Annals of Nuclear Medicine Vol. 17, No. 2, 123–130, 2003

# Effect of sabcomeline on muscarinic and dopamine receptor binding in intact mouse brain

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Sabcomeline [(*R*-(*Z*)-(+)-$\alpha$-(methoxyiamino)-1-azabicyclo[2.2.2]octane-3-acetonitrile)] is a potent and functionally selective muscarinic $M_1$ receptor partial agonist. However, little is known of the binding properties of sabcomeline under *in vivo* conditions. In this study, muscarinic receptor occupancy by sabcomeline in mouse brain regions and heart was estimated using [$^3$H]quinuclidinyl benzilate (QNB) and [$^3$H]*N*-methylpiperidyl benzilate (NMPB) as radioligands. In the cerebral cortex, hippocampus, and striatum, the estimated $IC_{50}$ value of sabcomeline for [$^3$H]NMPB binding was almost 0.2 mg/kg. Sabcomeline was not a selective ligand to $M_1$ receptors as compared with biperiden *in vivo*. In the cerebral cortex, maximum receptor occupancy was observed about 1 hr after intravenous injection of sabcomeline (0.3 mg/kg), and the binding availability of mACh receptors had almost returned to the control level by 3–4 hr. These findings indicated that the binding kinetics of sabcomeline is rather rapid in mouse brain. Examination of dopamine $D_2$ receptor binding revealed that sabcomeline affected the kinetics of both [$^3$H]raclopride and [$^3$H]*N*-methylspiperone (NMSP) binding in the striatum. It significantly decreased the $k_3$ and $k_4$ of [$^3$H]raclopride binding resulting in an increase in binding potential ($BP = k_3/k_4 = B_{max}/K_d$) in sabcomeline-treated mice, and an approximately 15% decrease in $k_3$ of [$^3$H]NMSP binding was also observed. Although the mechanism is still unclear, sabcomeline altered dopamine $D_2$ receptor affinity or availability by modulations via neural networks.

**Key words:** sabcomeline, mice, *in vivo*, muscarinic acetylcholine receptor, dopamine $D_2$ receptor

## INTRODUCTION

WIDESPREAD DEGENERATION of ascending cholinergic projections from the basal forebrain to the hippocampus and cerebral cortex has been well documented in Alzheimer's disease (AD),[1–3] and a selective loss of presynaptic nicotinic and muscarinic $M_2$ receptors is observed, whereas postsynaptic $M_1$ receptors are unaffected.[4–6] Muscarinic $M_1$ receptors have therefore been suggested as a primary target for selective muscarinic agonist replacement therapy in AD, but it has proved difficult to achieve receptor selectivity with full agonists, which are also prone to produce adverse effects. Sabcomeline is a potent and functionally selective muscarinic-$M_1$-receptor partial agonist, and has been reported to improve cognitive function in rodents and non-human primates.[7–10] Despite its potency at muscarinic $M_1$ receptors, sabcomeline has been found to evoke only minimal changes in heart rate and blood pressure,[7,10] suggesting lower efficacy in the cardiovascular system than in the central nervous system.

Recent advances in neuro-receptor imaging techniques by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have facilitated direct measurement of drug-receptor interactions in the living human brain. Several radioligands have been developed for muscarinic acetylcholine (mACh) receptor mapping by PET, and clinically important observations have been reported. Approximately 30% mACh receptor

Received October 18, 2002, revision accepted January 16, 2003.

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occupancy throughout the brain with [$^{11}$C]*N*-methylpiperidyl benzilate (NMPB) as radioligand was reported in patients with Parkinson's disease treated with a therapeutic dose of trihexyphenidyl.[11] Estimates of mACh receptor occupancy and the pharmacokinetics of sabcomeline in both the central and peripheral tissues *in vivo* are of great value in the evaluation of its efficacy. The relationship between the pharmacological profile and actions of sabcomeline at receptor sites can be analyzed.

