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Angiotensin-Converting Enzyme Inhibition-Induced Changes in Hippurate Renography and Renal Function in Renovascular Hypertension

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We studied the mechanism of angiotensin-converting enzyme (ACE) inhibition-induced changes in hippurate renography of the poststenotic kidney. **Methods:** Ten male mongrel dogs, six with unilateral and four with bilateral renal artery stenosis, were equipped with renal artery blood flow probes and catheters in the aorta, atrium and both renal veins. **Results:** Enalaprilat (10 mg intravenously) in conscious dogs with renal artery stenoses produced changes in all stenotic (n = 11) but not in nonstenotic kidney ^{123}I-hippurate renograms (n = 6). Renographic changes correlated significantly with initiation of intrarenal ^{131}I-hippurate retention, a decrease in mean arterial pressure (MAP), renal extraction of ^{131}I-hippurate and ^{125}I-iothalamate (r = 0.68, r = 0.62, r = 0.84, r = 0.83, respectively) but not with renal blood flow changes (r = 0.34). Furthermore, renal uptake of ^{131}I-hippurate and ^{125}I-iothalamate decreased in stenotic kidneys with a grade II renogram (−52 ± 11% and −79 ± 6%, respectively). Iodine-125-hippurate autoradiograms of stenotic kidneys during ACE inhibition showed tracer retention mainly in the proximal tubular cells. Results during osmotic diuresis supported our findings. **Conclusion:** Angiotensin-converting enzyme inhibition-induced hippurate retention curves of poststenotic kidneys appear to result from a sequence of events. A decrease in MAP combined with efferent vasodilation leads to a decrease in intraglomerular capillary pressure. This decrease in pressure causes a decease in glomerular filtration rate and proximal tubular urine flow. This decrease in turn hampers tubular hippurate transit and transport across the luminal membrane, leading to intrarenal hippurate retention and, in more severe cases, decreased renal hippurate uptake.

Key Words: renovascular hypertension; glomerular filtration rate; radioisotope renography; renal artery stenosis; renin-angiotensin system

J Nucl Med 1996; 37:482–488

Renovascular hypertension, the most common form of secondary hypertension, is potentially curable by surgical and radiologic intervention, but a reliable screening test is needed. The best screening test to date appears to be hippurate renography during angiotensin-converting enzyme (ACE) inhibition (*1*). Technetium-99m-MAG3, a radiopharmaceutical with properties similar to hippurate, has been recently introduced in lieu of hippurate (*2*). ACE inhibition considerably enhances the sensitivity of hippurate renography for detection of significant renal artery stenosis because ACE inhibition induces a typical pattern of delayed time-to-peak or slowed tracer excretion in the kidney behind a narrowed renal artery (*1–4*). The mechanism for this altered tracer handling is not completely understood. Several explanations have been advanced, such as a reduced tubular extraction of hippurate (*5*) or a delay of tracer in the tubular lumen (*2,6*). A decrease in renal tracer uptake does not explain the change in shape of the renographic curve, which is most likely caused by tubular tracer delay or a combination of tubular tracer delay and reduced renal tracer uptake. To identify the mechanism of ACE inhibition-induced altered hippurate handling, it is necessary to measure simultaneously several

Received Feb. 6, 1995; revision accepted Jul. 14, 1995.

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parameters likely to determine the fate of hippurate in the kidney.

We therefore studied ACE inhibition-induced changes in renal blood flow, renal extraction of hippurate and iothalamate, and plasma and renal clearance of hippurate and iothalamate in instrumented conscious dogs with renovascular hypertension. Changes in these parameters and in total intrarenal hippurate retention were compared with changes in simultaneously performed hippurate renography.

MATERIALS AND METHODS

Animals

Ten male mongrel dogs (25–35 kg) were selected by renal angiography to exclude pre-existing renal artery stenosis, double renal arteries or insufficient length of the renal artery stem for instrumentation. After angiography, dogs were kept on a salt-restricted diet containing less than 1 g salt/day and were trained to become accustomed to the experimental situation.

Instrumentation

Instrumentation was performed in two phases under general anesthesia (induction with thiopental; maintenance anesthesia with nitrous oxide, halothane and fentanyl). First, the right and left renal arteries were exposed retroperitoneally through flank incisions. The arteries were dissected from surrounding tissue, and either an electromagnetic blood flow probe (n = 8) or a transit time flow probe (n = 3) of appropriate size was placed around the right and left renal arteries. Distal to the flow probe an externally controllable constrictor device was placed around the right (n = 8) or left (n = 3) renal artery. This constrictor device has been described in detail elsewhere (*7*). Furthermore, Tygon catheters (Norton, Akron, OH; outside diameter 1.8 mm, inside diameter 1.0 mm) were placed in the descending aorta and right atrium through the omocervical artery and vein, respectively (*8*). The leads of the flow probes, the constrictor device and the arterial and venous catheters were tunneled subcutaneously to a location high on the back of the animal. The external parts of the leads were covered by a jacket. The zero level of the flow probes was verified at the end of each experiment by intra-arterially injected angiotensin II (5–10 μg), as described elsewhere (*9*).

