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Note

Analysis of the dopamine agonist N-0437 in rat serum using reversed-phase high-performance liquid chromatography with electrochemical detection

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In recent years there has been considerable interest in the substituted 2-aminotetralins because of their high affinity for dopamine receptors. Various studies have shown 2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin (N-0437, I, Fig. 1) to be a particularly interesting member of this group because of its potency and selective profile of activity as a D-2 dopamine receptor agonist in well established test models [1, 2]. The considerable first-pass effect through enzymic coupling of the free hydroxy group of I by glucuronic acid [3] made it necessary to study the serum levels of I and certain derivatives as part of a programme aimed at obtaining a clinically useful D-2 agonist.

In this report a simple and sensitive method is described for the quantification of I in rat serum, using reversed-phase high-performance liquid chromatography (RP-HPLC) with electrochemical detection. The analogue 2-(N-propyl-N-phenylethylamino)-5-hydroxytetralin (N-0434, II) was used as an internal standard.

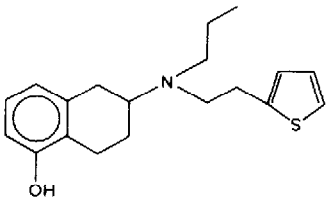

Fig. 1 Structure of I (N-0437)

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EXPERIMENTAL

Materials

I and II were synthesized in our laboratory and isolated as their hydrochloride salts [4]. I was dissolved before use in demineralized water. All reagents were of analytical grade and were used without further purification. Citric acid monohydrate, disodium hydrogenphosphate, 2-propanol, Na_2EDTA, acetonitrile and dichloromethane were obtained from Merck (Darmstadt, F.R.G.). Triethylamine was obtained from Janssen (Beerse, Belgium) and sodium hydroxide was obtained from Eka Nobel (Surte, Sweden).

Water filtered through a Milli-Q system (Millipore, Bedford, MA, U.S.A.) was used for the mobile phase. All other water used was demineralized. Stock solutions of the standards I and II were stored at 4°C. Storage of these solutions did not result in detectable decomposition. Centrifugation, for separation and extraction of the blood samples, was performed on an IEC Spinette centrifuge (Needham, MA, U.S.A.) at 600 *g*.

Chromatography

An LKB 2150 HPLC pump (Bromma, Sweden) was used as a solvent-delivery system with a Kipp (Delft, The Netherlands) 9209 autosampler connected to an ESA (Bedford, MA, U.S.A.) Coulochem Model 5100A electrochemical detector and a Model 5011 analytical cell. The potentials used were +250 mV for detector 1 and +550 mV for detector 2. Chromatograms were recorded on a Spectra-Physics (San Jose, CA, U.S.A.) SP 4270 integrator. Separation was performed on a 250 mm × 4.6 mm I.D. Chrompack (Middelburg, The Netherlands) ChromSpher C_8 reversed-phase column with 5 μm particle size.

The mobile phase (pH 4.3) consisted of a solution of 65 m*M* citric acid and 70 m*M* dibasic sodium phosphate, 20% (v/v) 2-propanol, 0.1% (v/v) triethylamine and 100 mg/l Na_2EDTA. The mobile phase was filtered through a 0.45-μm filter (Millipore) before use. The assays were performed at ambient temperature (21–22°C) with a flow-rate of 0.8 ml/min.

Animal procedure

Male Wistar rats were given an intraperitoneal (i.p.) injection of I (20 μmol/kg), and blood samples were taken via a cannula that had been placed in the jugular vein [5]. Blood samples of 0.5 ml were taken 15, 30, 60, 120, 240 and 360 min after i.p. injection. A 0.5-ml solution of 0.9% (w/v) sodium chloride was added, and the samples were centrifuged for 10 min, at ambient temperature, to separate the serum from the blood cells.

Extraction

To 0.45 ml of the diluted serum was added 0.05 ml of II, at a concentration of 40.0 nmol/ml, as internal standard. Then 2.0 ml of acetonitrile were added, and the solution was centrifuged for 5 min to separate the proteins. After separation, 5.0 ml of dichloromethane were added, and the samples were vortex-mixed for 1.5 min. After 10 min of centrifugation, the aqueous phase was separated from the

organic layer. The organic phase was washed, mixed, centrifuged and separated first with a sodium hydroxide solution (0.1 *M*) and then with water. After evaporation with nitrogen to dryness at 60°C the residue was taken up in 1.0 ml of the mobile phase, of which 75 μl were injected into the HPLC column.

RESULTS AND DISCUSSION

Representative chromatograms, obtained from I and II dissolved in water for standardization, blank rat serum and rat serum 30 min after i.p. injection (30 μmol/kg), are shown in Fig. 2. The retention time of I was 15 min and that of II was 20 min. There were no interfering peaks in the blank serum chromatograms in the range 13–30 min. An attempt to shorten the retention time by increasing the concentration of 2-propanol in the mobile phase was not successful because of overlapping with the other unknown peaks in the chromatogram.

To investigate the extraction efficiency, known amounts of I were added to drug-free serum. All the samples were extracted as previously described. The results were compared with those obtained from known amounts of the compound dissolved in water directly injected onto the column. The coefficients of variation (C.V.) between samples with the same concentration were calculated (Table I). The calibration curve was obtained by plotting the peak-height ratios of I to internal standard using drug-free serum containing I in the range 0.1–1.0 nmol/ml. A linear relationship was observed over this range ($y=23.528x+1.401$, $r=0.994$). The limit of detection was 0.05 nmol/ml at a signal-to-noise ratio of 3.

