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Note

Gas chromatographic method for the measurement of sodium valproate utilization by kidney tubules

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Sodium valproate (2-*n*-propylpentanoic acid, sodium salt), a widely used antiepileptic drug [1], has been reported to induce hyperammonaemia without clinical manifestations in most cases [2–5]. Recent arterio–venous measurements across the human [6] and rat [7] kidney have suggested that this hyperammonaemia may be due, at least in part, to an increased release of ammonia into the renal vein secondary to an increased renal production of ammonia from circulating glutamine.

In the search for the biochemical mechanisms of this hyperammonaemia, we investigated the effects of low concentrations of sodium valproate on the formation of ammonia by isolated rat kidney-cortex tubules. During this work, it was considered that sodium valproate, a branched-chain fatty acid, could be metabolized by the tubules used; to test this hypothesis, we were led to develop a method for the routine determination of low concentrations of sodium valproate.

Considering the physical properties of the free acid, most of the published procedures for the determination of sodium valproate in body fluids, which are derived from the classical methods used for the determination of free fatty acids in the derivatized and underivatized form [8, 9], are based on gas chromatography (GC) [10–19]; beside methods which use mass spectrometric [17, 19] or electron-capture [20] detection, only one of these methods allows the quantitation of concentrations of sodium valproate as low as 1 μg/ml [16].

In this paper, we describe a simple and sensitive GC method for sodium valproate determination. Briefly, valproic acid is extracted and injected without derivatization on a free fatty acid phase (FFAP) capillary column and detected

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with a flame ionization detector. With this technique, which allows accurate determination of 0.4 μg/ml of sodium valproate, it is possible to measure the utilization of this antiepileptic drug by isolated kidney tubules.

EXPERIMENTAL

Chemicals

Sodium valproate, kindly donated by the Centre de Recherche Clin-Midy (Montpellier, France) was stored under vacuum in a desiccator. Pure valproic acid was purchased from Ega (Strasbourg, France). Analytical-grade *n*-octanoic acid, obtained from Serva (Le Perray-en-Yvelines, France) and used as internal standard, was dissolved in ethyl acetate (Merck, Lyon, France) at various concentrations corresponding to the range of concentrations of sodium valproate to be determined.

Equipment

A gas chromatograph (Carlo Erba, 4200 Series) equipped with a flame ionization detector was used. The FFAP capillary column (length 36 m) was operated at constant temperature (125°C) with injector and detector temperatures of 240 and 270°C, respectively. Helium was used as carrier gas at a flow-rate of 3 ml/min. A Hewlett-Packard 3390A integrator was used for calculating the concentrations of sodium valproate in the samples by comparison with a calibration standard.

Incubation procedure

Kidneys used were from fed male rats (200–300 g) obtained from Iffa-Credo (Saint Germain-sur-l'Arbresle, France). Kidney-cortex tubules, prepared by collagenase treatment as previously described [21], were incubated for 60 min in 4 ml of Krebs–Henseleit buffer (pH 7.40) in the presence of various concentrations of sodium valproate; incubations were carried out at 37°C in a shaking water-bath in 25-ml stoppered Erlenmeyer flasks in an atmosphere of oxygen–carbon dioxide (95:5). The flasks were prepared in duplicate for all experimental conditions. Incubations were terminated by adding perchloric acid (2%, w/v, final concentration). In all experiments, zero-time flasks were prepared with sodium valproate by adding perchloric acid before the tubules. Media were centrifuged for 10 min at 4000 *g* in a refrigerated centrifuge; the supernatant obtained was neutralized with 20% (w/v) potassium hydroxide and used for sodium valproate determination.

Valproate utilization was calculated as the difference between the total flask content at the start of the experiment and after 60 min of incubation; the metabolic rates are expressed in micromoles of valproate removed per gram dry weight of tubules per hour.

Extraction procedure

To 0.5 ml of standard or sample in a 10-ml PTFE-lined screw-capped tube were added 0.1 ml of a 0.6 *M* hydrochloric acid solution and 0.5 ml of ethyl acetate

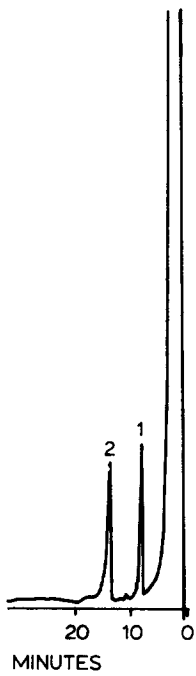

Fig. 1. GC separation of valproic acid after incubation with isolated rat kidney-cortex tubules and extraction by ethyl acetate with *n*-octanoic acid as internal standard. Peaks: 1 = valproic acid (17.45 pmol injected); 2 = *n*-octanoic acid (52 pmol injected).

containing *n*-octanoic acid as internal standard. Then, valproic acid was extracted by shaking the tube on a vortex for 1 min. Standards and samples were then simultaneously centrifuged for 10 min at 3000 *g*, and the organic phase was transferred to another tube for direct GC analysis.

Gas chromatography

Aliquots (2 μl) of the organic phase were injected into the gas chromatograph by means of a splitless injector. For each range of concentrations to be determined, calibration standards were prepared by spiking blank material with known amounts of sodium valproate solutions.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram obtained following this procedure for an extract of Krebs–Henseleit medium containing rat kidney-cortex tubules and sodium valproate after the extraction by ethyl acetate with *n*-octanoic acid as internal standard. Sharp peaks were obtained for valproic acid and *n*-octanoic acid.

