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Reconstitution of Hepatic Uricase in Planar Lipid Bilayer Reveals a Functional Organic Anion Channel

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Abstract. Rat renal proximal tubule cell membranes have been reported to contain uricase-like proteins that function as electrogenic urate transporters. Although uricase, *per se,* has only been detected within peroxisomes in rat liver (where it functions as an oxidative enzyme) this protein has been shown to function as a urate transport protein when inserted into liposomes. Since both the uricase-like renal protein and hepatic uricase can transport urate, reconstitution studies were performed to further characterize the mechanism by which uricase may function as a transport protein. Ion channel activity was evaluated in planar lipid bilayers before and after fusion of uricase-containing proteoliposomes. In the presence of symmetrical solutions of urate and KCl, but absence of uricase, no current was generated when the voltage was ramped between ±100 mV. Following fusion of uricase with the bilayer, single channel activity was evident: the reconstituted channel rectified with a mean slope conductance of 8 pS, displayed voltage sensitivity, and demonstrated a marked selectivity for urate relative to K^+ and Cl^-. The channel was more selective to oxonate, an inhibitor of both enzymatic uricase activity and urate transport, than urate and it was equally selective to urate and pyrazinoate, an inhibitor of urate transport. With time, pyrazinoate blocked both its own movement and the movement of urate through the channel. Channel activity was also blocked by the IgG fraction of a polyclonal antibody to affinity purified pig liver uricase. These studies demonstrate that a highly selective, voltage dependent organic anion channel is formed when a purified preparation of uricase is reconstituted in lipid bilayers.

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Key words: Bilayers — Channels — Organic anions — Reconstitution — Urate — Uricase

Introduction

Urate transport across brush-border and basolateral membranes of proximal tubules of rat kidney is consequent, in part, to a membrane protein which has a number of features similar to those of the hepatic peroxisomal enzyme uricase [1, 2]: the renal transporter and the hepatic enzyme have very similar affinities for urate, oxidize urate to allantoin, and are Cu^{++}-dependent proteins. Additionally, oxonate, a specific inhibitor of the enzymatic activity of uricase [11], inhibits urate transport in renal cortical membrane vesicles [1, 2, 23]. Although uricase, *per se,* has never been detected in rat kidney [9, 18], urate-binding proteins which have some homology to hepatic uricase have recently been affinity purified from renal cortical plasma membranes [24]. Uricase-like enzymatic activity of the purified renal membrane protein provided evidence of functional homology and immunoreactivity of the isolated renal protein to a polyclonal antibody made to enzymatically active, affinity-purified porcine liver uricase provided evidence of immunologic homology [24]. Importantly, this antibody to uricase has been documented to be a specific and potent inhibitor of urate transport in rat renal cortical membrane vesicles and, when used to localize urate-binding protein in intact rat renal cortex, selectively immunolabeled the proximal tubule [24], the nephron site of urate transport [reviewed in 3]. Finally, whereas uricase serves only as an oxidative enzyme when it resides within the core of hepatic peroxisomes [9, 18, 26], porcine liver uricase has been shown to be capable of functioning as a saturable, oxonate inhibitable, urate transporter when incorporated in the lipid bilayer of proteoliposomes [33].

The uricase-like urate transporter represents one of the two modalities of urate transport that have been reported in renal proximal tubules [1, 2, 6, 13, 20–23]. In contrast to the electroneutral urate/anion exchanger [6, 13, 20–22], the uricase-like transporter is a voltage sensitive urate uniporter [1, 2, 23]. Since urate that is transported via the uniporter carries a net negative charge [1, 2, 23], and authentic uricase can transport urate [33], electrophysiologic experiments were conducted using lipid bilayers to determine if uricase can function as an ion channel. These studies demonstrate that a highly purified preparation of uricase can function as a voltage sensitive, 8 pS anion channel when reconstituted in lipid bilayers. The channel is highly selective to the organic anions urate, oxonate, and pyrazinoate, but is minimally, if at all, permeable to chloride or potassium. Oxonate, pyrazinoate and a polyclonal antibody to hepatic uricase each reduced urate movement through the channel. Oxonate limited urate movement because the channel is more permeable to oxonate and therefore successfully competed with urate for movement through the channel, whereas pyrazinoate and the polyclonal antibody reduced urate movement by blocking ion channel activity.

Materials and Methods

Preparation of Uricase-Containing Proteoliposomes

A mixture of bovine brain phosphatidylcholine (10 mg/ml), phosphatidylethanolamine (10 mg/ml), phosphatidylserine (10 mg/ml), and cholesterol (10 mg/ml), (Avanti Polar Lipids, Birmingham, AL) in a ratio of 5:1:1:0.1 (wt/wt) was evaporated to dryness under nitrogen. The resultant 500 μg phospholipid pellet was suspended in 50 μl of a solution containing 220 mM KCl, 10 mM HEPES-KOH at pH 7.4. Following the addition of 25 μg of purified porcine uricase (Sigma Chemical, St Louis, MO) or the commercial purified porcine uricase that was repurified by affinity chromatography [24], proteoliposomes were formed by sonicating the mixture for 5 min at 80 kHz in a bath sonicator (Laboratory Supplies, Hicksville, NY) [33]. Fresh proteoliposomes were prepared for each experiment.

Polyclonal Antibody Production to Porcine Liver Uricase

Commercial pig liver uricase (Sigma Chemical) that was affinity purified [24] and shown to have enzymatic activity was used to generate polyclonal antibodies in rabbits (Pocono Rabbit Farm & Laboratories, Canadensis, PA). The IgG fraction of the polyclonal antibody to porcine uricase was obtained using a protein A antibody purification kit (Repligen, Cambridge, MA) and subsequently concentrated to 10 mg/ml with Centricon 30 concentrators (Amicon, Beverly, MA) [24].

