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Note

Application of high-performance liquid chromatography with electrochemical detection for monitoring the concentration of pentazocine in human blood

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Pentazocine is a non-narcotic analogue of morphine, which is widely used in the management of patients with pain. Determination of this compound by techniques in which the gas chromatograph was applied with a mass spectrometer [1] or an electron-capture detector [2] has been reported. However, the application of gas chromatography is not considered reasonable for a non-volatile compound because of the difficulty of derivatization. Recent developments in electrochemical detection (ED) have extended the application of column liquid chromatography to many drugs. ED is basically sensitive to substances with a phenolic hydroxy group in the molecule. This property has been widely utilized for the determination of monoamine transmitters [3–6]. Morphine, which possesses such a hydroxy group, has been determined by a combination of high-performance liquid chromatography (HPLC) and ED [7–9]. As pentazocine also has a phenolic hydroxy group (see Fig. 1), this drug can theoretically be expected to be amenable to HPLC–ED determination. It was demonstrated recently that pentazocine gave an electrochemical response while a real chromatogram was not apparent [10]. This study was undertaken to develop a simple and sensitive procedure for the determination of pentazocine in the blood of patients that is applicable to pharmacokinetic studies of the drug.

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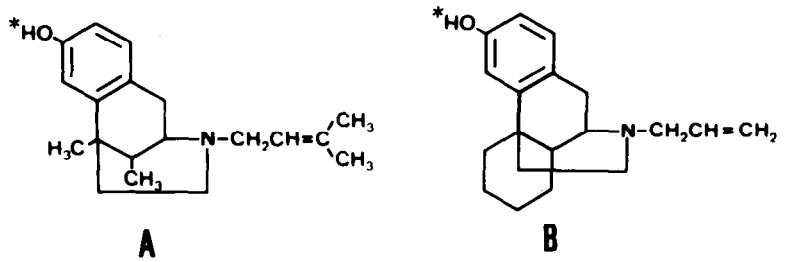

Fig. 1. Structures of (A) pentazocine and (B) levallorphan. Asterisks indicate the possible site of electrochemical reaction.

EXPERIMENTAL

Reagents

Pentazocine (Fig. 1A) was obtained from Otsuka Pharmaceuticals (Tokyo, Japan). The internal standard was levallorphan (Fig. 1B), which was purchased from Tadeka Chemical Industries (Osaka, Japan). All other reagents for extraction and chromatography were obtained from Wako (Osaka, Japan).

Chromatography

A Model 510 HPLC system (Waters Assoc., Milford, MA, U.S.A.) was used with a Model 7125 six-port injector (Rheodyne, Berkeley, CA, U.S.A.) and an EC 100 glassy carbon electrochemical detector (Eicom, Kyoto, Japan). The analytical column consisted of an Ultrasphere-ODS reversed-phase column (average particle size 5 μm; 250×4.6 mm I.D.) (Altex Scientific, Berkeley, CA, U.S.A.).

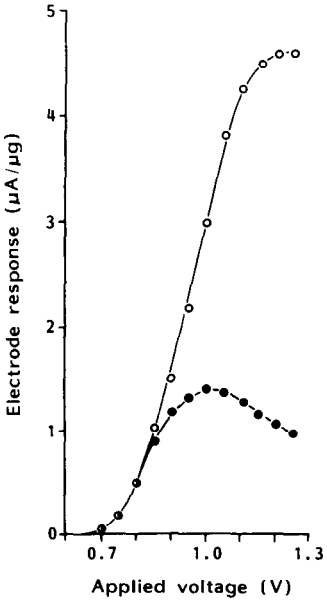

Fig. 2. Hydrodynamic voltammograms of (●) pentazocine and (○) levallorphan.

To protect the analytical column, a short ODS column (10×4.5 mm I.D.) was fitted. The detector potential was set at 1.0 V versus an Ag/AgCl reference electrode.

The mobile phase consisted of 0.05 *M* citrate buffer (pH 4.0) containing 30% acetonitrile and 0.2% pyridine. The flow-rate was set at 1.4 ml/min.

Samples

The blood samples were collected during surgical operations under anaesthesia with nitrous oxide and diazepam. No anticoagulant agent was added to the blood, which was centrifuged at 2000 *g* for 30 min. The serum was then stored in a deep-freezer (−80°C) until the assay was performed. The latter was, in general, completed within one week after sample collection.

Extraction procedure

The blood sample (500 μl) was transferred into a glass-stoppered tube containing 0.6 ml of 1.5 *M* Tris–HCl buffer (pH 8.5) and internal standard (levallorphan). A 10-ml volume of *n*-hexane–ethyl acetate (9:1) was added to the tube. The tube was agitated vigorously on a G-560 vortex (Scientific Industries, Bohemia, NY, U.S.A.) for 1 min and then centrifuged for 1 min to separate the organic layer, of which 9 ml were transferred to another tube. Then, 100 μl of 0.1 *M* hydrochloric acid were added to the tube and the mixture was vortexed for 1 min. After a brief centrifugation, a portion (80 μl, in general) of the acid layer was used as the chromatographic sample.

