Opening of Ca\(^{2+}\)-Dependent K\(^+\) Channels by Nordihydroguaiaretic Acid in Porcine Coronary Arterial Smooth Muscle Cells

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Received February 2, 1996   Accepted February 22, 1996

ABSTRACT—Effects of nordihydroguaiaretic acid (NDGA) on the Ca\(^{2+}\)-dependent K\(^+\) channel (BK channel) were examined by the patch clamp technique in single smooth muscle cells of porcine coronary artery. The open probability of BK channels in inside-out patches increased by about 30 times at the holding potential of 0 mV, when 10µM NDGA was added to the bathing solution (pCa 7.0). The effect of NDGA was concentration-dependent in the range of 1–100µM and partly removed by washout. The enhancement of BK channels by NDGA was not observed when the cytosolic Ca\(^{2+}\) concentration was very low (pCa > 8.5). These results clearly indicate that NDGA possesses a BK channel opening property in coronary arterial myocytes.

Keywords: Ca\(^{2+}\)-dependent K\(^+\) channel, Nordihydroguaiaretic acid, Coronary arterial smooth muscle

The first generation of K\(^+\) channel openers is a group of heterogeneous chemical compounds that activate the ATP-sensitive K\(^+\) channel (K\(_{ATP}\)), and these drugs have been introduced or under development for the treatment of angina, hypertension, asthma etc. (1). It has been reported that the activity of large conductance Ca\(^{2+}\)-dependent K\(^+\) channels (BK channel) is enhanced by niflumic and mefenamic acids (2). BK channels exist abundantly on the plasma membranes of various types of smooth muscles. Although the BK channel activities may not be high under resting conditions in normal vascular smooth muscle, its functional role in the regulation of vascular tone via the control of membrane potential may be more significant under hypertension (3). Much attention has been directed towards the discovery of compounds that can open BK channels; and so far, some newly synthesized openers such as NS 004 (4), NS 1619 (5) and MCI-154 (6) have been introduced. We report here for the first time that nordihydroguaiaretic acid (NDGA) is a potent opener of BK channels in coronary arterial myocytes.

Fresh hearts of young pigs (3- to 6-months-old) were obtained from a local slaughterhouse and transported to the laboratory in ice-cold oxygenated Krebs' solution. Single smooth muscle cells were isolated from the coronary artery by enzymatic dispersion (7). Single channel recordings from inside-out and outside-out patches were performed by a single suction micropipette method and the previously described system (7). Pipette resistance ranged from 7 to 10 Mohm. The standard bathing solution contained: 140 mM KCl, 5.9 mM NaCl, 1.2 mM MgCl\(_2\), 14 mM glucose and 10 mM HEPES; The pH was adjusted to 7.4 with NaOH. The pipette solution was the same as the bathing solution, whereas the K\(^+\) concentration was occasionally changed by replacing the K\(^+\) with equimolar Na\(^+\) in some experiments. The pCa of these solutions was adjusted to the range of 5 to 9 with Ca\(^{2+}\)EGTA buffer using 0.5 mM Ca\(^{2+}\) and the corresponding EGTA according to the methods used by Benham et al. (8). The pCa of the pipette solution for outside-out patch recording was adjusted to a certain value using 4 mM EGTA and the corresponding Ca\(^{2+}\). Data storage and analyses were performed as described previously (7). The experiments were carried out at room temperature (23±1°C). Pooled data are expressed as values of the mean±S.E.M. NDGA (from Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) at the concentration of 10⁻³ M and stored as a stock solution. The final concentrations of DMSO were 0.1%, 0.01% and 0.001%, when those of NDGA were 100, 10 and 1µM, respectively. When NDGA was applied, the entire experiment was performed in the presence of the corresponding concentration of DMSO. The statistical significance was determined by Student's t-test.

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The unitary current amplitude was approximately 6 pA when the pipette and bathing solutions contained 40 and 140 mM K+, respectively. The pCa in the bathing solution was 7.0. The increase in [Ca2+]o from pCa 7.0 to 5.0 resulted in marked enhancement of the activity and showed that there were three channels included in the patch; the open probability of the channel (Popen) increased by 70-100 times (not shown in Fig. 1A).

When the holding potential was changed from −80 to +50 mV by the ramp pulse, the channel current reversed from inward to outward at around −35 mV and the channel conductance was 146 ± 1 pS (n = 4). The averaged Popen at pCa 7.0 and 0 mV was 0.0074 ± 0.0048 (n = 6). The Ca2+-sensitivity and the conductance were roughly measured in each patch to confirm that the channels were BK channels.

