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Note

Gas chromatographic—mass spectrometric determination of nomifensine using a stable isotope-labeled analogue as an internal standard

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Nomifensine, 8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline, has a pharmacological profile similar, but not identical, to that of known antidepressants such as imipramine and amitriptyline [1]. Chemically unrelated to and unlike the tricyclic antidepressants, nomifensine possesses a definite, centrally stimulating effect at higher dosages which could be due to either a strong inhibition of dopamine re-uptake [2, 3], an agonistic effect on the dopaminergic receptor [4] or a reserpine-sensitive intraneuronal dopaminergic action [5]. In preliminary observations in humans nomifensine has shown an antidepressant action similar to that of imipramine [6, 7]; however, the incidence of side-effects observed with nomifensine seems to be less than that of imipramine and onset of the therapeutic effect begins earlier.

A highly sensitive and selective analytical methodology to quantitate nomifensine is essential for experimental studies on specific brain regions and for clinical monitoring and pharmacokinetics. The earlier methods of analysis based on gas chromatography (GC) [8], thin-layer chromatography [9], liquid chromatography [10] and radioimmunoassay [11] lack sensitivity, precision and selectivity. This report describes a gas chromatographic—mass spectrometric (GC—MS) assay of nomifensine in rat brain and plasma. Nomifensine can be extracted from either aqueous solution, plasma or brain homogenates with organic solvents and treated with trifluoroacetic anhydride (TFAA). The resulting trifluoroacetanilide (nomifensine-TFA) has excellent GC and MS characteristics. Selected-ion monitoring (SIM), the technique built on combined GC—MS [12, 13], was used to develop a sensitive and selective assay for nomifensine in plasma and brain tissue extract with site-specific ^{2}H-labeled nomifensine as internal standard.

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EXPERIMENTAL

Analytical-grade nomifensine maleate (Hoechst-Roussel, Frankfurt-am-Main, F.R.G.), ^2H_2O (Aldrich, Milwaukee, WI, U.S.A.; 99.8 atom% ^2H), pentafluoroacetic anhydride and trifluoroacetic anhydride (Aldrich, Milwaukee, WI, U.S.A.) were used without further purification. All solvents were of analytical-reagent grade (Fisher Scientific, Pittsburg, PA, U.S.A.); silanized tubes (10 ml) with screw caps were used for extraction and final solvent evaporation was performed in 5-ml glass-stoppered centrifuge tubes (Kimble Owens, Toledo, OH, U.S.A.). Pasteur pipets with hand-drawn constricted tips were utilized for all solvent transfers.

Nomifensine-d_2

Nomifensine (5 mg) in 10 ml of anhydrous tetrahydrofuran and 1 ml of ^2H-labeled pentafluoropropionic acid was heated under reflux for 3 h. After this, the material was cooled to room temperature, adjusted to pH 9.5 and extracted with benzene. The *ortho* and *para* H atoms in the aminophenyl moiety undergo typical electrophilic exchange [14] resulting in the formation of nomifensine-d_2. The product was chromatographically identical to the authentic unlabeled material. A selected-ion detection analysis of the material showed the presence of an ion equivalent to 98.5 ± 0.2% (n = 5) nomifensine-d_2.

Instrumentation

Preliminary GC was performed on a Perkin-Elmer Model 3920 instrument (Norwalk, CT, U.S.A.), equipped with a silanized 1.8-m column packed with 1% OV-17 and maintained at 190°C with a detector temperature of 300°C. The carrier gas flow-rate was 30 ml/min. GC—MS was carried out on an LKB-9000 instrument (Stockholm, Sweden) equipped with a multiple-ion detector—peak matcher accessory [12, 13]. GC column temperature was 190°C, the flash heater was at 220°C and the helium flow-rate was 20 ml/min. The retention time of nomifensine-TFA was 2 min. The ionization potential was 70 eV in the scan mode, 20 eV in the multiple-ion detection (MID) mode and the trap current was set at 60 uA.

Extraction and derivatization of nomifensine

Drug-free plasma (1 ml) or rat brain extract in 0.1 *M* hydrochloric acid (1.0 ml) was spiked with 10 ng of nomifensine and to the samples were added 25 μl of a stock solution of nomifensine-d_2 (concentration of stock solution 1 ng/μl). Following the addition of 1.0 ml of pH 9.2 carbonate buffer (1.0 *M*), the samples were thoroughly mixed on a vortex mixer and extracted with 10 ml of benzene. From each sample, the organic layer was separated and to it 1 ml of 0.1 *M* hydrochloric acid was added and the mixture was shaken for 15 min. The organic layer was discarded and the aqueous phase was mixed with 1.0 ml of carbonate buffer and extracted with 5 ml of benzene. The organic layer was evaporated to dryness under a gentle stream of nitrogen at 50°C, to the dried residue 100 μl of TFAA were added and the material was left at 25°C for 1 h. After this period, the material was evaporated to dryness under a gentle stream of nitrogen at room temperature. The material was taken up in 50 μl of benzene and 1.5 μl were injected in the GC—MS system.

RESULTS AND DISCUSSION

The mass spectrum of nomifensine-TFA (Fig. 1A) shows a molecular ion at *m/e* 334, fairly significant M − 1 ion at *m/e* 333, base peak at *m/e* 222 and peak of modest intensity at *m/e* 178. The fragmentation mechanism is readily discernible. The molecular ion formed by loss of a lone-pair electron from either nitrogen atom undergoes a typical β-cleavage fragmentation process [8, 15] to give the observed ions. The mass spectrum of nomifensine-d_2-TFA (Fig. 1B) shows a molecular ion at *m/e* 336, M − 1 ion at *m/e* 335, and is similar to that of nomifensine-TFA. Most ions are shifted to higher mass by 2 a.m.u.

