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In-vivo Microdialysis Measurement of 5-Hydroxytryptamine and its Metabolites, 5-Hydroxyindoleacetic Acid and *N*-Acetyl 5-Hydroxytryptamine, in Rat Blood: Effects of Histamine-receptor Antagonists

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Abstract

The blood concentrations of 5-hydroxytryptamine (5-HT) and its metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and *N*-acetyl 5-HT were assayed by in-vivo microdialysis and a highly sensitive HPLC procedure that was originally developed to analyse CNS mediators. We investigated the effects of histamine-receptor antagonists on 5-HT metabolism and its release into the blood of rats.

The mean basal levels of 5-HT, 5-HIAA and *N*-acetyl 5-HT in the blood measured by in-vivo microdialysis were $77{\cdot}2 \pm 9{\cdot}4$, $20{\cdot}3 \pm 1{\cdot}5$ and $1{\cdot}89 \pm 0{\cdot}15$ pmol mL^{-1}, respectively. These levels were not significantly affected by an intraperitoneal injection of saline, and remained at constant levels for at least 8 h after administration of saline. After an intraperitoneal injection of 5-HT hydrochloride (0·5 mg kg^{-1}), 5-HT was soon detected in the blood of the jugular vein. 5-HIAA also quickly appeared in the blood and declined monoexponentially from 60 min after injection. In contrast, *N*-acetyl 5-HT slowly appeared in the blood and it reached a maximal level at 270 min. The 5-HT and *N*-acetyl 5-HT levels in dialysates from rat jugular vein were significantly increased by intraperitoneal pyrilamine (2·0 mg kg^{-1}), (+)-chlorpheniramine (2·0 mg kg^{-1}) and cimetidine (20·0 mg kg^{-1}). However, there was no increase in the 5-HIAA concentration after an intraperitoneal injection of these histamine-receptor antagonists, demonstrating that the 5-HT released from various cells containing 5-HT was predominantly metabolized to *N*-acetyl 5-HT by *N*-acetyltransferase. Moreover, thioperamide did not affect the basal levels of 5-HT, 5-HIAA or *N*-acetyl 5-HT.

Because the recovered 5-HT, 5-HIAA and *N*-acetyl 5-HT in the dialysate is directly proportional to the free fraction in the blood, in-vivo microdialysis is a reliable method of examining 5-HT metabolism and its release into the blood.

5-Hydroxytryptamine (5-HT) is widely distributed in mammalian tissues including platelets, enterochromaffin cells and neurons in the central or peripheral nervous system. Therefore, an increase in the plasma 5-HT concentration may reflect the release of this amine from platelets, enterochromaffin cells or neurons. However, released 5-HT is actively taken up again into blood platelets and 5-HT nerve endings of synaptosomes, and is predominantly metabolized to 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase (MAO). 5-HT is also converted to *N*-acetyl 5-HT by *N*-acetyltransferase. The analysis of 5-HT and its metabolites in plasma provides an important tool in elucidating the pathophysiological significance of 5-HT in various psychiatric illnesses (Fowler et al 1982).

Microdialysis in-vivo has been widely applied for measuring the extracellular concentrations of many substances in the brain and other tissues. In this study, we establish an in-vivo microdialysis method for measuring 5-HT and its metabolites in blood and use it to determine the effect of histamine receptor antagonists on 5-HT release and metabolism.

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Materials and Methods

Animals

Male Wistar rats (180-200 g, Japan SLC Inc., Hamamatsu, Japan) were housed in an environmentally controlled room (23 ± 1 °C, 55 5% relative humidity, illuminated from 0700 h to 1900 h) with water and diet freely available.

Microdialysis

The rats were anaesthetized with intraperitoneal urethane (1·2 g kg^{-1}) and placed on their backs. The pectoral muscle was exposed, and the guide cannula was inserted into the jugular vein through the muscle. The microdialysis probe (CMA/10, membrane length 10 mm, Carnegie Medicin, Stockholm, Sweden) was introduced inside the guide, and was perfused with saline at a flow rate of 2 μL min^{-1}. Sample dialysates were collected every 30 min, and were directly subjected to an HPLC system. The recovery of 5-HT, 5-HIAA and *N*-acetyl 5-HT from the surrounding fluid in the dialysate (relative recovery) were $37{\cdot}5 \pm 5{\cdot}2$ (n = 4), $57{\cdot}5 \pm 3{\cdot}6$ (n = 4) and $42{\cdot}5 \pm 4{\cdot}5$% (n = 4), respectively, by an in-vitro perfusion test, in which the probe was placed in a test tube containing 10 nM 5-HT, 5-HIAA and *N*-acetyl 5-HT dissolved in saline and perfused at 37°C at a flow rate of 2 μL min^{-1}.

