J. Membrane Biol. 55, 141–147 (1980)

The Journal of
Membrane Biology

Effect of Quinidine on Na, H^+, and Water Transport by the Turtle and Toad Bladders

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Summary. The effect of quinidine on Na and H^+ transport by the turtle bladder and water transport by the toad bladder was examined. Quinidine inhibited the short-circuit current and the potential difference in a dose-dependent fashion. The effect of quinidine on the short-circuit was not dependent on extracellular calcium concentration and was not reversible with removal of the drug. Quinidine inhibited H^+ secretion in a dose-dependent fashion. The effect of quinidine on H^+ secretion also was not dependent on extracellular calcium concentration and was not reversible, either with removal of the drug or with stimulation of H^+ secretion with 5% CO_2. The effect of quinidine on Na or H^+ transport could not be elicited by an equivalent dose of tetracaine, suggesting that the inhibitory effect of quinidine is not dependent on its anesthetic properties. Quinidine also inhibited vasopressin and cyclic AMP stimulated water flow in the toad bladder. Quinidine did not alter calcium uptake by the turtle bladder but increased calcium efflux by the turtle and toad bladders. These observations suggest that a rise in cytosolic calcium is responsible for the inhibitory effect of quinidine on Na, H^+, and water transport.

Quinine and its isomer quinidine are compounds well known to cause skeletal muscle contraction and smooth muscle relaxation [3, 5, 8, 11, 12]. These effects of quinidine and quinine are thought to result from an increase in cytosolic calcium concentration [3, 5, 8, 10, 11]. The evidence available supports the concept that quinidine or quinine increases cytosolic calcium concentration both by preventing calcium uptake by intracellular organelles as well as by releasing calcium accumulated by these organelles [3, 5, 8, 10, 11]. Quinidine and quinine have been, therefore, extensively used in assessing the role of intracellular calcium in the process of muscle contraction and relaxation [3, 5, 8, 10, 11].

The role of intracellular calcium in sodium transport, urinary acidification, and water transport has not been completely defined. *In vivo* studies aimed at studying the role of intracellular calcium in these processes are difficult to evaluate both because of low permeability of cell membranes to calcium as well as because of renal hemodynamic alterations caused by hypercalcemia [1, 2, 24]. These hemodynamic alterations can, by themselves, alter Na, H^+ and water transport thus making it impossible to evaluate the direct role of calcium in these processes. Based on studies utilizing the ionophore A23187, a substance known to increase the permeability of artifical and living membranes to calcium, we as well as other investigators have provided evidence for a role of intracellular calcium over Na transport and urinary acidification [1, 2, 24].

The bladder of the fresh water turtle and that of the toad are capable of transporting Na and secreting H^+ *in vitro* [1, 2, 15, 18–22, 24]. The toad bladder is also capable of water transport in response to vasopressin [4, 7, 9]. These membranes are therefore ideal models in which to examine the factors influencing Na, H^+ and water transport. In the present study we examined the effect of quinidine, and inferentially the role of intracellular calcium, on Na and H^+ transport by the turtle bladder and on water transport by the toad bladder.

Materials and Methods

Effect of Quinidine on Na and H^+ Transport

Urinary bladders of fresh water turtles were removed, divided in two halves, and each hemibladder was mounted in a Lucite chamber. The exposed area of the bladder was 8 cm^2, and the

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0022-2631/80/0055-0141 $01.40

