Mechanisms of Veratramine-Induced 5-HT Syndrome in Mice

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ABSTRACT—Regional monoamine assays revealed that during veratramine-induced myoclonic movements, the contents of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex were reduced with a slight increase in dopamine metabolites in the midbrain and brainstem. A similar tendency to decrease 5-HT and 5-HIAA contents was observed in the hypothalamus and hippocampus without increase in the contents of dopamine and its metabolites. Norepinephrine levels were not modified in any brain region at any time after the administration of the veratrum alkaloid. It was found that the veratramine evoked 3H-5-HT release from the frontal cortical slices was Ca++-independent and persistent, and it continued approximately 20 min after the 2-min exposure to veratramine. The uptake of 3H-5-HT into the frontal cortical slices was inhibited competitively by veratramine. These results suggest that veratramine is both a releaser and uptake inhibitor of 5-HT and that the veratramine-induced involuntary movements may be mediated by serotonergic hyperfunction.

The administration of veratramine produces a syndrome characterized by generalized tremor, myoclonic movement of four extremities, hindlimb abduction, backward gait and Straub tail (1-4), resembling the "5-hydroxytryptamine (5-HT) syndrome" (5-7). Previous studies demonstrated that veratramine-induced tremor and struggling were suppressed by methysergide (3, 8), a non-selective 5-HT antagonist, but not by haloperidol, phenoxybenzamine or atropine (8, 9). Prior administration of phenytoin or morphine was also ineffective (4). These findings are in favor of the hypothesis that veratramine may alter serotonergic functions in the central nervous system (CNS); and thus, Izumi et al. (8) suggested that veratramine may act as a 5-HT agonist. However, the precise mechanism of action of veratramine remains to be elucidated. In the present study, we studied the effects of veratramine on the 5-HT system in mice.

MATERIALS AND METHODS

Animals

Male ddY mice, weighing 40-50 g, were used. Animals were housed at the Institute of Laboratory Animal Sciences, Kagoshima University, at a room temperature of 22-24°C, humidity of 60-70% and lighting from 6:00-18:00.

Monoamine assay

This experiment was done between 9:00 and 12:00, since the amines show a circadian rhythm. Veratramine was dissolved in 0.1 N HCl, and the solution was diluted with distilled water. Mice were decapitated 2 min (before tremor), 6 min (during tremor), or 12 min (during myoclonus) following the treatment with veratramine in a dose of 3.0 mg/kg, i.p., equivalent to the ED99 for myoclonus and tremor. Control animals were treated with ve-
hicle solution and sacrificed on a similar time-
schedule. The brain was dissected into seven
regions on a saline-ice plate according to the
guidelines of Glowinski and Iversen (10): cere-
bral cortex, striatum, hypothalamus, hippocam-
pus, midbrain, brainstem and cerebellum. Sam-
pies were frozen on dry ice until monoamine assay was carried out; the assay
was usually started within 12 hr. The tissue
sample was homogenized in 0.1 M perchloric
acid solution containing 0.1 M EDTA-2Na and
3,4-dihydroxy-benzylamine (30 mg/ml), using a polytron homogenizer at setting 6 for
10 sec (Kinematica) and then centrifuged at
25,000 x g for 20 min (Kubota 20000). The supernatant was filtered through a mem-
brane filter (FR-20, 0.2 μm, 13 mm; Fuji Photo Film
Co., Tokyo); and a 10-μl aliquot of the fil-
tered solution was injected into a high per-
formance liquid chromatograph (HPLC;
Bioanalytical System, Inc.). The HPLC system
was composed of a reverse phase column
(Spherisorb ODS-II; length: 250 mm, internal
diameter: 4.6 mm) packed with 5 μm of
Wakopak® and an electrochemical detector
(LC-4B amperometric detector) set at a poten-
tial of 0.8 V. The mobile phase, consisting of
a 0.15 M monochloroacetate buffer (pH 3.0)
containing 2 mM EDTA-2Na, 0.01% sodium octydyl sulfate and 10% methanol, was
pumped through the column at a rate of 1
ml/min.

