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Cyclic GMP-activated channel activity in renal epithelial cells (A6)

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We studied the effects of guanosine 3′, 5′-cyclic monophosphate (cGMP) and nitroprusside on ion channels in the apical membrane of confluent A6 cells (a distal nephron cell line) cultured on permeable supports for 10–14 days using patch clamp techniques. In cell-attached patches without any detectable channel activity, activity of a non-selective cation channel with a single-channel conductance of 1 pS was observed after adding nitroprusside. After adding cGMP to the cytosolic surface of inside-out patches with no detectable channel activity, we observed single channel activity similar to the channel observed after adding nitroprusside. These observations imply that nitroprusside activates a non-selective cation channel with small single channel conductance (1 pS) via an increase in cGMP which activates the channel.

Introduction

Guanosine 3′,5′-cyclic monophosphate (cGMP) affects ion channels in various ways in different tissues. Haynes et al. [1] and Zimmerman and Baylor [2] have reported that in retinal rods, cGMP activates cation channels with single-channel conductances of 8 and 25 pS, respectively. In contrast, Light et al. [3] have reported that cGMP reduces the open probability of an amiloride-blockable cation channel in renal epithelial cells. We had previously observed a strong stimulation of sodium and chloride channels by cyclic AMP in a distal nephron cell line (A6) [4,5]. Since cAMP and cGMP often have antagonistic effects on a system, we thought that cGMP might also modulate channel activity in these same cells. In the present study, we investigated the effects of sodium nitroprusside, an agent that increases intracellular levels of cGMP, and the effects of cGMP, itself, on single channels in A6 cells using patch clamp techniques. Both sodium nitroprusside and cGMP activated 1 pS non-selective cation channels in the apical membrane of A6 cells.

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Materials and Methods

Renal distal nephron cells (A6 cells) used in the present study were cultured on permeable supports (either collagen-coated CM filters from Millipore or polycarbonate filters from Nucleopore) for 10–14 days with 10% fetal calf serum and 1.5 μM aldosterone [6]. Isolated membrane patches were formed on the apical surface of cells from a confluent monolayer. Single channel currents from cell-attached and inside-out patches were recorded with a List EPC-7 patch clamp amplifier (Guilford, CT, U.S.A.). Only currents from patches that had 150–400 gigaohm seals were used for the present study. Single-channel currents shown in the present study were low-pass filtered at 100 Hz. The channel activity is represented as NP_o (N, the number of channels in a patch; P_o, the open probability of an individual channel).

$$NP_o = \sum_{i=1}^{N} i \cdot P_i \qquad (1)$$

where i is the number of channels simultaneously open and P_i is the probability of i channels being simultaneously open.

Transepithelial electrical parameters were measured from confluent cell monolayers cultured under conditions similar to those used for single-channel recordings. Transepithelial electrical measurements were ob-

tained using an Ussing chamber and a voltage clamp device (designed by Dr. S.I. Helman). The short-circuit current was defined as the current to clamp the transepithelial potential to zero. The transepithelial conductance was calculated from the transepithelial current response to a small step change in the transepithelial potential. The baseline transepithelial potential was calculated as the continuous short-circuit current divided by the transepithelial conductance. The cell monolayer was continuously 'short-circuited' except for the short duration pulses to assess transepithelial conductance and potential.

A freshly prepared, aqueous solution of sodium nitroprusside (Sigma Chemical Co.) was added to the apical bath to obtain a final concentration of 30 μM in both patch clamp and Ussing chamber experiments.

