Effects of WEB 1881 FU, a Novel Nootropic, on Cholinergic and Adrenergic Receptors in the Rat Brain: Action on M₁-Muscarinic Receptors

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Abstract—Effects of 4-aminomethyl-1-benzylpyrrolidin-2-one-hemifumarate (WEB 1881 FU), a novel pyrrolidinone nootropic, on acetylcholine (ACh) receptors and adrenoceptors were investigated using crude membranes of the rat brain. The affinity order of WEB 1881 FU was: M₁-muscarinic (m) ACh receptor > M₂-mACh receptor > α_2 -adrenoceptor > β -adrenoceptor > α_1 -adrenoceptor > nicotinic ACh receptor. The WEB 1881 FU-competition curve for [³H]pirenzepine binding in hippocampal membranes was rightward-shifted by GTP γ S; such behavior was also observed in the case of oxotremorine but not with scopolamine. The effects of long-term administration of WEB 1881 FU (30 mg/kg/day, i.p.) for 21 days resulted in a significant decrease in the B_{max} of [³H]quinuclidinyl benzilate binding in the cerebral cortex, hippocampus and striatum. In addition, the B_{max} of [³H]pirenzepine binding to hippocampal and striatal membranes and that of [³H]AF-DX 116 binding to cerebellar membranes were significantly decreased as well. From these results, WEB 1881 FU seems to act on M₁-mACh receptors, and its long-term administration probably induces the down-regulation of mACh receptors, mainly M₁-mACh receptors in the hippocampus and striatum and M₂-mACh receptors in the cerebellum.

Recent investigations have demonstrated cholinergic dysfunctions in the cerebral cortex and hippocampus of patients with Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT). The block of central muscarinic acetylcholine (mACh) receptors by scopolamine results in similar loss of memory and cognition to that in patients with AD and/or SDAT (1, 2). Scopolamine also caused the dysfunctions of learning and memory in mice (1). In addition, the nor-adrenergic system has an important role in learning and memory (3, 4), and α_1 -, α_2 - and β -adrenoceptors are also changed in the CNS of AD (2).

WEB 1881 FU, 4-aminomethyl-1-benzylpyrrolidin-2-one-hemifumarate, has been developed as a novel nootropic drug (5). Two pharmacological studies have showed that

the drug ameliorates the impairment of working memory induced by scopolamine and that by cerebral ischemia in rats (6) and facilitates the muscarinic transmission via action on presynaptic muscarinic receptors in dogs (7). However, the nootropic action of WEB 1881 FU has not been characterized in detail, and the following remain to be elucidated: 1) which type of acetylcholine (ACh) receptors and adrenoceptors, and 2) which subtypes of muscarinic ACh (mACh) receptors are responsible for its action in the brain. In addition, it is important to determine the influences of chronic administration of the nootropic to determine the toxicology of this agent in clinical pharmacology. The repeated administration of WEB 1881 FU for 7 and 21 days did not cause behavioral change in rats. We also examined the effects of repeated administration of this drug on ACh receptors and adrenoceptors in different brain regions.

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Here, we examined the effects of WEB 1881 FU on the specific bindings of pharmacologically selective, radiolabeled ligands: [^3H]nicotine, [^3H]quinuclidinyl benzilate (QNB), [^3H]pirenzepine, [^3H]AF-DX 116, [^3H]prazosin, [^3H]rauwolscine and [^{125}I]-iodocyanopindolol (ICYP) to whole brain membranes of rats. Furthermore, to determine the influences of repeated administration of the nootropic on the muscarinic subtypes of ACh receptors, we examined if the K_i and B_{max} values of [^3H]QNB, [^3H]pirenzepine and [^3H]AF-DX 116 binding are changed after long-term dosing of WEB 1881 FU to normal rats and compared the results to the effects of a control nootropic, calcium (Ca) hopantenate.

Materials and Methods

Animals and Materials: Male Wistar rats aged 6–9 weeks, weighing 190–470 g (Hokudo), were used for the long-term dosing study. These rats were bred under conventional conditions, housed at $23 \pm 1^\circ\text{C}$, and allowed free access to food and water; the light-dark cycle was set at 12 hours.

