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Short communication

Liquid chromatographic determination of myocardial interstitial epinephrine

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Abstract

This study describes a high-performance liquid chromatographic method with electrochemical detection (HPLC–ED) for monitoring of epinephrine (Epi) in the myocardial interstitial space. The in vitro detection limit for Epi was 200 fg in a 50-μl injection. Using a cardiac dialysis technique, 60-μl dialysates were sampled from the myocardial interstitial space (6-min fractions). After an alumina procedure, the dialysate Epi concentration was measured using the HPLC–ED system. Although the basal Epi concentration was undetectable, local administration of desipramine increased Epi concentration of the dialysate to 38.1±18.5 pg/ml. This system affords a new possibility for estimating myocardial interstitial Epi level.

Keywords: Epinephrine

1. Introduction

Epinephrine (Epi) is a potent agonist of cardiac α- and β-receptors. Although a large portion of plasma Epi arises from the adrenal medulla [1], several investigations have suggested that local cardiac Epi storage and synthesis also play important roles in regulating cardiac function [2,3]. In this regard, cardiac Epi kinetics have been assessed by cardiac extraction and spillover using radiotracer techniques [4,5]. Although such techniques revealed Epi kinetics across the whole heart, more localized Epi kinetics should be clarified in order to identify spatial heterogeneity of Epi concentration in the heart, such as the differences between the atrium and ventricle. Furthermore, if the radiotracer technique was applied together with systemic administration of pharmacological drugs to modulate cardiac Epi kinetics, the central modulation of the local Epi kinetics via the sympathetic system was inevitable. Thus, the purpose of this study was to apply the technique of cardiac dialysis to the measurement of myocardial interstitial Epi and examine the effects of desipramine, a neuronal uptake blocker, on the local Epi kinetics.

Advantages of the microdialysis system are

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numerous. Previous studies described an HPLC–ED system enabling the routine measurement of the low levels of norepinephrine [6,7] and dihydroxyphenylglycol [8] found in the myocardial interstitial space. Furthermore, local administration of pharmacological drugs through a dialysis probe offers a new approach to study function of sympathetic nerve terminals without interfering with systemic hemodynamics or the central modulation of neuronal regulation [9]. In this study, we have applied the cardiac dialysis approach with this system to measure myocardial interstitial Epi levels before and after local administration of desipramine. The Epi concentration of the dialysate was measured as an index of myocardial Epi concentration.

2. Experimental

2.1. Reagents and chemicals

Distilled water and methanol of HPLC grade and 1-octanesulfonic acid sodium salt were obtained from Nacalai Tesque (Kyoto, Japan). Standard Epi, 3,4-dihydroxybenzylamine (DHBA), tris(hydroxymethyl)aminomethane (Tris), ethylendiaminetetraacetic acid (EDTA) and acid-washed alumina were obtained from Wako Pure Chemical (Osaka, Japan). All other chemicals were of analytical grade and were used without any further pretreatment.

Stock solutions of Epi and DHBA were prepared separately at a concentration of 1 mg/l in 0.1 *M* perchloric acid. Stock solutions were stable at 4°C for 1 month. For a working standard solution, these stock solutions were mixed in 2% acetic acid to contain 40 ng/l of Epi and 80 ng/l of DHBA. The stock solution of DHBA was diluted with 2% acetic acid to give a concentration of 80 ng/l and served as a working internal standard (I.S.) solution for each sample.

