Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 843 (2006) 194–201
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Site-specific sampling of taurine from rat brain followed by on-line sample pre-concentration, throughout in-capillary derivatization and capillary electrophoresis

Shigeyuki Oguri *, Michiko Nomura, Youko Fujita

Laboratory of Food Hygiene, Department of Home Economics, Aichi-Gakusen University, 28 Kamikawanari, Hegoshi-cho, Okazaki-city 444-8520, Japan

Received 4 November 2005; accepted 1 June 2006
Available online 18 July 2006

Abstract

A method of pinpoint-sampling followed by on-line pre-concentration of the sample, throughout in-capillary derivatization and capillary electrophoretic separation was evaluated by demonstrating the detection of taurine, 2-aminoethanesulfonic acid at a specific location of a rat brain. The direct sampling of taurine from the rat brain was accomplished by using voltage injection associated with two kinds of driving forces, electrophoretic flow and electroosmotic flow (EOF). The capillary tube (75 μm of inner diameter × 375 μm of outer diameter) of the capillary electrophoresis (CE) apparatus was already filled with a CE run buffer, viz., 40 mM phosphate–borate buffer (pH 10) containing 2 mM *o*-phthalaldehyde (OPA)/*N*-acetylcysteine (NAC) as the derivatization reagent. One end of a platinum wire (0.5 mm o.d.), used as the anode, and the inlet end of capillary tube (from which a 1.0 cm long polyimide coating was removed), were pricked down onto the surface of either the cerebrum or cerebellum of a rat brain at a location of very small dimension. When a low voltage (5 kV, 30 s) was applied, taurine began to move from the rat brain into the capillary tube, and, simultaneously, electric focusing of taurine occurred by the action of "the pH-junction effect" at the inlet end of the capillary tube. After completing the injection, both the platinum wire and capillary tube were detached from the brain and dipped into the run buffer in an anode reservoir filed with the same solution as that in the capillary tube for the CE apparatus. Then, by applying a high voltage (20 kV) between the ends of the capillary tube, taurine was automatically derivatized to yield the fluorescent derivative, separated and detected with fluorescence (E_x = 340 nm, E_m = 455 nm) during migration throughout the capillary tube. The migration profiles obtained from cerebrum and cerebellum appeared to be different, but the peak corresponding to taurine was identified on both electropherograms. The efficacy of the present method including sample on-line pre-concentration prior to throughout in-capillary derivatization CE was first verified with several preliminary experiments by using samples of taurine in water, saline and a piece of 1.5% agar–gel block, as an alternate standard for the rat brain used in this study.

Keywords: Capillary electrophoresis; On-line pre-concentration; Taurine; Throughout in-capillary derivatization; Rat brain

1. Introduction

For the past several decades [1], in vivo microdialysis or its variations have become the method of choice in the study of unbound tissue concentrations of both endogenous and exogenous substances at site-specific locations of the brain or other organs. For example, in 1958, Kalant [2] first described the microdialysis procedure for extraction and isolation of corticosteroids from peripheral blood plasma. More recently, have used methods combined with on-line coupling to micro separation technique such as capillary electrophoresis (CE) [3], because less than 1 μl of dialysate or perfusion solution could be examined. Shippy and co-workers [4] and his group introduced a low flow push–pull perfusion system that provided chemical information from small volumes of the central nervous system (CNS) tissue without sacrificing neurochemical recovery. They succeeded in the in vivo monitoring of glutamate levels with CE examination of a 0.5 μl perfusion solution in the striatum of an anesthetized rat. Kennedy and co-workers [5,6] developed a method for monitoring primary amines in vivo using the microdialysis technique coupled with an on-line CE and micellar electrokinetic chromatograph (MEKC) with

Abbreviations: CE, capillary electrophoresis; EOF, electroosmotic flow; 2ME, 2-mercaptoethanol; NAC, *N*-acetylcysteine; OPA, *o*-phthalaldehyde

* Corresponding author. Tel.: +81 465 34 1212; fax: +81 564 34 1270.
E-mail address: s-oguri@gakusen.ac.jp (S. Oguri).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.06.008

laser-induced fluorescence detection. Dialysates were derivatized on-line with OPA/2-mercaptoethanol (2ME) and automatically transferred to a separation capillary by a flow-gated interface. By using such a system, they succeeded in monitoring taurine (2-aminoethanesulfonic acid) in rat ventral striatum [7]. This systematically designed method seemed to be very attractive and potentially effective for in vivo monitoring of biological components at a small-sized locations. However, the system was much too big and required using special techniques during its operation. Furthermore, the microdialysis method might not be proper for a reliable distribution in vitro study of a compound in a mammalian tissues or organs. For example, the distribution study in each organ (or tissue) should be carried out when the pre-clinical test for a new therapeutic drug enrollment is scheduled. In such a case, each organ is separated form the body and the target compound is separately extracted from each organ after administration of the compound to the body. After chemical modification of the compound (if necessary), an instrumental analysis is carried out by one method or another. By using this off-line method, the time required for analysis for is quite long. Moreover, there is currently an interest in analytical biochemistry regarding just how much of a biological compound is distributed at a restricted or regional site in an individual organ or tissue sample, rather than in the organ or sample as a whole, because the biological compound may not be distributed uniformly throughout. To meet the demands of speed, accuracy and efficiency, and to overcome the problems associated with the previously employed methods, it is important to reduce equipment size and to streamline the system by unifying the individual steps, e.g., sampling, chemical modification, separation and detection. In the work described in this paper, we designed a direct sampling method of the rat brain at small, site-specific location by mean of a voltage injection method that is generally used for the CE method. Then, the method was further modified to add sample on-line pre-concentration and throughout in-capillary derivatization CE method employed to uncouple the special on-line derivatization device from the method. The sample on-line pre-concentration prior to throughout in-capillary derivatization method was also evaluated with reference to our previous work, throughout in-capillary derivatization CE [8,9] and sample on-line pre-concentration prior to on-column derivatization capillary electrochromatography (CEC) [10,11], in order to apply them to the present method. By using the new method, we demonstrated an in vitro detection of taurine at small-sized site-specific locations in rat cerebrum and cerebellum for the first time.

