Kidney International, Vol. 27 (1985), pp. 9–16

Is ^{125}I iothalamate an ideal marker for glomerular filtration?

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Is ^{125}I iothalamate an ideal marker for glomerular filtration? The triiodinated angiographic contrast medium, iothalamate, has (usually labelled ^{125}I) been used extensively as a marker for glomerular filtration. We have studied the renal handling of ^{125}I iothalamate (IOT) in vivo and in vitro in several species. In renal cortical slices from chicken, rabbit, rat, and monkey, the tissue-to-medium ratio of IOT was twice that of ^{51}Cr-EDTA (EDTA) at 37°C; a difference that was abolished at 0°C and markedly reduced by added o-iodohippurate or iodipamide. In five chickens the steady-state renal clearance of IOT (C_{IOT}) was twice ($P < 0.05$) that of EDTA (C_{EDTA}) or ^{3}H inulin (C_I); a difference that was abolished by administration of 100 mg/kg/hr of novobiocin, an organic anion transport inhibitor. C_{EDTA} was similar to C_I before as well as after transport inhibition. Utilizing the Sperber technique the mean apparent tubular excretion fraction (ATEF) of IOT was 8%, while that of EDTA was 1% ($P < 0.01$; $N = 10$). After novobiocin coinfusion (new steady-state) $ATEF_{IOT}$ was significantly reduced ($P < 0.01$) and not different from that of EDTA (−1%). In the same animals the total urinary recovery of IOT was 84 and 57% ($P < 0.01$) before and after novobiocin, respectively, while corresponding values for EDTA was unchanged by the inhibitor. In seven rats the renal extraction of IOT was reduced from 29 to 17% ($P < 0.05$) by coinfusion of probenecid (5 mg/kg/hr). Corresponding extractions were 82 to 34% ($P < 0.005$) and 22% (unchanged) for PAH and EDTA, respectively. In six healthy volunteers the renal clearance of unlabelled IOT (HPLC method) equated that of creatinine but exceeded that of inulin with 38% ($P < 0.01$). This difference was reduced 34% ($P < 0.05$) by probenecid (1 g i.v.). In nineteen patients with a single or two kidneys the average plasma clearance (single injection technique; slope-intercept method) of IOT was 13% higher than that of EDTA ($P < 0.001$); a difference which was significantly ($P < 0.01$) reduced to half after pretreatment with probenecid (1 g i.v.); in some patients this difference was marked. The results show that IOT is subject to a significant and in some cases marked renal tubular secretion in chicken, rats, and humans. IOT, therefore, is not an ideal marker for glomerular filtration.

Le ^{125}I iothalamate est-il un marqueur idéal de la filtration glomérulaire? L'iothalamate, un produit de contraste angiographique tri-iodé a été largement utilisé (habituellement marqué à ^{125}I) comme marqueur de la filtration glomérulaire. Nous avons étudié l'excrétion rénale du ^{125}I iothalamate (IOT) in vivo et in vitro dans différentes espèces. Dans des tranches corticales de rein de poulet, de lapin, de rat, et de singe, le rapport tissu sur milieu de l'IOT était le double de celui du ^{51}Cr-EDTA (EDTA) à 37°C; une différence abolie à 0°C et réduite de façon marquée par l'addition de O-iodohippurate ou d'iodipamide. Chez cinq poulets la clearance rénale à l'équilibre d'IOT (C_{IOT}) était le double ($P < 0,05$) de celle de l'EDTA (C_{EDTA}) ou de ^{3}H inuline (C_I); une différence abolie par l'administration de 100 mg/kg/hr de novobiocine, un inhibiteur du transport des anions organiques. C_{EDTA} était identique à C_I avant comme après l'inhibition du transport. En utilisant la technique de Sperber, la fraction d'excrétion tubulaire apparente moyenne (ATEF) de l'IOT était de 8%, alors que celle de l'EDTA était de 1% ($P < 0,01$; $N = 10$). Après coperfusion de novobiocine (nouvel équilibre) $ATEF_{IOT}$ était significativement réduite ($P < 0,01$) et non différente de celle de l'EDTA (−1%). Chez les mêmes animaux, la récupération urinaire totale d'IOT était de 84 et 57% ($P < 0,01$) avant et après novobiocine, respectivement, alors que les valeurs correspondantes pour l'EDTA étaient inchangées par l'inhibiteur. Chez sept rats, l'extraction rénale d'IOT était réduite de 29 à 17% ($P < 0,05$) par coperfusion de probénécide (5 mg/kg/hr). Les extractions correspondant étaient de 82 à 34% ($P < 0,005$) et de 22% (inchangées) pour le PAH et l'EDTA, respectivement. Chez six volontaires sains, la clearance rénale de l'IOT non marqué (méthode HPLC) était égale à celle de la créatinine mais dépassait celle de l'inuline de 38% ($P < 0,01$). Cette différence était réduite de 34% ($P < 0,05$) par le probénécide (1 g i.v.). Chez dix-neuf malades avec un ou deux reins, la clearance plasmatique moyenne (technique par injection unique; méthode d'interception de la courbe) de l'IOT était 13% plus élevée que celle de l'EDTA ($P < 0,01$); une différence qui était significativement ($P < 0,01$) réduite de moitié après prétraîtement par le probénécide (1 g i.v.); chez certains malades cette différence était marquée. Ces résultats montrent que l'IOT est sujet à une sécrétion tubulaire rénale significative et parfois marquée chez le poulet, le rat, et l'homme. L'IOT n'est donc pas un marqueur idéal de la filtration glomérulaire.

