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Short communication

Microdialysis for the determination of acetaldehyde and ethanol concentrations in the striatum of freely moving rats

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Abstract

The subject of the present paper is the simultaneous determination of ethanol (EtOH) and acetaldehyde (AcH) concentrations in the striatum of freely moving rats using an in vivo microdialysis followed by head-space gas chromatography (GC). Major operation conditions of GC were as follows: column, injector and detector temperatures 90, 110 and 200 °C, respectively; Supelcowax™ wide bore capillary column (60 m length, 0.53 mm i.d., 2 μm film thickness); carrier gas, nitrogen; flow rate, 20 ml/min. The recovery of EtOH and AcH at a concentration 40 mM and 250 μM, respectively, by microdialysis showed a maximum of 83.8 ± 12.2 and 51.2 ± 6.5%, respectively, at a flow rate of 0.8 μl/min. A good linear calibration curve in the concentration range of 5–50 mM for EtOH ($r = 0.998$), and 10–250 μM for AcH ($r = 0.988$) was observed. Microdialysates were collected for 10 min each after insertion of probe into the striatum. Rats were treated with cyanamide (100 mg/kg, a potent aldehyde dehydrogenase inhibitor) and 60 min later with EtOH (1 g/kg) intraperitoneally. A 10 min sample was about 8 μl. This volume was mixed with 40 μl of 0.002% *t*-butanol as an internal standard in 0.6N perchloric acid, and then analyzed by head-space GC method. The peak EtOH and AcH concentrations in the striatal dialysates reached maximum at 30 min, and then gradually decreased. This method represents a reasonable tool to quantify in vivo both AcH and EtOH levels simultaneously in rat brain.

Keywords: Microdialysis; Acetaldehyde; Ethanol

1. Introduction

Microdialysis is an useful in vivo sampling technique, based on the passive diffusion of compounds down a concentration gradient across the semipermeable membrane of a dialysis fiber. The samples are relatively clean and protein free because the membrane pores are impermeable to macromolecules. Minimal tissue damage occurs in the location where it is inserted [1,18] which allows studies in alive, freely moving animals.

After alcohol intake, specific physiologic effects attributed to the action of acetaldehyde (AcH) occur in the peripheral and central nervous system. AcH in high doses can induces depression, ataxia, sleep, etc. and reduces motor activity [2]. In addition, in alcohol abusing individuals, AcH has been reported to mediate addiction, aldehydism [3], craving [4] and to produce derivatives with various neurotransmitters [5,6]. Several observations showed that elevation of AcH and catecholamines may occur in Asian flushers suffering from uncomfortable symptoms [10,11] after alcohol intake due to the deficient activity of aldehyde dehydrogenase (ALDH) [7–9].

Several animal studies have reported that blood AcH after alcohol intake can be detected in the brain [12,14,22,23]. The majority of these experiments were based on brain homogenates. On the other hand, only few authors have so far investigated ethanol (EtOH) levels in animal brain using an in vivo microdialysis technique [15,18,19].

The present article describes a microdialysis technique to evaluate simultaneously both EtOH and AcH levels in the striatum of awake rats which is based on microdialysis followed by head-space gas chromatography (GC).

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2. Materials and methods

2.1. Reagents

Cyanamide (CY) and perchloric acid (PCA, 60%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). AcH was purchased from Merck-Schuchardt (Hohenbrunn, Germany). All reagents used were of the highest pure grade available. Stock solution of 40 mM EtOH and 250 μM AcH were prepared in distilled water, *t*-butanol used as an internal standard (IS) was dissolved in 0.002% normal saline containing 0.6N PCA. All prepared solutions were stored at 4 °C. Ringer's solution (147 mM NaCl, 4 mM KCl, 2.25 mM $CaCl_2$) was prepared in distilled water.

