The stability of the antidepressive agent nomifensine in human plasma

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Nomifensine is a novel antidepressant drug which is rapidly metabolized in man, predominantly by the formation of an acid-labile nomifensine conjugate (Heptner et al 1978). Discrepancies arising when repeating analyses prompted us to investigate the stability of this entity in human plasma.

For this purpose, 60 ml heparinized blood was taken by venepuncture on two occasions from a subject 2 h after the ingestion of a single 200 mg dose of nomifensine maleate. The plasma was immediately separated by centrifugation and stored in 2·5 ml aliquots either on the bench at room temperature (20 °C), or at 4 °C in a refrigerator, for different periods up to a maximum of one week (168 h). At the end of the incubation period, the samples were quickly deep frozen and stored in this way until analysed for both free and conjugated nomifensine.

The analytical technique used for free nomifensine was an adaptation of an established gas-liquid chromatographic method with nitrogen-sensitive detection (Dawling & Braithwaite 1978; Braithwaite 1979). For the analysis of total (free plus conjugated) nomifensine, plasma samples (100 μl) were first hydrolysed with 1M HCl (1·0 ml) for 30m at room temperature. Following alkalinization with 4 M NaOH (350 μl) and addition of maprotiline internal standard, the drugs were extracted in the same way as for free nomifensine. The conjugated nomifensine was readily calculated by subtraction of the free concentration from that of the total. The decomposition of the acid-labile conjugate was assessed as the increase in free nomifensine concentration over its original value.

At room temperature, the rate of decomposition was considerable (Fig. 1), the free nomifensine concentration having doubled after only 5 h incubation. By the end of the week, there had been a 16-fold increase in the free nomifensine concentration, although the total nomifensine had remained constant (2·7 mg litre^{-1}). The rate of hydrolysis was drastically retarded when the incubation was carried out at 4 °C (Fig. 2). Nevertheless, after one week, the free nomifensine concentration was 1·5 times its original value. Again, the total nomifensine remained constant throughout (4·4 mg litre^{-1}). This phenomenon was not observed in samples that had been kept at −20 °C for a similar period.

Heptner et al (1978) have shown that the acid-labile nomifensine conjugate can be readily hydrolysed below pH 7·1. However, the results of our stability study in plasma show that this phenomnon occurs at physiological pH (7·4) even at 4 °C, while at room temperature the decomposition is extremely rapid. This finding raises questions about the reliability of previously published data and could offer some explanation for the reported poor agreement between various analytical methods (Chamberlain & Hill 1977). This instability makes sample collection impractical in the clinical setting, and measurement of total nomifensine in both the overdose and therapeutic situation may be the most acceptable compromise. For pharmacokinetic studies, where

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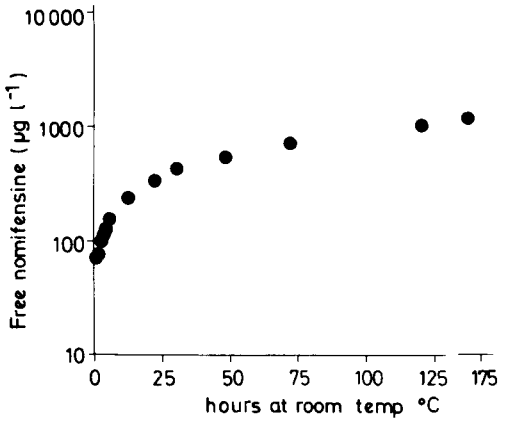

FIG. 1. Plasma concentration of free nomifensine (μg litre^{-1}) with time (h) during incubation at room temperature (20 °C).

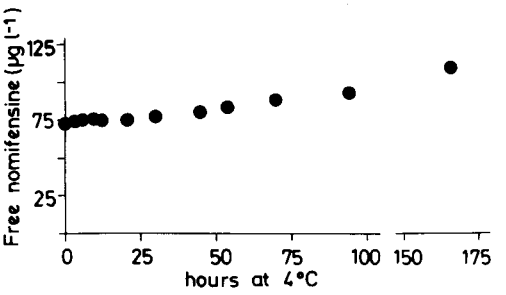

FIG. 2. Plasma concentration of free nomifensine (μg litre^{-1}) with time (h) during incubation at 4 °C.

both free and conjugated nomifensine concentrations are required, plasma samples must be deep frozen immediately after collection to prevent hydrolysis. Failure to observe these rigorous sample handling conditions will render any plasma drug concentration data invalid.

