Polyneuropathy is a common complication of chronic renal failure. The extent of the damage can be assessed by measurement of nerve conduction velocity (Johnston & Olsen, 1960) and vibratory perception threshold (Nielsen, 1972). Disease of the autonomic nervous system is not presumed to occur, possibly because objective function tests are still lacking. Recently, however, Winckler et al. (1973) presented evidence from fluorescence histochemical studies for a noradrenaline depletion of adrenergic nerve terminals in parotid biopsy specimens of uremic patients. The results were reproducible in subtotally nephrectomised rats. The reason for this phenomenon could be either a decrease of noradrenaline storage, eg a block of noradrenaline uptake, or an increase of metabolic turnover of noradrenaline in uremia. The present study was performed in order to examine both possibilities.

Our experiments on the neuronal uptake and metabolism of noradrenaline in uremia were carried out with subtotally nephrectomised Wistar rats, whose residual kidneys had been resected 14 days after the first operation. This two-stage procedure permits degrees of renal insufficiency with serum urea nitrogen concentrations varying between 40 and 200mg/100ml. In all experiments the control animals underwent two analogous sham operations.

In order to evaluate the statistical significance of noradrenaline depletion in uremia, the noradrenaline concentration was measured fluorimetrically in tissue homogenates according to Crout (1961). After 10 days of uremia the noradrenaline content had decreased to 80% in the submaxillary glands and to 72% in the hearts of the rats (p<0.005). The weight of the organs was not significantly different in both groups.

The neuronal uptake of noradrenaline in uremia was calculated from isolated perfused Langendorff hearts of uremic animals. Perfusion medium was a low calcium Krebs Henseleit solution (Ca$^{++}$ 2.3 mEq/l). In modification of the Iversen-technique (Iversen, 1963) the hearts were perfused at a constant rate (8ml/min), and the noradrenaline splitting enzymes COMT and
MAO were blocked by U 0521 (18 µg/ml)* and Pargyline (100 µg/ml). The Iversen technique allows discrimination between the neuronal and extraneuronal uptake of $^3$H-noradrenaline from their different half lives. Furthermore, initial rates of noradrenaline removal can be calculated for various perfusate concentrations and submitted to a Michaelis Menten analysis. In the uraemic group the Km of the neuronal uptake of $^3$H(+)noradrenaline was significantly reduced from 12.7 to $10^{-7}$ to $4.4 \times 10^{-7}$ M. The quotient Km/Vmax, however, remained unchanged, thus indicating an uncompetitive inhibition of the noradrenaline carrier in uraemic nerve endings (Figure 1).

![Figure 1. Michaelis Menten analysis of neuronal $^3$H(+)noradrenaline uptake in uraemia. In this plot of S/V against A the intersection of the regression line with the abscissa means the negative Km, the intersection with the ordinate the quotient Km/Vmax. The figure shows the uncompetitive inhibition of neuronal noradrenaline uptake in uraemia.](image)

In an analogous technique the tyramine induced release of $^3$H(+)noradrenaline from uraemic Langendorff hearts was measured under continuous stimulation with 100 µg/ml of tyramine. The hearts had been saturated by perfusion with 100 ng/ml of $^3$H(+)noradrenaline for 60 min before the experiment. At the onset of stimulation the control hearts contained 1.76 ± 0.36 µg/g of the tritiated amine, the uraemic ones 1.16 ± 0.38 µg/g. The cumulative noradrenaline release after 60 min of tyramine stimulation was calculated to 0.96 ± 0.18 µg/g for the control hearts and to 0.69 ± 0.24 µg/g for the hearts of uraemic rats. Although the total activity of $^3$H(+)noradrenaline at the onset of stimulation as well as the tyramine induced noradrenaline release were significantly reduced in the uraemic hearts (p <0.01, analysis of variance), the correlation with the actual size of the endogenous noradrenaline pool, i.e. to the total amount of available noradrenaline molecules, gave nearly identical results for both groups. In the control group the noradrenaline content

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*3,4-dihydroxy-2-methyl-propiophenone; the substance was kindly placed at our disposal by Dr Garland A Johnson from the Upjohn Company, Michigan
had decreased to 44.8 ± 3.3% of the initial value and in the uraemic group to 40.4 ± 4.1%. The results indicate that the tyramine induced release of noradrenaline is not severely impaired in uraemia.

The block of noradrenaline uptake is also evident in isolated adrenergic storage vesicles from myocardial homogenates of uraemic rats. The vesicles were obtained by sucrose gradient centrifugation (Whittaker et al, 1963) and incubated with 200ng/ml of \(^3\)H(t)-noradrenaline for 20 min. A second centrifugation at 280,000 x g for 60 min allowed separation of the storage vesicles from the supernatant. The noradrenaline uptake of the granular fraction was calculated from the difference of noradrenaline activity of the sediment and the supernatant. The noradrenaline uptake in the storage vesicles of uraemic rats was significantly decreased from 12.0 ± 2.9 to 6.5 ± 1.5 ng/mg protein. It may be of interest that addition of guanidinosuccinic acid (5 x 10^{-4} M) and methylguanidine (5 x 10^{-4} M), but not urea (4 x 10^{-2} M), also resulted in a similar inhibition of noradrenaline storage.

Data on noradrenaline metabolism in uraemia can be obtained either by administration of labelled noradrenaline precursors in vivo or by measurement of the enzyme activities of synthesis and catabolism in vitro. Administration of labelled precursors, however, would include the disadvantage that the larger part of the injected activity would disappear in other metabolic pathways than noradrenaline synthesis. Therefore we decided to measure the enzymes of noradrenaline synthesis in vitro.

Tyrosine hydroxylase, the rate limiting enzyme of noradrenaline synthesis, was measured in myocardial homogenates by a radiochemical assay according to Levitt et al (1967). The reaction is based on the formation of tritiated water from tyrosine-3,5-\(^3\)H and its separation by passage through a Dowex-50 H^+ column. Tyrosine hydroxylase activity was found to be significantly diminished with advancing renal failure (Figure 2). This finding may be of additional interest with respect to the fact that the formation of tyrosine from phenylalanine is also severely impaired in uraemia (Giordano et al, 1966; Giordano et al, 1969; Gulyassy et al, 1970; Bergström et al, 1972; Kopple et al, 1972).

Monoamine oxidase, the enzyme which catabolises noradrenaline to DOMA, and normetanephrine to VMA, was measured radiochemically in myocardial homogenates by the formation of indolacetic acid (IAA) from \(^14\)C-tryptamine according to Wurtman and Axelrod (1963). Surprisingly, the activity of the enzyme was positively correlated to the degree of renal failure, as measured by the serum urea nitrogen of the animals (Figure 2). In vitro incubation with urea was without any effect on the activity of monoamine oxidase.

It is too early to draw conclusions on intraneuronal noradrenaline metabolism from homogenates of the total tissue, but possibly more sensitive
determinations of metabolites from isolated perfused Langendorff hearts will help to reveal alterations of noradrenaline metabolism in uraemia.

SUMMARY

Evidence for a uraemic sympathicopathy was obtained in subtotally nephrectomised rats. Characteristic features are an inhibition of neuronal and granular noradrenaline uptake with consecutive noradrenaline depletion of the terminal reticulum. In isolated adrenergic storage vesicles a block of noradrenaline uptake was obtained by incubation with guanidinosuccinic acid and methylguanidine. The tyramine induced release of noradrenaline is not impaired in uraemia. Tyrosine hydroxylase activity was found to be decreased with advancing renal failure, whereas monoamine oxidase activity was elevated.
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