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Characterization of aconitine-induced block of delayed rectifier K^+ current in differentiated NG108-15 neuronal cells

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Abstract

The effects of aconitine (ACO), a highly toxic alkaloid, on ion currents in differentiated NG108-15 neuronal cells were investigated in this study. ACO (0.3–30 μM) suppressed the amplitude of delayed rectifier K^+ current ($I_{K(DR)}$) in a concentration-dependent manner with an IC_{50} value of 3.1 μM. The presence of ACO enhanced the rate and extent of $I_{K(DR)}$ inactivation, although it had no effect on the initial activation phase of $I_{K(DR)}$. It could shift the inactivation curve of $I_{K(DR)}$ to a hyperpolarized potential with no change in the slope factor. Cumulative inactivation for $I_{K(DR)}$ was also enhanced by ACO. Orphenadrine (30 μM) or methyllycaconitine (30 μM) slightly suppressed $I_{K(DR)}$ without modifying current decay. ACO (10 μM) had an inhibitory effect on voltage-dependent Na^+ current (I_{Na}). Under current-clamp recordings, ACO increased the firing and widening of action potentials in these cells. With the aid of the minimal binding scheme, the ACO actions on $I_{K(DR)}$ was quantitatively provided with a dissociation constant of 0.6 μM. A modeled cell was designed to duplicate its inhibitory effect on spontaneous pacemaking. ACO also blocked $I_{K(DR)}$ in neuroblastoma SH-SY5Y cells. Taken together, the experimental data and simulations show that ACO can block delayed rectifier K^+ channels of neurons in a concentration- and state-dependent manner. Changes in action potentials induced by ACO in neurons *in vivo* can be explained mainly by its blocking actions on $I_{K(DR)}$ and I_{Na}.

Keywords: Aconitine; NG108-15 cells; Delayed rectifier K^+ current; Na^+ current; Action potential

1. Introduction

Aconitine (ACO) is a highly toxic diterpenoid alkaloid occurring in plants of the *Aconitum* genus. ACO and its structurally related analogs are recognized for their phytomedical effects on the heart, central nervous system, and skeletal muscle (Ameri, 1998). The effects on Na^+ channels have thus been demonstrated to underlie toxicological or antinociceptive effects of this compound (Friese et al., 1997; Xu et al., 2006). It was thought that ACO binds with high affinity to the open state of voltage-gated Na^+ channels, thus causing a persistent activation of Na^+ channels by blocking their inactivation (Ameri, 1998; Wang and Wang, 2003; Fu et al., 2006). It has also been demonstrated that the increased amplitude of persistent Na^+ currents by ACO underlies the prolongation of action potentials (Ameri, 1998; Wright, 2001; Amran et al., 2004). Methyllycaconitine, its structurally-related analog, was reported to be effective in antagonizing α_7-nicotinic receptors (Hardick et al., 1995; Ameri, 1998). However, little information is available regarding the actions of ACO-related alkaloids on voltage-gated K^+ channels, although ACO was able to affect the repolarization phase of action potentials.

NG108-15 cells have been used as a neuron model in neurophysiology and pharmacology research (Brown and Higashida, 1988; Meves et al., 1999). It is a hybrid cell line

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derived from the fusion of two separate cell lines, mouse neuroblastoma (N18TG-2, a subclone of mouse C1300 neuroblastoma cells) and rat glioma (C6BV-1, a subclone of rat C6 glioma) (Brown and Higashida, 1988). This cell line was reported to express Kv3.1a mRNA and to exhibit the activity of delayed rectifier K^+ (K_{DR}) channels (Brown and Higashida, 1988; Yokoyama et al., 1989; Wu et al., 2001; Lo et al., 2003). In addition, the expression levels of $Na_v1.7$ were elevated in NG108-15 cells when neuronal differentiation was induced by pretreatment with a cyclic AMP analogue (Kawaguchi et al., 2007).

A characteristic feature of delayed rectification for K^+ channels (i.e., K_{DR} channels) is that brief depolarization activates the channel, but sustained depolarization can produce a slow inactivation (Baukrowitz and Yellen, 1995; Rasmusson et al., 1998; Fernandez et al., 2003). Inactivation is a basic conformational change intrinsic to most of the K^+ channels that controls repolarization. Inactivation has been observed for many cloned K^+ channels of the Kv superfamily, and is generally termed ''C-type inactivation'', to be distinguished from ''N-type'' inactivation by the ''ball-and-chain'' mechanism (Baukrowitz and Yellen, 1995; Rasmusson et al., 1998). K_{DR} channels with slow inactivation have been reported to affect the fluctuation in resting membrane potential (Marom, 1998). Moreover, the role of K_{DR} channels, particularly members of the Kv3 superfamily present in time-coding neurons is to stabilize the resting membrane potential and effectively reduce the broadening of high-frequency action potentials (Hernandez-Pineda et al., 1999; Rudy and McBain, 2001; Lien and Jonas, 2003; Tateno and Robinson, 2007). A similar type of inactivation has been characterized in Kv3.1 channels (Marom et al., 1993; Klemic et al., 2001).