Lipid Bilayer Chamber

A Teflon film (Type C-20, 12.5 μm thick, gift from Dupont Electronics, Wilmington, DE) in which a 50 μm hole was formed by an electrical arc was tightly fitted with silicone grease between two cups that were carved into each half of a plexiglass chamber. The faces of the Teflon film were exposed to the solutions in each cup via overlapping 5 mm i.d. holes on the internal aspects of each cup. One cup had a glass window on its exterior aspect which allowed visualization of the Teflon with a horizontally directed dissecting microscope. In addition, both cups were equipped with ports which permitted solution changes via a push-pull syringe system. Magnets, fixed to voltage regulated electric motors beneath each cup, permitted rapid mixing of the solutions via rotation of stirring bars. Solutions in each cup (1 ml) were connected to Ag-AgCl electrodes via salt bridges containing 3 M KCl in 2% agar. The electrodes were connected to a patch clamp amplifier (Model PC-501, Warner Instrument) through a head stage with a 10 gigohm feedback resistor and frequency bandwidth of 10 kHz (Model PN5101, Warner Instrument, Hamden, CT). The plexiglass chamber and electrodes were electrically shielded within an aluminum box with a removable lid. The entire assembly was mounted on an air-suspended optical bench (Micro-G, Technical Manufacturing, Peabody, MA). The cis side was defined as the chamber connected to the voltage-holding electrode and all voltages were referenced to the trans (ground) side.

Formation of Lipid Bilayer and Channel Reconstitution

The cups of the plexiglass chamber were each filled with 1 ml of a solution containing (in mM): 2.5 urate, 220 KCl, 10 HEPES-KOH, and 0.25 $CaCl_2$ at pH 7.4. A 1:1 (wt/wt) mixture of bovine brain phosphatidylethanolamine (10 mg/ml) and phosphatidylserine (10 mg/ml) (Avanti Polar Lipids) was dried with a nitrogen stream, dissolved in decane (Sigma Chemical) to 50 mg lipid/ml and then painted with a club-shaped glass rod onto the 50 μm diameter hole in the Teflon film. Subsequent to the formation of the bilayer, its capacitance was compensated for by minimizing the overshoot of the current produced by a 100 Hz-voltage pulse applied across the bilayer. Offset potentials due to all junction potentials were corrected using the zero adjust system of the patch clamp amplifier. Thereafter, the voltage was clamped at varying levels (−100 to +100 mV): voltage was generated and controlled by the patch clamp amplifier. If a stable resistance of at least 100 gigohms and a noise of less than 0.2 pA were maintained, the experiments were initiated by adding 5 μl of proteoliposomes to the trans chamber. The solution was vigorously stirred until fusion occurred. Fusion usually occurred within 5–10 min as detected by the presence of channel gating (clear transitions between the closed and open states).

Once fusion occurred, the stirrers were stopped and the solution in the trans chamber was replaced with the same volume of a uricase-free solution (in mM): 2.5 urate, 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4 to limit further channel incorporation. In all experiments, channel activity was evaluated in the presence of this 2.5 mM urate solution in both chambers (symmetrical urate). In one group of experiments, the channel was reexamined after a 10:1 urate gradient was created by changing the solution in the cis chamber to (in mM): 0.25 urate, 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4. In a second group of studies, the channel was reevaluated after fluid in the trans chamber was replaced with a solution containing (in mM): 0.25 urate, 2.5 oxonate (Aldrich Chemical, Milwaukee, WI), 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4 and an aliquot of oxonate was added to the cis chamber to yield an oxonate concentration of 0.25 mM. In other studies, the channel was reassessed after fluid in the trans chamber was replaced with a solution containing (in mM): 0.25 urate, 2.5 pyrazinoate (Aldrich Chemical), 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4 and an aliquot of pyrazinoate was added to the cis chamber to yield a pyrazinoate concentration of 0.25 mM. In a final group of experiments,

channel activity was compared in symmetrical solutions of (in mM) 2.5 urate, 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4 before and after an increasing amount of nonimmune IgG (up to 200 μg) or immune IgG (up to 15 μg) was sequentially added to each side of the lipid bilayer.

Data Analysis

Current output of the patch clamp was collected unfiltered onto VCR tapes (Sharp model VCA 5240). For analysis, the taped data were filtered at 1 kHz through an eight-pole filter (Bessel filter Model 902, Frequency Devices, Haverhill, MA) that was digitized at 0.4 ms/point (Labmaster DMA Interface model TL-1, Axon Instruments, Burlingame, CA). Data were analyzed with commercial software (Pclamp, Version 5.5, Axon Instruments) on a microcomputer after additional digitized filtering at 100 Hz. A channel event with an open time greater than 1.0 msec and a noise level at the open state less than 1.5 times the noise level of the baseline were analyzed. Records at each voltage were 1–2 min in duration. Data analysis was confined to those traces in which a single conductance was present. Open probability is defined as the fraction of the total time that the channel spent in the open state.

Results

Characteristics of the Reconstituted Channel

As depicted in Fig. 1*A*, single channel activity (evidenced by clear transitions between open and closed states) was detected in symmetrical urate solutions following fusion of uricase containing proteoliposomes with the lipid bilayer. In most instances, single channels were evident, however, as many as three channels were occasionally detected: when multiple channels were observed all had the same conductances (*not shown*). Single channel current traces obtained at different holding potentials revealed that the channel gated at positive voltage, but was closed and gated minimally if at all at negative voltages (Fig. 1*A*). This relationship between gating and the holding potential was consistently observed in 11 studies. (In a single experiment the channel was active at negative, but not positive voltage, implying that the channel had fused into the bilayer in the opposite direction.) The channel also reproducibly displayed a tendency to remain open for long periods of time interrupted by short closing periods: the mean closed and open times derived from the histogram distribution of events at +100 mV were 8 and 215 msec, respectively (Fig. 1*B* and *C*). Since both the closed and open time histograms fit single exponential curves (Fig. 1*B* and *C*), it is suggested that only one type of channel was present in the bilayer and that this channel has a single open and single closed state. Further evidence for a single type of channel is provided in Fig. 2 which depicts 45-sec traces obtained at three separate membrane potentials in the presence of symmetrical urate solutions.