Release study

The frontal cortex was dissected and the tis-
ue chopped into slices (approximately 1.0 mm
thick, 0.4 x 0.4 mm) using a McIlwain chop-
per. The slices were immediately transferred
to an incubation medium containing a cold
Krebs-Ringer bicarbonate (KRB) solution with the following composition: 118 mM NaCl,
4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄,
25 mM NaHCO₃, 1.2 mM KH₂PO₄ and 11
mM glucose. Pargyline (10 μM) was included
in solutions throughout the release and uptake
studies. Following a 10-min preincubation at
37°C, the slices were incubated for 20 min at
37°C in a water-bath in an atmosphere of 95% O₂ and 5% CO₂, with 3H-5-HT
(5-hydroxytryptamine creatinine sulfate, 0.1
μM) and ascorbic acid (0.6 μM). Following
the incubation, the slices were gently washed
three times with a warm KRB solution, and
then three slices were mounted on a superfu-
sion chamber. The preparation was superfused
at a rate of 0.12 ml/min by means of a peri-
staltic pump having three channels (Pharmacia,
Sweden), with continuous aeration with 95%
O₂ and 5% CO₂. Five different solutions were
prepared for superfusion: solution A (normal
KRB solution) was used for basal efflux of tri-
gium; solution B (high 25 mM K⁺ solution)
was obtained by equimolar replacement of
NaCl by KCl; solution C (veratramine solu-
tion) contained 100 μM veratramine in the
KRB; Solution D (vehicle solution) was KRB
containing 0.1 N HCl used for dissolution of
veratramine in (C); and in solution E (Ca²⁺-
free veratramine solution), CaCl₂ was omitted
from solution C and an identical volume of
EGTA (10 μM) was added.

The solution was removed from the cham-
ber by means of a second or third channel rol-
er. A three-way stopcock placed upstream of
the first channel tube allowed rapid switching
from solution A to solution B, C, D, or E.
The basal and stimulated efflux of total tritium
was collected, using a fraction collector, at 2-
min interval for estimation of total radioactiv-
ity. At 30 min after the initiation of superfu-
sion when the basal efflux was equilibrated,
the slices were stimulated with high K⁺ (solu-
tion B) or exposed to vehicle solution (solu-
tion D) or the veratramine solution without
CaCl₂ (solution E), for 2 min, followed by a
second 36-min period of basal efflux. The tis-
sues were then exposed to veratramine (solu-
tion C) for 2 min, followed by a third basal
efflux of 28 min. At the end of the super-
fusion, the slices were solubilized, and the
radioactivity in the slices and in each fraction of superfusate was measured in a liquid scin-
tillation spectrometer (Aloka, LSC-903), after
vigorous shaking with 3 ml of scintillation fluid
(3.0 g Permablend® III in a mixed solution of
333 ml Triton X-100 and 667 ml toluene).
Automatic quench correction was achieved with the external standard method. The amount of radioactivity released into each fraction was expressed as a percentage of the total tritium taken up into the tissue. The total tritium content remaining in the tissue at the time of sampling was determined in each experiment by adding to the \(^3\)H-content at the end of the superfusion the radioactivity collected in all the samples.

**Uptake study**

The uptake of \(^3\)H-5-HT in the frontal cortical slices was measured according to a slight modification of the method of Shaskan and Snyder (11). Briefly, cortical slices (approximately 1-mm thick, 0.4 × 0.4 mm) were placed in 1 ml of medium of the same composition as that employed in the preloading of \(^3\)H-5-HT for the release study, and preincubated for 10 min at 37°C. The uptake was started by adding 10 \(\mu\)l of \(^3\)H-5-HT (0.1 \(\mu\)M) and continued for various times as indicated in figures under the atmosphere of 95% O\(_2\) and 5% CO\(_2\). The reaction was stopped by adding 4 ml of ice-cold KRB solution, and the tissues were immediately blotted onto a glass microfiber filter (GF/B filter, Whatman Int., Ltd., England) and rapidly filtrated by a Millipore filtration unit. The washing procedure was repeated three times with an indentical volume of ice-cold KRB solution. Each washed filter was dried for 5 min at 60°C and extracted with 0.5 ml soluene in a counting vial. After adding 10 ml of scintillation liquid, the samples were allowed to stand for at least 48 hr before counting in order to minimize the chemical luminescence. The active uptake of \(^3\)H-5-HT

