Effect of Long-Term Fluvoxamine Treatment on Endogenous Serotonin Uptake Inhibitor-Like Substance in Monkey Cerebrospinal Fluid

Toru Egashira, Shinichiro Goto, Yuji Wada, Fusako Takayama and Yasumitsu Yamanaka

Department of Pharmacology, Oita Medical University, 1-1, Idaigaoka, Hasama-machi, Oita 879-55, Japan

ABSTRACT—We found that substances present in monkey cerebrospinal fluid (CSF) could inhibit [3H]-paroxetine binding in monkey brain preparations. The molecular weight of one of these inhibitory substances was approximately 2000, which is in agreement with earlier studies using human CSF. We also found that the inhibitory effect of the substances present in monkey CSF on [3H]-paroxetine binding decreased after 8 weeks of chronic fluvoxamine (5 mg/kg day, p.o.) treatment. These results suggest that the ability of a drug to decrease the activity of endogenous 5-HT uptake modulators may related to its anti-depressive effects.

Keywords: 5-HT uptake inhibitor, Fluvoxamine, Cerebrospinal fluid (monkey)

Many laboratories have reported that endogenous substances that modulate the 5-hydroxytryptamine (5-HT, serotonin) transport systems in the central nervous system (CNS) are present in rat plasma and brain (1, 2), monkey brain (3), human plasma (4, 5) and human cerebrospinal fluid (CSF) (6). Recently, [3H]-paroxetine has been used as a new and highly selective ligand for the study of neuronal 5-HT transport. Moreover, [3H]-paroxetine binding sites appear to be associated with neuronal 5-HT uptake sites while [3H]-imipramine binding sites are not (7, 8).

We previously reported that human CSF contains substances that modulate [3H]-paroxetine binding sites and 5-HT uptake sites and that the molecular weight of one of these substances was about 2000 (9). However, there are no studies on the changes in the amounts of these endogenous substances in depressive animal models. In this study, we investigated the presence of 5-HT uptake inhibitor-like substances in monkey CSF, by measuring the ability of CSF samples to inhibit [3H]-paroxetine binding. We then studied the effect of chronic treatment with fluvoxamine (10) (a selective 5-HT uptake-inhibiting antidepressant) on the ability of monkey CSF to inhibit [3H]-paroxetine binding.

Six Japanese monkeys (Macaca fuscata, male, 6–8 years old; donated from the Animal Center, Oita Medical University) were kept in individual cages for 30 days before the experiments were begun in order to acclimate them to the experimental conditions. They were given 5 mg/kg/day of fluvoxamine (p.o. in their drinking water; donated by Solvay-Meiji Yakuhin Co., Ltd., Tokyo) for 8 weeks. A sample of CSF was taken from each animal on the day before fluvoxamine treatment was begun and at 2, 4, 6 and 8 weeks after treatment was begun. The procedure was performed under Ketalar (Sankyo Co., Ltd., Tokyo) anesthesia (30 mg/kg, s.c.), between 10:00 a.m. and 11:00 a.m. Throughout the treatment period, the animals had free access to food and water, and no changes in body weight, appetite, or tissue hydration were observed. There was no indication of meningeal irritation. Our study was performed according to the Oita Medical University guide for the care and use of laboratory animals.

[3H]-Paroxetine binding to monkey cortical membrane was performed according to the method of Harbert et al. (11). Monkey frontal cortices were homogenized in 25 volumes of ice-cold 50 mM Tris-HCl buffer, containing 100 mM NaCl and 5 mM KCl (pH 7.4). The P2 fractions obtained by centrifugation of this homogenate were used as the crude membrane preparations for the assays (final concentration of approximately 0.1 mg protein/tube). Aliquots of the crude membrane suspension were incubated with 50 pM [3H]-paroxetine (New England Nuclear Corp., Tokyo) at 22°C, at a final volume of 250 μl, for 180 min. Fluoxetine (final concentration of 10 μM; a gift from Lilly Research Laboratories, Indianapolis, IN, USA) was used to determine non-specific binding. For the investigation of the effect of endogenous substances in CSF on paroxetine binding, the crude membrane prepara-
tions were preincubated with a CSF sample before the \[^{3}H\]-paroxetine was added. The incubation was terminated by rapid filtration of the membrane suspension (under reduced pressure) through Whatman GF/B glass fiber filters. Each filter was rapidly washed three times with 5 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The filters were then dried, and radioactivity was determined in Triton X-100-toluene scintillation fluid using a liquid scintillation spectrometer (Pharmacia 1409, Uppsala, Sweden).

