Glomerular Filtration and Tubular Secretion of MAG-3 in the Rat Kidney

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Technetium-99m mercaptoacetyltriglycine (MAG-3) has recently been introduced as a new radiopharmaceutical for dynamic renal scintigraphy. To elucidate the mechanism of renal excretion, micropuncture experiments were performed in rat kidneys for direct measurements of glomerular filtration and tubular secretory capacity. Fluid of Bowman space was collected from superficial glomeruli and analyzed for its contents of [^{99m}Tc]MAG-3, [^{125}I]hippurate and [^{3}H]inulin during constant infusion of these compounds. The ratio of activity of ultrafiltrate to that of arterial plasma was 0.23 for MAG-3, 0.68 for hippurate and 1.04 for inulin which demonstrates that the filtrated amount of MAG-3 is only 23% of that of inulin, presumably because of higher plasma protein binding which was also measured in vitro and found to be 80 ± 1.5% for MAG-3 and 32 ± 2% for [^{125}I]hippurate. Proximal and distal tubules were also micropunctured and their tubular fluid as well as the final urine analyzed for the activity of hippurate and MAG-3. The tubular fluid to plasma ratio values along the nephron and in the final urine were all lower for MAG-3 than for hippurate, indicating a lower secretory capacity. From measurements of whole renal clearance, GFR and plasma protein binding the filtered amount of MAG-3 was 0.26 and of hippurate 0.87 ml/min·g kidney weight ($p < 0.001$) and the secreted amount 2.01 and 2.38 ml/min·g kidney weight ($p < 0.05$), respectively. We conclude that MAG-3 is predominantly excreted by tubular secretion and that the lower renal clearance of MAG-3 as compared with that of hippurate is a result both of a substantially decreased glomerular filtration and of a lower tubular secretion.

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Recently, a new promising renal imaging radiopharmaceutical, technetium-99m mercaptoacetyltriglycine (MAG-3) has been introduced (*1,2*) and is undergoing clinical trials (*3–10*) as well as investigations in animal experiments (*1,11–14*). Most authors outline the similarity between MAG-3 and hippurate, since renal excretion of both is inhibited by probenecid and both have higher renal clearance than glomerular markers, indicating secretion in the kidney. MAG-3 is thus recommended as a substitute for hippurate for renal function studies. Some authors correct the MAG-3 clearance by a simple factor to get renal plasma flow (*15*) and others use it as a measure of tubular excretion rate (*4*). However, several differences between these two substances have been reported (*3,5,10,13,14,16,17*) and the present study was performed to further characterize the renal excretion mechanism for MAG-3 in comparison with hippurate. In the present study micropuncture technique was applied on the glomerular level for direct measurement of glomerular filtration and on proximal and distal tubules to outline the secretory capacity along the nephron. Plasma protein binding measurements together with whole kidney clearances were also used to estimate the filtered and secreted part of the total excretion.

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METHODS

Male Wistar rats of a special strain with surface glomeruli (strain H, Versuchstierzucht, Hannover, FRG) were used with body weights of 180–300 g and free access to normal rat chow and water. After anesthesia with thiobarbital (Inaktin, Byk, Constanz, FRG) 100—120 mg/kg i.p. the trachea was cannulated and polyethylene catheters placed into the femoral artery for continuous blood pressure monitoring and arterial blood sampling. The left jugular vein and/or the right femoral vein were also cannulated for infusion of radionuclide solutions and 0.9% Ringer solution to give a total infusion rate of

1 ml/h·100 g BW [^{99m}Tc]MAG-3 (kit prepared according to Ensing et al. (*18*) Mallinckrodt, Petten, Netherlands) and 125-I-hippurate (solution for ERPF measurements, Amersham International, New Hampshire, England) were diluted in the final solution to give ~18 MBq/h (500 μCi/hr) and 2.7 MBq/hr (70 μCi/hr), respectively. In two rats [^{3}H]inulin (Amersham International as above) was infused solely at a rate of 2.7 MBq/h (70 μCi/hr). A catheter was also placed into the left ureter for sampling of urine. The left kidney was prepared free of connecting tissue and placed in a lucite chamber fixed with cotton tissue and superfused with prewarmed mineral oil. The kidney surface was illuminated by a fiber optic light source.

