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Quantitative determination of the dopamine agonist lisuride in plasma using high-performance liquid chromatography with fluorescence detection

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ABSTRACT

An HPLC method for the determination of lisuride hydrogen maleate in plasma is described. After addition of ergotamine tartrate as internal standard, plasma is extracted with diethyl ether. Following evaporation of the solvent and redissolving in methanol the extract is injected on a silica HPLC column and lisuride is monitored by fluorescence detection using an excitation wavelength of 322 nm and an emission wavelength of 405 nm. The method is sufficiently accurate and precise with a detection limit of 20 pg/ml lisuride in plasma. The usefulness of the method is demonstrated by measurements of lisuride levels after oral intake of a 0.6 mg dose of the drug by a healthy male volunteer, showing a peak level of 1266 pg/ml, 45 min after intake.

INTRODUCTION

The ergot compound lisuride (for the structure see Fig. 1), belonging to a group of so called 8α-amino-ergolines, has strong dopaminergic properties; many clinical applications of this drug (available as the hydrogen maleate derivative) have been described [1]. It is approximately ten times as potent as the most commonly used related ergot derivative bromocryptine [2]. The drug has mainly been applied as adjuvant therapy in Parkinsonian patients [3,4] and in the treatment of hyperprolactinemia [5].

The drug can be administered either orally or intravenously [6]. When given in therapeutic doses, lisuride strongly suppresses synthesis of the pituitary hormone prolactin [6]. In pharmacokinetic studies the behaviour of the drug can be monitored by measuring the serum

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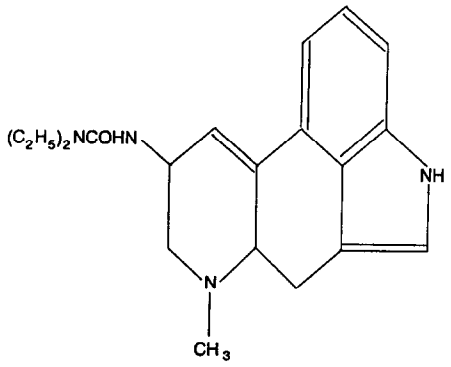

Fig. 1. The structure of lisuride.

prolactin concentration [6] or by measuring the concentration of the drug itself [6,7] in the course of time, apart from clinical effects. A specific radioimmunoassay method has been developed by Hümpel *et al.* [6] for measuring the plasma concentration of lisuride, having a detection limit of 20 pg/ml. Measurements of plasma lisuride after oral or intravenous administration in man demonstrate a half life of lisuride of approximately 1.9 h and a plasma clearance of *ca.* 800 ml/min (corresponding to liver "plasma flow") due to the fact that the drug undergoes quick biotransformation by the liver [6]. For the same reason the bioavailibility of the drug, given orally, is not more than 10% for a 100-μg dose or 22% for a 300-μg dose [6].

Although the drug has proven to be useful in the treatment of Parkinsonism its rather short life in patients presents difficulties because of the relatively short duration of clinical effects. To arrive at an optimal clinical effect, oral intake several times a day or constant subcutaneous pump infusion [8] would be required, imposing practical problems. For that reason we presently are studying other ways of administration such as transdermal application of the drug. In studies performed with rats it has been observed, that this mode of application resulted in measurable effects [9]. To evaluate the effectivity of such modes of administration also in man the plasma level of the drug and of prolactin have to be assayed and compared with oral or intravenous administration. Because of the fact that the antibody needed for radioimmunoassay of lisuride was not available to us we developed an HPLC method for its measurement in plasma, taking into account a published HPLC method for the determination of other ergot alkaloids [10] and the strongly specific fluorescent properties of this class of compounds. By applying a relatively simple analytical procedure it appeared possible to determine the plasma level of lisuride down to the low picogram range using HPLC with fluorescence detection. The method was tested by assaying consecutive plasma samples of a volunteer to whom the drug was given orally.

EXPERIMENTAL

Chemicals

Lisuride hydrogen maleate was provided as a kind gift by Schering AG (Berlin, Germany). Methanol and acetonitrile were from Rathburn Chemicals (Walkerburn, GB), ergotamine tartrate was from OPG Farma Groothandel (Utrecht, Netherlands), diethylether was from E. Merck Nederland (Amsterdam, Netherlands) and acetic acid (Baker Analyzed HPLC reagent) from J.T. Baker (Deventer, Netherlands).