Figure 3 demonstrates the mean current/voltage relationship in 11 experiments performed in symmetrical 2.5 mM urate solutions. The amplitude of the current increased linearly with increments in positive voltage, but remained relatively constant at 0.2–0.3 pA despite changes in the negative voltage. Thus, this channel displays rectification in symmetrical urate solutions. When only the current at positive voltage was fit by linear regression, the slope conductance was 8 ± 0.1 pS (r = 0.99).

The kinetic parameters of the reconstituted channel are depicted in Fig. 4. The open probability of this channel is less than 20% at holding potentials at or below −25 mV, but greater than 80% at voltages of +25 mV or higher (Fig. 4*A*). The relationship between open probability and voltage is described by a very steep sigmoid curve, with the steepest part of the slope close to 0 mV (Fig. 4*A*). Based on the sigmoid curve, which was fit with a modification of the Boltzmann equation [29], the channel would be open 50% of the time at 0 mV. The steepness of the slope implies that there is a highly charged region inside the channel that is responsible for the channel's marked voltage sensitivity [17]: the equivalent gating charge, calculated from the Boltzmann equation, is 3.5 ± 0.5. The voltage sensitivity of this channel is also evident from the relationship between mean open time and voltage. The mean open time increased exponentially with increases in voltage (Fig. 4*B*). In contrast, the mean closed time appears to be independent of voltage within the bandwidth limits of the system (Fig. 4*B*). These findings indicate that both the open probability and the mean open time of this channel are voltage-dependent phenomena.

To assess the channel's selectivity to urate, the current/voltage relationship was examined in the presence of a 10:1 urate gradient, trans:cis. As depicted in Fig. 5, in the presence of the urate gradient the current was greater at positive holding potentials than in symmetrical urate solutions (Fig. 3). The slope conductance (calculated from a linear regression analysis of the currents at positive voltage) was 23 ± 0.2 pS (n = 3), a value significantly different from that in symmetrical urate solutions ($P < .001$). Moreover, significant current was present at 0 mV and the reversal potential was shifted from 0 to −43 mV, a value that approaches the equilibrium potential for urate. When the selectivity of the channel was examined in the presence of symmetrical 2.5 mM urate concentrations but a 2:1 KCl gradient, trans:cis, the current/voltage relationship was indistinguishable from that recorded in the absence of a KCl gradient and the reversal potential remained at 0 mV (n = 3, *not shown*). Insofar as the channel is permeable to urate (Fig. 5) and the reversal potential was not measurably shifted (the expected reversal potentials for ideal K^+ or Cl^- conductances would be −17 and +17 mV, respectively), the channel is estimated [12] to be at least 80 times more selective for urate than for K^+ or Cl^-. These cumulative observations indicate that a purified prepara-

