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Short communication

In vivo microdialysis determination of collagen-induced serotonin release in rat blood

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Abstract

We developed a sensitive microbore HPLC method coupled with an on-line microdialysis system to simultaneously measure endogenous 5-hydroxytryptamine (serotonin; 5-HT) and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the rat blood in vivo. A dialysis tube was placed in the right jugular vein. The validity of the procedure is demonstrated because analysis of the aggregating agents, collagen (1 mg/kg) plus epinephrine (0.3 mg/kg) after intravenous injection, showed that they induced an increase in 5-HT and 5-HIAA levels in the jugular vein of the rat.

Keywords: Microdialysis; Serotonin; 5-Hydroxyindoleacetic acid; Collagen; Epinephrine

1. Introduction

In plasma, 5-hydroxytryptamine (serotonin; 5-HT) is taken up avidly by the platelets and stored in dense granules [1]. Whenever platelet aggregation is initiated, the dense granules release their stored 5-HT into the blood [2]. Once released, the first targets for 5-HT are probably the platelets themselves [3]. In order to measure blood levels of 5-HT and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), we developed a rapid, simple and sensitive method consisting of both automatic on-line microdialysis and a sensitive microbore high-performance liquid chromatography–electrochemical detection (HPLC–ED) system. In the present study, we evaluated our system by implanting a microdialysis probe in the jugular vein of the rat. The rat was then injected intravenously with aggregation agent, collagen (1 mg/kg) plus epinephrine (0.3 mg/kg).

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2. Experimental

2.1. Materials

Epinephrine, 5-HT, 5-HIAA, and collagen (type I, bovine achilles tendon), were purchased from Sigma (St. Louis, MO, USA). HPLC reagents and buffer reagents were obtained from Merck (Darmstadt, Germany). Triple-deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2. In vivo microdialysis experiments

Experiments were carried out in adult, male Sprague–Dawley rats (250–320 g) that were initially anesthetized with chloral hydrate (0.4 g/kg, i.p.). Routine surgical preparations included cannulation of both right and left femoral veins for infusion of chloral hydrate (40 mg/kg/h). A microdialysis probe (CMA 20), dialysing length 10 mm; I.D., 0.5 mm; (CMA/Microdialysis AB, Stockholm, Sweden) was cannulated to the right jugular vein and then perfused

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with ACD solution (citric acid 3.5 m*M*; sodium citrate 7.5 m*M*; dextrose 13.6 m*M*) at a flow-rate of 0.5 μl/min, by a microdialysis pump (CMA 100). The outflow of the dialysis sample was connected to an on-line injector (CMA 160). The injection volume was measured with a 10-μl sample loop. A sample was injected every 20 min and controlled by the microinjection pump. After dialysate levels had stabilized (approximately 2 h), two basal samples were collected and then the aggregation agent, collagen (1 mg/kg) plus epinephrine (0.3 mg/kg; Sigma) was intravenously administered.

2.3. Apparatus and chromatography

The HPLC–ED system consisted of a syringe pump (ISCO, Lincoln, NE, USA) at a flow-rate of 0.055 ml/min for 5-HT and 5-HIAA analysis. The dialysate was separated by a reversed-phase microbore column (100×1 mm I.D., particle size 5 μm, BAS; Bioanalytical System, West Lafayette, IN, USA). The mobile phase consisted of 80 ml acetonitrile, 2.2 m*M* sodium 1-octanesulfonate, 14.7 m*M* monosodium dihydrogenorthophosphate, 30 m*M* sodium citrate, 0.027 m*M* EDTA, and 1 ml diethylamine in 1 liter of double-distilled water. The solution was adjusted to pH 3.5 by orthophosphoric acid (85%). The mixture was filtered with a 0.22 μm Millipore membrane. 5-HT and 5-HIAA were detected by ED (BAS-4C) coupled to a glassy carbon working electrode (cell volume: 1.2 μl) and referenced to a Ag/AgCl electrode at +0.6 V. Output of peak area from ED was amplified and recorded by Waters Millennium 2010 software [4]. Data are expressed as mean±S.E.M. of the percentage increase over basal concentration. Basal condition values were calculated as the mean of two sequential samples.