Since the renal clearance concept was developed by Homer Smith [1], monitoring the glomerular filtration has played a central role in renal physiology and clinical nephrology. An ideal marker for glomerular filtration should be metabolically inert, non-toxic, and should not alter the renal function. Furthermore, it should be freely filtrable, not bound to plasma proteins or blood cells and, of course, it should neither be reabsorbed nor secreted by the renal tubules. Inulin is the classic marker for glomerular filtration [2] whether analyzed by chemical or radioactive methods. Presently, however, other radioactive tracers are usually preferred. The tracers available are ^{51}Cr-labelled EDTA (ethylene diamine tetraacetic acid), ^{99m}Tc-labelled DTPA (diethylene triamine pentaacetic acid), and iodine-labelled diatrizoate and iothalamate (Fig. 1). In preliminary studies in our laboratory we used the Sperber technique and found that renal clearance values for iothalamate were higher than those expected. This prompted us to investigate further the renal handling of iothalamate versus that of other markers for glomerular filtration in the Sperber preparation, in rats and in healthy volunteers and in patients with a single kidney or two kidneys. We also studied the in vitro accumulation of iothalamate and ^{51}Cr-EDTA in renal cortical slices from several species. Our results unequivocally demonstrate an active tubular secretion and perhaps also reabsorption

Received for publication February 21, 1983,
and in revised form March 27, 1984

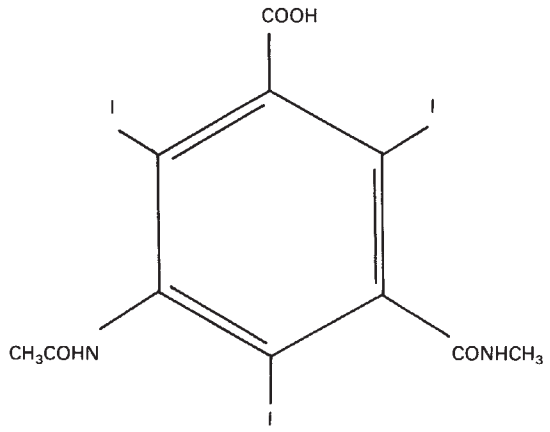

Fig. 1. *Chemical structure of iothalamic acid.* In diatrizoate and iodamide $CONHCH_3$ at position three is substituted by $NHCOCH_3$ and $CH_2NHCOCH_3$, respectively.

of iothalamate, which therefore is considered unsuitable as an ideal marker for glomerular filtration.

Methods

Studies with renal cortical slices

The in vitro accumulation of ^{125}I iothalamate and ^{51}Cr-EDTA in renal cortical slices from chicken ($N = 2$), rabbit ($N = 2$), rat ($N = 3$), and cynomolgus monkey ($N = 1$) was studied, using the technique previously described by Bárány [3, 4].

The tissue-to-incubation medium (T/M) ratio was calculated from results in experiments at 0°C (ice) and at 37°C without and in the presence of the transport competitive inhibitors sodium-o-iodohippurate (10^{-3} or 10^{-2} M) and iodipamide (10^{-3} or 10^{-2} M), respectively.

Experiments with the Sperber technique

Five Rhode Island Red hens were used in clearance experiments using a similar technique as described earlier [5]. This technique enables urine to be collected separately from the two kidneys through plastic funnel cones sewn over each ureteral opening. Tracer amounts of ^{125}I iothalamate, ^{51}Cr-EDTA, and ^{3}H-inulin were continuously infused via a polyethylene catheter (PP 50) into the right (ipsilateral) medial tarsal vein, that is, at least in part into the right renal portal system [5]. One hour was allowed to obtain steady-state conditions. Blood samples (0.5 to 0.8 ml) were then drawn from a polyethylene catheter (PP 50) placed into the left (contralateral) medial tarsal vein in the middle of each 10-min clearance period and centrifuged immediately.

After six 10-min clearance periods in steady-state, sodium novobiocin (100 mg/kg body weight and hour) was coadministered for another six 10-min periods via a polyethylene catheter (PP 50) placed into the left brachial vein.

The above procedure allows calculation of the clearance of each marker by the left (contralateral) kidney before and after the inhibition of organic anion transport, because the blood samples should give the systemic concentration of each substance, which is presented to this kidney by the arterial and the renal portal circulations [5].

In the above five animals and in an additional five animals the renal portal infusion of ^{125}I iothalamate allowed the apparent tubular excretion fraction (ATEF) to be calculated as the difference between ^{125}I activity excreted into the urine on the ipsilateral (right) and contralateral (left) side, respectively, divided by the amount infused [5]. ^{51}Cr-EDTA was used as the control marker. In four separate experiments only the free ^{125}I isotope was administered. The effect of novobiocin coadministration (as above) was determined in all experiments.

