Urinary Excretion of Chlorothiazide in Rats Before and After Phenobarbitone Administration

Changes in the microsomal enzyme system in the kidneys were not seen following phenobarbitone administration (GILETTE[1]), and no connection between the renal excretion of any drug and this mechanism has been described. On the other hand, a marked increase in liver blood flow was found following phenobarbitone administration in rats (OHNHAUS et al.[2]). These blood flow changes are regulated by receptors in the splanchnic area. Therefore, an increased flow to the kidneys is possible and might influence the renal excretion of drugs. To test this hypothesis, we investigated the urinary excretion rate of chlorothiazide in rats.

Method. 4 rats given a dose of labelled C^{14} chlorothiazide mixed with 'cold' drug (5.5 mg in 0.2 ml) i.p. were kept in a metabolic cage with water and food. Urine was collected after 12, 24 and 48 h. The volume of the collected urine was measured, the pH estimated and 0.5 ml of each sample added to 2.0 ml of scintillation bray. The samples were counted for 20 min in a liquid scintillation counter (Packard) and corrected by a standard curve. The calculated results are expressed as dpm/ml. This group of rats served as a control.

Another group of 4 rats were given 30 mg/kg phenobarbitone i.p. daily for 4 days. Thereafter a dose of C^{14} chlorothiazide was administered i.p. and the rats kept in a metabolic cage. Urine was collected and radioactivity measured as described above.

To test the metabolic degradation of chlorothiazide in rats, thin layer chromatography was performed. Normal rat urine and added C^{14}-chlorothiazide was run over a Kieselgel-plate (Merck, Darmstadt) in a chloroform/methanol (8:2) mixture as a control. The urine excreted during the experiment was run in the same way. The plates were then divided in centimeters and the Kieselgel scratched off. Each centimeter Kieselgel was added to liquid scintillation bray and counted in the scintillation counter.

Results. The results are shown in the Table and graphically in Figure 1. In 3 rats, before phenobarbitone administration, there was an excretion of about 45% of the given chlorothiazide within 48 h and most of this within 12 h. In comparison, in 3 rats, following phenobarbitone administration an excretion of about 85% was observed within the same period of time. One rat showed only a very low urinary excretion of 5.9% in 48 h and was therefore induced over a period of 4 days. Following the phenobarbitone administration, this rat showed an increased urinary chlorothiazide excretion of about 30.2%. pH and

[1] J. R. GILLETTE, Progr. Drug Res. *6*, 11 (1963).
[2] E. E. OHNHAUS, S. S. THORGEIRSSON, D. S. DAVIS and A. BRECKENRIDGE, Biochem. Pharmac. *20*, 2561 (1971).

A) Control rats

Rat I	i.p. injection	12 h	24 h	48 h	Total amount
Dose (dpm/ml)	931,040	376,744	49,158	13,434	439,336
Dose excreted (%)		40.5	5.3	1.4	47.2
Urinary output (ml)		4.1	4.5	6.0	14.6
Rat II					
Dose (dpm/ml)	636,066	286,640	2,544	6,765	295,949
Dose excreted (%)		45.1	0.4	1.0	46.5
Urinary output (ml)		8.0	6.0	11.6	25.6
Rat III					
Dose (dpm/ml)	137,646	61,463	1,738	2,426	65,627
Dose excreted (%)		44.6	1.3	1.8	47.7
Urinary output (ml)		5.5	3.0	18.0	26.5
Rat VII					
Dose (dpm/ml)	357,013	8,348	8,270	4,501	21,119
Dose excreted (%)		2.3	2.3	1.3	5.9
Urinary output (ml)		5.4	3.8	3.6	12.8

B) Rats after phenobarbitone administration (30 mg/kg phenobarbitone daily)

Rat IV	i.p. injection	12 h	24 h	48 h	Total amount
Dose (dpm/ml)	522,385	322,719	8,849	9,505	341,073
Dose excreted (%)		61.8	1.7	1.8	65.3
Urinary output (ml)		12.0	7.2	19.5	38.7
Rat V					
Dose (dpm/ml)	477,079	—	371,779	11,216	382,995
Dose excreted (%)		—	77.9	2.4	80.3
Urinary output (ml)		—	7.2	4.5	11.7
Rat VI					
Dose (dpm/ml)	374,760	—	305,104	3,881	308,985
Dose excreted (%)		—	81.4	1.0	82.4
Urinary output (ml)		—	6.0	3.5	9.5
Rat VII					
Dose (dpm/ml)	578,535	—	171,144	3,540	174,684
Dose excreted (%)		—	29.6	0.6	30.2
Urinary output (ml)		—	9.6	7.5	17.1

urinary flow showed no significant changes. The metabolic fate of chlorothiazide is seen in Figure 2. There was no difference in the running on the thin layer plate between the chlorothiazide added to rat urine and the chlorothiazide excreted in the urine. Degradation of chlorothiazide does not therefore appear to occur in rats.