L-[N-Methyl- ^3H]nicotine (2960 GBq/mmol), L-[benzyl-4,4'- ^3H]QNB (1624.3 GBq/mmol), [N-methyl- ^3H]pirenzepine (3219 GBq/mmol), [piperidinyl- ^3H]AF-DX 116 (2212.6 GBq/mmol), [furoyl-5- ^3H]prazosin (962 GBq/mmol), [methyl- ^3H]rauwolscine (2719.5 GBq/mmol), [^{125}I]ICYP (81 TBq/mmol) were purchased from New England Nuclear. WEB 1881 FU and Ca hopantenate were generous gifts from Nippon Boehringer Ingelheim and Tanabe, respectively. Phentolamine and propranolol were also gifts from Ciba-Geigy and Ohtsuka Pharmaceutical, respectively. Atropine, nicotine and scopolamine (Wako Chemicals), and oxotremorine and 5-hydroxytryptamine (Sigma) were purchased.

Dosing schedule: For repeated administration, 5 rats in one group were intraperitoneally (i.p.) given WEB 1881 FU (30 mg/kg) or the control drug, Ca hopantenate (100 mg/kg), once a day for 7 days or 21 days. Administration of saline was carried out in parallel as the corresponding vehicle controls.

Preparation of the membranes from several brain regions: At 24 hours after the last ad-

ministration, the rat brains were rapidly removed and dissected into 7 regions on an ice-cold plastic plate according to the method of Glowinski and Iversen (8). Seven regions of the rat brain: 1) cerebral cortex (CC), 2) hippocampus (Hp), 3) striatum (St), 4) hypothalamus (Hy), 5) midbrain (MB), 6) medulla oblongata (MO) and 7) cerebellum (Cr) were homogenized with 10 volumes of 50 mM Tris-HCl buffer (pH 7.4), and these homogenates were centrifuged at $50,000 \times g$ for 20 min. The pellets thus obtained were washed three times with the same buffer. The sedimented membranes were suspended (5–10 mg/ml) in 50 mM Tris-HCl buffer (pH 7.4) and stored at -80°C .

Receptor binding assay: The total volume of the reaction mixture was 100 μl , and it contained 100 mM NaCl, 25 mM MgCl_2 in 50 mM Tris-HCl buffer (pH 7.4), the membranes (50–200 μg protein) and radioligand: [^3H]nicotine (3–100 nM, for nACh receptor), [^3H]QNB (0.05–4 nM, for M_1 - and M_2 -mACh receptors), [^3H]pirenzepine (3–50 nM, for M_1 -mACh receptor), [^3H]AF-DX 116 (3–100 nM, for M_2 -mACh receptor), [^3H]prazosin (0.5–20 nM, for α_1 -adrenoceptor), [^3H]rauwolscine (0.5–40 nM, for α_2 -adrenoceptor) and [^{125}I]ICYP (0.03–1 nM, for β -adrenoceptor). After the reaction mixture was incubated at 30°C for 40 min (for the binding of [^3H]nicotine, [^3H]prazosin, [^3H]rauwolscine or [^{125}I]ICYP) or for 90 min (for the binding of [^3H]QNB, [^3H]pirenzepine or [^3H]AF-DX 116), it was filtered under vacuum through a Whatman GF/C filter pretreated with 0.3% polyethylenimine and then washed three times with 1.5 ml of ice-cold buffer. Specific binding was defined as the radioactivity bound after subtraction of the nonspecific binding: in the presence of 1 mM nicotine (for [^3H]nicotine binding), 1 μM , 10 μM or 100 μM atropine (for the binding of [^3H]QNB, [^3H]pirenzepine and [^3H]AF-DX 116, respectively), 100 μM phentolamine (for both the binding of [^3H]prazosin and [^3H]rauwolscine) or 100 μM propranolol (for [^{125}I]ICYP binding), from the total binding. For the binding to β -adrenoceptors, [^{125}I]ICYP binding assay was performed in the presence of 10 μM 5-hydroxytryptamine (5-HT) to block the 5-

HT_{1B} receptor binding (9).

Protein concentration was determined by the method of Lowry et al. (10) with bovine serum albumin as a standard.