As previously reported, apparent mACh receptor occupancy depends greatly on the radioligand used as well as the target region or tissue. For example, [$^3$H]NMPB binding in cerebral cortex is significantly more sensitive to competitive inhibition by both the non-selective mACh receptor antagonist scopolamine and the $M_1$ selective antagonist biperiden[12] than [$^3$H]quinuclidinyl benzilate (QNB) binding,[13] whereas [$^3$H]QNB binding in the heart is much more sensitive to inhibition by scopolamine than [$^3$H]NMPB binding. Accordingly, in this study we used [$^3$H]NMPB and [$^3$H]QNB binding to estimate mACh receptor occupancy in the brain and heart, respectively, of mice with various doses of sabcomeline.

Another interesting application of PET technology is neural interaction studies employing pharmacological perturbations. For instance, Dewey et al.[14] have reported induction of a decrease in [$^{11}$C]raclopride binding to dopamine $D_2$ receptors in human brain by scopolamine. In the present experiment, we examined cholinergic and dopaminergic interactions in intact mouse brain by using two different $D_2$ radioligands, [$^3$H]*N*-methylspiperone (NMSP) and [$^3$H]raclopride.

## MATERIALS AND METHODS

### *Materials*

Male ddY mice (8–9 w) were purchased from Japan SLC (Shizuoka, Japan) and housed at 23°C on a 12-hr light-dark cycle. All mice were given free access to food and water. The studies were performed with the approval of the Institutional Animal Care and Use Committee, School of Allied Health Sciences, Osaka University.

[$^3$H]NMPB (specific radioactivity: 3.1 GBq/$\mu$mol), [$^3$H]QNB (specific radioactivity: 1.8 GBq/$\mu$mol), [$^3$H]NMSP (specific radioactivity, 3.0 GBq/$\mu$mol) and [$^3$H]raclopride (specific radioactivity: 2.9 GBq/$\mu$mol) were provided by New England Nuclear (Boston, MA, USA). Sabcomeline [SB 202026 (*R*-(*Z*)-(+)-$\alpha$-(methoxyiamino)-1-azabicyclo[2.2.2]octane-3-acetonitrile)] was kindly supplied by GlaxoSmithkline (UK). Other chemicals were the highest grade commercially available.

### *Methods*

*Inhibition of* in vivo *[$^3$H]NMPB and [$^3$H]QNB binding by sabcomeline*

Mice were intravenously co-injected with various doses of sabcomeline (0.01 to 3 mg/kg) and 185 kBq of [$^3$H]NMPB or [$^3$H]QNB, and 60 min after the tracer injection the mice were lightly anesthetized with ether and decapitated. Their brains and hearts were quickly removed, and the brains were dissected to obtain the cerebral cortex, hippocampus, striatum, and cerebellum. The samples were weighed, dissolved with 1 m*l* of tissue solubilizer (Soluene-350, Packard), and 5 m*l* of scintillator (Hionic-Fluor, Packard) was added. The radioactivity of each sample was measured with a liquid scintillation counter, and the values are expressed as percent injected dose per gram tissue (% dose/g). Specific binding in each brain region was estimated by subtracting the radioactivity concentration in cerebellum co-injected with 10 mg/kg of scopolamine, a dose that saturates muscarinic receptors in living brain.[13] Specific binding in the heart was estimated by subtracting the radioactivity concentration in heart co-injected with 10 mg/kg of scopolamine.

To compare short-term (30 min) and long-term (60 min) apparent muscarinic receptor occupancy in the brain by sabcomeline measured by *in vivo* [$^3$H]NMPB binding after tracer injection, mice were intravenously co-injected with various doses of sabcomeline and [$^3$H]NMPB, and decapitated 30 or 60 min after the tracer injection. Radioactivity concentration in cerebral cortex, hippocampus, and striatum was determined by the same method as described above, and apparent muscarinic receptor occupancy was estimated by inhibition values of [$^3$H]NMPB specific binding.

To determine the time course of mACh receptor occupancy in the brain after administration of sabcomeline, mice were intravenously injected with 0.3 mg/kg of sabcomeline, and [$^3$H]NMPB was injected at 0, 0.5, 1, 2, 3, 4, 5, and 6 hr later. Mice were decapitated 30 min after the tracer injection, and the radioactivity concentration in each brain region was measured by the same method as described above.

