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Short communication

Detection of 3-methoxy-4-hydroxyphenylglycol in rabbit skeletal muscle microdialysate

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Abstract

A high-performance liquid chromatography with electrochemical detection (HPLC–ED) method is described for determination of 3-methoxy-4-hydroxyphenylglycol (MHPG) in microdialysate from the skeletal muscle interstitial space. Using a microdialysis technique, we sampled 30 μl dialysate from the skeletal muscle interstitial space and injected dialysate directly into HPLC–ED system. The control MHPG concentration of dialysate was 213 ± 18 pg/ml. The MHPG concentrations were reduced by entacapone (catechol-*O*-methyltransferase inhibitor, COMT), augmented by local infusion of dihydroxyphenylglycol. This system offers a new possibility for simple, rapid monitoring of MHPG as an index of COMT activity in skeletal muscle.

Keywords: 3-Methoxy-4-hydroxyphenylglycol

1. Introduction

3-Methoxy-4-hydroxyphenylglycol (MHPG) is a major metabolite of norepinephrine (NE) [1]. MHPG is produced by extra-neuronal *O*-methylation of 3,4-dihydroxyphenylglycol (DHPG) formed intraneuronally from NE or by the extra-neuronal combination of catechol-*O*-methyltransferase (COMT) and monoamine oxidase on NE. Microdialysis technique with high-performance liquid chromatography (HPLC) has been applied to monitor interstitial MHPG levels in the brain [2]. It is important for understanding the metabolic inactivation of NE to monitor MHPG production. Recently, it has been suggested that MHPG is a major extra-neuronal metabolite of NE in peripheral other organs including skeletal muscle [3]. Actually, the COMT activity is found in not only brain but also peripheral organs and tissues [4]. However, there has been no report monitoring peripheral MHPG levels by microdialysis technique with HPLC.

We previously reported that microdialysis technique with HPLC made it possible to directly measure the low levels of another important NE metabolite, DHPG in the cardiac myocardial interstitial space [5]. In the present study, we extend microdialysis technique with HPLC to the measurement of MHPG in skeletal muscle. We directly injected dialysate sample obtained from skeletal muscle to HPLC and analyzed dialysate MHPG concentration without internal standard or extraction procedure.

2. Experimental

2.1. Reagents

Distilled water and methanol were of HPLC grade from Wako Pure Chemical (Osaka, Japan). Standard solution of MHPG was obtained from Sigma (St. Lois, MO, USA) and 1-octane-sulfonic acid sodium salt was Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical grade and were used without any further pretreatment. A stock solution of MHPG was prepared separately at a concentration of 1 mg/l in 0.1 M perchloric acid. A working standard mixture containing (per liter) 100 ng of MHPG was made in Ringer's solution. Stock solutions were stable at 4 °C for 1 month.

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2.2. Dialysis probe and in vivo skeletal muscle 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 mm length, 0.5 mm o.d., and 0.2 mm i.d.) [6]. Eighteen male Japanese white rabbits weighing 2.5–2.8 kg each were anesthetized with pentobarbital sodium (30–35 mg/kg, i.v.). 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 heating pad. All protocols were performed in accordance with the *National Cardiovascular Center Research Institute Animal Care Ethics Committee Guidelines*. Heart rate and arterial blood pressure were simultaneously monitored with a data recorder. After longitudinal skin incision of left groin, the dialysis probe was implanted in the left adductor muscle along with long axis. The dialysis probe was perfused with Ringer's solution at 10 μl/min using a microinjection pump (CMA 102, Carnegie Medicin, Stockholm, Sweden). We started the dialysate sampling followed by a stabilization period of 2 h. The concentrations of MHPG were measured at (1) the control state, (2) 60 min after intraperiotoneal injection of entacapone (COMT) inhibitor (10 mg/kg), and (3) 60 min after infusion of DHPG (25 ng/ml) through the dialysis probe. One sample period was 3 min (one dialysate sample volume = 30 μl). Each sample was collected in a 300 μl microtube containing 3 μl of 0.1N HCl to prevent amine oxidation.

2.3. Chromatographic and detection conditions

Using an autoinjector (CMA 200, Carnegie Medicin), 30 μl was injected into the liquid chromatograph. 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 mm × 2.1 mm i.d., Eicom), an electrochemical detector equipped with a graphite electrode (ECD-300, Eicom), a chromato-integrator (D-2500, Hitachi, Tokyo, Japan) and a degasser (DG-300, Eicom). The mobile phase consisted of 1-octane-sulfonic acid sodium salt (200 mg/l in final concentration) in 0.1 M phosphate buffer (pH 6.0) and methanol (97:3, v/v). The flow-rate was 0.23 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 MHPG was determined by measuring the peak height and corrected from the volume of the added HCl.

