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Note

Assay of γ-vinyl-γ-aminobutyric acid (4-amino-hex-5-enoic acid) in plasma and urine by automatic amino acid analysis

Application to human pharmacokinetics

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γ-Vinyl-γ-aminobutyric acid (γ-vinyl GABA, 4-amino-hex-5-enoic acid, GVG; MDL 71.754) is an enzyme-activated inhibitor of GABA-transaminase [1], the enzyme responsible for the degradation of the neurotransmitter, GABA. Oral administration of γ-vinyl GABA to man produces the expected increases in central nervous system GABA concentrations [2]. Further, γ-vinyl GABA treatment has been shown to be of benefit in patients with neuroleptic-induced tardive dyskinesia [3] and in patients with resistant forms of epilepsy [4, 5].

To carry out metabolic, pharmacological and distributional studies, an assay for γ-vinyl GABA in biological specimens was required. Extraction procedures, followed by subsequent concentration of the organic phase, were not suitable due to the hydrophilic character of γ-vinyl GAGA. Conventional amino acid analysis using colorimetry was found to be too time-consuming and insensitive for most samples. A method has therefore been developed where the chromatographic separation has been made with a single buffer so that only part of a normal "physiological" assay is utilized. By using fluorimetric detection of the *o*-phthaladehyde derivative instead of colorimetry, 0.1 nmol of γ-vinyl GABA is 100 μl of injected sample can be easily measured. This method and its application to human pharmacokinetics are herein described.

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METHODS

Analytical

All chemicals were the highest grade commercially available. They were purchased from E. Merck (Darmstadt, F.R.G.) except for the lithium citrate sample dilution buffer which was bought from Pierce (Rotterdam, The Netherlands). γ-Vinyl GABA was synthesized in our centre [1].

The amino acid analyzer was a Liquimat II (Kontron, Paris, France) having two 4-mm I.D. glass columns filled with DC-6 resin (Durrum, Palo Alto, CA, U.S.A.) to a bed height of 30 cm. Automatic valve-switching ensured that the samples were injected on to alternate columns and the effluent of the analysis column was directed to the fluorimeter. A fluoromonitor (American Instrument, Silver Spring, MD, U.S.A.) was used as detector, after changing the light source to a pen-ray lamp (11 LSC - 1 L, Ultra-Violet Products, San Gabriel, CA, U.S.A.). A Corning Glass 7-51 filter was fitted for excitation (340 nm) and a Wratten 2A filter for emission (440 nm). Samples were injected automatically by an APE-100 (Kontron) equipped with a 100-μl loop.

Separation was made with a single buffer of high molarity (lithium citrate, 0.668 *M* lithium, adjusted to pH 4.6 with hydrochloric acid) to elute γ-vinyl GABA rapidly. Buffer flow was 25 ml/h and the column temperature was 45°C. γ-Vinyl GABA eluted after 45 min. Regeneration of the column was carried out after each analysis by pumping lithium hydroxide (0.3 *M*) for 16 min.

Derivative formation with the *o*-phthalaldehyde (OPA) reagent (17 ml/h) was achieved by using a simple "T" to mix the reagent and eluting buffer before passing to the fluorimeter. A reaction coil between the "T" and the fluorimeter was found to have little influence on the fluorescence of the amino acids when using these high buffer flow-rates. The OPA reagent consisted of 200 mg of OPA dissolved in 3 ml of methanol added to 1 l of 0.4 *M* (pH 10.4) potassium borate solution containing 3 ml of Brij and 1 ml of mercaptoethanol. The injection of samples alternately on to two columns in a manner similar to previously reported [6] shortened the analysis time.

Human plasma samples were mixed with 0.5 volume of 20% trichloroacetic acid, allowed to stand for 30 min at 0°C and then centrifuged for 2 min in an eppendorf 3200 centrifuge. The supernatant was then injected directly, or further diluted with lithium citrate sample dilution buffer (0.2 *M*, pH 2.2) as required.

Urine was collected in polyethylene bottles to which 1 ml of 6 *M* hydrochloric acid was added. An aliquot (1 ml) was mixed with 250 μl of trichloroacetic acid (20%), allowed to stand as before to precipitate any proteins, and then centrifuged. The supernatant (200—500 μl) was further diluted to 10 ml with sample dilution buffer, before injection on the column.

Measurements

Sample volumes of 100 μl were injected and areas compared with previously injected reference solutions. Fluorescence was linear over the range 0.2—5 nmol of γ-vinyl GABA injected. When γ-vinyl GABA was added to drug-free plasma and carried through the procedure the recoveries ± S.E.M. for additions

of 0.2, 1 and 3 nmol/ml were 98.7% ± 4.8, 99.% ± 4.8 and 93.8% ± 2.4, respectively. When samples were stored at −20° C for several months no significant changes in the γ-vinyl GABA content were seen.

Subjects

Four healthy male volunteers, age 21—35 years (mean = 26 years), weighing 65—75 kg (mean = 69 kg) consented to be studied. While in the fasting state each subject swallowed four gelatin capsules, each capsule containing 250 mg of γ-vinyl GABA without excipient, with approximately 200 ml of water. To assess intra-individual variation, in two volunteers drug was administered on two separate occasions separated by at least two weeks. At various times up to 24 h after each dose, venous blood (10 ml) was sampled into heparinized tubes and plasma obtained by centrifugation; 24-h urine was collected as described above.

Pharmacokinetic data were evaluated by the strip method [7].

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms from specimens of human plasma carried through the analytical procedure. Similar chromatograms have been obtained with plasma and serum from other species.

