Effects of Bifemelane Hydrochloride (MCI-2016) on Acetylcholine Release from Cortical and Hippocampal Slices of Rats

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Abstract—Bifemelane hydrochloride (MCI-2016) increased high K^+ -evoked acetylcholine (ACh) release from cortical and hippocampal slices of rats. The increasing effect of MCI-2016 was abolished in the absence of Ca ion and by pretreatment of reserpine (2.5 mg/kg, i.p.). These results suggest that monoaminergic neurons may be related to the enhancement of ACh release by MCI-2016. High K^+ -evoked ACh release from cortical slices was significantly decreased during aging. The decrease in ACh release was ameliorated by pretreatment of MCI-2016 (30 mg/kg, i.p.).

Bifemelane hydrochloride (MCI-2016, 4-(*o*-benzylphenoxy)-*N*-methylbutylamine hydrochloride) 1) improved the scopolamine-induced amnesia (1), 2) had anti-hypoxic and anti-ischemic action (2, 3) and 3) had EEG arousal action (4). MCI-2016 potentiated the effects of physostigmine, and the effects of MCI-2016 were antagonized by atropine. Furthermore, MCI-2016 ameliorated the decreased acetylcholine (ACh) level in the brain of animals treated with scopolamine, hypoxia or ischemia (5). These results suggest that MCI-2016 may potentiate the cholinergic mechanism in the central nervous system. In the present study, the effects of MCI-2016 on ACh release from rat brain slices were examined.

Slices (0.4 mm) from the cerebral cortex and hippocampus of male Wistar rats (young adult: 2–3 months old, old: 24–28 months old) were prepared using a tissue chopper (Hotta Rika). They were preincubated at 37°C for 20 min in Krebs-Ringer-bicarbonate (KRB) solution (in mM: NaCl, 118.5; KCl, 4.5; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 2.5; NaH_2PO_4 , 1.2; and glucose, 10, pH 7.4) bubbled with a mixture of 95% O_2 –5% CO_2 and transferred to fresh KRB solution, and then incubated with 1 μ M [3H]choline (NEN, 20 Ci/mmol) at 37°C for 10 min. They were washed and transferred to the chambers

and superfused (1 ml/min) with 95% O_2 –5% CO_2 oxygenated KRB solution containing 50 μ M eserine with or without drugs for 43 min. Spontaneous efflux was collected for the following 3 min. Slices were then stimulated by perfusing an isotonic high K^+ (50 mM)-KRB solution with or without drugs for 5 min. In this study, total tritium of superfusate was estimated as [3H] ACh, because high K^+ -evoked tritium release was almost abolished in Ca -free KRB solution (Table 1), and this release could be thought to be the release of newly synthesized ACh from labelled choline.

In the cerebral cortex, MCI-2016 at 0.1–10 nM significantly increased high K^+ -evoked ACh release (Table 1). High dose of MCI-2016 tended to inhibit the ACh release. This effect may be through the blockade of the Ca channel, because 100 μ M MCI-2016 inhibited [^{45}Ca] uptake into the membrane (data not shown). However, this dose is so high that this effect may be nonspecific. ACh release from cortical slices of rats treated with MCI-2016 (30 mg/kg, i.p., 4 hr before sacrifice) was also enhanced. The magnitude of the increment was almost equal to that of scopolamine treatment (1 mg/kg, i.p., 30 min before sacrifice). The administration schedule and the doses of drugs were determined from the results of our previous study (5). The

Table 1. Effects of MCI-2016 on ACh release from cortical and hippocampal slices of rats

Drugs	Treatment	Cerebral cortex			Hippocampus		
		Spontaneous efflux (d.p.m. $\times 10^{-3}$ /g tissue/min)	High K ⁺ -evoked release (% of spontaneous efflux)	N	Spontaneous efflux (d.p.m. $\times 10^{-3}$ /g tissue/min)	High K ⁺ -evoked release (% of spontaneous efflux)	N
Control (saline)	—	14.62 $\pm$ 0.81	147.7 $\pm$ 4.4	17	13.47 $\pm$ 1.02	160.1 $\pm$ 4.7	11
MCI-2016 0.01 nM	—	15.31 $\pm$ 0.65	151.8 $\pm$ 7.8	6	15.76 $\pm$ 1.48	176.7 $\pm$ 9.3	6
0.1 nM	—	14.26 $\pm$ 0.79	189.9 $\pm$ 7.4**	6	15.32 $\pm$ 0.42	188.9 $\pm$ 13.1*	6
1.0 nM	—	17.24 $\pm$ 0.87	179.0 $\pm$ 10.5**	6	16.42 $\pm$ 1.20	165.3 $\pm$ 9.2	6
10.0 nM	—	16.00 $\pm$ 0.91	177.0 $\pm$ 10.2*	8	15.37 $\pm$ 1.86	164.5 $\pm$ 3.9	6
1.0 μ M	—	14.74 $\pm$ 1.31	158.9 $\pm$ 5.5	6	16.31 $\pm$ 2.31	167.2 $\pm$ 7.2	6
100.0 μ M	—	14.05 $\pm$ 0.90	133.0 $\pm$ 8.8	6	13.52 $\pm$ 1.25	145.3 $\pm$ 5.9	6
MCI-2016 30 mg/kg, i.p.	—	14.06 $\pm$ 0.66	175.6 $\pm$ 12.3*	6	14.77 $\pm$ 1.39	172.1 $\pm$ 8.7	8
Scopolamine 1 mg/kg, i.p.	—	14.96 $\pm$ 0.87	171.5 $\pm$ 7.0*	6	12.93 $\pm$ 1.08	174.4 $\pm$ 5.0	6
—	Ca free (+1 mM EGTA)	15.83 $\pm$ 1.34	112.0 $\pm$ 3.2**	9	—	—	—
MCI-2016 10.0 nM	Ca free (+1 mM EGTA)	14.44 $\pm$ 0.87	117.8 $\pm$ 4.2***	9	—	—	—
—	Reserpine (2.5 mg/kg, i.p.)	15.14 $\pm$ 1.02	165.0 $\pm$ 8.4	10	—	—	—
MCI-2016 0.1 nM	Reserpine (2.5 mg/kg, i.p.)	16.06 $\pm$ 1.23	147.3 $\pm$ 5.9§§	10	—	—	—

