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Note

Sensitive screening method for buprenorphine in urine

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Buprenorphine, N-cyclopropylmethyl-7α-[1-(5)-hydroxy-1,2,2 -trimethylpropyl]-6,14-*endo*-ethano-6,7,8,14-tetrahydronororipavine, is a new morphine-like drug with both agonist and antagonist properties at the μ-opiate receptor [1]. As an analgesic, it is some 25—40 times more potent than morphine [2] and has been used primarily in the post-operative period and in terminal cancer. Because of its long duration of action, its ability to antagonise opiates and its low dependence liability and lack of significant withdrawal symptoms, it has been suggested that buprenorphine might be an effective agent in the management of opiate dependants [3, 4]. However, buprenorphine has potential for abuse [5] and, as such, urine screening methods are required for its detection. A radioimmunoassay technique has been developed [6] but such techniques are sometimes non-selective and can suffer from interferences.

The present paper outlines a method where urine was first hydrolysed with β-glucuronidase, and following preliminary extraction with a C_{18} bonded silica column and purification by thin-layer chromatography (TLC) was identified by high-performance liquid chromatography (HPLC).

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EXPERIMENTAL

Standards and reagents

Buprenorphine hydrochloride and N-desalkylbuprenorphine were supplied by Reckitt and Colman (U.K.), β-glucuronidase/sulphatase (type H-1) was obtained from Sigma (St. Louis, MO, U.S.A.) and HPLC-grade methanol from Waters Assoc. (Sydney, Australia). All other chemicals were of analytical-reagent grade.

Materials

Disposable Bond-Elut C_{18} columns (6 ml) were obtained from Analytichem International (U.K.) and silica gel $60F_{254}$ TLC plates (10 cm × 0.25 mm thick) from Merck (Darmstadt, F.R.G.).

Apparatus

HPLC analyses were performed using a Waters Assoc. HPLC system consisting of a Model M6000A pump, a fixed-wavelength detector (Model 441) and a U6K injector.

Hydrolysis of urine

Urine (10 ml) was mixed with 1 ml β-glucuronidase/sulphatase solution containing 5000 Fishman units of β-glucuronidase (15 mg/ml in 2 *M* sodium acetate buffer, pH 5.0) and incubated for 18 h at 37°C.

Preliminary extraction

The Bond-Elut columns were washed with 6 ml of methanol followed by 6 ml water. Both solvents were gently sucked through the column using a vacuum. Hydrolysed urine samples were adjusted to pH 7.5 with 20% sodium hydroxide and then applied to the columns again using gentle suction. The columns were washed with 6 ml water, then 6 ml methanol—water (50:50) twice and the eluates discarded. Buprenorphine was then eluted from the columns with 6 ml diethyl ether and the water—diethyl ether eluate collected. The aqueous phase was immediately removed using a Pasteur pipette and the diethyl ether transferred to a pointed glass tube and evaporated to dryness under nitrogen at 50°C. The extract was concentrated at the base of the tube by rinsing the walls with a small quantity of diethyl ether.

Thin-layer chromatography

The residue from the preliminary extraction procedure was first reconstituted in 75 μl of methanol by vortexing and ultrasonicating the sample and then spotted onto a TLC plate using a 100-μl glass syringe. Known amounts of buprenorphine standard (3 μg) were also applied to each plate. The plate was scraped prior to spotting with 2 mm wide channels between test extracts and standards to avoid horizontal diffusion. Plates were developed in the TLC solvent *n*-butanol—glacial acetic acid—water (60:15:75) to a distance of 10 cm. (The solvent was first shaken in a separating funnel and the organic layer removed and centrifuged before decanting into the TLC tank. The TLC tank was allowed to equilibrate for at least 1 h at 22°C before use.) Buprenorphine

TABLE I

TLC R_F VALUES FOR VARIOUS DRUGS RELATIVE TO BUPRENORPHINE

Compound	R_F Value	Compound	R_F Value
Oxycodone	0.43	Quinine	0 80
Codeine	0.45	Ephedrine	0.81
Morphine	0.47	Dextromoramide	0.84
Cocaine	0.53	N-Desmethyldoxepin	0.86
Nalorphine	0.63	Caffeine	0.88
Pethidine	0.65	Desipramine	0.90
Methadone metabolite*	0.65	Norpropoxyphene amide	0.94
Norpropoxyphene	0.65	Buprenorphine	1.00**
Lignocaine	0.65	Nortriptyline	1.03
Doxepin	0.72	Pentazocine	1.06
Imipramine	0.76	N-Desalkylbuprenorphine	1.10
Amitriptyline	0.77	Diazepam	1.39
Propoxyphene	0.78	Oxazepam	1.43
Methadone	0.78		

*2-Ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine.
**The absolute R_F value was approximately 0.65.

was detected by viewing under UV light at 254 nm. The approximate R_F values for buprenorphine and other likely interfering drugs are shown in Table I. A section of the plate at the R_F value for buprenorphine plus 0.5 cm above and below was scraped into a centrifuge tube, solubilised with 2 ml methanol and centrifuged. The methanolic extract was decanted into another tube and evaporated to dryness at 50°C under a stream of dry nitrogen.