2.2. Quantitation of AcH and EtOH by head-space GC

A Gas chromatograph equipped with flame ionization detector (Perkin-Elmer, Norwalk, CT, USA) combined with head-space auto sampler (Perkin-Elmer, HS-40) was used throughout the study. Head-space conditions including the use of 0.002% *t*-butanol in 0.6N PCA were set according to previous report [21]. Chromatographic conditions, in short, were as follows: column, injector and detector temperatures 90, 110 and 200 °C, respectively. The separation column was a Supelcowax^{TM} wide bore capillary column (60 m length, 0.53 mm i.d., 2 μm film thickness, Supelco, Bellefonte, PA, USA). Nitrogen was used as carrier gas at a flow rate of 20 ml/min.

2.3. Sample collection

After surgical operation, a guide cannula (8 mm long; AG-8, Eicom, Kyoto, Japan) was implanted into the striatum of male Wister rats (250–300 g) according to a previous report [17]. The day after surgery, a concentric dialysis probe (8 mm long, A-I-8-3, Eicom) with an active dialysis membrane (3 mm long, 0.20 mm i.d., 0.22 mm o.d., cut-off value 50 kDa) was inserted into the striatum and the microdialysis experiments begun. Ringer's solution was pumped through the dialysis tube at a constant flow rate of 0.8 μl/min. Rats were treated intraperitoneally (i.p.) with CY (100 mg/kg) and 60 min later with EtOH (20%, 1 g/kg). During sample collection, the glass vials (2.0 ml volume) were kept on ice. The perfusates were collected through a Teflon tube (0.1 mm i.d.) into a vial containing 40 μl PCA solution, and sealed tightly by parafilm. After sample collection for 10 min each, the parafilm was removed and the sample vial immediately put into a 30 ml head-space GC vial, and then sealed tightly by cap. The samples were collected at 15, 30, 60, 90, 120, 150, 180, and 240 min, for 10 min each after EtOH injection.

2.4. Calibration curve of AcH and EtOH

Calibration curves were constructed with six EtOH and AcH solutions. EtOH curves were prepared at concentrations 5, 10, 20, 30, 40, and 50 mM EtOH and AcH curves were prepared at 10, 25, 50, 100, 125, and 250 μM with 40 μl IS solution. The calibration curves were obtained by plotting the peak area ratio of EtOH or AcH divided by peak area of IS against the EtOH or AcH concentrations.

2.5. Percent recovery of AcH and EtOH by microdialysis probe

To determine the effect of flow rate on the recovery of EtOH and AcH, a probe with a 3.0 mm length of dialysis tubing was initially tested in vitro at different perfusion rates (0.8, 2.0 and 4.0 μl/min). The probe was immersed in a sealed vial containing known concentrations of EtOH (20 or 40 mM) and AcH (125 or 250 μM) and in a thermostated at 37 °C. Ringer's solution was perfused through the dialysis tube at constant flow rate of 0.8, 2.0 and 4.0 μl/min and the dialysates were collected for 10 min each into a vial containing PCA solution at a ratio of PCA solution to dialysate 5:1. The vials were sealed tightly and processed as described earlier. The levels of EtOH and AcH in the perfusates were compared with the concentrations of EtOH and AcH used in calibration curve.

2.6. Statistical analysis

The statistical software StatView (J-4.5, Berkeley, CA, USA) was used in the present work. All data were expressed as mean ± S.D.

3. Results and discussion

Microdialysis has been used in many in vivo studies on neurotransmitters [24]. More recently, we have used this technique for the determination of dopamine, serotonin and salsolinol in the striatum and nucleus accumbens of free moving rats [17]. AcH even in low concentrations has been reported to reinforce the effects of alcohol in brain [16]. On this basis, here we present an in vivo brain microdialyis study to quantify EtOH and AcH concentrations simultaneously in the striatum of free moving rats. Previous applied methods for extracellular fluid sampling in brain such as push–pull cannula [13,25] or prototype microdialysis technique [26] which can induce remarkable tissue damage in the site of sampling. This is the first report to our knowledge, using microdialysis to determine simultaneously both EtOH and AcH concentrations in the striatum of freely moving rats.

