Nortriptyline and 10-hydroxynortriptyline plasma concentrations

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Nortriptyline is a frequently prescribed tricyclic antidepressant. Although the major nortriptyline metabolite in human cerebrospinal fluid, plasma and urine has been identified as 10-hydroxynortriptyline (Hammar, Alexanderson & others, 1971) there are no published quantitative reports of 10-hydroxynortriptyline in plasma. Knapp, Gaffney & others (1972) in addition to identifying 10-hydroxynortriptyline in urine also identified it in bile. In quantitative studies of human urine, Alexanderson & Borgå (1973) concluded hydroxylation of nortriptyline to be the major determinant of disappearance of nortriptyline from plasma. Bertilsson & Alexanderson (1972) had previously concluded that there was no correlation between steady-state nortriptyline concentrations and the proportion of *cis*- and *trans*-10-hydroxynortriptyline isomers found in urine. To examine the relation between nortriptyline concentrations and therapeutic response, and side effects, it is necessary to quantitate the amount of 10-hydroxynortriptyline in patients' plasma.

Heparinized plasma from patients undergoing treatment for at least one week with a level dose of nortriptyline was assayed. Nortriptyline was measured by gas chromatography-mass fragmentography as previously described (Biggs, Holland & others, 1976). All samples were run in duplicate. Separate samples were hydrolysed in acid at 95° for 60 min as described by Borgå & Garle (1972). This frees the conjugated metabolite, and produces a maximum yield of 10-hydroxynortriptyline which undergoes dehydration when exposed to acid to form 10,11-dehydronortriptyline—the product measured (Hammer & others, 1971; Knapp & others, 1972). As Bertilsson & Alexanderson (1972) found that less than 1% of the total 10-hydroxynortriptyline excreted in urine was accounted for by 10,11-dehydronortriptyline, measurement of the 10,11-dehydro compound should be an accurate reflection of the 10-hydroxynortriptyline concentrations. Standard curves were prepared by adding known amounts of 10-hydroxynortriptyline to pooled human plasma, and treating them to the same procedures as the samples from patients. This technique controls for the dehydration reaction, the *N*-trifluoroacetyl derivative formation, and losses occurring during the extraction procedure. Desmethyl doxepin was added to nortriptyline and 10-hydroxynortriptyline samples to serve as an internal standard. The samples which were subjected to acid hydrolysis had the internal standard added after the samples were neutralized, but before they were carried through the extraction procedure. The desmethyl doxepin, nortriptyline and 10-hydroxynortriptyline were derivatized with trifluoroacetic anhydride and injected as the *N*-trifluoroacetyl derivatives onto a 3% OV-17 on Gas-Chrom Q column.

Fig. 1 shows the mass fragmentogram from a patient plasma sample after acid hydrolysis. The 10-hydroxynortriptyline measured as the *N*-trifluoroacetyl 10,11-dehydrocompound *m/e* 229, 230 is shown with a retention time of 2·3 min. The ratio of the two ions remained constant. Nortriptyline is measured at *m/e* 232 with a retention time of 1·9 min also as the *N*-trifluoroacetyl compound. For quantitative purposes nortriptyline was assayed separately from the acid hydrolysed 10-hydroxynortriptyline since the amount of nortriptyline after acid hydrolysis was slightly reduced. The internal standard was also measured as the *N*-trifluoroacetyl derivative *m/e* 234 and was represented by the peak at 2·3 min.

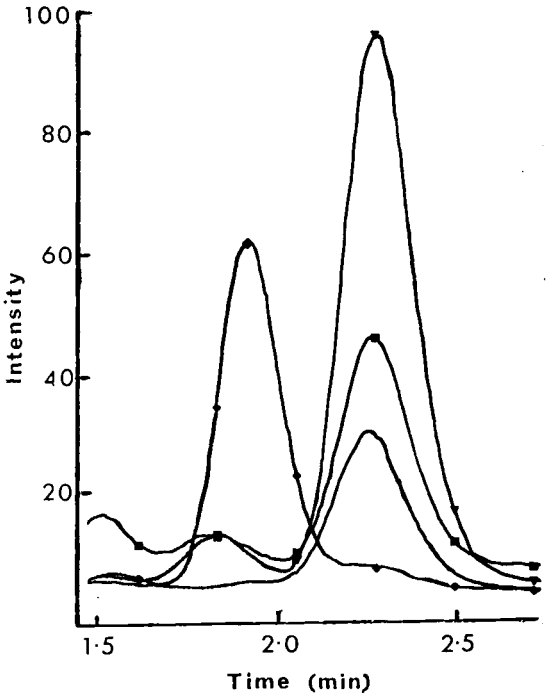

FIG. 1. Mass fragmentation from patient plasma showing ion intensities of the *N*-trifluoroacetyl derivatives of 10-hydroxynortriptyline, *m/e* 229, 230 (2·3 min); nortriptyline, *m/e* 232 (1·9 min); and desmethyldoxepin, *m/e* 234 (2·3 min) (4 ng of the nortriptyline derivative injected; electron multiplier at 2·7 kV). ■ 229 × 1, ▼ 230 × 1, ◆ 232 × 1, — 234 × 1.

