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Note

Determination of oxypertine in human serum by high-performance liquid chromatography

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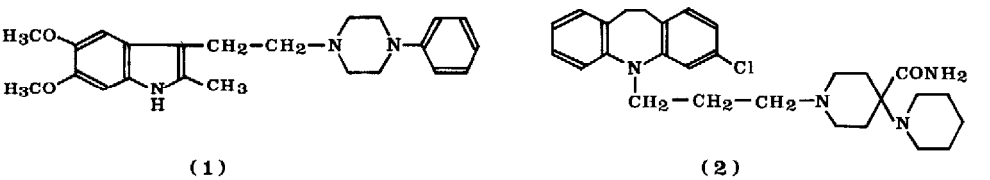
Oxypertine is different from previous antipsychotic drugs in that it has a chemical structure similar to that of serotonin (Fig. 1).

It is presumed that the main mechanism of action of oxypertine is a marked depletion of the noradrenaline level at the nerve terminals by acting on the noradrenergic neurons with a slight depletion of serotonin and dopamine levels [1—3]. In general, antipsychotic drugs act primarily on dopaminergic neurons and decrease the dopamine level at the nerve terminals. There are also some reports that dopamine or serotonin levels in the rat brain are reduced as a result of oxypertine administration [4—6].



(1) (2)

Fig. 1. Structures of oxypertine (1) and clocapramine (2).

When oxypertine is administered to patients, there is a minute or no increase in the serum prolactin level [7], which reflects the dopamine receptor blocking activity of the antipsychotic drug in the central nervous system. To date the correlation between serum oxypertine concentration and prolactin levels in humans has not been established.

Several assays for oxypertine have been described [8, 9], but they were not applicable to our laboratory. Thus we developed a high-performance liquid chromatographic method for measuring oxypertine in human serum.

EXPERIMENTAL

Materials

Oxypertine was obtained from Daiichi Seiyaku (Tokyo, Japan) and clocapramine from Yoshitomi Pharmaceutical (Osaka, Japan). Sodium carbonate, sodium acetate, acetic acid, isopropanol, diethyl ether and methanol were purchased from Wako Pure Chemical (Tokyo, Japan).

Oxypertine standards

Oxypertine (20 mg) is dissolved in methanol to a final volume of 20 ml. This solution is diluted to concentrations of 10, 25, 50 and 100 mg/l. The solutions were stored at 4°C.

Clocapramine standards

Clocapramine (20 mg) is dissolved in 20 ml of methanol. Store the solution at 4°C. An internal standard is prepared by diluting 1 ml of the stock solution to 10 ml with methanol when needed.

Instrumentation

A Hitachi 635 high-performance liquid chromatograph equipped with an ultraviolet detector (Hitachi, Tokyo, Japan) was used for the analysis. The column employed was 30 cm × 4 mm I.D. packed with Zorbax Sil 7–8 μm (Du Pont, Wilmington, DE, U.S.A.). The analysis was performed at room temperature. The effluent was monitored at 252 nm. The composition of the mobile phase was methanol—acetic acid—sodium acetate (200:0.3:0.1) with a flow rate of 0.8 ml/min. The chromatogram was recorded on an Hitachi 065 recorder and peak areas were calculated with a Chromatopac C-E1B (Shimadzu, Kyoto, Japan).

Procedure

In the case of an acute study, 50 mg of oxypertine were administered to a schizophrenic patient and blood samples were obtained at 0, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 h. Then, 150 mg of oxypertine were administered daily for eight weeks and blood samples were obtained at intervals of three or four days.

The serum concentration of prolactin was determined by radioimmunoassay using Prolactin Kit "Daiichi".

The method of measuring serum oxypertine levels was as follows. To 0.5 or 1 ml of serum in a glass centrifuge tube, was added 0.5 μg per 5 μl clocapramine (internal standard) along with 0.5 ml of 1 *M* sodium carbonate to make it basic. After the addition of 5 ml of diethyl ether containing 0.05 ml of isopropanol, the tube was shaken for 10 min. The organic layer was filtered through a cotton filter for dehydration into a pear-shaped flask then evaporated to dryness in vacuo at room temperature. The residue was dissolved in 50 μl of methanol and 5 μl were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Fig. 2 shows chromatograms of blank serum, serum spiked with 0.5 μg/ml

oxypertine and serum from a patient. Oxypertine was well separated from the internal standard and serum constituents. The retention times of oxypertine and internal standard were 6.4 and 8.8 min, respectively.

The calibration curve passes through the origin and is linear within the range 0—2 μg/ml. The lower limit of quantitation of oxypertine was 20 ng/ml in serum. The absolute recovery of oxypertine was 79.1 ± 3.5% (C.V. = 4.37%) at a concentration of 0.1 μg/ml. There was no remarkable increase in recovery of oxypertine when other solvents were used as extractants such as ethyl acetate, benzene, *n*-pentane or diethyl ether with a higher concentration of isopropanol. In the cases of ethyl acetate and diethyl ether with a higher concentration of isopropanol, additional peaks derived from serum were observed on the chromatograms.

