

## Changes of 5-Hydroxytryptamine Release by Different Glucose Concentration from Hippocampal Slices Exposed to Hypoxia

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We have previously reported that the spontaneous release of [<sup>3</sup>H]5-hydroxytryptamine(5-HT) was markedly decreased by hypoxic insult in rat hippocampal slices. In the present study, the effect of glucose on 5-HT release was examined. Fractional release of [<sup>3</sup>H]5-HT was measured from an incubation medium exchanged every 10 min for 140 min and after stabilization of [<sup>3</sup>H]5-HT release, 10 or 20 min period of hypoxia was induced by exchanging the media which have been previously saturated by 95% N<sub>2</sub>/5% CO<sub>2</sub> gas. In the media containing 1, 2, 5, 10 or 20 mM glucose, [<sup>3</sup>H]5-HT release was stabilized after 40 min of incubation. Exposure to hypoxia decreased [<sup>3</sup>H]5-HT release up to 60% of the control level in a glucose concentration-dependent manner and recovered gradually after hypoxic periods. However, in the media containing no glucose, the spontaneous release of [<sup>3</sup>H]5-HT increased continuously during incubation. Moreover, when hippocampal slices were exposed to hypoxia, the [<sup>3</sup>H]5-HT release increased up to 150% of the control level and recovered gradually to the control level after hypoxic periods. These results demonstrate that hypoxia inhibits or enhances 5-HT release in the presence or absence of extracellular glucose respectively and suggest that the availability of extracellular glucose is a key factor to determine the direction of 5-HT release under hypoxic condition.

**Key Words:** 5-hydroxytryptamine, glucose, hypoxia, hippocampal slice

After ischemia, a massive increase of neurotransmitters is observed in the extracellular fluid of the brain. Much attention has been made to the neurotoxic action of synaptically released excitatory amino acids such as glutamate in the development of ischemic neuronal damage. It is well established that glutamate is a major factor which mediates the neurotoxic actions during ischemia(Greenamyre *et al.*, 1983; Benveniste *et al.*, 1984; Graham *et al.*, 1993).

Altered concentrations of monoamine neurotransmitters are also observed after brain ischemia. The changes of the levels of these neurotransmitters such as dopamine, norepinephrine, and 5-hydroxytryptamine(5-HT) suggest their additional role in neuronal injury induced by ischemia. Reduction of neuronal damage was noted in the dopamine-depleted striatum prior to ischemia(Globus *et al.*, 1987). Treatment of pargyline, a monoamine oxidase inhibitor, before transient forebrain ischemia increased the survival rate of rats(Damsma *et al.*, 1990). Koide *et al.*, (1986) reported that the infusion of norepinephrine during the early recirculation period ameliorated the neuronal damage in the hippocampus and other regions of previous ischemia-induced rats.

5-HT has been known to exert modulatory

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effects on neuronal excitability and on responses to excitatory amino acids in various regions of mammalian brain (Lee *et al.*, 1986; Nedergaard *et al.*, 1986; Reynolds *et al.*, 1988). Therefore, 5-HT may affect the neurologic outcome in an ischemic brain injury. This has been supported by several studies that 5-HT antagonist prevented or reduced the ischemia-induced neuronal damages (Zivin, 1985; Globus *et al.*, 1992; Klisch and Bode-Greuel, 1992).

In spite of the reports suggesting the detrimental role of 5-HT in ischemia, much less has been known about the regulating mechanisms of 5-HT release induced by hypoxia or changes of glucose concentration appearing in brain ischemia.

To elucidate the mechanism of 5-HT release related with ischemic conditions in the brain, changes of 5-HT release from rat hippocampus, a region known as highly vulnerable to ischemia, were observed under combined conditions of hypoxia and various concentrations of glucose, being the major stressors in an ischemic environment.

## MATERIALS AND METHODS

### Animals

Adult male Sprague-Dawley rats (200 ~ 250 g) adapted to laboratory environments for at least 7 days were used for the experiments.