After a period of about 2 wk, flow measurements had stabilized, and a mild stenosis was induced during consciousness, defined as a 30% reduction in renal blood flow (RBF). In some cases, renal artery stenosis had occurred as a result of the implanted flow probe. The presence of such a stenosis, as indicated by abnormal ^{123}I-hippurate renographic changes during ACE inhibition and an increase in systemic blood pressure, was verified angiographically (see later). After a subsequent period of about 3 wk, the instrumentation was completed by bilateral renal vein catheterization. Both renal veins were exposed through a midline incision with minimal damage to the surrounding tissue. The left spermatic vein was ligated to prevent sampling of nonrenal blood. Heparin-coated Tygon tubing catheters (see above) were placed in both renal veins through and fixed onto the inferior caval vein. Catheters were tunneled as previously described. Before each experiment, the position of these catheters was verified by oxygen saturation measurements (*10*). All catheters were kept patent by refilling them daily with a fresh heparin solution (2000 IU/ml). After the experiments, stenoses were angiographically confirmed in all dogs.

Experimental Design

Before the experiments, dogs had free access to water but were deprived of food for at least 8 hr. Experiments were performed in six dogs with unilateral and four dogs with bilateral renal artery stenoses at least 4 days after implantation of the renal vein catheters. During the entire experiment, the trained dog was conscious and stood quietly in a hammock. To provide adequate urine production during the experiments, a sustained infusion of 5% glucose was administered at a rate of 250 ml/hr. Urine was collected by bladder catheterization. After a stabilization period of 110 min, control measurements were performed 40 and 20 min before ACE inhibition, followed by experimental measurements 20 and 40 min after ACE inhibition [10 mg enalaprilat intravenously, as previously described (*11*)]. Control and experimental measurements included ^{123}I-hippurate renography, blood pressure, RBF and renal extraction and plasma clearance of ^{131}I-hippurate and ^{125}I-iothalamate.

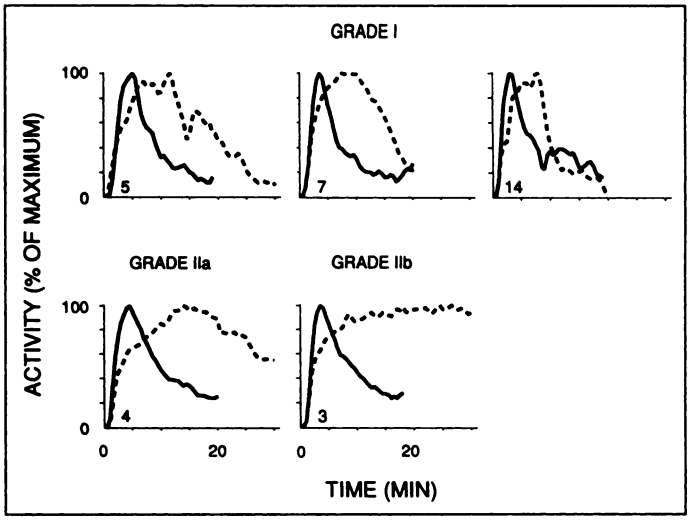

FIGURE 1. Three Grade I stenotic kidney renograms (renograms 5, 7 and 14) and grades IIa and IIb stenotic kidney renogram (renograms 4 and 3). Solid lines = before ACE inhibition; dashed lines = after ACE inhibition.

Experiments were repeated in seven dogs (four with unilateral, three with bilateral renal artery stenoses) during 5% mannitol infusion, instead of 5% glucose infusion, to induce osmotic diuresis. Between these and the previous experiments, at least 3 days were allowed for the animals to recover.

Renographic Protocol

The renographic protocol has been described in detail elsewhere (*11*). Briefly, data were recorded in 30-sec frames during a 20-min period after intravenous injection of approximately 5 MBq ^{123}I-iodohippurate with a large field of view gamma camera above the back of the animal. Kidney time-activity curves were plotted after correction for background activity and evaluated according to the recommendations of the Cleveland Consensus Meeting (*4*): grade I- a mild delay in time to peak activity and excretion; grade II- a delayed time to peak either with slow excretion within 30 min (grade IIa) or without signs of excretion within 30 min (grade IIb). Examples of the renographic curves are shown in Figure 1. Each kidney time-activity curve was scored by five independent observers who were not aware of the experimental circumstances. If disagreement occurred, the grade that received the most votes was used for further data analysis.