Fig. 1 shows a chromatogram (A) of pure sample obtained from EtOH 40 mM and AcH 250 μM, and a chromatogram (B) obtained from dialysate of rat treated with CY (100 mg/kg) + EtOH (1 g/kg) i.p. The retention time of AcH and EtOH was about 1.63 and 2.53 min, respectively, in both the pure sample and the microdialysate.

Fig. 2 shows the calibration curve of EtOH and AcH constructed over concentrations ranging 5–50 mM for EtOH and

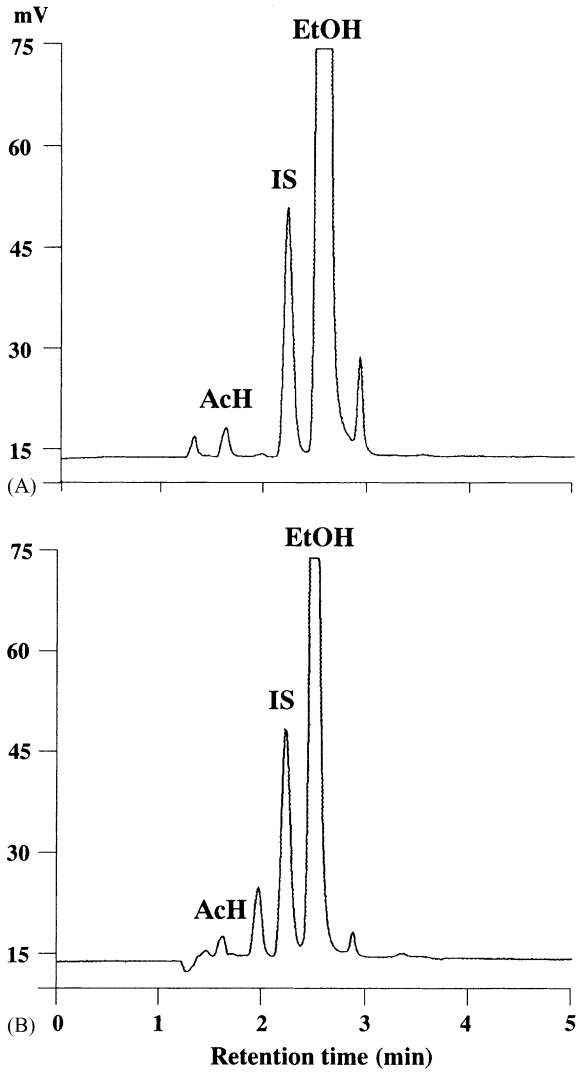

Fig. 1. Gas chromatograms obtained from standard solutions of EtOH (40 mM) and AcH (250 μM) (A), and the microdialysates of rats treated with CY (100 mg/kg) and 60 min later with EtOH (B). EtOH: ethanol; AcH: acetaldehyde.

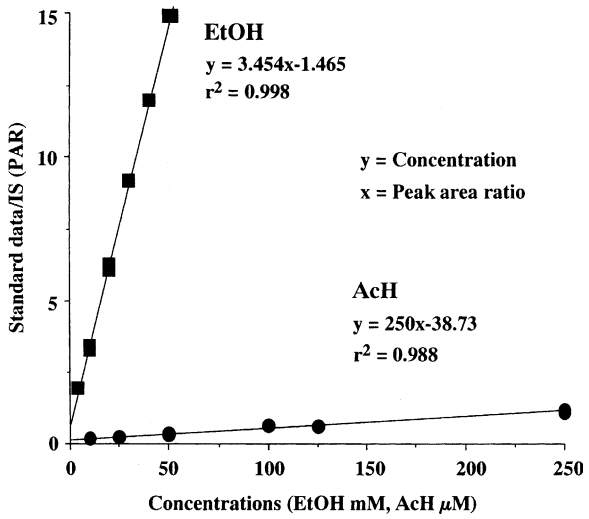

Fig. 2. Calibration curve plotted from standard solutions of EtOH and AcH in a range of concentrations 5–50 mM for EtOH and 10–250 μM for AcH. EtOH: ethanol; AcH: acetaldehyde; IS: internal standard; PAR: peak area ratio.