Experiments with rats

The renal handling of ^{125}I iothalamate was also investigated in seven rats, which was done by measuring the extraction of ^{125}I iothalamate as well as that of ^{14}C PAH and ^{51}Cr-EDTA before and during treatment with probenecid. A Sprague-Dawley strain (Anticimex, Stockholm, Sweden) of rats was used. The rats, each weighing 300 to 370 g, were kept on an Ewos standard rat diet (Södertälje, Sweden) and water ad libitum until the day of the experiment. Anesthesia was induced by an intraperitoneal injection of Inactin® at a dose of 120 mg/kg body wt. The body temperature was kept at 37.5°C through a servo-controlled heating table. The left kidney was reached by a flank incision. The renal vein was first perforated with a needle instrument and a fibrin membrane-covered hole was formed. A polyethylene catheter was then introduced through this hole without any ligatures. The left renal ureter and the bladder were catheterized; the bladder was catheterized only for drainage of the right kidney. The femoral vein and artery were cannulated for infusion and specimen sampling, respectively. The rats were continuously infused with a Ringer solution (150 mM/liter NaCl and 10 mM/liter $NaHCO^3$) at a rate of 3 ml/kg body wt per hour. ^{125}I iothalamate, ^{14}C PAH, and ^{51}Cr-EDTA were added to the solution to give a concentration of 2, 5, and 2 μCi/ml, respectively. When probenecid was given, it was added into the infusion solution and administered at a rate of 50 mg/kg body wt and hour.

The rats were allowed to equilibrate for 45 min, whereafter double clearance periods of 20 min were started. The means from these periods were used to calculate the extraction values.

Study in healthy volunteers

Six healthy males volunteered for a renal clearance study with 30-min urinary collection periods and spontaneous voiding. They drank water and soft drinks ad libitum which kept urine volumes to 200 to 450 ml/30 min. After a bolus injection of 50 mg/kg inulin and 100 mg sodium iothalamate, a continuous intravenous infusion of 28 mg/min and 1.5 mg/min, respectively, was administered through an indwelling cannula in one brachial vein. Two hours were allowed to achieve a steady-state. Three 30-min clearance periods were then performed before and after the intravenous injection of 1 g probenecid (injection time, 5 to 40 min), respectively. Blood samples were drawn from a cannula placed in the opposite brachial vein. Plasma and urine samples were analyzed for inulin (spectrophotometric method), iothalamate (using the HPLC method of Boschi and Marchesini [6]), and creatinine (according to Jaffe).

Clinical study

The glomerular filtration rate (GFR) was also measured on 23 occasions in 19 patients with different degrees of stable renal

Table 1. Simultaneously determined renal clearance of ^{51}Cr EDTA (C_{EDTA}) and ^{125}I iothalamate (C_{IOT}) and the difference between them (Δ) in patients without and after probenecid pretreatment

Patient no.	Diagnosis	Without probenecid			After probenecid		
		C_{EDTA}	C_{IOT}	Δ	C_{EDTA}	C_{IOT}	Δ
Group 1							
1	Donor	93	104	11			
2	Hydronephrosis	97	111	14			
3	Arteriolar thrombosis	63	79	16			
4	Hypernephroma	123	136	13			
5	Donor	96	108	12			
6	Renal tumor				63	65	2
7	Donor				59	66	7
8	TBC				57	68	11
9	Donor				85	91	6
10	Donor				90	95	5
11	Hypernephroma				132	142	10
Group 2							
12	Diabetes mellitus				25	34	9
13	Diabetes insipidus	99	131	32	103	104	1
14	Glomerulonephritis				61	63	2
15	Glomerulonephritis	123	130	7	126	139	13
16	Glomerulonephritis	76	87	11	95	94	−1
17	IgA nephropathy	124	132	8	96	100	4
18	SLE	37	47	10			
19	Glomerulonephritis	137	139	2			

functional impairment (see Table 1). Eleven patients had unilateral nephrectomies more than 1 year prior to the present investigation, and 12 patients were pretreated with probenecid (1 g) intravenously 1 to 2 hr prior to the first blood sample.

Four of these patients were also tested on a separate occasion without pretreatment. The test protocol was approved by the local ethical and isotope committees, respectively.

Glomerular filtration rate measurements in this part of the study were performed by the single injection method using ^{51}Cr-EDTA and ^{125}I-sodium iothalamate. Ten microcuries of ^{125}I-sodium iothalamate and 100 μCi ^{51}Cr-EDTA were mixed with 10 ml of physiological saline and given as a rapid intravenous injection. Venous blood samples (2 × 5 ml) were drawn 180, 210, and 240 min after the injection. The ^{51}Cr-EDTA and ^{125}I-sodium iothalamate plasma clearances were calculated according to the slope-intercept method described by Brøchner-Mortensen [7]. The clearance values were corrected to 1.73 m^2 body surface.

Isotope analysis

^{125}I and ^{51}Cr activities were determined in a two-channel scintillation spectrometer. ^{14}C or tritium activity was determined in 0.1 ml plasma and urine, respectively, added to 5 ml of a toulene scintillation mixture with 6 vol % Bio-solv BBS3 (Beckman Instruments Inc., Fullerton, California, USA) using a liquid scintillation spectrometer. Appropriate crossover corrections were made for simultaneously determined isotopes using a specially designed computer program.

