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Short communication

Determination of ketamine and norketamine enantiomers in plasma by solid-phase extraction and high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method is described for determination of sub-anaesthetic concentrations of the enantiomers of ketamine and its metabolite norketamine in plasma. The samples are purified by reversed-phase solid-phase extraction. The enantiomers are separated on a Chiral AGP column with a mobile phase containing 16% methanol and a 10 m*M* phosphate buffer at pH 7.0, and measured by UV-detection at a wavelength of 220 nm. Linear calibration curves with correlation coefficients better than 0.995 have been obtained in the range 10–320 ng/ml. Minimum detectable concentrations were about 2 ng/ml.

Keywords: Enantiomer separation; Ketamine; Norketamine

1. Introduction

Ketamine (K) is commercially available as a racemate and mainly used as a dissociative anaesthetic agent [1]. In doses 1/10–1/5 of those used for anaesthesia K induces analgesia by non-opioid mechanisms [1–3], binding to the phencyclidine site of the NMDA-receptor gated channel. The *S*-K enantiomer is four times more potent in affinity for this site than the *R*-K enantiomer [3]. This difference explains why the *S*-K enantiomer is a more potent analgesic agent than the *R*-K enantiomer [2]. K also binds to opioid receptors and interacts with the non-opioid sigma receptor site which may mediate psychomimetic effects even after low K doses [4,5].

This enantioselective method for determination of plasma *S*-K and plasma *R*-K concentrations was developed for use in concentration effect studies in humans. The method has been applied to measure drug concentrations after low doses of the enantiomers and of the racemate with simultaneous registration of analgesic and psychomimetic effects.

2. Experimental

2.1. Materials

Racemic K and norketamine (NK) were obtained from Parke-Davis (Morris Palins, NJ, USA). Pure enantiomers of K and NK were obtained from Prof. Ivar Öye, Oslo University, School of Medicine,

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Department of Pharmacology (Oslo, Norway). Acetonitrile was of HPLC-grade. All other chemicals were analytical reagents. Sep-Pak Light C_{18} cartridges (10 mm×5 mm I.D., 130 mg) were obtained from Waters (Milford, MA, USA). The water used was deionized.

2.2. Apparatus

The chromatographic system consisted of a LKB 2150 pump (Pharmacia Biotech, Solna, Sweden), a 7125 injector (Rheodyne, Berkeley, CA, USA) equipped with a 500 μl loop, a Chiral AGP 150×4.0 mm column with 5 μm particles (Chrom Tech, Hägersten, Sweden), a column oven (Microlab, Aarhus, Denmark), a Spectromonitor 3200 UV detector (Thermo Separation Products, Riviera Beach, FL, USA) and a 3396A integrator (Hewlett-Packard, Avondale, PA, USA). A Speed Vac Plus vacuum centrifuge (Savant Instruments, Farmingdale, NY, USA) was used for the concentration of samples before injection on HPLC.

2.3. Chromatographic conditions

The eluent was a 10 mmol/l potassium dihydrogen phosphate buffer pH 7.0 (adjusted with potassium hydroxide), containing 16% methanol. The flow-rate was 1.0 ml/min and the temperature was 40°C. The detector wavelength was set at 220 nm.

2.4. Sample purification

A 1-ml plasma sample was passed through a Sep-Pak light C_{18} cartridge (pre-treated with 1 ml of methanol and 1 ml of water). The cartridge was washed with 1 ml of water, with 2 ml of a 5 mmol/l ammonium sulphate buffer pH 9.6 (adjusted with ammonia) containing 3% acetonitrile, and with 1 ml of a 5 mmol/l ammonium sulphate buffer pH 9.6 containing 20% acetonitrile. The washing solution was displaced with 200 μl of a 20 mmol/l phosphoric acid buffer pH 2.1. (adjusted with ammonia) containing 25% acetonitrile, and K and NK were eluted with 500 μl of the same solution. The elute was mixed with 1 ml of 40 mmol/l sodium hydroxide (resulting pH ca. 11.5). This mixture was treated on a second Sep-Pak the same way as on the first, but omitting the wash with water. The flow-rate at all steps was approximately 1.5 ml/min. The elute from the second Sep-Pak was evaporated in a vacuum centrifuge to about 150 μl. A 12-μl volume of 1 mmol/l sodium hydroxide was added immediately before injection, and the whole volume was injected on the HPLC column.