| Region      | Time | 5-HT (ng/g tissue) Vehicle | 5-HT (ng/g tissue) Veratramine | 5-HIAA (ng/g tissue) Vehicle | 5-HIAA (ng/g tissue) Veratramine | NE (ng/g tissue) Vehicle | NE (ng/g tissue) Veratramine |
|-------------|------|---------------------------|-------------------------------|-----------------------------|---------------------------------|---------------------------|--------------------------|
| Cortex      | 2 min| 898 ± 136                 | 808 ± 129                     | 450 ± 32                    | 402 ± 19                        | 314 ± 56                  | 281 ± 41                 |
|             | 6 min| 802 ± 79                  | 810 ± 75                      | 463 ± 22                    | 467 ± 12                        | 262 ± 20                  | 299 ± 36                 |
|             | 12 min| 888 ± 67                 | 589 ± 45**                    | 535 ± 30                    | 419 ± 31*                       | 336 ± 37                  | 291 ± 38                 |
| Hypothalamus| 2 min| 1663 ± 241                | 1589 ± 120                    | 1450 ± 217                  | 1242 ± 63                       | 1525 ± 63                 | 1357 ± 160               |
|             | 6 min| 1190 ± 111                | 1479 ± 381                    | 1070 ± 62                   | 1250 ± 170                      | 1631 ± 154                | 1089 ± 273               |
|             | 12 min| 1573 ± 146               | 1422 ± 63                     | 1367 ± 106                  | 1186 ± 153                      | 1676 ± 231                | 1193 ± 106               |
| Hippocampus| 2 min| 872 ± 137                 | 695 ± 61                      | 801 ± 78                    | 667 ± 63                        | 405 ± 75                  | 339 ± 44                 |
|             | 6 min| 723 ± 93                  | 758 ± 116                     | 771 ± 70                    | 797 ± 67                        | 317 ± 41                  | 373 ± 40                 |
|             | 12 min| 772 ± 65                 | 609 ± 68                      | 810 ± 18                    | 686 ± 79                        | 342 ± 23                  | 346 ± 61                 |
| Striatum    | 2 min| 862 ± 116                 | 814 ± 69                      | 774 ± 77                    | 749 ± 26                        | 224 ± 20                  | 153 ± 16                 |
|             | 6 min| 816 ± 73                  | 626 ± 83                      | 776 ± 52                    | 680 ± 36                        | 191 ± 39                  | 161 ± 19                 |
|             | 12 min| 802 ± 74                 | 756 ± 72                      | 810 ± 63                    | 846 ± 84                        | 224 ± 18                  | 176 ± 28                 |
| Midbrain    | 2 min| 1194 ± 183                | 1419 ± 131                    | 1026 ± 119                  | 1237 ± 71                       | 456 ± 37                  | 490 ± 49                 |
|             | 6 min| 1260 ± 103                | 1041 ± 250                    | 1222 ± 78                   | 1034 ± 138                      | 459 ± 46                  | 485 ± 71                 |
|             | 12 min| 1130 ± 155               | 1147 ± 17                     | 1179 ± 93                   | 1249 ± 92                       | 481 ± 36                  | 464 ± 39                 |
| Brainstem   | 2 min| 800 ± 114                 | 790 ± 80                      | 635 ± 55                    | 684 ± 27                        | 465 ± 54                  | 492 ± 50                 |
|             | 6 min| 827 ± 68                  | 692 ± 76                      | 786 ± 34                    | 715 ± 38                        | 498 ± 37                  | 560 ± 54                 |
|             | 12 min| 768 ± 73                 | 696 ± 35                      | 718 ± 35                    | 713 ± 51                        | 560 ± 61                  | 486 ± 28                 |

Mice were decapitated 2 min (before tremor), 6 min (during tremor), or 12 min (during myoclonus) following the treatment with veratramine in a dose of 3.0 mg/kg, i.p., which is equivalent to the ED99 for myoclonus and tremor. Control animals were treated with vehicle solution and sacrificed on a similar time-schedule. Values are expressed as the mean ± S.E. (n = 6). *P < 0.05, **P < 0.01, as compared to each control.
was obtained by subtraction of uptake values at 0°C from those at 37°C.

