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ISOPROTERENOL AND NUCLEOTIDE INDUCED STIMULATION OF Ca^{2+} UPTAKE BY MICROSOMAL FRACTIONS FROM KIDNEY AND ISOLATED GLOMERULI

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Abstract— Renal cortical microsomal vesicles possess an ATP-dependent Ca^{2+} uptake system which is two to three times more active in accumulating Ca^{2+} than are the microsomes prepared from the outer medulla or papilla of the cat kidney. The microsomal Ca^{2+} uptake system was unaffected by sodium azide, and electron microscopy confirmed the absence of intact mitochondria. Ca^{2+} accumulating activity was significantly increased by 13 per cent in cortical microsomes prepared from kidneys which had been perfused with isoproterenol (2×10^{-7} M), whereas medullary or papillary microsomal Ca^{2+} accumulation was unaffected. Perfusate containing a higher isoproterenol concentration (2×10^{-6} M) stimulated cortical as well as papillary microsomal Ca^{2+} uptake by 13 and 31 per cent respectively, but had no effect on medullary microsomal Ca^{2+} accumulation. A lower isoproterenol concentration (2×10^{-8} M) did not change the Ca^{2+} uptake activity of microsomes isolated from either region of the cat kidney. The isoproterenol concentrations (2×10^{-7} and 2×10^{-6} M) which activated Ca^{2+} uptake in microsomes produced graded increases in renin secretion, whereas 2×10^{-8} M isoproterenol was relatively inactive in eliciting renin secretion. Renal cortical tissue exposed to cyclic AMP (cAMP) and 5′-AMP during subcellular fractionation showed significant increases in microsomal Ca^{2+} uptake. However, microsomes exposed to cAMP or 5′-AMP in the Ca^{2+} uptake medium were not stimulated. Isoproterenol also activated Ca^{2+} uptake by microsomes prepared from isolated glomeruli, and this stimulation was blocked by propranolol. We conclude that the cat renal cortex possesses specific receptors for isoproterenol which activate Ca^{2+} transport through a nucleotide mediated mechanism.

Catecholamines mediate renin secretion and regulate renal tubular function by activation of beta-adrenergic receptors [1, 2]. Beta-adrenergic stimulation also activates renal adenylate cyclase and increases the level of cAMP in kidney tissue slices [3, 4], suggesting that cAMP mediates the biological effects of catecholamines in the kidney. Exogenous cAMP added to renal cell suspensions [5] or infused into the renal artery [6] also stimulates renin secretion. Ca^{2+} may also mediate renal beta-adrenergic stimulation and renin secretion, since exogenous catecholamines or sympathetic nerve stimulation affect the mobilization of intracellular Ca^{2+} in the perfused cat kidney [7].

The interaction of Ca^{2+} with renal membranes has been described as being an ATP-dependent Ca^{2+} binding or uptake system in microsomal membranes prepared from rabbit and rat renal cortex [8, 9]. Cat renal cortical microsomes isolated from hormonally stimulated tissue have an increased capacity to accumulate Ca^{2+} [10]. Since, in the liver, adrenal cortex and aorta, Ca^{2+} uptake into microsomes is stimulated by hormones, cAMP and other nucleotides [11, 12], the microsomal membranes isolated from renal tissue appear to resemble microsomes isolated from other tissues in that they contain an activatable Ca^{2+} pumping system capable of participating in the regulation of cell Ca^{2+} levels.

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The present study describes and compares the Ca^{2+} uptake system in microsomal fractions from inner and outer medullary and cortical regions of the cat kidney, and reports that in two of these fractions Ca^{2+} accumulation is responsive to the beta-receptor agonist, isoproterenol. In addition, the stimulation of Ca^{2+} uptake by renal cortical microsomes isolated from tissue homogenates containing exogenous nucleotides is described. Finally, we also report the Ca^{2+}accumulating activity of a renal microsomal preparation from isolated glomeruli, which provides information on a Ca^{2+} uptake system derived from nontubular elements.

MATERIALS AND METHODS

Renal perfusion. Cats (2–4 kg) were anesthetized with pentobarbital, and left and right kidneys were perfused with Locke's solution as described previously [7]. All perfusions were done at room temperature using bicarbonate-buffered Locke's solution containing 3.0% dextran. Paired kidneys were perfused for 100 min, and then samples of perfusate were collected for four 10-min intervals. In certain experiments, one of the paired kidneys was exposed to isoproterenol during the final two 10-min intervals of perfusion; this period of exposure to isoproterenol was selected because its stimulant action on renin secretion reaches peak levels within 20 min [13].

