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A Method of *In Vivo* Simultaneous Measurements of Dopamine, Serotonin and Their Metabolites Using Intracerebral Microdialysis System

Kanji YOSHIMOTO[1], Setsuo KOMURA[1] and Tomio FUJITA[2]

1) Department of Legal Medicine, Kyoto Prefectural University of Medicine, Kawaramachi, Kamigyo-ku, Kyoto 602, Japan
2) Kyoto Analytical Application Laboratory, Shimadzu Co., Ltd., Nakagyo-ku, Kyoto 604, Japan

ABSTRACT

The present study demonstrated that it is possible to measure the endogenous extracellular dopamine (DA) and its metabolite dihydroxyphenylacetic acid (DOPAC), endogenous extracellular 5-hydroxytryptamine (5HT), its metabolites 5-hydroxyindoleacetic acid (5HIAA), 5-hydroxytryptophol (5HTOL) and its precursor 5-hydroxytryptophan (5HTP) simultaneously from the striatum of freely moving rats using intracerebral dialysis combined with high performance liquid chromatography (HPLC) with electrochemical detector (ECD).

Basal extracellular levels of DA, DOPAC, 5HTP, 5HT, 5HIAA and 5HTOL in the striatum were estimated to be 18.89 ± 2.23 (pg/25 μl/60 min), 7.25 ± 0.32 (ng/25 μl/60 min), 13.35 ± 3.01 (pg/25 μl/60 min), 5.32 ± 0.92 (pg/25 μl/60 min), 2.41 ± 0.08 (ng/25 μl/60 min) and 7.69 ± 0.45 (pg/25 μl/60 min), respectively. Pargyline (75 mg/kg i.p.) and tryptophan (100 mg/kg i.p.) increased the 5HT level in the striatum perfusates by 3-fold, whereas DOPAC and 5HIAA levels decreased by approximately 20% and 8%, respectively. In addition, DA and 5HTOL levels were not changed.

This method is suitable for monitoring dopaminergic and serotonergic activities dynamically.

Key words: *Brain dialysis, Dopamine, Serotonin*

Norepinephrine, dopamine (DA) and serotonin (5HT) are the major neurotransmitters in the CNS. In our laboratory we are interestied in the relationship between voluntary alcohol consumption and brain monosmine levels. In mice, a clear negative relationship was found between alcohol intake (g/kg/day) and brain 5HT level. We showed that in mice voluntary alcohol preference is influenced by brain 5HT levels genetically[7, 8]. However there are some problems. It is often difficult to relate the changes of the brain monoamine levels directly to behaviors, and it is therefore important to know the effects of voluntary alcohol consumption on the CNS. Many biochemical investigations of the brain have usually carried out by analyzing the brain tissue content of various chemicals[2]. Whoel tissue measurements represent a mixture of the intracellular as well as extracellular content.

Intracerebral dialysis is a new technique for investigating the behavior and the *in vivo* release of neurotransmitters such as DA[9,10], acetylcholine[1] and amino acids[4] in freely moving rats. Microdialysis is used to quantitate extracellular neurotransmitters and their metabolites and to correlate changes in their concentrations with behavior. However, there has been no report related the *in vivo* release of 5HT in freely moving rats. Also, *in vivo* voltammetry is a novel alternative approach for detection of endogenous brain monoamines *in vivo*[3, 5]. This *in vivo* voltammetry technique does not allow the distinction between parent monoamines and their metabolites; moreover, it is often difficult to identify the detected chemical materials.

In the present study, we have applied the microdialysis probe with HPLC and electrochemical detector (ECD) to estimate endogenous extracellular levels of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptophan (5HTP), 5-hydroxytryptamine (5HT), 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophol (5HTOL) simultaneously in rat brain regions *in vivo*.

MATERIALS AND METHODS

A microdialyis probe (CMA/10, the length of the membrane; 3 mm, Carnegie Medicin, Sweden) was used in this experiment. Male Wistar rats (245—280 g, SLC, Japan) were anestheized with chloral hydrate (400 mg/kg, i. p.) and placed in a stereotaxic frame (Takahashi Instruments, Japan). The skull was exposed and a small hole drilled to allow implantation of the microdialysis probe into the caudate nucleul (A, +1.6; L, +3.5; V, −8.0 mm: relative to the bregma)[6]. After the rat was allowed 36—48 hrs for surgical recovery, the microdi-

alysis probe was perfused with Ringer's solution (147 mM Na^+, 4.5 mM Ca^{2+}, 4 mM K^+ and 155.5 mM Cl^-, pH 6.5) at a rate of 1 μl/min using polyethylene tubing and connected to a microfusion system (Medical Agent, Kyoto, Japan). The perfusate was collected in a small polyethylene tube containing 5 μl 1 N HCl and 0.1 mM EDTA-2Na. Samples were collected at 60 min intervals, and immediately 25 μl was injected into the HPLC-ECD.