Data analysis: The data from the receptor binding assay were subjected to Scatchard analysis (11) to determine the dissociation constant (K_D) and the number of the binding sites (B_{max}). The displacement curves of WEB 1881 FU were computer-analyzed with a one-site or two-site model using the modified LIGAND program (12) by a nonlinear least-squares curve-fitting procedure, the simplex method (13). In addition, the K_i value of WEB 1881 FU to each ligand binding was estimated by the formula: $K_i = IC_{50} / (1 + [L] / K_D)$ (the IC_{50} is the 50% inhibitory concentration for each ligand-binding, and $[L]$ is the concentration of each radioligand). Statistical differences between drug-treated and vehicle groups were analyzed by Student's t -test.

Results

Effects of WEB 1881 FU on the binding of cholinergic and adrenergic receptor labeling

ligands to the whole brain membranes in vitro: Using whole brain membranes of rats, the effects of WEB 1881 FU on specific binding of several cholinergic and adrenergic receptor labeling ligands were examined. WEB 1881 FU inhibited specific binding of each ligand to whole brain membranes in the order of $[^3H]$ pirenzepine $> [^3H]$ AF-DX 116 $> [^3H]$ -QNB $> [^3H]$ rauwolscine $> [^{125}I]$ ICYP $> [^3H]$ -prazosin $> [^3H]$ nicotine (Fig. 1). To estimate the K_i values, the K_D and B_{max} values were determined from Scatchard analysis of each ligand and shown in Table 1. The K_i values of WEB 1881 FU as well as those of Ca hopantenate on the specific binding of each ligand are shown in Table 2. Ca hopantenate affected only the binding of $[^3H]$ AF-DX 116 and $[^3H]$ rauwolscine and required higher concentrations for the inhibition than WEB 1881 FU (Table 2).

Effects of GTP γ S on the displacement curve of WEB 1881 FU for $[^3H]$ pirenzepine binding to hippocampal membranes: Since WEB 1881 FU affected $[^3H]$ pirenzepine binding (probably to M_1 -mACh receptors)

Fig. 1. Effects of WEB 1881 FU on specific binding of several ligands to whole brain membranes in vitro. A, Effects on acetylcholine receptor binding: $[^3H]$ nicotine (final concentration: 17–19 nM), $[^3H]$ QNB (0.5–0.7 nM), $[^3H]$ pirenzepine (19–20 nM) and $[^3H]$ AF-DX 116 (14–18 nM) were used. B, Effects on adrenoceptor binding: $[^3H]$ prazosin (1–2 nM), $[^3H]$ rauwolscine (15–19 nM) and $[^{125}I]$ ICYP (0.1–0.2 nM) were used. The IC_{50} s were estimated from each curve: 6,360 μ M for $[^3H]$ nicotine binding, 207 μ M for $[^3H]$ QNB binding, 16.3 μ M for $[^3H]$ pirenzepine binding, 60.7 μ M for $[^3H]$ AF-DX 116 binding, 2,940 μ M for $[^3H]$ prazosin binding, 452 μ M for $[^3H]$ rauwolscine binding, and 2,510 μ M for $[^{125}I]$ ICYP binding (average of IC_{50} from 3 curves, respectively). QNB, quinuclidinyl benzilate; PZP, pirenzepine; PRZ, prazosin; RAU, rauwolscine. ICYP, iodocyanopindolol.

Table 1. The K_D and B_{max} value in specific binding of cholinergic and adrenergic receptor labeling ligands to whole brain membranes of rats

Receptor subtype	Ligand	K_D (nM)	B_{max} (pmol/mg protein)	(n)
Nicotinic	[³ H]Nicotine	58.7±4.1	0.126±0.19	(3)
Muscarinic (M_1+M_2)	[³ H]QNB	0.16±0.03	1.19±0.15	(3)
Muscarinic (M_1)	[³ H]Pirenzepine	12.1±5.6	1.11±0.04	(3)
Muscarinic (M_2)	[³ H]AF-DX 116	21.2	0.056	(1)
α_1 -Adrenergic	[³ H]Prazosin	1.56±0.56	0.172±0.25	(3)
α_2 -Adrenergic	[³ H]Rauwolscine	13.7±2.7	0.219±0.016	(3)
β -Adrenergic	[¹²⁵ I]ICYP	0.06±0.004	0.075±0.001	(3)

The equilibrium binding of each radiolabeled ligand in the membranes of rat whole brains were performed by the procedure described under "Materials and Methods". These data were analyzed by the method of Scatchard (11) and the values of K_D and B_{max} were determined. QNB, quinuclidinyl benzilate; ICYP, iodocyanopindolol; n, the number of experiments.

