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Myocardial interstitial serotonin and its major metabolite, 5-hydroxyindole acetic acid levels determined by microdialysis technique in rat heart

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ABSTRACT

Aims: The aim of this study was to elucidate myocardial interstitial serotonin (5-HT) kinetics in the heart, including 5-HT reuptake and enzymatic degradation to 5-hydroxyindole acetic acid (5-HIAA) via monoamine oxidase (MAO).
Main methods: Using microdialysis technique in anesthetized rats, we simultaneously monitored myocardial interstitial levels of 5-HT and its major metabolite, 5-HIAA, in the left ventricle and examined the effects of local administration of a MAO inhibitor, pargyline, or a 5-HT uptake inhibitor, fluoxetine.
Key findings: Pargyline increased dialysate 5-HT concentration from 1.8 ± 0.3 at baseline to 3.9 ± 0.5 nM but decreased dialysate 5-HIAA concentration from 20.7 ± 1.0 at baseline to 15.8 ± 1.4 nM at 60–80 min of administration. Fluoxetine increased dialysate 5-HT concentration from 1.9 ± 0.4 at baseline to 6.5 ± 0.9 nM at 60–80 min of administration, but did not change dialysate 5-HIAA concentration. Local administration of ADP (100 mM) increased dialysate 5-HT and 5-HIAA concentrations. Pargyline did not affect ADP-induced increase in dialysate 5-HT concentration but suppressed ADP-induced increase in dialysate 5-HIAA concentration during 60 min of ADP administration. Fluoxetine increased dialysate 5-HT concentration at 40–60 min of ADP administration, but did not affect ADP-induced increase in dialysate 5-HIAA concentration.
Significance: Simultaneous monitoring of myocardial interstitial 5-HT and 5-HIAA levels provides valuable information on 5-HT kinetics including reuptake and enzymatic degradation by MAO, which play a role in the regulation of myocardial interstitial 5-HT levels at baseline and when 5-HT levels are elevated.

Introduction

Various types of 5-hydroxytryptamine (5-HT; serotonin) receptors are expressed in the heart. Serotonin has important physiological effects on cardiovascular regulation, and these effects are receptor-dependent (Villalón and Centurión, 2007; Ramage and Villalón, 2008). Serotonin activates afferent cardiac vagal nerves via 5-HT_3 receptors and inhibits the release of norepinephrine from cardiac sympathetic nerve terminals via 5-$HT_{1B/1D}$ receptors. Serotonin induces vasoconstriction via 5-HT_{1B} and 5-HT_{2A} receptors located on the membrane of smooth muscle cells and also releases nitric oxide from endothelial cells via 5-HT_{2B} receptors. Moreover, 5-HT regulates cardiac structure and function via 5-HT_{2B} receptors (Nebigil et al., 2001). Serotonin accumulates in myocardial interstitium during ischemia–reperfusion and contributes to the progression of cardiomyocyte injury. Serotonin activates platelets via 5-HT_{2A} receptors leading to coronary thrombosis (Willerson et al., 1989), activates afferent cardiac sympathetic nerves via 5-HT_3 receptors (Longhurst et al., 2001; Fu and Longhurst, 2002), and induces coronary vasoconstriction (Golino et al., 1989; Métais et al., 2001). Meanwhile, 5-HT transporter (SERT) (Mekontso-Dessap et al., 2006; Ni and Watts, 2006) and monoamine oxidase (MAO) (Lowe et al., 1975; Kaludercic et al., 2011) have been identified in the heart.