### Preparation of hippocampal slices

Each animal was decapitated and its brain was rapidly removed. The brain was dissected along the midline into two halves. Each hippocampus isolated from both sides of the brain was sliced transversely at 400  $\mu\text{m}$  with a McIlwain mechanical tissue chopper (The Mickle Laboratory Engineering Co., Gomshall, Surrey, UK). All the procedures described above were performed on ice and the tissues were rinsed repeatedly with ice-cold (2 ~ 4°C) standard incubation media.

The standard incubation medium contained NaCl 124 mM, KCl 4 mM, CaCl<sub>2</sub> 2 mM, KH<sub>2</sub>PO<sub>4</sub> 1.25 mM, NaHCO<sub>3</sub> 25 mM, glucose 10 mM, adjusted to pH 7.3 ~ 7.5 with 95% O<sub>2</sub>/5% CO<sub>2</sub>.

Nialamide (Sigma Chemical Company, St. Louis, MO, USA) 12.5  $\mu\text{M}$  was added as a final concentration in order to prevent the metabolic destruction of 5-HT.

### Experimental protocol

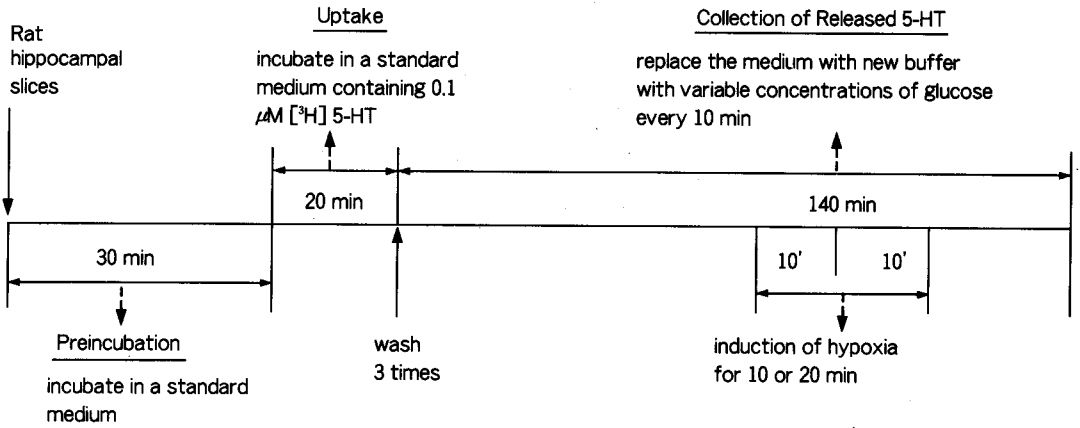
Rat hippocampal slices were allowed to equilibrate for 30 min in a standard incubation medium warmed to 37°C saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Following the equilibration period, the incubation medium was replaced with a new medium containing [<sup>3</sup>H]5-HT (0.1  $\mu\text{M}$ , 74  $\mu\text{Ci}$ ; Amersham International plc., Buckinghamshire, UK) for uptake. After 20-min of incubation, the slices were washed 3 times with standard incubation media. Ten to fifteen slices were transferred to a glass vial containing 3 ml of incubation medium. From that time each vial was drained off and refilled with the same volume of fresh media at 10-min interval for 140 min.

Drained media were used to count the radioactivity of released 5-HT. Zimelidine 10  $\mu\text{M}$  (Research Biochemical International, Natick, MA, USA), a 5-HT uptake inhibitor was included in the media throughout the experiment. To observe the effects of different glucose concentration on the release of 5-HT from the hippocampal slices, the glucose concentrations in the media were changed from 0 mM (glucose-deprived), 1, 2, and 5 mM (low-glucose), 10 mM (normal-glucose) to 20 mM (high-glucose).

After the lapse of 50 min from the initiation of the observation period, hypoxia was induced for 10 or 20 min by replacing the media saturated with 95% N<sub>2</sub>/5% CO<sub>2</sub>. The experimental protocol is diagrammed as shown in Fig. 1.