Table 2. The K_i value of WEB 1881 FU and Ca hopantenate on ligand-binding to whole brain membranes in vitro

Ligand (Receptor subtype)	WEB 1881 FU K_i (μ M)	Ca Hopantenate K_i (μ M)
[³ H]Nicotine (N)	6,360±1,840	n.c.
[³ H]QNB (M_1+M_2)	43.9±9.1	n.c.
[³ H]Pirenzepine (M_1)	6.3±0.9	n.c.
[³ H]AF-DX 116 (M_2)	35.4±21.4	375±108
[³ H]Prazosin (α_1)	1,520±160	n.c.
[³ H]Rauwolscine (α_2)	209±62	20,600±7,200
[¹²⁵ I]ICYP (β)	623±33	n.c.

The K_i value was calculated by the equation: $K_i = IC_{50}/(1 + [L]/K_D)$. The K_D s are shown in Table 1; [L] the concentration of each ligand used, and the IC_{50} are shown in the legend of Fig. 1. The results are expressed as the mean±S.E. of three experiments in each group. n.c.: not calculated; M_1 and M_2 : M_1 - and M_2 -mACh receptor, respectively; N, nACh receptor; α_1 , α_2 and β : α_1 -, α_2 - and β -adrenoceptor, respectively.

with relatively high affinity (Table 2), we then examined whether WEB 1881 FU acts on M_1 -mACh receptors as an agonist or an antagonist by the following experiments. It is well-known that guanine nucleotides potently and selectively affect agonist competition curves without affecting antagonist competition in experiments on the binding of radiolabeled antagonist in the presence of $MgCl_2$. Effects of GTP γ S on the displacement curve of WEB 1881 FU as well as oxotremorine and scopolamine for [³H]pirenzepine binding using hippocampal membranes were examined in the presence of 5 mM $MgCl_2$. Oxotremorine and WEB 1881 FU competed for [³H]pirenzepine binding with shallow slopes (Fig. 2); i.e., the Hill coefficients (nH) were significantly lower than unity (Table 3).

GTP γ S at 20 μ M did not shift the competition of the antagonist scopolamine, but clearly affected the competition of the agonist oxotremorine (Fig. 2). In contrast, GTP γ S partially induced a rightward-shift in the WEB 1881 FU competition curve for [³H]pirenzepine binding. The competition curves of oxotremorine and WEB 1881 FU in the presence of 20 μ M GTP γ S and the curve of scopolamine in the presence or absence of GTP γ S were steep, showing that nH is close to unity (Table 3). In addition, the K_i values of oxotremorine and WEB 1881 FU in the presence of GTP γ S were significantly ($P < 0.05$) increased as compared to those in the control (Table 3). Therefore, the competition curves of WEB 1881 FU and oxotremorine were computer-analyzed with the two-site

Fig. 2. Displacement curves of scopolamine, oxotremorine and WEB 1881 FU for [³H]pirenzepine binding to hippocampal membranes in the presence or absence of GTP γ S. Each curve was computer-analyzed by a one-site model (shown in Table 3) and a two-site model (shown in Table 6). SCO, scopolamine; OXO, oxotremorine. Scopolamine ($\diamond$, $\blacklozenge$); oxotremorine ($\star$, $\blackstar$); WEB 1881 FU ($\circ$, $\bullet$); control ($\diamond$, $\star$, $\circ$); 20 μ M GTP γ S ($\blacklozenge$, $\blackstar$, $\bullet$).

Table 3. Displacement curves of [³H]pirenzepine binding to hippocampal membranes

Drug	Control		20 μ M GTP γ S	
	K _i (nM)	nH	K _i (nM)	nH
Scopolamine	0.397 $\pm$ 0.023	1.38 $\pm$ 0.04	0.458 $\pm$ 0.022	1.30 $\pm$ 0.10
Oxotremorine	248 $\pm$ 9	0.68 $\pm$ 0.02	703 $\pm$ 48***	1.08 $\pm$ 0.11*
WEB 1881 FU	7,300 $\pm$ 640	0.91 $\pm$ 0.04	9,850 $\pm$ 540*	1.10 $\pm$ 0.04*

Competition experiments were performed with 7 or 8 concentrations of the competing drug. Each competition curve for [³H]pirenzepine binding to rat hippocampal membranes (from Fig. 3) was computer-analyzed with a one-site model as described under "Materials and Methods". Values show the mean $\pm$ S.E. of three experiments. The K_i values were calculated by $K_i = IC_{50}/(1 + [L]/K_D)$. The IC₅₀ value was estimated from each displacement curve (in Fig. 2) for the binding of [³H]pirenzepine, the concentration of which is expressed by [L] (8–10 nM), and the K_D value of [³H]pirenzepine binding is 12 nM (from Table 1). nH, pseudo Hill coefficient.