Preparation of subcellular fractions. At the end of the 20-min perfusion period (see above), kidneys were decapsulated at 4° and, beginning at the capsular margin, were sectioned inward to isolate 4–5 mm of cortex,

3–5 mm of medulla, and the papilla. Each renal zone was then minced separately and 0.6 to 1 g of tissue was homogenized at 4° in 0.25 M sucrose using 6–14 strokes of a Potter–Elvehjem homogenizer with a Teflon pestle (0.18 mm clearance). The homogenate was filtered through monofilament nylon mesh (100 μm) and sequentially centrifuged at 4° at 1475 *g* (10 min), 15,000 *g* (10 min) and 27,000 *g* (30 min). In certain experiments, the minced tissue was incubated for 15 min with isoproterenol, cAMP, 5′-AMP, or NaCl in 0.25 M sucrose, at 4°, prior to homogenization which was then carried out in the presence of the agent. In other experiments, renal cortical tissue was minced, homogenized, and then cAMP , 5′-AMP, or NaCl was added immediately to the homogenate. The 15,000 *g* (mitochondrial) fraction and 27,000 *g* (microsomal) fraction were resuspended in ice-cold 0.25 M sucrose to attain a final protein concentration of 0.6 mg/ml. Protein was assayed by the method of Lowry *et al.* [14].

Isolation of glomeruli. Cat glomeruli were isolated by a modification of the method of Meezan *et al.* [15]. Following renal perfusion as described above, a 40–60 ml suspension of magnetic iron oxide (15 mg/ml) [15] was infused through the cat renal artery. The renal capsule was removed and the kidneys were hemisected. The cortex was minced in ice-cold Locke's solution, forced through a stainless steel sieve (30 mesh, 500 μm), and then rinsed with Locke's solution. The tissue suspension was then sequentially passed through a 60-mesh (250 μm), 100-mesh (155 μm) and 230-mesh (63 μm) sieve. Tissue retained on the 230-mesh sieve was transferred to a small beaker which was placed atop a 2 kg magnet. The suspended tissue was swirled and allowed to settle; after the supernatant fraction was aspirated, the suspension was washed several times. The final tissue precipitate, which took 40–60 min to isolate, was designated as the glomerular preparation; it was resuspended in Locke's solution at a concentration of 5×10^5–8×10^5 glomeruli per 10 ml, and incubated for 30 min at 37°. In certain experiments, agents were added to the glomeruli during the last 20 min of incubation. Following the incubation, the glomeruli were homogenized in Locke's solution, filtered, centrifuged, and resuspended in 0.25 M sucrose as described above. In other experiments, intact glomeruli were placed in a 1 ml plastic syringe plugged with glass-wool and superfused with Locke's solution containing 0.2% bovine serum albumin (0.4–0.6 ml/min). The superfusate was collected at 10-min intervals and assayed for renin as described below.

Assay for Ca^{2+}*accumulation.* Ca^{2+} accumulation by 0.4 or 0.2 ml aliquots of subcellular fractions of homogenates of kidney was measured by a Millipore filtration technique as originally described by Moore *et al.* [16] for liver microsomes. The incubation medium contained (mM): imidazole–histidine buffer (pH 6.8), 30; KCl, 100; $MgCl_2$, 5; Na_2ATP in 100 mM Tris buffer (pH 6.8), 5; NaN_3, 5; potassium–oxalate, 5; $CaCl_2$, 0.01; and $^{45}CaCl_2$, in a final concentration of 0.1 μCi/ml. In some assays, 5 mM cAMP or NaCl was included. Ca^{2+} accumulation was initiated by addition of subcellular fraction protein. Equal protein concentrations were used in the assay of microsomal fractions from cortex, medulla and papilla. Aliquots (0.5 ml) of the $^{45}Ca^{2+}$ incubation medium were removed at timed intervals and the $^{45}Ca^{2+}$ accumulated by microsomal vesicles was collected by filtration of the assay medium through a Millipore filter under slight negative pressure. The filters were rinsed with 2 ml of isotonic sucrose and counted in ACS Scintillar by liquid scintillation spectrometry. Ca^{2+} accumulation is expressed as nmoles of Ca^{2+}/mg of protein, and represents total Ca^{2+} accumulation, including ATP-independent Ca^{2+} binding in membranes and/or filters.

Determination of renin. Samples of renal perfusate or superfusate were converted to angiotensin I, and renin activity was determined by radioimmunoassay, using renin substrate obtained from plasma of anesthetized cats whose renal vessels had been ligated for several hours prior to exsanguination [13].