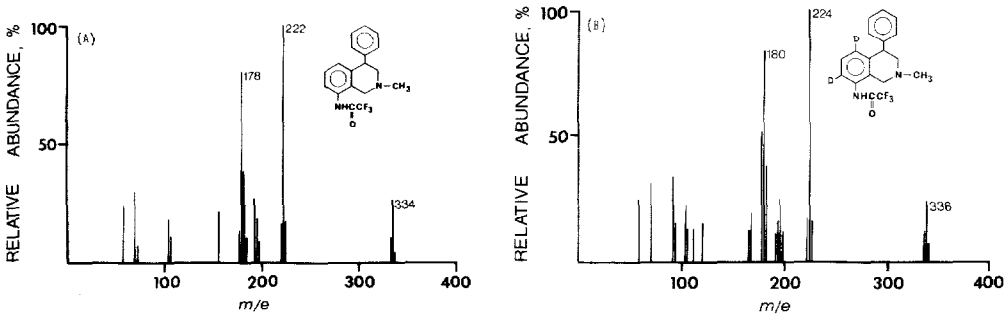

Fig. 1. Electron-impact mass spectrum of (A) nomifensine-TFA and (B) nomifensine-d_2-TFA.

Selected-ion monitoring assay

The ion at *m/e* 222 is selective for the nomifensine-TFA (*m/e* 224 for nomifensine-d_2) and is also the base peak and also a convenient working mass for SIM assay. Furthermore, control plasma or tissue extract, subjected to the described procedure for nomifensine, showed no significant background ions at *m/e* 222 and 224. Consequently, biological extract, along with labeled nomifensine, were processed as described above. An aliquot of the material was injected into the gas chromatograph—mass spectrometer and nomifensine was quantitated by measuring the ion intensities at *m/e* 222 and 224, respectively, and standard curves were established in the usual manner. Analysis of the data gave a slope of 0.98 ± 0.03 and an intercept of 0.2 ± 0.1 ng. These data affirm a simple linear relationship between the appropriate ion intensity ratios and concentration of nomifensine and exclude any isotopic exchange or any significant kinetic isotope effect in the fragmentation process.

Recovery and precision

Six samples containing 10 ng of nomifensine were analyzed as above using 25 ng/ml nomifensine-d_2 as internal standard. The results of these samples were 9.7 ± 0.35 ng/ml. These samples were analyzed in a second set. Exactly the same amounts were taken as above but the internal standard was added after the extraction. The recoveries for these samples, based on comparison of the ion intensity ratios of the two sets, were 78 ± 9.2%. The wide range of recoveries observed is expected in trace analysis and is attributed to variable

TABLE I

ANALYSIS OF NOMIFENSINE IN CONTROL PLASMA

Sample No.	n	Concentration added (ng/ml)	Concentration found (ng/ml)	Recovery (%)
1	6	5	4.82 ± 0.72	96
2	5	10	8.90 ± 0.61	89
3	5	20	19.32 ± 0.35	97
4	5	50	48.36 ± 0.50	97
5	5	100	97.70 ± 1.60	98

glassware, GC column adsorption and possible thermal decomposition. The limit of determination of the assay, being a function of extraction efficiencies, GC column conditions and ion source, cannot be quoted in absolute terms. With good mass spectrometer performance, clean and freshly silanized GC column and glassware and better than 60% recoveries, a limit of determination of approximately 1—2 ng/ml nomifensine is possible. Table I gives the assay precision in the control plasma at various levels of concentration of nomifensine.

The method described above was used for the analysis of the free drug in the whole brain of rats given 10 mg/kg nomifensine intraperitoneally and decapitated after 20 min. The analysis of a 0.1 *M* hydrochloric acid extract of whole brain tissue (1.0 ml) after the addition of nomifensine-d_2 was as described in Experimental and the tissue content of the drug was found to be 5.24 μg/g. The mass fragmentograms obtained from biological extracts (Fig. 2) are clean, show symmetrical peaks and obviously no interference from

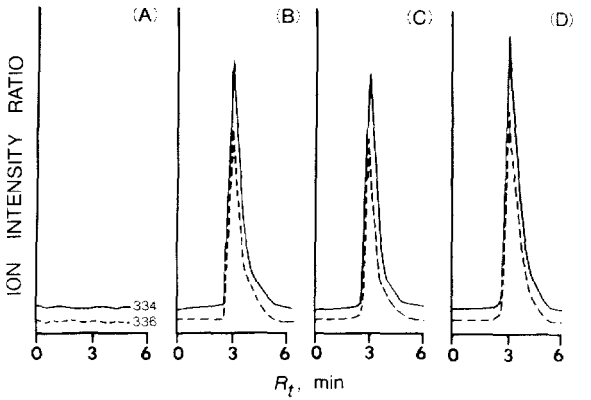

Fig. 2. Selected-ion fragmentograms of nomifensine-TFA (*m/e* 334) (——) along with nomifensine-d_2-TFA (*m/e* 336) (- - - - -). (A) Drug-free control plasma extract (1 ml). (B) Plasma extract containing 10 ng/ml nomifensine and 25 ng/ml nomifensine-d_2. (C) Extract from brain homogenate with added nomifensine-d_2 (1 μg). Rat was given nomifensine (10 mg/kg) in isotonic saline intraperitoneally. After 20 min the animal was decapitated, brain taken out (brain tissue 2.03 g), homogenized in 15 ml ice cold 0.1 *M* hydrochloric acid and an aliquot of this was taken and processed as above; nomifensine found was 625 ng for the aliquot and 5.24 μg/g of brain tissue. (D) Extract from brain homogenate as above, represents a duplicate assay.

extraneous materials is indicated. The analytical method described here will permit further detailed pharmacokinetic studies of this novel antidepressant in man.

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