

CHROMBIO. 5043

Note**Determination of nomifensine in rat brain by high-performance liquid chromatography with fluorescence detection**

V. D'ARANNO* and A. MANCINELLI

Department of Pharmacology, "Menarini Ricerche Sud", Via Tito Speri 10, 00040 Pomezia, Rome (Italy)

F. BORSINI

"A. Menarini" Pharmaceuticals, Via Sette Santi 3, 50131 Florence (Italy)

M. FURIO

Department of Pharmacology, "Menarini Ricerche Sud", Via Tito Speri 10, 00040 Pomezia, Rome (Italy)

and

A. MELI

"A. Menarini" Pharmaceuticals, Via Sette Santi 3, 50131 Florence (Italy)

(First received October 5th, 1988; revised manuscript received September 19th, 1989)

Although nomifensine has been recently withdrawn from the market as an antidepressant [1] because of its side-effects [2,3], it possesses pharmacological properties that can be used to investigate the role of the brain catecholaminergic system. Nomifensine blocks both dopamine and noradrenaline uptake [1,4].

Many different methods for estimating nomifensine in biological samples and pharmaceutical preparations have been described [5–16]. Only one of these determined nomifensine levels in the central nervous system, using gas chromatography–mass spectrometry (GC–MS) [14]. We have developed a method for the detection of nomifensine in brain using an organic extraction and the more common and less expensive high-performance liquid chromatography (HPLC) with fluorimetric detection.

EXPERIMENTAL

Reagents and chemicals

Nomifensine maleate (8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline) was kindly supplied by Hoechst (Milan, Italy) and prazosin [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine] was purchased from Pfizer (Latina, Italy). Disodium hydrogenphosphate 12-hydrate, sodium hydroxide, hydrochloric acid, orthophosphoric acid, benzene and acetonitrile were purchased from Merck (Darmstadt, F.R.G.) and nonylamine from Fluka (Buchs, Switzerland).

Solvents and other chemicals were of chromatographic grade. Water was bidistilled and filtered through a 0.2- μm Nucleopore[®] filter.

Instrumentation and chromatographic conditions

The HPLC system consisted of an HPLC pump (Model 114M, Beckman, Berkeley, CA, U.S.A.) equipped with a sample injection valve (Model 210 A, Beckman) and a 20- μl loop, a $\mu\text{Bondapak C}_{18}$ column (30 cm \times 3.9 mm I.D., 10 μm particle size) (Waters Assoc., Milford, MA, U.S.A.) and a fluorescence HPLC monitor (Model RF-530, Shimadzu, Kyoto, Japan) operated at excitation and emission wavelengths of 292 and 360 nm, respectively. A Perkin-Elmer LC-100 laboratory computing integrator was used to calculate peak areas.

The mobile phase consisted of 0.01 M disodium hydrogenphosphate buffer (pH 9.2)–acetonitrile–nonylamine (69.999:29.999:0.0020, v/v). The eluent was adjusted with 83% (w/v) orthophosphoric acid to pH 6.1, and the flow-rate was 1.8 ml/min.

Extraction procedure

One half of a rat brain (1 g) was homogenized in polypropylene tubes containing 2.5 ml of 0.5 M disodium hydrogenphosphate buffer (pH 9.2) and 20 μl of a methanolic solution of prazosin (10 $\mu\text{g}/\text{ml}$). Samples were immediately extracted twice with 4.5 ml of benzene by shaking for 30 min; this was done because double extraction increased the recovery of nomifensine by 30–35%. After 15 min centrifugation at 4275 g at 4°C, the combined organic phases were transferred to a polypropylene tube containing 1 ml of 0.1 M hydrochloric acid, and the mixture was shaken for 15 min and centrifuged as before. The supernatant was discarded, and 1 ml of 0.5 M disodium hydrogenphosphate buffer and 20 μl of 5 M sodium hydroxide were added to the aqueous phase. The aqueous phase was re-extracted with 5 ml of benzene. Following centrifugation the organic phase was transferred to polypropylene tubes and evaporated to dryness at room temperature (22–24°C) under a gentle stream of nitrogen. The dry residue was reconstituted with 100 μl of HPLC eluent, and 20 μl were injected into the HPLC column.