Substances

^{125}I sodium iothalamate, ^{51}Cr EDTA (etylene-diamino tetraacetic acid), ^{14}C PAH (paraminohippurate), and ^{3}H-inulin were obtained from the Radiochemical Centre, Amersham, England; inulin from Loevosan Gesellschaft, Linz, Austria; iothalamate (Conray) from Astra-Meditec, Mölndal, Sweden; o-iodohippurate from Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, USA; iodipamide (Biligrafin Forte) from Schering AG, Berlin, Germany; sodium novobiocin from Merck Sharp and Dohme, Rahway, New Jersey, USA; and probenecid was prepared for intravenous use in humans by Astra AB, Södertälje, Sweden.

Statistical analysis

Results are given as the arithmetic means ± SD or ± SEM. Differences were analyzed statistically with Student's *t* test for paired or unpaired observations and were considered significant at the 0.05 level.

Results

Experiments with renal cortical slices

In all four species (chicken, rabbit, rat, and monkey) tested the tissue-to-medium (T/M) ratio for ^{125}I iothalamate was more than twice those of ^{51}Cr-EDTA at 37°C (Fig. 2). At 0°C, however, the T/M ratio was equal for the two markers and not different from that of ^{51}Cr-EDTA at 37°C. Moreover, in the presence of o-iodohippurate or iodipamide, inhibitors of organic anion transport [4, 8], the T/M ratio for ^{125}I iothalamate was markedly depressed, approaching that of ^{51}Cr-EDTA. Taken together these results indicate an energy-dependent accumulation of ^{125}I iothalamate in renal cortical slices of chickens and three mammalian species, probably reflecting organic anion transport [9].

Experiments with the Sperber technique

Clearance experiments. The renal clearance of ^{125}I iothalamate in the contralateral kidney in the steady-state was about twice that of the other markers, ^{51}Cr-EDTA and ^{3}H-inulin, when simultaneously determined. The clearance values for the latter markers did not differ (the small differences observed were not statistically significant) and agreed with values found in previous studies [10]. During coadministration of novobiocin, an inhibitor of organic anion transport [8, 11], the renal clearance values of ^{125}I iothalamate were markedly reduced and approached those of the other markers. Furthermore, during control periods the apparent ipsilateral renal clearance values of ^{125}I iothalamate were significantly higher than those on the contralateral side—a difference that was markedly reduced during novobiocin coadminstration (data not shown). Contrary to this finding, the renal clearance values of ^{51}Cr-EDTA and ^{3}H-inulin were always equal for the two kidneys (data not shown). These results show a significant active tubular secretion of ^{125}I iothalamate in the chicken kidney, probably involving an anion transport system. As shown in Figure 3, when a new steady-state was reached during novobiocin coadministration, the mean renal clearance value for ^{125}I iothalamate tended to be lower than that of the other markers—for one such clearance period even significantly so, possibly suggesting that ^{125}I iothalamate is subject also to some degree of tubular reabsorption in the chicken. During novobiocin coinfusion the