Statistical analysis. Analysis of variance (split plot analysis) was used to assess the significance of differences in Ca^{2+} uptake over time by microsomes from control and isoproterenol-treated kidneys or glomeruli. The paired *t*-test was applied to evaluate significance of differences at a single time point by comparing Ca^{2+} uptake in microsomes from isoproterenol-treated kidney, glomeruli, or homogenate, as a percent of the uptake by microsomes from the control kidney of the same animal, or the control glomeruli or homogenate of the same kidney. Significance of difference is indicated in the text as values of probability (P).

Chemicals and reagents. Isoproterenol (Sigma Chemical Co., St. Louis, MO) and propranolol (a generous gift from Ayerst Laboratories, New York, NY) were stored in powder form and dissolved in appropriate medium just prior to use. $^{45}CaCl_2$ (20 μCi/μg) and [^{125}I]angiotensin I (500 μCi/μg) were obtained from New England Nuclear, Boston, MA. Cyclic AMP and 5′-AMP dihydrate (yeast) were used as the sodium salt in aqueous medium. Organic compounds, purchased from the Sigma Chemical Co., were of the highest purity available.

RESULTS

Calcium uptake by microsomal fractions of renal cortex, medulla and papilla. Figures 1 and 2 demonstrate the calcium accumulating activity of microsomal vesicles isolated from the cortex, medulla and papilla of the cat kidney. Ca^{2+} uptake in the different preparations of microsomal vesicles increased linearly during the 60 min of incubation, with the cortical microsomes manifesting the greatest Ca^{2+} accumulating activity. After 60 min, Ca^{2+} uptake by cortical microsomes was approximately two and three times greater than Ca^{2+} uptake by microsomes from medulla and papilla, respectively.

Calcium uptake by isolated renal microsomes was demonstrable only in the presence of ATP. In the absence of ATP, Ca^{2+} uptake by cortical, medullary and papillary microsomes was only 5 per cent of the uptake obtained after 60 min in the presence of ATP. Ca^{2+} uptake was also abolished by removing oxalate from the incubation medium. The mitochondrial inhibitor, sodium azide, failed to alter the Ca^{2+} accumulating activity of cortical microsomes, as evidenced by the fact that microsomes incubated for 60 min in the absence of azide accumulated 99.8 per cent of the Ca^{2+} accumulated by microsomes incubated in the presence of azide.

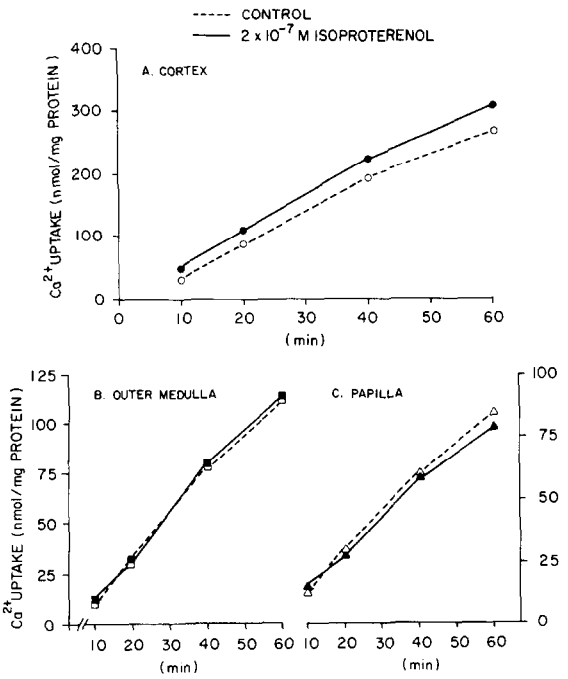

Fig. 1. Effect of isoproterenol (2×10^{-7} M) on Ca^{2+} uptake by cat renal microsomes isolated from (A) cortex, (B) outer medulla, and (C) papilla. Paired kidneys were perfused for 20 min in the presence or absence of isoproterenol. Microsomes prepared from each area of the kidney by centrifugation at 27,000 *g* were incubated at 37° with complete assay medium. Ca^{2+} accumulation was assayed as described in Materials and Methods. Each point represents the mean value from four different preparations.