Results

Sodium nitroprusside is known to increase intracellular levels of cGMP in many preparations [7]. To study the effect of cGMP on ion channels, we compared single-channel currents in cell-attached patches before and after adding sodium nitroprusside to the apical surface of the cell outside the patch (Figs. 1 and 2). Application of sodium nitroprusside (30 μM) in the apical bath activated channels in 4 out of 5 patches. Fig. 1 shows a typical time course of nitroprusside action on channel activity in a cell-attached patch. Fig. 1 indicates that before addition and for about 10 min after addition of nitroprusside, no channel activity was observed; but about 10 min after initial addition of

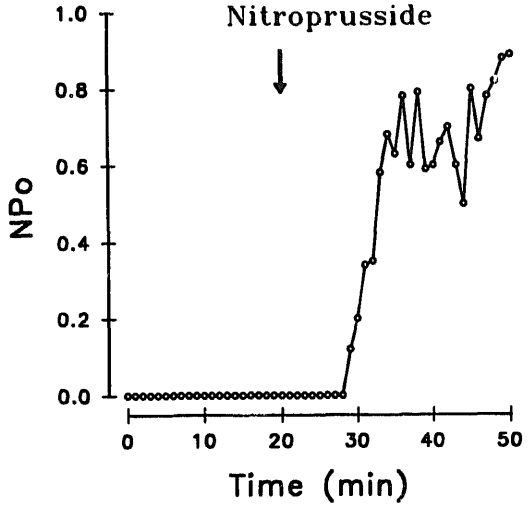

Fig. 1. The time course of nitroprusside action on NP_o. Before and for a 10-min period after adding sodium nitroprusside (30 μM) to the apical bath, no detectable channel activity was observed; i.e., NP_o was zero. About ten minutes after addition of sodium nitroprusside, channel activity appeared. Sodium nitroprusside activated channels in four out of five cell-attached patches. Both the pipette and bathing solutions had 120 mM NaCl, 3.4 mM KCl, 0.8 mM $CaCl_2$ and 0.8 mM $MgCl_2$, and the pH was adjusted to 7.4 with Hepes.

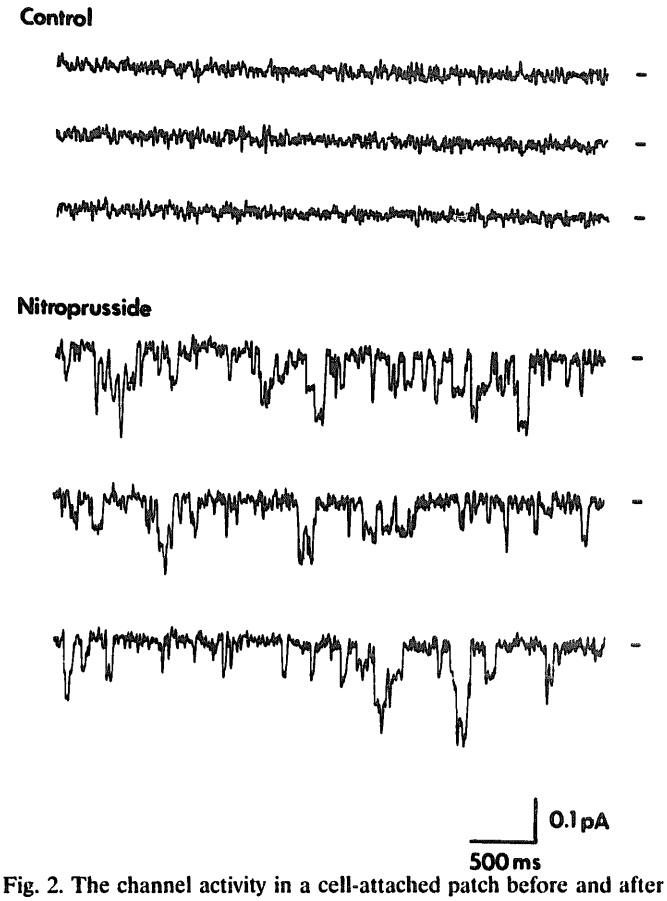

Fig. 2. The channel activity in a cell-attached patch before and after adding sodium nitroprusside (30 μM) to the luminal solution outside of the patch pipette with no potential applied to the patch ($V_p = 0$ mV, downward deflections are current from the patch pipette to cell interior). The upper three traces were recorded from a cell-attached patch before adding nitroprusside. The lower three traces were recorded from the same cell-attached patch about fifteen minutes after adding nitroprusside. Both the pipette and bathing solutions had 120 mM NaCl, 3.4 mM KCl, 0.8 mM $CaCl_2$ and 0.8 mM $MgCl_2$, and the pH was adjusted to 7.4 with Hepes. The horizontal bar beside each current trace shows the zero-current level.