The perfusates were assayed for DA, DOPAC, 5HTP, 5HT, 5HIAA and 5HTOL simultaneously by HPLC (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan). These compounds were separated by a reversephase column (Shim-pack CLC-ODS, 150 × 6.0 mm I.D., 5 μm) and connected precolumn with 0.05 M citric acid monohydrate / 0.05 M tris-sodium citrate dihydrate buffer, pH 4.5, containing 5% acetonitril, 24 mg sodium l-octanesulfonate and 37.2 mg EDTA-2Na per one litter buffer. Electrochemical measurements were made using glassy carbone working electrodes set at +0.5 V vs an Ag/AgCl reference electrode (L-ECD-6A, Shimadzu Co., Ltd., Kyoto, Japan). Parent monoamine and metabolite levels in the brain perfusate were calculated from the chromatographic peak areas and recorded by a recorder (C-RIB, Shimadzu Co., Ltd., Kyoto, Japan). To estimate the recovery of these compounds through the membrane, microdialysis probes were perfused *in vitro* (1 μl/min) and immersed in Ringer's solution containing of 10 pg/μl of these compounds. The amount of substance in the perfusate was compared with the amount outside the dialysis probe and expressed as percent recovery. These studies were performed during the day time.

RESULTS AND DISCUSSION

Fig.1 shows the standard pattern of monoamines and their related metabolites (75 pg) on HPLC-ECD apparatus. The % (concentration of solute in the perfusate / concentration of solute in the surrounding medium) of the these compounds through the microdialysis probe was estimated to be 28—34% at the perfusion speed of 1 μl/min. Fig.2 shows the typical elusion pattern on HPLC-ECD in the dialysate of the striatum. With the brain microdialysis probe implanted into the striatum of freely moving rats it was possible to measure basal levels of DA (18.89 ± 2.23 pg/25μl/60 min), DOPAC (7.25 ± 0.32 ng/25μl/60 min), 5HTP (13.35 ± 3.01 pg/25μl/60 min), 5HT (5.32 ± 0.92 pg/25 μl/60 min), 5HIAA (2.41 ± 0.08 ng/25μl/60 min) and 5HTOL (7.96 ± 0.45 pg/25μl/60 min). Fig.3 shows the results of the studies *in vivo* on the effect of the monoamine oxidase inhibitor and L-tryptophan on rat brain 5HT synthesis. The level of 5HT in the striatum perfusate was increased by 3-fold, whereas the levels of DOPAC and 5HIAA decreased by approximately 20% and 8% in rats treated with pargyline (75 mg/kg i. p.) and tryptophan (100 mg/kg

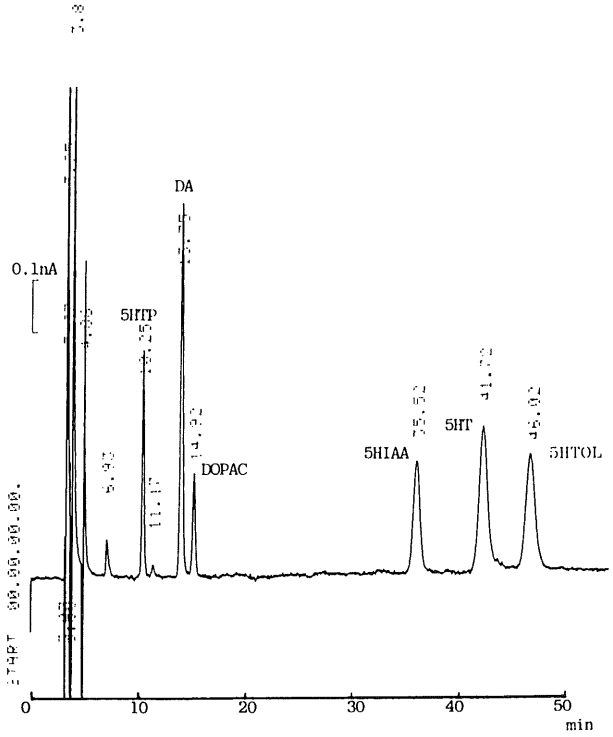

Fig. 1. Standard pattern of monoamines and their metabolites (75 pg) on HPLC-ECD. 5HTP: 5-hydroxytryptophan, DA: dopamine, DOPAC: dihydroxyphenylacetic acid, 5HIAA: 5-hydroxyindoleacetic acid, 5HT: 5-hydroxytryptamine, 5HTOL: 5-hydroxytryptophol.

