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Inhibitory effect of memantine, an NMDA-receptor antagonist, on electoporation-induced inward currents in pituitary GH_3 cells

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ABSTRACT

The membrane electroporation-induced inward current (I_{MEP}) in pituitary tumor (GH_3) cells was characterized. This current emerges irregularly when membrane hyperpolarizations to −200 mV with a holding potential of −80 mV were elicited. Neither E-4031 (10 μM), glibenclamide (30 μM), nor ZD7288 (30 μM) caused any effects on I_{MEP}. The single-channel conductance and pore radius were estimated to be around 1.12 nS and 1.7 nm, respectively. $LaCl_3$- and memantidine (MEM)-induced block of this current was also examined. The IC_{50} value for $LaCl_3$- and MEM-induced inhibition of I_{MEP} was 35 and 75 μM, respectively. However, unlike $LaCl_3$, MEM (300 μM) did not exert any effect on voltage-gated Ca^{2+} current. In inside-out configuration, MEM applied to either external or internal surface of the excised patch did not suppress the activity of ATP-sensitive K^+ channels expressed in GH_3 cells, although glibenclamide significantly suppressed channel activity. This study provides the first evidence to show that MEM, a non-competitive antagonist of *N*-methyl D-aspartate receptors, directly inhibits the amplitude of I_{MEP} in pituitary GH_3 cells. MEM-mediated block of I_{MEP} in these cells is unlinked to its inhibition of glutamate-induced currents or ATP-sensitive K^+ currents. The channel-suppressing properties of MEM might contribute to the underlying mechanisms by which it and its structurally related compounds affect neuronal or neuroendocrine function.

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1. Introduction

Membrane electroporation (MEP) is recognized to exert a considerable increase in the electrical conductivity and permeability of the plasma membrane by the use of an externally applied electrical field [1]. By applying a strong electric current pulse, it has been widely used to electrotransfer large, membrane impermeant molecules such as DNAs, anticancer drugs or antibodies into cells [2–4]. Several studies have demonstrated that MEP in whole cell-configured heart cells can be induced by electrical fields applied in the form of voltage- or current-clamp commands [5–7]. However, despite its growing use, the electrical and pharmacological properties of MEP-induced inward current (I_{MEP}) remain largely unknown.

Mementine (MEM), is a derivative of amantadine, has been used to treat neurological disorders associated with excitotoxic cell death, including Parkinson's disease and vascular dementia [8–10]. The therapeutic effect of MEM is due to its ability to bind preferentially to *N*-methyl-D-aspartate (NMDA) receptor-operated cation channels [11]. The unique pharmacological properties enable MEM to be beneficial because it blocks excessive NMDA receptor activation with no interference with other physiological function. Besides that, unlike other NMDA receptor antagonists, it is a well tolerated NMDA receptor antagonist that can reduce or prevent excitotoxic damage without producing undesired adverse effects.

Despite the fact that NMDA receptors constitute the main target of MEM, there exist several studies reporting other underlying mechanism of its actions. For example, it was shown to reduce action potential firing in cultured neurons [12], to block 5-HT_3 and nicotinic receptors [13,14], and to inhibit ATP-sensitive K^+ (K_{ATP}) channels in substantia nigra neurons [15]. MEM is long recognized as an antiviral agent [16]. Taken together, these results suggest the presence of additional mechanisms of action whose relative importance may be responsible for its beneficial effects.

In this study, we attempted to characterize electrical properties of I_{MEP} in pituitary GH_3 cells and to examine whether $LaCl_3$ or MEM has any effects on I_{MEP}. Our results demonstrate that $LaCl_3$ or MEM can produce a depressant action on I_{MEP} in a concentration-dependent manner in these cells.

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2. Materials and methods

2.1. Drugs and solutions

MEM (Memantine, 1-amino-3,5-dimethyl-adamantane), diazoxide, glibenclamide, tetraethylammonium chloride and tetrodotoxin were obtained from Sigma–Aldrich (St. Louis, MO). E-4031 was purchased from Enzo (Plymouth Meeting, PA) and ZD7288 was from Tocris (Bristol, UK). All culture media, fetal calf serum, horse serum, L-glutamine, trypsin/EDTA, and penicillin–streptomycin were obtained from Invitrogen (Carlsbad, CA). All other chemicals, including CsCl, $LaCl_3$ and *N*-methyl-D-glucamine$^+$ ($NMDG^+$), were commercially available and of reagent grade.

The composition of normal Tyrode's solution is as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). To record I_{MEP}, the patch pipette was filled with a solution (in mM): K-aspartate 130, KCl 20, KH_4PO_4 1, $MgCl_2$ 1, Na_2ATP 3, Na_2GTP 0.1, EGTA 0.1, and HEPES-KOH buffer 5 (pH 7.2). To measure voltage-gated Ca^{2+} currents (I_{Ca}), K^+ ions inside the pipette solution was replaced with equimolar $NMDG^+$ ions and pH was adjusted to 7.2 with HCl.

2.2. Cell preparation

GH_3 pituitary tumor cells, obtained from the Bioresources Collection and Research Center ([BCRC-60015]; Hsinchu, Taiwan), were maintained in Hams'F-12 medium supplemented with 15% horse serum, 2.5% fetal calf serum and 2 mM L-glutamine in a humidified environment of 5% CO_2/95% air [17]. The experiments were performed 5 or 6 days after cells had been cultured (60–80% confluence).

