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Sodium cyanate-induced opening of calcium-activated potassium currents in hippocampal neuron-derived H19-7 cells

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Abstract

We investigated the chemical toxic agent sodium cyanate (NaOCN) on the large conductance calcium-activated potassium channels (BK_{Ca}) on hippocampal neuron-derived H19-7 cells. The whole-cell and cell-attach configuration of patch-clamp technique were applied to investigate the BK_{Ca} currents in H19-7 cells in the presence of NaOCN (0.3 mM). NaOCN activated BK_{Ca} channels on H19-7 cells. The single-channel conductance of BK_{Ca} channels was 138 ± 7 pS. The presence of NaOCN (0.3 mM) caused an obvious increase in open probability of BK_{Ca} channels. NaOCN did not exert effect on the slope of the activation curve and stimulated the activity of BK_{Ca} channels in a voltage-dependent fashion in H19-7 cells. The presence of paxilline or EGTA significantly reduced the BK_{Ca} amplitude, in comparison with the presence of NaOCN. These findings suggest that during NaOCN exposure, the activation of BK_{Ca} channels in neurons could be one of the ionic mechanisms underlying the decreased neuronal excitability and neurological disorders.

Keywords: Sodium cyanate; Calcium-activated potassium current

Sodium cyanate (NaOCN), as a mild chemical toxin, has been used to study hypoxia and known to increase the hypoxia tolerance of animals by increasing the affinity to hemoglobin to O_2 [19,20]. Its selective inhibition of protein and DNA synthesis such as carbamylation makes it employed widely as chemical toxic, herbicides, and an inhibitor for tumor growth and erythrocyte sickling [2], though detailed mechanism not completely understood. NaOCN was demonstrated to deplete ascorbate in the matrix which involves ascorbate-dehydroascorbate redox cycle in inflicting oxidative stress [9]. It has also been noted that NaOCN may induce inhibition of glutathione reductase activity, which is suggested as one of the possible pathophysiologies related to non-lethal cyanogen intoxication with spastic paraparesis [22], an acquired human neurological disorder with mostly unknown in its pathophysiology [1,4,24]. In addition, peripheral motor and/or sensory neuropathy is reported secondary to NaOCN without a clear mechanism [3,15].

Large conductance calcium-activated potassium channels (BK_{Ca}) are present in many tissues and participate in a variety of cellular processes. They play an important role in the regulation of neuronal excitability, cell volume and excitation–contraction coupling [8,26]. In neurons, BK_{Ca} channel are largely responsible for the generation of the fAHP [18] and regulation of neuronal excitability and maintenance of membrane potential [25]. The gating of the channels can

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be controlled by intracellular Ca^{2+} and/or membrane depolarization [23]. Reduced oxygen tension has been shown to induce inhibition of BK_{Ca} channels in cells from carotid body [16], pulmonary smooth muscle [28], and the adrenal gland [21]. However, other studies have shown that reduced oxygen tension increases the activity of BK_{Ca} channels in cat cerebral arterial smooth muscles [5]; besides, the activities of BK_{Ca} channel during reduced oxygen tension in central neurons were not consistent between studies [6,29]. Therefore, the main objective of this study was to address the question of whether NaOCN could affect calcium-activated potassium current in these cells. There is so far no electrophysiological study concerning NaOCN reported. We provide evidence that NaOCN can have an increasing effect on BK_{Ca} activity in H19-7 cells, which could then decrease neuronal excitability.

The H19-7 cell line, originally derived from hippocampi dissected from embryonic day 17 (E17) Holtzman rat embryos and immortalized by retroviral transduction of temperature-sensitive tsA58 SV40 large T antigen, was obtained from American Type Culture Collection ([CRL-2526], Manassas, VA) [17]. It has been confirmed that it possesses the characteristics of hippocampal neurons [13]. H19-7 cells were maintained in Dulbecco's modified Eagle's media with 4 mM L-glutamine adjusted to contain 1.5 g/l sodium bicarbonate and 4.5 g/l glucose supplemented with 10% fetal bovine serum, 200 μg/ml G418 and 1 μg/ml puromycin in flasks coated with 15 μg/ml poly-L-lysine. They were equilibrated in a humidified atmosphere of 5% CO_2/95% air at temperature 34 °C. The experiments were performed after 5 days of subcultivation (60–80% confluence) with cells obtained passages 2 and 4.