Table 4. Quantitative parameters of oxotremorine and WEB 1881 FU for [³H]pirenzepine binding to hippocampal membranes in the absence of GTP γ S

Drug	K _H (nM)	K _L (nM)	K _L /K _H	% of K _H
Oxotremorine	23.2 $\pm$ 6.9	630 $\pm$ 35	27	28.3 $\pm$ 5.1
WEB 1881 FU	1,109 $\pm$ 816	8,520 $\pm$ 540	8	6.4 $\pm$ 2.7

Competition binding data of oxotremorine and WEB 1881 FU for [³H]pirenzepine binding in the absence of GTP γ S were computer-analyzed with a two-site model as described under "Materials and Methods".

model, indicating that the high affinity sites of WEB 1881 FU binding (6.4%) to M₁-mACh receptors are less than the sites of oxotremorine binding (28%) (Table 4).

Effects of 7 days administration of WEB 1881 FU on [³H]nicotine, [³H]QNB, [³H]-prazosin, [³H]rauwolscine and [¹²⁵I]ICYP binding to membranes in brain regions: An i.p.-administration, once a day, of WEB 1881

FU (30 mg/kg/day) or Ca hopantenate (100 mg/kg/day) for 7 days caused a significant inhibition of the [³H]QNB binding to the membranes of the cerebral cortex, hippocampus, striatum and cerebellum (Fig. 3B), but did not affect the binding in other regions. Bindings of [³H]nicotine and [³H]prazosin were not changed by the treatment with WEB 1881 FU or Ca hopantenate in all regions

(Fig. 3, A and C). Ca hopantenate-treatment decreased the [3H]rauwolscine binding in the hypothalamus and midbrain and increased

it in the cerebellum (Fig. 3D). [125I]ICYP binding in the hypothalamus was increased by the repeated administration of WEB 1881 FU

(Fig. 3E).

Effects of 21-days administration of WEB 1881 FU on $[^3\text{H}]\text{QNB}$, $[^3\text{H}]\text{pirenzepine}$ and $[^3\text{H}]\text{AF-DX 116}$ binding: The B_{max} values of $[^3\text{H}]\text{QNB}$ binding decreased in the cerebral cortex, hippocampus and striatum of rats repeatedly treated with WEB 1881 FU (30 mg/kg/day) for 21 days (Table 5). The treatment with Ca hopantenate (100 mg/kg/day)

for 21 days also caused a significant ($P < 0.05$) decrease in the B_{max} of $[^3\text{H}]\text{QNB}$ binding in the hippocampus, striatum and hypothalamus (Table 5). The B_{max} values of $[^3\text{H}]\text{pirenzepine}$ binding were significantly decreased in the hippocampal ($P < 0.05$) and striatal ($P < 0.01$) membranes of WEB 1881 FU-treated rats and in the striatal membranes ($P < 0.05$) of Ca hopantenate-treated rats

Fig. 3. Changes in specific binding of [3 H]nicotine (A), [3 H]QNB (B), [3 H]prazosin (C), [3 H]rauwolscine (D) and [125 I]ICYP (E) by 7 days administration of WEB 1881 FU (30 mg/kg/day) or of Ca hopantenate (100 mg/kg/day) in 7 brain regions. Rats were sacrificed 24 hours after the last administration. White column, vehicle group; hatched column, Ca hopantenate-treated group; black column, WEB 1881 FU-treated group. A, [3 H]nicotine binding. The binding assay was carried out with 50 nM [3 H]nicotine in the presence or absence of 1 mM nicotine. B, [3 H]quinuclidinyl benzilate (QNB) binding. The assay was carried out with 1.5 nM [3 H]QNB in the presence or absence of 1 μ M atropine. C, [3 H]prazosin binding. The assay was carried out with 2.5 nM [3 H]prazosin in the presence or absence of 100 μ M phenolamine. D, [3 H]rauwolscine binding. The assay was carried out with 20 nM [3 H]rauwolscine in the presence or absence of 100 μ M phenolamine. E, [125 I]iodocyanopindolol (ICYP) binding. In the presence of 10 μ M 5-HT, the assay was carried out with 0.5 nM [125 I]ICYP in the presence or absence of 100 μ M propranolol. Values are the means $\pm$ S.E. * P < 0.05, ** P < 0.01 versus vehicle administration.