2. Experimental

2.1. Chemicals

Taurine, OPA and *N*-acetyl cysteine (NAC) were of the highest grade. These reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan), and used without further purification. The other reagents used were of the HPLC grade or the highest grade commercially available. All aqueous solutions were prepared by using water purified with a Milli-Q purifier system (Millipore, Milford, MA, USA). Taurine and a 100 μM 17-component amino acid solution were prepared by diluting a 2.5 mM 17-component L-amino acid solution (Type H; Wako Pure Chemical Co.) with water to make an adequate concentration. The run buffer was prepared by mixing equal molar amounts of 20 mM (or 40 mM) sodium dihydrogenphosphate and 20 mM (or 40 mM) sodium tetraborate, and was adjusted to pH 10, with 1 M sodium hydroxide. The phosphate–borate buffers solution were stored at room temperature prior to use. Then, 13.4 mg of OPA and 16.3 mg of NAC were each separately added to 50 ml quantities of the phosphate–borate buffers (pH 10) and dissolved by sonification for 3 min, respectively. A portion of each solution was filtered with a disposable syringe filter unit DISMIC-13cp (ADVANTEC, Tokyo, Japan). The run buffer was prepared just before use. The 1.5% agar–gel block containing taurine was prepared as follows: 3 g of granulated agar (Wako Pure Chemical) was suspended in 100 ml of 0.9% sodium chloride (or saline) in a glass flask. The glass flask was then heated until the suspension became clear. Subsequently, a 10 mM taurine solution in 0.9% sodium chloride solution was added to make the adequate concentration. Approximately 5 ml of the solution was poured into a 10 ml plastic vessel, and placed at room temperature to make an agar–gel block (ca. 2 cm circle in diameter, 3 cm in height).

2.2. Apparatus

The CE system consisted of an 890-CE stabilized high voltage power supply (Jasco, Tokyo, Japan) with a FP-920 fluorescence detector (Jasco) equipped with a capillary flow-cell unit for CE and a model 807-IT data processor (Jasco). A 75 μm (i.d.) × 375 μm (o.d.) capillary tube of fused silica (Polymicro Technologies, Phoenix, AZ, USA) was used throughout the work. The detection window (1.0 cm) was made by removing the polyimide coating at the 20 cm position from the cathodic end of the tube. The electropherograms were recorded by monitoring the fluorescence intensity at 455 nm after excitation with light of 340 nm. For homogenization of the rat brains, an Iuchi model HOM digital homogenizer (AsOne Co., Osaka, Japan) was used. For centrifugation of the samples, a Hitachi model Himac-CR 20 refrigerated centrifuge (Ibaragi, Japan) was employed.

2.3. CE set-up

Two reservoirs were placed in the CE system, one at the anodic site and another at the cathodic site. The anodic reservoir contained 1 ml of run buffer in which the derivatizing reagent (2 mM OPA/NAC) was present, while the cathodic reservoir contained 1 ml of run buffer that lacked the derivatizing reagent. Both the ends, one of a piece of platinum wire (0.5 mm of outer diameter, anode) and one end of the capillary tube (from which a 1.0 cm long polyimide coating was removed) at the anodic site of CE apparatus were fixed in a Teflon-holder as shown in Fig. 1. Before the sample solution was injected into the capillary at the anodic end, any remaining residue in the capillary was swept out with the run buffer and subsequently filled with the same buffer at the cathodic end. When the CE system was not in the

