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Short communication

# High-performance liquid chromatographic determination of myocardial interstitial dihydroxyphenylglycol

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**Abstract**

This study describes a high-performance liquid chromatographic method with electrochemical detection (HPLC–ED) for monitoring dihydroxyphenylglycol (DHPG) in the myocardial interstitial space. Using a cardiac dialysis technique, 10-μl dialysates were sampled from the myocardial interstitial space (1-min fractions) and were injected directly into the HPLC–ED system. The in vitro quantification limit for DHPG was 250 fg in a 10-μl injection. The basal DHPG concentration of dialysate was 181±46 pg/ml. This system offers a new possibility for monitoring myocardial interstitial DHPG levels.

*Keywords:* Dihydroxyphenylglycol

## 1. Introduction

Dihydroxyphenylglycol (DHPG) is a deaminated metabolite of norepinephrine (NE). Several studies have suggested that DHPG is a prominent intraneuronal metabolite of NE, and that the DHPG level serves as an index of the cytosolic (neuroplasm) NE level [1–5]. DHPG can easily pass through the cell membrane and diffuse into the myocardial interstitial space. Therefore, measurement of cardiac DHPG may be particularly appropriate for providing information about intraneuronal NE disposition at the cardiac sympathetic nerve terminals [3,4]. Up to now, cardiac DHPG output has been estimated only through the plasma level. Recently, due to the development of a dialysis technique [6], cardiac dialysis has made it possible to measure myocardial interstitial DHPG levels.

Previous studies described an HPLC–ED system for the routine measurement of the low levels of NE found in the myocardial interstitial space [7,8]. We have applied cardiac dialysis with this system to measure myocardial interstitial DHPG concentrations. The DHPG concentration of dialysate was measured as an index of myocardial interstitial DHPG concentration. Furthermore, local administration of neuropharmacological drugs through a dialysis probe offers a new approach to study sympathetic nerve terminal function [9]. This approach does not interfere with systemic hemody-

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namics or central modulation of neuronal regulation. The DHPG concentration of dialysate was measured before and after local perfusion of a monoamine oxidase (MAO) inhibitor. From this experiment, we investigated whether the cardiac dialysis method makes it possible to detect the low levels of DHPG found in the myocardial interstitial space.

## 2. Experimental

### 2.1. Reagents and chemicals

Distilled water and methanol were of HPLC grade from Wako Pure Chemical (Osaka, Japan). Standard DHPG was obtained from Sigma (St. Louis, MO, USA) and 1-octane-sulfonic acid sodium salt was from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical grade and were used without any further pretreatment.

A stock solution of DHPG was prepared at a concentration of 1 mg/l in 0.1 *M* perchloric acid. A working standard containing (per liter) 200 ng of DHPG was made in Ringer's solution. The stock solution was stable at 4°C for one month.

### 2.2. Dialysis probe and in vivo cardiac dialysis

For cardiac dialysis, we designed a transverse dialysis probe. The dialysis fiber (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 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, each weighing 2.1–4.5 kg, were anesthetized with pentobarbital sodium (30–40 mg/kg, i.p.). The level of anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (1–2 mg/kg·h). 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 left 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 speed of 10 μl/min using a microinjection pump (CMA 102, Carnergie Medicin, Stockholm, Sweden). The dialysate was sampled and the concentration of DHPG was measured in (1) the control state, (2) 30 min after beginning local perfusion of the MAO inhibitor, pargyline (1 m*M*, Sigma). One sampling period was 1 min (one dialysate sample volume=10 μl). Each sample was collected in a 300-μl microtube containing 1 μl of 0.1 *M* HCl, to prevent amine oxidation. A microtube contained 11 μl of sample. We measured the dead space between the dialysis fiber and the sample tube, taking into account this space at the start of each dialysate sampling.

### 2.3. Chromatographic and detection conditions

Using an autoinjector (CMA 200, Carnergie Medicin), 10 μl was directly injected into the liquid chromatograph. The HPLC system consisted of a pump with a pulse dampener (EP-300, Eicom, Kyoto, Japan), a guard column (CA-ODS, 5×4 mm I.D., Eicom), two analytic reversed-phase columns (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 980 ml of 0.1 *M* phosphate buffer (pH 5.1) containing 1-octanesulfonic acid sodium salt (27 mg/l final concentration) and 20 ml of methanol. The flow-rate was 0.2 ml/min. The electrochemical detector was operated at +550 mV versus an Ag–AgCl reference electrode. The HPLC separation was performed at 25°C.

The concentration of DHPG was determined by measuring the peak height and correcting for the volume of the added HCl.

