Dual effect of insulin-like growth factor on the apical 70-pS K channel in the thick ascending limb of rat kidney

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Wei, Yuan, Yu-Jung Chen, Dimin Li, Ruimin Gu, and Wen-Hui Wang. Dual effect of insulin-like growth factor on the apical 70-pS K channel in the thick ascending limb of rat kidney. Am J Physiol Cell Physiol 286: C1258–C1263, 2004; 10.1152/ajpcell.00441.2003.—We used the patch-clamp technique to study the effect of insulin-like growth factor I (IGF-I) on the apical 70-pS K channel in the isolated thick ascending limb (TAL) of the rat kidney. The isolated TAL was cut open to gain access to the apical membrane. Addition of 25 nM IGF-I stimulates the apical 70-pS K channel and increases channel activity, defined by the product of channel open probability and channel number, from 0.31 to 1.21. The stimulatory effect of IGF-I is not mediated by nitric oxide- or protein tyrosine phosphatase-dependent mechanisms, because inhibition of nitric oxide synthase or blocking protein tyrosine phosphatase did not abolish the stimulatory effect of IGF-I on the 70-pS K channel. In contrast, inhibition of mitogen-activated protein (MAP) kinase with PD-98059 or U0126 abolished the stimulatory effect of IGF-I. This suggests that MAP kinase is responsible for mediating the effect of IGF-I on the apical K channels. Moreover, the effect of IGF-I on the apical 70-pS K channel is biphasic because high concentrations (>200 nM) inhibit apical 70-pS K channels. Application of 400 nM IGF-I decreased channel activity from 1.45 to 0.2. The inhibitory effect of IGF-I is not blocked by calphostin C (an inhibitor of PKC), but inhibition of protein tyrosine kinase with herbimycin A abolished the IGF-induced inhibition. We conclude that IGF-I has a dual effect on the apical 70-pS K channel. Moreover, the effect of IGF-I on the apical 70-pS K channel is not completely understood, it is most likely that MAP kinase is responsible for mediating the effect of IGF-I on the apical K channels. Therefore, the goal of the present study is to explore the effect of IGF-I on the apical K channels. There are three types of K channels: renal outer medulla K+ (ROMK)-like (30 pS), the intermediate conductance (70 pS), and the Ca2+-dependent, large-conductance K channel (5, 31, 37). However, the 30- and 70-pS K channels are mainly responsible for K recycling across the apical membrane, whereas the Ca2+-dependent, large-conductance K channel is involved in the regulation of cell volume (31). Although the molecular nature of the 70-pS K channel is not completely understood, it is most likely that ROMK is a key component of the 70-pS K channel because no 70-pS K channel has been found in the ROMK (-/-) mouse (19). Thus we investigated the effect of IGF-I on the apical 70-pS K channel, which plays an important role in K recycling across the apical membrane, and to delineate the mechanism by which IGF-I regulates the 70-pS K channel.

METHODS

Preparation of medullary TAL. Pathogen-free Sprague-Dawley rats (8 wk old) were obtained from Taconic Farms (Germantown, NY) and kept on a normal rat chow and free access to water. The rats were killed by cervical dislocation, and the kidneys were removed immediately. Several thin coronal sections were cut with a razor blade, and medullary TAL (mTAL) tubules were dissected. The dissection buffer solution contained (in mM) 140 NaCl, 5 KCl, 1.8 MgCl2, 1.8 CaCl2, 5 glucose, and 10 HEPES (pH 7.4 with NaOH). The isolated TAL tubules were placed on a 5 × 5-mm cover glass coated with Cell-Tak (Collaborative Research, Bedford, MA). The cover glass was transferred to a chamber mounted on an inverted microscope (Nikon, Melville, NY), and the tubules were superfused with the bath solution as described above for the dissection. We used a sharpened pipette to open the mTAL to gain access to the apical membranes.