### Counting of the radioactivity of [<sup>3</sup>H] 5-HT

One milliliter of medium drained from the vial every 10 min was mixed with 9 ml of liquid scintillation cocktail (READY SAFE, Beckman Instruments Inc., Fullerton, CA, USA). The radioactivity of the mixture was counted with a liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA). To measure the radioactivity of [<sup>3</sup>H]5-HT left in the hippocampal slices, the tissues were treated with 1 ml of tissue solubilizer



**Fig. 1.** Diagram illustrating the experimental protocol. Experiments were performed over 3 steps. During the first 30 min, slices were preincubated in a standard incubation medium. [<sup>3</sup>H]5-HT uptake into slices was done for the following 20 min, and observation of 5-HT release was carried out for the last 140 min.

(SOLUENE™ 100, Packard Instrument Company, Inc., Downers Grove, IL, USA) and incubated for 2 hrs at 37°C. Then, 70 μl of glacial acetic acid (Merck & Co., Inc., NJ, USA) was added to neutralize the tissue solubilizer and 100 μl of the completely solubilized sample was mixed with 9 ml of liquid scintillation cocktail for counting.

**Data analysis**

All the results were expressed as fractional release, which was defined as shown below.

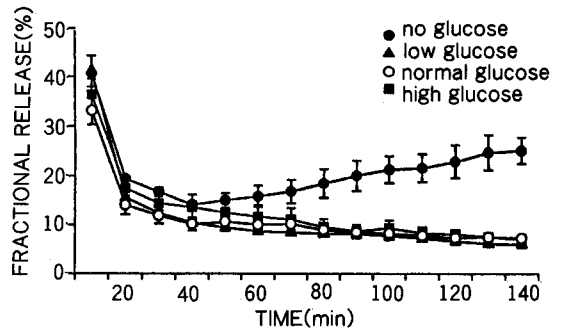
$$\text{Fractional release(\%)} = \frac{\text{cpm}(\text{medium})_{\text{FRAC}} \times 100}{\text{cpm}(\text{medium})_{\text{TOTAL}} + \text{cpm}(\text{tissue})}$$

cpm(medium)<sub>FRAC</sub>: the radioactivity (counts per min, cpm) of [<sup>3</sup>H]5-HT released into the media collected during the given 10-min time fraction

cpm (medium)<sub>TOTAL</sub>: the sum of the radioactivity (cpm) of [<sup>3</sup>H]5-HT released into the media collected every 10 min from the given time fraction to the end of the observation period

cpm (tissue): the radioactivity(cpm) of [<sup>3</sup>H]5-HT remaining in hippocampal slices after 140-min observation period

Statistical comparisons among multiple groups were carried out using analysis of variances (ANOVA) followed by Dunnett's test. The level of significance was set at 0.05.



**Fig. 2.** Spontaneous release of [<sup>3</sup>H]5-HT from the rat hippocampal slices at different concentrations of glucose. 2 mM glucose group was selected for plotting the changes of 5-HT release in a low glucose condition. Each point was calculated as percent when the radioactivity of released 5-HT during the fifth 10-min time fraction was set to control value. The vertical bars indicate S.E.M.

**RESULTS**

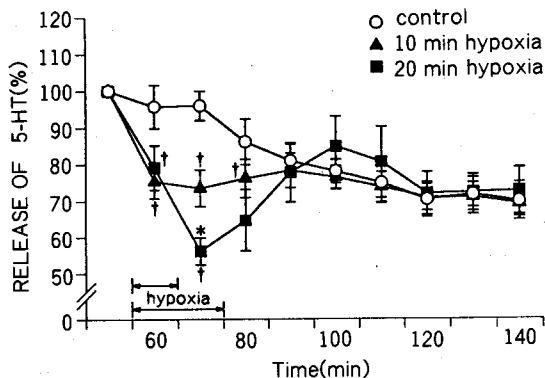
**Spontaneous release of [<sup>3</sup>H]5-HT in different glucose concentrations**

The hippocampal slices showed spontaneous 5-HT release throughout the experiment. 5-

