Effects of Idebenone (CV-2619) on Metabolism of Monoamines, Especially Serotonin, in the Brain of Normal Rats and Rats with Cerebral Ischemia

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Abstract—The effects of 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619) on the contents, turnover, release and uptake of monoamines, especially serotonin (5-HT), in various brain regions of Wistar rats were studied in vivo and in vitro. In normal rats, an intraperitoneal (i.p.) dose of 100 mg/kg of CV-2619 had no significant effect on the levels of norepinephrine (NE), dopamine (DA) and their metabolites, and 5-HT in the brain regions examined, but it increased the levels of 5-hydroxyindole-3-acetic acid (5-HIAA), the main metabolite of 5-HT, in many brain regions. In rats with cerebral ischemia, a low dose (10 mg/kg, i.p.) of CV-2619 normalized the decreased levels of 5-HIAA in the cerebral cortex, hippocampus, diencephalon and brain stem. A 5-HT biosynthesis inhibitor, DL-p-chlorophenylalanine (PCPA, 150 mg/kg, i.p.), decreased the levels of 5-HT in all brain regions to one-third of the control levels 24 hr after administration in normal rats. CV-2619 (10, 30 or 100 mg/kg, i.p.), administered 24 hr after the treatment with PCPA, accelerated the PCPA-induced 5-HT decreases in the hippocampus, diencephalon and brain stem in a dose-dependent manner. In vitro CV-2619, like p-chloroamphetamine (PCA), stimulated 5-HT release from slices of the hippocampus and diencephalon. CV-2619 slightly inhibited and PCA markedly inhibited 5-HT uptake into hippocampal slices. The mechanism of the 5-HT releasing action of CV-2619 in hippocampal slices seems to be mediated through endogenous calcium. These results suggest that CV-2619 has an enhancing effect on the turnover of 5-HT in the hippocampus, diencephalon and brain stem of rats.

A novel quinonyl compound, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619), is being developed as a drug that improves brain metabolism and that has unique pharmacological actions such as protective action on spontaneous occurrence of stroke and cerebral ischemia induced-stroke in stroke-prone spontaneously hypertensive rats, and has an ameliorating effect on cerebral ischemia-induced learning and memory disturbances in rats (1, 2). A neurochemical study of the effects of CV-2619 on monoamine metabolism in rat brain has not been undertaken.

In the present study, we used high performance liquid chromatography (HPLC) with electrochemical detection (ECD) to examine the effect of CV-2619 on monoamine metabolism in normal rats and rats with cerebral ischemia. As it was found in the present examination that CV-2619 increased 5-HT turnover in various brain regions of normal rats, the effect of CV-2619 on the release and uptake of radiolabelled 5-HT in rat brain slices was also investigated; the results obtained were compared to those obtained with a 5-HT releasing agent, p-
chloroamphetamine (PCA).

Materials and Methods

Animals: Male Wistar rats (9–11 weeks old) were used for all experiments.

Materials: The following drugs and analytical grade of reagents were used: CV-2619 [6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone)] (Takeda); DL-p-chlorophenylalanine (PCPA), and dihydroxyphenylacetic acid (DOPAC) (Aldrich Chemical); p-chloroamphetamine (PCA), norepinephrine (NE), 5-hydroxytryptamine creatinine sulfate complex (5-HT), isoproterenol HCl, 3-methoxy-4-hydroxyphenylacetic acid (HVA), tetrodotoxin, ouabain octahydrate, and sulfatase (type H-1) (Sigma); Alumina (neutral activity grade 1) (Woelm); pargyline HCl (Nakarai Chemical); Amberlite CG-50 (type-2) (Rohm and Harse); ethylenediaminetetraacetic acid (EDTA), and glycoletherdiamine-N,N,N',N'-tetraacetic acid (EGTA) (Dojin Yakukagaku); dopamine HCl (DA) and 5-hydroxyindole-3-acetic acid (5-HIAA) (Tokyo Kasei); (3-methoxy-4-sulfoxyphenyl)-glycol K (MHPG-SO_4) (Fluka); 3-methoxy-4-hydroxyphenyglycol piperazine (MHPG) (Calbiochem-Behring); Pic B-7 (1-heptane-sulfonic acid) (Waters); atropine sulfate (Merck); imipramine HCl (Fujisawa); [14C]-5-HT (hydroxytryptamine binoxalate, 5-[2-14C])., specific activity 50.7 mCi/mmol (New England Nuclear).