Micropuncture was performed after a 60 min equilibration time (Fig. 1). Surface glomeruli appearing as clearly visible capillary balls at the surface of the kidney were punctured with sharpened glass pipets of 5–7 μm outer diameter filled with sudan stained paraffin oil. Oil was injected into Bowman space and the proximal tubule and ultrafiltrate collected by applying slight suction to the pipet, monitoring the meniscus of the oil block just at the glomerular neck of the proximal tubule. If by chance a glomerular capillary loop was punctured and erythrocytes aspirated, the sample was discharged and another glomerulum punctured. Late proximal tubules and early distal tubules were identified by a single shot injection of lissameen-green coloured Ringer solution into a randomly selected proximal tubule by the aid of a micropuncture pipet with a tip diameter of 1–2 μm. They were then punctured with the collection pipet as above and tubular fluid was collected in front of an injected oil block (compare Fig. 1). In three nephrons, only collections at one site of the nephron were successful. Glomerular collection and tubular collections were not performed in the same individual nephron.

Collection time was 2–5 min giving samples of 10 to 100 nl. They were transferred to precalibrated parallel bore capillaries (Microcaps, Drummond, USA) to measure the volume. After transfer into counting vials filled with 0.5 ml water their radioactivity was counted in a well type counter. The ^{99m}Tc activity was counted the same day and recounted after the decay of ^{99m}Tc for the ^{125}I activity. The micro-samples of the two separate rats, where [^{3}H]inulin was infused solely, were counted in a scintillation counter (Beckman LS 3800, Fullerton, USA) after transferring them into 0.5 ml water and 3 ml scintillation cocktail (Picofluor 40, Packard Instruments, Groningen, Netherlands). Corresponding plasma samples (P) taken immediately after each puncture and timed final urine samples (U) were also analyzed for the radioactivity as above. The amount of filtrated MAG-3 and hippurate is given by the ratio UF/P, where UF is the radioactivity of ultrafiltrate from the Bowmann space. The filtration rate of the single glomerulum (SNGFR) equals the flowrate of the sample.

For measurement of plasma protein binding of MAG-3 and hippurate plasma was centrifuged in special vials (Amicon, Centrifree, MA) at 3000 *g*. Radioactivity of plasma before centrifugation and of the ultrafiltrate gave the amount of protein binding from the formula (1—F/P)·100, where F is filtrate activity and P plasma activity.

For whole kidney clearances six separate rats were used with the same animal preparation as above. 1.8 MBq/hr [^{99m}Tc]MAG-3, 360 KBq/hr [^{125}I]hippurate and 320 KBq/hr 51 Cr EDTA (Behringwerke, Marburg, FRG) were infused simultaneously at a rate of 1 ml/hr·100 g BW. Plasma and urine aliquots were measured for their radioactivity in a well-type gamma counter with background, crosstalk and decay correction. MAG-3 and hippurate clearance and GFR, i.e., EDTA clearance were calculated in the usual manner (i.e., U·V/P) corrected for kidney wet weight. Two to four sampling periods of 20–30 min were averaged for every rat. The amount of filtrated and secreted MAG-3 or hippurate could be calculated from the protein free tracer fraction times GFR and total clearance minus filtrated amount, respectively.

Statistics

Analysis was performed using the students t-test for pairs (MAG-3 versus hippurate in the same rats) or unpaired samples (MAG-3 or hippurate versus inulin in different rats). Means ± s.e.m. are given. Two-tailed P (2p) <0.05 was taken as significant.

RESULTS

Table 1 gives the data from glomerular micropuncture and Table 2 those of micropuncture at early distal