TABLE I

RETENTION TIMES OF COMPOUNDS INJECTED UNDER THE SAME ANALYTICAL ELUTION CONDITIONS AS NOMIFENSINE

Compound	Retention time (min)
2-Hydroxydesipramine	7
Desmethyldesipramine	17
Desipramine	19
Trimipramine	36
Nortriptyline	> 60
Amitriptyline	> 60
Clomipramine	> 60

Recovery and internal standard curves

The recovery was determined by adding known amounts of nomifensine to the brain of untreated rats in the range 5–2000 ng/g and comparing the peak areas obtained after extraction with those obtained after injection of various concentrations of nomifensine into the chromatographic column. Internal standard curves were constructed by adding to the brain of untreated animals known amounts (5–2000 ng) of nomifensine and analysing concurrently with brain samples from treated animals. Concentrations of nomifensine in brain samples were calculated by plotting the ratio of the peak area of nomifensine to the peak area of prazosin on the internal standard curve. The within-assay precision was determined by assaying nomifensine in six samples for each concentration in the range 5–2000 ng.

Selectivity/interference studies

The retention times of some antidepressant drugs, which were injected under the analytical conditions for nomifensine, are shown in Table I. None of these compounds interfered with nomifensine or the internal standard.

Application of the method

Nomifensine (2.5 mg/kg) was administered intraperitoneally to rats three times (24, 5 and 1 h) before killing. This dosing schedule is normally used to detect antidepressant properties in this animal species [17].

RESULTS AND DISCUSSION

Nomifensine is a fluorescent substance, and the excitation and emission wavelengths for its detection were set at 292 and 360 nm, respectively [15]. Although prazosin is not a structural analogue of nomifensine, it was chosen as internal standard because it fluoresces under the same analytical conditions.

Nomifensine and prazosin gave symmetrical, well separated peaks, with re-

retention times of 5.5 and 3.7 min, respectively (see Fig. 1a). The limit of detection (signal-to-noise ratio of 3) was 5 ng/g of tissue (Fig. 1b and c). Under our experimental conditions the peak-area ratio nomifensine to prazosin was linear in the range 5–2000 ng/g of tissue ($y=0.0088+0.494x$; correlation coefficient=0.9978). The average extraction efficiency was 96% (see Table II). A typical chromatogram of a brain sample from a rat treated with nomifensine is shown in Fig. 1d.

We also tested the chromatographic performance of the metabolite 4'-hydroxynomifensine (retention time 2.5 min; Fig. 1a) because it may have some pharmacological activity [18]. Under our experimental conditions it can be recovered (75%) with a sensitivity of 15 ng/g of tissue. However, no detectable levels of 4'-hydroxynomifensine were found in nomifensine-treated rats. This casts some doubts on the role of this metabolite in mediating the pharmacological effect of nomifensine, at least as far as rats are concerned.

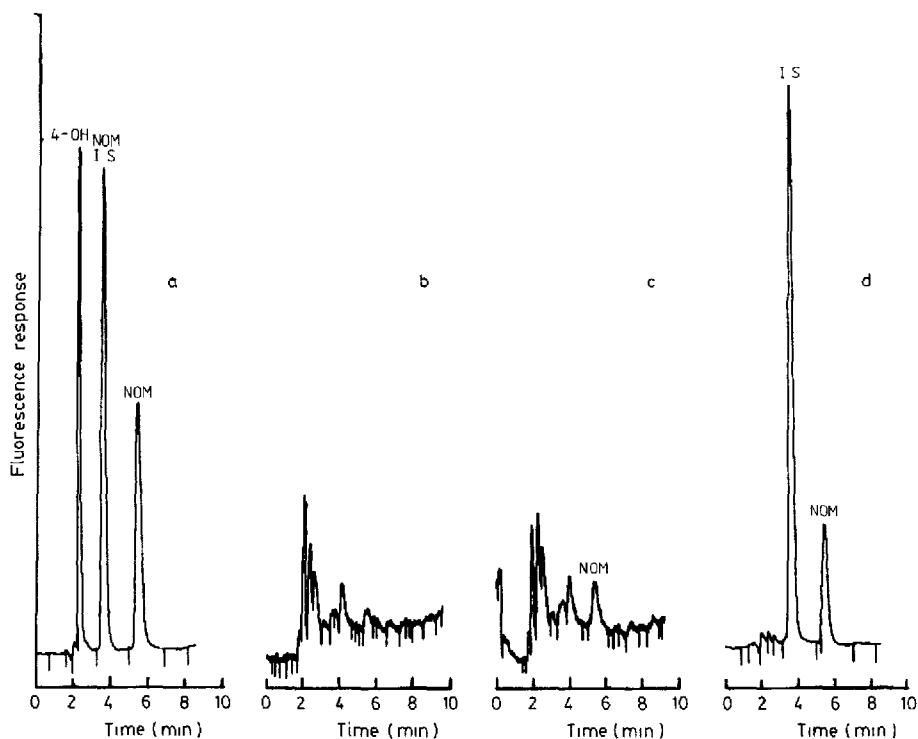


Fig. 1. Chromatograms of (a) 100 ng of 4-hydroxynomifensine (4-OH NOM, external standard), 40 ng of prazosin (I.S.) and 100 ng of nomifensine (NOM) with retention times of 2.4, 3.7 and 5.5 min, respectively, (b) brain extract from an untreated rat, (c) brain extract treated with 5 ng of NOM added at the beginning of the extraction and (d) brain extract after intraperitoneal injection of nomifensine (the concentration of nomifensine is 390 ng/g).