Drug treatments: The time-course effect of CV-2619 on the levels of NE, DA, 5-HT and their metabolites was assessed at 30, 60 and 120 min after administration (i.p.) in a dose of 100 mg/kg. Control animals were administered with its vehicle (5% arabic gum saline). The levels of monoamines and their metabolites were examined for a dose dependency at 60 min after CV-2619 was administered at doses of 10, 30 and 100 mg/kg (i.p.). The levels of monoamines, especially 5-HT, in the hippocampus, diencephalon and brain stem were also studied after pretreatment with PCPA (150 mg/kg, i.p.). The rats received PCPA 24 hr before and were killed 30 min after being treated with CV-2619 (10, 30 or 100 mg/kg, i.p.).

Preparation of rats with cerebral ischemia: Cerebral ischemia was induced by the method of Pulsinelli and Brierley (3). Briefly, under pentobarbital anesthesia, the bilateral vertebral arteries were cauterized by a bipolar coagulator (Mizuho Ika Kogyo, Micro-1D), and silk threads were placed around both common carotid arteries without interrupting carotid blood flow. On the next day, the rats were restrained by hand, subjected to bilateral common carotid artery occlusion for 200 sec by pulling the silk threads, and then killed by microwave irradiation (5 KW for 1.5 sec) (Toshiba, TMW-6402). CV-2619 (3 and 10 mg/kg, i.p.) was administered 30 min before bilateral carotid occlusion.

Determination of cerebral monoamines and their metabolites: The head of control or drugs-treated rats was exposed to the microwave irradiation and then cooled in an ice-water bath for 30 min to prevent water loss from the brain tissue. The brain was removed and dissected into 6 regions: cerebral cortex, hippocampus, nucleus accumbens+striatum, hypothalamus+thalamus (diencephalon), brain stem and cerebellum. These tissues were weighed and homogenized in 5–30 volumes of 0.05 M perchloric acid containing 0.1 M EDTA, and glycoaldehydeimine-N,N,N',N'-tetraacetic acid (EGTA) (Dojin Yakukagaku); dopamine HCl (DA) and 5-hydroxyindole-3-acetic acid (5-HIAA) (Tokyo Kasei); (3-methoxy-4-sulfoxyphenyl)-glycol K (MHPG-SO_4) (Fluka); 3-methoxy-4-hydroxyphenyglycol piperazine (MHPG) (Calbiochem-Behring); Pic B-7 (1-heptane-sulfonic acid) (Waters); atropine sulfate (Merck); imipramine HCl (Fujisawa); [14C]-5-HT (hydroxytryptamine binoxalate, 5-[2-14C]), specific activity 50.7 mCi/mmol (New England Nuclear).
was transferred to a glass-stoppered test tube containing ascorbic acid (100 µg) and (NH₄)₂SO₄ (2 g), and extracted with three successive 5 ml portions of diethylether. The diethylether extract was centrifuged at 1,000 g for 5 min at 4°C and evaporated under N₂ gas to a small volume. The residue was then shaken for 10 min with 10 ml of n-heptane and 0.2 ml of 0.1 M perchloric acid containing isoproterenol as an internal standard. After the mixture was centrifuged at 1,000 g for 5 min at 4°C, an aliquot of the aqueous layer was used for an assay.

The hydrolysis of conjugated MHPG (MHPG-SO₄) was performed using sulfatase as follows: The acidic supernatant of the tissue homogenate was adjusted to pH 4.5 with 0.2 M NaOH and 0.1 M acetate buffer (pH 4.5), and then it was incubated for 2 hr at 37°C with sulfatase (10 mg per tube). After hydrolysis, the pH was adjusted to 2.3 with HCl, and the diethylether extraction was carried out as described above. The MHPG levels in the results refer to a total of the free and conjugated forms. Recoveries of metabolites in the whole procedures were DOPAC, 66.9%; MHPG, 86.5%; MHPG-SO₄, 78.4%; 5-HIAA, 63.0%, and HVA, 84.1%. The final concentration of monoamines and their metabolites were corrected by the percent recoveries of the respective monoamine and metabolite and percent recovery of isoproterenol as an internal standard.