Renal function tracers (^{131}I-hippurate and ^{125}I-iothalamate) were infused at a constant rate after a priming dose (*12*), starting at the beginning of the stabilization period. To measure renal extraction of the tracers, arterial and renal venous blood samples were drawn, always in the same order, within 3 min of each other. Blood samples were centrifuged at 4°C (480 × g), and plasma was removed immediately to prevent diffusion of hippurate from the red cells into plasma. Because ^{123}I has a half-life of only 13 hr, interference of ^{123}I-hippurate with measurements of ^{125}I-iothalamate and ^{131}I-hippurate was prevented by delaying the

TABLE 1
Control Values of RBF, E_{hip} and E_{iot} in Nonstenotic and Stenotic Kidneys During Glucose and Mannitol Infusion

Parameter	Glucose infusion		Mannitol infusion	
	Nonstenotic kidneys (n = 6)	Stenotic kidneys (n = 11)	Nonstenotic kidneys (n = 4)	Stenotic kidneys (n = 9)
RBF (ml/min)	213 ± 37	150 ± 18	170 ± 41	160 ± 31
E_{hip}	0.84 ± 0.02	0.84 ± 0.01	0.83 ± 0.02	0.82 ± 0.01*
E_{iot}	0.33 ± 0.02	0.33 ± 0.02	0.27 ± 0.02	0.22 ± 0.02*

*$p < 0.05$ versus glucose infusion.
Values are expressed as mean ± s.e.m.
RBF = renal blood flow; E_{hip} = renal hippurate extraction; E_{iot} = renal iothalamate extraction.

measurement of ^{125}I and ^{131}I in the blood and urine by at least 72 hr.

Autoradiography

Autoradiography of water-soluble compounds was used to determine the location of hippurate retention in the kidney (*13*) and was performed in four dogs with a unilateral renal artery stenosis subsequent to the previously described experiments. The experimental design was comparable to that previously described. The dog was again placed in the hammock and received a sustained infusion of 5% glucose at a rate of 250 ml/hr. During the entire experiment, blood pressure and RBF were measured. After a stabilization period of 150 min, ACE inhibition was injected 20 min later, followed by an injection of 4 MBq ^{125}I-hippurate. The dog was subsequently killed with an overdose of barbiturate at a previously established time (such that the amount of hippurate in the stenotic kidney was maximal according to the ACE inhibition ^{123}I-hippurate renogram; i.e., 8–10 min after injection of ^{125}I-hippurate). Both kidneys (nonstenotic and stenotic) were quickly excised, and pieces of each kidney were frozen in liquid isopenthane. Autoradiograms were subsequently prepared according to the method of Rogers (*13*). Tissue sections were stained with hematoxylin and eosin.

Data Analysis

The extraction ratio of hippurate (E_{hip}) by the stenotic and nonstenotic kidneys was calculated as the difference between the arterial (A_{hip}) and renal venous ^{131}I-hippurate concentration (V_{hip}) divided by the arterial ^{131}I-hippurate concentration: $E_{hip} = (A_{hip} - V_{hip})/A_{hip}$. The same factor applied to the extraction ratio of iothalamate: $E_{iot} = (A_{iot} - V_{iot})/A_{iot}$. Thus, E_{hip} represents the fraction of hippurate taken up by the kidney. The total amount of hippurate extracted from the blood, however, should include the blood supplied to the kidney because changes in RBF can cause substantial variation in the amount of hippurate supplied to the kidney. Renal uptake of hippurate (RU_{hip}) was therefore calculated as $RU_{hip} = E_{hip} \times RBF \times (1 - Hematocrit)$ (i.e., hippurate cleared from plasma in milliliters per minute). An identical approach was used to establish the amount of iothalamate cleared from plasma (RU_{iot}). To establish whether any hippurate was removed from the blood but retained in the kidneys instead of being excreted into the urine, the effective renal plasma flow (ERPF) of both kidneys together was calculated in two ways (*6*): (a) by multiplying the hippurate concentration in the infusate (I) with the infusion rate (Vi) divided by the plasma tracer concentration (P), $ERPF_i = I \times Vi/P$; (b) by multiplying the hippurate concentration in the urine (U) with the urine flow rate (Vu) divided by the plasma concentration, $ERPF_u = U \times Vu/P$. The difference between $ERPF_i$ and $ERPF_u$ reflects the amount of hippurate retained in the kidney per dog, at least for the case that no extrarenal plasma clearance occurs. The $ERPF_i$ could also have been calculated by summing the RU_{hip} of both kidneys in each dog. In three dogs, however, only RU_{hip} from a single kidney could be calculated because of unilateral flow probe failure and renal venous catheter occlusion; therefore, we chose the method described above.

Data are expressed as mean ± s.e.m. Control values presented are the means values at 20 and 40 min before ACE inhibition. Because control values varied among kidneys and experiments, ACE inhibition-induced changes are expressed as percentage of control values. Statistical analysis was performed with the commercially available software program Instat (GraphPad Software, San Diego, CA). Differences were tested using one-way analysis of variance with a Bonferoni correction for multiple comparisons and, if appropriate, a Student's t-test for paired observations. Correlations between changes in the aforementioned parameters and changes in renographic time-activity curves were assessed by Spearman's rank correlation analysis. Differences and correlations were considered significant at a level of 5%.

RESULTS

Control Values

Induction of renal artery stenosis resulted in an increase in mean arterial pressure (MAP) in all dogs, from an average of 111 ± 7 to 133 ± 3 mmHg ($p < 0.01$). The increase was comparable in dogs with either a unilateral (22 ± 9 mmHg) or a bilateral (21 ± 11 mmHg) stenosis. Control values of MAP were comparable during glucose and mannitol infusion (n = 10, 133 ± 4 mmHg; n = 7, 130 ± 5 mmHg).