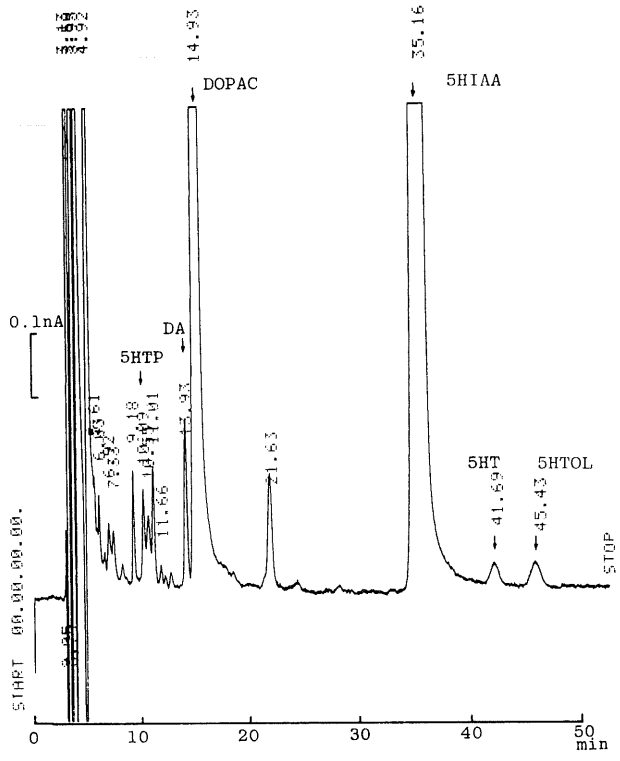

Fig. 2. Typical pattern of monoamines and their metabolites on HPLC-ECD in the striatum dialysate. A 25 μl amount of the striatum dialysate was assayed simultaneously by the HPLC-ECD under the condition mentioned above at the same time as sample collection.

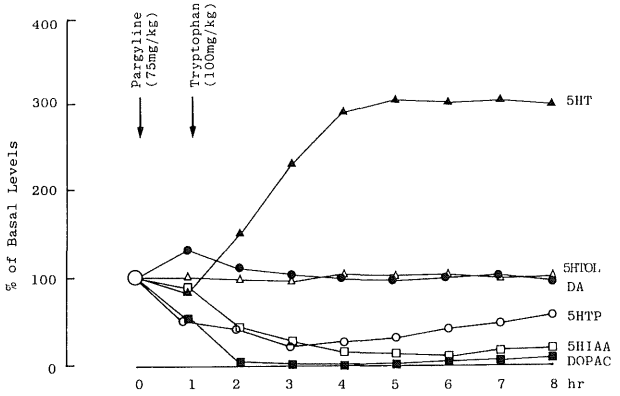

Fig. 3. Effect of pargyline (75 mg/kg i.p.) and tryptophan (100 mg/kg i.p.) on the levels of 5HTP, 5HT, its metabolites and DA, and its metabolites. Results are means of 3 rats.

i. p.), respectively. The relationship between tryptophan and brain 5HT synthesis and hyperactivity was determined by this brain microdialysis technique *in vivo* in the freely moving rats. Furthermore, the 5HTP level increased by 4-fold at one hour after the administration of tryptophan (100 mg/kg i. p.) alone. Changes in the extracellular concentration of the metabolites, DOPAC, 5HIAA and 5HTOL, have been used as an index of dopaminergic and serotonergic activities.

We could determine the levels of the compounds related to the dopaminergic and serotonergic systems simultaneously in the freely moving rats. The dialysis technique can be applied to a large number of problems in pharmacology and physiology. It is useful for the study of mechanism of *in vivo* neurochemical reaction such as appetitive behavior, drinking, rhythmic behavior, and memory which are related mainly to the serotonergic neurons.

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