Condition of HPLC/ECD: The analysis was performed by separation on a reversephase column (Nucleosil 5C-18, 4 mm i.d. × 15 cm), with a mobile phase of 0.05 M citrate-acetate buffer (pH 4.8) containing 3.5% acetonitrile for the analysis of metabolites or 1% acetonitrile and 0.005 M 1-heptanesulfonic acid for the analysis of monoamines, pumped at a flow rate 0.8 ml/min. The mobile phase buffer was degassed using a vacuum pump before use. The potential of the ECD was set at 0.8 V vs. a Ag/AgCl reference electrode. The retention time of each peak was confirmed by those of authentic monoamines and their metabolites. Measurement of 5-HT release from brain slices: 5-HT release from brain slices was measured using the method reported previously, with a minor modification (4). Ten rats were decapitated for each experiment, and the hippocampus and diencephalon were excised from each one. These brain regions were sliced to a thickness of 0.5 mm with a McIlwain tissue chopper and dispersed in 95% O₂ - 5% CO₂ saturated Krebs-Ringer phosphate buffer (KRPB) containing 0.1 mM pargyline and 10 mM glucose, and were then centrifuged at 2,000 g for 5 min at 4°C. Slices of 40 mg wet weight were put into test tubes containing 2 ml of KRPB, incubated with 1 µCi of [¹⁴C]-5-HT for 60 min at 37°C, repeatedly rinsed with fresh KRPB, and centrifuged three times. The slices, thus preloaded with [¹⁴C]-5-HT were superfused with KRPB at a constant rate of 0.2 ml/min in a superfusion apparatus. The radioactivities of [¹⁴C] in the superfusate effluents collected every 10 min for 30 min and every 5 min from 30 to 90 min after the start of superfusion and radioactivity in the supernatant (3,000 g for 10 min at 4°C) of the 5% trichloroacetic acid homogenized slices were counted using a liquid scintillation spectrophotometer with ACS® II scintillation. CV-2619 dissolved in 1% ethanol and other agents dissolved in KRPB or their vehicles were superfused from 60 to 75 min after the start of superfusion.

Measurement of 5-HT uptake into hippocampal slices: The 0.5 mm thick slices (20 mg wet weight) of hippocampus were incubated for 10 min at 37°C with 0.5 µCi of [¹⁴C]-5-HT in 95% O₂-5% CO₂ saturated KRPB. Immediately after the incubation, the slices were centrifuged at 3,000 g for 5 min at 4°C and then repeatedly rinsed with fresh KRPB, and this procedure was repeated three times. The radioactivities in the supernatant of the 0.2 N NaOH homogenized slices were counted by liquid scintillation. An aliquot of the same supernatants was assayed for protein using the Folin reagent (5).

Statistics: Statistical comparisons between different treatments were made using Student’s t-test (two-tailed).

Results
1. Effect on the levels of monoamines and their metabolites in various brain regions of normal rats: As shown in Table 1, when compared
Table 1. Effects of CV-2619 on the levels of monoamines and their metabolites in various brain regions of rats

| Brain regions     | Treatments (mg/kg, i.p.) | Monoamines | Metabolites | Metabolites |
|-------------------|--------------------------|------------|-------------|-------------|
|                   |                          | NE         | DA          | 5-HT        | Total MHPG  | DOPAC       | HVA         | 5-HIAA      |
|                   |                          | (ng/g wet weight) |            |             |             |             |             |             |
| Cerebral cortex   | Saline                   | 351±24     | 571±36      | 1206±10     | 112±10      | 109±12      | 107±7       | 167±11      |
|                   | CV-2619 (100)            | 322±32     | 576±44      | 1105±127    | 150±22      | 130±14      | 136±14      | 276±38*     |
| Hippocampus       | Saline                   | 443±51     | 331±33      | 1296±60     | 94±5        | 75±7        | 55±4        | 332±34      |
|                   | CV-2619 (100)            | 447±29     | 346±12      | 1260±61     | 121±11      | 104±17      | 70±7        | 549±52**    |
| N. Accumbens      | Saline                   | 514±50     | 5404±310    | 1718±61     | 120±15      | 993±67      | 696±37      | 534±58      |
| + Striatum        | CV-2619 (100)            | 481±24     | 6116±549    | 1593±92     | 119±14      | 1175±155    | 869±107     | 617±54      |
| Hypothalamus      | Saline                   | 699±61     | 384±44      | 1697±96     | 150±16      | 108±11      | 73±5        | 268±12      |
| + Thalamus        | CV-2619 (100)            | 661±21     | 448±29      | 1705±64     | 181±19      | 129±15      | 97±6*       | 592±25**    |
| (Diencephalon)    |                          |            |             |             |             |             |             |             |
| Brain stem        | Saline                   | 453±38     | 87±13       | 1802±99     | 141±23      | 43±7        | 42±2        | 268±12      |
|                   | CV-2619 (100)            | 476±34     | 117±18      | 1798±84     | 122±16      | 68±7*       | 54±5        | 639±50***   |
| Cerebellum        | Saline                   | 235±24     | 36±3        | 300±42      | 94±8        | 38±7        | 21±2        | 54±3        |
|                   | CV-2619 (100)            | 239±23     | 37±2        | 258±45      | 78±3        | 64±8        | 26±3        | 97±5*       |