In contrast, mitochondrial-enriched (15,000 *g*) cortical fractions had a lower Ca^{2+} accumulating ability compared to the microsomal fractions, and sodium azide depressed the Ca^{2+} uptake activity of the mitochondrial fraction to 19 per cent of that measured in the absence of azide. Electron microscopic examination showed that the 27,000 *g* fraction was composed almost entirely of rounded membrane vesicles, a few ribosomes,

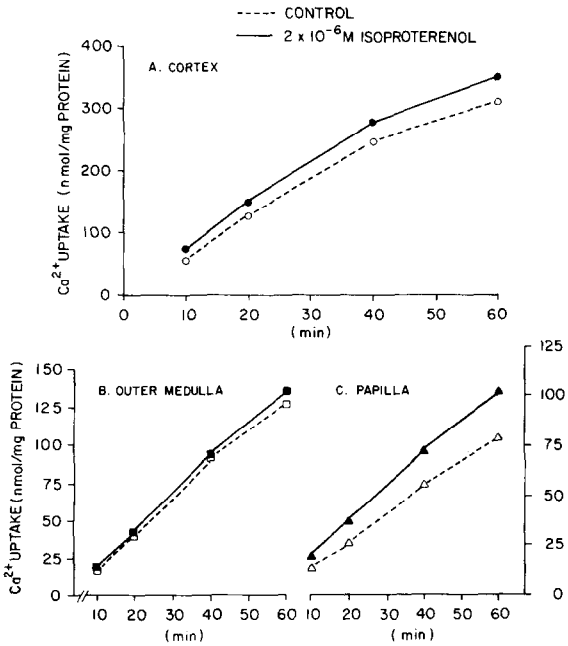

Fig. 2. Effect of isoproterenol (2×10^{-6} M) on Ca^{2+} uptake by renal microsomes isolated from (A) cortex, (B) outer medulla, and (C) papilla. Experiments were carried out as described in the legend of Fig. 1.

some tubular structures and Golgi bodies, but no intact mitochondria, confirming the absence of an azide effect in this fraction.

Effect of isoproterenol on Ca^{2+} *uptake.* Cortical microsomes prepared from homogenates of kidneys perfused with isoproterenol (2×10^{-7} M) showed enhanced Ca^{2+} accumulating activity (Fig. 1A). Analysis of variance demonstrated that the Ca^{2+} uptake curves generated from control and isoproterenol-treated kidney microsomes were parallel ($P < 0.0001$) and were significantly different ($P < 0.05$). By contrast, isoproterenol failed to augment Ca^{2+} uptake in medullary or papillary microsomes ($P > 0.5$) (Fig. 1, panels B and C). Isoproterenol (2×10^{-6} M) elicited a similar increase in Ca^{2+} accumulating activity by cortical microsomes ($P < 0.05$) (Fig. 2A); however, this higher isoproterenol concentration was less specific in its stimulatory effects than the lower concentration (2×10^{-7} M), since it also produced a highly significant increase in Ca^{2+} uptake by papillary microsomes ($P < 0.002$) (Fig. 2C). This higher isoproterenol concentration had no discernible effect on medullary microsomes ($P > 0.5$) (Fig. 2B). Thus, isoproterenol (2×10^{-7} and 2×10^{-6} M)-treated kidney cortical microsomes showed a significant increase of 13 per cent above control Ca^{2+} uptake values after 60 min of incubation (Table 1), and isoproterenol (2×10^{-6} M) produced a greater increase (31 per cent) in Ca^{2+} uptake by papillary microsomes (Table 1). By contrast, a lower isoproterenol concentration (2×10^{-8} M) failed to elicit an increase in Ca^{2+} accumulation in any of the three microsomal fractions (Table 1). Isoproterenol (5×10^{-6}–5×10^{-7} M) when added to minced renal medullary or cortical tissue in 0.25 M sucrose at 4°, 15 min prior to homogenization, failed to enhance the Ca^{2+} accumulating ability of microsomes isolated from the treated tissue (data not shown).

The isoproterenol concentrations (2×10^{-7} and 2×10^{-6} M) which activated Ca^{2+} uptake in microsomes produced graded increases in renin secretion over a 20-min perfusion period, whereas the isoproterenol concentration (2×10^{-8} M) which failed to elicit any increase in Ca^{2+} uptake elicited only a marginal increase in renin release (Fig. 3A).

Table 1. Effect of isoproterenol on Ca^{2+} uptake by microsomal fractions from cortex, medulla and papilla*

Isoproterenol	Cortex	Medulla	Papilla
2×10^{-8}	99 ± 3	107 ± 7	97 ± 5
2×10^{-7}	113 ± 2†	103 ± 15	95 ± 8
2×10^{-6}	113 ± 2†	119 ± 22	131 ± 5†

* Paired kidneys were perfused with Locke's solution for 20 min in the presence or absence of isoproterenol. The kidneys were sectioned into three regions and separately homogenized, and microsomal vesicles were prepared. Ca^{2+} uptake by microsomes from isoproterenol-perfused kidneys was determined after 60 min and is represented as a percentage of the microsomal fraction prepared from the corresponding unstimulated kidney. Each value is the mean (± S.E.) of four separate experiments. See Figs. 1 and 2 for absolute values.