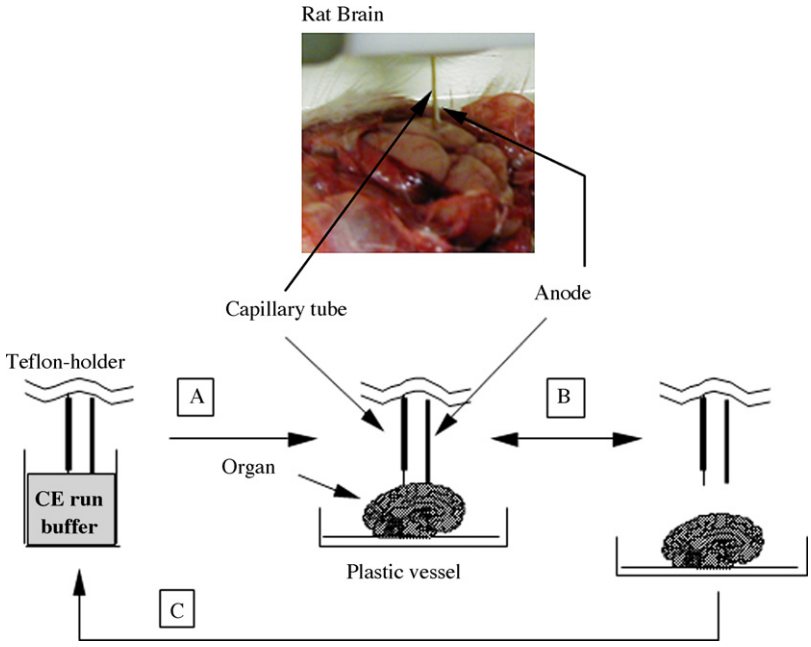

Fig. 1. Schematic diagram illustrating the sequence of procedural steps of site-specific detection. (A) Rat brain or other sample in a plastic vessel is put onto the stage. A capillary tube (75 mM inner diameter) is filled with a phosphate–borate buffer (pH 10) containing 2 mM OPA/NAC. Saline solution is nebulized onto the surface of the sample before proceeding to the following step. (B) The stage is raised by a hand-manipulator until both the end of platinum wire (anode) and of the capillary tube just prick the surface of the sample. Low voltage is applied to inject and pre-concentrate taurine. (C) The stage is lowered, thereby exposing the both platinum wire and capillary tube, which are then dipped into the anode reservoir containing the same solution as that in the separation capillary tube of CE. The electrokinetically concentrated taurine is automatically derivatized, separated and detected by the fluorescence detector of the CE apparatus when the high voltage is subsequently applied for electrophoresis.

use, the run buffer in the capillary tube and in both reservoirs was removed and the capillary was washed out several times with, in the following order: 1 M sodium hydroxide, water and methanol.

2.4. Animal sample preparation

Male Wistar rats were purchased from Chubukagaku-shizai Co. (Nagoya, Japan), and were housed with free access to water and regular food (CE-2, Chubukagaku-shizai Co.) at 25 ± 1 °C prior to experiments. At 8 weeks of age, a rat was anesthetized with ether and was killed by cutting the carotid artery. The skull of the rat was opened and the detection of taurine at site-specific locations of the cerebrum or cerebellum was done by using the method described below. After completion of taurine detection, both cerebrum and cerebellum were carefully and separately removed from the skull. During this operation, a saline solution was sprayed onto the tissues to prevent surface dryness. To determine the taurine content in throughout the whole cerebrum and cerebellum, 100 mg of each brain tissue was transferred to a 2 ml glass vessel, placed in an ice–water bath, and then homogenized with 1.0 ml of methanol. Subsequently, the homogenized solution was transferred into a polypropylene microcentrifuge tube and centrifuged at 30,000 rpm for 5 min at 4 °C. The supernatant was evaporated to dryness in vacuo, and, subsequently, the resulting residue was reconstructed with 100 μl of saline. The solution was examined by means of the optimized CE apparatus as described above.

2.5. Direct sampling and detection

The brain, contained in a plastic vessel or seat, was placed on the sample stage beside the CE apparatus. Then, the stage was carefully lifted up by a hand-manipulator until either an agar–gel block or the rat brain (either cerebrum or cerebellum) were just barely pricked by the platinum wire and the end of the capillary tub at a small-sized location on their surface. After applying 2 kV for several seconds in the case of agar–gel block or 5 kV for several seconds in the case of the rat brain, the power supply was then turned off and the sample stage was subsequently lowered. An anode reservoir containing CE run buffer was set up onto the holder to receive both the end of capillary tube and that of the anodic platinum wire into the run buffer prior to performing throughout in-capillary derivatization CE. Then, 7 kV was applied to the agar–gel block (20 kV for the rat brain). Note: the inlet end of the capillary was fixed horizontally at the position of the surface of run buffer in the cathodic reservoir. In addition, all parts of the system, including the stage, were completely insulated from the ground. The schematic diagram for this method is shown in Fig. 1.

3. Results and discussion

To perform the site-specific sampling of a biological compound from a rat organ with the present method, we chose taurine and the rat brain as a standard, representative bioactive compound and organ for this study, because taurine is ubiq-

uitous and is the most abundant, free, semi-essential amino acid in the mammalian heart [12], retina [13,14], skeletal muscle [15], brain [16,17] in leukocytes [18], etc. [19]. It is believed that taurine is an especially important component of the developing brain and must be supplied to humans in their diet [20]. Furthermore, taurine is an interesting compound because it has wide variety of roles in the mammalian body, acting, for example, as a neurotransmitter, as an antioxidant [21], as a modulator of intracellular calcium levels, as an osmolyte [22], etc. Currently, the most frequently applied techniques for determining the presence of taurine include column chromatography either via ion-exchange [23–26] or reverse-phased mode [27–39] after pre-column derivatization with *o*-phthalaldehyde (OPA) [23,27–29,31,32,36,37,39], dansyl chloride [34], fluorescamine [38] or with detection by means of a mass-spectrometer [33], electrochemical detection [27,39] or by other methods [24,26]. With the remarkable progress of micro-analytical techniques over the past several decades, e.g., CE or microchip CE, the time required for analysis of biological specimens has become greatly reduced. However, despite the reduction in required sample size and the separation time for analysis, as just mentioned above, gross analysis time, which includes procedures for pre-treatment of a biological or food sample, has not shown much reduction. Furthermore, the verified reported methods do not solve the problem of making a determination at a restricted or regional site in an individual organ. To overcome these problems, we demonstrated the detection of taurine by the method described and optimized by following steps.