3. Results and discussion

Chromatograms of blank serum, and of blank serum spiked with racemic K and NK are shown in Fig. 1. Chromatograms of plasma before dose, and of plasma 10 min after i.v. injection of 0.3 mg/kg bodyweight of racemic K (healthy volunteer) are shown in Fig. 2. Calibration curves were linear for all components in the range 10–320 ng/ml. Correlation coefficients better than 0.995 were obtained for all components. Minimum detectable concentrations were about 1.6 ng/ml for *S*-NK, 1.9 ng/ml for *R*-NK, 1.7 ng/ml for *S*-K and 2.0 ng/ml for *R*-K. Recovery was 81% for K and 87% for NK. Coefficients of variation (day to day) at 40 ng/ml were 8.5% for *S*-NK, 4.1% for *R*-NK, 4.8% for *S*-K and 6.0% for *R*-K ($n=6$).

Geisslinger et al. [6] analyzed K and NK enantiomers in plasma using solvent extraction and HPLC on an AGP-column. The mobile phase consisted of 20 m*M* phosphate buffer pH 7.0 containing 2.5% 2-propanol. The minimum detectable concentrations were about 20 ng/ml. The method was used for pharmacokinetic studies of K after high doses [7]. For low concentrations, in our experience solvent extraction gave insufficient purification and problems with adsorption to glass.

The solid-phase extraction was optimised to give maximal purification from both protein and small-molecule impurities without significant loss of recovery.

HPLC with methanol as organic modifier instead of 2-propanol resulted in higher efficiency and eliminated the risk of bacterial growth. The use of potassium phosphate instead of sodium phosphate gave higher efficiency and changed selectively. Raising the temperature from ambient to 40°C also resulted in higher efficiency and changed selectively.

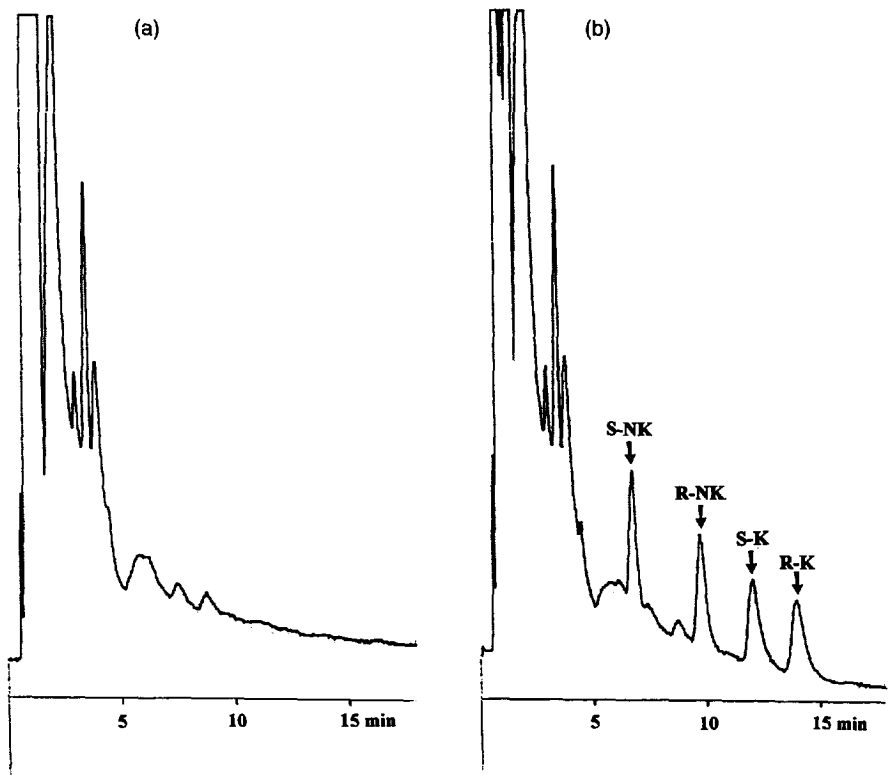

Fig. 1. Chromatograms from (a) blank plasma and (b) blank plasma spiked with 20 ng/ml of each enantiomer of NK and K. 0.004 AUFS.