*Effect of sabcomeline on [$^3$H]raclopride and [$^3$H]NMSP binding in intact brain*

Mice were injected with 0.3 mg/kg (i.p.) of sabcomeline 30 min prior to tracer injection. [$^3$H]raclopride (185 kBq) or [$^3$H]NMSP (185 kBq) was intravenously injected, and mice were lightly anesthetized with ether and decapitated at various intervals after tracer injection (1, 5, 10, 15, 20 and 30 min for [$^3$H]raclopride binding, 1, 10, 20, 30, 45 and 60 min for [$^3$H]NMSP binding). The cerebral cortex, striatum, and cerebellum were quickly removed and weighed. The radioactivity concentration in each sample was measured by the same method as described above. The cerebellum was used as a reference region to estimate the amount of non-specific binding plus free ligand concentration in the brain. Specific binding in the striatum or cerebral cortex at each time point was determined by subtracting the corresponding radioactivity concentration in the cerebellum from the total radioactivity concentration in the striatum or cerebral cortex.

**Table 1** Inhibition of *in vivo* [$^3$H]NMPB binding by sabcomeline

| Sabcomeline (mg/kg) | Radioactivity Concentration (% dose/g) | | | | |
|---|---|---|---|---|---|
| | Cerebellum | Hippocampus | Striatum | Cerebral cortex | Heart |
| 0 | 2.41 ± 0.248 | 8.87 ± 0.525 | 10.8 ± 0.60 | 12.1 ± 1.07 | 1.25 ± 0.253 |
| 0.01 | 2.08 ± 0.181 | 6.95 ± 1.068* | 9.14 ± 0.974* | 9.51 ± 0.744** | 1.55 ± 0.238 |
| 0.03 | 1.99 ± 0.219* | 6.24 ± 0.639*** | 8.74 ± 0.851* | 7.76 ± 0.348** | 1.30 ± 0.096 |
| 0.1 | 2.06 ± 0.064 | 6.45 ± 0.882** | 7.99 ± 0.661*** | 8.21 ± 0.459** | 1.20 ± 0.234 |
| 0.3 | 1.92 ± 0.090* | 4.79 ± 0.048*** | 6.53 ± 0.659*** | 6.18 ± 0.403*** | 1.18 ± 0.265 |
| 1 | 2.01 ± 0.326 | 3.91 ± 0.407*** | 4.91 ± 0.369*** | 4.35 ± 0.269*** | 1.19 ± 0.108 |
| 3 | 1.80 ± 0.189** | 2.87 ± 0.244*** | 3.22 ± 0.321*** | 3.05 ± 0.298*** | 1.02 ± 0.088 |
| Scopolamine (10 mg/kg) | 1.42 ± 0.308** | 1.86 ± 0.590*** | 1.55 ± 0.327*** | 1.82 ± 0.430*** | 0.82 ± 0.047* |

Data are means ± s.d. of the values in 3–4 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to control mice.

**Table 2** Inhibition of *in vivo* [$^3$H]QNB binding by sabcomeline

| Sabcomeline (mg/kg) | Radioactivity Concentration (% dose/g) | | | | |
|---|---|---|---|---|---|
| | Cerebellum | Hippocampus | Striatum | Cerebral cortex | Heart |
| 0 | 2.68 ± 0.307 | 3.29 ± 0.410 | 3.68 ± 0.490 | 3.85 ± 0.541 | 23.4 ± 1.30 |
| 0.01 | 2.59 ± 0.192 | 3.32 ± 0.405 | 3.28 ± 0.219 | 3.65 ± 0.151 | 22.0 ± 1.41 |
| 0.03 | 2.37 ± 0.172 | 2.84 ± 0.471 | 3.13 ± 0.526 | 3.34 ± 0.332 | 20.0 ± 0.48** |
| 0.1 | 2.29 ± 0.232 | 2.89 ± 0.298 | 3.16 ± 0.344 | 3.29 ± 0.305 | 19.0 ± 0.91** |
| 0.3 | 1.71 ± 0.135** | 2.63 ± 0.296* | 2.87 ± 0.290* | 2.95 ± 0.074* | 12.5 ± 1.17*** |
| 1 | 1.55 ± 0.092** | 2.44 ± 0.198* | 2.85 ± 0.285* | 2.76 ± 0.069* | 10.8 ± 0.75*** |
| 3 | 0.96 ± 0.057** | 2.09 ± 0.194** | 2.32 ± 0.162** | 2.04 ± 0.125** | 4.84 ± 0.467*** |
| Scopolamine (10 mg/kg) | 0.50 ± 0.009*** | 0.60 ± 0.025*** | 0.62 ± 0.057** | 0.59 ± 0.015** | 0.96 ± 0.047*** |

Data are means ± s.d. of the values in 4 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to control mice.