3.1. On-line pre-concentration prior to throughout in-capillary derivatization CE

Detection of the small amount of taurine present in rat brain by means of a normal CE apparatus is more difficult as compared with HPLC due to reduced detection sensitivity, which, in turn, is caused by the short length of the light-path at the detection window. One solution is to use a technique called on-line pre-concentration, which has enjoyed some degree of success with CE in the past decade [40]. On-line pre-concentration techniques induce electrokinetic focusing of large volumes of injected sample based on differences in local analyte velocity within two or more distinct buffer segments in a capillary. In our previous papers [10,11], we demonstrated the efficacy of using on-line pre-concentration prior to on-column derivatization CEC. This technique produces a remarkable enhancement of the detection sensitivity for biogenic amines, even those which are lacking in either chromophore or fluorophore in their molecules. Thus, this on-line pre-concentration method was applied to the method of throughout in-capillary derivatization CE, which was also developed by our group [8,9]. On-line pre-concentration can be done simply by making the injection time much longer than usual. In this study, a 40 cm capillary (20 cm of effective length) and 2 mM OPA/NAC–20 mM phosphate–borate buffer (pH 10) were employed for the separation capillary tube and run buffer, respectively. The throughout in-capillary derivatization CE was carried out at 7 kV.

First, on-line pre-concentration prior to throughout in-capillary derivatization CE was evaluated by using a 10 μM solution of taurine in water. When electrophoresis began after introducing the solution into the separation capillary tube by means of hydrostatic injection (5 cm high; 5, 30, 60 and 150 s), sample stacking occurred at the border between the sample plug (low conductivity) and the run buffer plug (high conductivity) based on the so-called "field-amplified sample stacking effect" and, subsequently, taurine was derivatized during migration through the capillary tube filled with the run buffer containing the derivatization reagent, OPA/NAC. As shown in Fig. 2A, the sample became 20 times more concentrated relative to the conventional method (5 cm high; 5 s) and gave a sharp peak, even when the sample was injected for 150 s. The migration time of taurine decreased with increasing injection time. This shift in migration time in Fig. 2A is caused by the longer injection time, which makes the center of the stacking plug closer to the detection window.

Next, because taurine or other biological compounds are usually present in an isotonic environment, we felt it necessary to evaluate the present method using taurine in a saline solution (0.9% sodium chloride solution). Ten micromolar of taurine in saline was also examined by the same manner as described above, and the various results are shown all together in Fig. 2B. Although the pre-concentration of taurine in the saline solution resulted in approximately a 23-fold enhancement in concentration, which was almost the same level of enhancement of detection sensitivity, the shift in migration time in this case was less than that in the case of taurine in water.

In this case, we believe pre-concentration occurred by means of a different mechanism, namely, the "dynamic pH-junction effect" [41]. Usually, sample pre-concentration by the dynamic pH-junction method can be performed by making two kinds of plugs, "sample plug" and "run buffer plug", in a capillary tube, each with a pH different from the other. Upon application of voltage, stacking is due to decreases in the velocity of the sample analyte when migrating from the low pH sample zone to a relatively high pH buffer zone. Although the sample plug in this study was composed of taurine ($pK_a = 4.99$) [44] in an unbuffered saline solution, it is likely that a pH boundary formed between the sample plug and the run buffer plug (pH 10). Application of the voltage generates motion in the boundary between the two plugs, resulting in electrokinetic focusing of taurine bands towards the front end of the sample zone.

Finally, because our final goal in this study was to perform direct sampling and detection of taurine in a rat brain, we knew that, in such a detection, it would be better to employ a voltage injection technique, rather than hydrostatic injection. The 10 μM taurine in saline solution was also evaluated using the 2 kV injection for the same injection times as the method mentioned above. The results are shown in Fig. 2C, and from these it was recognized that the sample pre-concentration prior to throughout in-capillary derivatization occurred just as well as if the hydrostatic injection method had been employed. In the present case, we anticipated that the sample plug would be formed by EOF and, simultaneously, electrokinetic focusing of taurine by