Data from poststenotic kidneys of the dogs with bilateral and

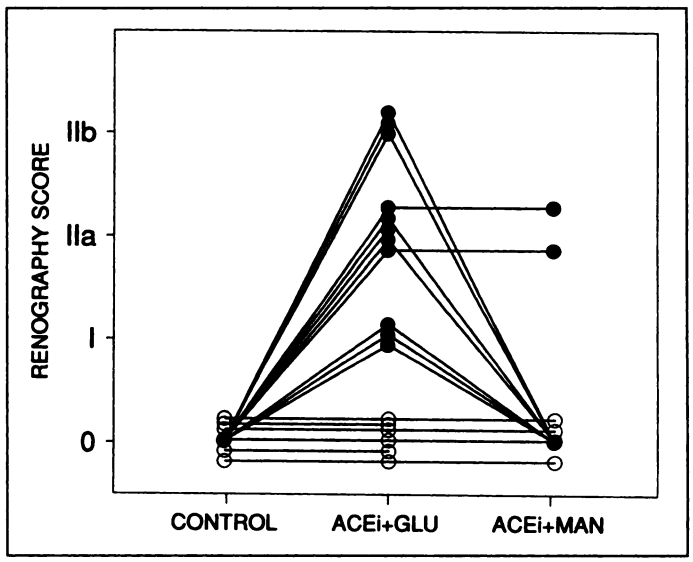

FIGURE 2. Renographic scores before (CONTROL) and after ACE inhibition during glucose (ACE inhibition + GLU) and mannitol infusions (ACE inhibition + MAN). Open circles = nonstenotic kidneys; solid circles = stenotic kidneys.

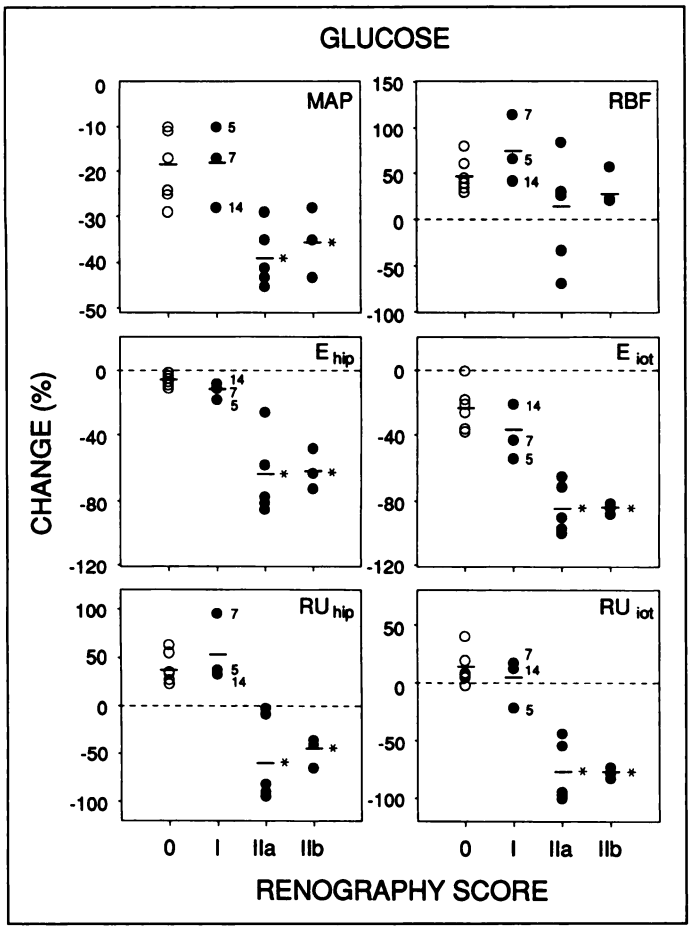

FIGURE 3. ACE inhibition-induced changes (% of control) in MAP, RBF, renal hippurate extraction (E_{hip}), renal iothalamate extraction (E_{iot}), renal hippurate uptake (RU_{hip}) and renal iothalamate uptake (RU_{iot}) versus renographic changes during glucose infusion. Open circles = nonstenotic kidneys; solid circles = stenotic kidneys; horizontal lines = mean changes (*$p < 0.01$ versus nonstenotic kidneys); numbers = renograms shown in Figure 1.

unilateral stenoses are discussed together (n = 11 during glucose, n = 9 during mannitol infusion). Control values of RBF, E_{hip} and E_{iot} of the nonstenotic and stenotic kidneys during glucose and mannitol infusion are shown in Table 1. Control values of these parameters during glucose infusion were not significantly different in the nonstenotic and stenotic kidneys, although RBF in the nonstenotic kidneys tended to be higher. This was also the case during mannitol infusion.

In the control period, during glucose as well as during mannitol infusion, the amount of hippurate cleared from the plasma (298 ± 21 and 287 ± 27 ml/min, respectively) was comparable to the amount of hippurate excreted in the urine (295 ± 31 and 287 ± 33 ml/min, respectively), indicating that without ACE inhibition, no hippurate is retained in the kidneys.