**Table 3** Estimated $IC_{50}$ values for inhibition of [$^3$H]QNB and [$^3$H]NMPB specific binding *in vivo*

| | Scopolamine ($\mu$g/kg)[a] | | Biperiden ($\mu$g/kg)[a] | | Sabcomeline ($\mu$g/kg)[b] | |
|---|---|---|---|---|---|---|
| | [$^3$H]QNB | [$^3$H]NMPB | [$^3$H]QNB | [$^3$H]NMPB | [$^3$H]QNB | [$^3$H]NMPB |
| Cerebral cortex | 420 | 110 | 2500 | 210 | 2500 | 160 |
| Cerebellum | 65 | n.d. | 1100 | n.d. | 550 | n.d. |
| Heart | 30 | n.d. | 1200 | n.d. | 450 | n.d. |

[a]Calculated from the data of Hosoi et al.[13] [b]Calculated from the data in Table 1 and Table 2. n.d., not determined.

*Kinetics analysis*

A simplified two compartment model[15] and the graphical method (Patlak Plot)[16] were employed in quantitative analyses of [$^3$H]raclopride binding and [$^3$H]NMSP binding *in vivo*, respectively. In both cases, the time course of radioactivity in the cerebellum was used as an input function. The least square method was applied for curve fitting. The forward rate constant ($k_3$) parallels the product of two components, the maximum number of binding sites available ($B_{max}$) and the bimolecular association rate constant ($k_{on}$). The reverse rate constant ($k_4$) is equal to the dissociation rate constant ($k_{off}$).

*Statistical analysis*

The differences between the control and sabcomeline groups were examined by two-factor factorial ANOVA. Group differences after significant ANOVAs were subjected to Student's t-test. The correlation between [$^3$H]NMPB specific binding 30 min and 60 min after tracer injection was assessed by Spearman rank correlation analysis.

## RESULTS

*Inhibition of* in vivo *specific binding of [$^3$H]NMPB and [$^3$H]QNB by sabcomeline*

Inhibition of [$^3$H]NMPB and [$^3$H]QNB binding in mouse brain and heart by different doses of sabcomeline is shown in Table 1 and Table 2, respectively. In control mice, high accumulation of [$^3$H]NMPB was observed in the cerebral cortex, striatum and hippocampus, whereas low accumulation of radioactivity was seen in the