3. Results and discussion

When mobile phase was injected into HPLC, there was no peak corresponding in retention time to that of standard MHPG in chromatogram. A chromatogram of MHPG standard solution (60 pg/30 μl) is shown in Fig. 1. The calibration curve for MHPG peak height was linear in the concentration range of 0.9–60 (0.9, 3, 9, 30, and 60) pg per 30 μl injection. The r^2 value for MHPG was 0.9997. We could not prepare MHPG-free dialysate in the present study. Therefore, the accuracy and precision were studied with MHPG-free perfusate. Mean concentrations and coefficient variation (C.V.) with MHPG-free perfusate are presented in Table 1. In intra-day measurement, mean concentration ranged from 86 to 107% of theory and C.V. from 1 to 6% over 0.9 to 60 pg per injection of MHPG base concentration range. In inter-day measurement, mean concentration ranged form 93 to 105% of theory and C.V. from 3 to 10% over 0.9 to 60 pg per injection of MHPG base concentration.

When perfusate solution was injected into HPLC, there was no peak corresponding in retention time to that of standard MHPG in chromatogram. A peak corresponding in retention time to standard MHPG was estimated as MHPG peak. Fig. 1 shows typical chromatograms obtained from dialysate samples. MHPG peak height of dialysate was reduced by entacapone, and augmented by DHPG infusion. Furthermore, this DHPG-induced increment in MPHG peak height was canceled with the pretreatment of entacapone. The MHPG concentration of control dialysate was 213 ± 18 pg/ml ($n = 6$) (Fig. 2). Administration of entacapone significantly decreased the MHPG concentration of dialysate to 133 ± 16 pg/ml ($n = 5$). Local administration of DHPG increased the MHPG concentration of dialysate to 697 ± 265 pg/ml ($n = 5$). Thus, dialysate MHPG concentration reflects MHPG production and COMT activity at the skeletal muscle.

Using dialysate samples, we carried out preliminary validation study. We validated the accuracy of the method by spiking skeletal muscle dialysate with known amount of MHPG and calculate recovery. Dialysate samples were obtained from a rabbit, and we spiked the dialysate samples with known amount of MHPG (0.9, 3.0, and 6.0 pg). Peak corresponding in retention time to standard MHPG were linear and r^2 value was 0.982. The basal dialysate samples were spiked with MHPG in LOQ level (0.9 pg). Their MHPG levels with and without spiked MHPG (0.9 pg) were 5.6 ± 0.1, 6.5 ± 0.2 pg per injection ($n = 4$). Inter-day precision data were calculated from dialysate samples in two rabbits. Their C.V. values were 3.8 and 3.5%, respectively. Furthermore, the potential for electrochemical detector was re-set at 500 mV, dialysate peak corresponding in retention time to standard MHPG and standard MHPG peak were reduced in parallel. There are several peaks eluting between 4.5 and 5.5 min in dialysate samples. Changes in the concentration of anion-paring agent did not alter the retention