nitroprusside, channel activity increased. Fig. 2 shows single-channel current traces from the same patch shown in Fig. 1 before and after nitroprusside addition. For 20 min prior to adding sodium nitroprusside (upper three traces in Fig. 2), this patch had no detectable channel activity for a range of applied potentials from -100 mV to 100 mV. As mentioned above, after adding sodium nitroprusside, there was no immediate increase in channel activity. However, about 10 min after adding sodium nitroprusside, channel activity appeared in the patch (lower three traces in Fig. 2). In four patches which responded to nitroprusside, the time between application and response varied from a low of 10 to a high of 15 min. After treatment, the patch shown in Fig. 2 contained at least three channels with comparable, but very small, single-channel currents when no potential was applied (about 0.07 pA). NP_o was about 0.6. If there are actually three channels in this patch, then the mean open probability of each

channel is 0.2. The single-channel current-voltage relationship shows that the sigle-channel conductance of the nitroprusside-activated channel is about 1 pS in cell-attached patches and the reversal potential is about 40 mV more depolarized than the apical membrane potential (Fig. 3A).

To determine the ion selectivity of the nitroprusside-activated channel, we made inside-out patches after channel activity appeared. Fig. 3B shows the current-voltage relationship with various cytosolic ionic conditions. When the patch pipette solution contained 120 mM NaCl and the cytosolic solution contained 20 mM Na, 100 mM *N*-methyl-D-glucamine and 120 mM Cl, we observed some inward current with no potential applied to the patch membrane (i.e., membrane potential = 0 mV). This suggests that the nitroprusside-activated channel is permeable to Na. When the cytosolic solution contains 120 mM KCl and the pipette solution contains 120 mM NaCl, the reversal potential is close to 0 mV. This suggests that either the nitroprusside-activated channel has similar permeabilities to Na and K or is highly permeable to chloride. However, when cytosolic chloride was reduced to 20 mM by the replacement with gluconate, the current-voltage relationship was unchanged from the relationship in symmetric 120 mM NaCl solutions. This implies that the nitroprusside-activated channel has very low permeability to Cl compared to Na or K. In other words, the nitroprusside-activated channel is a non-selective cation channel. In addition, some patches with channel activity were excised to form inside out patches in solutions with varying Ca^{2+} concentrations; however, the activity of the nitroprusside-induced channel in the present study did not change even if the cytosolic Ca^{2+} concentration was varied from 10^{-7} M to 10^{-3} M (data not shown).