Drug administration

The concentrations of 5-HT, 5-HIAA and *N*-acetyl 5-HT at 60–90 min after insertion of the probe, decreased to a steady state. Therefore, immediately after the first three fractions had been collected, drugs were administered intraperitoneally at doses as follows: 5-HT hydrochloride (0·5 mg kg^{-1}, Sigma Chemicals, St Louis, MO, USA), mepyramine maleate (2·0 mg kg^{-1}, Sigma Chemicals), (+)-chlorpheniramine maleate (2·0 mg kg^{-1}, Yoshitomi Pharmac. Ind., Osaka, Japan), (−)-chlorpheniramine maleate (2·0 mg kg^{-1}, Schering Corporation, NJ, USA), cimetidine (Tagamet injection, 10 mg kg^{-1}, Fujisawa Pharmac. Co., Tokyo, Japan), and thioperamide (10 mg kg^{-1}, Sumitomo Pharmaceutical. Co., Osaka, Japan). Doses of the salt forms of drugs are expressed as weights of the salts.

Measurements of 5-HT and metabolites

The 5-HT, 5-HIAA and *N*-acetyl 5-HT concentrations of the dialysate were determined by HPLC with electrochemical detection. The dialysate (60 μL) was mixed with 20 μL 0·02 M acetic acid, and 50 μL of the mixture was injected onto a Biophase ODS-IV column (110 × 4 mm i.d., particle size 3 mm, Bioanalytical Systems, W. Lafayette, IN, USA) connected to an LC-4B amperometric detector (Bioanalytical Systems) equipped with a glassy carbon electrode set at a potential of 0·7 V relative to the Ag/AgCl reference electrode. The retention times of 5-HIAA, *N*-acetyl 5-HT and 5-HT were 8·4, 11·2 and 15·9 min, respectively, and detection limit of the system was about 20 fmol per injection.

Statistical analysis

In each experiment, the mean of the first three fractions was defined as the mean basal level. Dialysate values were corrected for in-vitro recovery and are presented as means ± s.e. for n experiments. The significance of differences between drug-treated and control animals was analysed by Student's *t*-test.

Results

The mean basal levels of 5-HT, 5-HIAA and *N*-acetyl 5-HT in the blood were 77·2 ± 9·4, 20·3 ± 1·5 and 1·89 ± 0·15 pmol mL^{-1}, respectively. These levels were not appreciably affected by intraperitoneal injections of saline, and remained constant for at least 8 h after saline administration.

As shown in Fig. 1, 5-HT was quickly absorbed after injection of 5-HT hydrochloride (0·5 mg kg^{-1}), and the 5-HT concentration in dialysates from the blood declined monoexponentially after 150 min. 5-HIAA soon appeared in the blood and also declined monoexponentially from 60 min after injection. In contrast, *N*-acetyl 5-HT slowly appeared in the blood, and reached a maximal concentration at 240 min after administration.

Figs 2 and 3 show the time course of changes in the concentrations of 5-HT, 5-HIAA and *N*-acetyl 5-HT in dialysates from the rat jugular vein after intraperitoneal injection of histamine-receptor antagonists. The 5-HT and *N*-acetyl 5-HT concentrations increased to about 250 and 200%, respectively, of the control value at 120 min after injection of pyrilamine, and continued to increase thereafter. However, there was no significant change in the concentration of 5-HIAA. The 5-HT

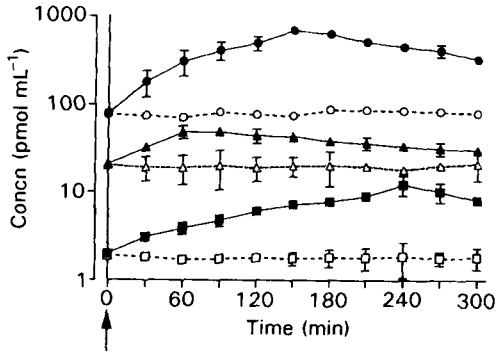

FIG. 1. 5-HT (●, ○) and its metabolites, 5-HIAA (▲, △) and *N*-acetyl 5-HT (■, □) in microdialysates from rat blood after intraperitoneal injection of 5-HT hydrochloride (0.5 mg kg^{-1}). 5-HT hydrochloride (−) or saline (---) was immediately injected after collecting the first three fractions as indicated by the arrow. Each point is the mean ± s.e. of 4 experiments.