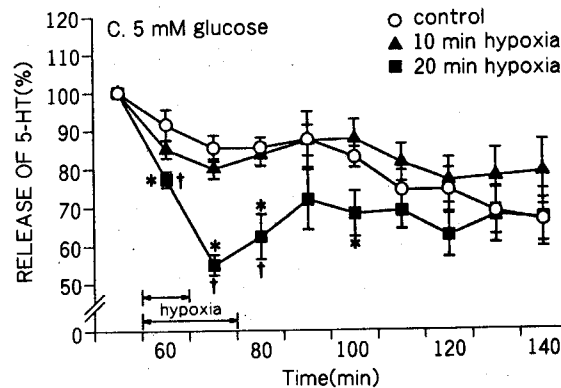
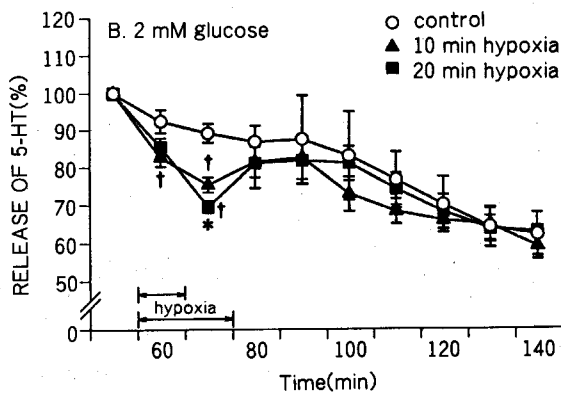
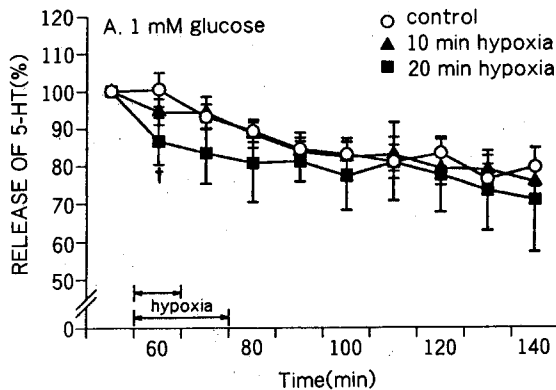
HT release decreased rapidly during the first 40 min, irrespective of glucose concentrations in the media. Thereafter, a steady release of 5-HT was observed up to 140 min. Therefore, 5-HT release at 5th 10-min period was used as a control and the changes of 5-HT release were expressed as percent values compared to the control. When the glucose was present in the media, even in low concentration (1mM), [<sup>3</sup>H]5-HT release decreased gradually with time. However, the glucose-deprived group showed sustained increase in the fractional release of [<sup>3</sup>H]5-HT (Fig. 2).

**Changes of [<sup>3</sup>H]5-HT release in normal-glucose concentration**

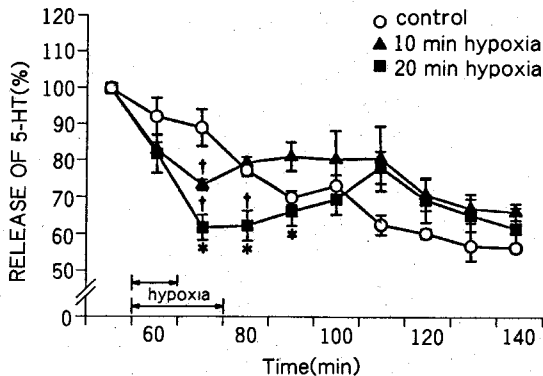
As shown previously, progressive decrease in the fractional release of [<sup>3</sup>H]5-HT was observed when no hypoxic insult was made. Hypoxia intensified the decrease in fractional [<sup>3</sup>H]5-HT release during its exposure to hippocampal slices. The degree of a hypoxia-in-



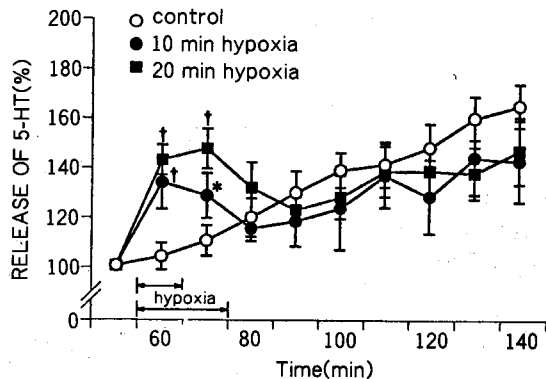
**Fig. 3.** Effects of hypoxia on the release of [<sup>3</sup>H]5-HT from the rat hippocampal slices in normal-glucose concentration (10 mM). Hypoxia was induced for 10(▲) or 20(■) min by replacing the media with 95% N<sub>2</sub>/5% CO<sub>2</sub>-saturated buffer. Each point was calculated as percent when the radioactivity of released 5-HT during the fifth 10-min period was set to control value. The vertical bars indicate S.E.M. ANOVA followed by Dunnett method for multiple comparisons shows significant differences (\*p < 0.05 compared to control, †p < 0.05 compared to 10 min hypoxia).