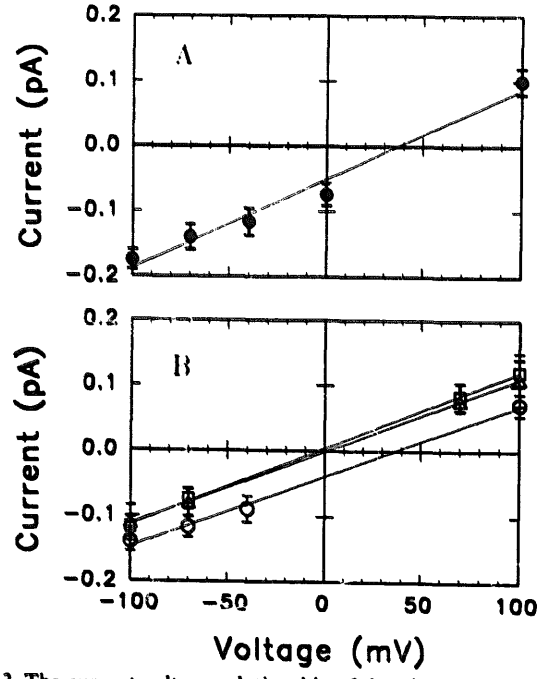

Fig. 3. The current-voltage relationship of the nitroprusside-activated channel. The upper panel (A) shows the current-voltage relationship of the channel in a cell-attached patch. The lower panel (B) shows the current-voltage relationship of the channel in an inside-out patch which was made from the cell-attached patch shown in Fig. 2A. The pipette solution contained 120 mM NaCl. The cytosolic solution contained 20 mM Na, 100 mM *N*-methyl-D-glucamine and 120 mM Cl (circles), 120 mM KCl (squares) or 120 mM Na, 20 mM Cl and 100 mM gluconate (triangles). The pH of the solution was adjusted to 7.4 with Hepes. Symbols and vertical bars, respectively, show the mean values ± S.D. of three experiments.

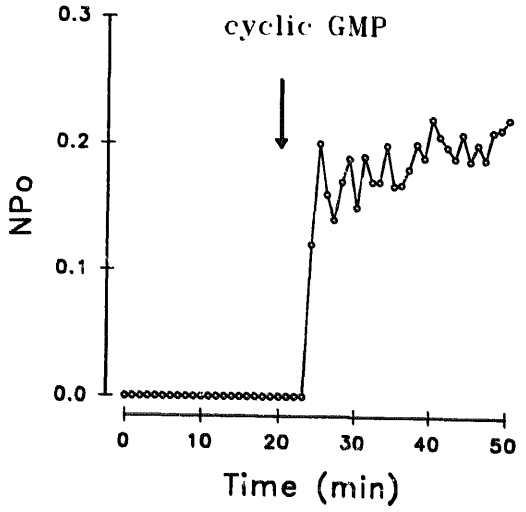

Fig. 4. The time course of cGMP action on NP_o. Before and 4 min after adding cGMP (0.1 mM), no detectable channel activity was observed; i.e., NP_o was zero. About 4 min after adding cGMP (0.1 mM), some channel activity appeared. Cyclic GMP (0.1 mM) activated channels in all three inside-out patches tested in the present study.

We also examined the effects of membrane-permeable analogues of cGMP (dibutyryl-cGMP and 8-bromo-cGMP) on ion channels in cell-attached patches using the same protocol as was used for sodium nitroprusside. However, we only detected channels similar to nitroprusside-activated channels in 2 out of 9 patches exposed to dibutyryl-cGMP (1 mM). 8-Bromo-cGMP (1 mM) was ineffective in inducing 1 pS non-selective cation channel activity.

The results with the membrane permeable analogues raised the possibility that sodium nitroprusside might not be acting via an increase in intracellular levels of cGMP. To clarify the effect of cGMP, we applied cGMP (0.1 mM) directly to the cytosolic surface of excised patches that, prior to application of cGMP, had no detectable channel activity for a 20-min period after making the inside-out patch (Fig. 4). After adding cGMP, there was no imr diate increase in channel activity. However, about min after adding cGMP, channel activity appeared in the patch (Fig. 4). Fig 5 shows the single-channel currents before and afte r adding cGMP from the same patch as that shown in Fig. 4. The single-channel current before adding

