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Short communication

Routine high-performance liquid chromatographic determination of myocardial interstitial norepinephrine

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Abstract

The present study describes a high-performance liquid chromatographic–electrochemical detection (HPLC–ED) system for routine measurement of the low levels of norepinephrine (NE) found in the myocardial interstitial space. In this system, an in vivo detection limit of 100 fg in a 50-μl injection was achieved for NE. Using cardiac dialysis technique, 20-μl dialysates were sampled from the myocardial interstitial space at 2-min intervals. The basal dialysate NE concentration was 16.6 ± 4.0 pg/ml. This low detection limit allowed the dialysate NE concentration to be monitored for dysfunction of the cardiac sympathetic nerve terminal. This system offers a new possibility for routine analysis of myocardial interstitial NE levels.

1. Introduction

The assessment of organ-specific sympathetic nerve activity is of great value in understanding the pathophysiology of cardiac disease [1,2], although up to the present, no research has focused directly on this issue. Recently, owing to the development of the dialysis technique [3], cardiac dialysis has made possible the detection of norepinephrine (NE) levels in the local myocardial interstitial space [4].

Previous studies demonstrated the responses of dialysate NE to electrical stimulation of the stellate ganglion or myocardial ischemia [4,5]. We have extended these studies to routinely measure dialysate NE as an index of cardiac sympathetic nerve activity. An improved high-performance liquid chromatographic–electrochemical detection (HPLC–ED) method was used to measure the dialysate NE with a time course corresponding to the changes in cardiac sympathetic nerve activity. Dialysate NE levels were measured before and after transection of the stellate ganglion. In addition, the dialysate NE was measured by perfusate containing a neural uptake inhibitor.

2. Experimental

2.1. Reagents and chemicals

Distilled water and methanol were HPLC grade from Wako Pure Chemical (Osaka, Japan). Norepinephrine (NE), 3,4-dihydroxybenzylamine (DHBA), tris(hydroxymethyl)aminomethane

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(Tris), ethylenediaminetetraacetic acid (EDTA), and acid-washed alumina were obtained from Wako Pure Chemical, and 1-octanesulfonic acid sodium salt from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical grade and were used without any further pretreatment.

Stock solutions of NE and DHBA were prepared separately at a concentration of 1 mg/l in 0.1 *M* perchloric acid. A working standard mixture containing (per litre) 40 ng of NE, 80 ng of DHBA was made in 2% acetic acid. The stock solution of DHBA was diluted with 2% acetic acid to give a 80 ng/l working internal standard solution. Stock solutions were stable at 4°C for one month.

2.2. Dialysis probe and in vivo cardiac dialysis

For cardiac dialysis, we designed a long transverse dialysis probe. The dialysis fibre (13 mm length, 0.31 mm O.D., and 0.2 mm I.D.; PAN-1200, 50 000-molecular mass cut-off, Asahi Chemical, Tokyo, Japan) was bundled and glued at both ends into a polyethylene tube (25 cm length, 0.5 mm O.D., and 0.2 mm I.D.). Ten adult cats of either sex weighing 2.1–4.5 kg each were anaesthetized with pentobarbital sodium (30–35 mg/kg i.p.). The level of anaesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (1–2 mg kg^{-1} h^{-1}). The animals were intubated and ventilated with room air mixed with oxygen. Body temperature was maintained with a heated pad and lamp. Electrocardiogram, heart rate, and arterial blood pressure were simultaneously monitored and recorded with a data recorder for analysis. After a left thoracotomy and incision of the pericardium, the dialysis probe was implanted in the midwall of the left ventricle. The dialysis probe was perfused with Ringer's solution at a speed of 10 μl/min using a microinjection pump (CMA 102, Carnegie Medicin, Stockholm, Sweden). The dialysate was sampled and dialysate NE was measured in (1) the control state, (2) 30 min after transection of the bilateral stellate ganglions, and (3) 30 min after beginning local perfusion of neural uptake inhibitor, desipramine (10^{-4} *M*, Sigma, St. Louis, MO, USA) following the transection of the stellate ganglions. Each sample was collected in a 300-μl microtube containing 5 μl of 1.0 *M* perchloric acid using a microfraction collector (CMA 142, Carnegie Medicin). One sampling period was 2 min (1 sample volume = 20 μl). We measured a dead space of 10 μl between the dialysis fibre and sample tube, taking this space at the start of each dialysate sampling.

2.3. Sample preparation

Each dialysate sample was transferred into a 1.5-ml polypropylene conical tube. A 50-μl volume of working internal standard 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) was added to the vial and shaken for 15 min. After shaking, the alumina was washed three times with distilled water, transferred into a microfilter tube (Ultrafree C3, Millipore, Bedford, MA, USA), and centrifuged for drying (600 *g*, 5 min). The NE 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 standard solutions (NE 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 NE and DHBA were 64 ± 2% and 65 ± 3%, respectively.

