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Letter to the Editor

Rapid and simple procedure for the determination of salsolinol in urine using high-performance liquid chromatography with electrochemical detection

Sir,

Salsolinol (6,7-dihydroxy-1-methyl-1,2,3,4,tetrahydroisoquinoline) is an endogenous tetrahydroisoquinoline alkaloid that has been detected in human urine, cerebrospinal fluid and brain tissue [1–5]. Salsolinol is derived from dopamine, which reacts with acetaldehyde, a metabolite of alcohol [1,6,7] and displays neurotransmitter-like activity [8].

It has been reported that alcohol intake modulated urinary salsolinol levels, although this point remains controversial [8–12]. Furthermore, exogenous salsolinol may be provided by dietary sources, such as chocolate and bananas, leading to disturbed endogenous salsolinol detection in urine [12,13].

Gas chromatography, mass spectrometry [12,14] and high-performance liquid chromatography (HPLC) [3,4,10] have provided the possibility of measuring low levels [2–5] of endogenous salsolinol in biological samples.

In order to test daily urinary levels of salsolinol in healthy volunteers under a chocolate, banana and/or alcohol diet, we have developed a sensitive, rapid and simple assay using high-performance liquid chromatography (HPLC) with electrochemical detection.

EXPERIMENTAL

Materials

Salsolinol hydrochloride and 3,4-dihydroxynorephedrine were obtained from Sigma (La Verpilliere, France). All other chemicals and solvents used were of analytical-reagent grade. Columns packed with cation-exchange resin (Biorex 70) (40 mm × 8 mm I.D.), and recommended for catecholamine extraction, were obtained from Bio-Rad Labs. (Paris France) (Ref. 189-2202). A Chrompack (Les Ulis, France) Chromsep Microspher HPLC C_{18} (particle size 3 μm) column (100 mm × 4.6 mm I.D.) was used.

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Collection and treatment of urine samples

Urine samples were collected during 24 h and supplemented with 15 ml of concentrated hydrochloric acid. Samples were stored at −20°C. Before analysis, they were centrifuged for 10 min at 200 *g* and 2.5 ml of supernatant were transferred into a test-tube containing 250 μl of 3,4-dihydroxynorephedrine (40 ng/ml) as internal standard (I.S.) and 4 ml of 0.1 *M* hydrochloric acid. After adding 15 ml of EDTA (0.1%, w/v), samples were adjusted to pH 6.5 with sodium hydroxide solution and passed through a Bio-Rad column. The effluent was discarded and, after two washes with distilled water (10 ml), the absorbed material was eluted with 5 ml of boric acid (4%, w/v).

HPLC separation and detection

HPLC was performed using a system consisting of a Waters Assoc. (St. Quentin en Yveline, France) Model 510 pump, a pulsation absorber (Touzart et Matignon, Vitry, France), a Rheodyne injector with a 20-μl sample loop and a Waters Assoc. Model 460 electrochemical detector equipped with a graphite cell. A 40-μl aliquot of eluate from the Bio-Rad column was injected into the HPLC system. Elution was carried out at a flow-rate of 0.8 ml/min with a solution containing KH_2PO_4 (0.0347 *M*), citric acid (0.03 *M*) sodium octanesulphonate (0.66 g/l) and EDTA (0.11 *M*) (pH 4.85), mixed with 8% (v/v) methanol. The detector potential was set at +0.7 V *versus* an Ag/AgCl reference electrode and the sensitivity was adjusted to 2 nA.

RESULTS AND DISCUSSION

The extraction and HPLC conditions were adapted from those for a routine assay for catecholamine: methanol adjusted from 10 to 8%, column particle size decreased to 3 μm and detector potential set from +0.6 to +0.7 V. A one-step extraction was achieved by cation-exchange chromatography. Ten samples can easily be prepared in less than 90 min. Following extraction, a 93% absolute recovery of salsolinol added to urine was obtained (Fig. 1a, b and d). Peak-area ratios (analyte to I.S.) were plotted *versus* the concentration (ng/ml). Linearity of the detection procedure was obtained between 0 and 50 ng/ml ($y = 0.077x + 0.006$; $r = 0.9993$). The limit of detection was 1 ng/ml of extracted salsolinol in supplemented urine (signal-to-noise ratio of at least 3).

