Modification by cGMP of Glibenclamide-Sensitive $K^+$ Currents in Xenopus Oocytes

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ABSTRACT—Effects of sodium nitroprusside, 8-bromo cGMP and methylene blue on the glibenclamide-sensitive $K^+$ current evoked by $K^+$ channel openers in Xenopus oocytes were studied. Sodium nitroprusside (0.1–1 mM, an activator of guanylate cyclase) enhanced by 20–50% the $K^+$ currents induced by KRN2391, nicorandil and cromakalim ($K^+$ channel openers). 8-Bromo cGMP (1 mM) also increased the $K^+$ current by 40–60%. Methylene blue (10 μM, an inhibitor of guanylate cyclase) irreversibly blocked the $K^+$ current by about 20–30%. These results suggest that the activation of glibenclamide-sensitive $K^+$ channels by $K^+$ channel openers is modulated either positively or negatively by intracellular cGMP in oocytes.

Keywords: K$^+$ channel, Glibenclamide, cGMP

The ATP-sensitive $K^+$ channel is the ion channel that is activated by the deficiency of intracellular ATP and by $K^+$ channel openers, and it is blocked selectively by antidiabetic sulfonylureas such as glibenclamide and by various drugs including verapamil (1), phentolamine (2), chlorpromazine (3) and trifluoperazine (3).

The oocyte of Xenopus laevis shows an outward current when a $K^+$ channel opener such as cromakalim, pinacidil, nicorandil, KRN2391 and Y-26763 is externally applied (4–7). These outward currents are carried by the efflux of $K^+$ ions, because their reversal potentials are close to the $K^+$ equilibrium potential in oocytes (about −100 mV), and they are sensitive to tetroethylammonium (4, 5, 7). The ion channel mediating these $K^+$ currents is thought to be ATP-sensitive $K^+$ channels, since the currents are suppressed by the selective blocker of ATP-sensitive $K^+$ channels, glibenclamide (4–7), and by drugs such as verapamil, phentolamine, chlorpromazine and trifluoperazine (5–7).

The glibenclamide-sensitive $K^+$ current induced by a $K^+$ channel opener in oocytes is facilitated by such manipulations as raising the intracellular level of cAMP, namely, by the application of cyclic nucleotide phosphodiesterase inhibitor xanthine derivatives such as 3-isobutyl-1-methylxanthine and theophylline, or by the application of forskolin, an adenylate cyclase activator (1, 2).

cGMP, another cyclic nucleotide, is known to directly activate ion channels in sensory receptor cells (8) and to induce, at a high concentration, a $K^+$ current in Xenopus oocytes (9). However, little is known about the effect of cGMP at a subthreshold concentration on the $K^+$ current induced by $K^+$ channel openers. Knowledge of a modulatory effect of cGMP, if present, might offer some information about the possible interaction between two antihypertensive drugs, a nitrate and a $K^+$ channel opener. Thus, we have investigated the effects of an activator and an inhibitor of guanylate cyclase on the glibenclamide-sensitive $K^+$ current induced in Xenopus oocytes by $K^+$ channel openers, cromakalim, nicorandil and KRN2391 in the present study. The results suggest the modulatory role of cGMP itself or cGMP-dependent processes in the activation of the $K^+$ channel.

Follicle-enclosed oocytes were collected from female frogs (Xenopus laevis; Hamamatsu Biological Research Service, Inc., Hamamatsu) anesthetized in ice and then preincubated in modified Barth's medium for 1–4 days (5–7). For electrophysiological recording, each oocyte was placed in a recording well (about 0.2 ml in capacity) and superfused at a constant rate of 3 ml/min with frog Ringer solution consisting of 120 mM NaCl, 2 mM KCl, 1.8 mM CaCl$_2$ and 5 mM HEPES (pH 7.4). The oocyte membrane potential was voltage-clamped routinely at −20 mV with two glass microelectrodes each filled with 3 M KCl (1–2 Mohm) using a voltage-clamp amplifier, CEZ-1200 (Nihon Kohden, Tokyo), and current
responses were directly recorded by a Thermal-array Recorder, RTA-1100 (Nihon Kohden), as previously described (5–7). All electrophysiological experiments were carried out at room temperature (19–23 °C).

Cromakalim (Kirin Brewery Co., Takasaki), KRN2391 (Kirin Brewery Co.) or nicorandil (Chugai Pharmaceutical Co., Tokyo) was dissolved in frog Ringer solution and applied to a voltage-clamped oocyte for a constant period of 20 sec by constant flow superfusion (3 ml/min) to evoke an outward current response. Each agent was repetitively applied to the same oocyte with 6-min intervals. 8-Bromo cGMP (Sigma Chemical Co., St. Louis, MO, U.S.A.), methylene blue (Sigma Chemical Co.) or sodium nitroprusside (Wako Pure Chemical Co., Osaka) was dissolved in frog Ringer solution and applied to a voltage-clamped oocyte by superfusion (3 ml/min). Sodium nitroprusside was kept in dim light at room temperature for at least 1 hr before use.