2.4. Chromatographic and detection conditions

The HPLC system consisted of a pump with a pulse dumper (EP-300, Eicom, Kyoto, Japan), guard column (AC-ODS, 5 × 4 mm I.D., Eicom), analytic reversed-phase column (Eicompak CA-5ODS, 150 × 2.1 mm I.D., Eicom), electrochemical detector equipped with a graphite electrode (ECD-300, Eicom), chromato-integrator (D-2500, Hitachi), and degasser (DG-300, Eicom). The mobile phase consisted of 2700 ml of 0.1 *M* phosphate buffer (pH 6.1), 300 ml methanol, and 1-octanesulfonic acid sodium salt (600 mg/l final concentration). The flow-rate was 0.25 ml/min.

The electrochemical detector was operated at +400 mV vs. an Ag/AgCl reference electrode. The HPLC separation was performed at 25°C.

Norepinephrine and DHBA concentrations were determined by measuring the peak height under the chromatogram.

3. Results and discussion

A standard chromatogram showing resolution of 2 pg/50 μl NE and 4 pg DHBA under 18 min is presented in Fig. 1A. Linear regression analysis of the peak height versus concentration demonstrated linearity for both NE and DHBA in the 0 to 20 pg per 50 μl injection range. The r values for NE and DHBA were 0.999 and 0.999, respectively.

The lower limit of NE detection was 100 fg for dialysate sample per 50-μl injection at a signal-to-noise ratio of 3:1. The inter-day peak-height precision for 2 and 4 pg standards analyzed over six consecutive days was 555 ± 6 (mean ± S.D.) and 1030 ± 7 for NE and DHBA, respectively.

Fig. 1B illustrates a typical chromatogram obtained from a dialysate sample. Control dialysate NE levels averaged 16.6 ± 4.0 pg/ml (n = 10) in the midwall of the left ventricle (Fig. 2). Transection of the bilateral stellate ganglions significantly decreased the dialysate NE to 8.6 ± 4.7 pg/ml (n = 10). Local administration of desipramine significantly increased the dialysate NE concentration to 76.6 ± 7.6 pg/ml (n = 6).

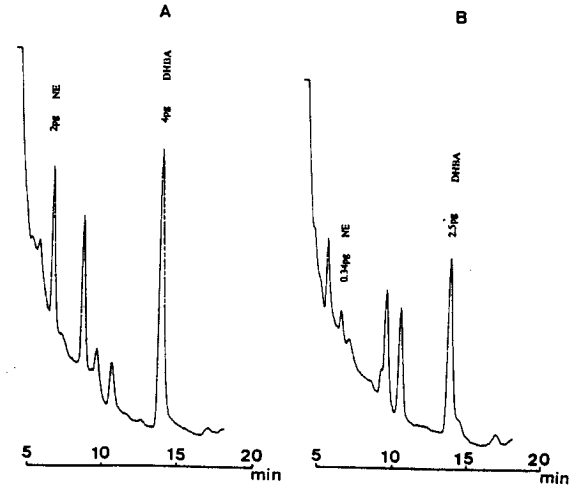

Fig. 1. (A) Chromatogram of norepinephrine and DHBA (internal standard). (B) Typical chromatogram of dialysate sample. NE = norepinephrine; DHBA = 3,4-dihydroxybenzylamine.

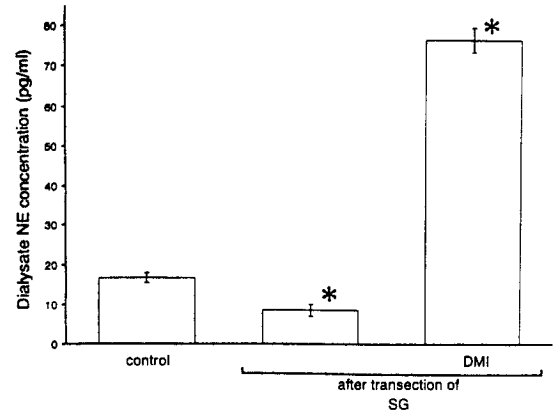

Fig. 2. Dialysate NE concentration before and after transection of stellate ganglions and during local administration of DMI. Transection of stellate ganglions decreased dialysate NE levels, and subsequent local administration of DMI increased the dialysate NE levels. NE = norepinephrine; DMI = desipramine; SG = stellate ganglions. * = $p < 0.05$ vs. control value.

As a direct result of the system's high sensitivity, 2-min sampling makes it possible to detect the low levels of dialysate NE after the transection of the stellate ganglions. In addition to improving the HPLC–ED, we also modified the alumina extraction [6] to achieve a smaller sampling volume. The NE was optimally eluted from the alumina (5 mg) using 60 μl of 2% acetic acid.

Control dialysate NE levels averaged 16.6 ± 4.0 pg/ml in the midwall of the left ventricle. A previous study indicated that dialysate NE reflected the concentration of myocardial interstitial NE which originated mainly from the cardiac sympathetic nerves [4]. The control value was similar to the values reported in our previous studies [4,5]. Cousineau et al. [7] have developed a different method to measure myocardial interstitial NE levels, a multiple tracer dilution bulk technique. Data from their studies cannot be compared directly to dialysate NE, as their technique involves a potentially significant infusion of NE.

In this system, an in vivo detection limit of 100 fg was achieved for NE. The low detection limit allowed the dialysate NE concentration to be monitored for the functioning of the cardiac sympathetic nerve terminal. This system offers a new possibility for routine analysis of myocardial interstitial NE levels.

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