Within-run and between-run precision values are summarized in Table I. These results indicate that the reproducibility of the procedure is satisfactory.

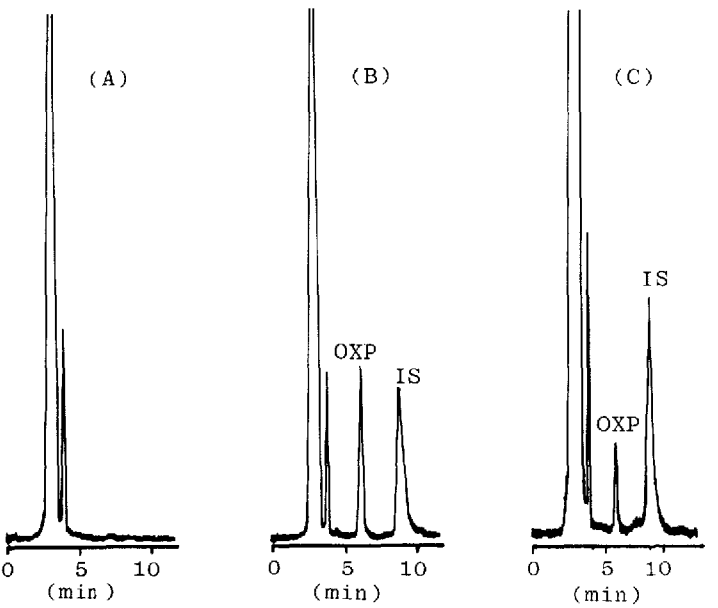


Fig. 2. Typical chromatograms of (A) blank serum, (B) serum spiked with 0.5 μg of oxypertine (OXP) and clocapramine (IS), (C) serum obtained from a patient 3 h after oral administration of oxypertine.

TABLE I

VALUES OF PRECISION

	Within-run		Between-run	
n	10	10	5	5
Mean (ng)	91.47	483.93	100.47	449.85
S.D. (ng)	4.27	18.96	4.66	17.97
C.V. (%)	4.43	3.92	4.63	3.99

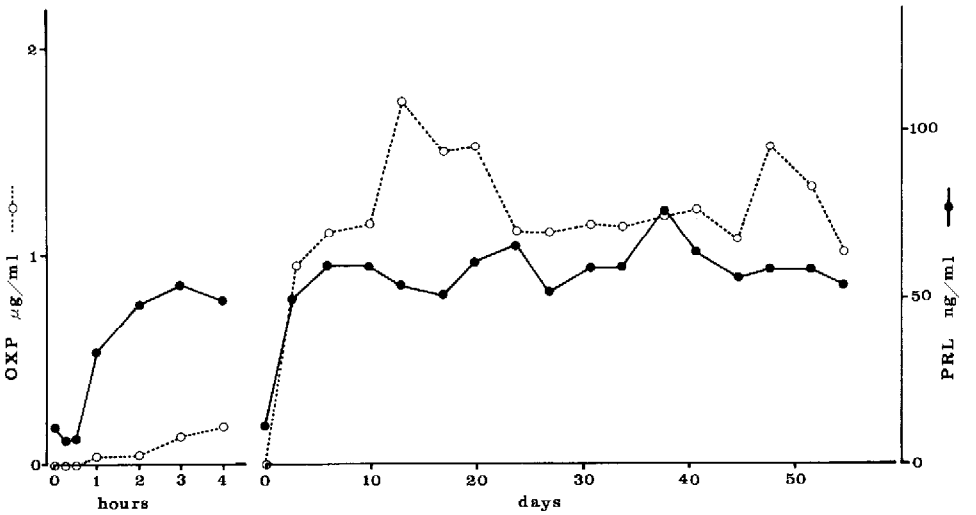


Fig. 3. Serum levels of oxypertine (OXP) and prolactin (PRL) after oral administration of oxypertine to a patient.

Some commonly prescribed antiparkinsonian drugs such as trihexyphenidyl and biperiden were chromatographed after extraction by the method described in this paper. No peaks interfered with the measurement of oxypertine.

The simplicity and precision of the extraction procedure leads us to conclude that the procedure is acceptable as a routine method for measuring oxypertine.

Fig. 3 shows serum levels of oxypertine and prolactin after acute and chronic administration of oxypertine. When oxypertine was administered acutely, increased serum oxypertine and prolactin levels were seen between 1 and 4 h. In the case of chronic treatment, the serum concentration of oxypertine reached a steady state within four days. In addition, parallel changes in serum prolactin levels were observed.

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