results are expressed for this area. The two sides of the bladder were bathed in 10 ml of bicarbonate and magnesium-free Ringer's solution containing the following composition in millimoles/liter: NaCl, 114.4; KCl, 3.5; Na_2HPO_4, 2.0; dextrose, 5; and either 1.8 or 0.2, $CaCl_2$. In all experiments the mucosa was bubbled with compressed air which had been passed through three KOH traps to remove all Co_2 and the serosa was bubbled with a gas mixture containing 99% air and 1% CO_2. The spontaneous potential difference (PD) was measured through the use of 3 M KCl-agar bridges and calomel half cells which were connected to a Keithley model 600B Voltmeter (Keithley Instruments, Cleveland, Ohio). An automatic voltage clamp was used to supply enough current via KCl-agar bridges and Ag-AgCl electrodes to nullify the spontaneous PD. The short-circuit current (SCC) was measured by a Simpson microammeter. All experiments were performed in a short circuited state. Bladders that failed to maintain a spontaneous PD greater than 10 mV during the first hour were discarded. The rate of Na transport was measured as the SCC. The rate of H^+ secretion was measured as the SCC after the sodium transport was completely abolished by 5×10^{-4} M ouabain added to the serosal side. Schwartz [19] has shown that the addition of ouabain to the serosa results in a reversal of SCC (mucosa positive-serosa negative). The reverse SCC (RSCC) was identical to the rate of H^+ secretion measured by the pH-stat method. We have measured the rate of H^+ secretion simultaneously with the pH stat method and with the RSCC method and found it to be not significantly different (pH stat – 25.0 ± 2.64 and RSCC – 23.7 ± 2.31 μA in 18 hemibladders). In the experiments reported here the RSCC or the SCC was recorded cortinuously except for brief intervals when the PD was measured. In all experiments the pH of the mucosa was maintained at 7.4. The pH of the serosa was also maintained at 7.4. After the RSCC or SCC was stable for at least 30 min, quinidine hydrochloride (dissolved in turtle Ringer's solution) was added to either the serosal or mucosal side of one hemibladder (experimental), and an equal amount of choline chloride was added to the serosal side of the other hemibladder (control). In some experiments cesium chloride, instead of choline chloride was added to the control hemibladder. No difference between choline chloride or cesium chloride was noted, i.e., neither affected SCC or RSCC. The RSCC or the SCC was measured continuously for 90–120 min after the addition of the quinidine. In some experiments in which the serosa was bubbled with 1% CO_2, 5% CO_2 was used after 2 hr in an attempt to reverse the effect of the quinidine on the RSCC. In some experiments we attempted to reverse the effect of quinidine on SCC or RSCC by washing the serosal solution twice with fresh Ringer's solution and observing the SCC or the RSCC for an additional 60 min.

Effect of Quinidine on Water Flow

These experiments were performed on bladders isolated from toads, *Bufo marinus*, obtained from Mogul Ed, and said to originate from Mexico. Paired hemibladders were excised (one serving as control and the other as experimental) and mounted as sacs on glass tubing. The bladders were filled either with 5 ml of diluted Ringer's solution (diluted 1:5) and suspended in bath containing 100 ml of full strength Ringer's containing the following composition in mmol/liter: Na, 111.2; Cl, 113; K, 5.4; Ca, 0.9; HPO_4, 2.4; and H_2PO_4, 0.6; dextrose, 5; pH 7.4; osmolality, 220 mOsm/kg H_2O. The bathing solution in which the bladders were suspended was fully aerated with compressed air and vigorously stirred. Water flow was measured gravimetrically at 30-min intervals; the bladders were removed from the bathing solution, blotted gently, and weighed on a Mettler scale. After an equilibration period two baseline weight measurements at 30-min intervals were obtained. Quinidine hydrochloride diluted in Ringer's solution was then added to the serosal solution and an equal amount of diluent was added to the control hemibladder. After two additional weight measurements, at 30-min intervals, vasopressin (20 mU/ml) or cyclic adenosine monophosphate (AMP) (10 mM) was added to the serosal solution of both control and experimental hemibladders; weight loss was measured at 30 min after the addition of vasopressin or cyclic AMP. Weight changes were corrected for the surface area of hemibladders by assuming that each hemibladder represented a perfect sphere.