**Fig. 4.** Effects of hypoxia on the release of [<sup>3</sup>H]5-HT from the rat hippocampal slices in low-glucose concentration. A. 1 mM glucose, B. 2 mM glucose, C. 5 mM glucose. The vertical bars indicate S.E.M. ANOVA followed by Dunnett method for multiple comparisons shows significant differences (\*p < 0.05 compared to control, †p < 0.05 compared to 10 min hypoxia). Other legends are the same as Fig. 3.



**Fig. 5.** Effects of hypoxia on the release of [<sup>3</sup>H]5-HT from the rat hippocampal slices in high-glucose concentration. The vertical bars indicate S.E.M. ANOVA followed by Dunnett method for multiple comparisons shows significant differences (\*p < 0.05 compared to control, †p < 0.05 compared to 10 min hypoxia). Other legends are the same as Fig. 3.



**Fig. 6.** Effects of hypoxia on the release of [<sup>3</sup>H]5-HT from the rat hippocampal slices in glucose-deprived media. The vertical bars indicate S.E.M. ANOVA followed by Dunnett method for multiple comparisons shows significant differences (\*p < 0.05 compared to control, †p < 0.05 compared to 10 min hypoxia). Other legends are the same as Fig. 3.

duced decrease was more profound in 20-min hypoxic exposure than in the 10-min one. Soon after the hypoxic period, a rebound rise in the fractional release was observed requiring more than 20 min to be normalized to control levels(Fig. 3).

**Changes of [<sup>3</sup>H]5-HT release in low-glucose concentration**

When exposed to hypoxia, the tissues showed rapid decrease in fractional [<sup>3</sup>H]5-HT release. After replacing the media with oxygen-saturated buffer, a rebound increase in fractional release was observed. Hypoxia-induced decrease of 5-HT release was smaller at lower concentration of glucose than normal-glucose group. However, the overall pattern of release and the changes of release according to the duration of hypoxic period were similar to those of normal-glucose group (Fig. 4).

**Changes of [<sup>3</sup>H]5-HT release in high-glucose concentration**

Fractional [<sup>3</sup>H]5-HT release at 20 mM-glucose group decreased gradually with time as observed in the normal- and low-glucose con-

ditions. Hypoxic exposure induced a similar pattern of fractional [<sup>3</sup>H]5-HT release to that of normal-glucose group(Fig. 5).

**Changes of [<sup>3</sup>H]5-HT release in glucose-deprived media**

In the glucose-deprived condition, the fractional [<sup>3</sup>H]5-HT release rose continuously with time throughout the observation period. The fractional [<sup>3</sup>H]5-HT was increased by hypoxic insult and hypoxia-induced increase of [<sup>3</sup>H]5-HT release was greater in 20-min exposure group than in 10-min exposure one. Immediately after the hypoxic period, the hippocampal slices showed a rebound decrease in fractional 5-HT release. Thereafter, the values returned approximately to control levels(Fig. 6).

**DISCUSSION**

Our present study shows that 5-HT release from hippocampal slices is very dependent upon the hypoxia or changes of glucose concentrations. High levels of fractional release of [<sup>3</sup>H]5-HT were observed in all experimen-

tal groups during the initial 40-min period. This can be ascribable mainly to the washed [ $^3\text{H}$ ]5-HT remaining on the surfaces of the hippocampal slices as observed in the dopamine release curves reported by Baker and Dyck (1985).