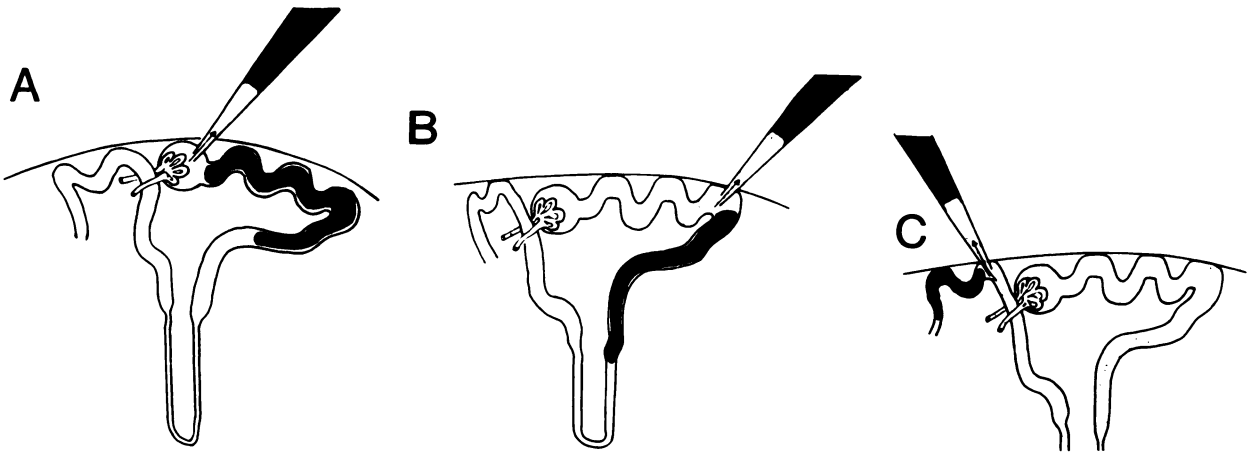

FIGURE 1
Schematic drawing of the micropuncture technique for collection of ultrafiltrate from Bowman space (A), endproximal site (B) or from the early distal tubule (C).

TABLE 1
Glomerular Micropuncture Data*

Rat	SNGFR nl/min	UF/P MAG-3	UF/P OIH	UF/P inulin
1	11.7	0.21	0.62	
	27.5	0.15	0.43	
	16.9	0.19	0.47	
2	18.6	0.16	0.76	
	18.8	0.28	0.86	
	27.9	0.18	0.57	
	20.4	0.24	0.76	
	21.4	0.32	0.87	
	29.9	0.23	0.62	
3	33.0	0.19	0.48	
	23.0	0.18	0.56	
	24.8	0.24	0.48	
	27.1	0.26	0.77	
4	13.7	0.28	0.58	
	23.6	0.30	0.66	
	20.5	0.27	0.63	
	17.2	0.34	0.68	
	26.6	0.23	0.70	
5	23.2			1.19
	24.0			1.01
	17.5			1.17
6	15.8			0.92
	15.0			1.05
	17.8			1.01
	16.6			0.91
Mean	21.3	0.24	0.64	1.04
s.e.m.	1.1	0.01	0.03	0.04
n	25	18	18	7
2P<			0.001	

* SNGFR = single nephron filtration rate, UF/P = ratio between activity of the fluid in Bowman space to that of arterial plasma. Values below 1.0 indicate restricted filtration and 1.0 free filtration.

and late proximal site as well as of the activity in the final urine. The ratio of activity of ultrafiltrate in Bowman space to that of arterial plasma was 1.04 for inulin, 0.64 for OIH and 0.24 for MAG-3, which demonstrates, that only 23% of MAG-3 in the arterial plasma is free filtrated, whereas 61% of OIH and all inulin is free filtrated.

In vitro measurements showed that MAG-3 was bound to proteins in rat plasma at 80 ± 1.5% as compared to 32 ± 2% for OIH ($2P < 0.001$).

Table 2 shows the results of micropuncture at tubular sites and the activity in the final urine. Both the tubular fluid to plasma ratio, TF/P, at late proximal sites, early distal sites and in the final urine were lower for MAG-3 than that for hippurate ($2P < 0.001$, 0.01 and 0.05, respectively). A considerable increase of the TF/P values along the nephron from the glomerulus to the final urine was observed for both substances.

Table 3 gives the results of six separate rats where simultaneous measurements of glomerular filtration rate by 51-Cr-EDTA and of the OIH and MAG-3 clearance provided means of 1.28 ± 0.05, 3.25 ± 0.08 and 2.27 ± 0.08 ml/min·g kidney weight, respectively. The MAG-3 clearance was significantly lower than that of OIH ($2P < 0.001$) and higher than that of EDTA ($2P < 0.001$). The amount of filtration calculated from GFR and the protein free plasma fraction was 0.26 ± 0.01 ml/min·g kidney weight for MAG-3 and 0.87 ± 0.04 for OIH ($2P < 0.001$). The amount of secretion of MAG-3 and OIH, i.e., the difference between total clearance and filtrated amount was 2.00 ± 0.10 and 2.39 ± 0.08 ml/min·g kidney weight ($2p < 0.05$, respectively.