**Chemicals**

Veratramine was purchased from Aldrich Chemical Co. (London), and 3H-5-HT was obtained from New England Nuclear (Boston).

**Statistical analysis**

Statistical analysis was done by one-way or two-way analysis of variance.

**RESULTS**

**Effects of veratramine on monoamine contents**

The results are summarized in Tables 1 and 2. No change in the levels of monoamines or their metabolites was seen in any of the discrete brain regions at 2 min after the veratramine injection (before tremor). At 6 min (during tremor), the content of 5-HT was slightly decreased in the striatum, midbrain and brainstem by 25%, 17% and 16%, respectively. In these regions, the contents of 5-HIAA were also slightly decreased (9-15%). During myoclonus (12 min after the injection), 5-HT and 5-HIAA levels in the cortex were significantly decreased by 37% and 20%, respectively. A similar tendency toward decreasing 5-HT and 5-HIAA contents was noted in the hypothalamus and hippocampus. The concentration of DOPAC and/or HVA was significantly increased in the midbrain and brainstem, without alteration of dopamine content. In the hypothalamus and hippocampus, there were no consistent changes in the contents of dopamine and its metabolites. Norepinephrine levels were not modified in any brain region in the animals sacrificed of any time following the administration of the veratrum alkaloid. No significant changes were noted in the cerebellum (data not shown).

| Table 2. Effects of veratramine on contents of DA, DOPAC and HVA in discrete mouse brain regions |
| Region       | Time  | DA (ng/g tissue) | DOPAC (ng/g tissue) | HVA (ng/g tissue) |
|--------------|-------|------------------|---------------------|------------------|
|              |       | Vehicle          | Veratramine         | Vehicle          | Veratramine         | Vehicle          | Veratramine         |
| Cortex       | 2 min | 893 ± 138        | 879 ± 33            | 224 ± 20         | 219 ± 11            | 164 ± 18         | 143 ± 11            |
|              | 6 min | 981 ± 68         | 1011 ± 63           | 257 ± 15         | 262 ± 15            | 168 ± 9          | 137 ± 21            |
|              | 12 min| 1167 ± 45        | 1049 ± 141          | 289 ± 20         | 293 ± 18            | 186 ± 10         | 190 ± 17            |
| Hypothalamus | 2 min | 661 ± 68         | 539 ± 64            | 430 ± 28         | 354 ± 67            | 331 ± 37         | 273 ± 24            |
|              | 6 min | 599 ± 48         | 604 ± 65            | 406 ± 28         | 403 ± 30            | 369 ± 47         | 332 ± 49            |
|              | 12 min| 743 ± 106        | 597 ± 66            | 414 ± 49         | 423 ± 54            | 351 ± 58         | 342 ± 32            |
| Hippocampus  | 2 min | 98 ± 17          | 62 ± 13             | 80 ± 5           | 65 ± 11             | 110 ± 20         | 65 ± 9              |
|              | 6 min | 71 ± 8           | 58 ± 10             | 74 ± 5           | 72 ± 16             | 88 ± 11          | 75 ± 9              |
|              | 12 min| 123 ± 52         | 89 ± 14             | 90 ± 13          | 90 ± 15             | 88 ± 4           | 97 ± 15             |
| Striatum     | 2 min | 10963 ± 1268     | 10700 ± 503         | 1611 ± 244       | 1483 ± 123          | 747 ± 55         | 721 ± 37            |
|              | 6 min | 9589 ± 544       | 10406 ± 628         | 1317 ± 136       | 1513 ± 137          | 752 ± 65         | 691 ± 71            |
|              | 12 min| 11315 ± 763      | 11323 ± 998         | 1471 ± 157       | 1900 ± 182          | 823 ± 33         | 947 ± 106           |
| Midbrain     | 2 min | 267 ± 26         | 316 ± 37            | 191 ± 17         | 204 ± 23            | 136 ± 15         | 145 ± 10            |
|              | 6 min | 298 ± 36         | 283 ± 28            | 206 ± 19         | 211 ± 13            | 149 ± 21         | 130 ± 11            |
|              | 12 min| 260 ± 20         | 298 ± 19            | 181 ± 16         | 252 ± 19*           | 155 ± 11         | 204 ± 14*           |
| Brainstem    | 2 min | 73 ± 7           | 82 ± 10             | 82 ± 5           | 76 ± 8              | 62 ± 10          | 56 ± 10             |
|              | 6 min | 78 ± 9           | 100 ± 15            | 85 ± 10          | 122 ± 15            | 77 ± 12          | 83 ± 14             |
|              | 12 min| 94 ± 9           | 89 ± 14             | 92 ± 6           | 133 ± 11**          | 65 ± 8           | 94 ± 13             |