Patch-clamp technique. Patch pipettes were pulled with a Narishige model PP83 vertical pipette puller and had resistances of 4–6 MΩ when filled with 140 mM NaCl. The channel current was amplified by an Axon 200A patch-clamp amplifier and was low-pass filtered at 1 kHz by using an eight-pole Bessel filter (902LPF, Frequency Devices, Haverhill, MA). The current was passed through an Axon interface (Digitada1200) to digitize the signal, collected by an IBM-compatible Pentium computer (Gateway 2000) at a rate of 4 kHz and analyzed by using the pClamp software system 6.04 (Axon Instruments, Burlingame, CA). Channel activity was defined as NPo, a product of channel open probability and channel number, from 0.31 to 1.21. The stimulatory effect of IGF-I on the 70-pS K channel is biphasic because high concentrations (>200 nM) inhibit apical 70-pS K channels. Application of 400 nM IGF-I decreased channel activity from 1.45 to 0.2. The inhibitory effect of IGF-I is not blocked by calphostin C (an inhibitor of PKC), but inhibition of protein tyrosine kinase with herbimycin A abolished the IGF-induced inhibition. We conclude that IGF-I has a dual effect on the apical 70-pS K channel. Moreover, the effect of IGF-I on the apical 70-pS K channel is not completely understood, it is most likely that MAP kinase is responsible for mediating the effect of IGF-I on the apical K channels. Therefore, the goal of the present study is to explore the effect of IGF-I on the apical K channels. There are three types of K channels: renal outer medulla K+ (ROMK)-like (30 pS), the intermediate conductance (70 pS), and the Ca2+-dependent, large-conductance K channel (5, 31, 37). However, the 30- and 70-pS K channels are mainly responsible for K recycling across the apical membrane, whereas the Ca2+-dependent, large-conductance K channel is involved in the regulation of cell volume (31). Although the molecular nature of the 70-pS K channel is not completely understood, it is most likely that ROMK is a key component of the 70-pS K channel because no 70-pS K channel has been found in the ROMK (-/-) mouse (19). Thus we investigated the effect of IGF-I on the apical 70-pS K channel, which plays an important role in K recycling across the apical membrane, and to delineate the mechanism by which IGF-I regulates the 70-pS K channel.

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open probability ($P_o$) and channel number ($N$). The $NP_o$ was calculated from data samples of 60-s duration in the steady state as follows

$$NP_o = \sum (t_1 + 2t_2 + \ldots + it_i)$$

where $t_i$ is the fractional open time spent at each of the observed current levels. Because three types of K channels have been identified in the mTAL, we measured the channel current at three different membrane potentials in each patch to estimate the conductance of the K channel in the patch. From the calculated conductance, the channel type is determined. All experiments were performed in cell-attached patches.

**Solution and statistics.** The pipette solution contained (in mM) 140 KCl, 1.8 MgCl$_2$, and 5 HEPES (pH 7.4). IGF-I, calphostin C, $N^\text{ω}$-nitro-$L$-arginine methyl ester, and phenylarsine oxide (PAO) were obtained from Sigma, whereas PD-98059 and herbimycin A were purchased from Biomol (Plymouth Meeting, PA). PAO and herbimycin A were dissolved in DMSO. The final concentration of DMSO was $<0.1\%$ and had no effect on channel activity. The data are presented as means ± SE. We used paired Student’s $t$-test to determine the statistical significance. If $P$ value is $<0.05$, the difference is considered to be significant.

**RESULTS**

Figure 1 is a typical recording from 18 experiments showing the effect of IGF-I on the apical 70-pS K channel in the TAL. Addition of 25 nM IGF-I significantly stimulates the activity of the 70-pS K channel and increases $NP_o$ from $0.31 \pm 0.06$ to $1.21 \pm 0.2$ ($P < 0.01$). Figure 2 is a dose-response curve of the IGF-I effect, and it yields a $K_D$ (8 nM), a concentration required for stimulation of channel activity to 50% of the maximal value. However, the effect of IGF-I on the 70-pS K channel is biphasic: IGF-I at concentrations $<50$ nM stimulates the apical 70-pS K channel, whereas IGF-I at higher than 200 nM inhibits the channel activity. Figure 3 is a recording demonstrating dual effect of IGF-I on the 70-pS K channel. Addition of 25 nM IGF-I increases the channel activity from $0.35 \pm 0.05$ to $1.45 \pm 0.2$ ($P < 0.01$), and a further increase in IGF-I concentration to 400 nM reduced $NP_o$ from $1.45 \pm 0.2$ to $0.2 \pm 0.05$ ($P < 0.01, n = 6$). The inhibitory effect of IGF-I was reversible because washout restored the channel activity to the control level ($NP_o = 0.5 \pm 0.1$) ($N = 3$).