HPLC instrumentation

The eluent was delivered by a Pharmacia LKB 2150 pump (Uppsala, Sweden); samples were injected by means of a WISP autosampler (Waters, Milford, MA, USA); the detector was a Shimadzu spectrofluorometer, type RF-10A (Kyoto, Japan). HPLC chromatograms were recorded using a Philips PM 8252 dual pen recorder (Eindhoven, Netherlands). Isocratic separation was achieved using a Silica Newguard, particle size 7 μ, 15 × 3.2 mm I.D. guard column and a Spheri-5 Silica, particle size 5 μ, 100 × 4.6 mm I.D. column, both from Applied Biosystems (San Jose, CA, USA).

HPLC conditions

The eluent was a mixture of methanol–distilled water–acetic acid–acetonitrile (40:2:10, v/v/v). The flow-rate was 1 ml/min; 50 μl of sample was injected. The excitation wavelength of the fluorescence detector was set at 322 nm, the emission wavelength at 405 nm.

Standard solutions

To prevent decomposition by light all solutions were prepared and stored in vessels wrapped in aluminium foil. Approximately 4.5 mg of lisuride was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, working solutions were made in methanol by 250 fold (≈180 pg/μl) or 2500 fold dilution (≈18 pg/μl). As internal standard ergotamine tartrate was used. Preliminary investigations showed that the fluorometric response of ergotamine was approximately 1.5 times as low as that of an equivalent amount of lisuride. Approximately 9 mg of ergotamine tartrate was accurately weighed and dissolved in 100 ml of methanol. A working solution, containing internal standard, was made from this stock solution by 250 fold (≈360 pg/μl) dilution with methanol. All stock solutions were stored at −20°C; working solutions were prepared freshly just before carrying out the analytical procedure.

Analytical procedure

All procedures were carried out in brown glass reagent tubes. To 2 ml of plasma 10 μl of working internal standard solution (≈3600 pg) was added. (Less internal standard solution, *i.e.* 2 μl, was added, when the lisuride concentration in plasma was expected to be less than 100 pg/ml.) After mixing the sample was allowed to stand for *ca.* 20 min. Diethyl ether (5 ml) was added and extraction was performed by placing the closed tubes on a rotary shaker for 30 min. After centrifugation the ether layer was collected and the extraction procedure was repeated with another 5 ml of ether. The second ether extract was added to the first one and the 10-ml ether extract was evaporated to dryness at room temperature by means of a stream of nitrogen. The residue was redissolved in 100 μl of methanol and from this sample 50 μl was injected onto the HPLC by means of the WISP autosampler on the same day, as extraction was performed.

Calibration was performed by adding varying known amounts of lisuride and the same amount of internal standard (10 μl) as used for patient plasma samples, to pooled lisuride-free plasma (aliquots of 2 ml) and carrying out the above described procedure. The lowest amount of lisuride added to these plasma samples was 8 μl 2500-fold diluted stock standard solution (≈144 pg) and the highest amount 50 μl 250-fold diluted standard solution (≈9000 pg). Thus a plasma lisuride range of 70–4500 pg/ml was covered. The concentration of lisuride in the patient samples was calculated by comparison of the obtained peak-height ratios lisuride/internal standard with those of the calibration (spiked plasma) samples. When the lisuride plasma concentration was expected to be lower than 100 pg/ml, a calibration line, using 2 μl of internal standard solution (≈720 pg) and a lowest amount of lisuride of *ca.* 18 pg and highest amount of *ca.* 360 pg added to 2-ml aliquots of plasma, was constructed. The latter calibration plot is not shown in the Results and Discussion.

Plasma samples

EDTA plasma samples (10 ml of full blood) were collected from a healthy male volunteer, who took an oral dose of 0.6 of mg lisuride hydrogen maleate. After 15 min the first blood sample was drawn and the next five samples at half-hour intervals. After centrifugation of the blood samples, plasma samples were stored in brown reagent glass tubes at −20°C until analysis.

RESULTS AND DISCUSSION

The HPLC method was developed making use of lisuride-free pooled plasma, to which varying amounts of lisuride were added, apart from a constant amount of the internal standard, ergotamine tartrate. These samples were processed as described in the Experimental section. Fig. 2A shows a chromatogram of such a plasma sample, containing 18 pg lisuride/2 ml, in which a small, but significant peak of lisuride is visible. Since an aliquot of 50 μl is injected from 100 μl of an ether extract obtained from a 2-ml plasma sample (see above), this small peak represents an amount of 9 pg of lisuride, present in 1 ml of plasma. This peak is completely absent when lisuride-free plasma is extracted. Thus the detection limit of the assay can be estimated to be at least in the order of 20 pg/ml of plasma, which is more than sufficient in studies of pharmacologi-

cal effects of the drug and is comparable to the formerly described immunoassay of lisuride [6]. From Fig. 2A it can also be concluded that in spite of the relatively simple clean-up procedure a chromatogram is obtained, in which practically no interference from endogenous compounds is noticed, owing to the very specific fluorometric detection. To obtain chromatograms as depicted in Fig. 2 it should be noted that the diethyl ether used for extraction should be of recent date,