2.2. Dialysis probe and in vivo cardiac dialysis

We designed a transverse dialysis probe for cardiac dialysis. The dialysis fiber (8 mm×0.31 mm O.D. and 0.2 mm I.D.; PAN-1200, 50 000 molecular mass cut-off, Asahi Chemical, Tokyo, Japan) was glued contiguously at both ends to polyethylene tubing (25 cm×0.5 mm O.D. and 0.2 mm I.D.). Six Japanese white rabbits, each weighing 2.3–3.2 kg, were anesthetized with a mixture of urethane (500 mg/kg) and α-chloralose (80 mg/kg). Additional anesthetics were given as necessary to maintain an appropriate depth of anesthesia. The animals were intubated and ventilated with room air mixed with oxygen. Body temperature was maintained at around 38°C with a heating pad and lamp. Arterial blood pressure was monitored through a catheter inserted via the right femoral artery. After midline thoracotomy and incision of the pericardium, the dialysis probe was implanted in the left ventricular myocardium. The dialysis probe was perfused with Ringer's solution at a rate of 10 μl/min using a microinjection pump (CMA 102, Carnergie Medicin, Stockholm, Sweden). The dialysate was sampled and the concentration of Epi measured in (1) the control state, and (2) 30 min after beginning local perfusion of desipramine (10 μ*M*). Each sample was collected in a 300-μl microtube containing 6 μl of 0.1 *M* HCl, to prevent amine oxidation. The collection period for each sample was 6 min.

2.3. Sample preparation

Each dialysate sample was transferred into a 1.5-ml polypropylene conical tube. A 50-μl volume of the working I.S. solution (4 pg of DHBA), acid-washed alumina (5 mg), and 1.0 ml of 1 *M* Tris buffer (pH 8.6, containing 0.2% disodium EDTA) were added to the vial which was shaken for 15 min. After shaking, the alumina was washed three times with distilled water, transferred into a microfilter (Ultrafree C3, Millipore, Bedford, MA, USA), and centrifuged to remove excess fluid (600 *g*, 5 min). The Epi and DHBA were then eluted from the alumina using 60 μl of 2% acetic acid. Using an autosampler (CMA/200, Carnegie Medicin), 50 μl was injected into the liquid chromatograph. Alumina recoveries of the standard solutions (Epi, 2 pg; DHBA, 4 pg) were calculated, by comparison with height of chromatographic peaks corresponding in retention times to those of the directly injected standards. The average recoveries of the alumina procedure were 61.3±5.0% and 74.8±4.5% for Epi and DHBA, respectively ($n=6$).

2.4. Chromatographic and detection conditions

Interfering compounds in the dialysate were removed by the above-mentioned alumina procedure. Using an autoinjector (CMA 200, Carnegie Medicin), 50 μl was injected into the liquid chromatograph. The HPLC system consisted of a pump with a pulse dampener (EP-300, Eicom, Kyoto, Japan), a guard column (AC-ODS, 5×4 mm I.D., Eicom), an analytical reversed-phase column (Eicompack CA-5ODS, 150×2.1 mm I.D., Eicom), an electrochemical detector equipped with a graphite electrode (ECD-300, Eicom), a chromato-integrator (D-2500, Hitachi) and a degasser (DG-300, Eicom). The mobile phase consisted of 900 ml of 0.1 *M* phosphate buffer (pH 6.1) containing 1-octanesulfonic acid sodium salt (550 mg/l final concentration) and 100 ml of methanol. The flow-rate was 0.25 ml/min. The electrochemical detector was operated at +400 mV versus an Ag–AgCl reference electrode. The HPLC separations were performed at 25°C.