As previously described (9), 5 ml of pooled monkey CSF was chromatographed on a Sephadex G-25 column (1.9 x 87 cm) with 1 mM phosphate buffer (pH 7.4), at a flow rate of 103 ml/hr, and effluent fractions of 5.15 ml/tubes were collected. Column effluent fractions were monitored by measuring the absorbance at 280 nm. Each fraction was tested for its ability to inhibit \[^{3}H\]-paroxetine binding, as described above. We found five different effluent fractions that inhibited \[^{3}H\]-paroxetine binding. This profile was very similar to that which we obtained for human CSF (9). On the basis of it's Ve/Vo ratio, the first fraction may contain a high molecular weight protein (Fig. 1) such as albumin or \(\alpha_1\)-acid glycoprotein. These substances can alter imipramine binding and 5-HT uptake (5, 12, 13). The third, fourth and fifth inhibitory fractions probably represented endogenous substances of low molecular weight, so we analyzed these peaks by HPLC with electrochemical detection (Eicom Co., Ltd., Kyoto) to determine if any monoamines were present. We found 5-HT, 5-HIAA and several unidentified compounds (data not shown); and we suspect that the inhibition of \[^{3}H\]-paroxetine binding by these fractions may be due to monoamines, their metabolites or some other unidentified compound.

The second inhibitory fraction (F-2) was at a Ve/Vo of 1.80, with a molecular weight of about 2000. Addition of a small aliquot of F-2 markedly inhibited \[^{3}H\]-paroxetine binding. Under the assay conditions described, 25 \(\mu\)l of F-2 inhibited \[^{3}H\]-paroxetine binding by 44% in a non-competitive manner (data not shown). In addition, we found fractions from the same CSF sample that enhanced \[^{3}H\]-paroxetine binding. Endogenous substances that enhance \[^{3}H\]-paroxetine binding have previously been reported (5, 14), so we did not study these fractions in detail.

We investigated the effects of chronic fluvoxamine treatment (5 mg/kg/day, p.o., for 8 weeks) on the ability of the F-2 fraction to inhibit \[^{3}H\]-paroxetine binding. As shown in Fig. 2, addition of 100 \(\mu\)l of the F-2 fraction obtained from gel chromatography of control monkey CSF inhibited the \[^{3}H\]-paroxetine binding by 45%. However, when each F-2 fraction obtained from gel chromatography of fluvoxamine-treated monkey CSF was added, the ability of F-2 to inhibit \[^{3}H\]-paroxetine binding was significantly decreased.

The present study demonstrates that substances capable of inhibiting \[^{3}H\]-paroxetine binding are present in monkey CSF. These results indicate that an endogenous 5-HT uptake inhibitor-like substances exist in monkey brain and they may play a role in the regulation of the serotonergic system. Moreover, chronic administration of fluvoxamine, a selective 5-HT uptake-inhibiting antidepressant, decreases the ability of F-2 in monkey CSF to inhibit \[^{3}H\]-paroxetine binding. It is possible that the amount of 5-HT uptake inhibitor-like substances in monkey CSF decreased by chronic administration of

![Fig. 1. Typical profile of gel filtration of monkey CSF on Sephadex G-25. Each monkey CSF sample was chromatographed on a Sephadex G-25 column with 1 mM phosphate buffer, pH 7.4, at a flow rate of 103 ml/hr, and fractions of 5.15 ml/tube were collected. Column effluents were monitored by measuring the absorbance at 280 nm (---). An aliquot (100 \(\mu\)l) of each fraction was tested for its ability to inhibit \[^{3}H\]-paroxetine binding in monkey brain (○○). The \[^{3}H\]-paroxetine binding assay was conducted as described in the text. The control value was 24 fmol/mg cortical membrane.](image-url)
fluvoxamine. This might be a homeostatic change related to the balance between the administered 5-HT uptake inhibitors (antidepressant) and endogenous 5-HT uptake inhibitor-like substances. The data presented in the present study suggest that chronic treatment with fluvoxamine modulates the level of endogenous 5-HT uptake inhibitor-like substances and that this could be responsible for its antidepressive effect. However, it is not clear whether such actions are characteristic of fluvoxamine alone or of all selective 5-HT uptake-inhibiting antidepressants. Further studies of these endogenous substances and their modulation by antidepressants will be necessary before this distinction can be made.

REFERENCES