DISCUSSION

Since MAG-3 has recently been introduced as a new radiopharmaceutical for renal dynamic scintigraphy there is much interest on the renal excretion mechanism for this compound. Already in early studies, the similarity of MAG-3 to hippuran concerning its high renal clearance and the inhibitory effect of probenecid (*1,2,17,19*) led to the conclusion that the same transport mechanism may be involved for the two substances. The high amount of binding to plasma proteins of MAG-3 was taken as an argument, that very low filtration takes place in the glomerulus and that tubular secretion of MAG-3 is in the range of OIH to explain the final clearance values (*1,5,17*). Both in animal experiments (*11–14*) and all human clinical trials (*2–6,8,10*), the renal clearance of MAG-3 was shown to be considerably less than that of OIH. There exists also experimental evidence that the affinity of MAG-3 to rat tubules is less than for OIH (*13*) and also in patients a lower affinity to the tubular transport system compared with [^{131}I]hippurate has been shown (*17*). The present study adds more evidence to these observations. We thus found that the amount of filtered MAG-3 is considerable less than that of inulin and that of OIH. Since it correlates strongly with the amount of protein free MAG-3 in arterial plasma the glomerular filtration barrier seem to permit all protein free MAG-3 to be filtered. The same conclusion is valid for the 125-I-hippurate preparation used in the present study. Its protein binding was 32% in rat plasma and the ultrafiltrated amount was 64% of the whole plasma activity. In human plasma and for other preparations of hippurate other values have been observed (*10*), MAG-3, however, showed in human studies similar high protein binding and consequently a restricted glomerular filtration. The present findings further demonstrate a lower secretory capacity for MAG-3 than for hippurate at two tubular sites (compare Table 2 and Fig. 2) and also in the final urine (compare Table 3 and Fig. 2). From whole clearance and measurements of plasma protein binding in patients (*9,10*) it is evident that similar conditions will exist also in the human kidney. Accord-

TABLE 2
Tubular Micropuncture and Final Urine Data*

Rat	Nephron	TF/P prox MAG-3	TF/P prox OIH	TF/P dist MAG-3	TF/P dist OIH	U/P final urine MAG-3	U/P final urine OIH	Clearance MAG-3	Clearance OIH
1	1	3.4	6.8	17.1	32.8	589	858	2.413	3.518
	2	3.2	4.5						
2	1	3.5	5.1	10.4	21.3	588	998	1.885	3.200
3	1	2.1	4.7	16.6	33.6	720	1553	2.090	4.506
	2	1.9	3.0						
4	1	1.1	3.4	7.4	13.0	869	2093	1.652	3.978
7	1	1.7	4.5	16.2	37.1	145	266	1.995	3.381
	2	4.6	9.8	10.8	19.2				
8	1	2.1	4.1			595	799	2.103	2.807
	2	3.2	8.7	17.8	22.8				
	3			9.1	12.3				
Mean		2.68	5.46	13.17	24.01	584	1094	2.023	3.565
s.e.m.		0.34	0.71	1.47	3.36	99	261	0.103	0.245
n	11	10	10	8	8	6	6	6	6
2P<			0.001		0.01		0.05		0.01

* TF/P = ratio between activity of tubular fluid to that of arterial plasma at proximal (TF/P_{prox}) or distal (TF/P_{dist}) level. U/P = ratio of activity in the final urine to that of arterial plasma. Clearance given in ml/min·g KW.

ing to the tubular reabsorption or secretion the concentration of various substances changes along the length of the nephron (*20*). Inulin starts at the glomerulus with a concentration equal to that of the plasma water and concentrates along the nephron based on the removal of water from the tubular fluid. The resulting profile is shown in Figure 2 according to the data from rats (*21,22*). P-aminohippuric acid, which is secreted activily predominantly by the proximal tubulus (*23*) results in a profile higher than that of inulin (*20,23*). Fitting the present data into a graph of the concentration profile along the nephron (Fig. 2), it becomes obvious that MAG-3 places in between [^{125}I]hippurate and inulin, arguing for a lower tubular secretion rate of MAG-3 than that of hippurate. For technical reasons we were not able to simultaneously determine inulin in our micropuncture samples to correct directly in every individual sample for the effect of water reabsorption on the tubular concentration ratio. It is difficult to assay [^{3}H]inulin in the presence of [^{125}I]hippurate. The inulin concentration profile along the nephron is, however, well known both from earlier experiments in other laboratories (*21*) and from our experiments (*22*). Separate experiments in two rats were, however, performed on the glomerular level in the present study since we had no earlier data. The observed inulin concentration in Bowman space is in agreement with free filtration of all inulin in the plasma water through the glomerular filtration barrier whereas hippurate and MAG-3 are restricted.