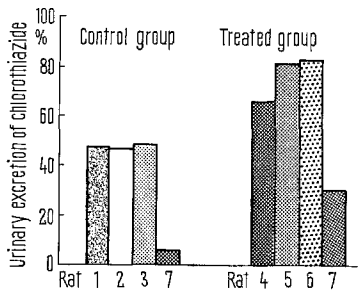

Fig. 1. This graph shows the urinary excretion of chlorothiazide in percent in the control and phenobarbitone treated group.

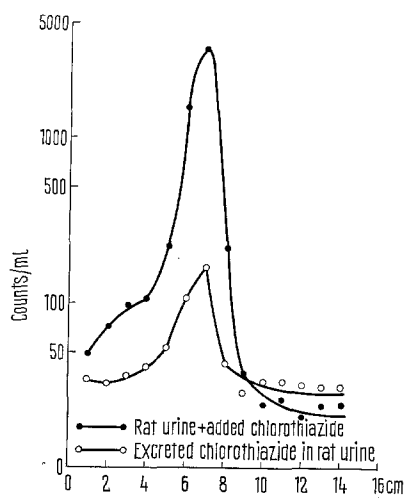

Fig. 2. Chlorothiazide dissolved in rat urine and excreted chlorothiazide were run by thin layer chromatography. Control and excreted drug show a peak at the same place on the plate.

Discussion. Chlorothiazide is a diuretic compound which is not metabolized in mice, dogs and man (BRETTELL et al.[3]) and is excreted mainly by glomerular filtration and tubular secretion (BEYER[4]). Usually 90% of the chlorothiazide given is excreted by the kidneys, but nevertheless complete biliary excretion is described in nephrectomized dogs (BAER et al.[4]). Nothing is known about the route of excretion in rats, but in view of our results a significant biliary excretion appears more likely.

The drug/creatinine clearance ratio of chlorothiazide is similar to that for *p*-aminohipparate (PAH) and therefore equivalent to renal plasma flow (BEYER[4]). After phenobarbitone administration, the urinary excretion of chlorothiazide increased from about 40 to 80%, which would be comparable to a doubling of the renal plasma flow. This could contribute to an increased blood flow in other organs besides the liver. On the other hand, chlorothiazide is also secreted via the tubules and phenobarbitone administration could influence the enzyme system and the protein content in the tubular epithelium. MÜLLER and KLINGER[6], however, found no increase in cytochrom P 450 and protein content in rats following phenobarbitone administration. Therefore, an increased excretion of chlorothiazide due to an increased plasma flow through the kidneys appears more likely.

Zusammenfassung. In 8 Ratten wurde vor und nach Phenobarbiton-Behandlung die Urinausscheidung von markiertem Chlorothiazid als Mass des Nierenplasmadurchstromes gemessen. Es zeigte sich eine Verdoppelung der Chlorothiazid-Ausscheidung in den ersten 12 Stunden nach Phenobarbital-Behandlung. Dieser Effekt ist nicht auf eine Erhöhung microsomaler Enzyme oder Carrierproteine zurückzuführen, sondern möglicherweise auf eine Erhöhung der Durchblutung.

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[3] H. R. BRETTELL, J. K. AIKAWA and G. S. GORDON, Archs. int. Med. *106*, 57 (1960).
[4] K. H. BEYER, Ann. N.Y. Acad. Sci. *71*, 363 (1958).
[5] J. E. BAER, H. L. LEIDY, A. V. BROOKS and K. H. BEYER, J. Pharm. exp. Ther. *125*, 295 (1959).
[6] D. MÜLLER and W. KLINGER, Congress of the Hungarian Pharmacologial Society 1971, Abstract.

Comparison of β-Adrenergic Blocking Activity of Eight Blockers in the Excised and Blood-Perfused Canine Sino-Atrial Node Preparation

Previously we compared effects of seven well-known β-adrenergic blockers on the chronotropic response to isoproterenol with the blood-perfused in situ SA node preparations in dogs[1]. In this study, using excised and blood-perfused canine SA node preparations[2], we compared the blocking activity of eight β-adrenergic blockers.

The heart was removed from the dog, anesthetized with ether, and plunged into cold Tyrode's solution. The sinus node artery was cannulated at its origin of the right coronary artery. The excised right atrium was placed in the funnel-shaped double wall glass jacket which was kept at 38°C by circulating warm water. The preparation was perfused at a constant pressure of 100 mm Hg by the aid of a peristaltic pump with the arterial blood of a donor dog anesthetized with sodium pentobarbital. Sodium heparin was used for preventing blood coagulation. The sinus rate was recorded on an ink-writing oscillograph through a tachometer triggered by an atrial electrogram.

Drugs used are as follows: L-norepinephrine (Fluka AG), DL-5-methyl-8-(2-hydroxy-3-*tert*-butylaminopropoxy)

[1] K. HASHIMOTO, K. OHKUDA, S. CHIBA and N. TAIRA, Experientia *25*, 1156 (1969).
[2] S. CHIBA, K. KUBOTA and K. HASHIMOTO, Tohoku J. exp. Med., in press.