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Note

Determination of *m*-iodobenzylguanidine in serum and urine by high-performance liquid chromatography

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m-Iodobenzylguanidine (MIBG) has a structural similarity to epinephrine and norepinephrine (Fig. 1) and acts as a blocker of catecholamine synthesis. ^{131}I-Radiolabelled MIBG ([^{131}I]MIBG) was first used to image the adrenal medulla by Wieland et al. [1]. Later, ^{123}I- or ^{131}I-labelled MIBG was used for diagnostic scintigraphy of pheochromocytomas and neuroblastomas [2,3]. At present, first trials are done to evaluate the benefit of [^{131}I]MIBG in the radiotherapy of advanced pheochromocytomas and neuroblastomas [4–6]. For pharmacokinetic in-

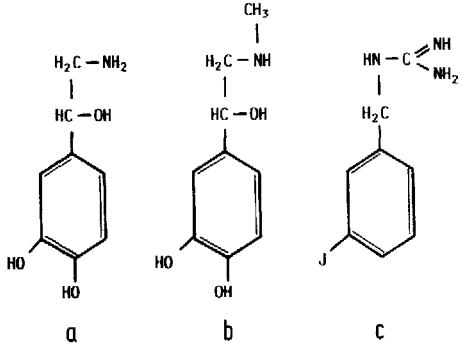

Fig. 1. Chemical structures of norepinephrine (a), epinephrine (b) and *m*-iodobenzylguanidine (c).

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vestigations in the patients treated, MIBG concentrations in serum or urine are determined by counting the ^{131}I activity. Prior to this analysis, a separation of [^{131}I]MIBG and liberated free ^{131}I is necessary. The analysis has to be done rapidly after application because ^{131}I has a half-life of 8.04 days. A specially equipped and controlled laboratory is necessary to handle the radioactivity. Even with high specific activity only a small proportion of the normal iodine atoms are replaced by ^{123}I or ^{131}I in labelled MIBG and thus the decay of the radioactivity does not significantly change the total amount of MIBG in the specimen. This means that [^{123}I]- or [^{131}I]MIBG is best monitored by measuring the concentration of unlabelled MIBG, which is present in excess in any radioactive MIBG preparation. This paper presents a simple and sensitive high-performance liquid chromatographic (HPLC) assay for the determination of unlabelled MIBG in biological samples, such as serum and urine.

EXPERIMENTAL

Materials

[^{131}I]MIBG for therapeutic use and unlabelled MIBG were purchased from Amersham and Buchler (Braunschweig, F.R.G.). Acetonitrile was obtained from Rathburn (Maidenhead, U.K.). Potassium dihydrogenphosphate, disodium hydrogenphosphate, ethanol, glacial acetic acid and chloroform were from Merck (Darmstadt, F.R.G.). Methanol was from Baker (Gross Gerau, F.R.G.) and heptanesulphonic acid (sodium salt) from Fluka (Neu-Ulm, F.R.G.). All solvents were HPLC grade, the other reagents were analytical grade. Analytical columns (250 mm×4.0 mm I.D.) were purchased from Knauer (Berlin, F.R.G.) and were packed with 5-μm LiChrosorb RP-8 material. Pre-columns (30 mm×4.0 mm I.D.), also packed with 5-μm LiChrosorb RP-8, were obtained from Knauer or Bischoff (Leonberg, F.R.G.).

Instrumentation

The HPLC apparatus consisted of three LDC/Milton Roy Model ConstaMetric pumps, an LDC/Milton Roy SpectroMonitor D spectrophotometer, an LDC/Milton Roy Model MP 3000 computer–integrator, two Besta Model H automatic valves and an automatic Waters WISP 710 B chromatographic injection system. Two detection wavelengths, 232 and 254 nm, were used. The flow-rate of the mobile phase was 1.0 ml/min. The configuration of the three pumps, the injection system, the two columns, the motor valves and the detection system (Fig. 2) allowed for automatic pre-column purification and enrichment of unlabelled MIBG and regeneration of the pre-column every five determinations.

Patients

Fourteen children with advanced neuroblastoma were treated with [^{131}I]MIBG after relapse or unresponsiveness to conventional therapy, as published elsewhere [6].

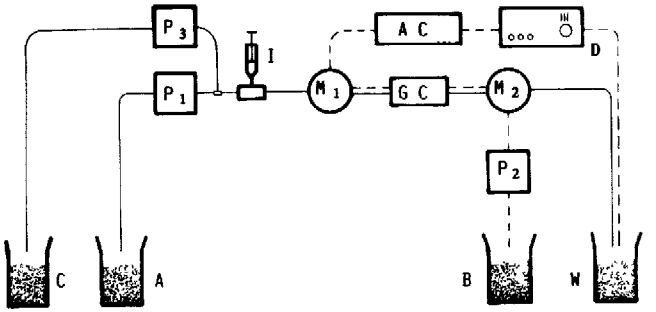

Fig. 2. Configuration of the analytical system. The solid lines show the direction of the solvent flow during enrichment on the pre-column and the dashed lines show the direction of the solvent flow for separation on the analytical column. P_1 = pump 1 delivering solvent A (phosphate buffer), P_2 = pump 2 delivering solvent B (phosphate buffer–acetonitrile); P_3 = pump 3 delivering solvent C (methanol); I = automatic injection system; M_1 and M_2 = automatic motor valves; GC = pre-column; AC = analytical column; D = detector; W = waste.