10–250 μM for AcH. Good linearity was observed in EtOH ($r = 0.998$, $y = 3.454x - 1.465$) and AcH ($r = 0.988$, $y = 250x - 38.73$) solutions.

Table 1 shows the probe recovery of EtOH at concentrations 20 and 40 mM and AcH at concentrations 125 and 250 μM in the perfusates. We found that the recoveries at concentrations 20 and 40 mM EtOH and 125 and 250 μM AcH did not change significantly at a same flow rate (0.8, 2.0 and 4.0 μl/min). The maximum recovery of 83.8 ± 12.2 and 51.2 ± 6.5%, respectively, for EtOH at a concentration 40 mM and AcH at a concentration 250 μM was observed at a flow rate of 0.8 μl/min, whereas at higher flow rates it decreased considerably. Probe recoveries of EtOH and AcH were inversely proportional to the speed of perfusion in the dialysates. In the present study, therefore, we chose perfusion speed at 0.8 μl/min to get a maximum probe recovery of EtOH and AcH in the dialysates.

The present method was applied to the rats administered with CY (100 mg/kg, ALDH inhibitor) plus EtOH i.p. Fig. 3 represents the time courses of concentrations of EtOH and AcH in the striatal dialysates. Each data as shown in Fig. 3 was calculated by correcting for the recovery of EtOH and AcH. The peak concentration of EtOH and AcH in the striatal dialysates reached maximum of 23.3 ± 3.1 mM and 191.2 ± 34.0 μM (mean ± S.D.), respectively, at 30 min after EtOH dosing, and then decreased gradually. Previously, we have reported that the peak levels of EtOH and AcH in blood were about 18.5 ± 2.5 mM and 687 ± 96.5 μM in rats treated with CY (100 mg/kg) + EtOH (1 g/kg) [20]. We observed that EtOH concentration in the striatum was comparable to that in the blood, but AcH concentration in the

Table 1
Probe recovery of EtOH (20 and 40 mM) and AcH (125 and 250 μM) at different speeds of perfusion (0.8, 2.0 and 4.0 μl/min)

Perfusion rate (μl/min)	Recovery (%)			
	AcH (250 μM)	AcH (125 μM)	EtOH (40 mM)	EtOH (20 mM)
0.8	51.2 ± 6.5	49.4 ± 4.5	83.8 ± 12.2	70.5 ± 8.1
2.0	36.5 ± 4.4	33.9 ± 3.6	44.1 ± 2.3	45.8 ± 35
4.0	19.8 ± 1.7	22.2 ± 2.4	32.1 ± 2.3	33.6 ± 3.3

All data (mean ± S.D., $n = 5$) were obtained from dialysates. EtOH: ethanol; AcH: acetaldehyde.

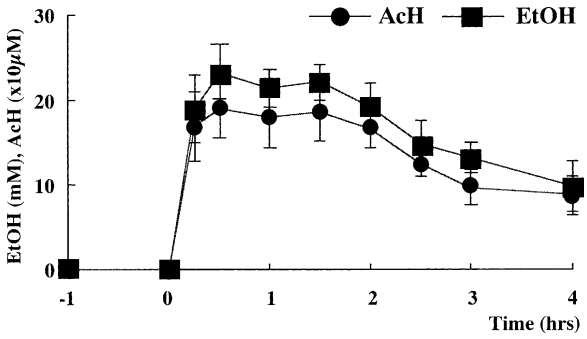

Fig. 3. Time courses of dialysate concentrations of EtOH and AcH in the striatum of rats treated with CY (100 mg/kg) + EtOH (l g/kg). Values represent mean ± S.D. ($n = 4$). Perfusate values were corrected for probe recovery. EtOH: ethanol; AcH: acetaldehyde.

striatum was about four times lower compared to the blood. Our hypothesis is that the low concentration of AcH in the striatum may be due to the existence of aldehyde dehydrogenase in the blood–brain barrier that can inhibits diffusion of low blood AcH concentration through the capillary endothelium in the brain. In conclusion, the present study suggests that the microdialysis tool can be applied in vivo to quantify both EtOH and AcH levels simultaneously in animal brain.