All values are the mean±S.E.M. of 6 rats. *P<0.05, **P<0.01, ***P<0.001 vs. each saline group. CV-2619 (100 mg/kg, i.p.) was administered 30 min before microwave irradiation (5 KW, 1.5 sec). Total MHPG=conjugate+free MHPG.
with the saline control. CV-2619 (100 mg/kg, i.p.) suspended in 5% arabic gum saline (saline) had no significant effects on the levels of NE, DA and 5-HT 30 min after administration. It slightly increased DA metabolites, DOPAC and HVA in the diencephalon and brain stem, but not the NE metabolite. However, it markedly increased the 5-HT metabolite 5-HIAA in the brain stem, diencephalon, hippocampus and cerebellum. The increase was 155–238% of the saline control, and it was largest in the brain stem.

The time-course and dose-response studies of the effects of CV-2619 on the levels of monoamine metabolites, especially 5-HIAA, were examined. Time-course: The time-course study (30, 60 and 120 min after the administration) of the effect of CV-2619 (100 mg/kg, i.p.) on the levels of monoamine metabolites in various brain regions is summarized in Table 2. CV-2619 markedly increased the 5-HIAA levels in the cerebral cortex, hippocampus, diencephalon and brain stem 30 to 60 min after administration. The maximum effect was observed at 30 min in the brain stem and at 60 min in the other brain regions; control levels were recorded at 120 min. CV-2619 produced a slight increase in the levels of the DA metabolites DOPAC, in the hippocampus, and HVA, in the cerebral cortex and brain stem, 60 min after administration, but had no effect on the NE metabolite MHPG.

Dose-response: CV-2619 in doses of 10, 30 and 100 mg/kg (i.p.) did not affect the levels of MHPG, DOPAC and HVA in this experiment (data not shown), but the highest dose of the drug increased the levels of 5-HIAA in the hippocampus, diencephalon and brain stem (Table 3).

Hence, CV-2619 was found to increase selectively the 5-HIAA levels in the hippocampus, diencephalon and brain stem 30–60 min after the treatment.

2. Effect on the levels of monoamines and their metabolites in various brain regions of rats with cerebral ischemia

CV-2619 at doses of 3 and 10 mg/kg, i.p., were given 30 min before bilateral carotid occlusion. As shown in Table 4, 5-HIAA levels in the cerebral cortex, hippocampus, diencephalon and brain stem of the cerebral

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Table 2. Effects of CV-2619 on the levels of monoamine metabolites in various brain regions of rats: time-course study