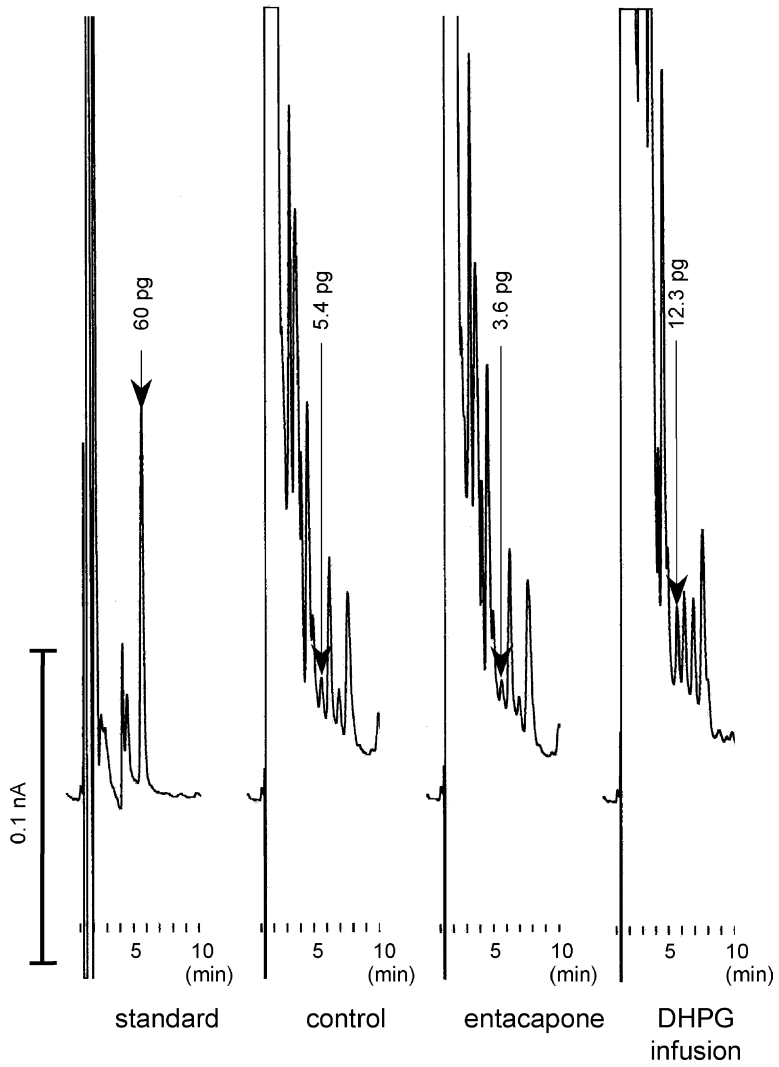

Fig. 1. Chromatograms of 3-methoxy-4-hydroxyphenylglycol (MHPG). The injection volume was 30 μl standard, 60 pg MHPG; control, skeletal muscle dialysate; entacapone, skeletal muscle dialysate after administration of entacapone; dihydroxyphenylglycol (DHPG) infusion, skeletal muscle dialysate after local infusion of DHPG through the dialysis probe.

time of MHPG but altered the retention times of interfering cross-peaks. Therefore, we carefully chose the concentration of anion-paring agent to avoid interfering cross peaks. Up to now, we have measured >200 dialysate samples and can say unequivocally that these peaks do not interfere the measurement of MHPG peak.

Thus, our method detects as little as 900 fg of MHPG per injection. Several studies on measurement of MHPG in plasma or urine were published [7–10]. Dialysate MHPG levels in the skeletal muscle range below their detection limit because their employed methods need complicated procedures, which attenuate their sensitivity. In this system,

Table 1
Intra- and inter-day accuracy and precision

Nominal/added concentration (pg per injection)	Intra-day			Inter-day		
	No. of values	Accuracy	Precision C.V. (%)	No. of values	Accuracy	Precision C.V. (%)
0.9	5	86	6	5	93	10
3	5	99	2	5	105	6
6	5	107	2	5	102	4
30	5	99	1	5	100	3
60	5	103	2	5	96	5

Accuracy: mean found concentration (% of nominal). C.V.: coefficient variation (CV) of the mean concentration. Standard MHPG was dissolved in Ringer's solution.

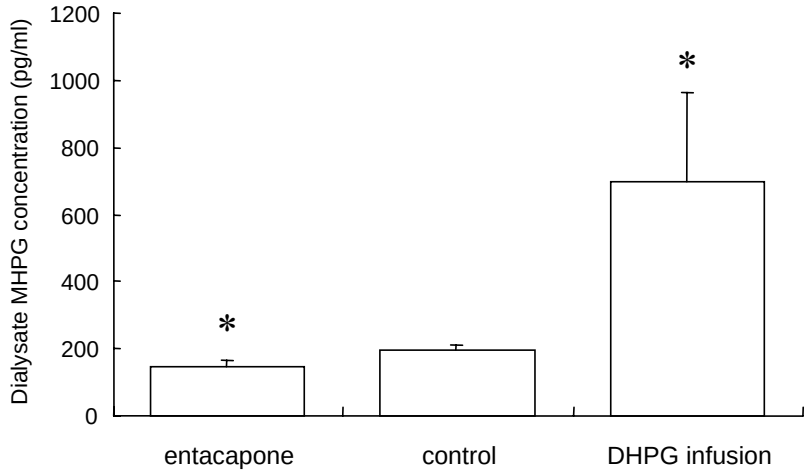

Fig. 2. Effects of entacapone (COMT inhibitor) or dihydroxyphenylglycol (DHPG) on dialysate 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations. Intraperitoneal administration of entacapone decreased dialysate MHPG concentrations and DHPG infusion through dialysis probe increased dialysate MHPG concentrations. Values are means ± S.E. $^{*}P < 0.05$ vs. value of control.

quantification limit of MHPG was comparable to those of NE and DHPG. For each measurement of NE, DHPG and MHPG, we employed the same HPLC–ED apparatus but separate own system. Chromatographic condition in mobile phase component and flow rate, were chosen for highly sensitive measurement of MHPG. Further, sample from plasma or urine need extraction procedure for eliminating many possible interferences [7]. Extraction procedure restricts highly sensitive measurement. When dialysate from the skeletal muscle was injected to HPLC–ED, the low concentration of MHPG was detectable and reproducible without extraction or internal standard.

To our knowledge, this is the first report on the in vivo measurement of MHPG by direct injection of dialysate obtained from skeletal muscle to HPLC–ED system without internal standard or complex extraction procedure. Early study suggested that majority of MHPG in plasma was derived from skeletal muscle in human study [3]. Therefore, measurement of MHPG in skeletal muscle may be particularly appropriate for providing information about the mechanism of peripheral MHPG production. In this study, administration of COMT inhibitor significantly decreased the MHPG concentrations of dialysate and local administration of DHPG significantly increased the MHPG concentrations. Therefore, we consider that the concentration of MHPG in the skeletal muscle dialysate might correspond to the COMT activity in the skeletal muscle. Taken together with measurement of NE and DHPG, these analyses yield new approach to norepinephrine kinetics in the skeletal muscle. This system offers a simple, rapid monitoring of skeletal muscle MHPG concentrations as an index of in vivo COMT activity.

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