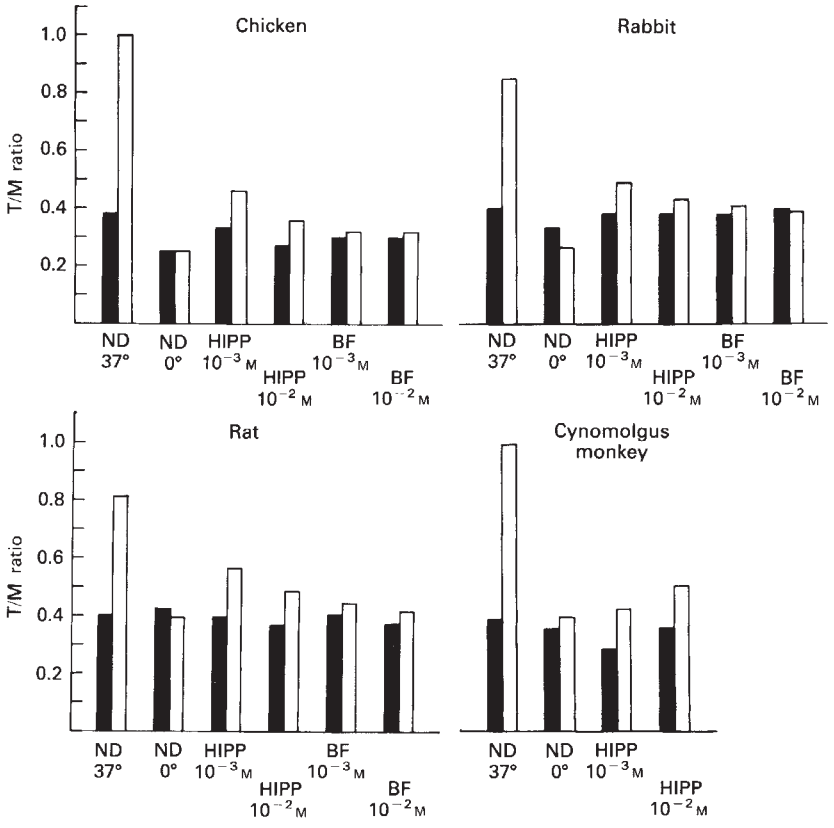

Fig. 2. *Tissue to medium* (T/M) *ratios for* ^{51}*Cr-EDTA* (filled columns) *and* ^{125}*I iothalamate* (open columns) *in renal cortical slices from chicken, rabbits, rats, and one cynomolgus monkey at 0° and 37°C without* (ND) *and with inhibitors of organic anion transport.* Abbreviations are: HIPP, o-iodo-hippurate; BF, iodipamide. Mean values of chicken (N = 2), rabbit (N = 2), and rat (N = 3) experiments are given.

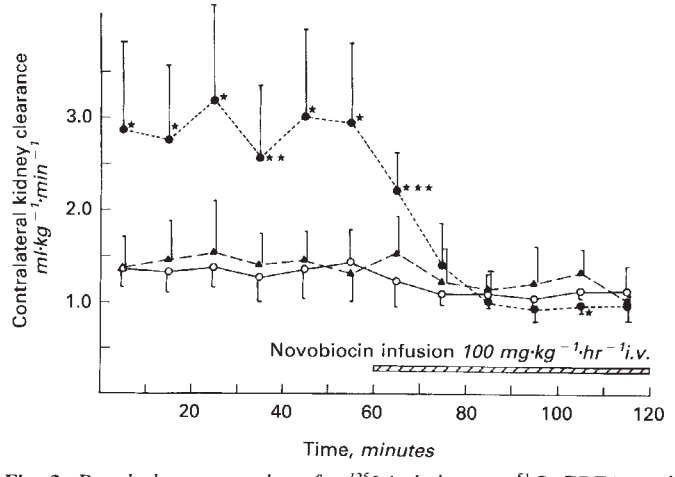

Fig. 3. *Renal clearance values for* ^{125}*I iothalamate,* ^{51}*Cr-EDTA, and* ^{3}*H-inulin (continuous infusion technique) in the contralateral kidney of five hens before and during coadministration of novobiocin.* Results are given as mean values and 1 SD (*vertical lines*). Statistically significant differences between ^{125}I iothalamate and simultaneously determined ^{3}H-inulin clearances are indicated: $*P < 0.05$; $**P < 0.02$; $***P < 0.01$. Symbols are: ●---●, ^{125}I iothalamate; ○–○, ^{51}Cr-EDTA; ▲—▲, ^{3}H-inulin.

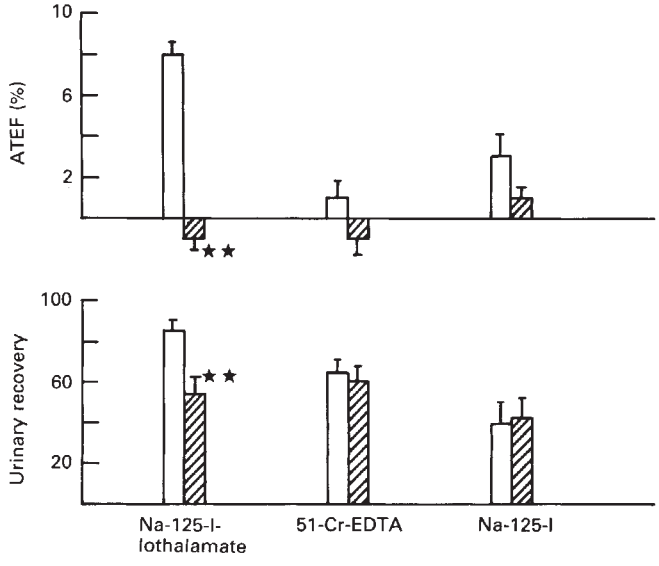

Fig. 4. *Results from experiments with the Sperber technique in the chicken.* Apparent tubular excretion fraction (ATEF) values and total urinary recovery (percentage of infused amount) of ^{125}I iothalamate, ^{51}Cr-EDTA (N = 20) and ^{125}I (N = 4) in steady-state before (*open columns*) and after (*striped columns*) coadministration of novobiocin (100 mg/kg/hr). Results are given as mean values and 1 SD (*vertical lines*). Statistically significant reduction by novobiocin is shown by $**P < 0.01$.

clearance values of ^{51}Cr-EDTA and ^{3}H-inulin still did not differ from each other, but were 18% lower than during control periods. This agrees with previous findings for ^{51}Cr-EDTA [8] and suggests that novobiocin slightly reduces glomerular filtration.