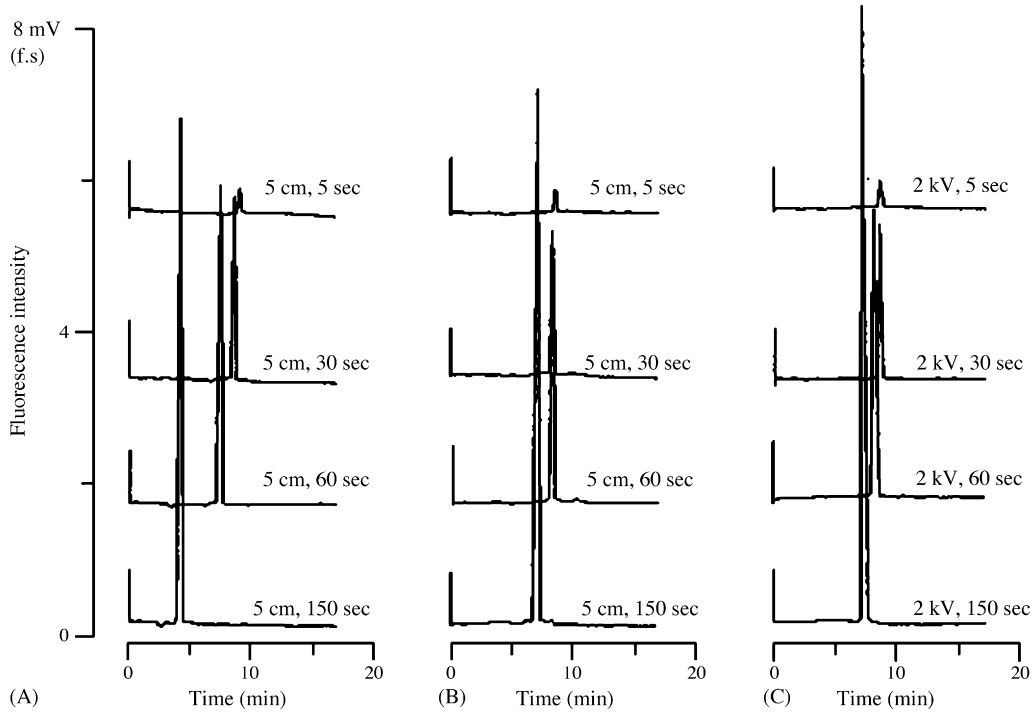

Fig. 2. Effect of the sample matrix and injection method on on-line pre-concentration. Conditions: sample consisting of 10 μM taurine in water (A) or 10 μM taurine in saline (B and C); sample injection, 5 cm high hydrostatic injection for 10, 30, 60 and 150 s (A and B) or 2 kV voltage injection for 5, 30, 60 and 150 s (C); capillary, a 40 cm fused silica capillary tube (75 μm inner diameter, 20 cm effective length); CE run buffer, 2 mM OPA/NAC–20 mM phosphate–borate buffer (pH 10); CE applied voltage, 7 kV. For other CE conditions, please refer to Section 2.

dynamic pH-junction would occur at the inlet site of the capillary tube.

In addition, the effect of the presence of Mg^{2+} and Ca^{2+}, which are major cations present in biological samples, on the performance of voltage injection used here was examined, because we expected that their presence would disrupt the EOF used to inject taurine. It was observed that there was no effect on the performance of taurine injection from those cations under the condition of the present injection method when 10 μM taurine in Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2{\cdot}6H_2O$, 1.8 mM $CaCl_2{\cdot}2H_2O$, 0.4 mM $NaH_2PO_4{\cdot}2H_2O$, 11.9 mM $NaHCO_3$ and 5.6 mM D-glucose) and the 10 μM taurine in 0.9% NaCl solution were compared with each other.

3.2. Detection of taurine in an agar–gel block

Given the above results, a direct sampling of taurine was carried out using a 1.5% agar–gel block as a substitute for rat brain. Just before pricking the agar–gel block with the platinum anode wire and the capillary tube, it was necessary to spray a saline solution onto the surface of the agar–gel block to prevent accidentally inserting an air-plug into the capillary tube during performance of site-specific detection due to the drying out of the agar–gel surface from evaporation. The 10 μM taurine concentration in the agar–gel block was tested with a 2 kV injection voltage for 5, 30, 60 and 150 s. The taurine effectively moved from the agar–gel matrix to the separation capillary tube and electrokinetically focused on the inlet site of the capillary tube by application of the same voltage and injection times shown in Fig. 2C. The peak area corresponding to taurine increased with increasing injection time as shown in Fig. 3. In addition, the same results were given when a 1.5% agar–gel block made with Tyrode's solution was used instead of a 1.5% agar–gel block made with 0.9% saline. This result showed that pre-concentration prior to in-capillary derivatization takes place even if the taurine is mixed in an agar–gel block, as well as in saline solution or Tyrode's solution. Therefore, we concluded that taurine present in an immobilized matrix such as agar–gel or mammalian tissues can be introduced into a capillary tube by means of voltage injection. It should be noted that the distance between the end of the platinum wire and capillary tube had an effect on the reproducibility of the assay results, hence, this distance, as shown in Fig. 1, was fixed in this study.

3.3. Assay validations

To study the peak area responses to the different concentrations of taurine (10, 100, 500 and 1000 μM) in 1.5% agar–gel blocks, a linearity test was carried out by using a 2 kV injection for 30 s. Taurine in the agar–gel block was smoothly introduced into the capillary tube in each case as expected and showed increasing peak area counts with increasing concentration of taurine. The parameters of the linearity was calculated by the least squares regression method for $y = ax + b$, where y, x, a and b are peak area counts, concentration of taurine (mM) used in the agar–gel plate, intercept at y-axis and slope, respectively.

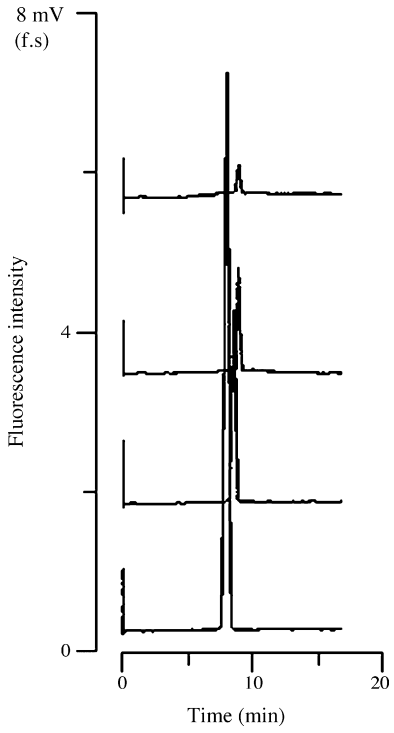

Fig. 3. Effect of different 2 kV injection times (10, 30, 60 and 150 s) on the electropherograms of 10 μM taurine in a 1.5% agar–gel block. CE conditions as described in Fig. 2.