Radioactive Calcium Uptake and Calcium Efflux

These experiments were performed on the stripped mucosa of the turtle bladder utilizing the method of Nagakawa et al. [15] to separate the mucosa from the remaining tissues. For the calcium uptake experiments, all hemibladders were preincubated in 10 ml of oxygenated turtle Ringer's solution containing 5×10^{-4} M ouabain for 10 min. The hemibladders were incubated for an additional 10 min. After 10 min of incubation with either the quinidine or the diluent 0.5–0.9 μCi of $^{45}CaCl_2$ was added and after 5 min of exposure to ^{45}Ca the hemibladders were removed, blotted, and digested in 1 ml of 1 N NaOH overnight. The dissolved tissue was neutralized in 200 ml of 5 N HCl and in 10 ml of Scintiverse (Fisher Scientific). Aliquots of the digested tissue were removed for determination of protein and counting in a liquid scintillation counter. Appropriate corrections for quenching and machine efficiency were made. Based on the specific activity of ^{45}Ca in the incubation medium and in the tissue ^{45}Ca uptake was calculated and the results are expressed in nmol/mg protein per 5 min. The method utilized to measure calcium efflux was adapted from the method utilized previously by one of us to measure calcium efflux in the heart [17]. For the efflux measurements the hemibladders were loaded for 30 min in 5 ml of oxygenated turtle Ringer's solution containing 0.9 μCi/ml of ^{45}Ca. After this time, the tissues were removed and passed sequentially through a series of prewashed scintillation vials containing 5 ml of nonradioactive turtle Ringer's solution which was continuously oxygenated. The efflux was measured at 1-min intervals during the first 10 min, and then at 15, 20, 25, and 30 min, and thereafter at 10-min intervals until 120 min had lapsed. During the first 5 min the experimental hemibladders were not exposed to quinidine; after this time all subsequent vials of experimental hemibladders contained 10^{-3} M quinidine. 1 ml aliquots were dissolved in 10 ml of Bray's solution (Isolab) and counted in a liquid scintillation counter. The amount of ^{45}Ca remaining in the tissue at the end of the experiment was determined as described above. Appropriate quenching corrections were made. The amount of ^{45}Ca effluxed relative to the 1 min count was calculated and expressed per mg protein per min. Identical experiments were performed in the toad bladder except the whole bladder, rather than mucosa, was used.

Quinidine hydrochloride, tetracaine, dinitrophenol, and cyclic AMP were obtained from Sigma Co. (St. Louis, Mo.), and vasopressin from Parke Davis. The data were normalized by expressing the experimental values of Na or H^+ transport as percentage of baseline values [1, 2, 24]. Unless otherwise specified, the data of Na and H^+ transport refer to values obtained before and 90 min after addition of quinidine or other drugs. The data on water transport refer to values obtained 30 min after addition of AVP. Data are presented as mean ± SEM. The *t* test for paired and unpaired groups was used to analyze the data wherever appropriate.

Results

Effect of Quinidine on Na Transport

The effect of quinidine, 10^{-3} M in the serosal solution, on Na transport (measured as the SCC) is shown