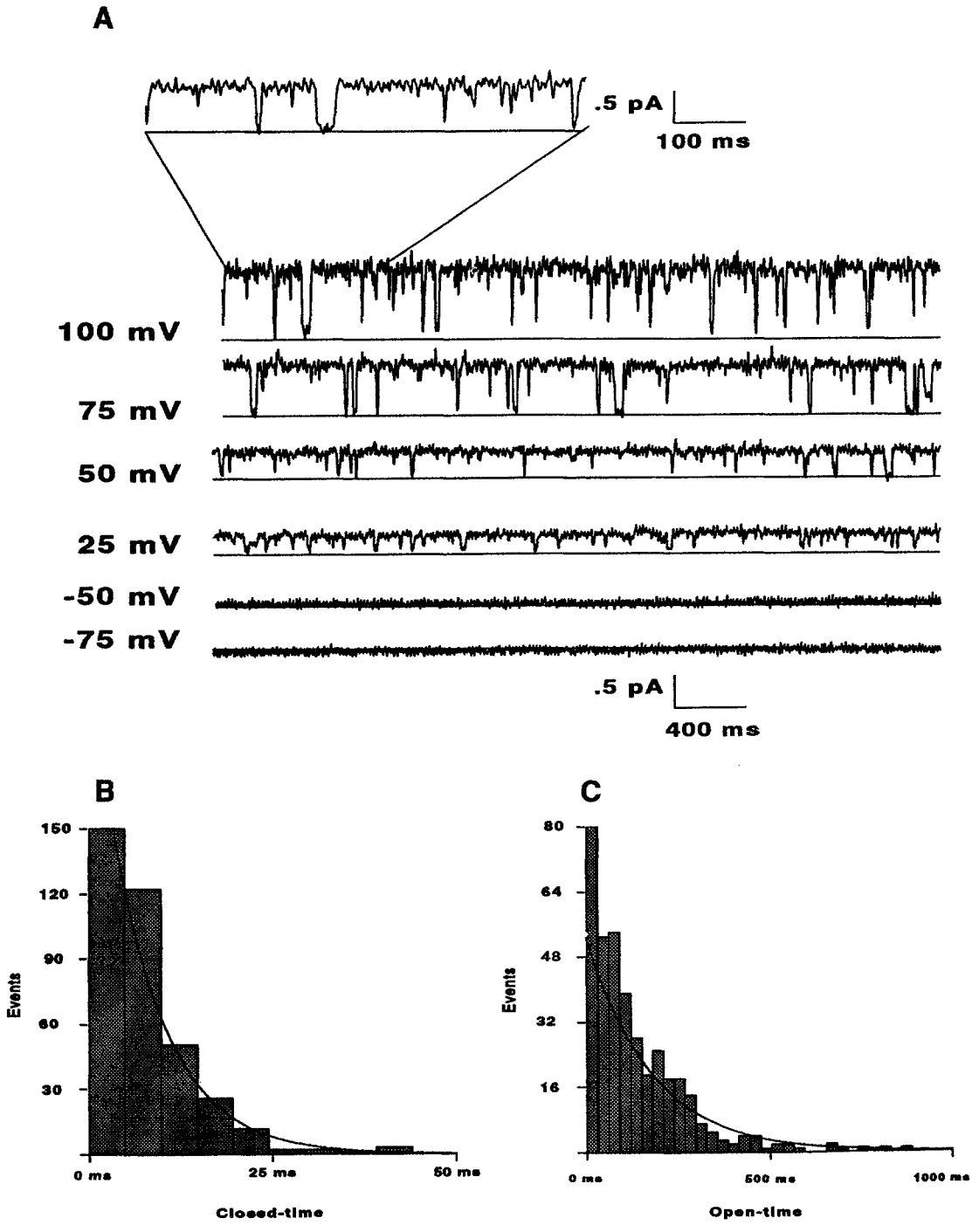

Fig. 1. (*A*) Representative 5-sec traces obtained at different holding potentials in an individual experiment. Channel activity was recorded in symmetrical urate solutions (in mM) 2.5 urate, 220 KCl, 10 HEPES-KOH, 0.25 $CaCl_2$ at pH 7.4 following fusion of uricase containing proteoliposomes with the planar lipid bilayer. Solid horizontal lines indicate the closed state. The upper insert represents a magnification of a portion of the upper trace. (*B*) depicts the histogram of the closed time and (*C*) the histogram of the open time of the channel recorded in *A* at a holding potential of 100 mV: the solid lines represent the best fits to single exponentials ($r = 0.99$ in *B* and in *C*).

tion of uricase can function as a voltage-sensitive channel that is highly selective for urate, relative to K^+ and Cl^-, when reconstituted in a lipid bilayer.

In an attempt to maximize the purity of the commercially purified uricase, the commercial preparation was subjected to an additional round of purification by affinity chromatography [24]. This affinity-purified protein was enzymatically active and, after fusion with the

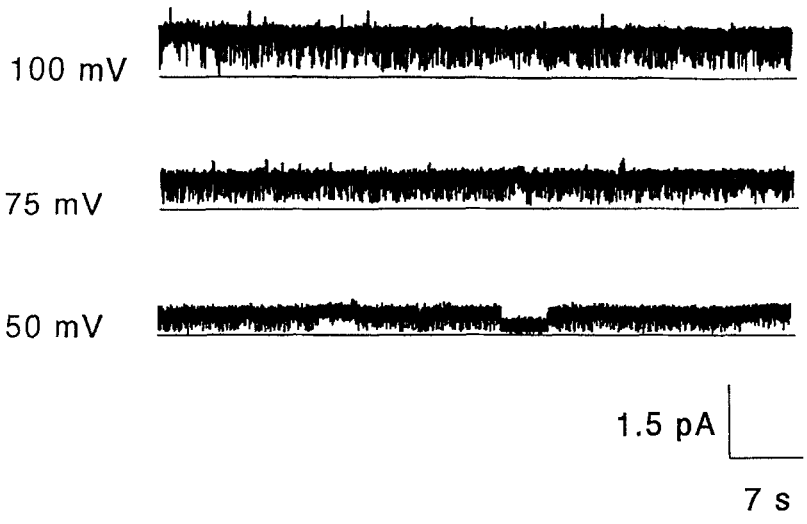

Fig. 2. Representative 45-sec traces obtained at different holding potentials in an individual experiment. Channel activity was recorded in symmetrical 2.5 mM urate solutions. Solid horizontal lines indicate the closed state.

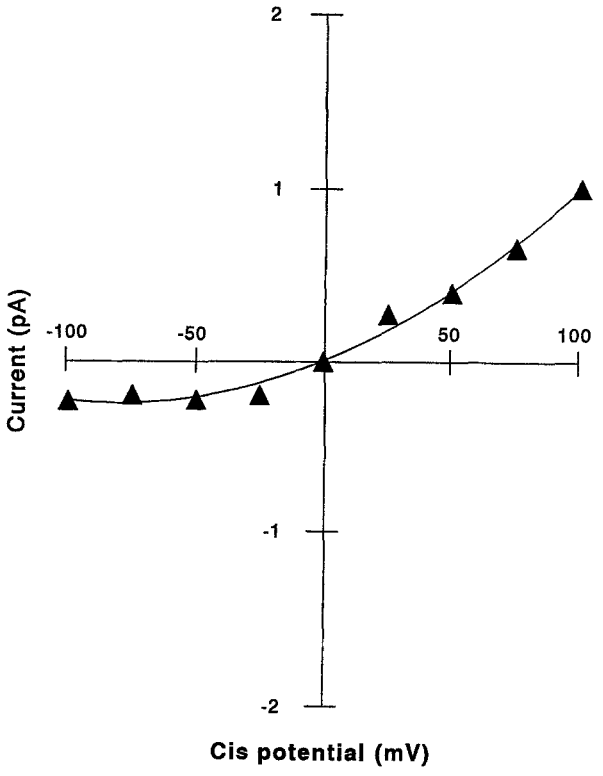

Fig. 3. Current/voltage relationship of the channel in symmetrical 2.5 mM urate solutions. Data represent the mean ± SE in 11 experiments; where not depicted, the SE is included within the space of the symbol. The solid line represents the best fit to a single exponential ($r = 0.99$).

lipid bilayer, formed a channel with characteristics (conductance, kinetics and selectivity to urate) that were indistinguishable from those detected with the purified commercial preparation (n = 3). This finding implies that uricase (or a protein of similar size that copurifies with uricase, independent of the method of protein purification) is responsible for the observed channel activity.