concentration in the blood increased to about 220% of the control value within 210 min after injection of cimetidine, then returned to the basal level. Although the 5-HIAA concentration was not significantly influenced by cimetidine, the *N*-acetyl 5-HT concentration also continued to increase from 60 min after an injection of cimetidine. However, thioperamide did not affect the basal levels of 5-HT, 5-HIAA or *N*-acetyl 5-HT. A significant increase in the 5-HT concentration was produced by (+)-chlorpheniramine (200–500%) at 60–210 min after administration when compared with the saline-treated group, but the 5-HT concentration was not significantly affected by its (−)-isomer. Moreover, the *N*-acetyl 5-HT concentration in the blood continued to increase for at least 240 min after injection of either chlorpheniramine enantiomer, and its concentration after administration of the (−)-form was higher than that of the (+)-form. However, the 5-HIAA concentration was not significantly increased by either enantiomer of chlorpheniramine (Fig. 3).

Discussion

Assuming that the relative recovery of 5-HT, 5-HIAA and *N*-acetyl 5-HT through the membrane under in-vivo dialysis conditions is the same as that in-vitro, the basal concentrations of 5-HT, 5-HIAA and *N*-acetyl 5-HT in the blood can be estimated as 77·2, 20·3 and 1·89 nM, respectively. Microdialysis also showed that 5-HT was quickly absorbed from the abdominal cavity after intraperitoneal administration. Simultaneously, 5-HT and its metabolites, 5-HIAA and *N*-acetyl 5-HT were detected in the blood. These results suggested that the absorbed 5-HT was quickly metabolized to 5-HIAA by MAO, but, the metabolism of 5-HT by *N*-acetyltransferase was slower. It is also likely that the recovered 5-HT and its metabolites in the dialysate are directly proportional to the free fraction in the blood, thus indicating that in-vivo blood microdialysis is a reliable means of examining 5-HT release and metabolism.

In our experiments using this in-vivo blood microdialysis method, the 5-HT concentration was shown to increase after intraperitoneal administration of mepyramine, an H_1-receptor antagonist and cimetidine, an H_2-receptor antagonist, demonstrating 5-HT release into the blood. It was also suggested that the 5-HT released from various cells containing 5-HT is pre-

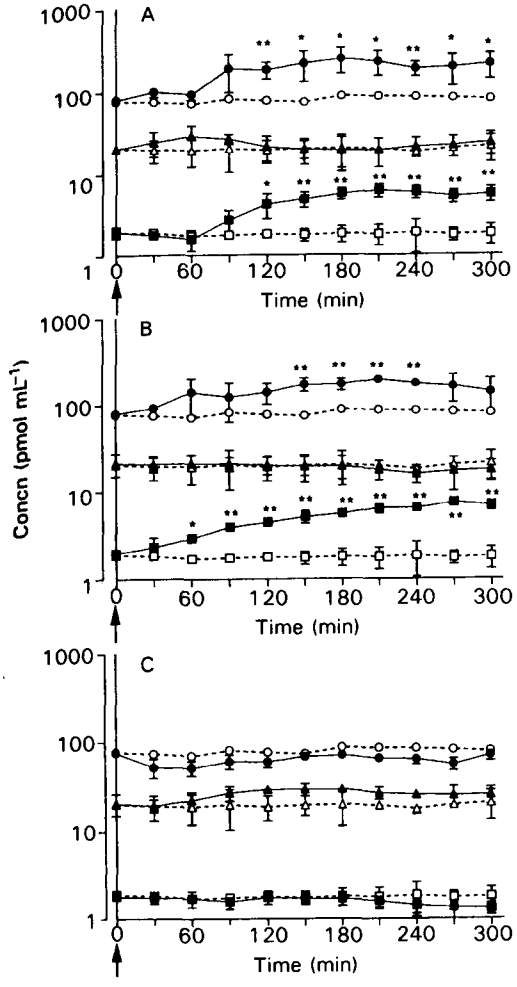

FIG. 2. Changes in the concentrations of 5-HT (●, ○) and its metabolites, 5-HIAA (▲, △) and *N*-acetyl 5-HT (■, □), in microdialysates from the rat jugular vein after intraperitoneal injection of histamine-receptor antagonists. Pyrilamine (A), cimetidine (B), thioperamide (C) or saline was immediately injected after collecting the first three fractions as indicated by the arrow. – Drug administration, -·- saline. Each point is the mean ± s.e. of 4 experiments. $^{*}P<0.05$, $^{**}P<0.01$ compared with saline-treated rats.