ATEF and urinary recovery studies (Fig. 4). The mean ATEF value of ^{125}I iothalamate in steady-state was 0.08, a value significantly different ($P < 0.01$) from that of ^{51}Cr-EDTA (mean ATEF, 0.01). During novobiocin coinfusion (new steady-state)

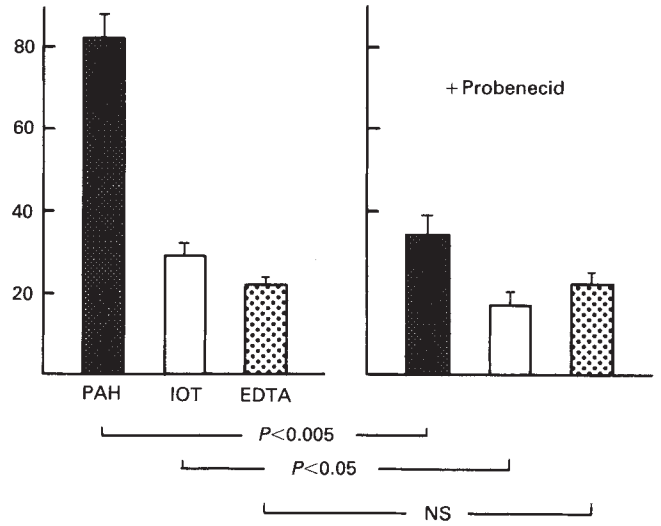

Fig. 5. *Renal extractions (%) of ^{14}C paraaminohippurate* (filled columns). *^{125}I iothalamate* (open columns) *and ^{51}Cr-EDTA* (dotted columns) *in seven rats in steady-state before and after infusion of probenecid (50 mg/kg/hr).* Results are given as mean values + 1 SEM. Statistically significant reduction by probenecid is shown.

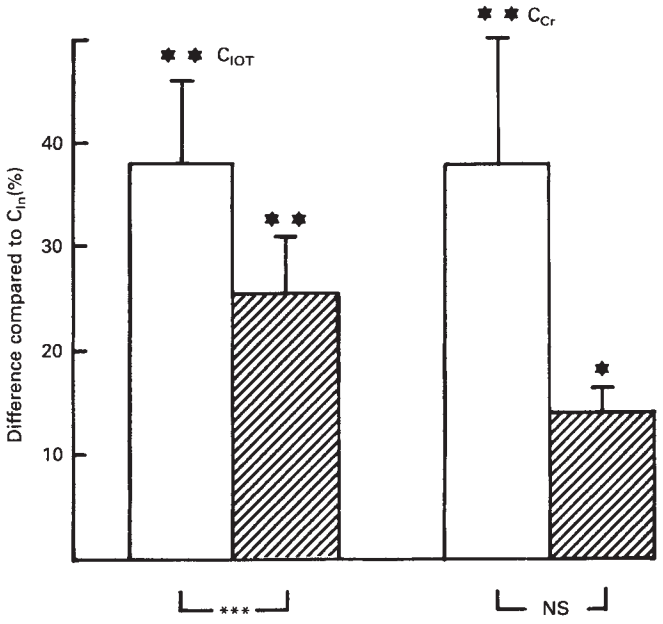

Fig. 6. *Percentage difference in steady-state renal clearance values (calculated as the average of three clearance periods in each of six healthy volunteers) of iothalamate* (C_{IOT}) *and creatinine* (C_{Cr}) *compared to that of inulin* (C_{In}) *before* (open columns) *and after* (filled columns) *probenecid injection. Results are shown as mean values + 1* SEM. $*P < 0.05$ and $**P < 0.01$ denotes significant difference compared to C_{In} and $***P < 0.05$ denotes significant reduction by probenecid.

ATEF values for ^{125}I iothalamate and ^{51}Cr-EDTA were equal and averaged -0.01. Corresponding values for ^{125}I (infused as the sodium salt in solution) were 0.03 and 0.01, respectively. The mean total urinary recovery in the steady-state of ^{125}I iothalamate was 84% of the amount infused, a value which was significantly reduced to 57% during novobiocin coinfusion. The corresponding recoveries for ^{51}Cr EDTA and ^{125}I, respectively, were not changed by novobiocin. Together these results support the concept that ^{125}I iothalamate (but not ^{51}Cr-EDTA or free ^{125}I) is subject to active tubular secretion in the chicken (compare with [12]).

Experiments with rats

The renal extraction of ^{51}Cr-EDTA in the rat averaged 22%, a value which was not changed during coadministration of probenecid, an organic anion transport inhibitor [13]. This indicates that probenecid did not affect the GFR in the rat kidney. As expected, probenecid reduced the ^{14}C PAH extraction by 80% (Fig. 5). Moreover, the ^{125}I iothalamate extraction was reduced by 41%, presumably by interference with its tubular secretion. It is obvious in Figure 5 that some secretion of PAH prevailed; it was not possible, however, to further increase the dose of probenecid due to adverse effects (drop in mean blood pressure). Furthermore, during probenecid treatment the renal extraction of ^{125}I iothalamate was 23% lowe than that of ^{51}Cr-EDTA. Although this difference was not statistically significant, it may suggest a tubular reabsorption of ^{125}I iothalamate also in the rat kidney.