2.3. Electrophysiological measurements

Before each experiment, an aliquot of cell suspension was transferred to a recording chamber positioned on the stage of a DM-IL inverted microscope (Leica, Wetzlar, Germany). Cells were bathed at room temperature (25 °C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Patch electrodes were made from Kimax-51 capillaries (Kimble Glass, Vineland, NJ) using a PP-830 puller (Narishige, Tokyo, Japan), and had a resistance of 3–5 MΩ when filled with different pipette solutions described above. Patch-clamp recordings were made in cell-attached, inside-out, or whole-cell configurations using an RK-400 amplifier (Bio-Logic, Claix, France) [17].

2.4. Data recordings and analyses

The data were stored online in a TravelMate-6253 computer (Acer, Taipei, Taiwan) at 10 kHz through a Digidata-1322A interface (Molecular Devices, Sunnyvale, CA). The interface device was equipped with an Adaptec SlimSCSI card (Milpitas, CA) via a PCMCIA slot and controlled by pCLAMP 9.2 (Molecular Devices). Concentration–response data for $LaCl_3$- or MEM-induced block of I_{MEP} were fitted with a modified form of the Hill equation. That is,

$$y = 1 - \frac{(1-a) \times [C]^{n_h}}{IC_{50}^{n_h} + [C]^{n_h}},$$

where y is the relative amplitude of I_{MEP}; [C] is the concentration of $LaCl_3$ or MEM; IC_{50} and n_h are concentrations required for a 50% inhibition and the Hill coefficient, respectively. Maximal inhibition (i.e., 1-a) of I_{MEP} in the presence of $LaCl_3$ or MEM was also estimated.

Values are provided as the mean values ± SEM with sample sizes (n) indicating the number of cells from which the data were taken. The paired or unpaired Student's t-test and one-way analysis of variance with the least significant difference method for multiple comparisons were used for the statistical evaluation of difference among means. A P value of less than 0.05 was considered to indicate statistical difference.

3. Results

3.1. Electrical properties of I_{MEP} in pituitary GH_3 cells

In the first series of experiments, the properties of I_{MEP} in these cells were characterized. Cells were bathed in Ca^{2+}-free Tyrode's solution which contained 10 mM CsCl. To preclude the contamination of Cl^- currents, Cl^- ions included in the recording pipette were replaced with aspartate. During whole-cell recordings, when membrane hyperpolarizations from −80 to −200 mV with a duration of 300 ms at a rate of 0.1 Hz were applied to the cell, an irregular and transient inward current was elicited (Fig. 1). Membrane hyperpolarizations to −200 mV were found to induce the mean maximal amplitude of 1354 ± 206 pA (n = 21). These inward currents were not suppressed by E-4031 (10 μM), glibenclamide (30 μM) or ZD7288 (30 μM). E-4031 is a blocker of *erg*-mediated K^+ current [18,19], glibenclamide suppresses K_{ATP} channels [17,20], and ZD7288 is known to block the amplitude of hyperpolarization-activated cationic current [21]. When bathing solution was replaced with $NMDG^+$ solution, this current could still be induced, although the magnitude of inward currents was diminished. Therefore, this type of inward current found in GH_3 cells is thus referred to as an MEP-induced inward current (I_{MEP}) [6,7,22]. Both hyperpolarization-induced cationic current and *erg*-mediated K^+ current that are important determinants of resting potential are not responsible for I_{MEP} observed in these cells.

These inward currents in response to membrane hyperpolarization often comprised multiple smaller currents occurring asyn-

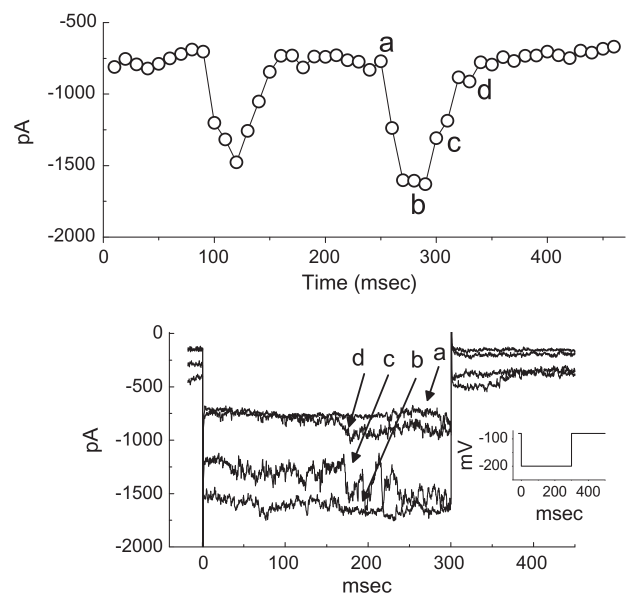

Fig. 1. Electrical properties of I_{MEP} in pituitary GH_3 cells. In these experiments, cells were bathed in Ca^{2+}-free Tyrode's solution containing 10 mM CsCl. The cell was held at −80 mV and hyperpolarizing pulses to −200 mV with a duration of 300 ms at a rate of 0.1 Hz were applied. Current amplitude at each trace was measured at the end of hyperpolarizing pulse. The time course of current amplitude in response to membrane hyperpolarizations indicates an episodic change of I_{MEP}. The lower part shown in a, b, c, and d denotes superimposed current traces corresponding to those labeled a, b, c, and d in the upper part. Inset indicates the voltage protocol used. During hyperpolarizing stimuli, I_{MEP} was generated with a waxed and waned pattern.

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