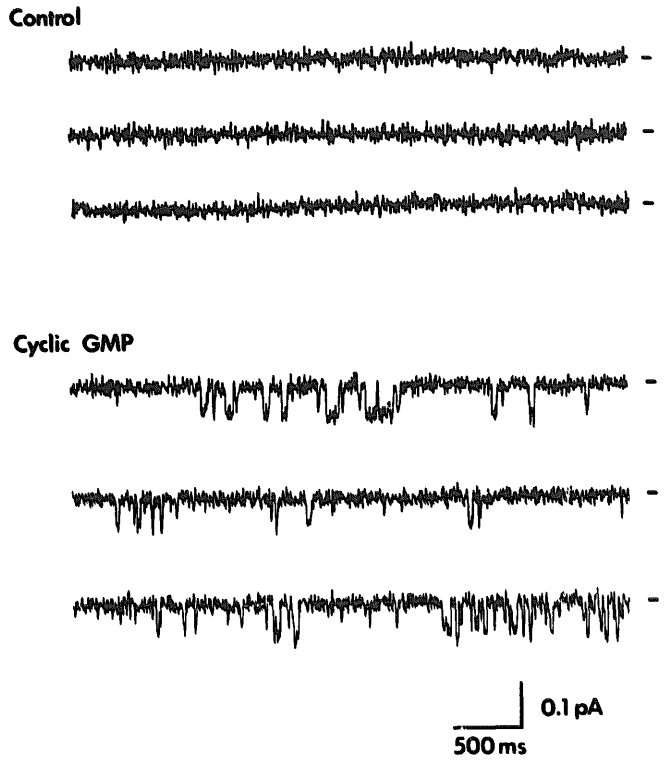

Fig. 5. The channel activity in an inside-out patch before and after adding cGMP (0.1 mM) to the cytosolic surface with no potential applied to the patch ($V_p = 0$ mV, downward deflections are current from the patch pipette to the cell interior). The upper three traces were recorded from an inside-out patch before adding cGMP. The lower three traces were recorded from the same insideout patch about seven minutes after adding cGMP. The pipette solution had 120 mM NaCl, 0.8 mM $CaCl_2$ and 0.8 mM $MgCl_2$, while the cytosolic (bathing) solution had 10 mM NaCl, 110 mM *N*-methyl-D-glucamine, 0.8 mM $CaCl_2$ and 0.8 mM $MgCl_2$. The pH of the both solutions was adjusted to 7.4 with Hepes. The horizontal bar beside each current trace shows the zero current level.

cGMP is shown in the upper three traces in Fig. 5. Current traces of the channel activity in the patch 4 min after adding cGMP are shown in the lower three traces in Fig. 5. This result shows that cGMP, like sodium nitroprusside, activates channel activity. The single-channel conductance of the cGMP-activated channel was about 1 pS and the channel had an ion selectivity that was similar to the nitroprusside-activated channel. In all three inside-out patches tested, we observed that, 4–6 min after adding cGMP, channel activity similar to that shown in Fig. 5 appeared in the patches.

To investigate the physiological significance of the nitroprusside-activated channel, we applied sodium nitroprusside to monolayers of A6 cells placed in an Ussing chamber and measured changes in short-circuit current and transepithelial conductance.

Based upon the single-channel properties and the relative frequency of highly-selective sodium and nitroprusside-induced channels within patches, any nitroprusside-induced change in transepithelial current is likely to be only 10–20% of the maximal sodium transport observed in cells exposed to high concentrations of aldosterone (> 500 nM). Indeed, application of sodium nitroprusside (30 μM) to monolayers of A6 cells grown in the presence of 10% serum and 1.5 μM aldosterone produced no detectable change in the short-circuit current or transepithelial conductance (data not shown). However, for monolayers in which the baseline levels of sodium transport have been reduced by removing serum and aldosterone for 3 days, nitroprusside application significantly increased the short-circuit current in 3 out of 8 experiments. In monolayers which responded to nitroprusside, the response began shortly after the addition of nitroprusside and stabilized after a variable time (from 20 min in one cell to about an hour in the other cells). Fig. 6A shows the magnitude of the conductance response to application of nitroprusside in the monolayers and suggests that monolayers with low basal transepithelial conductance respond to nitroprusside more strongly than monolayers with higher basal transepithelial conductance. There is a similar relationship between basal transepithelial current and the transepithelial current response to nitroprusside (Fig. 6B).