## 3. Results and discussion

A standard chromatogram of 2 pg/10 μl DHPG eluted in less than 15 min is presented in Fig. 1A. The calibration curve for DHPG was linear in the concentration range of 0.4 to 10 (0.4, 2, 10) pg/ml ($y=407.1x+10.9$). The $r^2$ value for DHPG was greater than 0.99. To examine the peak height precision for 2 pg of DHPG, repeatability and

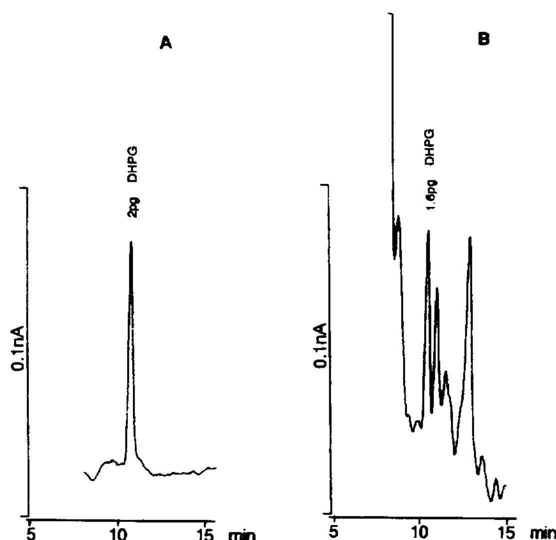


Fig. 1. (A) Chromatogram of a dihydroxyphenylglycol standard (2 pg/10 μl injection). (B) Typical chromatogram of an injected dialysate sample (10-μl injection). DHPG=dihydroxyphenylglycol.

reproducibility were calculated. The intra-day coefficient of variation (C.V.) was 1.4% ($n$=11) and the inter-day C.V. was 1.6% (six consecutive days). The limit of detection for DHPG was determined using two criteria; (1) a signal-to-noise ratio that was higher than three and (2) a C.V. that was lower than 10%. This limit was 100 fg per 10-μl injection. The limit of quantification was 250 fg per 10-μl injection, estimated at a signal-to-noise ratio of ten.

The dialysis probe was perfused with Ringer's solution at 10 μl/min in the DHPG solution (20 pg/ml), and the DHPG concentration of the dialysate was measured. The average recovery of the dialysis fiber was 16.7±1.6%, by calculating the ratio of dialysate–DHPG concentration of the solution.

A chromatogram of injected Ringer's solution had no peak corresponding in retention time to that of standard DHPG. A peak corresponding in retention time to standard DHPG was estimated as DHPG peak and dialysate DHPG level. Fig. 1B shows a typical chromatogram obtained for a dialysate sample. The DHPG concentration of control dialysate was 181±46 pg/ml ($n$=10) (Fig. 2). Local administration of pargyline (1 m$M$) significantly decreased the DHPG concentration of dialysate to 50±19 pg/ml ($n$=7).

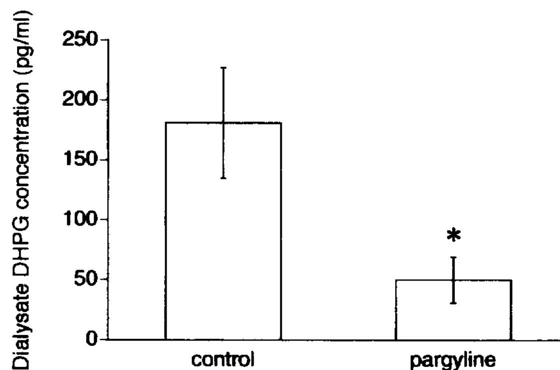


Fig. 2. DHPG concentration of dialysate before and after local administration of pargyline (mean±S.D.). Local administration of pargyline decreased the DHPG levels of dialysate. DHPG= dihydroxyphenylglycol. * $p$<0.05 vs. control value.

In this system, an in vitro detection limit of 100 fg, which was almost the same as that for NE in a previous report [7,8], was achieved for DHPG, and the DHPG concentration of dialysate was measured by direct injection into a HPLC system, without the alumina procedure, unlike NE measurements of dialysate [7,8]. With this highly sensitive HPLC system, ghost peaks were included in a chromatogram of dialysate sample. However, there were no endogenous interferences found in the DHPG region on injection of mobile phase or Ringer's solution. This system and cardiac dialysis made it possible to detect low levels of DHPG in myocardial dialysate and to monitor myocardial interstitial DHPG levels in 1-min fractions.

To our knowledge, this is the first report on the in vivo monitoring of myocardial interstitial DHPG levels. The control DHPG concentration of dialysate was 181±46 pg/ml. This value was nine times higher than that of the control dialysate's NE concentration, which we reported for the same preparation [7,8].

Myocardial interstitial DHPG levels are predominantly determined by production in the cardiac sympathetic nerve terminals and extraction from the blood circulation [3]. In this study, local administration of a MAO inhibitor, pargyline, caused a decrease in the DHPG levels of dialysate. Furthermore, regional production of DHPG by cat heart is exclusively dependent on intra-neuronal sources [3,10]. Therefore, we consider that the measurement of

myocardial interstitial DHPG levels provides information about intraneuronal disposition in cardiac sympathetic nerve terminals. This system offers a new possibility for monitoring the levels of myocardial interstitial DHPG.

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