High-performance liquid chromatography

The residue was reconstituted with 200 µl of HPLC mobile phase (35%, v/v, acetonitrile in 0.01% phosphoric acid and 0.01% sodium chloride; final pH 2.8) and 20—100 µl aliquots were injected onto a 30 cm × 4 mm I.D. µBondapak C_{18} HPLC column (Waters Assoc.). The solvent was pumped at a flow-rate of 1.5 ml/min and peaks were detected by their ultraviolet absorbance at 214 nm (0.02 a.u.f.s.). Under these conditions, buprenorphine had a retention time of 6.0 min (see Fig. 1). Relative retention times for other likely interfering drugs are shown in Table II.

RESULTS AND DISCUSSION

In man, around 15—27% of a dose of buprenorphine appears in the urine, mainly in the form of glucuronide conjugates of the parent compound and its N-desalkyl metabolite [2]. To gain the sensitivity necessary for the detection of unchanged buprenorphine, urine samples were first incubated with β-glucuronidase to hydrolyse conjugates. Preliminary extraction of buprenorphine and its metabolite was achieved using solvent partition on a Bond-Elut C_{18} column, followed by a TLC step, with final separation and detection by HPLC. The intermediate TLC step was found to be particularly necessary for the removal of endogenous urine constituents which interfered in the final

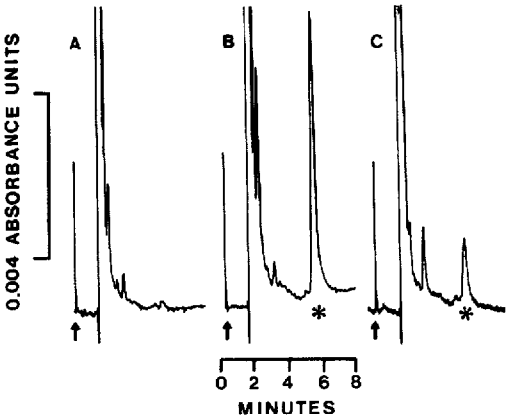

Fig. 1. Chromatograms showing (A) an extract of a blank urine sample, (B) an extract from urine spiked with buprenorphine (100 ng/ml) and (C) an extract of urine from a patient taking 4 mg buprenorphine sublingually per day (equivalent to approximately 31 ng/ml). Samples were injected as indicated by the arrows and the buprenorphine peak is identified by an asterisk. HPLC conditions as specified in Experimental.

TABLE II

HPLC RETENTION TIMES FOR VARIOUS DRUGS RELATIVE TO BUPRENORPHINE

Compound	Retention time	Compound	Retention time
Caffeine	0.27	Buprenorphine	1.00*
Nalorphine	0.30	Desipramine	1.38
Ephedrine	0.31	Diazepam	1.41
Quinine	0.46	Imipramine	1.54
N-Desalkylbuprenorphine	0.53	Nortriptyline	1.54
Pethidine	0.54	Propoxyphene	1.58
Pentazocine	0.68	Dextromoramide	1.73
Oxazepam	0.68	Amitriptyline	1.77
N-Desmethyldoxepin	0.85	Methadone	1.88
Doxepin	1.00	Norpropoxyphene amide	1.93

*The absolute retention time was approximately 6.0 min using the HPLC conditions specified in the text.

HPLC analysis. N-Desalkylbuprenorphine is partially separated from buprenorphine during the TLC stage of the procedure and completely separated by HPLC. A range of other compounds was also tested for possible interference in the method (Tables I and II). These were chosen on the basis of our past experience in screening urine samples from a population of addicts participating in a methadone maintenance programme. Commonly prescribed medications (e.g. tricyclic antidepressants and benzodiazepines), drugs of abuse (e.g. pethidine, pentazocine etc.) and others such as caffeine and quinine were tested. Where possible, commonly encountered metabolites of these drugs (e.g. norpropoxyphene, methadone metabolite) and chemical breakdown products (e.g. norpropoxyphene amide) were also included. Selectivity of the method for buprenorphine was greatly enhanced by the intermediate TLC step. Moreover, urine samples from some 60 known addicts on methadone maintenance were analysed and no interfering peaks were observed.

To test the reproducibility of the method, buprenorphine was spiked into urine at concentrations of 20 and 100 μg/l and quantified by peak-height measurements after separation as outlined above. Peak height for buprenorphine was linear over the range 20—200 ng injected onto the column. The recoveries were 21 ± 4% and 35 ± 6% (mean ± S.E.M.; n = 10), respectively. The limit of detection for the method for urine was approximately 7.5 μg/l buprenorphine.

In several cases semi-quantitative estimation of buprenorphine concentration in the urine was undertaken. Urine samples from seven patients who had received treatment for two weeks with 4 mg of buprenorphine daily (sublingually) were screened 24 h after the last dose and all showed buprenorphine with concentrations ranging from 54—260 μg/l. Buprenorphine was also detected in urine from two of these same patients 72 h after dosage (37 and 144 μg/l). Samples from four other patients on a similar treatment protocol were only tested 48 h after the last dose when concentrations ranging from 21 to 126 μg/l were found.

CONCLUSION

A sensitive and selective method for the identification and semi-quantitative determination of buprenorphine in urine has been developed and provides a procedure for urine screening in cases of suspected abuse.

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