August 30, 1979

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Molecules related to Tris and some derivatives that protect haemoglobin during freeze-drying

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Freeze-drying is a classic stabilizing process for preserving fragile compounds. However, it cannot be used with haemoglobin without precautions, because during the desiccation a large amount of the ferrous iron of the heme oxidizes, forming methaemoglobin. Drying becomes possible if one or several adjuvant substances are added to the solution.

We have demonstrated a protection of haemoglobin by Tris [tris (hydroxymethyl) aminomethane, or 'THAM'] (Labrude et al 1976). This effect led us to seek other 'active' agents among related compounds. Seven molecules related to Tris were tested in 1976. We have now enlarged that study in order to demonstrate the chemical groups that are responsible for the protection Tris gives, in the hope of finding the most simple active molecule and also of understanding the mechanism of the protection which is, at now, unknown.

Materials and methods. Haemoglobin solutions, prepared as for transfusion, were obtained by haemolysis of washed red blood cells, two centrifugations and decantations to eliminate the stromata, dialysis against distilled water, adjustment of the haemoglobin concentration to 75 $\pm$ 3 g litre^{-1}, and storage at +4 °C for no more than 3 to 4 days. The 27 compounds studied are shown in Table 1, classified according to their similarity to Tris. The following modifications to this amine buffer were studied: replacement or removal of the alcohol functions (compounds 2 to 6); substitution (compounds 7 to 11), removal (compounds 12 to 14), or replacement (compound 15) of the amine function; or modification of both alcohol and amine functions (compounds 16 to 18). Finally, compounds not closely allied to Tris were studied as checks on the hypothesis about the specificity of action: compounds without an amine function (compounds 19 to 22), with a preponderant amine function (compounds 23 to 25), or containing heteroatoms (compounds 26 to 28).

Our nomenclature for these molecules does not conform to the usual norms because we have chosen to consider them all as derived from 1,3-propanediol: thus Tris is 2-amino-2-hydroxymethylpropane-1,3-diol. Among these compounds, several are used as amine buffers in biology: 2-amino-2-methylpropane-1,3-diol (AMPD), 2-amino-2-methylpropanol, mono-Tris, bis-Tris, Tricine, bis-Tris-propane, bis-AMP, Bicine, and triethanolamine.

The compounds were made up in 0·3 M stock solutions. We had already noticed in the previous study (Labrude et al 1976) that Tris is more effective in these conditions than at higher concentrations. Five ml of haemoglobin solution was mixed with 5 ml of an aqueous solution of the molecule being studied, oxygenated by bubbling with oxygen, and frozen at −40 °C under oxygen. A control was performed by adding 5 ml of demineralized water to the haemoglobin solution. The results are the means of 30 measurements for the control, 9 for the Tris, 3 for each of the other compounds studied.

Before each solution was frozen, its oxyhaemoglobin saturation and its pH were measured (with a Radiometer Hemoximeter OSM2 and a Beckman Phasar II pH meter, respectively), See Table 1.

The samples were freeze-dried in a Chaix-Meca apparatus (Nancy, France) in the following conditions: freezing to −40 °C; primary desiccation for 16 h up to −10 °C; secondary desiccation for 8 h up to +5 °C. Then the apparatus was opened to the atmosphere. The flasks, immediately stoppered, were kept under air at 4 °C for the several hours that preceded the analyses.

The freeze-dried materials were redissolved in 10 ml of demineralized water at room temperature (20 °C), and then for each sample the amount of methaemoglobin (method of Evelyn & Malloy 1938), the oxyhaemoglobin saturation, the pH, the appearance of the freeze-dried material and of the solution, and the time required for redissolution were noted.

Results (Table 1). Since Tris was taken as the model, the activities of the other compounds are reported relative to its activity. Three classes can be distinguished:

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