RESULTS AND DISCUSSION

In ED, the electrode response depends on the applied voltage. Current–voltage curves (hydrodynamic voltammograms) of pentazocine and the internal standard (levallorphan) are shown in Fig. 2. Both pentazocine and levallorphan initiated their electrochemical response at an applied voltage of +0.7 V. In previous studies [5,6], monophenolic amino acids such as tyrosine and tryptophan were found to initiate their response at almost the same voltage. This probably implies that the responses were due to the same site of reaction, the phenolic hydroxy group in their molecules. The electrochemical response of pentazocine reached a plateau at about 1.0 V and that of levallorphan was at 1.2 V. This difference may reflect the stability of electrochemical reactability. Further, the response was unfortunately about four times higher with levallorphan than pentazocine. Nevertheless, the determination could be carried out for pentazocine with a sensitivity of 1 ng.

The recoveries in the extraction procedure were calculated, after adjusting for solvent loss, to be 90±5 and 76±4% for pentazocine and levallorphan, respectively. The intra- and inter-assay coefficients of variation were also calculated to be less than 6 and 4%, respectively. The quantitation was based on the peak heights of the resulting chromatograms. The ratios of the peak heights for pentazocine and the internal standard were compared for samples and standards taken through the entire extraction procedure. In the range from 1 ng to 1 μg, the

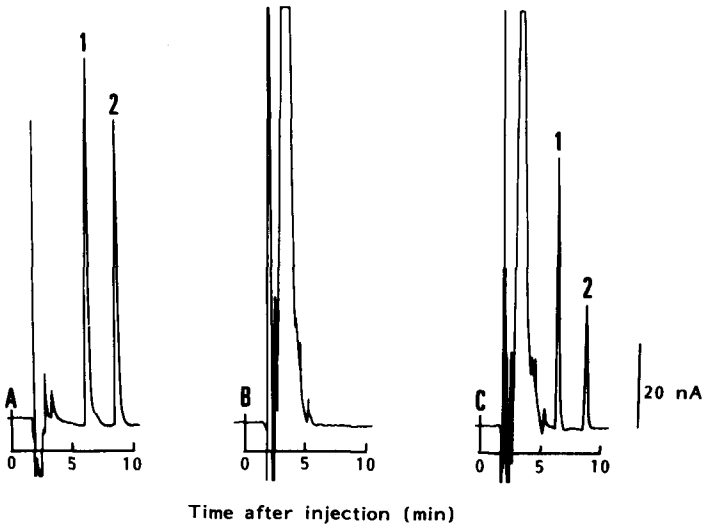

Fig. 3. Typical chromatograms of serum extracts. (A) Standard substances (50 ng of pentazocine and 25 ng of levallorphan); (B) control blank serum; and (C) 30 min after a 30-mg intravenous dose of pentazocine to a patient. Note that no interference is observed on the chromatogram of the blank serum. Chromatographic conditions: stationary phase, Ultrasphere-ODS (average particle size 5 μm); mobile phase, 0.05 *M* citrate buffer (pH 4.0) containing 30% acetonitrile and 0.2% pyridine; detector voltage, +1.0 V versus Ag/AgCl reference electrode. Peaks: 1 = levallorphan (internal standard); 2 = pentazocine.

ratios of pentazocine and levallorphan varied linearly ($r = 0.998, P < 0.001$). This made it possible to calculate the concentration from a simple measurement of the ratio. The detection limit of pentazocine was 1 ng at a signal-to-noise ratio of 5.

On the reversed-phase column, the concentrations of organic solvent influenced the retention time of the basic substances. The concentration of acetonitrile was varied so as to obtain the most appropriate separation. The mobile phase finally selected was a 0.05 *M* sodium citrate–citric acid buffer (pH 4.0) containing 0.2% pyridine and 30% acetonitrile. The retention times of levallorphan and pentazocine were 5.5 and 7.7 min, respectively, in this mobile phase, which provided a complete separation of both substances (Fig. 3). No biological substances

TABLE I

DETERMINATION OF PENTAZOCINE IN THE BLOOD OF FOUR PATIENTS

Patient No.	Concentration (ng/ml)		
	1 min	30 min	60 min
1	3180	139	130
2	3229	125	102
3	3520	102	68
4	2802	184	98
Mean ± S.D.	3182 ± 295	137 ± 34	99 ± 25

interfered with the peaks of levallorphan or pentazocine. One chromatographic run was finished within 10 min and 45 samples were processed within a routine 8-h working day.

The present assay system was applied to blood samples obtained from patients. A 30-mg amount of pentazocine was injected intravenously during the course of a surgical operation for the purpose of analgaesia. When the blood collected at 1 min after the injection was assayed, the concentration was calculated to be 3.2 μg/ml (Table I). The concentration then fell to 137 ng/ml 30 min after the injection. This rapid decline reflected the distribution of the drug within the body (α-phase). When the drug was monitored after 60 min, the decrease in concentration from the value at 30 min was not so great, implying that the elimination (β) phase had begun during this period. As the limit of detection of the present system was calculated to be 1 ng, determinations might be possible up to 6 h after a single injection of 30 mg of pentazocine.

In conclusion, a simple and sensitive procedure has been developed for the determination of pentazocine employing HPLC–ED. The detection limit was 1 ng and one chromatographic run was completed within 10 min.

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