Effect of Oxonate on Channel Activity

In the presence of a 10:1 oxonate gradient (trans:cis) and a 10:1 urate gradient (cis:trans) the channel also rectified (Fig. 6*A*); however, at each positive voltage the current was significantly greater than in symmetrical urate solutions (Fig. 3). In the presence of oxonate, the mean slope conductance (calculated from a linear regression analysis of the currents at positive voltages) was significantly greater than in symmetrical urate solutions (10 ± 0.2 *vs.* 8 ± 0.1 pS, $P < .05$). Additionally, there was a significant current at 0 mV and the reversal potential was shifted to −25 mV, a value that approached the equilibrium potential for oxonate. These findings are consistent with a channel that is more permeable to oxonate than urate: utilizing the Goldman equation [12] the calculated oxonate/urate permeability ratio is 4.5. An evaluation of the kinetics of the channel in the presence of oxonate reveals that the open probability of the channel is not significantly altered by oxonate (Figs. 4*A* and 6*B*). Similarly, oxonate did not produce a significant change in the relationship between mean open or closed time and voltage (Figs. 4*B* and 6*C*). These observations suggest that oxonate does not block the channel, but rather moves through the channel.

Effect of Pyrazinoate on Channel Activity

The current/voltage relationship was initially unaffected when the symmetrical urate solutions were replaced with

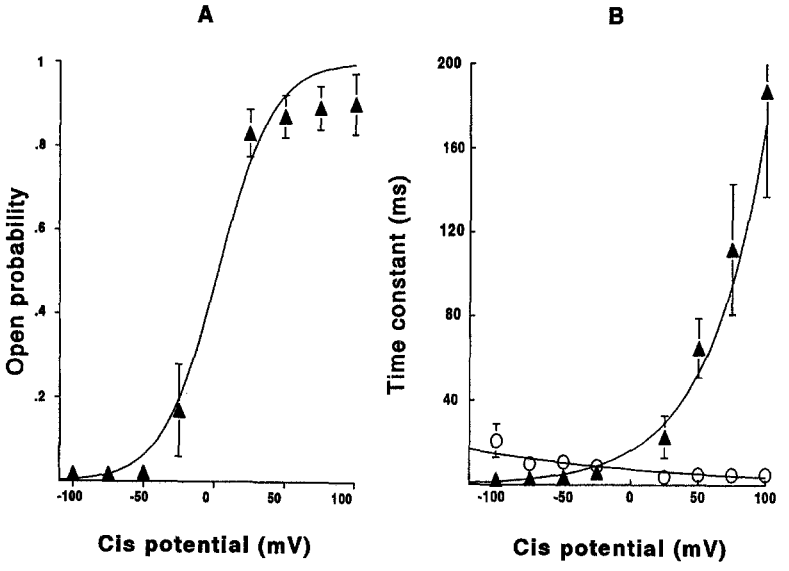

Fig. 4. Voltage sensitivity of the channel in symmetrical 2.5 mM urate solutions. Data represent the mean ± SE in 11 experiments; where not depicted, the SE is included within the space of the symbol. (*A*) Relationship between open probability of the channel and voltage. The solid line represents the best fit of the data estimated from the Boltzman equation ($r = 0.98$). (*B*) Relationship between mean open (solid triangles) and mean closed (open circles) times of the channel and voltage. The solid lines represent the best fits to single exponentials ($r = 0.99$ and 0.89 for the mean open and closed times, respectively).

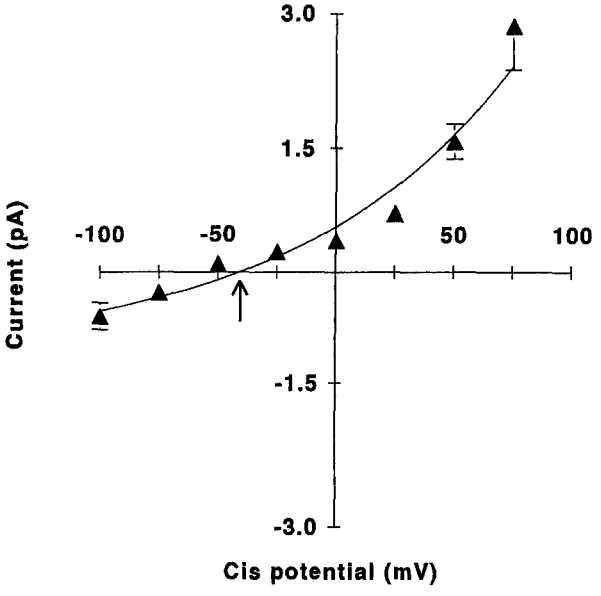

Fig. 5. Current/voltage relationship of the channel in the presence of a 10:1 urate gradient (2.5 mM trans:0.25 mM cis). Data represent the mean ± SE in 3 experiments; where not depicted, the SE is included within the space of the symbol. The solid line represents the best fit to a single exponential ($r = 0.99$). The arrow indicates the reversal potential at −43 mV.

solutions that provided a 10:1 pyrazinoate gradient (trans:cis) and a 10:1 urate gradient (cis:trans) (not depicted). However, after a period of time of exposure to pyrazinoate (5–10 min), channel activity completely disappeared (n = 4, Fig. 7). This loss of channel activity was not consequent to channel rundown since activity was readily restored when the pyrazinoate-containing solution in the trans chamber was replaced with (in mM): 2.5 urate, 220 KCl, 10 HEPES-KOH 0.25 $CaCl_2$ at pH 7.4 (n = 2, Fig. 7). These findings indicate that pyrazinoate has an effect on the channel that is distinctly different from that of oxonate: initially pyrazinoate moves through the channel with a permeability that is indistinguishable from urate, however, pyrazinoate ultimately interacts with the channel, blocking its own movement as well as that of urate.