As the result of this, a and b were given as 4.5×10^5 and 138, respectively. The correlation factor ($r = 0.997$) implied a strong linear relationship between peak area response and concentrations between 10 and 1000 μM solution of taurine in agar–gel plates. Reproducibility tests, based on five individual assays of 10 μM solution of taurine in an agar–gel plate showed 2.8% c.v. The recovery of taurine from the agar–gel block with the voltage injection method (5 kV, 30 s) was tested by comparing both peak areas obtained from the standard taurine solution and the agar–gel block at concentration of 10 μM taurine, and resulted at 96.7%.

3.4. Separation of taurine from a 17-component amine solution

It was first necessary to separate taurine from other kinds of amines, such as amino acids, before proceeding to taurine analysis in a rat brain, because the throughout in-capillary derivatization CE method employed in this study can only detect amino compounds, such as amino acids, present in a sample. In the technique of throughout in-capillary derivatization CE, the pH of the run buffer is a dominant factor, because the run buffer serves both as a separation buffer and as a derivatization buffer. Previously, OPA/NAC and a phosphate–borate buffer at pH 10 were employed as the basic derivatizing reagent and run buffer, respectively. OPA/NAC reacts with amino groups very quickly, even at room temperature, and, whereas OPA/NAC-amine derivatives show strong fluorescence, OPA/NAC itself does not. Considering their performance from an examination of their peaks on a CE electropherogram, a run buffer consisting of sodium dihydrogenphosphate and sodium tetraborate was chosen as the most suitable run buffer. Next, certain chemical modifiers, such as cyclodextrin (alpha, beta and gamma), sodium dodesyl sulfate (SDS), and methanol were evaluated to obtain the optimal separation between taurine and 17 kinds of amino acids (used as the standard amino acids). An 80 cm capillary tube (60 cm of effective length) was used for the separation capillary tube instead of the previous 40 cm capillary, and a 2 mM OPA/NAC–40 mM phosphate–borate buffer (pH 10) was used for the run buffer. To obtain the best separation of taurine from the other amines, a 5 kV/30 s. voltage injection and 20 kV CE running voltage were employed. Under optimized conditions, the presence and absence of taurine at 10 μM level in a 10 μM 17-component amino acid saline solution were examined, and typical electropherograms were obtained, as shown in Fig. 4A and B, respectively. By comparing both electropherograms, the peak corresponding to taurine required 43.0 min to migrate, and its complete separation from other peaks corresponding to the various amino acids was confirmed in this study.

3.5. Detection of taurine in rat brain at site-specific locations

As described above, our experiments showed that taurine was detectable even when immobilized in a matrix such as agar–gel matrix; it was subsequently separated and differentiated from 17 kinds of common amino acids using the present equipment. Next, both the end of a capillary tube and of a platinum wire connected to CE apparatus were directly pricked onto the sur-

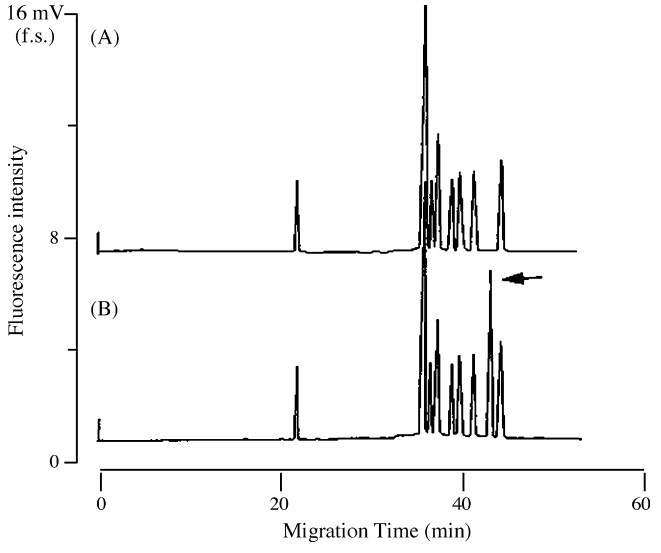

Fig. 4. Typical electropherograms (A) and (B) obtained from a mixture of 17 amino acids (10 μM each) and a mixture of 17 amino acids (10 μM in each) plus taurine (10 μM), both mixtures in saline solution, respectively. The arrow in panel (B) indicates the peak corresponding to taurine. Conditions: capillary, an 80 cm fused silica capillary tube (75 μm of inner diameter, 60 cm of effective length); injection, 5 kV, 30 s; CE run buffer, 2 mM OPA/NAC–40 mM phosphate–borate buffer (pH 10); CE applied voltage, 20 kV. For other CE conditions, please refer to Section 2.

face of either the cerebrum or the cerebellum of a rat brain. In order to prevent the release of taurine caused by damaging either brain tissue or blood capillaries during the pricking operation, extreme caution was taken to insure that the capillary tube just barely broke the surface of the brain tissue to a minimal depth. If the capillary tube is to be inserted deeply into the brain tissue, like a microdialysis probe, use of a tapered capillary tube is recommended. Unfortunately, we cannot make such a capillary tube in our machine shop at this moment.