Study in healthy volunteers

The renal clearance of inulin (C_{In}) in the steady-state averaged 120 ± 33.2 and 94 ± 15.5 ml/min/1.73 m^2 body surface before and after the injection of probenecid, respectively (means ± SD; all clearance values calculated from the average of two to three clearance periods before and after probenecid in each of six volunteers); a mean reduction of 21.7% ($P < 0.05$) was found. This agrees with our earlier findings [14]. The average renal clearance of unlabelled iothalamate (C_{IOT}) in steady-state before probenecid was equal to that of creatinine (C_{Cr}) and exceeded that of inulin with 38% ($P < 0.01$ in both cases, compare Fig. 6). It is well documented that the renal clearance of creatinine will overestimate the true GFR due to active tubular secretion of creatinine [9, 23]. Our results in healthy volunteers strongly imply that this is the case also for iothalamate. After probenecid, C_{IOT} and C_{Cr} still significantly exceeded C_{In} (Fig. 6). More importantly, probenecid reduced C_{IOT}/C_{In} by 34% ($P < 0.05$) and C_{Cr}/C_{In} by 63% (NS) compared to control ratios (Fig. 6). It seems therefore that the tubular secretion of iothalamate in humans involves an organic anion transport system.

Clinical study

The simultaneously determined plasma clearance values for ^{51}Cr-EDTA and ^{125}I iothalamate are presented in Table 1. Those patients who had a unilateral nephrectomy are presented as group 1, the others as group 2. Calculated on all patients, the clearance values for ^{125}I iothalamate were significantly higher than those of ^{51}Cr-EDTA ($P < 0.001$). This was true for patients with, as well as without, probenecid pretreatment. Without pretreatment the plasma clearance of ^{125}I iothalamate was on the average 13% higher than that of ^{51}Cr-EDTA. Probenecid administration did not abolish the difference between the two filtration estimates but reduced the difference significantly to an average of 7% ($P < 0.01$). These results indicate that discrepancies between ^{51}Cr-EDTA and ^{125}I iothalamate clearance values also in humans may depend on a tubular secretion of ^{125}I iothalamate. The fact that probenecid did not completely abol-

ish the difference between ^{51}Cr-EDTA and ^{125}I iothalamate clearance values is possibly due to insufficient inhibition by probenecid of tubular secretion; in a previous study in humans it was found that a corresponding dosage of probenecid reduced the average renal clearance of unlabeled PAH by only 17.4% [14].

Discussion

The initial studies of the validity of iothalamate as a marker for glomerular filtration were performed by Griep and Nelp [15]. In stop-flow studies in dogs they found that the urine to plasma ratios for cobalt 57-labelled vitamin B_{12} parallelled those of ^{125}I iothalamate, and they concluded that there was no significant tubular reabsorption nor secretion of iothalamate in this species. Moreover, they found negligible urinary excretion of ^{131}I iothalamate in aglomerular fish over a 24-hr observation period whereas 30% of the infused amount of phenol red was excreted. Oester, Olesen, and Madsen [16] found a good correlation between simultaneously determined renal clearances of inulin and ^{125}I iothalamate in dogs using a constant infusion technique. Further studies in humans showed an excellent correlation between the renal clearance of ^{125}I- or ^{131}I-labelled iothalamate and that of inulin, when iothalamate was administered as a constant intravenous infusion [17–19] or subcutaneously [19]. It should be realized that an average renal clearance value of iothalamate similar to that of inulin, is still compatible with a tubular secretion of the former, if there is a simultaneous, and quantitatively similar, tubular reabsorption. In such a case, however, individual values would be expected to differ more. In the study by Anderson, Sawyer, and Cutler [20], although an acceptable overall correlation was found, there is a notable scattering of simultaneously obtained clearance measurements of ^{125}I iothalamate and inulin. The same is true in the study of Gagnon et al [21], especially in the patients with inulin clearance values above 80 ml/min. This tendency was even more pronounced in dogs, where the renal clearance of ^{125}I iothalamate was significantly lower than that of inulin; this was true whether inulin was analyzed with the anthrone method or according to Heyrovsky [21]. In the same study it was also found that the renal extraction of ^{125}I iothalamate in dogs was lower than that of inulin, 0.37 versus 0.43%, respectively. Some of our findings after transport inhibition in chickens (Fig. 3) and rats (Fig. 5) are consistent with these results, and could be the result of a small tubular reabsorption of ^{125}I iothalamate in these species. In the study of Gagnon et al [21], however, the ratio of ^{125}I iothalamate to inulin clearance was not changed when unlabelled iothalamate was added as a carrier, which was considered as evidence against a tubular reabsorption of iothalamate in dogs. Interestingly, Sigman et al [22] in their earliest paper found the average ^{131}I iothalamate renal clearance value in humans to be about 10% higher than that of inulin (continuous infusion technique). This result is comparable to the 13% difference that we found for ^{125}I iothalamate versus ^{51}Cr-EDTA plasma clearance in patients (Table 1). In healthy volunteers, however, we found the *renal* clearance of unlabelled iothalamate to be on an average 38% higher than that of inulin. It is at this time unclear whether this difference depends on the subjects tested (patients vs. healthy volunteers), the clearance method (plasma vs. renal clearance), the marker (^{125}I vs. unlabelled iothalamate) or on the reference marker (^{51}Cr-EDTA vs. inulin). It strongly indicates, however, that the higher ^{125}I iothalamate plasma clearance obtained in patients compared to that of ^{51}Cr-EDTA (Table 1) most likely reflects a higher renal clearance, probably due to tubular secretion of ^{125}I iothalamate in these patients.