without changing the K_D values (Table 6). In addition, the B_{max} value of [3 H]AF-DX 116 binding to the cerebellar membranes of rats treated with WEB 1881 FU was significantly (P < 0.05) decreased without changing K_D , but the B_{max} and K_D of [3 H]AF-DX 116 binding were not changed by Ca hopantenate (Table 6).

Discussion

The results presented here suggest that WEB 1881 FU could directly bind to brain receptors with the following potency order: M_1 -mACh receptor > M_2 -mACh receptor > α_2 -adrenoceptor > β -adrenoceptor > α_1 -adrenoceptor > nACh receptor. Recently, Haga et al. reported that mACh receptor agonists

have the GTP-effect in a reconstitution experiment using purified M_1 -mACh receptors and GTP-binding protein (G_i and G_o) (14). To clarify whether WEB 1881 FU acts as an agonist or an antagonist on M_1 -mACh receptors, we examined the effects of GTP γ S. GTP γ S did not induce the shift of the scopolamine-competition curve for [3 H]pirenzepine binding to hippocampal membranes, but induced the rightward-shift of the oxotremorine-competition curve (Fig. 2). The WEB 1881 FU-competition curve for [3 H]pirenzepine binding was also rightward-shifted by GTP γ S, like that for oxotremorine, although the extent for WEB 1881 FU was less than that for oxotremorine. Therefore, WEB 1881 FU may be regarded as a partial

Table 5. Effects of WEB 1881 FU and Ca hopantenate administration for 21 days on K_D and B_{max} of [³H]QNB binding to membranes of 7 brain regions

Region	Vehicle (Saline)		WEB 1881 FU (30 mg/kg/day)		Ca Hopantenate (100 mg/kg/day)	
	K _D (nM)	B _{max} (pmol/mg)	K _D (nM)	B _{max} (pmol/mg)	K _D (nM)	B _{max} (pmol/mg)
CC	0.29±0.02	2.31±0.04	0.23±0.03	1.91±0.33**	0.32±0.07	2.34±0.08
Hp	0.37±0.02	2.14±0.07	0.34±0.03	1.77±0.05*	0.44±0.02	1.92±0.03*
St	0.34±0.04	2.39±0.03	0.25±0.03	1.91±0.04***	0.34±0.03	2.06±0.05*
Hy	0.09±0.02	0.84±0.03	0.05±0.01	0.75±0.01	0.04±0.01	0.68±0.01*
MB	0.27±0.04	0.95±0.02	0.22±0.03	0.93±0.01	0.20±0.02	0.99±0.01
MO	0.08±0.02	0.48±0.05	0.10±0.01	0.54±0.01	0.11±0.04	0.52±0.03
Cr	0.15±0.05	0.14±0.01	0.11±0.03	0.14±0.01	0.10±0.01	0.14±0.01

WEB 1881 FU (30 mg/kg/day) or Ca hopantenate (100 mg/kg/day) was administered for 21 days. The animals were sacrificed 24 hours after the last injection for the binding assay. The results are expressed as the mean±S.E. of three experiments in each group. CC, cerebral cortex; Hp, hippocampus; St, striatum; Hy, hypothalamus; MB, midbrain; MO, medulla oblongata; Cr, cerebellum. *P<0.05, **P<0.01, ***P<0.001 versus vehicle administration.