† Significantly different from control, using paired Student's *t*-test ($P < 0.01$).

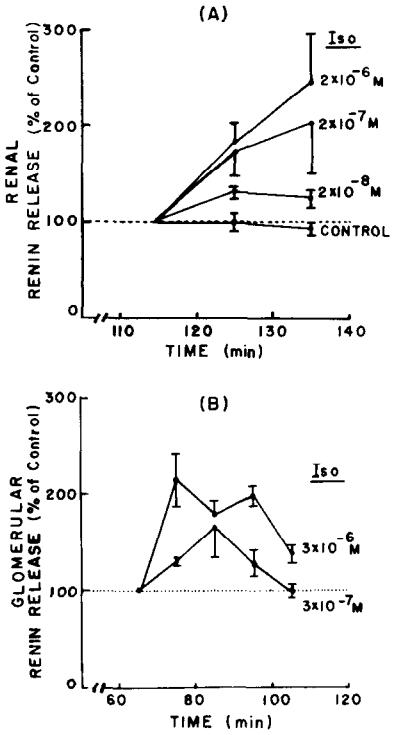

Fig. 3. Effect of isoproterenol on renin release from (A) perfused kidneys and from (B) superfused glomeruli. Panel A: kidneys were perfused with Locke's solution for 110 min and then varying concentrations of isoproterenol were added to the perfusion medium for an additional 20 min. Panel B: glomeruli were superfused with Locke's solution for 110 min. Isoproterenol was added during min 70–90 of perfusion. Each point represents the average rate of renin release (± S.E.) during a 10-min collection period calculated from at least four different preparations. Renin release is expressed as a percentage of the output rate during the control collection period immediately preceding exposure to isoproterenol. The mean values for basal renin release were (A) 178 (± 56) ng/min and (B) 26 (± 0.5) ng/min.

Effect of nucleotides on Ca^{2+} *uptake.* Renal cortical tissue was incubated with cAMP and 5′-AMP in order to determine whether the isoproterenol stimulation of Ca^{2+} uptake could be reproduced by other agents. Tissue which had been exposed to 5×10^{-3} M cAMP or 5′-AMP prior to and during homogenization, or immediately following homogenization, showed significant increases in renal cortical microsomal Ca^{2+} uptake during 60 min of incubation. Table 2A shows that tissue treated with cAMP or 5′-AMP prior to homogenization produced microsomes which had a 47 and 48 per cent greater Ca^{2+} accumulating ability, respectively, than control Ca^{2+} uptake values. However, when tissue was treated with NaCl prior to homogenization in order to evaluate the possible effects of the nucleotide salt content on Ca^{2+} uptake properties, microsomes isolated from the NaCl-treated tissue showed a significant increase of 26 per cent above the untreated microsomal Ca^{2+} accumulation (Table 2A). The nucleotide-stimulated Ca^{2+} uptake in microsomes isolated from renal cortical tissue exposed to cAMP or 5′-AMP prior to homogenization was significantly greater than the NaCl-stimulated Ca^{2+} uptake values of similarly treated microsomes after 60 min of incubation. Analy-

Table 2. Effect of nucleotides on Ca^{2+} uptake by cat renal microsomes

(A)

Agent	Ca^{2+} uptake* (nmoles Ca^{2+}/mg protein)	% of control†	P‡
None	202	100	
cAMP	289	147 ± 8	< 0.02
5′-AMP	278	148 ± 15	< 0.01
NaCl	251	126 ± 5	< 0.05

Cat renal cortical tissue was preincubated for 15 min, at 4° in 0.25 M sucrose, or sucrose containing 5 mM cAMP, 5′-AMP or NaCl. The tissue was homogenized and Ca^{2+} uptake by the isolated microsomes was determined as described in Materials and Methods. Values for NaCl-treated tissue were significantly ($P < 0.05$) different from values for nucleotide-treated tissue ($n = 4$).

(B)

Agent	Ca^{2+} uptake* (nmoles Ca^{2+}/mg protein)	% of control†	P‡
None	167	100	
cAMP	227	133 ± 7	< 0.02
5′-AMP	211	124 ± 7	< 0.05
NaCl	186	109 ± 4	> 0.3

Cat renal cortical tissue was homogenized in 0.25 M sucrose, at 4° and 5 mM cAMP, 5′-AMP or NaCl was immediately added to the crude homogenate. The microsomal fraction from control or treated homogenates was isolated and the Ca^{2+} uptake activity was determined as described in Materials and Methods ($n = 5$).