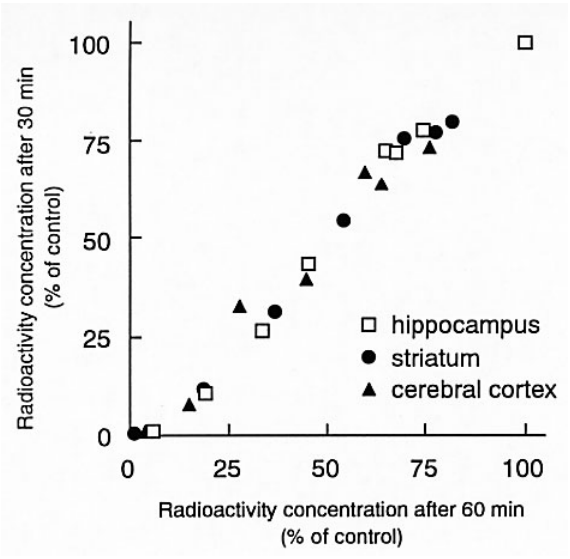


**Fig. 1** Correlation between [$^3$H]NMPB specific binding 30 min and 60 min after tracer injection.

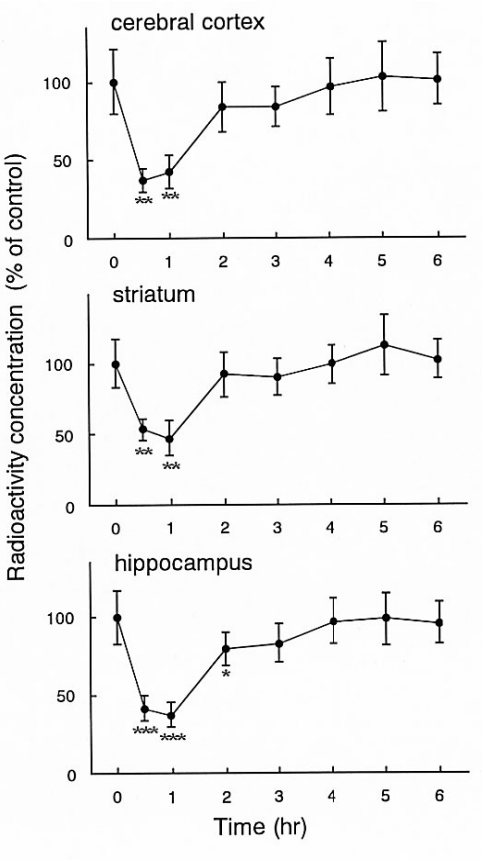


**Fig. 2** Time course of inhibition of [$^3$H]NMPB-specific binding following i.v. injection of sabcomeline (0.3 mg/kg). Specific binding of [$^3$H]NMPB was measured in mouse cerebral cortex, hippocampus, and striatum at 30 min after tracer injection. Results are means ± s.d. of data from 4 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared to control mice.

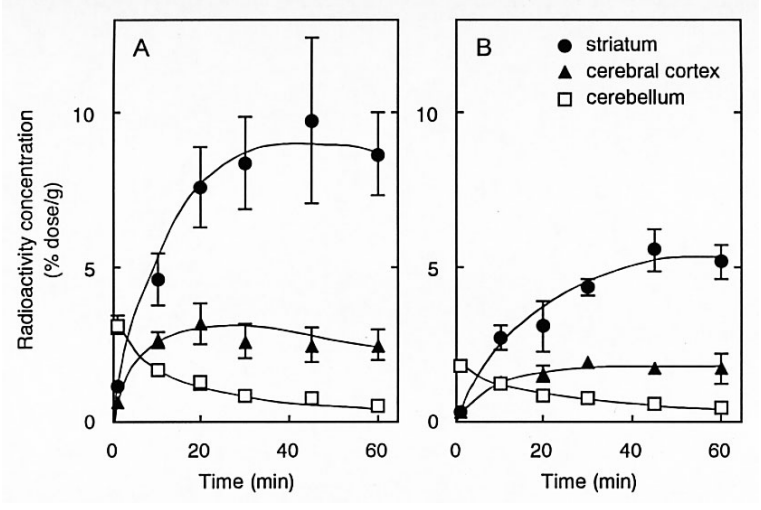


**Fig. 3** Time course of radioactivity concentrations in the striatum (specific binding), cerebral cortex (specific binding), and cerebellum (total binding) following i.v. injection of [$^3$H]NMSP. Mice were injected with vehicle (A) and sabcomeline (B), respectively, 30 min prior to tracer injection. Results are means ± s.d. of data from 3–4 animals. There were significant differences between vehicle and sabcomeline injected mice in the striatum, cerebral cortex, and cerebellum [$F(1, 25) = 46.5$, 35.5, and 62.7, respectively, $p < 0.001$].

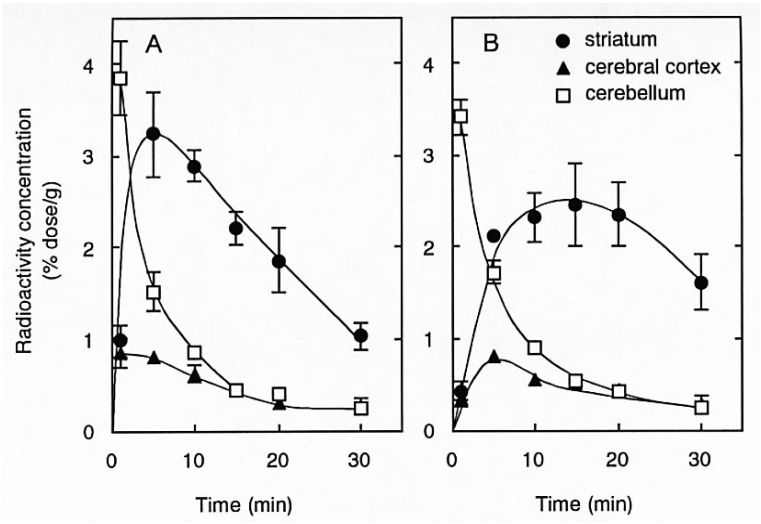


**Fig. 4** Time course of radioactivity concentration in the striatum (specific binding), cerebral cortex (specific binding), and cerebellum (total binding) following i.v. injection of [$^3$H]raclopride. Mice were injected with vehicle (A) and sabcomeline (B), respectively, 30 min prior to tracer injection. Results are means ± s.d. of data from 3 animals.