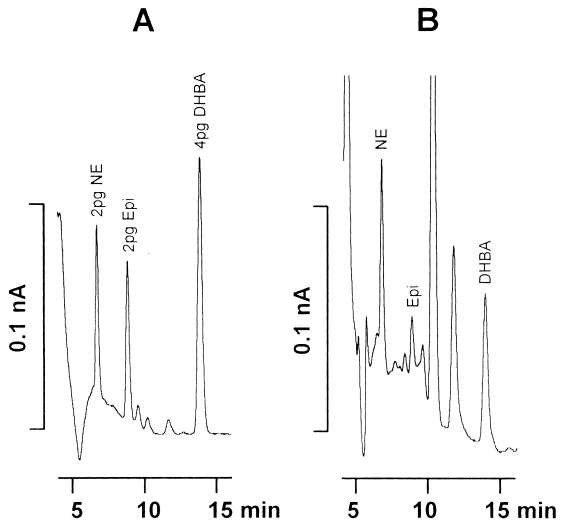

Fig. 1. (A) Chromatogram of a 50-μl injection of standard solution containing 2 pg of norepinephrine (NE), 2 pg of epinephrine (Epi) and 4 pg of 3,4 dihydroxybenzylamine (DHBA); (B) Typical chromatogram of an injected dialysate sample after local administration of desipramine.

3. Results and discussion

A standard chromatogram of 2 pg of Epi in a 50-μl injection required less than 15 min as shown in Fig. 1A. The calibration curve for Epi was linear in the concentration range from 0.5 to 5 (0.5, 1, 2, 5) pg with an r^2 value of 0.99 ($y=217.9x+35.4$). To examine the precision of peak height for 2 pg of Epi, repeatability and reproducibility were calculated. The intra-day coefficient of variation (C.V.) was 1.5% ($n=12$) and the inter-day C.V. was 2.7% (6 consecutive days). Since basal Epi exists in the dialysate sample from the heart, a limit of quantification cannot be strictly determined by using a spiked dialysate with a known amount of Epi. If we spiked the 60 μl of perfusate with 400 fg of Epi and performed the alumina procedure, however, the intra-day C.V. was 6.7% with the measured value of 405±27 fg ($n=6$). Thus the limit of quantification was around 400 fg per 50-μl injection. The limit of detection for Epi was determined using two criteria: (1) a signal-to-noise ratio of higher than three, and (2) a C.V. of lower than 10%. This limit was 200 fg per 50-μl injection.

In order to determine the average Epi recovery ratio of the dialysis fiber, we subjected the dialysis probe — perfused with Ringer's solution at 10 μl/min — to a test solution of Epi (20 pg/ml), while the Epi concentration of the dialysate was measured. The average recovery of the dialysis fiber was 6.4±0.7%, as determined by calculating the ratio of the Epi concentration of the dialysate to that of the test solution.

A chromatogram of injected Ringer's solution alone had no peak corresponding in retention time to standard Epi. The dialysate Epi level could therefore be reliably estimated from the observed peak corresponding in retention time to standard Epi. Fig. 1B shows the typical chromatogram of a dialysate sample obtained after a desipramine injection. The dialysate Epi level increased to 38.1±18.5 pg/ml by local administration of desipramine, whereas the Epi level was undetectable under control conditions.

To our knowledge, this is the first report on the in vivo monitoring of myocardial interstitial Epi levels. Although Eisenhofer et al. [5] using a radiotracer method showed a decrease in Epi spillover with systemic desipramine administration, inhibition of neuronal uptake (uptake 1) clearly increased the dialysate Epi level in this study. Thus, uptake 1

appears to effectively lower myocardial interstitial Epi concentration under basal conditions. The apparent contradiction between the results of Eisenhofer et al. and the present ones could come from differences in methodologies of Epi estimation and desipramine administration. As discussed by the authors, since desipramine suppresses the sympathetic outflow through central mechanisms [4], the local increase in Epi concentration by inhibition of neuronal uptake might have been counterbalanced in their study. Additionally, according to in vitro experiments, 10 μM of desipramine was needed to increase the net efflux of endogenous Epi, which was 1000-fold greater than that needed to increase the net efflux of endogenous norepinephrine [10]. Thus, a systemic administration of desipramine might not be sufficient to achieve such a concentration.

Basal Epi production in the heart has been explained by several mechanisms; extraction of plasma Epi, neuronal Epi synthesis, and non-neuronal Epi synthesis. Although further studies are clearly needed to identify the relative importance of these mechanisms on Epi production, the cardiac dialysis technique explores a new possibility for monitoring the levels of myocardial interstitial Epi.

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