ACE Inhibition-Induced Renographic Changes

The hippurate renographic scores of the nonstenotic and stenotic kidneys before (control period) and after ACE inhibition during glucose and during mannitol infusion are shown in Figure 2. For the control period during glucose as well as mannitol infusion, nonstenotic and stenotic kidney time-activity curves were comparable (i.e., normal). In response to ACE inhibition during glucose infusion, the time-activity curves of the nonstenotic kidneys remained normal, whereas those of all stenotic kidneys showed a pattern of delayed hippurate handling that varied from grade I to grade IIb. The grade I stenotic kidney renographic curves were derived from two dogs with unilateral (Fig. 1, renograms 5 and 7) and from one dog with bilateral renal artery stenosis (Fig. 1, renogram 14). With respect to the latter, it can be seen that ACE inhibition induced only a mild delay in time to peak and an early retention at 10 min. Retention could not be appreciated at 20 min in renograms 7 and 14 because of motion artifacts in the baseline graphs. Osmotic diuresis abolished the ACE inhibition-induced delayed renographic hippurate handling in seven of nine stenotic kidneys (Fig. 2). In the two stenotic kidneys in which ACE inhibition still induced altered hippurate handling, the renographic pattern was comparable the nonmannitol setting (grade IIa).

Renographic Changes in Relation to Changes in Renal Function

ACE inhibition-induced changes in MAP, RBF, E_{hip}, E_{iot}, RU_{hip} and RU_{iot} versus renographic changes during glucose infusion are shown in Figure 3. Renographic ACE inhibition-induced changes appeared to be related to a decrease in MAP ($r = 0.62$, $p < 0.05$) such that this decrease was most pronounced in stenotic kidneys with a grade IIa or IIb pattern (−37 ± 2%) compared with nonstenotic kidneys (−19 ± 3%, $p < 0.01$).

Although renal processing of hippurate is presumed to reflect RBF, ACE inhibition-induced changes in renographic hippurate handling were not related to changes in RBF ($r = 0.34$, p = ns): The RBF response was not significantly different in both stenotic (+33 ± 15%) and nonstenotic kidneys (+48 ± 8%). ACE inhibition-induced renographic changes were not only related to the decrease in MAP but to a decrease in E_{hip} and E_{iot}

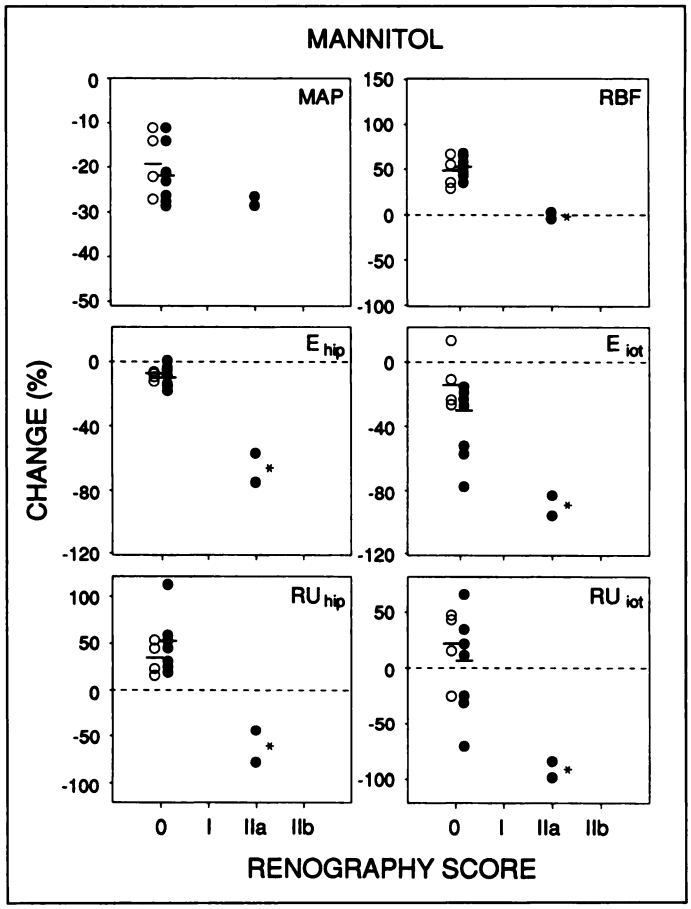

FIGURE 4. ACE inhibition-induced changes (% of control) in MAP, RBF, renal hippurate extraction (E_{hip}), renal iothalamate extraction (E_{iot}), renal hippurate uptake (RU_{hip}) and renal iothalamate uptake (RU_{iot}) versus renographic changes during mannitol infusion. Open circles = nonstenotic kidneys; solid circles = stenotic kidneys; horizontal lines (not shown for grade IIa kidneys) = mean changes (*$p < 0.05$ versus nonstenotic kidneys).