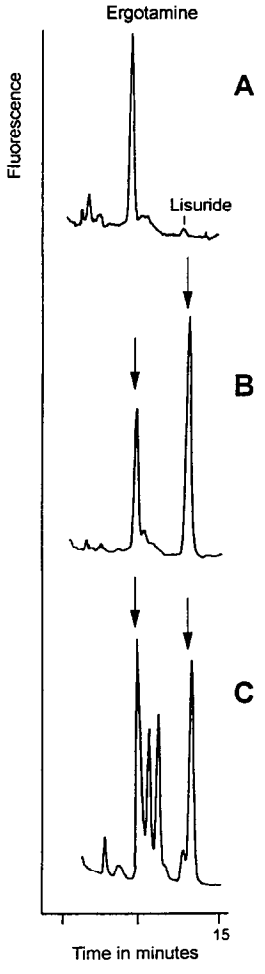

Fig. 2. Examples of HPLC chromatograms of the plasma lisuride assay, showing the fluorescence signal as function of time. A: plasma sample to which 18 pg of lisuride/2 ml was added (2 μl of internal standard solution added). B: plasma sample to which 2400 pg of lisuride/ml was added. C: one of the plasma samples of the volunteer who orally took 0.6 mg of lisuride (see text). Arrows indicate peaks of lisuride or internal standard.

because longstanding diethyl ether contains impurities giving rise to spurious peaks or to chromatograms with peaks on a descending background. Every new batch of diethyl ether should therefore previously be tested on its suitability as extractant.

Furthermore the method allows to measure plasma lisuride quantitatively. The calibration curves obtained after analysis of plasma samples to which varying amounts of lisuride had been added are depicted in Fig. 3, showing a linear relationship between peak-height ratio lisuride/internal standard and plasma lisuride in at least the range 70–4500 pg/ml. Reproducibility was tested by repeated analysis of two specific spiked pooled plasma samples within one single run and analyses of another spiked sample in different runs performed during some weeks. The results obtained from these samples were: 47.8 pg/ml ± 8.71 pg/ml, $n = 8$ (C.V. = 18.3%) and 484 pg/ml ± 36 pg/ml, $n = 10$ (C.V. = 7.4%) respectively for the within-run analyses and 165 pg/ml ± 22 pg/ml, $n = 7$ (C.V. = 13.3%) for the analyses between runs, which was considered sufficient. It was also observed that repeated freezing and thawing of the same sample three times a week did not influence the analytical results beyond the normally observed analytical variation. Thus the plasma lisuride concentration can be reliably measured or measured for a second time after storage of frozen plasma during a number of weeks, provided that samples are protected from light. The extraction recovery of lisuride was tested by comparing the peak-height ratio obtained for a spiked plasma samples to which the internal standard was added before extraction (the normal procedure) with the peak height-ratio of the same plasma samples to which the same amount of internal standard was added after extraction (after evaporation of the solvent). In all cases the peak-height ratios of these samples were practically the same, from which it was concluded, that the extraction recovery of lisuride was close to 100%.

Clinical applicability of the assay was assessed by analyzing a series of plasma samples obtained from a healthy male volunteer, who took an oral dose of 0.6 mg of lisuride (see Experimental). The obtained plasma lisuride levels as a function