Procedure

Calibration standards of unlabelled MIBG in human serum and urine were prepared by spiking with the desired amount of unlabelled MIBG in ethanol. They were freshly prepared each time from an MIBG–ethanol stock solution stored at −20°C. Patient sera and urine specimens were also stored at −20°C; they were thawed immediately before analysis and filtered through a 0.22-μm filter. Aliquots of 200 μl of the standard, urine or serum specimen were injected and enriched on the pre-column equilibrated with 0.06 *M* phosphate buffer (pH 6.0) (solvent A). The solvent flow-rate through the pre-column to the waste was maintained for 5 min after injection, allowing for elution of most of the serum proteins and other contaminants from the pre-column. The unlabelled MIBG was firmly bound to the stationary phase under these conditions. Column-switching and flow-reversal took place after 5 min. Mobile phase B, consisting of 35% (v/v) acetonitrile and 1% (w/v) heptanesulphonic acid (sodium salt) in 0.06 *M* phosphate buffer (pH 6.0), was pumped in the reverse direction through the pre-column to the analytical column. The separation was terminated after 15 min and the pre-column was equilibrated with the phosphate buffer again for 5 min. Every five determinations, the pre-column was washed by flushing with methanol (solvent C).

RESULTS AND DISCUSSION

In aqueous solutions the analyte shows two absorption maxima in the UV region (232 and 254 nm). Detection at 232 nm yields a ten-fold increase of the MIBG signal over that at 254 nm, but with a marked increase in the background. Thus the better choice of wavelength is dependent on the matrix concerned. We suggest, that urine samples should be measured at 232 nm and serum samples at 254 nm. The chromatographic peak area was proportional to the unlabelled MIBG concentration, and the calibration curve was linear over the range 0–40 ng of unlabelled MIBG injected per test. The equation of the calibration line was

$y=0.781x+0.015$ (correlation coefficient = 0.999). The recovery of the pre-column enrichment proved to be quantitative (96.2%).

The limit of detection in serum and urine samples was 50 ng/ml when the injection volume was 200 μl at a signal to-noise ratio of 3. The intra-assay coefficient of variation (C.V.) was 3.3%, on the basis of five measurements of a 100-μl sample of 200 ng/ml solution of unlabelled MIBG injected into the system. The inter-assay C.V., based on measuring the same sample at 24 and 48 h, was 6.9%.

Fig. 3 shows typical chromatograms obtained from test s ra, spiked with 3 μg/ml unlabelled MIBG (Fig. 3b) and with 1.5 μg/ml unlab lled MIBG (Fig. 3c) and from a patient treated with [^{131}I]MIBG (Fig. 3d). Fig. 3a shows a blank. The unlabelled MIBG is clearly separated from the serum cc npounds. In addition seven drugs, commonly used in the treatment of patients, have been examined for interferences. The drugs tested were diazepam, dimenhydrinat, etoposide, metoclopramide, paracetamol, prolonium iodide and sulphamethoxazole/trimethoprim. Only metoclopramide and sulphamethoxazole/trimethoprim gave signals