Of special interest in the present context are the findings by Rosenbaum et al [23] that the renal clearance values for ^{125}I iothalamate (and creatinine) obtained in kidney donors and transplant recipients were significantly higher than those of inulin. Their conclusion was that inulin is inadequate as a marker for glomerular filtration in these subjects. An alternative explanation, and a more likely one in view of our results, is that their results reflect a tubular secretion of ^{125}I iothalamate and therefore incriminates this substance rather than inulin as a marker for glomerular filtration. In support of this, it is well known that creatinine which in our study (Fig. 6) as well as in the study of Rosenbaum et al [23] gave similar renal clearance values as (^{125}I) iothalamate, is clearly subject to tubular secretion in humans [9].

It is apparent from our clearance data that the renal tubular secretion was more pronounced in the chicken as compared to the mammalian species. This may reflect the difference in filtration fraction (GFR/RPF) in the three species which is about 10, 28, and 21% in chicken [24], rats [25], and humans, respectively. In other words, a tubular secretion of a substance is more readily detected in a species such as the chicken with a relatively low GFR, all other factors being equal. Considering also other advantages with the Sperber technique (compare [24]), it seems warranted to recommend a wider use of this technique in studies of the renal handling of organic substances, especially concerning their renal tubular secretion.

It may seem that the difference found in our patients between the plasma clearance of ^{125}I iothalamate and that of ^{51}Cr-EDTA is clinically unimportant. However, in some patients (compare patients 3 and 13 in Table 1) the difference is marked. Furthermore, it is well known that creatinine clearance tends to overestimate the GFR especially in patients with renal insufficiency presumably due to increased tubular secretion of this substance (compare [26]). Although it has not yet been tested, it is reasonable to assume that this may also apply to iothalamate, a substance that evidently also is subject to tubular secretion in humans. Moreover, the common clinical practice to determine glomerular filtration by the so-called single injection technique, where the clearance values are calculated from the elimination curve of the test substance in blood, may be unnecessarily complicated by the use of an inadequate marker [27]. For instance, in the extensive study of Hall, Guyton, and Farr [28] the renal clearance of inulin was related to the plasma clearance of ^{125}I iothalamate, as calculated by different formulae. The correlation was bad when using a slope intercept method for calculation of the plasma clearance of iothalamate; it was better when using a two-compartment model, while it was acceptable by using a method based on the total area under the plasma concentration time curve. This reasoning is based on the assumption that iothalamate is an ideal marker for glomerular filtration. Our results do not support this assumption.

Iothalamate and its isomers diatrizoate and iodamide (compare Fig. 1) are all derived from 2, 4, 6 triiodobenzoic acid; from a structural point of view, one would expect them to be subject to some degree of renal tubular secretion. Indeed, a renal

tubular secretion has been demonstrated in several mammalian species for diatrizoate [29, 30] and for iodamide [31–33]. Furthermore, it is well known that iothalamate [34, 35] and diatrizoate [34–36] significantly reduce the renal extraction of PAH in dogs and humans. Moreover, diatrizoate inhibits the accumulation of PAH in renal cortical slices [37]. These effects are most probably due to competitive inhibition of organic anion transport in the tubules and supports the idea of a tubular secretion of iothalamate and diatrizote.

Angiographic contrast media such as iothalamate and diatrizoate are potentially nephrotoxic [38] and occasionally produce acute renal failure [39]. The pathogenesis of contrast media-induced acute renal failure is unclear; renal ischemia, intraluminal obstruction, and direct toxic effects on tubular cells have been postulated as possible causes [40]. A tubular secretion of these contrast agents—and associated accumulation in proximal (?) tubular cells [9, 37]—creates the possibility of an intracellular mechanism behind the nephrotoxicity, perhaps similar to that of the cephalosporins [41].

Finally, an active tubular transport of the contrast medium may contribute to the appearance of a normal nephrogram during infusion urography in many patients with early oliguric renal failure [42]. This has been postulated to reflect persistent glomerular filtration and subsequent backleak of contrast molecules [42]. An alternative explanation is that the nephrogram appears normal because of active transport of contrast media from peritubular blood into (proximal?) tubular cells and still not obstructed tubular lumina.

In conclusion, results in the present study unequivocally show a marked tubular secretion of ^{125}I iothalamate in chickens and rats. It seems therefore that this substance is not a suitable marker for glomerular filtration in experimental studies. Furthermore, our results indicate a significant and sometimes marked tubular secretion of iothalamate in humans, giving plasma (^{125}I iothalamate) and particularly renal (iothalamate) clearance values that overestimate the true GFR. ^{125}I iothalamate, therefore, does not seem to be an ideal reference substance for determinations of glomerular filtration in clinical studies [23, 43, 44]. However, for clinical routine purposes ^{125}I iothalamate may be used as a filtration marker with an apparent accuracy comparable to that of creatinine.

Acknowledgment

Preliminary reports of part of this work have been presented in abstract form in *Acta Physiol Scand* 440(suppl):95, 1976, and at the XIXth EDTA Congress in Madrid, September 6–9, 1982. The authors thank S. Sandberg, L. Ekman, and I. Linderoth for technical assistance.

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