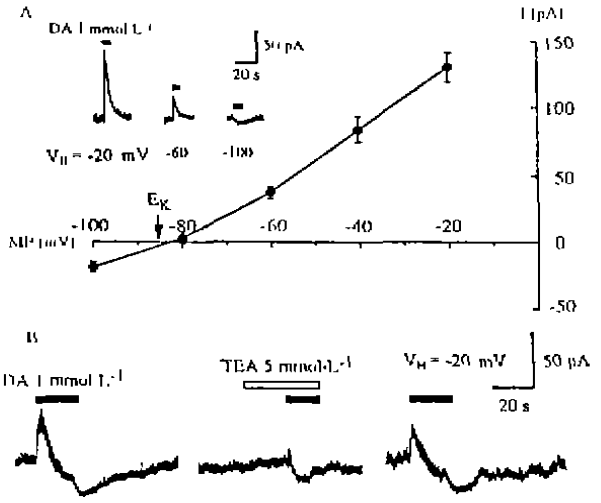

Fig 5. Current-Voltage (I-V) relationship of DA (1 mmol·L^{-1})-induced current (I_{DA}). Inside: I_{DA} at various V_H values in the same neuron. A: I-V relationship of I_{DA}. $n=3$ neurons. B: TEA blocked the DA-induced current, a typical case from 3 neurons.

DISCUSSION

The major finding of this study was that DA failed to evoke any obvious current responses in the single neurons freshly dissociated from rat striatum, VTA or SNC (only small outward current), but evoded 3 types of current responses, outward current, slow inward current, and outward followed by inward current in single hippocampal CA_1 pyramidal neurons. In the present investigation, caffeine, which induces the typical calcium dependent potassium currents[9], monitor the activity of examined neuron, indicating that the cellular condition is good enough to respond to agonist. The exactly cause that DA failed to evoked current responses in some brain area neurons is still not clear, but it seems to be associated with the following factors: (1) The DA receptors in brain of 7—14 day old rats might be not developed enough[10]. (2) The DA receptors located the SNC, VTA and striatum may be easily damaged sometimes for brain slice preparations[4,5,6].

Although the evidences have shown that DA-ergic afferents to the hippocampal formation originate from SNC[2] and VTA[3], and some DA receptor subtypes showed highest expression in hippocampus[11], whether or not DA gates the ionic current and what kind of ligand-gated ionic channel is activated by DA in this area are still poor understood. In the present study, it is firstly reported that these are DA-induced 3 types of ionic currents in single hippocampal CA_1 pyramidal neuron. The outward current response accompanied while membrane conductance increase indicates that the application of DA to the surface of CA_1 pyramidal neuron opens the ionic channel. The I-V relationship shows the reversal potential of DA-induced current is close to the E_K and it is sensitive to typical potassium channel blocker, TEA. This result clear indicates that DA-induced current response is carried by K^+. This finding is in agreement with the previous report by Benardo *et al*[7]. With regard to slow inward current accompanied with membrane decrease, it might be due to the M-current blockade. This phenomenon seems to be the same as the action of acetylcholine on muscurinic receptor of hippocampal CA_1 neuron[12].

In our experiments, the DA concentrations for evoking the marked current responses in CA_1 neurons were relatively high (0.1 mmol·L^{-1}). The response feature of CA_1 neuron to DA is needed further investigation with specific DA receptor antagonist. Gribkoff *et al* reported that 1 mmol·L^{-1} DA had a more pronounced suppressive action on the recorded population EPSP and spike than 0.1 mmol·L^{-1} DA had[13]. However, it is still controversial that the major electrophysiological actions of DA is diverse due to its action on D_1 and D_2 receptors or due to the cross-action

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1)

on the catechol amine receptors in the hippocampus[14].

Interestingly, we have found that tetrahydroberberine, a novel DA receptor antagonist[15], potently inhibits the I_{DA} in the hippocampal CA_1 pyramidal neurons in the current experiments (to be published).

ACKNOWLEDGMENT To Prof AKAIKA Norio, Department of Neurophysiology, Tohoku University School of Medicine, Sendai 980 Japan, for the helpful advice and comments.

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急性分离大鼠脑内神经元上由多巴胺引起的离子电流

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关键词 多巴胺; 钾通道; 海马; 锥体细胞; 膜片箝技术

目的: 研究多巴胺(DA)诱发神经元的电流反应. **方法**: 制霉菌素打孔的膜片箝全细胞记录. **结果**: 在 −20 mV 箝制电压下, DA (0.1—1 mmol·L^{-1})对26 %黑质神经元(5/19个)引起外向电流; 对36 %海马 CA_1 锥体细胞(25/69个)出现3种反应; 外向电流伴有膜电导增加、缓慢内向电流伴随膜电导减小、外向—内向电流. DA 引起的 CA_1 锥体细胞电流反应的阈剂量为3 mmol·L^{-1}, 无电压依赖关系. 翻转电位(E_{DA})接近 K^+ 平衡电位, 为 TEA 抑制. **结论**: DA 诱发海马 CA_1 锥体细胞的外向电流可能是 K^+ 电流.