* Correspondence.

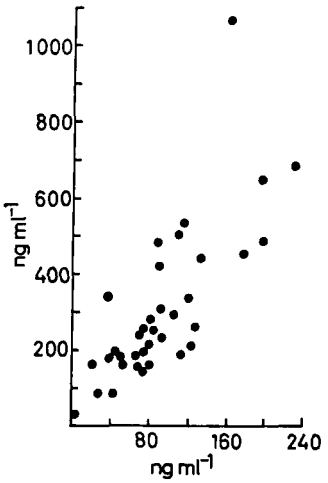

FIG. 2. Plasma concentrations of nortriptyline (ng ml^{-1}) and of free and conjugated 10-hydroxynortriptyline (ng ml^{-1} on ordinate) in 36 patients treated with nortriptyline. r=0·75, $P < 0{\cdot}001$.

In the analysis of samples from 36 patients the nortriptyline concentration was (mean ± s.e.) 92 ± 86 ng ml^{-1} while the amount of acid hydrolysable 10-hydroxynortriptyline was 308·1 ± 34·2 ng ml^{-1} giving a 10-hydroxynortriptyline: nortriptyline ratio of 3·64 ± 0·29. The non-acid hydrolysable 10-hydroxynortriptyline: nortriptyline ratio was 0·80 ± 0·10 indicating that the major portion of the 10-hydroxynortriptyline in plasma (mean 78%) is conjugated. Fig. 2 shows the nortriptyline and total 10-hydroxynortriptyline concentrations in the 36 plasma samples examined. The correlation 0·75 $P < 0{\cdot}001$ is highly significant. In all cases, the amount of total 10-hydroxynortriptyline was higher than nortriptyline concentrations; however, on the basis of the previously mentioned data, most of the 10-hydroxynortriptyline is conjugated, and therefore would be assumed to be biologically inactive. At the time samples were collected patients were on different dose levels of nortriptyline. When correction for this difference is made by dividing the plasma nortriptyline concentration by the daily dosage, the resulting ratio corrects for the dosage discrepancy. If hydroxylation is the major reaction controlling the plasma nortriptyline concentration, subjects achieving high plasma nortriptyline concentrations compared to the amount of nortriptyline ingested, would be expected to have comparatively low 10-hydroxynortriptyline concentrations when compared to the amount of nortriptyline present. Such is the case as can be seen from a plot of the ratio of total 10-hydroxynortriptyline: nortriptyline against the nortriptyline plasma: daily dose ratio which gave a negative correlation of r = — 0·50, $P < 0{\cdot}01$ indicating that the major determinant of individual differences in plasma nortriptyline concentrations is the rate of hydroxylation, as has been suggested by the study of urinary metabolites.

The purpose of investigating 10-hydroxynortriptyline plasma concentrations was to determine if significant amounts of 10-hydroxynortriptyline are present in plasma. In 36 patients the mean total plasma 10-hydroxynortriptyline concentration was 3·64 times greater than that for nortriptyline however, the much smaller ratio (0·80) of free 10-hydroxynortriptyline: nortriptyline makes it unlikely that 10-hydroxynortriptyline plays a significant biological role in patients undergoing treatment with nortriptyline unless the 10-hydroxy compound is more potent. It has been suggested from animal studies and also from the multiple studies on human urine that hydroxylation is a factor controlling individual differences in plasma steady-state concentrations. The evidence presented demonstrates that hydroxylation is the major determinant of plasma steady-state nortriptyline concentrations, even when other factors such as individual differences in weight, body mass, and other routes of degradation are not considered.

Supported, in part, by Public Health Service Grant Nos. MH-25571, MH-13002, MH-26878, DA-00259, RR-00954 from the National Institutes of Health. We wish to express our appreciation to Merck, Sharp and Dohme for supplying the 10-hydroxynortriptyline used in this study and to Eli Lilly and Company for supplying the nortriptyline (Aventyl) used to treat the patients studied.

July 14, 1976

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