Sodium nitroprusside (SNP) is known to increase intracellular cGMP in smooth muscles by generating nitric oxide, an activator of guanylate cyclase (10). As shown in Fig. 1 (A and B), the K+ current induced by cromakalim was reversibly enhanced by SNP. This facilitation was concentration-dependent, that is, the amplitude of cromakalim responses was increased by 19.2 ± 4.2% (n = 5) (mean ± S.D.) and 56.9 ± 16.8% (n = 5) by 100 μM and 1 mM SNP, respectively. SNP at 1 mM also showed similar but weaker potentiating effects on the K+ currents induced by KRN2391 (37.2 ± 20.7%, n = 5) and nicorandil (21.3 ± 6.4%, n = 5) (Fig. 1, C and D). As shown in Fig. 1 (A, C and E), a high concentration of SNP (1 mM) often induced an outward current by itself. However, SNP facilitated cromakalim-induced currents either in the presence or in the absence of the outward current induced by SNP itself (Fig. 1, A and B). Although the property of this SNP-induced current is unclear at present, it was in-

![Fig. 1. The effect of sodium nitroprusside (SNP) on responses to cromakalim, nicorandil and SNP-induced currents in oocytes. (A and B) Facilitation by SNP (1 mM and 100 μM for 2 min, long bars) of responses to cromakalim (100 μM CK, 20 sec, short bars). (C and D) Facilitation by SNP (1 mM, 2 min, long bars) of responses to nicorandil (2 mM NCR, 20 sec, short bars) and KRN2391 (100 μM KRN, 20 sec, short bars). (E) The outward current induced by SNP (1 mM, 30 sec) and no effect thereon of glibenclamide (2 μM GCM, 2 min, long bar). Records A–E were from different oocytes voltage-clamped at −20 mV. Calibrations: 100 nA vertical and 1 min horizontal.](image)

![Fig. 2. The effect of 8-bromo cGMP on responses to nicorandil, KRN2391 and cromakalim in oocytes. (A–C) Potentiation by 8-bromo cGMP (BeGMP, 1 mM for 2 min, long bars) of responses to nicorandil (2 mM NCR, 20 sec, short bars), KRN2391 (200 μM KRN, 20 sec, short bars) and cromakalim (100 μM CK, 20 sec, short bars). Records A–C were from different oocytes voltage-clamped at −20 mV. Calibrations: 100 nA and 3 min. (D) Mean amplitudes (± S.D., n = 5) of responses to nicorandil (closed circles), KRN2391 (open circles) and cromakalim (open squares) before and after the application (arrowhead) of 8-bromo cGMP (1 mM, 2 min). The abscissa is the trial number at 6-min intervals. Cont 1 and Cont 2 are controls. Values plotted were from similar recordings to those in A–C, and each was normalized to Cont 2.](image)
sensitive to glibenclamide. As shown in Fig. 1E, the SNP-induced current was little affected by 2 µM glibenclamide, which is potent enough to abolish the K⁺ current induced by any one of the K⁺ channel openers used. This indicates that SNP-induced current is mediated either by a K⁺ channel other than the glibenclamide-sensitive K⁺ channel or by non-selective cation channels. A possible explanation for the results shown in Fig. 1 (A–D) is that SNP-derived nitric oxide activates guanylate cyclase and the resultant increase of cGMP potentiates the activity of K⁺ channel openers. To prove this possibility, however, it is necessary to examine whether SNP actually raises the cGMP level in oocytes.

To obtain more direct evidence for the potentiating effect of cGMP, the effect of 8-bromo cGMP on the K⁺ currents induced by K⁺ channel openers was then studied. Since cGMP at a high concentration is known to induce by itself a K⁺ current in oocytes (9), a subthreshold concentration of 1 mM was externally applied for 2 min to oocytes. 8-Bromo cGMP (1 mM) facilitated nicorandil-induced K⁺ currents by about 50% (Fig. 2, A and D), and this facilitation lasted for over 30 min. Similar potentiating effects of 8-bromo cGMP were also observed for the K⁺ currents induced by KRN2391 (Fig. 2, B and D) and by cromakalim (Fig. 2, C and D).

Methylene blue inhibits the nitric oxide-activated production of cGMP (10). Although its selectivity as a guanylate cyclase inhibitor is somewhat disputable, it is also known that methylene blue lowers the basal level of intracellular cGMP in neuronal cells (11). As shown in Fig. 3, the application of 10 µM methylene blue for 5 min suppressed by 20–25% the K⁺ current induced by all K⁺ channel openers used. This suppression was virtually irreversible.

These results suggest that cGMP itself or cGMP-dependent processes modify the sensitivity of glibenclamide-sensitive K⁺ channels to K⁺ channel openers.

At least two possibilities may be raised as the mechanism of the current-potentiating action of cGMP. The first possibility is that cGMP activates a cGMP-dependent protein kinase(s) which then phosphorylates glibenclamide-sensitive K⁺ channel protein(s). Phosphorylation is needed not only for the maintenance of the activity of K⁺ channels of this type (12) but also for the activation of the K⁺ channels by diazoxide, a K⁺ channel opener (13), and by ADP (14). The second possibility is that cGMP directly binds to the nucleotide binding site of the K⁺ channel and enhances the channel sensitivity to K⁺ channel openers. The binding site for a K⁺ channel opener in the ATP/glibenclamide-sensitive K⁺ channel is suggested to be the same as the ATP binding site (15). Thus, it is conceivable that cGMP might compete with ATP for the ATP binding site and enhances the sensitivity of K⁺ channels to K⁺ channel openers.

Since K⁺ channel openers are expected to protect the myocardium from ischemia, a K⁺ channel opener and a nitrate are likely to be used in combination. In this case, since the nitrate would potentiate the channel opening activity of the K⁺ channel opener, it might enhance the myocardial protecting effect of the K⁺ channel opener.

In conclusion, the sensitivity of the glibenclamide-sensitive K⁺ channel in Xenopus oocytes to K⁺ channel openers is modulated by cGMP itself or cGMP-dependent processes.

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