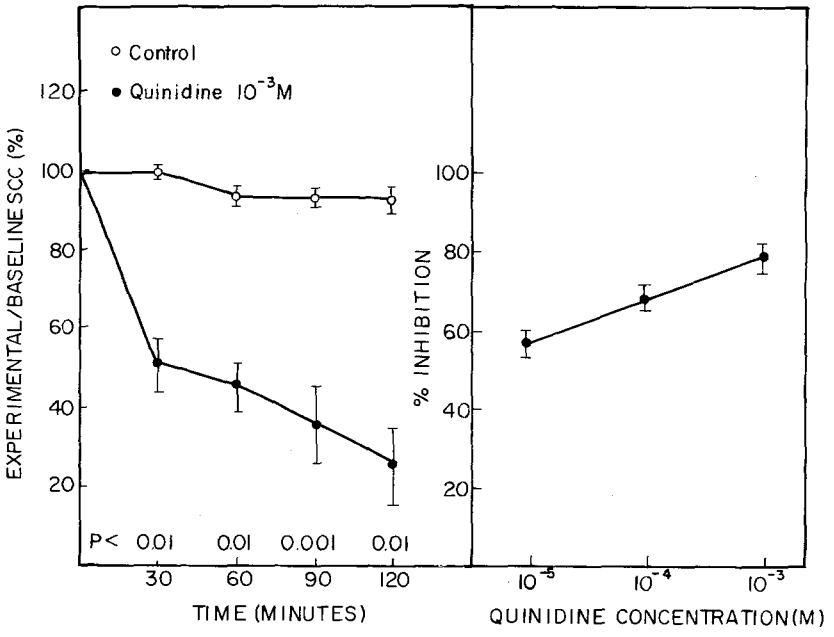

Fig. 1. Left panel shows SCC, expressed as percentage of baseline values, in control (open circles) and quinidine, 10^{-3} M in the serosal solution, treated hemibladders (filled circles) ($n=6$). The right panel shows the percent inhibition of SCC observed with different concentrations of quinidine in the serosol solution.

in Fig. 1 (left panel). The SCC is expressed as percentage of the baseline values. There was no difference in the baseline values of SCC between the two sets of hemibladders (371 ± 56.7 and 382 ± 23.2 μA, NS). In control hemibladders SCC and PD remained unchanged throughout the duration of the experiment. In the hemibladders treated with quinidine the SCC decreased significantly at 30 min, achieving a mean value of $24.6\pm10.5\%$, $P<0.01$ as compared to control hemibladders, at the end of 2 hr. The decline in SCC in the quinidine treated hemibladders was accompanied by a proportionally smaller decrease in PD thus leading to an increase in resistance (PD 27.6 ± 7.0 *vs.* 16.4 ± 5.35 mV, $P<0.05$). The effect of quinidine on Na transport could not be reversed by washing the serosal solution free of quinidine. Removal of quinidine, however, prevented a further decline in the SCC.

At 10^{-5} and 10^{-4} M, quinidine inhibited SCC to $58\pm4.3\%$ and to $65\pm5.9\%$ of baseline values, respectively ($P<0.001$, $n=5$) (Fig. 1, right panel). The effect of quinidine on Na transport in the presence of a low extracellular calcium concentration (0.2 mM, $n=3$) was also examined. The mean depression of the SCC elicited by 10^{-3} M quinidine after 90 min was the same magnitude in the presence of 1.8 mM Ca as in the presence of 0.2 mM Ca ($30\pm9.0\%$ *vs.* $42\pm3.2\%$, NS). Thus the inhibitory effect of quinidine on SCC is not dependent on extracellular calcium concentration.

Addition of 10^{-3} M quinidine in the mucosal solution also resulted, after 90 min, in a decrease in SCC and in PD, whereas in the control hemibladders the SCC and PD remained unchanged (control 91.6 ± 3.7 and quinidine $26\pm7.4\%$, $n=5$, $P<0.001$; control PD 31.4 ± 8.5 to 28.4 ± 8.3 mV, NS; quinidine 48.4 ± 7.6 to 23.0 ± 7.0 mV, $P<0.01$). Addition of quinidine to the serosal solution of hemibladders pretreated with quinidine in the mucosal solution did not yield an additive effect.

Effect of Tetracaine on Na Transport

Anesthetic agents have been shown to influence transepithelial transport [14]. Since quinidine has an anesthetic effect, we examined whether tetracaine, a compound well known for its anesthetic properties, would inhibit Na transport at a dose of 10^{-3} M. At this concentration tetracaine failed to alter SCC (control 92 ± 10.7 and tetracaine $84\pm4.5\%$, $n=5$, NS).

Effect of Dinitrophenol on Na Transport

These experiments were performed to determine whether quinidine inhibits Na transport by uncoupling oxidative phosphorylation. Dinitrophenol was added to either control hemibladders or to hemibladders pretreated with quinidine ($n=5$). There was no difference in the baseline SCC between the two sets of hemibladders. The decline in SCC is expressed as percentage ratio of the experimental period divided by the preceding period. Dinitrophenol alone, 10^{-4} M, added to the serosal solution caused a significant depression in SCC which achieved a stable value of $58.7\pm64\%$, $P<0.01$, by 30 min ($n=5$). Addition of quinidine, 10^{-3} M in the serosa, resulted in a depression of SCC to 54.8 ± 11.5, $P<0.01$, at 60 min. Addition of dinitrophenol, 10^{-4} M in the serosal solution, resulted in further depression of SCC achiev-