TABLE II

INTRA-ASSAY REPRODUCIBILITY FOR THE DETERMINATION OF NOMIFENSINE IN RAT BRAIN

Concentration (ng/g)	Coefficient of variation (%)	Recovery (mean \pm S.D., $n=6$) (%)
5	12.5	102.1 \pm 14.2
20	2.3	99.5 \pm 2.3
50	5.8	95.6 \pm 6.0
200	3.2	96.0 \pm 3.1
1000	2.9	94.1 \pm 3.0
2000	1.6	96.4 \pm 1.58

Benzene has also been used for the extraction of nomifensine from rat brain using GC-MS [14]. Because benzene is carcinogenic, we tried to use other solvents, such as hexane or diethyl ether, which have been utilized for nomifensine extraction from plasma [5,11,13], or ethyl acetate. None of them gave satisfactory results under our analytical conditions. Although the sensitivity for nomifensine detection from rat brain tissues using GC-MS [14] is higher (ca. five-fold) than using HPLC with fluorescence detection, the present method represents a reliable procedure for determining nomifensine in rat brain with more common and less expensive laboratory equipment.

ACKNOWLEDGEMENTS

The authors thank Mrs. Giovanna Guiso of "Mario Negri" Institute of Milan (Italy) for the generous gift of the 4-hydroxynomifensine. This work was supported by IMI Contract No. 45054.

REFERENCES

- 1 I. Hoffman, *Arzneim.-Forsch.*, 23 (1973) 45.
- 2 C. Mueller-Eckhardt, B. Allolio, A. Salama, V. Kiefel and U. Deuss, *Transfusion*, 27 (1987) 250.
- 3 G. Mueller-Eckhardt, G. Giers, A. Salama, D.J. Schendel, G. Fass, C. Mueller-Eckhardt, *Vox Sanguinis*, 54 (1988) 59.
- 4 H.J. Gerhards, A. Carezni and E. Costa, *Naunyn-Schmiedeberg's Arch Pharmacol.*, 286 (1974) 49.
- 5 L. Vereczkey, G. Bianchetti, V. Rovei and A. Frigerio, *J. Chromatogr.*, 116 (1976) 451
- 6 J. Chamberlain and H.M. Hill, *Br. J. Pharmacol.*, 4 (Suppl. 2) (1977) 117S.
- 7 W. Heptener, M.J. Badian and S. Baudner, *Br. J. Clin. Pharmacol.*, 4 (Suppl. 2) (1977) 123S.
- 8 M. Uilheim and P. Hajdu, *Arzneim.-Forsch.*, 27 (1977) 98.
- 9 I.M. McIntyre, T.R. Norman, G.D. Burrows and K.P. Maguire, *Br. J. Clin. Pharmacol.*, 12 (1981) 691.

- 10 C.T. Hung, R.B. Taylor and N. Peterson, *J. Pharm. Biomed. Anal.*, 1 (1983) 73.
- 11 R.L.P. Lindberg, J.S. Salonen and E I. Lislao, *J. Chromatogr.*, 276 (1983) 85.
- 12 M.P. Quaglio and A.M. Bellini, *Farmaco, Ed. Prat.*, 39 (1984) 222.
- 13 R.L.P. Lindberg, *J. Chromatogr.*, 341 (1985) 333.
- 14 S.P. Bagchi, T. Lutz and S.P. Jindal, *J. Chromatogr.*, 344 (1985) 362.
- 15 A.-A.M. Wahbi, M.A. Abounassif, E.-R.A. Gad-Kariem and H.Y. Aboul-Enei, *Talanta*, 34 (1987) 287.
- 16 C.T. Hung, R.B. Taylor and N. Paterson, *J. Chromatogr.*, 240 (1982) 61.
- 17 R.D. Porsolt, G. Anton, N. Blavet and M. Jalfre, *Eur. J. Pharmacol* , 47 (1978) 379.
- 18 S. Caccia, G. Guiso and M.G. Zanini, *J. Chromatogr.*, 190 (1980) 475.