as well ($r = 0.84$ and $r = 0.83$, respectively, $p < 0.001$). This decrease in E_{hip} and E_{iot} was most pronounced in stenotic kidneys with either a grade IIa or IIb renographic pattern ($-65 \pm 7\%$ and $-84 \pm 4\%$) compared with nonstenotic kidneys ($-6 \pm 1\%$ and $-23 \pm 6\%$, $p < 0.01$). The more pronounced ACE inhibition-induced decrease in E_{hip} and E_{iot} in grade II stenotic kidneys was manifested by a decrease in RU_{hip} ($-52 \pm 11\%$, $p < 0.01$) and RU_{iot} ($-79 \pm 6\%$, $p < 0.01$), in contrast to nonstenotic kidneys in which RU_{hip} increased ($+40 \pm 6\%$, $p < 0.01$) and RU_{iot} remained the same ($+14 \pm 6\%$, p = ns). ACE inhibition-induced changes in MAP, E_{hip}, E_{iot}, RU_{hip} and RU_{iot} (-18 ± 5, -12 ± 3, -39 ± 10, $+54 \pm 21$ and $+3 \pm 12\%$, respectively) in grade I stenotic kidneys were not different from those in nonstenotic kidneys.

ACE inhibition-induced versus renographic changes in MAP, RBF, E_{hip}, E_{iot}, RU_{hip} and RU_{iot} during mannitol infusion are shown in Figure 4. The disappearance of ACE inhibition-induced changes in the renogram during mannitol infusion is accompanied by a less pronounced decrease in MAP compared with that during glucose infusion ($-21 \pm 3\%$ versus $-28 \pm 4\%$, $p < 0.05$). Moreover, the two stenotic kidneys that still showed grade IIa renographic pattern belonged to those with the most pronounced reduction in MAP. This finding supports the existence of a causal relation between renographic abnormalities and the decrease in blood pressure. ACE inhibition-induced changes in RBF in the stenotic kidneys during osmotic diuresis were comparable to those during glucose infusion ($+42 \pm 9\%$ versus $+42 \pm 14\%$, p = ns). This finding again suggests that changes in RBF are of minor importance for the changes in renographic hippurate handling, although the observation that the two grade IIa stenotic kidneys were the only ones that showed no change in RBF remains intriguing. Together with the less pronounced ACE inhibition-induced decrease in MAP during mannitol infusion, the decrease in E_{hip} and E_{iot} in stenotic kidneys with a normalized renographic pattern became comparable to that in nonstenotic kidneys, whereas in the two stenotic kidneys that still showed delayed hippurate excretion, the decrease in E_{hip} and E_{iot} was still more pronounced than that in nonstenotic kidneys ($p < 0.05$). This was manifested by comparable ACE inhibition-induced changes in RU_{hip} and RU_{iot} in stenotic and nonstenotic kidneys with a normal renographic pattern, whereas in the two stenotic kidneys with a grade IIa renographic pattern, RU_{hip} and RU_{iot} decreased.

Autoradiography

During glucose infusion, ACE inhibition appeared to cause intrarenal hippurate retention: $ERPF_i$ exceeded $ERPF_u$ during ACE inhibition in all dogs (295 ± 31 versus 150 ± 28 ml/min, $p < 0.01$), including the dogs with an unilateral grade I renographic pattern. Mannitol infusion reversed this phenomenon: $ERPF_i$ did not differ significantly from $ERPF_u$ during ACE inhibition (327 ± 29 versus 354 ± 35 ml/min). Combination of the glucose and mannitol data indicated that the ACE inhibition-induced renographic changes per dog (the change in grade of both kidneys added together) were related to the degree of intrarenal hippurate retention for both kidneys together ($r = 0.68$, $p < 0.01$).

To localize intrarenal hippurate retention, autoradiographic studies were performed in two dogs with an ipsilateral grade II and a contralateral normal renographic pattern during ACE inhibition. Representative examples of ^{125}I-hippurate localization in the ipsilateral and the contralateral kidney of one dog are shown in Figure 5. Almost no grains could be detected in the nonstenotic kidney (Fig. 5A), whereas large numbers of grains were seen in the stenotic kidney (Fig. 5B). Most of the tracer in the stenotic kidney was associated with the proximal tubular cells, indicating that hippurate is retained in the stenotic kidney primarily inside these cells. The grains appeared to be homogeneously distributed over the various cortical and juxtamedullary tubuli, but the applied technique does not allow definite conclusions on the latter observation.

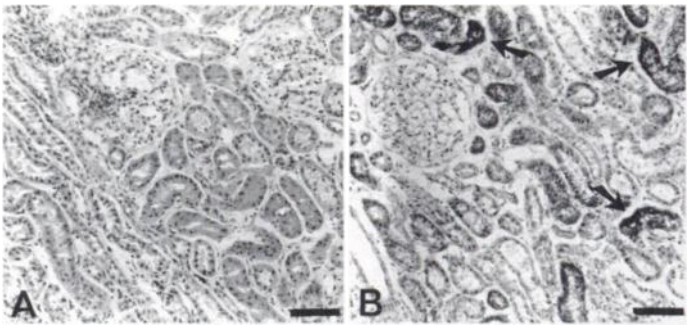

FIGURE 5. Iodine-125-hippurate autoradiograms of (A) nonstenotic and (B) grade II stenotic kidneys of one dog during ACE inhibition that was killed 10 min after ^{125}I-hippurate injection. Virtually no grains are present in the nonstenotic kidney, whereas most of the grains are associated with the proximal tubular cells in the stenotic kidney (arrows). Bar = 100 μm.