cerebellum and heart. When [$^3$H]NMPB was co-injected with 10 mg/kg of scopolamine, there were very low radioactivity concentrations in all regions, with no significant regional differences in the brain. The specific binding of [$^3$H]NMPB in cerebral cortex, striatum, and hippocampus was therefore estimated by subtraction of radioactivity in the cerebellum 60 min after injection of [$^3$H]NMPB with 10 mg/kg of scopolamine. More than 80% of the total radioactivity in these regions was found to be specific binding, and sabcomeline significantly and dose-dependently inhibited [$^3$H]NMPB binding in these mACh-receptor-rich regions (Table 1). [$^3$H]QNB binding in the heart was also significantly and dose-dependently inhibited by sabcomeline (Table 2).

*Time course of mACh receptor occupancy following intravenous injection of 0.3 mg/kg of sabcomeline*

The values of mACh receptor occupancy in the cerebral cortex, hippocampus, and striatum in mouse brain obtained 30 min after injection of [$^3$H]NMPB were compared with those obtained 60 min after injection of the tracer. As shown in Figure 1, a good correlation between the values at two different time points (30 min and 60 min) was obtained ($Rs = 0.98$, $p < 0.01$, in all regions). The time course of mACh receptor occupancy in the cerebral cortex, striatum, and hippocampus followed for 6 hr after the i.v. injection of sabcomeline (0.3 mg/kg) is shown in Figure 2. In all regions studied, maximum receptor occupancy was observed about 1 hr after injection of sabcomeline. The binding availability of mACh receptors had almost returned to the control level 3–4 hr after the injection of sabcomeline.

**Table 4** Kinetics properties of tracers in mouse striatum

| | [$^3$H]Raclopride | | | [$^3$H]NMSP |
|---|---|---|---|---|
| | $k_3$ ($min^{-1}$) | $k_4$ ($min^{-1}$) | BP | $k_3$ ($min^{-1}$) |
| Control | 0.296 | 0.121 | 2.45 | 0.121 |
| Sabcomeline | 0.175 | 0.059 | 2.96 | 0.104 |

Calculated from the data in Figure 3 and Figure 4.

*Effect of sabcomeline on the kinetics of [$^3$H]raclopride and [$^3$H]NMSP binding*

The effect of sabcomeline on the kinetics of [$^3$H]NMSP and [$^3$H]raclopride binding in mouse cerebral cortex and striatum is shown in Figure 3 and Figure 4, respectively. As shown in Figure 3, sabcomeline significantly decreased the radioactivity concentrations in all regions. The results of Patlak plot analysis of [$^3$H]NMSP binding in the striatum are shown in Table 4. Sabcomeline decreased the $k_3$ value of [$^3$H]NMSP binding by approximately 15%. In contrast, the kinetic analysis showed a decrease in both the $k_3$ and the $k_4$ values of [$^3$H]raclopride binding and an approximately 20% increase in [$^3$H]raclopride binding potential (BP) in the striatum of sabcomeline injected mice (Table 4).