* Data shown represent mean values after 60 min of incubation.

† Values are means ± S.E. and are represented as a percentage of Ca^{2+} uptake by corresponding microsomal fractions prepared from untreated homogenates.

‡ Significance (P) was determined using paired Student's *t*-test.

sis of variance of the data indicated that microsomes from cAMP- and 5′-AMP-, but not NaCl-treated tissue had Ca^{2+} uptake values significantly ($P < 0.01$) higher than control values throughout the 60 min of incubation. When cortical tissue was exposed to 5×10^{-4} M cAMP or 5′-AMP prior to and during homogenization, the resulting microsomal Ca^{2+} uptake values were not different from control (data not shown).

Renal cortical tissue was also exposed to cAMP and 5′-AMP immediately following homogenization, and the resultant microsomes had a higher Ca^{2+} accumulating ability than did microsomes not exposed to the nucleotides (Table 2B). After 60 min of incubation, cAMP- and 5′-AMP-treated microsomes had 33 and 24 per cent higher Ca^{2+} uptake values, respectively, than did their corresponding controls (Table 2B). When NaCl was added to the renal cortical tissue homogenate, however, there was no significant increase in the ability of the resulting microsomes to accumulate Ca^{2+} (Table 2B). Microsomes from both cAMP- and 5′-AMP-treated homogenates had significantly higher ($P < 0.05$) Ca^{2+} uptake activity than microsomes from NaCl-treated homogenates. When control microsomes from untreated renal cortical tissues were incubated in Ca^{2+} uptake medium containing 5×10^{-3} M cAMP, 5′-AMP or NaCl, no increase in Ca^{2+} uptake activity was observed during 60 min of incubation (data not shown).

Ca^{2+} uptake by microsomes prepared from isolated glomeruli. The technique of isolating glomeruli by a combination of graded sieving and magnetic iron oxide allows the preparation of 99–100 per cent pure glomeruli. Glomeruli were homogeneous in appearance, and completely free of tubular elements.

Microsomes prepared from isolated glomeruli actively accumulated Ca^{2+} in a time-dependent manner (Fig. 4 panels A and B). After 40 min of incubation, glomerular microsomes accumulated 16(±3) nmoles Ca^{2+}/mg of protein (Fig. 4A), which was less than 10 per cent of the amount of Ca^{2+} accumulated by cortical microsomes over the same time interval. Isoproterenol (10^{-6} M) significantly enhanced Ca^{2+} accumulation by glomerular microsomes during a 40 min incubation when statistical significance was assessed by analysis of variance ($P < 0.01$) (Fig. 4A). Similarly, when Ca^{2+}

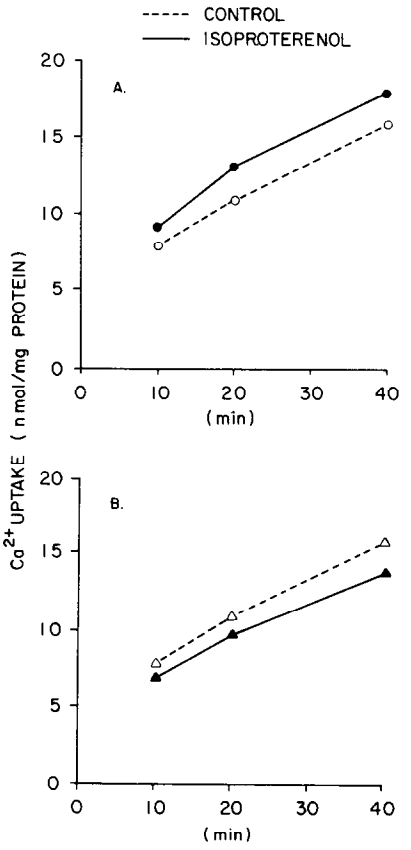

Fig. 4. Isoproterenol stimulation of Ca^{2+} accumulation by a microsomal fraction from isolated glomeruli and inhibition of this effect by propranolol. Glomeruli were isolated from cortical tissue and incubated for 20 min in Locke's solution in the presence or absence of isoproterenol (10^{-6} M). In panel B, propranolol (10^{-6} M) was added to both control and isoproterenol-treated fractions. The glomeruli were then homogenized, and isolated microsomal fractions were prepared and Ca^{2+} uptake was determined. Each point is the mean derived from four different preparations.

uptake by glomerular microsomes exposed to isoproterenol was compared with Ca^{2+} uptake by untreated microsomes from the same preparation after a 10-min incubation, an average increase of 15 ($\pm$ 3) per cent was observed which was highly significant ($P < 0.01$), as determined by the paired Student's *t*-test ($n = 4$). Propranolol, the beta-adrenergic receptor blocking agent, while not markedly affecting basal Ca^{2+} accumulation, completely abolished the stimulation of Ca^{2+} uptake induced by isoproterenol (Fig. 4B).