Table 6. Effects of WEB 1881 FU and Ca hopantenate administration for 21 days on parameters of [³H]pirenzepine and [³H]AF-DX 116 bindings to brain membranes

Region	Vehicle (Saline)		WEB 1881 FU (30 mg/kg/day)		Ca Hopantenate (100 mg/kg/day)	
	K _D (nM)	B _{max} (pmol/mg)	K _D (nM)	B _{max} (pmol/mg)	K _D (nM)	B _{max} (pmol/mg)
[³H]Pirenzepine binding						
CC	10.7±0.7	0.91±0.04	11.5±0.9	0.92±0.04	11.5±0.7	0.94±0.05
Hp	13.6±0.9	1.32±0.04	11.6±1.0	1.06±0.04*	14.2±0.9	1.23±0.04
St	14.9±0.8	1.49±0.04	12.9±0.7	1.17±0.04**	14.8±0.7	1.32±0.02*
[³H]AF-DX 116 binding						
Cr	60.2±11.8	0.078±0.011	46.0±12.3	0.046±0.002*	42.3±8.2	0.055±0.006

WEB 1881 FU (30 mg/kg/day) or Ca hopantenate (100 mg/kg/day) were administered for 21 days. The animals were sacrificed 24 hours after the last injection for the binding assay. The results are expressed as the mean±S.E. of three experiments in each group. CC, cerebral cortex; Hp, hippocampus; St, striatum; Cr, cerebellum. *P<0.05, **P<0.01 versus vehicle administration.

agonist of M₁-mACh receptors in the CNS. However, it will be interesting to determine if WEB 1881 FU has an intrinsic efficacy as a partial agonist of M₁-mACh receptors in the brain.

Scopolamine, a mACh receptor antagonist, is known to induce the impairment of learning and memory (1), while oxotremorine, a mACh receptor agonist, reverses the scopolamine- and fornix damage-induced impairment of learning and memory (15, 16). Recently, Hagan et al. reported that the intracerebro-ventricular injection of pirenzepine, a specific

M₁-mACh receptor antagonist, caused the blockade of spatial learning (17), suggesting that central M₁-mACh receptors are important to learning and memory. WEB 1881 FU ameliorates the impairment of working memory caused by scopolamine and by cerebral ischemia (2). Therefore, the pharmacological properties of WEB 1881 FU as a partial agonist of M₁-mACh receptors seem to underlie its therapeutic effects on the dementia induced by the dysfunction of cholinergic system.

After long-term administration of WEB

1881 FU to normal rats for 7 days or 21 days, the [^3H]QNB binding was significantly decreased in the cerebral cortical, hippocampal, striatal and cerebellar membranes; and these decreases were due to the reduced B_{max} without changing the K_D . Selective radioligands such as [^3H]pirenzepine for M_1 -mACh receptors and [^3H]AF-DX 116 for M_2 -mACh receptors have recently become available (18, 19). The B_{max} of [^3H]pirenzepine binding to hippocampal and striatal membranes and B_{max} of [^3H]AF-DX 116 binding to cerebellar membranes were significantly decreased by WEB 1881 FU-treatment. Thus the long-term administration of WEB 1881 FU probably induces the down-regulation of mACh receptors, mainly M_1 -mACh receptors in the hippocampus and striatum and M_2 -mACh receptors in the cerebellum. It is reported that long-term dosing of aniracetam, a similar pyrrolidinone derivative, at the dose of 50 mg/kg/2 dosing/day for 20 days also produced down regulation of [^3H]QNB binding to the dentate gyrus of the hippocampus, although this nootropic cannot inhibit [^3H]QNB binding in vitro (20). WEB 1881 FU, however, directly binds to M_1 -mACh receptors with high affinity, with a K_D of approximately 6 μM .

Ca hopantenate contains γ -aminobutyric acid (GABA) and pantoyl moiety in its chemical and has improving effects on cholinergic function in the CNS (21–23). The drug seems to be an agonist for GABA $_A$ receptors, since the increase of ACh release from brain slices evoked by Ca hopantenate is antagonized by bicuculline (21). In addition, Ca hopantenate could not bind to M_1 -mACh receptors in rat brain membranes (Fig. 1). Thus, the interaction of Ca hopantenate with GABA receptors is related to its pharmacological action as an antidementia drug (21). WEB 1881 FU is another pyrrolidinone derivative, like aniracetam with antidementia action, but may also have a partial M_1 -mACh agonistic effect compared to aniracetam.

In conclusion, WEB 1881 FU exerts its antidementia action by a different mechanism from Ca hopantenate and has different effects than aniracetam. WEB 1881 FU is probably effective on dementia due to the dysfunction of the cholinergic system in the CNS.

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