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IN VIVO RENAL SYNTHESIS AND RENAL TUBULAR SECRETION
OF PARA-TYRAMINE IN THE RABBIT

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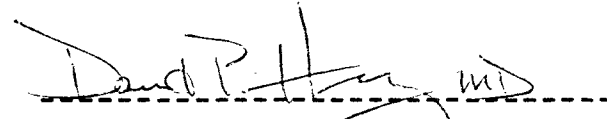
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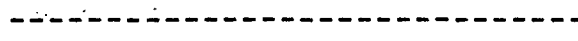


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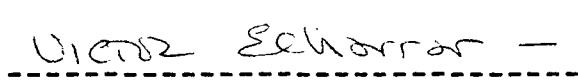


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ABSTRACT

The aromatic amine para-tyramine (p-TYM) is a predominant amine in the urine of man, rabbit, and dog. However, the origin of urinary p-TYM is not known. Renal clearance experiments were performed in order to determine the origins of urinary p-TYM. These experiments required the ability to measure p-TYM in plasma, and it was necessary to develop a means to measure plasma p-TYM accurately.

A radioenzymatic assay (REA) for p-TYM used an enzyme isolated from barley roots, tyramine N-methyltransferase (TNMT). TNMT catalyzed the N-methylation of p-TYM and phenylethylamine (PEA) to form [³H]-N-methyl p-TYM ([³H]-NMp-TYM) and [³H]-N-methyl PEA ([³H]-NMPEA), respectively, using [³H]-S-adenosyl-L-methionine as a [³H]-methyl donor. Thus, TNMT was used to develop an assay for both p-TYM and PEA. [³H]-NMp-TYM and [³H]-NMPEA were isolated by solvent extraction and thin-layer chromatography (TLC), and were quantified by liquid scintillation spectroscopy. The REA was linear from 5 to 1,000 pg, and the limit of sensitivity for p-TYM was 0.9 pg (36 pg/ml). Of 26 amines tested, only p-TYM, PEA, amphetamine and m-TYM were substrates for TNMT. After the N-methylated reaction products of these amines were separated with a TLC system, only phenylethanolamine produced greater than 1% of p-TYM CPM

(1.5%).

Renal clearance experiments were performed in the rabbit. During two consecutive control collection periods, the deliveries to the kidney of p-TYM in plasma were (mean \pm S.E.M., n=16) 10.2 ± 2.3 and 7.5 ± 1.1 ng/min., while the urinary outputs of p-TYM were 25.6 ± 3.7 and 27.4 ± 3.9 ng/min. during the same periods. Since p-TYM urinary output was 2.5 to 3.5 times greater than delivery of p-TYM in plasma to the kidney, at least 70 to 78% of urinary p-TYM must have been produced in the kidney.

A specific inhibitor of renal tubular cationic secretion, cyanine 863 (6 mg/Kg, n=7), was infused intravenously. Renal and systemic hemodynamics (mean arterial pressure, heart rate, glomerular filtration, renal plasma flow, and hematocrit) and urine flow rate were stable after vehicle (n=5) and after cyanine 863. The renal clearances of p-TYM (C_{p-TYM}) and NE (C_{NE}), and plasma levels of p-TYM (P_{p-TYM}) and NE (P_{NE}) were stable after vehicle. After cyanine 863, C_{p-TYM} was decreased by $59.6 \pm 6.6\%$ ($P < 0.02$), while C_{NE} was decreased by $70.9 \pm 5.8\%$ ($P < 0.01$). Thus, both NE and p-TYM are secreted by cationic transport.

Alpha-mono-fluoromethyldopa (FMD, 4 mg/Kg, n=5), a specific inhibitor of aromatic-amino-acid-decarboxylase (AADC), was infused intravenously, and renal function and systemic hemodynamics were monitored. Renal AADC was

completely inhibited after FMD, compared to that of animals treated with vehicle. C_{p-TYM} decreased by $95.6 \pm 1.4\%$ ($P < 0.002$), while C_{NE} decreased by $38.2 \pm 5.9\%$ ($P < 0.02$) after FMD. \dot{P}_{NE} did not change, while P_{p-TYM} was decreased ($25.4 \pm 8.5\%$, $P < 0.02$). Renal function and systemic hemodynamics were unchanged. Since C_{p-TYM} was decreased almost to zero following specific inhibition of AADC, nearly all of the urinary p-TYM must be synthesized by AADC. A decrease in C_{p-TYM} was accompanied by a decrease in C_{NE} , and thus renal p-TYM synthesis by AADC may modulate renal NE release.

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