Therefore, the objective of this study is to determine whether ACO, a highly potent neurotoxin, has any effects on ion currents and membrane potential in differentiated NG108-15 neuronal cells. Of note, we found that in these cells, in addition to inhibition of voltage-gated Na^+ current (I_{Na}), ACO could produce inhibitory effects on delayed rectifier K^+ current ($I_{K(DR)}$) in a concentration- and state-dependent fashion. Current inactivation of $I_{K(DR)}$ in the presence of ACO was also quantitatively characterized. Similar effects were also observed in neuroblastoma SH-SY5Y cells. The major action of ACO on $I_{K(DR)}$ is thought to be through an open-channel mechanism. Simulation studies can also duplicate experimental results.

2. Materials and methods

2.1. Cell preparation and differentiation

The clonal strain NG108-15 cell line, formed by Sendai virus-induced fusion of the mouse neuroblastoma clone N18TG-2 and the rat glioma clone Cg BV-1, was originally obtained from the European Collection of Cell Cultures (ECACC-88112302; Wiltshire, UK). NG108-15 cells were kept in monolayer cultures at a density of 10^6/ml in plastic disks containing Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Gaithersburg, MD, USA) supplemented with 100 μM hypoxanthine, 1 μM aminopterin, 16 μM thymidine, and 5% (w/v) fetal bovine serum (FBS) as the culture medium, in a humidified incubator equilibrated with 90% O_2/10% CO_2 at 37 °C (Wu et al., 2001). The experiments were generally performed after 5 days of subcultivation (60–80% confluence). To induce neuronal differentiation, culture medium was replaced with medium containing 1 mM dibutyryl cyclic-AMP and cells were cultured in the incubator for 1–7 days. NG108-15 cells proliferated well in the culture medium; however, they stopped proliferating and showed the growth of neurites in response to dibutyryl cyclic-AMP (Tojima et al., 2003). The numbers of neurites and varicosities were found to be significantly increased in NG108-15 cells treated with 1 mM dibutyryl cyclic AMP.

Human neuroblastoma SH-SY5Y cells were obtained from American Type Culture Collection ([CRL-2266]; Manassas, VA, USA). Cells were maintained in DMEM supplemented with 10% FBS, penicillin (200 U/ml), and streptomycin (100 μg/ml) in an incubator at 37 °C, containing 5% CO_2. To induce differentiation, SH-SY5Y cells were treated with all-*trans* retinoic acid (10 μM) and 2% fetal calf serum for 5–7 days before the experiments were made.

2.2. Electrophysiological measurements

Cells used for electrophysiological experiments were dissociated and an aliquot of cell suspension was transferred to a recording chamber mounted on the stage of an inverted DM-IL microscope (Leica Microsystems, Wetzlar, Germany). Cells were bathed at room temperature (20–25 °C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. Patch pipettes were pulled from Kimax-51 glass capillaries (1.5–1.8 mm o.d., Kimble; Vineland, NJ, USA) using a two-stage electrode puller (PP-830, Narishige, Tokyo, Japan) and the tips fire-polished with a microforge (MF-83; Narishige). Pipettes used had a resistance of 3–5 MΩ when immersed in normal Tyrode's solution. Ion currents were measured with glass pipettes in the whole-cell configuration of the patch-clamp technique, using an RK-400 (Biologic, Claix, France) or an Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA) (Wu et al., 2001).

2.3. Data recording and analyses

The signals were displayed on an analog/digital oscilloscope (HM-507; Hameg, East Meadow, NY, USA) and on a Dell 2407WFP-HC LCD monitor (Round Rock, TX, USA). The data were stored online in a Slimnote VX_3 computer (Lemel, Taipei, Taiwan) at 10 kHz through a Digidata-1322A interface (Molecular Devices). This device was controlled by pCLAMP 9.0 software (Molecular Devices). Currents were low-pass filtered at 1 or 3 kHz. Ion currents recorded during whole-cell experiments were digitally stored and analyzed subsequently by use of pCLAMP 9.0 (Molecular Devices), Origin 7.5 software (OriginLab, Northampton, MA, USA), SigmaPlot 7.0 software (SPSS, Apex, NC, USA), or custom-made macros in Microsoft Excel (Redmont, WA, USA). The pCLAMP-generated voltage-step protocols were generally used to measure the current-voltage (I–V) relations for ion currents (e.g., I_{Na} or $I_{K(DR)}$). Action potential duration was measured at 50% of repolarization.

The concentration-response data for inhibition of $I_{K(DR)}$ were fitted to the Hill equation:

$$\text{Percentage inhibition} = \frac{E_{max} + [C]^n}{[C]^n + IC_{50}^n},$$

where $[C]$ represents the concentration of ACO; and IC_{50} and n are the concentration required for a 50% inhibition and Hill coefficient, respectively; and E_{max} is ACO-induced maximal inhibition of $I_{K(DR)}$. Microsoft Solver in Excel was generally used to fit data by a least-squares algorithm.

Values were provided as means ± SEM with sample sizes (n) indicating the number of cells from which the data were obtained. The paired or unpaired Student's t-test and one-way analysis of variance with the least-significance-difference method for multiple comparisons were used for the statistical evaluation of differences among means. Statistical significance was determined at a P value of <0.05.

2.4. Drugs and solutions

ACO (aconitine or acetylbenzoylaconine, $C_{34}H_{47}NO_{11}$), dibutyryl cyclic AMP, orphenadrine citrate, tetraethylammonium chloride, tetrodotoxin,