3. Results and discussion

The method described in this paper provides an excellent separation of 5-HT and 5-HIAA. Peaks of 5-HT and 5-HIAA were well-resolved and their retention times were identical to those in Fig. 1. The

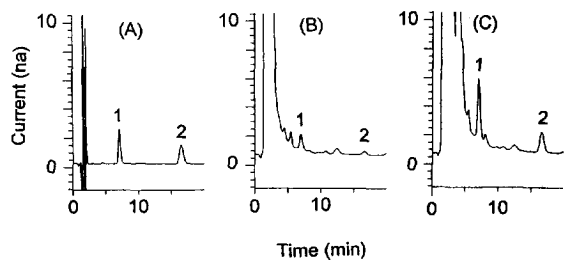

Fig. 1. (A) Typical chromatograms of 10 ng/ml standard compounds of 5-HT and 5-HIAA; (B) a basal condition of microdialysis sample from the jugular vein; (C) a microdialysis sample collected 20 min after intravenous injection of collagen (1 mg/kg) plus epinephrine (0.3 mg/kg). 1=5-HIAA; 2=5-HT; na=nanoampere.

in vitro recovery of 5-HT and 5-HIAA of the microdialysis probe based on the 100 ng/ml standard were 89% and 68%, respectively (n=4). Peak detection limits of 5-HT and 5-HIAA were 0.1 ng/ml at a signal-to-noise ratio of 3. This high recovery might be due to the slow infusion rate (0.5 μl/min), long active dialysing length (10 mm) and diameter (0.5 mm) of the microdialysis probe.

The calibration curves were linear for 5-HT and 5-HIAA (range 1–200 ng/ml). The linear regressions of 5-HT and 5-HIAA are $Y=4.52\cdot10^{-6}X-0.17$ (r=0.999) and $Y=3.43\cdot10^{-6}X+0.057$ (r=0.999), respectively, where Y is the concentration in ng/ml and X is the response in peak area. Intra- and inter-assay precision and accuracy were determined at three concentrations of 5-HT and 5-HIAA. The results are presented in Table 1.

The dialysate samples collected over the first 60 min were discarded to allow recovery from the acute effects of the surgical procedure. Control blood dialysate concentrations of 5-HT and 5-HIAA were 3.87±0.99 and 17.28±6.56 ng/ml, respectively. After intravenous administration of collagen (1 mg/kg) plus epinephrine (0.3 mg/kg), the extracellular concentrations of 5-HT and 5-HIAA were increased to 228% of basal conditions at 20 min (Fig. 2) These results are in agreement with previous in vivo [5] and in vitro [1] studies with respect to 5-HT release. Therefore, this method may provide for further platelet aggregation or 5-HT related study.

Table 1
Intra- and inter-assay precision and accuracy for 5-HT and 5-HIAA determination

	Nominal concentration (ng/ml)					
	5-HT			5-HIAA		
	2	5	20	2	5	20
Intra-assay						
Mean (n=4)	1.98	4.94	19.93	2.06	4.93	20.04
S.D.	0.11	0.18	0.21	0.06	0.08	0.05
% C.V.	5.55	3.64	1.05	2.91	1.62	0.25
Accuracy(%)	−1.0	−1.2	−0.35	3.0	−1.4	0.2
Inter-assay						
Mean (n=4)	2.08	5.03	19.81	1.94	5.13	19.92
S.D.	0.13	0.17	0.34	0.14	0.24	0.05
% C.V.	6.25	3.38	1.72	7.22	4.68	0.25
Accuracy(%)	4.0	6.0	−0.95	−3.0	2.6	−0.4

$$\text{Precision (\% C.V.)} = \frac{\text{Standard deviation (S.D.)}}{\text{Mean concentration}} \times 100$$

$$\text{Accuracy (\%)} = \frac{\text{Mean conc.} - \text{Actual conc.}}{\text{Actual conc.}} \times 100$$

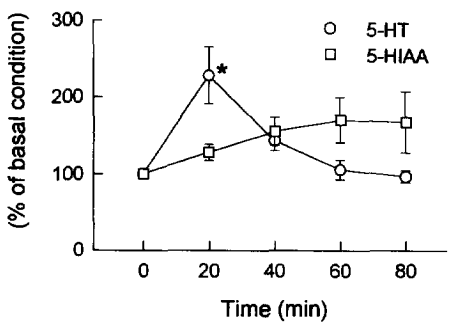

Fig. 2. The effect of intravenous administration of collagen (1 mg/kg) plus epinephrine (0.3 mg/kg) on the microdialysis output of 5-HT and 5-HIAA. Drugs were administered at time 0. Values represent the group mean±S.E.M. (n=5). The basal condition of dialysate concentrations were based on an average of the two samples prior to the injection of the drugs. *=Significantly different (Student's t test) from control (time=0), $p<0.05$.

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