| Brain region  | Treatments (mg/kg, i.p.) | Min after administration | Free MHPG (ng/g wet weight) | DOPAC (ng/g wet weight) | HVA (ng/g wet weight) | 5-HIAA (ng/g wet weight) |
|---------------|--------------------------|--------------------------|-----------------------------|-------------------------|----------------------|-------------------------|
| Cerebral cortex | Saline                   | 30–60                    | 102±11                      | 104± 5                  | 124± 7               | 143±16                  |
|               | CV-2619 (100)            | 30                       | 115±19                      | 118± 9                  | 141± 9               | 205±32                  |
|               |                          | 60                       | 88± 5                       | 118± 3                  | 143± 4*              | 221±18*                 |
|               |                          | 120                      | 69± 1                       | 101± 9                  | 123± 7               | 130±35                  |
| Hippocampus   | Saline                   | 30–60                    | 84± 9                       | 87± 7                   | 74± 8                | 321±33                  |
|               | CV-2619 (100)            | 30                       | 86±17                       | 85± 8                   | 73± 6                | 488±38**                |
|               |                          | 60                       | 73± 7                       | 108± 5*                 | 87± 7                | 598±64**                |
|               |                          | 120                      | 62± 8                       | 79±16                   | 78±6                 | 415±62                  |
| Diencephalon  | Saline                   | 30–60                    | 63± 8                       | 140±22                  | 89± 9                | 456±57                  |
|               | CV-2619 (100)            | 30                       | 76±13                       | 132± 9                  | 96± 4                | 670±34**                |
|               |                          | 60                       | 65± 5                       | 132± 5                  | 100± 6               | 779±53**                |
|               |                          | 120                      | 67± 4                       | 121±16                  | 92± 4                | 429±22                  |
| Brain stem    | Saline                   | 30–60                    | 104± 2                      | 51± 9                   | 44± 2                | 367±23                  |
|               | CV-2619 (100)            | 30                       | 125± 9                      | 62± 3                   | 52± 5                | 565±61**                |
|               |                          | 60                       | 117± 4                      | 56± 3                   | 60± 4**              | 511±57*                 |
|               |                          | 120                      | 116± 6                      | 52± 2                   | 49± 6                | 311±61                  |

All values are the mean±S.E.M. of 4–6 rats. *P<0.05. **P<0.01 vs. each saline group.
ischemic control group were decreased as compared with those of the sham-operated group, but other monoamines and metabolites were not significantly affected by the cerebral ischemic treatment (data not shown). CV-2619 (10 mg/kg, i.p.) normalized the decreased levels of 5-HIAA observed in the rats with cerebral ischemia, but 3 mg/kg had no significant effect (Table 4).

3. Effect on p-chlorophenylalanine (PCPA)-induced 5-HT decrease in normal rats

PCPA in doses of 150, 200 and 300 mg/kg (i.p.) dose-dependently decreased the levels of 5-HT, but not those of NE and DA, in the whole brain 24 hr after administration in the preliminary experiments. In subsequent experiments, the lowest dose of PCPA was administered i.p., and the rats were killed 24 hr later.

As shown in Table 5, PCPA decreased the 5-HT contents to about one-third of the normal values (Table 1) in many brain regions. CV-2619 (10, 30 and 100 mg/kg, i.p.) administered 24 hr after the treatment with PCPA accelerated the PCPA-induced 5-HT decrease in the hippocampus, diencephalon and brain stem in a dose-dependent manner.

These results suggest that CV-2619 has an enhancing effect on the turnover of 5-HT in the hippocampus, diencephalon and brain stem of rats.

4. Effect on the release of \(^{14}\text{C}\)-5-HT from brain slices

As shown in Fig. 1, the addition of CV-2619 (10\(^{-5}\), 3\times10\(^{-5}\) and 10\(^{-4}\) M) to the superfusion medium stimulated the \(^{14}\text{C}\)-5-HT release from slices of hippocampus (upper
Table 5. Effects of CV-2619 on p-chlorophenylalanine (PCPA)-induced 5-HT decreases in various brain regions of rats

| Brain region                  | Saline   | Dose of CV-2619 (mg/kg, i.p.) |
|-------------------------------|----------|------------------------------|
|                               | 5-HT levels (ng/g wet weight) | 10     | 30    | 100   |
| Cerebral cortex               | 500±15   | 586±140                      | 546±20 | 486±24 |
| Hippocampus                   | 470±24   | 454±24                       | 389±15*| 327±20**|
| N. Accumbens + Striatum       | 646±25   | 832±169                      | 655±22 | 735±30 |
| Diencephalon                  | 571±29   | 536±25                       | 495±15*| 444±19**|
| Brain stem                    | 571±29   | 516±18                       | 477±10**| 474±15**|
| Cerebellum                    | 192±13   | 193±4                        | 170±8  | 195±10 |

All values are the mean±S.E.M. of 5–6 rats. *P<0.05, **P<0.01, ***P<0.001 vs. each saline group. PCPA (150 mg/kg, i.p.) and CV-2619 were administered 24 hr and 30 min before the microwave irradiation, respectively.