Solid-phase extraction with one Sep-Pak did not always give sufficient purification. Due to these results and because of sometimes fast deteriorating column efficiency, further purification on a second Sep-Pak was added. This solved the purification problem, but the deterioration of column efficiency still occurred. The washing recommended by the manufacturer did not have any effect, nor did washing with 75% acetonitrile/water. If the column inlet was opened, and a thin layer (less than 1 mm) of the packing material was removed, and substituted by 40-μm glass beads, the efficiency was fully restored. The reason for the deterioration is still not known, though purification on two Sep-Pak columns, evaporation to 150 μl instead of the original 100 μl, and pH adjustment of the sample with sodium hydroxide instead of potassium hydroxide before injection seems to have reduced the problem. The detector wavelength 220 nm has been chosen because it gives high sensitivity and low background disturbances. At 210 nm, the sensitivity for K is about 30% higher, but the background disturbances made quantitation difficult. In the region 230–300 nm the sensitivity is less than a tenth of that at 220 nm.

This method has been used for determining the *S*- and *R*-enantiomers of K and its metabolite NK after administration of racemic K (0.15–0.45 mg/kg) in patients with severe pain due to ischemia in one of the lower limbs (Persson et al., unpublished). The assay method has also been used for enantioselective plasma analysis of *S*-K and its *S*-NK metabolite after administration of *S*-K (0.1 or 0.2 mg/kg) to healthy volunteers [8]. Our method showed adequate sensitivity for these studies in which sub-anaesthetic doses of K or its *S*-K enantiomer were given.

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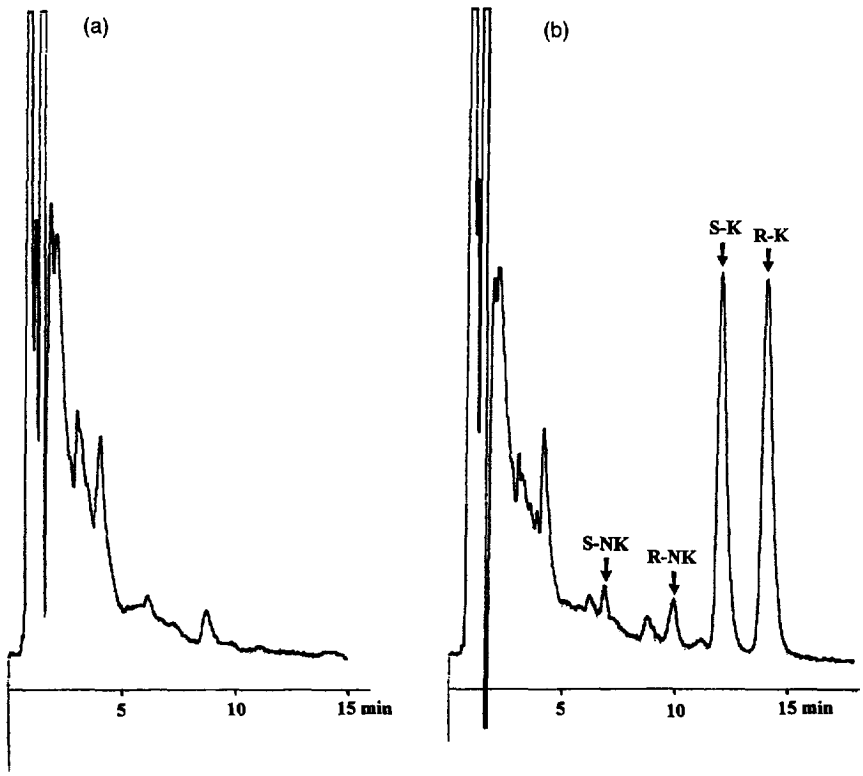

Fig. 2. Chromatograms from plasma sample taken (a) before dose and (b) 10 min after a sub-anaesthetic dose (0.3 mg/kg of racemic K). 0.004 AUFS. The concentrations are: *S*-NK 4.0 ng/ml, *R*-NK 7.0 ng/ml, *S*-K 70 ng/ml and *R*-K 80 ng/ml.

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