DISCUSSION

In the present study, we investigated the relation between the ACE inhibition-induced changes in renal hippurate handling as observed on the isotopic renogram and the changes in systemic and renal hemodynamics. As intended, ACE inhibition induced abnormalities on the hippurate renogram in all stenotic kidneys, and the pattern varied from delayed time to peak activity with early retention (grade I) to a rising graph without a peak (grade IIb). Renographic ACE inhibition-induced changes during glucose infusion were not related to changes in RBF but were related to a decrease in MAP, E_{hip}, E_{iot}, RU_{hip} and RU_{iot} and to intrarenal hippurate retention. Iodine-125-hippurate autoradiographic studies of grade II stenotic kidneys showed that hippurate is retained primarily within the proximal tubular cell. Mannitol infusion abolished the ACE inhibition-induced renographic changes in seven of nine stenotic kidneys, along with a normalization of the changes in MAP, E_{hip}, E_{iot}, RU_{hip}, RU_{iot} and intrarenal hippurate retention, thus supporting the existence of a causal relation between renographic changes and the decrease in MAP. ACE inhibition-induced changes in the renographic pattern of the poststenotic kidney therefore appear to be caused by a decrease in MAP, which leads to a sequence of events resulting in retention of hippurate in the proximal tubular cells.

How can we explain the relation between the ACE inhibition-induced decrease in MAP and the changes in renographic pattern? Normally, a decrease in systemic blood pressure will not lead to a decrease in intraglomerular capillary pressure (P_{GC}) because the kidney adapts with preglomerular vasodilation and postglomerular vasoconstriction. The latter is angiotensin II mediated. In the poststenotic kidney in contrast, P_{GC} will decrease because preglomerular vasodilation is probably already maximal, and the compensatory postglomerular vasoconstriction, which is already present, is abolished by ACE inhibition (*14,15*). The present observation that in response to ACE inhibition in the grade II stenotic kidney, E_{iot} decreased to a greater degree than in the nonstenotic kidney agrees with this result. That the relation between the decrease in MAP and the renographic changes induced by ACE inhibition is mediated by changes in P_{GC} is illustrated by findings that lowering blood pressure with other antihypertensive agents did not result, or resulted to a lesser extent, in delayed poststenotic tracer

handling in renographic studies (*14,16,17*). Although renal effects of mannitol probably also play a role (*18*), the observation that mannitol infusion abolished the ACE inhibition-induced delayed hippurate handling in most stenotic kidneys in parallel to a less pronounced decrease in MAP, stresses the existence of a relation between the decrease in blood pressure and the poststenotic changes in tracer handling. The failure of mannitol to reverse the ACE inhibition-induced renographic changes in the two dogs with the most pronounced decrease in MAP may indicate that the kidneys affected are those with the most severe stenosis, an assumption that appears to be confirmed by the fact that these dogs had the highest blood pressure.

How could a decrease in P_{GC} result in a renographic pattern of hippurate retention? The present data show that induction of a grade II renogram pattern occurs together with a decrease in RU_{iot}, which implies a decrease in glomerular filtration rate (GFR) resulting from the decrease in P_{GC}. A similar relation between a decrease in GFR and renographic delayed ^{99m}Tc-diethylenetriamine (DTPA) handling in response to captopril was found by Nally et al. (*16*). The importance of a decrease in GFR in this phenomenon is confirmed by the present observation that mannitol abolished the hippurate retention patterns in the stenotic kidneys if the decrease in RU_{iot} did not occur. A pronounced reduction in GFR will lead to a decrease in proximal tubular urine flow. Next to better maintaining the GFR, mannitol could exert its effect through increased proximal tubular flow due to decreased sodium and water reabsorption (*19*), as previously suggested (*6*). Either way, the role of proximal tubular flow appears to be essential. Reduced tubular flow could cause intrarenal hippurate retention in two ways (*20*):

1. Hippurate may reach the tubular lumen, but due to reduced tubular flow, the transit time of the tracer is increased, as was suggested in a recent review by Wilcox (*21*);
2. Hippurate may be retained in the proximal tubular cells. This retention may result from a reduced secretion of hippurate into the tubular lumen caused by high urine tracer concentration or a reduced supply of exchangeable anions (*22*) or an enhanced reabsorption of hippurate caused by a high urine tracer concentration or a prolonged tubule contact time (*23*). Because the autoradiographic data indicate that the retained radioactive hippurate is mainly located within the proximal tubular cells, both options may be in play. Either form of retention leads to a prolongation of the presence of hippurate in the kidney, causing the well-known delayed renographic pattern.