Fig. 1. Effects of CV-2619 on the release of [14C]-5-hydroxytryptamine (5-HT) from slices of hippocampus (upper panel) and diencephalon (lower panel) of rats. The drug was superfused from 60 to 75 min after the start of superfusion.

Fig. 2. Effects of p-chloroamphetamine (PCA) on the release of [14C]-5-hydroxytryptamine (5-HT) from slices of hippocampus (upper panel) and diencephalon (lower panel) of rats. The drug was superfused from 60 to 75 min after the start of superfusion.

A 5-HT releasing agent, p-chloroamphetamine (PCA) at 10^-7-10^-4 M, markedly stimulated the [14C]-5-HT release from slices of hippocampus (upper panel of Fig. 2) and diencephalon (lower panel of Fig. 2) in a concentration-dependent manner. The PCA-induced 5-HT release was much more marked in the diencephalon than the hippocampus.
Effect on the uptake of $[^{14}\text{C}]-5$-HT into brain slices

As shown in Table 6, the addition of CV-2619 (3 x 10^{-5} and 10^{-4} M) and PCA (10^{-5}-10^{-4} M) significantly inhibited the $[^{14}\text{C}]-5$-HT uptake into hippocampal slices.

The mechanism of 5-HT releasing action of CV-2619

Omitting of Ca^{2+} in the superfusate or adding a Na^{+} channel blocker (tetrodotoxin, 10^{-4} M), a (Na^{+}-K^{+})-stimulated ATPase inhibitor (ouabain, 5 x 10^{-3} M) or a muscarinic receptor blocker (atropine, 10^{-4} M) did not modify the 5-HT releasing effect of CV-2619 (10^{-4} M) from hippocampal slices (data not shown). However, the CV-2619-enhanced 5-HT release was markedly blocked by combining the addition of a Ca^{2+} chelator (EGTA, 5 x 10^{-3} and 10^{-2} M) and removal of Ca^{2+} from the superfusate (Fig. 3).

Discussion

The results presented above demonstrate that CV-2619 at doses of 100 mg/kg (i.p.) in normal rats and at 10 mg/kg (i.p.) in rats with cerebral ischemia produced marked increases in the 5-HT main metabolite 5-HIAA in the hippocampus, diencephalon and brain stem, but showed no effects on the monoamine contents and only slight effects on the DA metabolites DOPA and HVA in normal rats. CV-2619 (30 and 100 mg/kg, i.p.) stimulated PCPA-induced 5-HT decreases in the brain regions in which the increases in 5-HIAA were observed in normal rats. These results suggest that CV-2619 has an accelerating effect on the turnover of 5-HT in these brain regions. Interestingly, cerebral ischemia produced a marked

Table 6. Effects of CV-2619 and p-chloroamphetamine (PCA) on the uptake of $[^{14}\text{C}]-5$-hydroxytryptamine (5-HT) into hippocampal slices of rats

| Treatment       | Concentration (M) | Uptake of $[^{14}\text{C}]-5$-HT (dpm/mg protein/10 min) |
|-----------------|-------------------|----------------------------------------------------------|
|                 |                   | %                                                        |
| Control (1% ethanol) | 10^{-5}           | 2478±141 (100)                                           |
| CV-2619         | 3 x 10^{-5}       | 2443±117 (98)                                            |
|                 | 10^{-4}           | 2019±43** (81)                                           |
|                 |                   | 1920±72** (77)                                           |
| Control PCA     | 10^{-6}           | 2707±180 (100)                                           |
|                 | 10^{-5}           | 2356±75 (87)                                             |
|                 | 10^{-4}           | 1715±67** (63)                                           |
|                 |                   | 1601±140*** (59)                                         |

All values are the mean±S.E.M. of 8 determinations. **P<0.01, ***P<0.001 vs. each control group.

Fig. 3. Effects of Ca^{2+}-free medium plus EGTA on the CV-2619-induced release of $[^{14}\text{C}]-5$-hydroxytryptamine (5-HT) from hippocampal slices of rats. All values are the mean±S.E.M. of 4 determinations. *P<0.05, **P<0.01, ***P<0.001 vs. each control.