The viability of these isolated glomeruli was demonstrated by the ability of isoproterenol (3×10^{-7} to 3×10^{-6} M) to elicit a dose-dependent increase in renin release (Fig. 3B). In fact, with the higher isoproterenol concentration, secretion had a biphasic pattern (Fig. 3B), which was similar to that observed in the intact kidney during prolonged exposure to high concentrations of isoproterenol [13].

DISCUSSION

The present study demonstrates active Ca^{2+} uptake by isolated membrane vesicles (27,000 *g* fraction) prepared from various sections of the feline kidney. The lack of sensitivity to azide and the electron microscopic evidence confirmed that the microsomal Ca^{2+} accumulating activity in renal microsomes cannot be attributed to mitochondrial elements. The Ca^{2+} uptake activity of microsomal membranes from the rat kidney has been reported to be associated with the endoplasmic reticulum [9], which suggests that intracellular membranes are an important component of renal cortical microsomes. In the present study, Ca^{2+} sequestering activity was highest in cortical microsomes and lower in medullary and papillary microsomes. Various factors may contribute to the quantitative differences observed in Ca^{2+} uptake by microsomes from cortex, medulla and papilla. The kidney is morphologically a heterogeneous organ in which the cortex contains glomeruli, the juxtaglomerular apparatus and tubular elements (proximal and distal convoluted tubules) whereas the medulla and papilla (inner medulla) contain mainly tubular (Henle's loop and collecting ducts) and vascular elements [17]. Thus, each cell type upon homogenization may yield different membranes for vesicle formation. The finding that isoproterenol (2×10^{-7} M) specifically stimulated Ca^{2+} uptake in cortical, but not in medullary or papillary, microsomes isolated from the perfused kidney supports the concept of a heterogeneity of renal membrane vesicles and indicates that cortical microsomes contain specific catecholamine-sensitive receptor sites.

The observation that isoproterenol was able to stimulate Ca^{2+} uptake when perfused through the intact kidney is similar to the findings of an earlier study in which Ca^{2+} uptake was enhanced in microsomes prepared from rat adrenal cortical tissue which had been exposed to ACTH prior to homogenization [12]. In kidney, however, we were unable to enhance Ca^{2+} accumulation in microsomes after the tissue had been preincubated in ice-cold sucrose containing isoproterenol and then homogenized. The cell biochemistry, binding characteristics, or diffusibility of isoproterenol in the renal tissue under the pre-homogenization conditions may account for the absence of Ca^{2+} uptake stimulation. In any event, the lack of a stimulatory effect by isoproterenol added to the tissue prior to homogenization indicates that the enhanced Ca^{2+} uptake seen in microsomes isolated from the isoproterenol-perfused kidney is not due to differences in membrane recovery, but is the result of some enduring biochemical process activated by the interaction of the catecholamine with specific receptor sites. The same catecholamine-sensitive sites uncovered in cortical microsomes also seem to be present in glomerular membranes, as evidenced by the finding that isoproterenol, in only slightly higher concentrations than those used in the intact kidney, displayed a comparable degree of stimulation of Ca^{2+} uptake by glomerular microsomes. The fact that the stimulation of Ca^{2+} accumulation by isoproterenol was blocked by a concentration of propranolol which inhibited catecholamine-induced ^{45}Ca efflux and renin release in the intact kidney [7] confirms it was mediated by beta-adrenergic receptors. However, no firm conclusion can be drawn as to whether microsomes derived mainly from juxtaglomerular cells [7, 13] or from vascular smooth muscle elements [18, 19] account for isoproterenol stimulation of Ca^{2+} binding in these glomerular preparations. It should also be noted that Ca^{2+} uptake in glomerular microsomes was much lower than that found in similarly prepared membranes from intact kidney; this difference may be due to the absence of Ca^{2+} accumulation by tubular fragments and to the prolonged time and rather harsh preparative methods required to isolate the glomeruli. Glomeruli isolated by infusion of magnetic iron oxide do not, however, manifest marked alterations in metabolic activity [20], and their viability was readily demonstrated in the present study by their ability to secrete renin in response to isoproterenol.