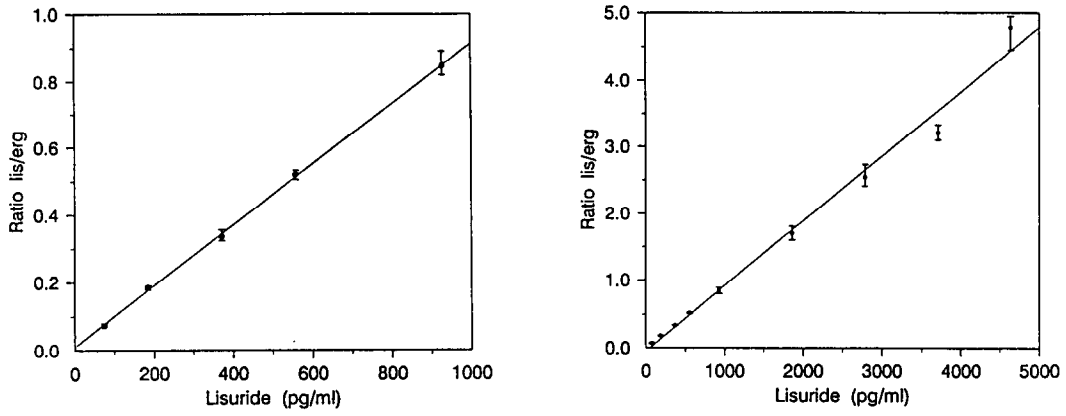

Fig. 3. Calibration curves representing peak-height ratios of lisuride/ergotamine (ratio lis/erg) in spiked samples of plasma to which a fixed amount of ergotamine and varying amounts of lisuride have been added in triplicate (see text). In this particular case the stock standard solutions contained 4.64 mg of lisuride/ml and 4.38 mg of ergotamine/ml. Error bars indicate the variations found in peak-height ratios at each level of lisuride concentration in the calibration mixtures, dots represent the mean ratios. Left: range 0–1000 pg lisuride/ml plasma. For these calibration points the linear regression equation was: y (peak-height ratio lisuride/ergotamine) = 0.000904x (concentration lisuride in pg/ml) + 0.0086 pg/ml; r (correlation coefficient) = 0.998. Right: range 0–5000 pg lisuride/ml plasma. The linear regression equation of this calibration line was: $y = 0.000969x - 0.0504$ pg/ml; $r = 0.991$.

of time are depicted in Fig. 4. In Fig. 2B a typical chromatogram of one of the calibration samples (spiked plasma) and in Fig. 2C a chromatogram of one of the plasma samples obtained from the volunteer are depicted. From comparison of these two chromatograms it is obvious that *in vivo* lisuride is converted into a number of metabolites, which appear, apart from intact lisuride, as separate (three) peaks in the chromatograms of persons to whom lisuride has been given. Some of these metabolites may be ascribed to metabolic changes in the urea part of the molecule, *e.g.* loss of one or more ethyl groups [6]. We did not attempt to establish the identity of any of these metabolites, as we considered this to be beyond the scope of the present investigation. A relatively small contribution of one of these metabolites in the peak of the internal standard is noticed, becoming apparent when no internal standard is added; however this does not substantially affect the calculation of the lisuride concentration in plasma as described above because of the relatively very large contribution of the internal stand to the peak height. Fig. 4 demonstrates, that already in the first plasma sample, taken 15 min after intake of lisuride, the presence of the drug can be observed. Approximately 45 min later the maximal plasma level of the drug, being 1266 pg/ml, after 75 min a level of 484 pg/ml and after 2 h of 400 pg/ml is reached. The pattern shown in Fig. 4 resembles the one found by Hümpel *et al.* [6], who followed, using their RIA method for the determination of plasma lisuride, the course of plasma lisuride after oral intake of 300 μg lisuride hydrogen maleate (a dose 2 times less compared to the dose taken in this paper). A

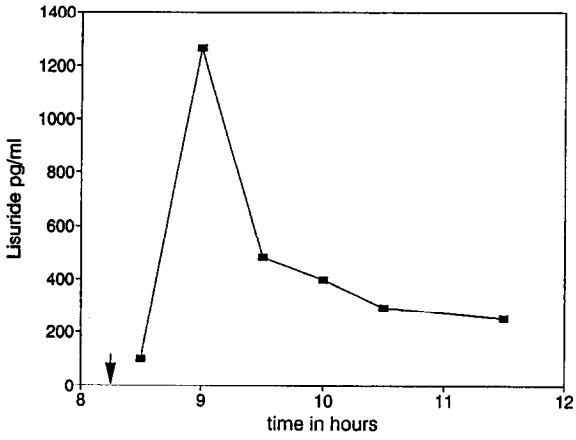

Fig. 4. Lisuride concentrations as function of time measured in consecutive plasma samples obtained from a volunteer who orally took 0.6 mg of lisuride. The arrow indicates the time, at which lisuride was orally taken.

peak level of approximately 250 pg/ml, one hour after intake, and of 150 pg/ml two hours after intake was observed in the latter case.

Summarizing it can be stated that a relatively simple and sensitive HPLC assay for plasma lisuride has been developed, useful in clinical studies. Currently the assay is applied in studies monitoring the penetrance of the drug when administered transdermally; the results of those studies will be reported elsewhere.

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