Absorption of γ-vinyl GABA following oral administration was rapid, with peak plasma concentrations reached between 0.75 and 2 h, ranging from 172 to 315 nmol/ml (Fig. 2). Mean peak plasma concentration was 192 nmol/ml at 1 h post drug. Thereafter, plasma concentrations declined but were still detectable

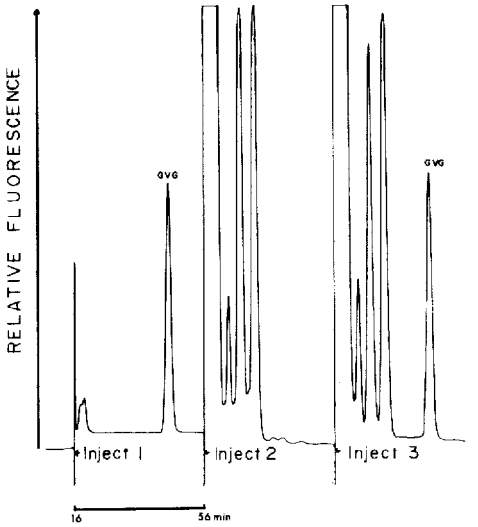

Fig. 1. Chromatograms of samples prepared as described in the text. In practice, the chromatogram is recorded between 16 and 56 min after each injection to avoid recording the early eluting acidic and neutral amino acids. The first injection (1) is of a 6.67% trichloroacetic acid solution containing 1 nmol of γ-vinyl GABA. Injection 2 is a sample of human plasma before administration of γ-vinyl GABA. Injection 3 is obtained from a plasma sample of the same subject 24 h after taking γ-vinyl GABA.

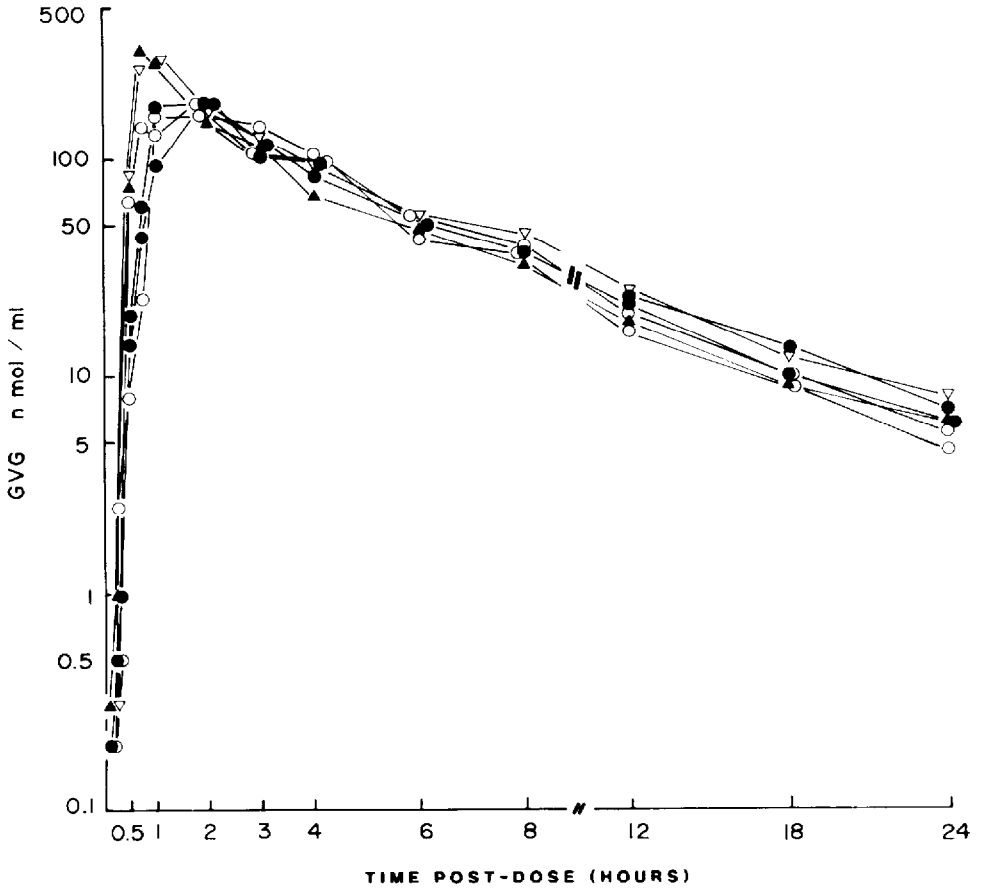

Fig. 2. Plasma concentrations of γ-vinyl GABA (GVG) as a function of time after a 1-g oral dose in four normal volunteers. Each symbol represents a single individual, with two subjects (open and closed circles) being studied twice.

in all cases at 24 h. Both intra- and inter-individual differences were remarkably small. Individual and mean plasma concentration curves could be fitted to an open two-compartment model. Terminal elimination half-lives of individual curves varied from 5.3 to 7.4 h (mean = 6.6 h) with the mean curve having a half-life of 6.8 h. 24-h urinary recovery of γ-vinyl GABA ranged from 746 to 951 mg (i.e. 75–95%; mean = 83%). Thus, γ-vinyl GABA is rapidly and almost completely absorbed following oral administration in man and is mostly eliminated unchanged in the urine within 24 h. No binding to human plasma proteins occurs [8].

The method described allows determination of plasma and urinary drug concentrations following therapeutic doses of γ-vinyl GABA [3—5]. Because of the nature of this drug's action, however, i.e. irreversible enzyme inhibition, it is likely that the kinetics of enzyme turnover will have a greater influence on the time course of the biochemical action than kinetics of the drug itself. Nevertheless, the method should prove useful in the further clinical development of this novel enzyme inhibitor.

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