However, the pattern of [ $^3\text{H}$ ]5-HT release during the following periods was different according to whether the glucose is present in the incubation media or not. The hippocampal slices in a normal glucose condition without hypoxic insult showed a fractional 5-HT release which decreased progressively with time. The pattern of [ $^3\text{H}$ ]5-HT release in a lower or higher concentration of glucose was similar to that in the normal glucose condition. Deprivation of glucose from the incubation media expressed the opposite pattern of [ $^3\text{H}$ ]5-HT release to that observed in the presence of glucose conditions, i.e., the hippocampal slices incubated without glucose showed continuously rising fractional release with time. These results show that the changes of glucose at a millimolar level may not be a critical factor for the mechanism of 5-HT release, indicating that the energy demand for the release of 5-HT may not be so high that its general pattern of release can be maintained. However, in the absence of glucose, the regulating mechanism of 5-HT seems to be greatly changed or broken due to the energy failure. Therefore, the availability of extracellular glucose is thought to be the key factor for determining the direction of 5-HT release.

Exposure to hypoxia decreased [ $^3\text{H}$ ]5-HT release up to 60% of the control level in the presence of glucose and recovered to control level after hypoxic periods. Although the hypoxia-induced decrease was glucose concentration-dependent, i.e., the degree of decrease and the changes by hypoxia got blunted as the concentration became lower, it was of the same pattern observed in all glucose-treated groups. Similar results were obtained in the experiments performed by Russ *et al.* (1991), although the tissues used for that experiment and the observed neurotransmitter were different from those of this experiment. After observing the combined effects of hypoxia and increasing concentrations (5 to 50 mM) of

glucose on the release of noradrenaline from isolated rat atria, they reported that the increase of the glucose concentration had no more than a very minor effect on the pronounced release of noradrenaline induced by hypoxia. On the contrary, the hypoxic insult in the glucose-deprived condition enhanced the fractional release of 5-HT. As observed in the groups without hypoxic exposure, the direction of 5-HT release, that is to say, enhancement or inhibition in the hypoxia-exposed condition was also determined by whether the glucose was treated in the media or not, not by the hypoxia itself. In addition, both the hypoxia-induced inhibition (as seen in the glucose-present media) and enhancement (as seen in the glucose-deprived media) of 5-HT release were dependent on the duration of hypoxic exposure.

These overall results observed either in normoxic or in hypoxic condition suggest that the hypoxia positively exaggerates the direction (enhancement or inhibition) of 5-HT predetermined by the status of the availability of the extracellular glucose, and that the mechanism of 5-HT release either induced by hypoxia or regulated by glucose concentration may be different and act independently each other.

The latter suggestion is further evidenced by the rebounding patterns of 5-HT after hypoxic exposure observed in the absence of glucose. While the sustained rise of fractional 5-HT release in the absence of glucose without hypoxic insult may be explained as a result of a progressively increasing energy deficiency during the experiment, the rebounding increase of the fractional 5-HT release during hypoxic exposure is not clearly explainable by this mechanism alone because the abrupt fall in the levels of 5-HT release appeared immediately after the hypoxic period which seemed to be reactive to the exaggerated increase of 5-HT release. As for the rebound pattern in the presence of glucose, we have formerly confirmed that the rebound increase of 5-HT during posthypoxic period in the presence of 10 mM glucose was mediated through the activation of *N*-methyl-D-aspartate (NMDA) receptor, i.e., the increase of 5-

HT release after hypoxic exposure was blocked by 2-amino-5-phosphonovaleric acid, an NMDA receptor blocker (Hwang *et al.*, 1994). This indicates that there may exist an additional releasing mechanism during hypoxic exposure in the absence of glucose which is different from that induced by glucose deprivation alone. In a similar way, we can assume that some neurotransmitters released by hypoxic insult may be involved in the mechanism of 5-HT release during hypoxic exposure in the absence of glucose.

It has been reported that the activity of a receptor for excitatory amino acids could affect the release of acetylcholine as well as norepinephrine or dopamine (Snell and Johnson, 1986; Clow and Jhamandas, 1989). These reports necessitate further investigations for the interactions between 5-HT and other neurotransmitters including their neurons in order to elucidate the mechanism of 5-HT release observed in the glucose-deprived condition.

In conclusion, these results demonstrate that hypoxia inhibits or enhances 5-HT release in the presence or absence of extracellular glucose respectively and suggest that the availability of extracellular glucose is a key factor to determine the direction of 5-HT release under hypoxic condition.

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