Fig. 3. Effects of Ca^{2+}-free medium plus EGTA on the CV-2619-induced release of $[^{14}\text{C}]-5$-hydroxytryptamine (5-HT) from hippocampal slices of rats. All values are the mean±S.E.M. of 4 determinations. *P<0.05, **P<0.01, ***P<0.001 vs. each control.
decrease in 5-HIAA in the cerebral cortex, diencephalon and brain stem. CV-2619 normalized the decrease in 5-HIAA at a lower dose (10 mg/kg, i.p.) compared with the dose (100 mg/kg, i.p.) required for enhancing the turnover of 5-HT in the brain regions of normal rats. This indicates that CV-2619 exerts a more prominent effect on 5-HT metabolism in rats with the cerebral ischemia than in normal rats. The addition of CV-2619 (3×10⁻⁵ and 10⁻⁴ M) to the superfusion medium markedly stimulated the prelabelled [¹⁴C]-5-HT release, and it slightly inhibited the [¹⁴C]-5-HT uptake in hippocampal slices. This in vitro result supports the above-mentioned in vivo findings.

A 5-HT releasing agent, PCA, facilitated 5-HT release and inhibited 5-HT uptake. The PCA-induced 5-HT release was much more marked in the diencephalon than in the hippocampus. PCA was reported to lower 5-HIAA and reduce the turnover of 5-HT (6). Although CV-2619 inhibited the 5-HT uptake, it enhanced the levels of 5-HIAA and turnover of 5-HT. Thus, the effects of CV-2619 on 5-HT metabolism seem to be different from those of PCA.

The fact that CV-2619 facilitated the 5-HT turnover suggests that the drug may stimulate the biosynthesis and release of 5-HT. The effect of CV-2619 on the biosynthesis of 5-HT has not been examined yet, but the release of 5-HT was confirmed in the present in vitro experiments. The mechanism of the 5-HT releasing action of CV-2619 was not mediated through exogeneous calcium, (Na⁺-K⁺)-ATPase or the cholinergic system, but through endogeneous calcium. However, the precise mechanism is likely to be very complicated and further study is needed to solve this problem.

Charney et al. reported that the intravenous infusion of L-tryptophan (TRP) caused robust increases in prolactin and growth hormone in healthy humans; the subjects were reported to feel significantly “higher”, more mellow and drowsier after the TRP infusion than after a placebo infusion (7). Studies conducted in both animals and humans have demonstrated that TRP administration increases the 5-HT turnover in the brain (8). These findings indicate an important role of 5-HT in mood regulation. Therefore, CV-2619 may elevate the feeling or improve the mood of patients with cerebrovascular disorders, because it improved the decrease in 5-HIAA in the rats with cerebral ischemia.

The exact mechanism of the improving effect of CV-2619 in the rats with cerebral ischemia is not clear. However, the drug had no effect on cerebral blood flow, but had an ameliorating effect on cerebral energy metabolism in the rats with cerebral ischemia (1). Therefore, the normalizing effect of CV-2619 on the decrease in 5-HIAA in the rats with cerebral ischemia seems to be due to both ameliorating cerebral energy metabolism and enhancing the turnover of 5-HT.

Weigartner et al. reported that subjects treated with low or high dose of ethanol demonstrated impaired memory, particularly in tests involving the recall of poorly learned information, and that zimelidine, a relatively specific 5-HT uptake blocker, reversed this ethanol-induced impairment (9). Winblad et al. reported that the levels of monoamines, especially 5-HT, in the cerebral cortex, hippocampus, striatum and hypothalamus were all reduced in senile dementia and chronic alcoholism (10). From these reports, it is speculated that the hypofunction of the 5-HT system may be closely related to the pathogenesis in senile dementia and memory impairment due to chronic alcoholism and cerebral stroke. As demonstrated in the present results, CV-2619 enhanced the 5-HT turnover in vivo and in vitro, especially in the hippocampus and diencephalon. Therefore, it is anticipated that CV-2619 may improve memory impairment in patients with senile dementia and cerebral stroke.

In conclusion, CV-2619 seems to exert an enhancing effect on the turnover of 5-HT in the hippocampus, diencephalon and brain stem of the brain in normal rats. The finding is consistent with the in vitro results indicating that CV-2619 selectively stimulates 5-HT release from hippocampal slices. The stimulation of 5-HT release seems to be mediated by endogeneous calcium. Furthermore, the compound improved the decrease in a 5-HT metabolite, 5-HIAA, induced by cerebral ischemia in rats.

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