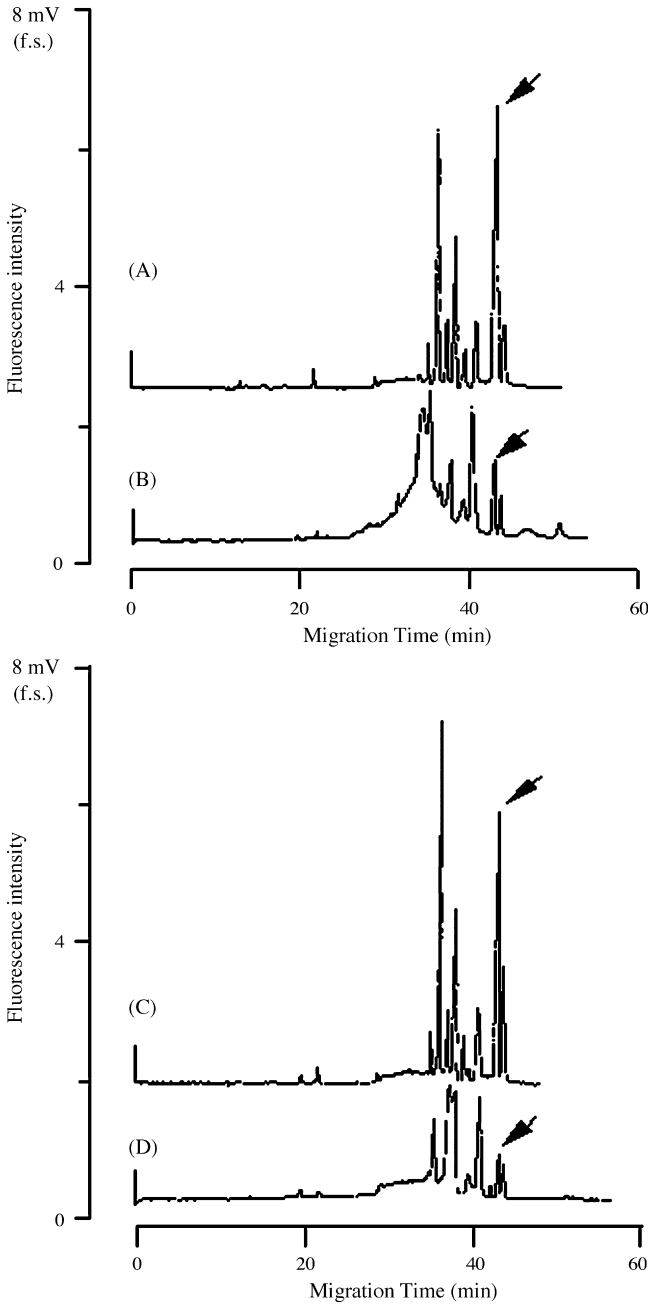

Fig. 5. Typical electropherograms obtained from rat cerebrum extract (A) and directly from a site-specific location of the cerebrum (B) with 5 kV injection for 30 s in both cases. The electropherograms and obtained from rat cerebellum extract (C) and directly from a site-specific location of the cerebellum (D) with 5 kV injection for 30 s in both cases. Other CE conditions as described in Fig. 4.

Then, with the application of a 5 kV injection voltage for 30 s between the anode in the brain and cathode, taurine began to move from the brain to the inlet of the capillary tube driven by both and electrophoretic force and by EOF. During this period, electrokinetic focusing of taurine simultaneously occurred via a dynamic pH-junction mechanism, as was also the case in the previous experiment which used the agar–gel block. After completing the injection of taurine, the throughout in-capillary derivatization CE was carried out under the optimized condition s as described at the previous section. When an injection time much longer than 30 s was employed, the peak corresponding to taurine did not appear separated from the other peaks even though the peak corresponding taurine increased. Samples collected from the methanolic extract of either the cerebrum or cerebellum were also analyzed by the same procedure. The resulting electropherograms obtained from the intact brain via site-specific detection and from the methanolic extract were compared with each other. Fig. 5A and B show the typical electropherograms obtained from the intact rat cerebrum and the methanolic extract of the rat cerebrum, respectively. Fig. 5C and D show the typical electropherograms obtained from the intact rat cerebellum and methanolic extract of the rat cerebellum, respectively. Although each electropherograms obtained from the cerebrum (Fig. 5A and B) and the cerebellum (Fig. 5C and D) show differences from each other, each electropherogram showed the peak corresponding to taurine identified with its migration time, without any other technique, e.g., CE-mass spectrometer. These results show that the method of throughout in-capillary derivatization CE coupled with on-line sample pre-concentration can be used effectively to perform detection of taurine at site-specific locations of a rat brain.

In this experiment of detecting taurine in a rat brain, the capillary was directly pricked on the surface of the brain without microscopic observation. We believe that the present method detects both intra- and extra-cellular taurine, as does the in vivo microdialysis method. Even though the sample used in this experiment was dead, this demonstration showed that the preset method can also be applied to living samples as well.

4. Conclusions

On-line sample pre-concentration prior to on-column derivatization CE has been previously present in other papers [10,11]. In the work presented in those papers, the on-line sample preconcentration technique for CE was applied to the throughout in-capillary derivatization CE method, and it was shown that this technique was able to separately detect free amino acids, including biogenic amines using OPA/NAC as a derivatization reagent. The present paper shows that the on-line sample pre-concentration prior to throughout in-capillary derivatization CE method makes it possible to perform detection of biogenic compounds (e.g., taurine) in biological organs at a site-specific location by means of the voltage injection method. This new result suggests the possibility of detecting analytes in living organs in situ, because this method does not require any operations that would involve isolating an organ from a body for the purpose of extraction of the target analyte.