The time-course of I found in serum following a single i.p. injection of 20 μmol/kg is shown in Fig. 3. Serum levels reached a peak at 30 min and declined steadily thereafter, reaching a plateau between 210 and 130 pmol/ml from 2 to 6 h post

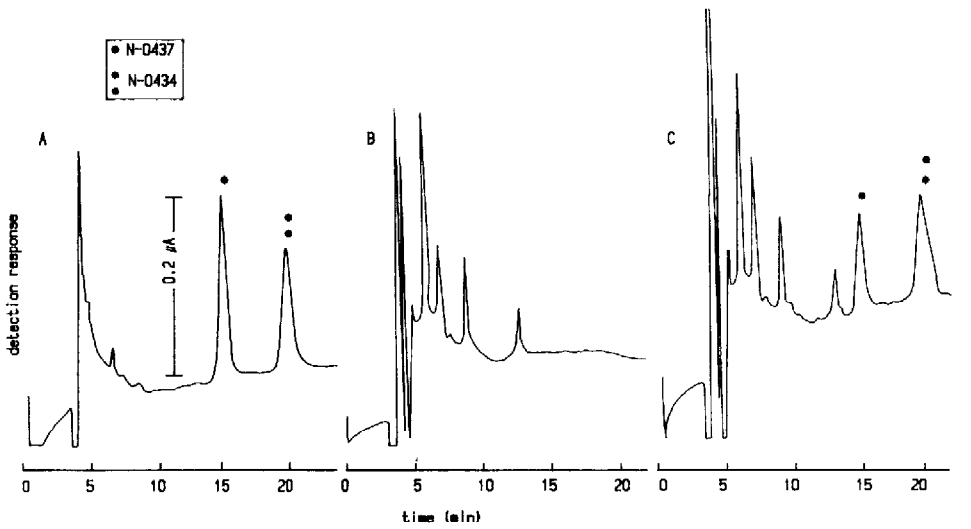

Fig. 2. Chromatograms of (A) I (N-0437) and II (N-0434) (1.0 nmol/ml) dissolved in water, (B) blank rat serum and (C) serum from a rat treated with an i.p. dose of I (20.0 μmol/kg) at 30 min post-injection.

TABLE I

ANALYTICAL RECOVERY AND PRECISION

Compound	Concentration (nmol/ml)	n	Recovery (%)	C.V. (%)
I	0.1	6	80 2	10.2
	0.25	6	86.9	8 1
	0.5	6	88.2	8.6
	1.0	6	84.3	4.9
Mean of total samples		24	84.9	8.4
II	1.0	24	85.9	7.8

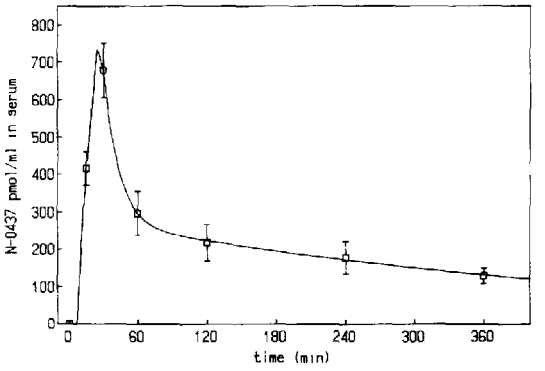

Fig 3. Time course of concentration of I in serum after i.p. injection (20 μmol/kg). Results are the means ± S.E.M. for four rats at each time interval. Curve-fitting was performed with the non-linear regression program CFT3 ($R = 0.9994$) [6]. (The data are not corrected for internal standard recovery as the internal standard was not available during these experiments. Additional experiments with the internal standard gave no significant differences from the results described here.)

injection. The early peak of I in plasma is to be expected because of its lipophilicity, and is consistent with other observations of lipophilic drugs such as the opiate agonist naltrexone [7, 8] and morphine [9].

In summary, this analytical method measures serum levels of the 2-aminotetralin (I) with nanomole sensitivity. This method should be useful for the determination of I in serum and brain tissue after peripheral administration of pharmacologically appropriate doses.

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REFERENCES

1 J.C. van Oene, J.B. de Vries, D Dijkstra, R.J.W. Renkema, P.G. Tepper and A.S. Horn, Eur. J. Pharmacol., 102 (1984) 101.
2 J. van der Weide, J.B. de Vries, P.G. Tepper, D N. Krause, M L. Dubocovitch and A.S. Horn, Eur. J. Pharmacol., 147 (1988) 249.
3 T. Gerding, in preparation.
4 A.S. Horn, P.G. Tepper, J. van der Weide, M. Watanabe, G. Grigoriadis and P. Seeman, Pharm. Weekbl. Sci., 7 (1985) 208.
5 A.B. Steffens, Phys. Behav., 4 (1969) 833.
6 P.G. Ruifrok, Biopharm. Drug Dispos., 3 (1982) 243.
7 C. Kim, R. Cheng and W.A. Corrigall, Chromatographia, 25 (1988) 94.
8 A.L. Misra, R. Bloch, J. Vardy, S.J. Mule and K. Verebey, Drug Metab Dispos., 4 (1976) 76
9 F. Molenaar, J.P. Yska, J. Visser and D K F. Meijer, Eur. J. Clin. Pharmacol., 29 (1985) 19.