After ten evaluations for each measurement, the inter-assay reproducibility gave a relative standard deviation (R.S.D.) of 6% (4.78 ± 0.29 ng/ml; mean ± S.D.) and the intra-assay repeatability gave an R.S.D. of 6.4% (5.1 ± 0.33 ng/ml) at lower values (calibration range 0–10) and 3% (20.11 ± 0.57 ng/ml) at higher values (calibration range 0–50).

Despite the co-extraction of dopamine, epinephrine and norepinephrine with salsolinol, no interference with detection was observed. In contrast, co-elution of endogenous and exogenous salsolinol was obtained from salsolinol-supplemented urinary extracts (Fig. 1c and d).

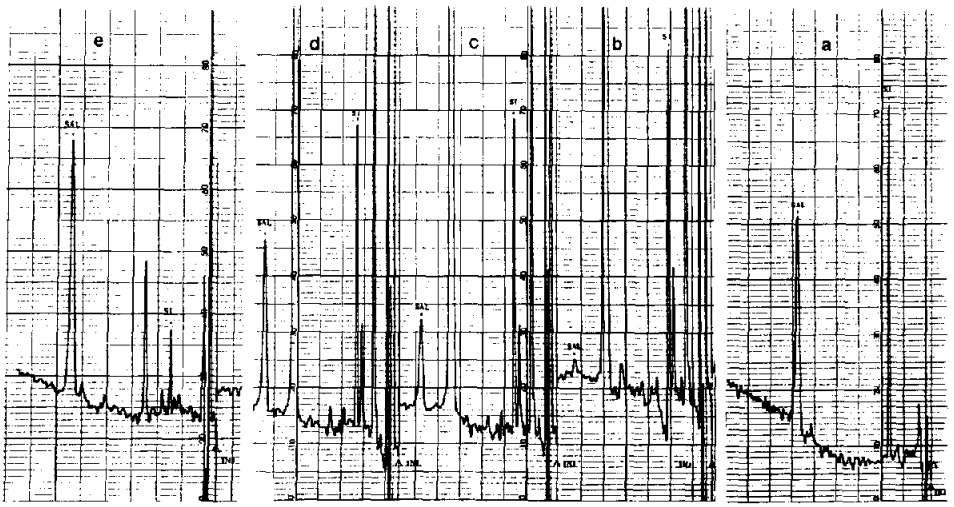

Fig. 1. HPLC profiles of (a) authentic compounds and (b–e) urine extracts. The extraction procedure and analytical conditions are described in the text. (a) 5 ng/ml salsolinol and 2 ng/ml I.S. dissolved in 0.1 *M* HCl (20 μl per injection). (b,c,d) Extracts from healthy volunteer urine: (b) before cocoa intake, (c) after cocoa intake and (d) the latter supplemented with 2 ng/ml salsolinol. (e) Diluted (1:5) cocoa extract (2.5 ng/ml).

The mean value of free salsolinol in urine from fourteen healthy volunteers (23–33 years old) under chocolate, banana and alcohol abstinence was 3043 ± 2068 ng per 24 h (1.89 ± 0.85 ng/ml) with a range of 1287–7840 ng per 24 h. These results agree with previous data [14] on five healthy volunteers (38–47 years old) with a mean value for free salsolinol in urine of 2 ± 0.171 ng/ml detected by gas chromatography–mass spectrometry. Using similar assay procedures, lower values (1100 ± 300 ng per 24 h) for free salsolinol detected in eight healthy subjects have been reported [2].

TABLE I

URINARY SALSOLINOL LEVELS BEFORE AND AFTER COCOA INTAKE BY THREE VOLUNTEERS

See text for methods.

Subject	Before cocoa intake		After cocoa intake	
	ng/ml	ng per 24 h	ng/ml	ng per 24 h
1	1.1	1665	7.1	13210
2	1.2	1287	25.5	28000
3	1	1500	7.8	11700

In order to test the influence of diet on salsolinol levels, we compared the daily urinary salsolinol excretion in three healthy volunteers before and soon after an intake of 10 g of cocoa. Preliminary studies detected 0.3 mg of salsolinol in 10 g of cocoa (Fig. 1e). Table I indicates a 780–2176% enhancement of salsolinol levels after cacao intake.

In conclusion, the present method is rapid, simple, sensitive and reliable. Further, this study confirms the absolute necessity to take into account dietary sources of tested subjects, including healthy volunteers and patients.

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Laboratoire d'Hormonologie and URA CNRS 1338,
CHRU La Miletrie, B.P. 577,
86021 Poitiers Cedex (France)

C. DUFAY
J. C. LECRON
F. DAUDON
J. GOMBERT*

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