Application of 1 μM NDGA increased the channel activity within 20 sec (Fig. 1). Since the unitary current amplitude was not affected by NDGA, the increase in
channel activities may have resulted from the enhancement of those of BK channels. \( P_{\text{open}} \) changed from 0.0028 to 0.0031 (see Fig. 1A: c and f). A further increase in \( P_{\text{open}} \) to 0.108 was observed when 10 \( \mu \)M NDGA was applied. The increased \( P_{\text{open}} \) was decreased to 0.0013 by changing the bathing solution containing nominally free \( \text{Ca}^{2+} \) and 0.5 mM EGTA (\( \text{pCa} \approx 8.5 \) assuming contamination of 50 \( \text{pM} \) \( \text{Ca}^{2+} \)). The \( P_{\text{open}} \) of BK channels under the conditions of \( \text{pCa} \) 7.0 and the holding potential of 0 mV was increased by 1-100 \( \text{pM} \) NDGA in a concentration-dependent manner. The effect of 10 \( \text{pM} \) NDGA was mostly removed by washout, whereas that of 100 \( \text{pM} \) NDGA substantially remained.

The effects of NDGA were also observed in outside-out patches (Fig. 1B). Since the number of channels in a patch could not be detected in this configuration, \( nP_{\text{open}} \) was measured by dividing the integrated current of an original recording by integration of unitary current for a certain recording period (20 sec). The \( nP_{\text{open}} \) was increased by 9.0 times by the application of 10 \( \mu \)M to the bathing solution (Fig. 1Bb). Such results were obtained in all three examined patches; \( nP_{\text{open}} \) was increased by 3, 9 and about 400 times, respectively, by 10 \( \mu \)M NDGA. The channel activity was completely blocked by addition of 100 \( \text{nM} \) iberiotoxin to the bathing solution (Fig. 1Bc).

The \( P_{\text{open}} \) of the channel under control conditions and also the change in \( P_{\text{open}} \) by NDGA varied widely from patch to patch, especially at low \( \text{Ca}^{2+} \) concentrations. In inside-out patches where channel activities were measured in the absence and presence of 1, 10 and 100 \( \mu \text{M} \) NDGA, the \( P_{\text{open}} \)s were 0.0074±0.0048 (n=6), 0.0174±0.0101 (n=5), 0.137±0.086 (n=6) and 0.389±0.285 (n=3), respectively (Fig. 2A). When the \( P_{\text{open}} \) in the absence of NDGA was taken as 1.0 in each patch, the averaged value of the relative \( P_{\text{open}} \) in the presence of 10 \( \mu \text{M} \) NDGA was 30.2±9.4 (n=6, \( P<0.05 \) vs control). Effects of NDGA on the current-voltage relationships of the BK channel current were measured using a ramp pulse protocol. Summarized data are shown in Fig. 2B. When \( \text{K}^+ \) concentrations in the pipette and bathing solutions were 40/140, 140/140 and 140/70 mM, the calculated \( \text{K}^+ \) reversal potentials were 31.6, 0 and +17.5 mV, respectively. The measured reversal potentials were −36.8±0.5 (n=4), +1.0±0.9 (n=3) and +14.4±0.8 mV (n=3) in the control (open symbols), respectively, and were not affected by 10 \( \mu \text{M} \) NDGA (closed symbols). The conductance was 146±1, 255±16 and 200±4 \( \text{pS} \) in the control and 152±7 (n=4), 263±9 (n=3) and 196±2 \( \text{pS} \) (n=3) in the presence of 10 \( \mu \text{M} \) NDGA, respectively (\( P>0.05 \), between the control and in the presence of NDGA).

The present study clearly indicates that NDGA acts as a potent BK channel opener in myocytes of the porcine coronary artery. The channel affected by NDGA was defined as the BK channel based upon the single channel conductance, \( \text{Ca}^{2+} \)-sensitivity (7, 8) and the blockade by iberiotoxin. NDGA did not affect channel conductance at any \( \text{K}^+ \) concentrations. NDGA is contained in the resinous exudate of plants and is used as an antioxidant in fats and oils. In pharmacological studies, it has been used as an inhibitor of 5-lipoxygenase. Although the regulation of voltage-dependent \( \text{Ca}^{2+} \) channel activity by lipoxygenase products has been reported in several types

![Fig. 2. Effects of NDGA on BK channel open probability and channel conductance. A: Summarized data about effects of NDGA on the open probability of BK channels recorded under the conditions shown in Fig. 1A (pCa=7.0) in inside-out patches. The numbers in the parentheses indicate the number of cells used. B: Current-voltage relationships measured using the ramp pulse protocol in the absence (open symbols) and presence of 10 \( \mu \text{M} \) NDGA (closed symbols) under the conditions in Fig. 1A (pCa=7.0) in inside-out patches. The \( \text{K}^+ \) concentrations in the pipette and bathing solutions were 40/140 (squares), 140/140 (circles) and 140/70 (triangles) mM.](image-url)
of preparations (9), it has been demonstrated that NDGA rather directly blocks the Ca\(^{2+}\) channel (10). The BK channel opening property of NDGA observed in this study may also be due to a direct action, since the effect was observed in inside-out patches in the absence of an activator of phospholipase A\(_2\).

It is notable that, at least in excised patches, the potency of NDGA to enhance BK channel activity may be apparently higher than that of niflumic acid (2) and comparable to or even higher than those of newly synthesized BK channel openers (4–6). The simple chemical structure of NDGA may possibly be a new prototype for the development of a more potent and selective opener of the BK channel, which may possibly be useful as a vasodilator and/or broncho-dilator.

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