Effect of Anti-uricase on Channel Activity

Addition of nonimmune IgG (up to 200 μg) did not significantly change the magnitude of the current or the kinetics of the channel (Fig. 8). In contrast, after the channel was exposed to the IgG fraction of the polyclonal antibody to uricase (up to 15 μg), the open probability of the channel progressively decreased until channel activity completely disappeared (n = 3, Fig. 8). Since this antibody was raised to enzymatically active, affinity-purified porcine uricase [24], the antibody-induced block in urate movement through the channel supports the suggestion that it is the uricase that functions as a channel when reconstituted in lipid bilayers.

Discussion

The present study has demonstrated that an anion channel is reconstituted when a purified preparation of uricase, an oxidative enzyme that normally resides within the core of hepatic peroxisomes, is fused with planar lipid bilayers (Figs. 1 and 2). When reconstituted, this protein was found to function as an 8 pS channel with a single open and single closed state that rectifies in symmetrical urate solutions (Fig. 3). Additionally, this channel was observed to display marked voltage sensi-

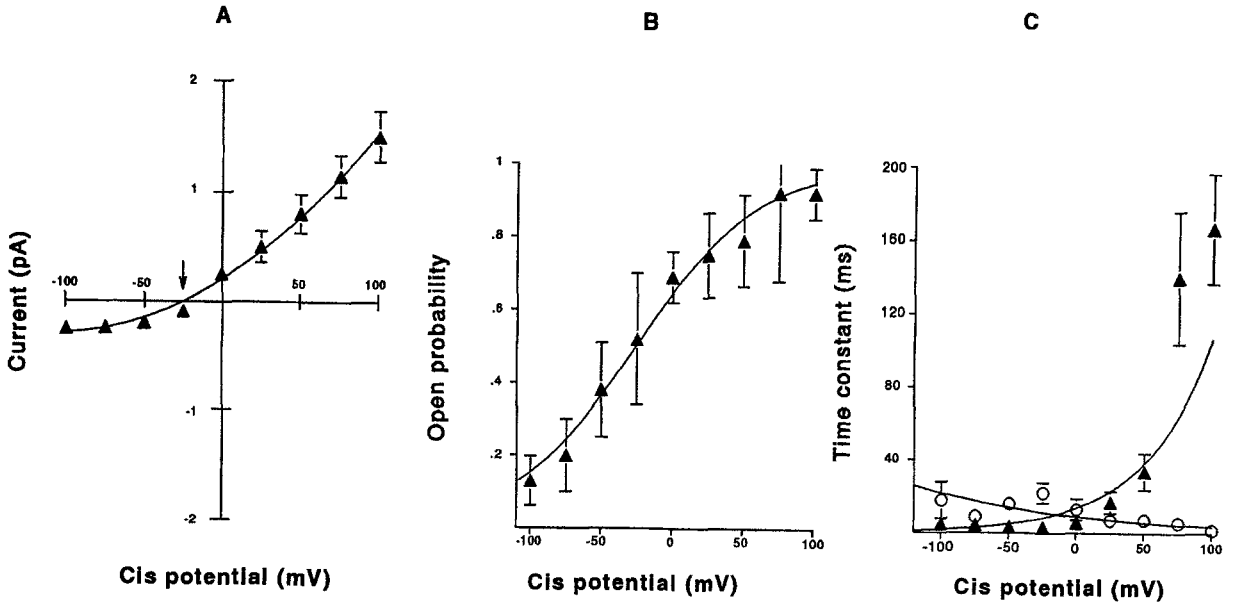

Fig. 6. Selectivity of the channel to urate relative to oxonate. Data represent the mean ± SE of data obtained in the presence of a 10:1 oxonate gradient (2.5 mM trans:0.25 mM cis) and a 10:1 urate gradient (2.5 mM cis:0.25 mM trans) in 3 experiments; where not depicted, the SE is included within the space of the symbol. (*A*) Current/voltage relationship of the channel: the arrow indicates the reversal potential at −25 mV. The solid line represents the best fit to a single exponential (r = 0.99). (*B*) Relationship between open probability of the channel and voltage. The solid line represents the best fit of the data estimated from the Boltzman equation (r = 0.95). The equivalent gating charge was 2.2 ± 0.9. (*C*) Relationship between mean open (solid triangles) and mean closed (open circles) times of the channel and voltage. The solid lines represent the best fits to single exponentials (r = 0.82 and 0.93 for the mean open and closed times, respectively).

tivity (Fig. 4), implying that the molecule contains a high density of charged amino acids at some site that is critical to the protein's ability to function as a channel. The channel was further characterized as being highly selective to the organic anion urate, relative to the inorganic anion chloride (Fig. 5), and at least as selective to the alternate organic acid anions oxonate (Fig. 6*A*) and pyrazinoate. Finally, a polyclonal antibody that was raised to enzymatically active hepatic uricase was found to block activity of the reconstituted urate channel.