The putative cardiac 5-HT pathways are illustrated in Fig. 1. Free 5-HT in circulation and 5-HT contained in platelets have been considered to be important sources of 5-HT in cardiovascular tissue (Watts et al., 2012; Ramage and Villalón, 2008), although 5-HT has been identified in cardiac tissues including sympathetic nerves (Cohen, 1985), mast cells (Parikh and Singh, 1997), and myocytes (Pönicke et al., 2012). Serotonin in myocardial interstitium is partly washed out into blood stream and partly taken up into cardiac tissues by SERT. Intracellular 5-HT, including the 5-HT taken up from the interstitium, is subjected to deamination by MAO and subsequently degraded to 5-hydroxyindole acetic acid (5-HIAA), which can move across cell membrane by diffusion. Thus 5-HT reuptake and degradation may play an important role in the regulation of myocardial interstitial 5-HT level. Moreover, myocardial interstitial 5-HIAA level is a potential index of 5-HT degradation via MAO. However, there is a paucity of reports investigating cardiac 5-HT kinetics including 5-HT reuptake and its degradation to 5-HIAA.

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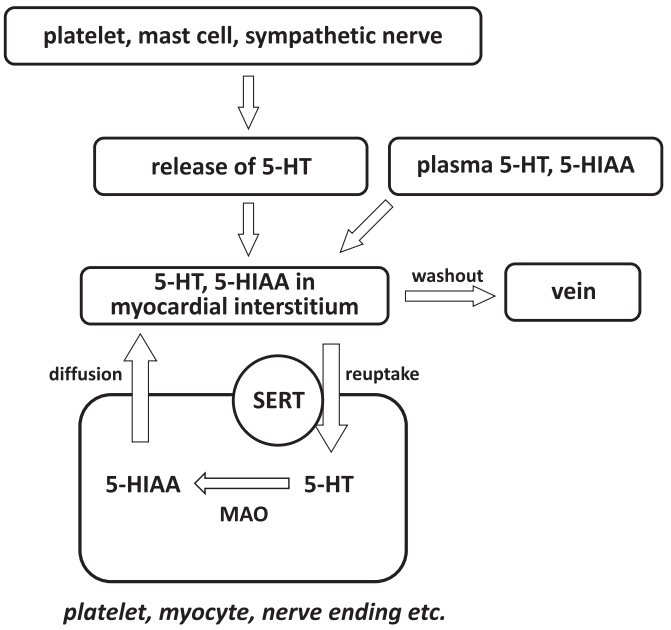

Fig. 1. Schematic illustration of putative cardiac 5-HT pathways. 5-HT; serotonin, 5-HIAA; 5-hydroxyindole acetic acid, SERT; serotonin transporter, MAO; monoamine oxidase.

In the present study, we applied microdialysis technique to the heart of anesthetized rats to investigate myocardial interstitial 5-HT kinetics. We simultaneously monitored dialysate 5-HT and 5-HIAA concentrations and pharmacologically evaluated myocardial interstitial 5-HT kinetics including 5-HT reuptake and degradation to 5-HIAA at baseline and when 5-HT is released in myocardial tissues.

Materials and methods

Animal preparation

Animal care was provided in accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, which is approved by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Twenty-one male Wistar rats aged 19 to 24 weeks and weighing 390 to 450 g were used in the present study. A rat was anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) and butorphanol tartrate (0.4 mg/kg), followed by continuous intravenous infusion of pentobarbital sodium (30 mg/kg/h) and butorphanol tartrate (0.2 mg/kg/h) via a catheter inserted into the right cervical vein to maintain an appropriate level of anesthesia. Heparin sodium (5 IU/kg/h) was infused intravenously as an anticoagulant. Then the rat was intubated and ventilated with room air mixed with oxygen. Tidal volume was set at 5 ml/kg and respiratory rate to 85 breaths/min. Systemic arterial pressure and heart rate were monitored by a catheter inserted into the right cervical artery. When necessary, arterial blood (100 μl) was sampled from a catheter inserted into the artery. Body temperature was maintained at 37.5–38 °C with a heating pad and a lamp, and monitored with a rectal thermistor.

With the animal in a lateral position, the 3rd to 5th ribs on the left side were partially resected to expose the heart for implantation of the dialysis probe (see "Dialysis technique" below). At the end of the experiment, the rat was euthanized with an overdose of pentobarbital sodium (>150 mg/kg), and a postmortem examination was conducted to confirm that the dialysis probe did not penetrate into the ventricular cavity.