We have also examined the effect of IGF-I on the 30-pS K channel in the TAL. IGF-I in the range from 5 to 200 nM failed to increase the activity of the 30-pS K channel (control, $P_o = 0.9 \pm 0.1$; IGF, $P_o = 0.9 \pm 0.1$; $n = 10$). Because the dual effect can be observed in the same cell, it excludes the possibility that the biphasic effect of IGF-I is the result of patching two types of cells. It has been reported that there are two types of cells, a smooth and a rough surface, in the TAL (32). Moreover, immunocytochemical staining has shown the presence of ROMK-positive and ROMK-negative cells (41). Because it is most likely that both the 70- and the 30-pS K channels contain the ROMK, it is certain that only ROMK-positive cells were patched in our study (19). In addition, we have carried out experiments to examine whether the channel activity could be detected in smooth-surface or rough-surface
We observed the channel activity from 8 of 20 cells in the TAL. Also, six cells have a clear, smooth surface, whereas the other two cells were hard to determine the cell type. Because the channel activity is observed mainly in these smooth-surface cells, it is most likely that the study was performed in ROMK-positive cells with smooth surface.

After demonstrating that IGF-I has a dual effect on the 70-pS K channel, we explored the mechanism by which IGF-I stimulates the 70-pS K channel. Stimulation of IGF-I receptor has been shown to activate protein tyrosine phosphatase (PTP) by enhancing tyrosine phosphorylation (2, 17, 23) or to increase nitric oxide (NO) release (38, 42). Our previous experiments have shown that an increase in NO release or activation of PTP stimulates the channel activity (12, 20). Therefore, we examined the effect of IGF-I on the 70-pS K channel in the presence of PAO, an inhibitor of PTP. Although the channel activity in the presence of PAO (1 μM) is lower than the control value (without PAO), inhibition of PTP did not abolish the stimulatory effect of IGF-I (Fig. 4). From inspection of Fig. 4, it is apparent that addition of 25 nM IGF-I increased \( N_P \) from 0.18 ± 0.04 to 1.27 ± 0.2 (\( P < 0.001, n = 6 \)). To determine whether the stimulatory effect of IGF-I is mediated by a NO-dependent pathway, the effect of IGF-I on the 70-pS K channel has been examined in the presence of 0.5 mM \( \text{N}^\text{\textregistered}\text{G}-\text{nitro-L-arginine methyl ester}, \) an inhibitor of nitric oxide synthase (NOS). Figure 5 is a typical recording showing the effect of 25 nM IGF-I on channel activity. We confirmed the previous finding that inhibition of NOS decreased the channel activity (20). However, inhibition of NOS also did not block the stimulatory effect of IGF-I because IGF-I significantly increased \( N_P \) from 0.11 ± 0.02 to 0.8 ± 0.2 (\( P < 0.05, n = 4 \)).

Stimulation of IGF-I receptor has been demonstrated to activate mitogen-activated synthase (MAP) kinase. Several studies have also demonstrated that MAP kinase is involved in the stimulation of K-channel activity. Therefore, we examined the effect of IGF-I on the 70-pS K-channel activity in the presence of PD-98059 or U0126, inhibitors of ERK-dependent MAP kinase (1, 10). Addition of PD-98059 (50 μM) or U0126 (1 μM) alone had no significant effect on the 70-pS K channel. This suggests that MAP kinase is not involved in the regulation of channel activity under control conditions. However, PD-98059 or U0126 completely abolished the effect of 25 nM IGF-I on the 70-pS K channel. Data summarized in Fig. 6 show that IGF-I did not significantly increased \( N_P \) (control value, 0.35 ± 0.1; IGF, 0.52 ± 0.15) (\( n = 10 \)).

After establishing the role of MAP kinase in mediating the stimulatory effect of IGF-I on the 70-pS K channel, we extended the study to exploring the mechanism by which IGF-I inhibits the channel activity. Because IGF-I at high concentrations inhibits the channel activity, we selected the patches with...
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NP

a typical recording demonstrating that 400 nM IGF-I reduced NP from 0.6 ± 0.15 to 0.15 ± 0.02 (P < 0.05, n = 4). In contrast, inhibition of PTK with herbimycin A not only increases channel activity as reported previously (12) but also completely abolished the effect of IGF because IGF-I did not alter NP (control, 1.2 ± 0.2; IGF-I, 1.3 ± 0.2; n = 5) (data not shown).