Our observations that cAMP and 5′-AMP stimulate renal cortex microsomal Ca^{2+} uptake are also similar to those of Laychock *et al.* [12] who found that cAMP, 5′-AMP and other nucleotides stimulate the Ca^{2+} uptake system in the adrenal cortex when the tissue is exposed to the nucleotides during subcellular fractionation. Why millimolar concentrations of nucleotides are required to stimulate renal, as well as adrenocortical [12], microsomal Ca^{2+} accumulating mechanisms is not known, but the fact that 5′-AMP and cAMP are required in high concentrations suggests that phosphodiesterase activity is not a limiting factor in maintaining the nucleotide levels in the homogenate. In addition, the ability of cAMP to stimulate microsomal Ca^{2+} uptake when added to the tissue after homogenization shows that the nucleotide activation is independent of the intact cell. On the other hand, the lack of effect of cAMP or 5′-AMP on Ca^{2+} uptake when added to isolated renal microsomes has also been observed in adrenal and aortic microsomes [12], and may be an indication of the fact that the enhancement of Ca^{2+} uptake in microsomes by nucleotides involves protein kinase activity which is present in intact tissue and the crude homogenate, but is relatively absent from the isolated microsomal preparation [11]. Although it is beyond the scope of the present study to account for the mechanisms by which nucleotides and isoproterenol stimulate Ca^{2+} uptake, recent studies have demonstrated that the addition of cAMP and protein kinase to microsomes from cardiac sarcoplasmic reticulum [21], aorta [11] and platelets [22] stimulates Ca^{2+} uptake,

presumably by a mechanism involving protein phosphorylation. Furthering the hypothesis that phosphorylation participates in the regulation of Ca^{2+} transport, Kirchberger and Raffo [23] demonstrated that phosphoprotein phosphatase activity determines the degree of phosphorylation of membrane proteins, which, in turn, is associated with changes in Ca^{2+} transport. It has been reported that millimolar concentrations of several nucleotides, in addition to AMP, stimulate phosphoprotein phosphatase activity [24]. Even salts such as NaCl can activate this enzyme [24]. Perhaps, in homogenates of renal cortex as well as adrenal cortex [12], high concentrations of nucleotides stimulate Ca^{2+} uptake through a complex process involving protein phosphorylation and dephosphorylation. Thus, the question as to whether the effects of nucleotides as well as isoproterenol on Ca^{2+} uptake activity in renal microsomes may be mediated by phosphorylation will be a subject for further study. Unlike the effect of nucleotides on Ca^{2+} uptake, however, the small increase in Ca^{2+} accumulation by microsomes isolated from cortical tissue exposed to NaCl prior to homogenization may be due to an effect upon the biochemistry of the intact cell or upon microsomal vesicle formation, since the effect of NaCl was lost when it was introduced to the vesicles following homogenization.

It has also been suggested that the increase in cAMP levels of tissues stimulated by beta-adrenergic agents [25] mediates effects on Ca^{2+} accumulating activity via phosphorylation mechanisms [26]. Beta-adrenergic agents stimulate cAMP formation in various segments of the kidney [3, 4], and the increased cAMP levels in turn could affect Ca^{2+} sequestration in the cell. Consistent with this idea is our previous demonstration that parathyroid hormone, which is also known to increase renal cAMP [3, 27], is able to stimulate Ca^{2+} uptake by feline cortical and papillary microsomes [10]. Moreover, parathyroid hormone, which is more efficacious as an activator of renal adenylate cyclase than isoproterenol [28, 29], is similarly more effective in stimulating Ca^{2+} uptake by renal microsomes [10]. An additional correlation between cAMP and Ca^{2+} uptake is indicated by the inability of angiotensin II, which is not a stimulator of adenylate cyclase [30–32], to increase renal microsomal Ca^{2+} uptake [10].

The functional correlation observed between isoproterenol-stimulated Ca^{2+} uptake and renin release prompts speculation that isoproterenol-activated Ca^{2+} accumulation by cortical microsomes may have physiological significance, since catecholamines enhance mobilization of cellular Ca^{2+} from juxtaglomerular cells of the intact kidney [7]. Ca^{2+} uptake by papillary microsomes is also stimulated by isoproterenol; this may be a reflection of the action of isoproterenol on cation and water transport in the renal tubules and collecting ducts [33–35]. The report that cAMP stimulates renal microsomal Ca^{2+} reabsorption by the intact kidney [36] provides further support for the physiological relevance of the present findings.

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