As shown in Table I, satisfactory coefficients of variation were obtained for the whole extraction and analysis procedure over a large range of concentrations of sodium valproate when repetitive samples ($n=6$) were analysed. Good reproducibility was obtained with concentrations of sodium valproate as low as 0.4

TABLE I

PRECISION OF THE DETERMINATION OF SODIUM VALPROATE ADDED TO BLANK SAMPLES (EXTRACTS OF KREBS-HENSELEIT MEDIUM CONTAINING RAT KIDNEY TUBULES)

Concentration of sodium valproate found (μmol/ml)	Coefficient of variation ($n=6$) (%)
$2.5 \cdot 10^{-3}$	3.7
$4.0 \cdot 10^{-3}$	4.4
$7.7 \cdot 10^{-3}$	3.3
$7.0 \cdot 10^{-2}$	2.5
$7.0 \cdot 10^{-1}$	2.8

μg/ml, which is the lowest concentration determined with good accuracy using underivatized sample and conventional flame ionization detection; the latter value indicates that the present procedure is more sensitive than previously published methods also based on GC with the same detection procedure [10–16, 18].

Table II indicates that good recovery was obtained at all the concentrations studied. Further extraction under basic or neutral conditions was not necessary since no interfering substances were observed when extracting blank material.

This method was applied to determine whether sodium valproate was metabolized by isolated rat kidney-cortex tubules. At 0.1 m*M*, a concentration at which sodium valproate exerts its maximal stimulatory effects on renal ammonia production from glutamine, the rate of sodium valproate metabolism by the tubules used was 3.6 ± 1.3 μmol/g dry weight of tubular tissue per hour (mean ± standard error of the mean; $n=6$; 23.6 ± 1.5 mg dry weight of tubules per flask); with 0.01 m*M* of sodium valproate as substrate, the corresponding value was 0.8 ± 0.2 ($n=6$; 20.2 ± 2.1 mg dry weight of tubules per flask). From these data, it can be calculated that 21.2 and 40.4% of the amount of valproate present at time zero were metabolized by the tubules, when the substrate concentration was 0.1 and 0.01 m*M*, respectively. These values, together with the good precision of the method used (see Table I), indicate that the valproate peaks obtained after 0 and 60 min of incuabtion were sufficiently different to allow accurate determinations of the valproate utilized by the tubules.

TABLE II

RECOVERY OF SODIUM VALPROATE ADDED TO BLANK MATERIAL (EXTRACTS OF KREBS-HENSELEIT MEDIUM CONTAINING RAT KIDNEY TUBULES)

Concentration of sodium valproate added (μmol/ml)	Recovery ($n=6$) (%)
$3.3 \cdot 10^{-3}$	94
$6.7 \cdot 10^{-3}$	92
$8.7 \cdot 10^{-2}$	95
$7.5 \cdot 10^{-1}$	93

In summary, this paper describes a simple and sensitive method for the determination of sodium valproate incubated with isolated rat kidney-cortex tubules. A single extraction without derivatization gives satisfactory recoveries and coefficients of variation. This method is applicable to a wide variety of isolated tissue preparations and in particular to isolated human kidney tubules.

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REFERENCES

1 J. Bruni, Arch. Neurol., 36 (1979) 393.
2 D.L. Coulter and R.J. Allen, Lancet, i (1980) 1310.
3 J.A. Sills, J.R.H. Trefor and W.H. Taylor, Lancet, ii (1980) 260.
4 C. Marescaux, J.M. Warter, M. Laroye, L. Rumbach, G. Micheletti, C. Koehl, M. Imler and D. Kurtz, J. Neurol. Sci., 58 (1983) 195.
5 C. Marescaux, J.M. Warter, L. Rumbach, G. Micheletti, G. Chabrier and M. Imler, World Rev. Nutr. Diet., 43 (1984) 174.
6 J.M. Warter, C. Marescaux, G. Chabrier, L. Rumbach, G. Micheletti and M. Imler, Rev. Neurol. (Paris), 5 (1984) 370.
7 J.M. Warter, M. Imler, C. Marescaux, G. Chabrier, L. Rumbach, G. Micheletti and J. Krieger, Eur. J. Pharmacol., 87 (1983) 177.
8 A.T. James and A.J.P. Martin, Biochem. J., 50 (1952) 679.
9 E.O. Umeh, J. Chromatogr., 56 (1971) 29.
10 S. Willox and S.E. Foote, J. Chromatogr., 151 (1978) 67.
11 R.N. Gupta, F. Eng and M.L. Gupta, Clin. Chem., 25 (1979) 1303.
12 N. Grgurinovich and J.O. Miners, J. Chromatogr., 182 (1980) 237.
13 M.I. Arranz Peña, J. Chromatogr., 225 (1981) 459.
14 R. Nishioka, S. Kawai and S. Toyoda, J. Chromatogr., 277 (1983) 356.
15 C.J. Jensen and R. Gugler, J. Chromatogr., 137 (1977) 188.
16 A. Sioufi, D. Colussi and F. Marfil, J. Chromatogr., 182 (1980) 241.
17 H. Nau, W. Wittfoht, H. Schäfer, C. Jakobs, D. Rating and H. Helge, J. Chromatogr., 226 (1981) 69.
18 R. Nishioka, M. Takeuchi, S. Kawai, M. Nakamura and K. Kondo, J. Chromatogr., 342 (1985) 89.
19 F.S. Abott, J. Kassam, A. Acheampong, S. Ferguson, S. Panesar, R. Burton, K. Farrell and J. Orr, J. Chromatogr., 375 (1986) 285.
20 S.C. Chan, Clin. Chem., 26 (1980) 1528.
21 G. Baverel, M. Bonnard, E. d'Armagnac de Castanet and M. Pellet, Kidney Int., 14 (1978) 567.