References

[1] N. Plock, C. Kloft, Eur. J. Pharm. Sci. 25 (2005) 1.
[2] H. Kalant, Biochem. J. 69 (1958) 99.
[3] S. Parrot, L. Bert, L. Mouly-Badina, V. Sauvinet, J. Colussi-Mas, L. Lambas-Senas, F. Robert, J.P. Bouilloux, M.F. Suaud-Chagny, L. Denoroy, B. Renaud, Cell. Mol. Neurobiol. 23 (2003) 793.
[4] S. Kottegoda, I. Shaik, S.A. Shippy, J. Neurosci. Methods 121 (2002) 93.
[5] M.W. Lada, R.T. Kennedy, Anal. Chem. 68 (1996) 2790.
[6] R.T. Kennedy, J.E. Thompson, T.W. Vickroy, J. Neurosci. Methods 114 (2002) 39.
[7] A. Smith, C.J. Watson, K.J. Frantz, B. Eppler, R.T. Kennedy, J. Peris, Alcohol Clin. Exp. Res. 28 (2004) 1028.
[8] S. Oguri, T. Fujiyoshi, Y. Miki, Analyst 121 (1996) 1683.
[9] S. Oguri, K. Yokoi, Y. Motohase, J. Chromatogr. A 787 (1997) 253.
[10] S. Oguri, Y. Yoneya, M. Mizunuma, Y. Fujiki, K. Otsuka, S. Terabe, Anal. Chem. 74 (2002) 3463.
[11] S. Oguri, H. Tanagaki, M. Hamaya, M. Kato, T. Toyo'oka, Anal. Chem. 75 (2003) 5240.
[12] A. Guidotti, A. Giotti, Recenti Prog. Med. 49 (1970) 61.
[13] J.D. Militante, J.B. Lombardini, Nutr. Neurosci. 5 (2002) 75.
[14] R. Salceda, Adv. Exp. Med. Biol. 526 (2003) 83.
[15] D. Conte Camerino, D. Tricarico, S. Pierno, J.F. Desaphy, A. Liantonio, M. Pusch, R. Burdi, C. Camerino, B. Fraysse, A. De Luca, Neurochem. Res. 29 (2004) 135.
[16] A. Barbeau, Can. J. Neurol. Sci. 2 (1975) 343.
[17] T.M. Foos, J.Y. Wu, Neurochem. Res. 27 (2002) 21.
[18] D.B. Learn, V.A. Fried, E.L. Thomas, J. Leukoc. Biol. 48 (1990) 174.
[19] S. Macaione, G. Tucci, R.M. Di Giorgio, Ital. J. Biochem. 24 (1975) 162.
[20] G.E. Gaull, J.A. Sturman, G.Y. Wen, H.M. Wisniewski, Prog. Clin. Biol. Res. 91 (1982) 349.
[21] O.I. Aruoma, B. Halliwell, B.M. Hoey, J. Butler, Biochem. J. 256 (1988) 251.
[22] P. Olivero, A. Stutzin, Neurochem. Res. 29 (2004) 169.
[23] B.R. Larsen, D.S. Grosso, S.Y. Chang, J. Chromatogr. Sci. 18 (1980) 233.
[24] S. Miyazaki, H. Tanaka, R. Horikawa, H. Tsuchiya, K. Imai, J. Chromatogr. 181 (1980) 177.
[25] C.A. Palmerini, C. Fini, M.G. Cantelmi, A. Floridi, J. Chromatogr. 423 (1987) 292.
[26] T.R. Cataldi, G. Telesca, G. Bianco, Anal. Bioanal. Chem. 378 (2004) 804.
[27] S. Murai, H. Saito, Y. Masuda, T. Itoh, J. Pharmacol. Methods 23 (1990) 195.
[28] A. Sano, S. Matsutani, S. Takitani, J. Chromatogr. 458 (1988) 295.
[29] D.W. Porter, M.A. Banks, V. Castranova, W.G. Martin, J. Chromatogr. 454 (1988) 311.
[30] M.H. Stipanuk, L.L. Hirschberger, J. De la Rosa, Methods Enzymol. 143 (1987) 155.
[31] L.L. Hirschberger, J. De La Rosa, M.H. Stipanuk, J. Chromatogr. 343 (1985) 303.
[32] B.R. Larsen, D.S. Grosso, S.Y. Chang, J. Chromatogr. Sci. 18 (1980) 233.
[33] E. Marchei, M. Pellegrini, R. Pacifici, I. Palmi, S. Pichini, J. Pharm. Biomed. Anal. 37 (2005) 499.
[34] H. Nakamura, T. Ubuka, Adv. Exp. Med. Biol. 526 (2003) 221.
[35] H. Inoue, K. Fukunaga, Y. Tsuruta, Anal. Biochem. 319 (2003) 138.
[36] T.P. Piepponen, A. Skujins, J. Chromatogr. B 757 (2001) 277.
[37] C.J. Waterfield, J. Chromatogr. B 657 (1994) 37.
[38] T. Sakai, T. Nagasawa, J. Chromatogr. 576 (1992) 155.
[39] S. Murai, H. Saito, E. Abe, Y. Masuda, T. Itoh, J. Neural Transm. Gen. Sect. 87 (1992) 145.
[40] P. Britz-McKibbin, S. Terabe, J. Chromatogr. A 1000 (2003) 917.
[41] J.B. Kim, P. Britz-McKibbin, T. Hirokawa, S. Terabe, Anal. Chem. 75 (2003) 3986.
[44] S. Mou, X. Ding, Y. Liu, J. Chromatogr. B 781 (2002) 251.