Mice were treated as described in Table 1. Values are expressed as the mean ± S.E. (n = 6). *P < 0.05, **P < 0.01, as compared to each control.
Effects of veratramine on release of $^{3}$H-5-HT

Figure 1 shows a typical pattern of tritium overflow from frontal cortical slices preloaded with $^{3}$H-5-HT stimulated with high K$^{+}$ and veratramine. Stimulation with 25 mM KCl for 2 min caused a short-lasting overflow of tritium. This release was Ca$^{++}$-dependent (data not shown). After cessation of the stimulus, the efflux returned to the prestimulation level. Exposure to veratramine (100 $\mu$M) evoked a persistent overflow of tritium, continuing for approximately 20 min (14.1 ± 0.21% of the tissue tritium content, $n = 4$, $P < 0.01$ as compared to 3.6 ± 0.43% in the vehicle control value). The release was not modified by a Ca$^{++}$-free medium (16.2 ± 0.50%, $n = 4$, Fig. 1).

Effects of veratramine on $^{3}$H-5-HT uptake

The uptake of $^{3}$H-5-HT into the frontal cortical slices was inhibited by veratramine in a concentration-dependent fashion with an IC$_{50}$ of 0.13 $\mu$M. This value appears to be slightly higher than the IC$_{50}$ of imipramine, a well-known 5-HT uptake inhibitor, as demonstrated in Fig. 2. Using the IC$_{50}$ of veratramine, kinetic studies were carried out to determine the value of K$_{m}$ and the nature of inhibition. Plots of the reciprocals of $^{3}$H-5-HT concentration (0.05–1.0 $\mu$M) against its accu-

![Figure 1](image-url)
mulation into the frontal cortical slices in the absence and presence of veratramine revealed that the $K_m$ value obtained by least square fitting of the Lineweaver and Burk (12) transformation is 0.30 $\mu$M, and the inhibition of $^3$H-5-HT uptake by veratramine is competitive in nature (Fig. 3). The $K_i$ value of veratramine determined by the graphic method of Lineweaver and Burk (12) is 0.25 $\mu$M, approximately equal to the $K_m$ value.