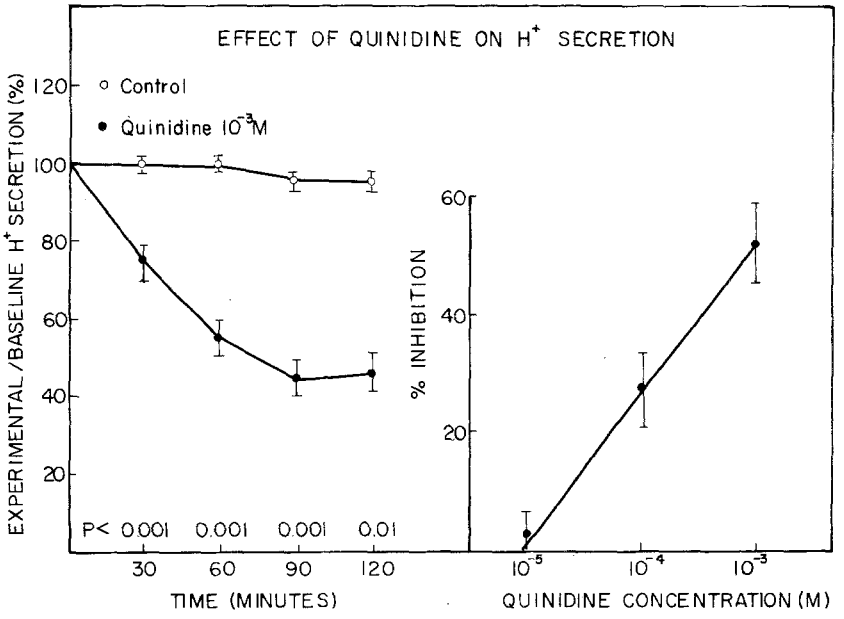

Fig. 2. Left panel shows H^+ secretion, expressed as percentage of the baseline values, in control (open circles) and quinidine, 10^{-3} M in the serosal solution treated hemibladders (filled circles) ($n=11$). The right panel shows the percent inhibition of H^+ secretion, as compared to control, elicited by various concentrations of quinidine in the serosal solution

ing a value of $24.6 \pm 10.0\%$, $P<0.05$, 30 min after addition of dinitrophenol. Thus dinitrophenol inhibits SCC in both control and quinidine-treated hemibladders.

Effect of Quinidine on H^+ Secretion

The effect of quinidine, 10^{-3} M in the serosa, on H^+ secretion (expressed as a percentage of baseline values) is shown in Fig. 2 (left panel). There was no difference in the baseline rate of H^+ secretion between the two sets of hemibladders (34.3 ± 3.8 *vs.* 36.7 ± 5.0 μA, $n=11$, NS). At 30 min H^+ secretion was significantly lower in quinidine-treated hemibladders than in controls; H^+ secretion continued to decline, achieving a maximum inhibition to $45.5 \pm 6.3\%$ of baseline values, $P<0.001$ at 90 min. Removal of the quinidine by washing the serosal solution with fresh Ringer's solution did not reverse the inhibitory effect of this compound on H^+ secretion, but it prevented a further decline in H^+ secretion. Addition of 5% CO_2, a potent stimulator of H^+ secretion, to the serosal phase resulted in a significant increase in H^+ secretion in control hemibladders but failed to elicit the same response in the quinidine treated hemibladders (control 139.2 ± 14 and quinidine $45 \pm 10.2\%$, $n=5$, $P<0.01$).

The right panel of Fig. 2 shows the effect of various concentrations of quinidine in the serosal solution in H^+ secretion. At 10^{-5} M quinidine failed to inhibit H^+ secretion ($3.0 \pm 6.0\%$, $n=5$, NS). The mean inhibition of H^+ secretion, as compared to controls, was $29 \pm 6.6\%$ at 10^{-4} M, $n=5$, $P<0.025$, and $53 \pm 7.6\%$ at 10^{-3} M, $n=11$, $P<0.001$.