The role of PTK in mediating the effect of IGF-I at high concentrations is further supported by experiments in which inhibition of PTK can restore the channel activity. Figure 8 is a typical recording demonstrating that 400 nM IGF-I reduced NP from 1.27 ± 0.2 to 0.2 ± 0.05 (P < 0.01, n = 5), and application of herbimycin A (1 µM) restored the channel activity (NP = 0.9 ± 0.2). Moreover, in the presence of herbimycin A, the dose-response curve of IGF-I effect shows only the stimulatory effect (data not shown).

DISCUSSION

The IGF-I system is composed of IGF-I, IGF-I receptor, and IGF-binding proteins (16). IGF-I and IGF-I receptor have been shown to be expressed in different nephron segments, including the TAL (7). However, the role of IGF-I in the regulation of renal function is not completely understood. IGF-I has been shown to increase blood flow and glomerular filtration rate (13, 14). In the present study, we have demonstrated that IGF-I also plays an important role in the regulation of the apical 70-pS K channel but does not affect the activity of the 30-pS K channel in the TAL.

Two types of K channels, 30 and 70 pS, have been identified to be the main contributors to the apical K conductance under physiological conditions (5, 36). Moreover, it is speculated from a rough estimation that the apical 70-pS K channel could contribute as much as 70% of the apical K conductance in the TAL from rats on a high-K diet (21). Although the molecular nature of the 70-pS K channel in the TAL is not clear, it is most likely that Kir1 (ROMK) is also an essential component of the 70-pS K channel because K-channel activity is absent in the apical membrane of the TAL in the ROMK-knockout mice (19). The apical K channel plays an important role in K recycling, which is essential for the function of the Na-K-Cl cotransporter. Na and K enter the cell via the Na-K-Cl cotransporter, and Na leaves the cell via a basolateral Na-K-ATPase. Because K concentration in the luminal fluid of the TAL is one order lower than that of Na and Cl, K must be recycled through the apical K channel. The importance of K recycling in maintaining NaCl absorption is best demonstrated in ROMK knockout mice in which Na transport in the loop of Henle is severely impaired (19). Because IGF-I has an effect on the 70-pS K channel in the TAL, we speculate that IGF-I should have an important effect on Na transport in the TAL.

The effect of IGF-I on the 70-pS K channel is biphasic: low concentrations of IGF-I (<100 nM) stimulate, whereas high concentrations (>200 nM) inhibit the 70-pS K channel. Relevant to this observation is the report that, in blastocyes, low concentrations of IGF-I or insulin stimulate glucose uptake, whereas high concentrations decrease glucose uptake (6). It has been proposed that high concentrations of IGF-I-induced decrease in glucose uptake are the result of a downregulation of IGF-I receptors. However, this mechanism is not supported by the observation that inhibition of PTK can abolish the IGF-induced inhibition of channel activity. Thus it is possible that high concentrations of IGF-I may activate additional signal transduction pathways, such as the PTK-dependent pathway. For instance, our laboratory (21) has shown previously that low

Fig. 7. Channel recording showing the effect of IGF-I (400 nM) on the 70-pS K channel in the presence of calphostin C (100 nM). The holding potential was 0 mV, and the channel closed level is indicated by “C.” Three parts of the recording indicated by nos. in the top trace are extended to show the fast time resolution. The experiment was conducted in a cell-attached patch.

Fig. 8. Channel recording showing the effect of IGF-I (400 nM) on the 70-pS K channel in the presence of herbimycin A (1 µM). The holding potential was 0 mV, and the channel closed level is indicated by “C.” Three parts of the recording indicated by nos. in the top trace are extended to show the fast time resolution. The experiment was performed in a cell-attached patch.
concentrations of angiotensin II inhibit the apical K channel in the TAL by stimulation of 20-hydroxyeicosatetraenoic acid formation, whereas high concentrations of angiotensin II lead to activation of the NO-dependent pathway and stimulation of channel activity. Also, the effect of angiotensin II on the Na/H exchanger in the rat proximal tubule has been found to be concentration dependent (18, 34, 35). Although herbimycin A is a specific PTK inhibitor (27), it is possible that herbimycin A may have an effect other than inhibition of PTK. However, the observation that the effect of herbimycin A was enhanced in K-restricted rats that have a high-PTK expression (12) suggests that the effect of herbimycin A is the result of inhibition of PTK.