*P<0.05, **P<0.01 vs. control ***P<0.01 vs. MCI-2016 (10 nM) §§P<0.01 vs. MCI-2016 (0.1 nM)

mechanism of increasing effects of both drugs may be different, because scopolamine decreased, but MCI-2016 slightly increased ACh content (5). Scopolamine inhibited specific [^3H]quinuclidinyl benzilate (QNB) binding by 50% at 0.8–0.9 nM (6), but the K_i value for MCI-2016 on [^3H]QNB binding to the rat cerebral membrane was 16.5 μM . The K_i value was calculated from the equation $K_i = \text{IC}_{50}/1 + ([^3\text{H}]\text{ligand}/K_d)$. The IC_{50} value refers to the concentration required to cause 50% inhibition of [^3H]QNB binding (117 pM). The K_d value was determined by linear regression analysis of the Scatchard plot which was obtained from 25.6–488 pM of [^3H]QNB. Stimulating effect of MCI-2016 on ACh release may not be through the binding to the muscarinic ACh receptors. MCI-2016 had little effect on spontaneous efflux. The addition of MCI-2016 (10 nM) in Ca-free KRB solution did not affect the high K^+ -evoked ACh release (Table 1), so the increasing effect of MCI-2016 on ACh release seems to require Ca ion in the medium.

In the rats pretreated with reserpine (2.5 mg/kg, i.p., 24 hr before sacrifice), high K^+ -evoked ACh release tended to be increased,

but the increase was not statistically significant. Under this condition, MCI-2016 (0.1 nM) did not enhance high K^+ -evoked ACh release. This drug was previously reported to enhance the norepinephrine (NE) content and NE turnover (7). These results suggest that monoaminergic neurons in the brain may be related to the enhancement of high K^+ -evoked ACh release by MCI-2016. It has been reported that NE inhibited the ACh release (8–10). On the other hand, Ho et al. (11) and Singer et al. (12) reported that centrally administered NE increased the choline acetyltransferase activity. Robinson et al. (13) indicated that the activation of the septal-hippocampal cholinergic neurons by amphetamine is most likely due to the increase in noradrenergic stimulation, and Waterhouse et al. (14) showed that iontophoretically applied NE enhanced the neural response to ACh. Beani et al. (15) reported that while NE inhibited the ACh release, NE at low dose produced a late increase in ACh outflow through the activation of dopaminergic neurons. From these respects, noradrenergic neurons certainly modulate cholinergic neurons, but the mechanism is

Fig. 1. Effects of MCI-2016 on ACh release from cortical and hippocampal slices of old rats. MCI-2016 (30 mg/kg, i.p.) was administered 4 hr prior to sacrifice. Each point represents the mean $\pm$ S.E.M. * $P < 0.05$ vs. old rats. $^{\#}P < 0.05$ vs. young adult rats.

complicated, and at present, it is not clarified whether the regulation is excitatory or inhibitory.

In the hippocampus, MCI-2016 also increased high K^+ -evoked ACh release, but this effect was small (Table 1). Attenuation of decreased ACh level by MCI-2016 was also more prominent in the cerebral cortex (5). Therefore, the sensitivity of MCI-2016 seems to be higher in the cerebral cortex than in the hippocampus.

In the senescent rats (24–28 months old), high K^+ -evoked ACh release from cortical slices was significantly lower than that in the young adult rats (Fig. 1), as described by other investigators (16–18). In the hippocampus, the decrease in ACh release was scarcely observed in the senescent rats. Pretreatment of MCI-2016 (30 mg/kg, i.p., 4 hr before sacrifice) ameliorated the decrease in high K^+ -evoked ACh release from cortical slices. It is very interesting that MCI-2016 potentiates the ACh release even in the hypofunction of the ACh release.

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