We observed pronounced ACE inhibition-induced decrease in E_{hip} and RU_{hip} in grade II stenotic kidneys during glucose infusion, which is quantitatively comparable with the findings of Wenting et al. (*5*) in humans. There are several possible explanations for this decrease:

1. Less hippurate was filtered because of a decrease in GFR. Although a higher fraction of hippurate entering the kidney is filtered in a dog than in humans [i.e. one third (*6*)], this finding is insufficient to explain the 65% decrease in E_{hip} in grade II stenotic kidneys.
2. Hippurate may be less efficiently extracted because of a shortened plasma transit time (*24*). This appears to be unlikely because the RBF response was comparable in both stenotic and nonstenotic kidneys, whereas the decrease in E_{hip} was most pronounced in stenotic kidneys.
3. Tubular transport of hippurate could be inhibited during ACE inhibition either by competition between ACE inhibition and hippurate for the organic anion transport system (*25*) or by a high concentration of tracer in the tubuli (*22*). Competition appears unlikely because the stenotic and nonstenotic kidneys then would have been affected to the same extent. Although the present data cannot prove that tubular hippurate concentration increases sufficiently, inhibition of transport due to a high tubular hippurate concentration cannot be excluded. The autoradiographic experiments indicate that most of the hippurate cleared from the plasma is retained in the proximal tubuli, and previous reports have shown that the excretory maximum of para-aminohippurate is depressed by glucose-infusion (*26*).
4. E_{hip} and RU_{hip} may decrease as a result of enhanced reabsorption of hippurate from a low urine flow rate (*23*). In previous experiments we showed that urine flow rate in the stenotic kidney during ACE inhibition can become almost zero (*6*); thus, this option is also a possibility.

The preceding discussion concerning the mechanism of ACE inhibition-induced renographic changes is mainly based on renal function data from grade II stenotic kidneys. Although ACE inhibition-induced changes in E_{hip}, E_{iot}, RU_{hip} and RU_{iot} in grade I stenotic kidneys were not significantly different from those in normal kidneys, these data do not appear to contradict our findings. In our opinion, ACE inhibition-induced changes in the most important parameters (i.e., E_{iot} and RU_{iot}) in these kidneys tended to be in between the nonstenotic and grade II stenotic kidneys, and the absence of a significant difference compared with normal kidneys is probably due to a paucity of data. There has been some discussion in published reports as to whether a grade I renographic pattern represents significant renal artery stenosis. In the present study, the recommendations of the Cleveland Consensus Meeting were followed, which resulted in three kidneys with an ACE inhibition-induced grade I renographic pattern. The finding that ACE inhibition-induced changes in renal function in these three kidneys were not different from those observed in the nonstenotic kidneys could, alternatively, be interpreted as an indication that no significant renal artery stenosis was present. The $ERPF_i$ and $ERPF_u$ data, however, showed ACE inhibition-induced intrarenal hippurate retention. Furthermore, in the two cases of unilateral renal artery stenosis, hypertension was present. Thus, in our view, a grade I renographic pattern represents significant renal artery stenosis.

Our data show that ACE inhibition-induced altered hippurate handling in the stenotic kidney is largely dependent on the decrease in GFR. Such a decrease in GFR is likely to occur because the handling of the GFR tracer DTPA is altered, as previously demonstrated by Nally et al. (*16*). It is therefore not surprising that ACE inhibition renography can be performed with both GFR and ERPF markers (*3,27–30*). It is of interest that RU_{iot} did not decrease in the kidneys in which ACE inhibition induced a grade I hippurate renographic pattern. The implication might be that these stenotic kidneys would not have been detected with renographic techniques using a glomerularly filtered tracer such as DTPA. Thus, the sensitivity of hippurate ACE inhibition renography to detect renal artery stenosis may in theory be higher, as was also claimed by Sfakianakis et al. (*30*), albeit for a different reason.

CONCLUSION

The present study shows that the ACE inhibition-induced hippurate retention curve by the poststenotic kidney is probably the result of a sequence of events. A decrease in systemic blood pressure in combination with efferent vasodilation will lead to a decrease in intraglomerular capillary pressure. This decrease in glomerular pressure will lead to a more or less pronounced decrease in GFR and proximal tubular urine flow. The latter may hamper tubular transit of the tracer and affect transport across the luminal membrane. This in turn will cause intrarenal hippurate retention and, most likely, in the more severe cases, a decrease in renal hippurate uptake. Because the decrease in GFR plays a crucial role in this phenomenon, our data also explain the finding that both ERPF and GFR markers perform well in the detection of renal artery stenosis by ACE inhibition renography.

ACKNOWLEDGMENTS

We are indebted to Mr. J.M. Elstrodt for technical assistance, Mr. W. Zeinstra for skillful construction of the constrictor, Mr. A. Nijmeijer for assistance in training the dogs, Dr. R.A.M. Kengen from the Department of Nuclear Medicine for advice and Ms. J.C.A.M. Bun from the Department of Histology for preparation of the autoradiograms. This study was made possible by grant C89.844 from the Dutch Kidney Foundation.

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