In the presence of 0.2 mM calcium, 10^{-3} M quinidine caused a significant decrease in H^+ secretion as compared to controls (control $90 \pm 4.5\%$ *vs.* quinidine $39 \pm 3.2\%$, at 90 min after addition of quinidine $n=5$, $P<0.001$). The mean inhibition of H^+ secretion observed with 10^{-3} M quinidine in the presence of 1.8 mM Ca (Fig. 2) was not significantly different from that observed in the presence of 0.2 mM Ca. The addition of 10^{-3} M quinidine to the mucosal solution resulted only in a small decrease in H^+ transport after 90 min (control 89 ± 2.8 and quinidine $76 \pm 6.7\%$, $n=5$, $P<0.025$).

Effect of Tetracaine on H^+ Secretion

Addition of 10^{-3} M tetracaine to the serosal did not alter H^+ secretion (control 95 ± 4.9, tetracaine $97 \pm 1.3\%$, $n=5$, NS). There was no difference in the baseline rate of H^+ secretion between the two sets of hemibladders (30.4 ± 6.8 *vs.* 32.2 ± 6.0 μA, NS).

Effect of Dinitrophenol and Quinidine on H^+ Secretion

Dinitrophenol, 10^{-4} M in the serosal solution, resulted in a significant decrease in H^+ secretion (control $84.2 \pm 5.1\%$, dinitrophenol $49.2 \pm 14.1\%$, $n=5$, $P<0.02$). Baseline H^+ secretion was not different between the two sets of hemibladders (25.0 ± 5.1 *vs.* 34.0 ± 5.1 μA, NS). Addition of quinidine to dinitrophenol-treated bladders resulted in an additional decrease in H^+ transport from 49.2 ± 14.1 to $17.2 \pm 10.7\%$, $P<0.01$.

Table 1. Effect of quinidine on baseline and vasopressin or cyclic AMP stimulated water flow

Quinidine concentration		n			Water flow (μl/cm²/hr)			% Inhibition
					Baseline	$P<$	Stimulated	
I	10^{-4} M	6	Q		5.1±0.65	0.005	177.6±23.9	
				$P<$	NS		NS	5.9± 5.8
			C		4.6±0.90	0.001	185.6±15.8	
II	10^{-3} M	5	Q		5.9±3.1	0.05	57.5±21.6	
				$P<$	NS		0.025	65.5±11.2[a]
			C		2.3±1.0	0.005	178.0±24.2	
III	5×10^{-3} M	6	Q		4.2±1.7	NS	12.1± 8.5	
				$P<$	NS		0.005	92.3± 6.1[b]
			C		3.8±0.4	0.001	219.2±28.0	
IV	10^{-3} M	5	Q		4.8±0.9	0.01	134.0±25.4	
				$P<$	NS		0.02	26.8± 6.8[c]
			C		5.1±1.2	0.001	181.0±19.1	

I, II, III = AVP, 20 mU/ml, in the serosal phase; IV = cyclic AMP (10 mM in the serosa).
[a] $P<0.001$ as compared to I.
[b] $P<0.05$ as compared to II.
[c] $P<0.01$ as compared to II.
n = number of experiments; Q = quinidine treated hemibladder; C = control hemibladder.

Effect of Quinidine on Water Flow

The effect of quinidine on baseline and vasopressin-stimulated water flow is shown in Table 1. Quinidine had no effect on baseline water flow at three different concentrations used; at 10^{-4} M quinidine did not alter the vasopressin-stimulated water flow. At 10^{-3} M and 5×10^{-3} M quinidine inhibited the vasopressin-stimulated water flow by 65.5±11.2 and 92.3±6.1%, respectively. The water flow stimulated by cyclic AMP was also inhibited by quinidine. Observe, however, that 10^{-3} M quinidine caused a greater inhibition of vasopressin than cyclic AMP-stimulated water flow (vasopressin 65.5±11.2% inhibition and cyclic AMP 26.8±6.8% inhibition, $P<0.01$).