The marked selectivity of the reconstituted channel for urate relative to chloride (Fig. 5) is quite striking in that it approximates 80:1. In prior studies of selectivity of anion channels, the channels have either been designated as being anion channels on the basis of their greater selectivity to anions relative to cations [7, 19, 27, 31, 35, 36] or a comparison has been made between the selectivity of the channels to chloride relative to other halides [4, 10, 14, 30, 34, 36] or the alternate inorganic anions, gluconate [27, 28] and bicarbonate [27]. The order of halide selectivity has varied considerably depending on the specific anion channel [4, 10, 14, 27, 30, 34, 36], and the selectivity for chloride has been found to exceed that of gluconate and bicarbonate in one type of anion channel [27, 28]. The selectivity to chloride has also been found to exceed that of several organic anions including the amino acids taurine in C6 glioma cells [19] and aspartate and glutamate in MDCK cells [5], and the organic acid anion, malate, in guard cells of plants [15, 16]. Thus, while the high degree of selectivity of the reconstituted channel for urate over chloride (Fig. 5) appears to be unique, it remains to be determined whether any of the previously described anion channels conduct urate and, if so, how selective the channel is to urate relative to chloride.

Oxonate, which is a specific inhibitor of the enzymatic activity of uricase [11], has also been demonstrated to be a potent inhibitor of urate uptake in uricase-containing proteoliposomes [33] and an equally potent inhibitor of urate uptake via a uricase-like transporter in rat [1, 2] and rabbit [23] renal cortical membrane vesicles. In rabbit, where urate uptake was shown to derive from both a channel-like urate transporter and a classical, saturable carrier, oxonate was found to inhibit both modes of urate transport [23]. However, the mechanism of the inhibitory effect of oxonate on urate uptake has not been defined. It is therefore of interest that the present study demonstrates that oxonate not only moves through the reconstituted urate channel but that the channel is more permeable to oxonate than it is to urate (Fig. 6*A*). Insofar as these findings may be applicable to urate uptake via the uricase-like transporter in renal cortical membranes, it seems likely that the observed inhibitory effects of oxonate on urate uptake [1, 2, 23] may result

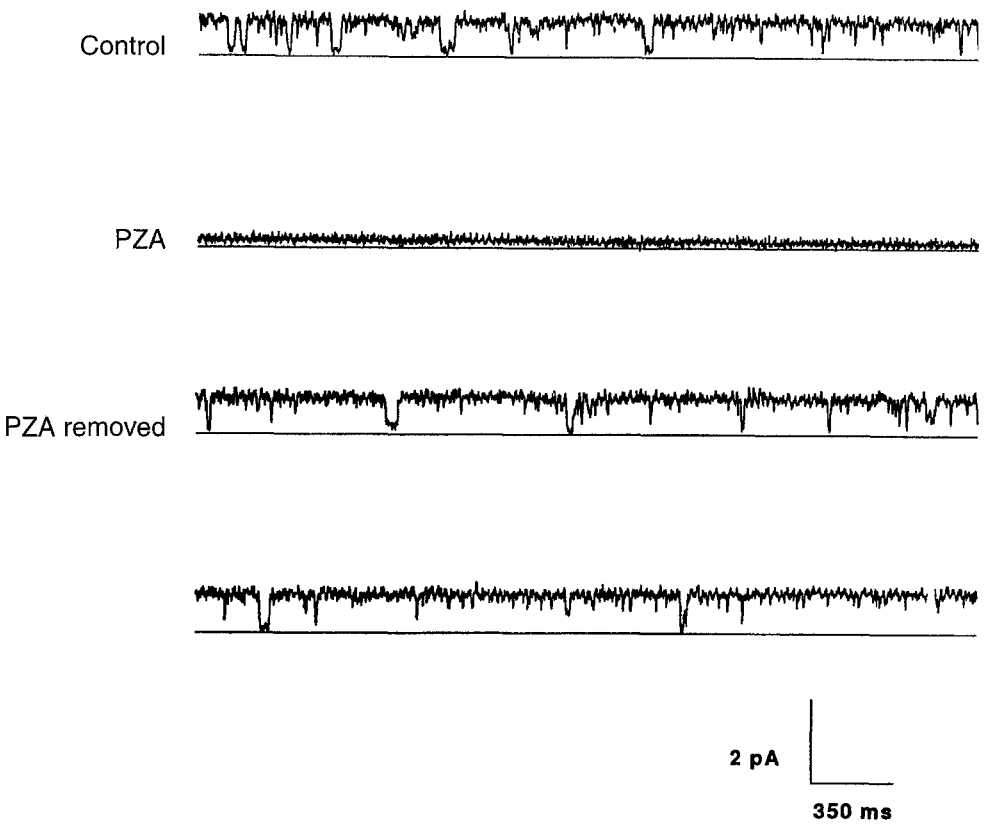

Fig. 7. Representative experiment demonstrating the effect of pyrazinoate (PZA) on channel activity. The upper trace, control, was obtained in symmetrical 2.5 mM urate solutions. The second trace, PZA, was obtained 5 min after the cis and trans solutions were changed to create a 10:1 pyrazinoate gradient (2.5 mM trans:0.25 mM cis) and a 10:1 urate gradient (2.5 mM cis:0.25 mM trans). The lower 2 traces, PZA removed, represent a continuous record of channel activity obtained immediately after the PZA-containing solutions were replaced with symmetrical 2.5 mM urate solutions. All traces were obtained at a holding potential of 50 mV.

from exclusion of the less permeant species (urate) from the channel-like transporter, rather than a direct blockade produced by an interaction between oxonate and some critical site in the channel.