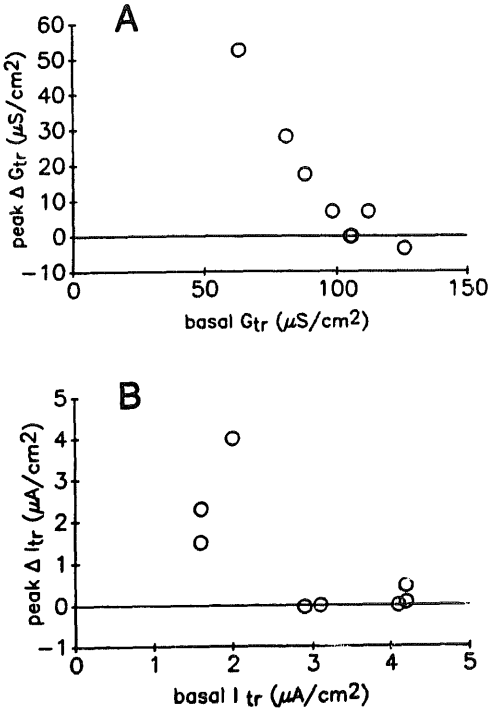

Fig. 6. (A) Peak transepithelial conductance change in response to application of nitroprusside (30 μM) as a function of basal transepithelial conductance. (B) Peak transepithelial current change to nitroprusside as a function of basal transepithelial current. In experiments with a response to nitroprusside, the response begins within 15 min, thus for experiments with no response within 20 min, the experiments were terminated.

Discussion

In retinal rods, cGMP induces cationic currents by increasing ionic conductance [8,9]. In addition, Haynes et al. [1] have reported that cGMP activates cation channels in retinal rods with a single-channel conductance about 25 pS. Zimmerman and Baylor [2] also report that cGMP activates or induces two types of channels with unit conductances of 8 and 24 pS. We also report that cGMP activates channel activity, but there are some obvious differences between the characteristics of the cGMP-activated channels in A6 cells and those in photoreceptors. The most obvious difference is the small single channel conductance of the channel in A6 cells (1 pS) compared to the channels in retinal rods (8 and 24–25 pS). An additional difference is the sensitivity to divalent cations. Cyclic-GMP-activated channels in retinal rods are blocked by Ca or Mg [1,2]. However, the activity of the cGMP-activated channel in patches excised from A6 cells did not change even if the cytosolic Ca^{2+} concentration was varied from 10^{-7} M to 10^{-3} M.

Stanton and co-workers [3,10] report the effect of cGMP on single channels in renal epithelial cells; i.e., cGMP inhibits an amiloride-blockable cation channel with a unit conductance of 28 pS. This result is clearly different from our result, but given the large difference in unit conductance of the channels examined in the two studies, it seems likely that we are dealing with different channel proteins.

Although cGMP consistently induced channel activity very similar to that induced by nitroprusside, membrane permeable analogues of cGMP did not consistently induce channel activity. This may mean that these compounds are not as permeable as expected through the apical membrane or that the phosphodiesterase activity of A6 cells is quite high.

The possible physiological role of the 1 pS cation channel reported here is interesting. Application of sodium nitroprusside to monolayers of A6 cells with high basal sodium transport rates produced little change in the short-circuit current or total tissue conductance, but in monolayers with low basal sodium transport there was a significant nitroprusside-induced current and conductance (up to a 3-fold increase in basal current). This difference could not simply be due to an inability to detect the relatively small nitroprusside currents in the face of large transepithelial sodium currents, since even though all nitroprusside responses are relatively small, the responses in high basal current monolayers would still be large enough to be easily detectable under any transport conditions. Therefore, it seems that there may be a reciprocal relationship (implied by Fig. 6) between the magnitude of the nitroprusside-inducible non-selective current and aldosterone-inducible sodium transport. Thus, the nitroprusside-activated channel represents a separately regulated apical permeability which could make a significant contribution to total apical permeability (and sodium reabsorption) under exactly those conditions when the aldosterone-inducible sodium channel would be expected to make little or no contribution.

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