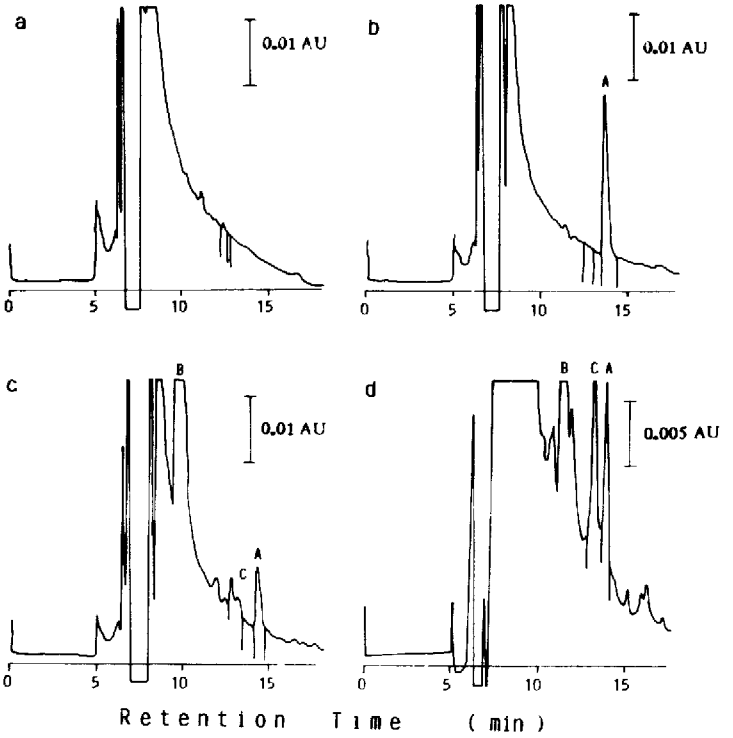

Fig. 3. HPLC profiles from (a) blank serum, (b) the same serum, spiked with 3 μg/ml unlabelled MIBG (A), (c) the same serum, spiked with 1.5 μg/ml unlabelled MIBG (A), 5 μg/ml sulphamethoxazole/trimethoprim (B) and 5 μg/ml metoclopramide (C), (d) serum sample from a patient. Conditions: pre-column, 5-μm LiChrosorb RP-8 (30 mm×4.0 mm I.D.), analytical column, 5-μm LiChrosorb RP-8 (250 mm×4.0 mm I.D.); mobile phase B, 35% acetonitrile and 1% heptanesulphonic acid (sodium salt) in 0 06 *M* phosphate buffer (pH 6.0); detection wavelength, 254 nm; flow-rate, 1.0 ml/min.

with retention times close to that of MIBG (Fig. 3c and d), but are completely separated. The calculated unlabelled MIBG concentration in the patient serum (Fig. 3d) was 1 μg/ml.

Fig. 4a shows a chromatogram, obtained from a test urine, spiked with 1 μg/ml unlabelled MIBG; Fig. 4b shows a chromatogram of a urine sample from a patient. The calculated concentration of this probe was 0.66 μg/ml.

Fig. 5 shows the concentrations of unlabelled MIBG from a female patient who was treated with [^{131}I]MIBG for advanced neuroblastoma and monitored over a period of 4 h. She received a dose of $3.7 \cdot 10^9$ Bq. The MIBG had a specific activity of $1.11 \cdot 10^9$ Bq/mg. The body weight was 16 kg. Thus the dose was 0.21 mg/kg body weight by intravenous infusion over a period of 3 h. The unlabelled MIBG level in the serum steadily increased over the period of infusion and then fell off with a half-life of ca. 40 min.

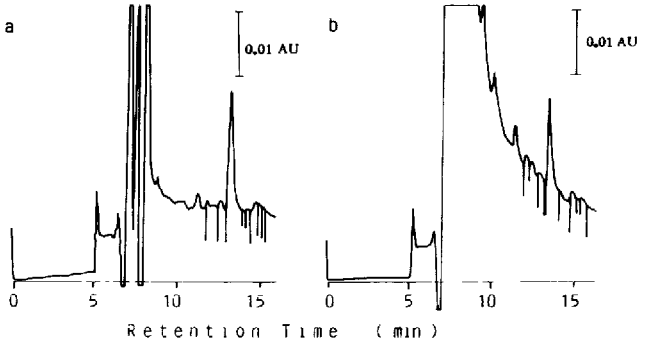

Fig 4. HPLC profiles from (a) test urine, spiked with 1 μg/ml unlabelled MIBG and (b) urine sample from a patient. Conditions as in Fig. 3, except that the detection wavelength was 232 nm.

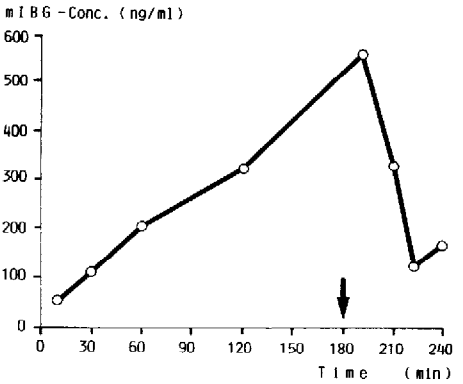

Fig. 5. Measured concentrations of unlabelled MIBG in serum of a patient during a 3-h infusion of [^{131}I]MIBG (dose 0.21 mg/kg body weight) and shortly after. The arrow indicates the end of the infusion period.

CONCLUSION

The method described here has been developed for the quantification of unlabelled MIBG in biological samples, such as serum or urine. A high sensitivity was achieved by the use of a column-switching system, which allows for separation from most of the serum proteins, for pre-column enrichment and for the use of relatively high sample volumes without band broadening.

MIBG was determined at two wavelengths, 232 nm for urine samples and 254 nm for serum samples. Our measurements showed no interference with the unlabelled MIBG peak from other drugs commonly administered to patients.

It has proved possible to monitor MIBG in the serum of patients after storage of the sample for nine months at $-20\,^{\circ}C$. The method may help to facilitate further studies of the pharmocokinetics of MIBG.

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