Pyrazinoate has also been shown to be a potent inhibitor of urate uptake via the uricase-like transporter in rat [1, 2] and rabbit [23] renal cortical membrane vesicles. As in the case of oxonate, the mechanism responsible for the inhibitory effect of pyrazinoate on urate uptake has not been described. However, on the basis of the disparate effects of pyrazinoate (Fig. 7) and oxonate (Fig. 6*A*) on the reconstituted urate channel, the present study suggests that the mechanism of inhibition of renal urate transport by pyrazinoate and oxonate may be distinct. First, in contrast to the preferential permeability of the channel to oxonate over urate, which limits urate flux through the channel, the channel displays very similar permeabilities to pyrazinoate and urate. Second, unlike oxonate, pyrazinoate ultimately reversibly blocks the channel, restricting its own movement as well as that of urate. Since pyrazinoate can move through the reconstituted channel, it is suggested that pyrazinoate blocks the channel when it binds to a site that is located outside of the channel's selective pore. It remains to be determined whether a similar mechanism is involved in pyrazinoate's inhibition of urate transport across renal cell membranes.

The activity of the reconstituted channel was also blocked by the IgG fraction of a polyclonal antibody that was raised to enzymatically active, affinity purified pig liver uricase (Fig. 8). Of note, this antibody inhibits transport via the electrogenic uricase-like urate transporter, but does not affect transport on the electroneutral urate/anion exchanger or the Na^+-dependent glucose transporter in membrane vesicles prepared from rat renal cortex [24]. Based on the specificity of inhibition of membrane transport by anti-uricase, it was suggested that some epitopes must be conserved between hepatic uricase and the renal membrane uricase-like transporter [24]. Insofar as this antibody also inhibits the activity of the reconstituted urate channel, it seems likely that the region of the protein that allows urate movement through the reconstituted channel and the region of the electrogenic uricase-like transporter that permits urate translocation across the renal proximal tubule cell membrane are highly conserved in the two proteins. Direct evi-

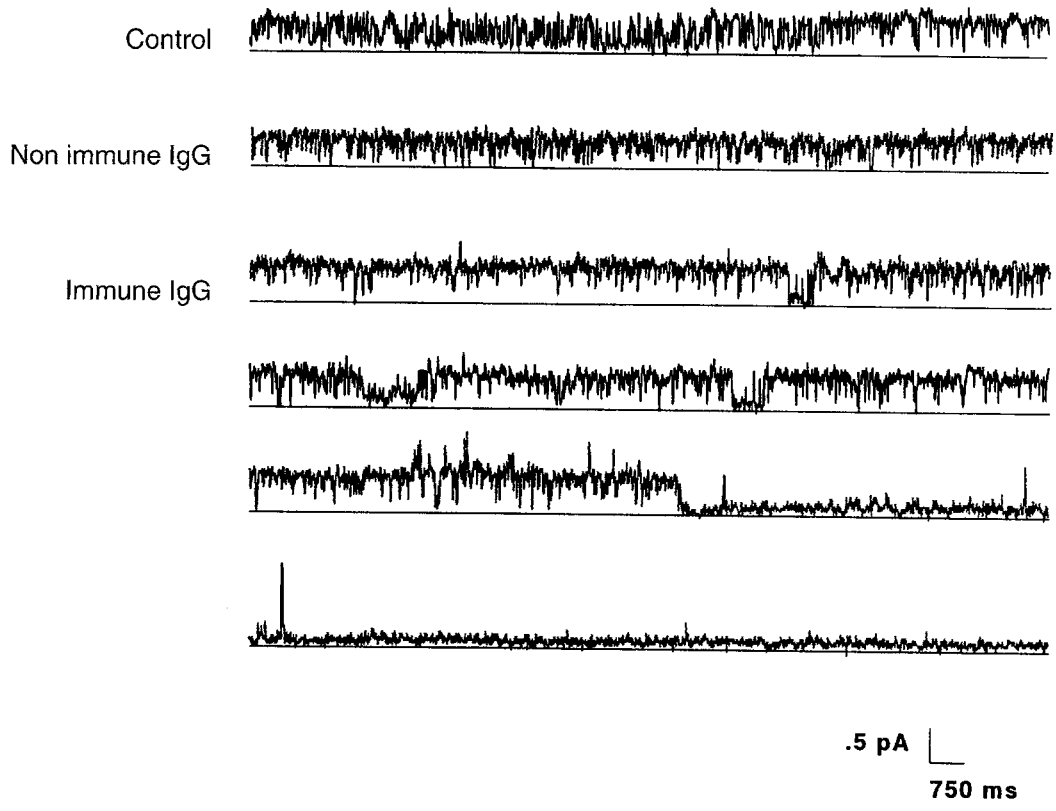

Fig. 8. Representative experiment demonstrating the effect of a polyclonal antibody to pig liver uricase on channel activity. The upper trace, control, was obtained in symmetrical 2.5 mM urate solutions. The second trace was obtained 10 min after 200 μg of non-immune rabbit IgG was added to the cis side of the chamber. The lower 4 traces represent a continuous recording of channel activity: the record was started 2 min following addition of 15 μg of the IgG fraction of the polyclonal antibody to pig liver uricase to the cis side of the chamber. All traces were obtained at a holding potential of 50 mV.

dence for this suggestion must await the cloning and sequencing of the uricase-like transporter and comparison with the sequence of porcine hepatic uricase [38].

Although the present studies have demonstrated that a purified preparation of uricase functions as a highly selective, voltage dependent anion channel when fused with lipid bilayers, it is of interest that no transmembrane spanning domains (either alpha helices or beta sheets) are predicted [8, 25] from the reported amino acid sequence of the 32 kDa monomer of pig liver uricase [38]. However, as uricase exists as a multimer of four polypeptide chains of approximately equal mass [32], the intact protein may assume a configuration that allows it to traverse the bilayer membrane as an anion channel. Alternatively, the lipid and/or solvent environments may modify the protein's tertiary structure to produce an ion channel [37]. Thus, while evidence has been provided that this protein can function as a channel, it remains to be determined how it forms a three-dimensional structure with a hydrophilic pore that behaves as an anion channel in a lipid bilayer membrane.

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