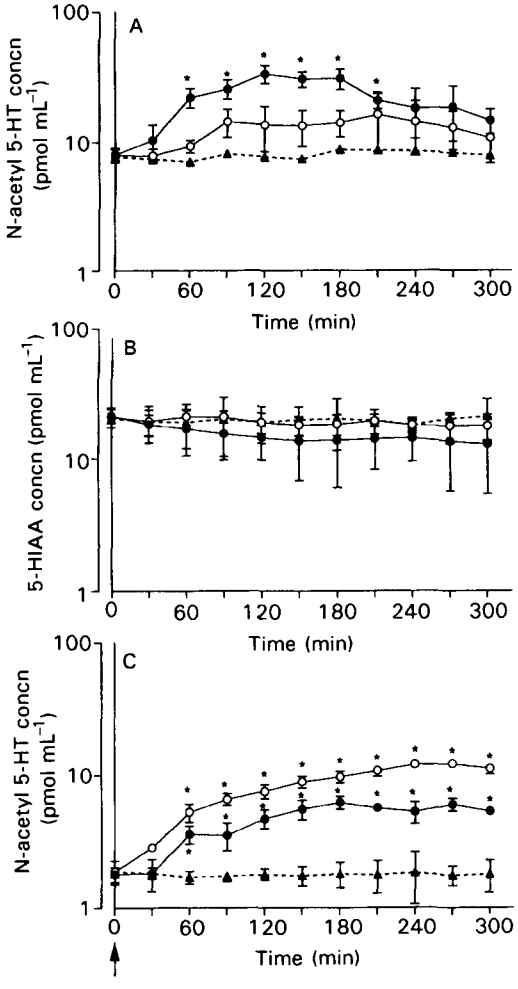

FIG. 3. Changes in the concentrations of 5-HT and its metabolites, 5-HIAA and *N*-acetyl 5-HT, in microdialysates from the rat jugular vein after intraperitoneal injection of chlorpheniramine enantiomers. (+)-Chlorpheniramine (●), (–)-chlorpheniramine (○) or saline (▲) was immediately injected after collection of the first three fractions as indicated by the arrow. Each point is the mean ± s.e. of 4 experiments. $^{*}P<0.01$ compared with saline-treated rats.

dominantly metabolized to *N*-acetyl 5-HT by *N*-acetyltrasferase in the liver or other tissues. However, there was no significant difference in the 5-HIAA concentration in rats given saline, mepyramine or cimetidine. Therefore, these compounds may inhibit MAO activity. On the other hand, the H_1 antagonist, (+)-chlorpheniramine produced a significant increase in the 5-HT concentration at 60–210 min after injection at a dose of 2·0 mg kg^{-1}, whereas l-chlorpheniramine, an inactive isomer, had little effect at the same dose. However, the concentration of *N*-acetyl 5-HT after injection of (–)-chlorpheniramine was higher than that after (+)-chlorpheniramine, suggesting that (–)-chlorpheniramine accelerates the *N*-acetylation of 5-HT as compared with (+)-chlorpheniramine. In contrast, the 5-HIAA concentration was not significantly changed by injection of either enantiomer of chlorpheniramine. The H_3-receptor antagonist, thioperamide facilitates the electrically evoked tritium overflow from superfused rat brain cortex slices that were first incubated with [^{3}H]5-HT (Fink et al 1990). Thioperamide generally increases the 5-HIAA/5-HT ratio (an increase in 5-HIAA and/or a decrease in 5-HT) and the pargyline-induced 5-HT accumulation in the rat and mouse brain (Oishi et al 1990). In this study, we examined 5-HT release and metabolism after injection of thioperamide (10 mg kg^{-1}) and found that thioperamide did not affect the basal levels of 5-HT, 5-HIAA or *N*-acetyl 5-HT in microdialysates. The reason for the discrepancy between these results and published data from studies of the brain is not known; further experiments on uptake and metabolism in-vitro will be necessary to support our data obtained using in-vivo microdialysis.

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