Immediately before each experiment, cells were dissociated and an aliquot of cell suspension was transferred to a recording chamber positioned on the stage of an inverted microscope (DM IL, Leica Microsystems, Wetzlar, Germany). The microscope was coupled to a video camera system with magnification up to 1500× in order to monitor cell during the experiments. Cells were bathed at room temperature (20–25 °C) in normal Tyrode's solution containing 1.8 mM $CaCl_2$. The recording pipettes were pulled from thin-walled borosilicate glass capillaries (Kimax-51, Kimble Glass, Vineland, NJ) using a two-stage microelectrode puller (PP-830, Narishige, Tokyo, Japan) and the tips were fire-polished with a microforge (MF-83; Narishige). When filled with pipette solution, their resistance ranged between 3 and 5 MΩ. Ion currents were recorded in the cell-attach and whole-cell configuration of the patch-clamp technique, using an RK-400 patch-clamp amplifier (Bio-Logic, Claix, France) [27]. All potentials were corrected for liquid junction potential, a value that would always develop at the tip of the pipette when the composition of the pipette solution was different from that in the bath. Tested drugs were applied by perfusion or added to the bath to obtain the final concentrations indicated.

The signals were displayed on an analog/digital oscilloscope (HM 507, Hameg Inc., East Meadow, NY). The data were on-line stored in a computer through a high-speed/low-noise analog/digital interface (Digidata 1322A, Axon Instruments, Union City, CA), controlled by a commercially available software (pCLAMP 9.0, Axon Instruments). The sampling rate for electrophysiological measurements was 10 kHz. Currents were low-pass filtered at 1 or 3 kHz. Ion currents recorded during whole-cell experiments were stored without leakage correction and analyzed subsequently using the pCLAMP 9.0 software (Axon Instruments), the Origin 6.0 software (Microcal Software, Inc., Northampton, MA), SigmaPlot 7.0 software (SPSS, Inc., Apex, NC), or custom-made macros in Excel (Microsoft, Redmont, WA). The pCLAMP-generated voltage-step protocols were used to examine the current–voltage (*I–V*) relations for ion currents [27]. The averaged results are presented as the mean values ± S.E.M. The paired or unpaired *t* test and one-way ANOVA with the least significance difference method for multiple comparisons were used for the statistical evaluation of differences among the mean values. Differences between values were considered significant when $p < 0.05$.

The composition of normal Tyrode's solution was 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 K^+ current, the patch pipette was filled with a solution (in mM): KCl 140, $MgCl_2$ 1, Na_2ATP 3, Na_2GTP 0.1, EGTA 0.1, and HEPES–KOH buffer 5 (pH 7.2). The pipette solution was filtered on the day of use with a 0.22-μm pore size syringe filter (Millipore). For single channel experiments, high K^+ bathing solution contained (in mM): KCl 145, $MgCl_2$ 2, and HEPES–KOH buffer 5 (pH7.2). Sodium cyanate, apamin, glibenclamide and paxilline were purchased from Sigma Chemical (St. Louis, MO).

In the patch-clamp technique, the whole-cell configuration was used to investigate the effect of NaOCN on macroscopic ionic currents in H19-7 cells. In these experiments, cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$. In order to inactivate other voltage-dependent K^+ currents, each cell was held at the level of 0 mV. When the cell was held at 0 mV, series of large outward currents were elicited in response to various voltage pulses ranging from −40 to +100 mV with 20-mV increments. These outward currents have been referred to as $I_{K(Ca)}$. When cells were exposed to NaOCN, the amplitude of outward currents was significantly enhanced throughout the entire voltage clamp step. When the voltage step from 0 to +100 mV was evoked, 0.3 mM NaOCN significantly increased current amplitude to 480 ± 70 pA from a control value to 248 ± 55 pA ($p < 0.05$, $n = 8$). After the washout of NaOCN, the amplitude returned to 265 ± 55 pA ($n = 6$). Neither glibenclamide (10 μM) nor apamin (200 nM) affected the NaOCN-induced increase in the amplitude of outward currents (data not shown). The average *I–V* relations for these currents in the absence and presence of NaOCN (0.3 mM) are shown in Fig. 1. These results