### DISCUSSION

The study on the effect of veratramine on regional monoamine metabolism in the brain exhibited rather specific action on the serotonergic system; veratramine decreased the endogenous levels of 5-HT by 34% and those of 5-HIAA by 20%, during myoclonus, in the cortex. A similar tendency was obtained in the hypothalamus and hippocampus. The reduction in 5-HT content is likely to be connected to the persistent depletion of the indoleamine into the synaptic cleft, as demonstrated in the release study. The decrease in 5-HIAA content may be related to the concurrent competitive inhibition of 5-HT uptake by veratramine, which prevents the intraneuronal deamination of 5-HT by monoamine oxidase (MAO). An alternative possibility that veratramine inhibits intraneuronal MAO activity remains to be elucidated in the present study. The most interesting finding in the regional study is that the major change was found in the frontal cortex which has been reported to have the highest density of 5-HT$_2$ sites in the brain of rats (13, 14) and guinea pigs (14). This may imply that veratramine-induced myoclonus might occur in association with the depletion of 5-HT and 5-HIAA contents in the frontal cortex, a region having a large population of 5-HT$_2$ binding site. On the other hand, Smith and Peroutka suggested that specific components of the 5-HT syndrome were mediated by 5-HT$_1A$ receptors (15). Therefore, it could be that 5-HT syndrome induced by veratramine was also related to 5-HT$_1$ receptors.

Minor increases in dopamine metabolites,
without changes in dopamine content, were observed during myoclonus in the midbrain and brainstem. These results suggest that the dopamine turnover rate increased during myoclonus induced by veratramine. The exact explanation for the increased dopamine turnover can not be given at the present time. However, there is some evidence showing the relationship between dopaminergic and serotonergic neuronal activity. Giambalvo and Snodgrass (16) demonstrated that a reduction in 5-HT content following unilateral lesions of the median raphe with 5,7-dihydroxytryptamine increased the concentration of DOPAC and HVA in the ipsilateral striatum without changes in dopamine contents. Similar dopamine-serotonin interactions have been shown by Nicolaou et al. (17) who demonstrated that the decreased concentration of 5-HT and 5-HIAA caused by unilateral lesions in the dorsal or median raphe in rats resulted in increasing DOPAC and HVA in the ipsilateral substantia nigra or striatum. These findings suggest that such projected serotonin neurons may exert a tonic inhibition of their respective dopaminergic terminals. The increased dopamine turnover in the midbrain and brainstem, where dopaminergic neurons are contained, may be the consequence of the reduced contents of 5-HT and 5-HIAA by veratramine. This hypothesis may be supported by the fact that dopamine metabolites were unchanged in the cortex, hypothalamus and hippocampus, the regions that are not rich in dopaminergic-serotonergic fiber connection.

It was found in the present study that 3H-5-HT preloaded in the frontal cortical slices was released in a Ca++-independent manner. The mode of release differed from that induced by depolarization in that the release by veratramine was long-lasting (approximately 20 min following the 2-min exposure), while the release by high 25 mM K+ was transitory. Therefore, the mechanism of 3H-5-HT release elicited by veratramine may be different from the Ca++-dependent depolarization mechanism. Veratramine may act like p-chloroamphetamine or fenfluramine releasing 5-HT through a carrier-dependent mechanism (18). Recently, it has been shown that MAO inhibitors are able to increase a component of non-calcium dependent, depolarization-induced release of 3H-5-HT through the plasma membrane carrier (19). Therefore, a part of the non-calcium dependent release of 3H-5-HT evoked by veratramine may be due to the concurrent use of pargyline which was applied throughout the present release study.

3H-5-HT uptake into the cortical slices was inhibited by veratramine in a concentration-dependent manner with an IC50 of 0.13μM. Based on these biochemical data, the most potent site of action for veratramine may be the 5-HT uptake system, since veratramine appears to be more potent at this site compared to its releasing agent effects. Thus, the excessive overflow of tritium observed in the release study may be largely due to the uptake inhibition. The Ks value (0.25μM) for the competitive inhibition of 3H-5-HT uptake by veratramine is close to the Km value (0.30μM), reflecting the affinity of veratramine for 5-HT uptake sites. Although these findings suggest that veratramine stimulates the release of 5-HT in addition to blocking its uptake, the observed persistent effect of tritium overflow appears to be more consistent with behavioral results in which myoclonic movements continued for approximately 24-26 min after the i.p. injection of veratramine (3.0 mg/kg).

In the present study, it is concluded that veratramine acts at presynaptic serotonin neurons and evokes a non-calcium dependent 5-HT release, together with a competitive uptake inhibition. This may be the primary mechanism by which veratramine produces myoclonus and tremor in mice.

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