Microdialysis technique

The materials and properties of the dialysis probe have been described previously (Sonobe et al., 2013, 2014; Kawada et al., 2012, 2013). Briefly, each end of a dialysis fiber (0.31 mm O.D., 0.2 mm I.D., 50,000 molecular weight cut off; PAN-1200, Asahi Chemical, Japan) was inserted into a polyethylene tube (25 cm length, 0.5 mm O.D., 0.2 mm I.D.) and glued. The length of the exposed dialysis fiber was 5 mm. When a dialysis probe was bathed in Ringer solution containing 5-HT (20 ng/ml) and 5-HIAA (20 ng/ml) at 38 °C and perfused with Ringers solution at a speed of 1 μl/min, the *in vitro* recovery rate was 21.9% ± 1.1% for 5-HT and 21.6% ± 1.0% for 5-HIAA (number of dialysis probes = 3). Given the recovery rates of around 20%, the dialysate concentrations of 5-HT and 5-HIAA were thus lower than the corresponding myocardial interstitial concentrations. When comparing with plasma concentrations, the interstitial concentrations were estimated from dialysate concentrations using these *in vitro* recovery rates (Fig. 2).

Using a fine guiding needle, two dialysis probes were implanted transversely into the left ventricular wall. The two dialysis probes were separated by a distance of at least 5 mm to avoid the influence of local infusion of pharmacological agents. Each dialysis probe was perfused at a rate of 1 μl/min with Ringer solution or Ringer solution containing pharmacological agent, using a microinjection pump (CMA/102, Carnegie Medicin, Sweden). Ringer solution consisted of 147.0 mM NaCl, 4.0 mM KCl, and 2.25 mM $CaCl_2$. Dialysate sampling was started from two hours after probe implantation. One sampling period was set at 20 min, which yielded a sample volume of 20 μl. For a given collection period, the actual dialysate sampling started after a lag of 10 min taking into account the dead space between the dialysis membrane and the sample tube.

Analytic procedure

5-HT and 5-HIAA assays were simultaneously conducted using high-performance liquid chromatography with electrochemical detection. A dialysate sample from the heart (10 μl) was injected directly into the liquid chromatograph using an autosampling injector (M-504; Eicom, Kyoto, Japan). For plasma level measurement, arterial blood was collected into a tube containing EDTA-2Na to prevent platelet aggregation. The plasma was transferred to a centrifugal filter device (Amicon Ultra-0.5, 10,000 MW cutoff, Millipore) and centrifuged at 10,000*g* for 20 min at 4 °C. The ultrafiltrate (10 μl) was injected into the liquid chromatograph using an autosampling injector.

The liquid chromatography system consisted of a pump with degasser (EP-700; Eicom), separation column (Eicompak SC-5ODS, ODS, 5 μm particle size, 3.0 mm ID × 150 mm length; Eicom), and electrochemical detector (ECD-700; Eicom). The temperature of the separation was maintained at 25 °C by a column oven (ATC-700; Eicom). The electrochemical detector was operated with a graphite electrode (WE-3G; Eicom) at +0.45 V vs. an Ag/AgCl reference electrode. Mobile phase consisted of 4% (vol/vol) methanol and 96% (vol/vol) 100 mM phosphate buffer adjusted to pH 5.40. The pump flow rate was 0.50 ml/min. Chromatograms were recorded and analyzed by an A–D converter (Power Chrom EPC-500; Eicom) with a computer. Concentrations of 5-HT and 5-HIAA were determined by measuring the peak areas. The absolute detection limits of 5-HT and 5-HIAA were 1.6 and 3.7 fmol/injection, respectively (signal-to-noise ratio = 3).

Experimental protocols

Protocol 1: Effects of pargyline and fluoxetine on basal 5-HT and 5-HIAA levels (n = 7)

To examine the myocardial interstitial 5-HT kinetics under basal conditions, we investigated the effects of a MAO inhibitor, pargyline, and a 5-HT reuptake inhibitor, fluoxetine. Baseline dialysate was