The mechanism for the low secretion of MAG-3 is of interest. In our first experimental study on MAG-3, we showed less penetration of MAG-3 into blood cells than was found for hippurate (*11*). The same has been

TABLE 3
Data on Clearance As Well As Filtrated and Secreted Amounts

Rat	Left renal clearance ml/min g KW OIH	Left renal clearance ml/min g KW MAG-3	Left renal clearance ml/min g KW EDTA	Secreted ml/min g KW OIH	Secreted ml/min g KW MAG-3	Filtered ml/min g KW OIH	Filtered ml/min g KW MAG-3
A	3.171	2.372	1.492	2.156	2.074	1.015	.298
C	3.339	1.929	1.260	2.482	1.677	.857	.252
D	3.127	2.310	1.127	2.361	2.085	.766	.225
E	3.518	2.593	1.300	2.634	2.333	.884	.260
F	3.458	2.040	1.336	2.550	1.773	.908	.267
G	2.933	2.301	1.163	2.142	2.068	.791	.233
Mean	3.258	2.258	1.280	2.388	2.002	0.870	0.256
s.e.m.	0.090	0.098	0.054	0.084	0.097	0.036	0.011
2P< (MAG-3 vs. OIH)		0.001			0.05		0.001

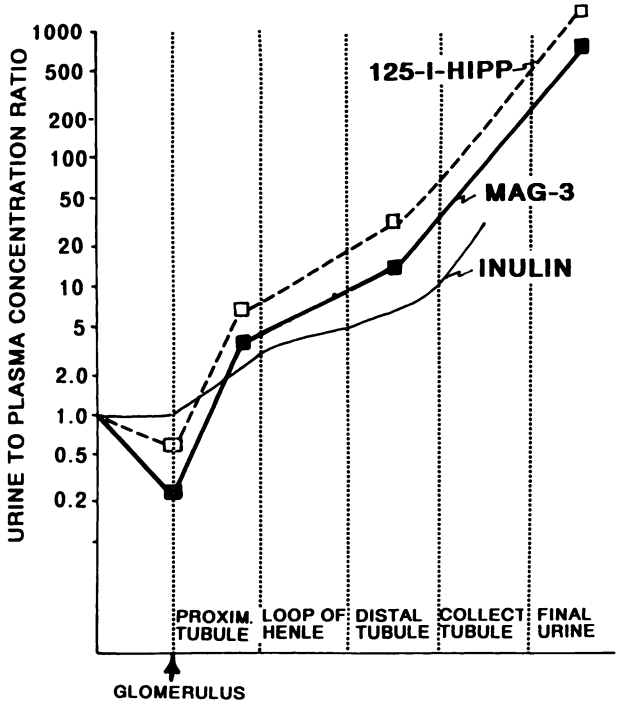

FIGURE 2
Change of concentration of inulin, hippurate and MAG_3 along the nephron. Observe the logarithmic scale. Filled and open squares give mean values of the present study. The profile for inulin is taken from the literature (*20–22*).

shown also in patients (*6,10*). That observation may also be true for penetration into tubular secretory cells which may be responsible for lower outward transport capacity into tubular lumen. The high protein binding of MAG-3 may be involved in such a hypothetical chain of events. However, other substances predominantly protein bound have not shown an increased clearance when their protein binding is reduced (*24*). No such experiments have been performed until now with MAG-3. Thus, the reason for a lower transport capacity for MAG-3 in the kidney is still unclear and further experiments are necessary.

To summarize, we have demonstrated that the lower clearance of MAG-3 is partly explained by a lower amount of filtration, due to a higher plasma protein binding but also by a lower tubular secretion rate both at the proximal and the distal tubular level.

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