Effect of Quinidine on Calcium Uptake and Calcium Efflux

Quinidine, 10^{-3} M, failed to alter calcium uptake by the turtle bladder (control 16.6±1.7, quinidine 17.3±1.9 nmol/mg protein per 5 min, $n=6$, NS). Figure 3 shows calcium efflux by control hemibladders and by hemibladders treated with 10^{-3} M quinidine at 5 min. Addition of quinidine promoted a significant efflux of calcium as compared to control hemibladders. The effect of quinidine on calcium efflux was also examined in the toad bladder. Identical results to those depicted in Fig. 3 were observed.

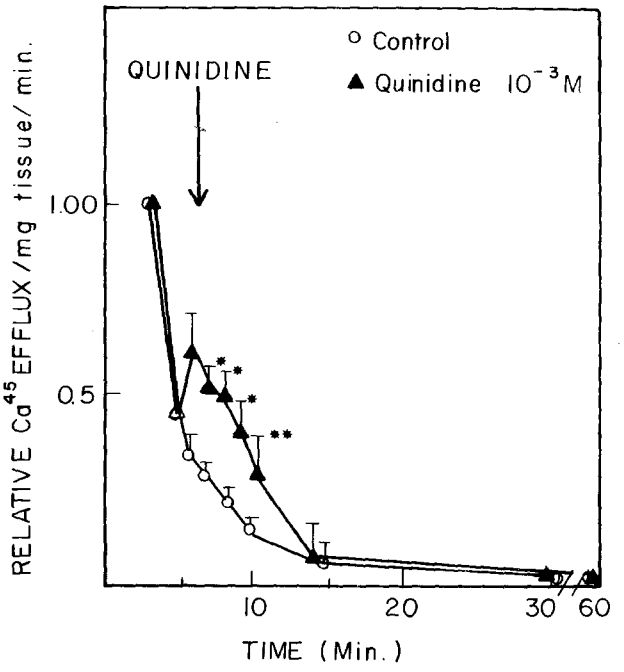

Fig. 3. Shows ^{45}Ca efflux in control and quinidine, 10^{-3} M, treated turtle hemibladders. Quinidine was added at 5 min and caused a significant increase in calcium efflux ($n=6$).

Discussion

The bladders of the fresh water turtle and the toad are capable of Na transport and urinary acidification *in vitro* [1, 2, 15, 18–22, 24]. The rate of Na transport

which can be accurately assessed by the SCC [15], is influenced by the availability of substrate and oxygen, and mucosal Na concentration. When Na transport is totally inhibited by ouabain the SCC reverses and the mucosa becomes positive in relation to the serosa [19]. This reverse SCC has been shown to be the equivalent of the rate of H^+ secretion measured with the pH stat technique [1, 19]. The reverse SCC is thus an accurate measure of H^+ secretion. The rate of H^+ secretion by the turtle bladder is influenced by the availability of substrate and CO_2, electrochemical gradients and aldosterone [21].

The present experiments demonstrate that quinidine exerted a profound inhibitory effect on the SCC. Thus inhibitory effect was dose dependent, not dependent on extracellular calcium concentration, and not readily reversible by removal of quinidine. The decrease in SCC was accompanied by a proportionally smaller decrease in PD leading to an increase in resistance of the membrane. Similar to its effect on Na transport, quinidine also inhibited H^+ secretion by the turtle bladder. The inhibitory effect of quinidine was dose dependent, not dependent on extracellular calcium concentration, and not readily reversible by either removal of quinidine or stimulation of H^+ secretion with 5% CO_2 in the serosal phase.

In the turtle bladder the rate of H^+ secretion in the short-circuit state is independent of the rate of Na transport provided CO_2 is available [18–21]. In the present experiments H^+ secretion was measured in the presence of CO_2 and substrate and in the absence of electrochemical gradients. Changes in these parameters or in Na transport cannot therefore account for the inhibitory effect of quinidine on H^+ transport. Among the known possibilities the inhibitory effect of quinidine on Na or H^+ transport could be due to its effect on intracellular calcium, or to its anesthetic properties. It is unlikely that the effects of quinidine on Na or H^+ transport is the result of anesthetic action since tetracaine, a substance known for its anesthetic properties, when used in comparable concentrations to quinidine failed to alter Na or H^+ transport.