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References

[1] F.X. Mathy, B. Vroman, D. Ntivunwa, A.J.D. Winne, R.K. Verbeeck, V. Preat, J. Chromatogr. B 787 (2003) 323.
[2] E.B. Truitt, M.J. Walsh, in: B. Kissin, H. Begleiter (Eds.), The Biology of Alcoholism, vol. 1, Plenum Press, New York, 1971, p. 161.
[3] J.P.V. Wartburg, R. Buhler, Lab. Invest. 50 (1984) 5.
[4] Z.M. Brown, Z. Amit, B. Smith, Behav. Neural Biol. 28 (1980) 150.
[5] A.A. Smith, Adv. Exp. Med. Biol. 56 (1975) 265.
[6] T.J. Peters, R.J. Ward, Mol. Aspects Med. 10 (1988) 179.
[7] I. Ijiri, Jpn. J. Stud. Alcohol. 9 (1974) 35.
[8] T. Kijima, Jpn. J. Stud. Alcohol. 14 (1979) 101.
[9] Y. Mizoi, I. Ijiri, Y. Tatsuno, T. Kijima, S. Fujiwara, J. Adachi, S. Hishida, Pharmacol. Biochem. Behav. 10 (1979) 303.
[10] S. Harada, D.P. Agarwal, H.W. Goedde, Lancet II (1981) 982.
[11] Y. Mizoi, Y. Tatsuno, J. Adachi, M. Kogame, T. Fukunaga, S. Fujiwara, S. Hishida, I. Ijiri, Pharmacol. Biochem. Behav. 18 (1983) 127.
[12] H.W. Sippel, J. Neurochem. 23 (1974) 451.
[13] J.Y. Westcott, H. Weiner, J. Shultz, R.D. Myers, Biochem. Pharmacol. 29 (1980) 411.
[14] K. Kiianmaa, P. Virtanen, Neurosci. Lett. 10 (1978) 181.
[15] K. Yoshimoto, S. Komura, Alcohol Alcohol. 28 (1993) 171.
[16] W.A. Hunt, Alcohol 13 (1996) 147.
[17] J. Mostofa, K. Ameno, S. Ameno, N. Okada, I. Ijiri, Alcohol. Clin. Exp. Res. 27 (2003) 795.
[18] T.N. Ferraro, P. Weyers, D.P. Carrozza, W.H. Vogel, Alcohol 7 (1990) 129.
[19] T.N. Ferraro, D.P. Carrozza, W.H. Vogel, Alcohol. Clin. Exp. Res. 15 (1991) 504.
[20] J. Mostofa, K. Ameno, S. Ameno, N. Okada, I. Ijiri, Legal Med. 5 (2003) S79.
[21] T. Okada, Y. Mizoi, Jpn J. Alcohol Drug Depend. 17 (1982) 141.
[22] B. Tabakoff, R.A. Anderson, R.G. Ritzmann, Biochem. Pharmacol. 25 (1976) 1305.
[23] D.J. Hoover, J.F. Brien, Can. J. Physiol. Pharmacol. 59 (1981) 65.
[24] L. Hernandez, B.G. Stanley, B.G. Hoebel, Life Sci. 39 (1986) 2629.
[25] J.M. Peinado, D.M. Collins, R.D. Myers, Neurobiol. Aging 8 (1987) 241.
[26] D.A. Kovacs, J.G. Zoll, C.K. Erickson, Pharmacol. Biochem. Behav. 4 (1976) 621.