IGF-I has been demonstrated to play an important role in the regulation of cell proliferation (16). In addition, IGF-I has also been suggested to regulate the function of ion transporters in a variety of tissues. IGF-I has been shown to stimulate the KCl cotransporter in skeletal muscle (40) and to increase transepithelial Na transport in the urinary bladder (3, 4). In addition, IGF-induced proliferation of HEK cells is related to activation of voltage-gated K channels (11). IGF-I has also been reported to stimulate gibberellic acid-sensitive K channels in follicle-enclosed Xenopus oocytes (26). Although IGF-I has been shown to regulate the function of a variety of ion transporters, the mechanism by which IGF-I regulates the ion carriers and channels is different. The effect of IGF-I on voltage-gated K channels is mediated by stimulation of phosphatidylinositol 3-kinase (11). IGF-I has been reported to decrease K-channel activity by a p38 MAP kinase-dependent pathway in rat brain stem neurons (24). The present observation, that inhibition of ERK-dependent MAP kinase abolished the stimulatory effect of IGF-I, strongly suggests that IGF-induced activation of the 70-pS K channel is mediated by ERK/MAP kinase. ERK/MAP kinase is a family of serine/threonine protein kinases (25) and has been shown to play an important role in cell proliferation, cell differentiation, and cell death. There are two possibilities by which MAP kinase regulates the 70-pS K-channel activity. MAP kinase may directly regulate the channel activity by phosphorylation of the channel proteins or associate protein. Alternatively, MAP kinase can modulate the activity of other signaling molecules, which, in turn, regulate the channel activity. Relevant to this possibility is the report that IGF-I enhances glucose transport in retinal endothelial cells by MAP kinase and PKC and that MAP kinase is the upstream of the PKC-dependent pathway (8). Further experiments are needed to delineate the mechanism by which MAP kinase regulates the 70-pS K channel. The role of ERK/MAP kinase in mediating the effect of IGF-I on the 70-pS K channel is established by experiments in which application of PD-98059 and U0126 abolished the effect of IGF-I. Although PD-98059 and U0126 are highly selective ERK1/2/MAP kinase inhibitors and have no significant effect on p38 MAP kinase and c-Jun NH2-terminal kinase. However, we cannot exclude the possibility that MAP kinase other than ERK1/2 is also involved in mediating the stimulatory effect of IGF-I on the 70-pS K channel.

Whereas the stimulatory effect of IGF-I is mediated by MAP kinase, three lines of evidence indicate that the inhibitory effect of IGF-I on the 70-pS K channel is mediated by stimulation of PTK but not by PKC. First, inhibition of PTK abolished the IGF-induced inhibition of the 70-pS K channel. Second, IGF can still inhibit the channel activity in the presence of calphos- tin C. Finally, herbimycin A treatment reversed the IGF-induced inhibition.

The physiological importance of biphasic effects of IGF-I on the 70-pS K channel in the TAL is not clear. It is possible that IGF-I stimulates under control conditions, whereas it inhibits Na absorption when IGF-I concentration is elevated. It has been reported that the plasma concentrations of IGF-I are between 340 and 400 ng/ml or 45 and 50 nM under physiological conditions (9). However, it has been shown that IGF-I peptide concentration increased more than twofold in the K-restricted rats by a mechanism involving augmentation of IGF-binding proteins in the kidney (15). Thus it is possible that IGF concentration may increase to the extent such that IGF-I may suppress the transport function in the TAL during hypokalemia in which Na and Cl transport in the loop of Henle were impaired (22, 30, 33). The second role of IGF-I may be involved in activation of PTK activity induced by low-K intake (39). We have previously shown that low-K intake increases activity of Src family PTK. Because high concentrations of IGF-I stimulate PTK activity, it is possible that an increase in the IGF-I signaling pathway may be the upstream of the c-Src-dependent signal transduction pathway. Further experiments are required to explore this possibility.

In conclusion, IGF-I at low concentrations stimulates, whereas at high concentrations it inhibits, the apical 70-pS K channel in the TAL. The stimulatory effect of IGF-I is mediated by MAP kinase, whereas the inhibitory effect is due to stimulation of PTK.

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