The results of the present experiments demonstrate that quinidine fails to inhibit calcium uptake but enhances calcium efflux. Release of calcium from intracellular organelles with a consequent increase in cytosolic calcium is associated with an increase in the rate of calcium efflux. Thus these observations suggest that in the turtle bladder, as in the muscle, quinidine increases cytosolic calcium concentration and that the latter event is responsible for the inhibition of Na and H^+ transport [4, 5, 8, 10, 12]. An increase in cytosolic calcium concentration induced by the ionophore A23187 has been reported to result in a decrease in Na and H^+ transport [1, 2, 24]. The mechanism whereby an increase in cytosolic calcium elicits these effects is not known.

We attempted to further clarify the mechanism whereby quinidine inhibits Na and H^+ transport with the use of dinitrophenol. Dinitrophenol uncouples oxidative phosphorylation and thus decreases the rate of Na and H^+ transport [22, 23]. We reasoned that of quinidine inhibited Na and H^+ transport by uncoupling oxidative phosphorylation, then it should have no effect on Na and H^+ transport in bladders pretreated with dinitrophenol. The results presented demonstrate that quinidine further inhibits Na and H^+ transport in bladders pretreated with dinitrophenol, suggesting that the effect of quinidine on Na and H^+ transport is not likely the result of uncoupling of oxidative phosphorylation. The results with dinitrophenol and quinidine, however, have to be interpreted with caution since it is possible that quinidine with dinitrophenol produced further uncoupling of oxidative phosphorylation and thereby further reduced H^+ and Na transport. It should be emphasized that the effect of quinidine on oxidative phosphorylation remains controversial; in the heart quinidine has been reported by some investigators [6, 13] either to uncouple oxidative phosphorylation, whereas other have failed to demonstrate such an effect [13]. This issue is further complicated by the possibility that an increase in cytosolic calcium may in itself uncouple oxidative phosphorylation [16, 23], and it is therefore difficult to ascertain whether quinidine previously inhibited calcium transport and then led to uncoupling or whether it caused uncoupling and then secondarily led to an increase in cytosolic calcium. Additional studies are necessary to elucidate this issue.

In addition to its inhibitory effect on Na and H^+ transport, quinidine also inhibited vasopressin and cyclic AMP-stimulated water flow in the toad bladder. The demonstration that quinidine inhibits both vasopressin as well as cyclic AMP-stimulated water flow indicates that quinidine also acts at a step beyond the generation of cyclic AMP. It should be emphasized that quinidine caused a greater inhibition of vasopressin than of cyclic AMP-stimulated water flow, suggesting that the main inhibitory effect precedes the generation of cyclic AMP. It would be tempting to speculate that the inhibitory effect of quinidine on water flow is also the result of an increase in intracellular calcium concentration. A similar inhibition of vasopressin-stimulated water flow has been observed when bladders were preincubated with the ionophore A23187, an agent which increases intracellular calcium concentration [9]. These observations suggest that a rise in intracellular calcium

modulates the response of the toad bladder to vasopressin and cyclic AMP.

It is important to point out that although a common mechanism, i.e., a rise in cytosolic calcium, is being postulated to explain the inhibition of Na, H^+ and water transport by quinidine, we do not know the reason for the different sensitivities of Na, H^+, and water transport to the same concentration of quinidine (Figs. 1 and 2 and Table 1).

In conclusion, quinidine, an agent which increases cytosolic calcium concentration, inhibits Na transport and urinary acidification by the turtle bladder and also inhibits vasopressin-stimulated water flow in the toad bladder. The data suggest that these findings are mediated through a rise in intracellular calcium.

The authors wish to thank George Dytko, Roger Mola, and Jell Hseih for technical assistance.

This research was supported in part by the following grants: VA Central Office Grant 7083. VA Basic Institutional Support Grant 3324, U.S. Public Health Service Grants AM20170 and AM22099, and Chicago Heart Association Grants A79-30 and A79-28.

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