## DISCUSSION

AD is characterized by progressive loss of muscarinic receptors on nerve terminals, whereas postsynaptic muscarinic $M_1$ receptors appear to largely remain intact. Sabcomeline, a functionally selective $M_1$ receptor partial agonist, has been developed as a therapeutic agent for AD, and an *in vitro* binding study using cloned human muscarinic receptors revealed that it possesses approximately

equal affinity for displacing [$^3$H]QNB binding from all muscarinic receptor subtypes.[17] By contrast, in a functional *in vitro* model, sabcomeline showed $M_1$ selectivity. It caused maximal depolarization of the rat superior cervical ganglion, an $M_1$-mediated effect, at a low concentration, but its effects on $M_2$-mediated release of acetylcholine and $M_3$-mediated smooth muscle contraction of guinea pig ileum were less than the effect of oxotremorine and carbachol, respectively.[7] The functional $M_1$ selectivity of this compound was also demonstrated by pharmacological studies in rodents and marmosets,[10] but little was known about the binding properties of sabcomeline under *in vivo* conditions. In the present study, the effect of intravenous injection of sabcomeline on muscarinic receptor occupancy in brain regions and the heart was estimated in mice using [$^3$H]QNB and [$^3$H]NMPB as radioligands.

As previously reported, apparent muscarinic receptor occupancy greatly depends on the radioligand used as well as the target tissue.[13] [$^3$H]NMPB seemed to be a superior tracer for estimation of muscarinic receptor occupancy in the cerebral cortex, striatum, and hippocampus. Approximately 50% of [$^3$H]NMPB binding was inhibited by 0.1–0.3 mg/kg of sabcomeline in the cerebral cortex, striatum, and hippocampus. It has been reported that sabcomeline reverses delay-induced deficits in the T-maze with 0.03–0.1 mg/kg in rat.[8] Considering sensitivity between rats and mice, sabcomeline has a beneficial effects on the cognitive process when it occupies more than 50% of mACh receptors in these regions estimated by [$^3$H]NMPB. Since the amounts of specific binding of [$^3$H]NMPB in the cerebellum and heart were very low, making estimation of muscarinic receptor occupancy in these regions with [$^3$H]NMPB is difficult, [$^3$H]QNB was used as the radioligand for these two tissues. The results of *in vivo* inhibition of [$^3$H]QNB and [$^3$H]NMPB binding by various doses of scopolamine, biperiden, an $M_1$ selective antagonist (data from Hosoi et al.[13]), and sabcomeline (present result) are shown in Table 3. In the cerebral cortex, sabcomeline has almost the same potency as biperiden in inhibition of [$^3$H]QNB binding. In the cerebellum and heart which regions are rich in $M_2$ receptors (more than 80%),[18–20] however, sabcomeline shows about two times higher potency for inhibition of [$^3$H]QNB binding than biperiden and almost the same degrees of $IC_{50}$ ratio cerebral cortex/cerebellum (ca. 4.5) as scopolamine (ca. 6.5). These *in vivo* inhibitory properties of sabcomeline with regard to [$^3$H]QNB binding in comparison with scopolamine and biperiden suggested that under *in vivo* conditions sabcomeline has no $M_1$ selectivity like biperiden. These results on *in vivo* binding properties of sabcomeline were consistent with *in vitro* binding characteristics as previously reported, although it has been functionally $M_1$ selective.

In order to examine the duration of the effect of a single injection of 0.3 mg/kg of sabcomeline on muscarinic receptors in mouse brain, we evaluated apparent receptor occupancy based on [$^3$H]NMPB binding 30 min after injection in comparison with the values for 60 min after injection. As shown in Figure 1, a good correlation between [$^3$H]NMPB binding at these two different intervals was obtained, and we assessed the kinetics of sabcomeline on the basis of *in vivo* [$^3$H]NMPB binding 30 min after injection of the tracer. Maximum inhibition of [$^3$H]NMPB binding in the cerebral cortex and other regions was observed 30 min and 60 min after intravenous injection of sabcomeline. Since the inhibition of [$^3$H]NMPB binding almost completely disappeared within 3–4 hrs after the injection, the binding kinetics of sabcomeline in mouse brain is rather rapid. Previous reports have also indicated short-term pharmacological effects of this compound in rodents,[7,21] whereas a prolonged ameliorating effect of sabcomeline on performance of a visual object discrimination task was reported in marmosets,[10] indicating a significant species difference in the binding kinetics of sabcomeline in the intact brain. PET with [$^{11}$C]labeled NMPB instead of [$^3$H]NMPB will be of great value in estimating the interaction between sabcomeline and muscarinic receptors in the intact brain of rodents and primates. In general, its sensitivity to centrally acting drugs in the human brain is significantly higher than in rodent brain. Human brain mapping by PET is also useful in determining appropriate doses of therapeutic agents.

Another important finding in the present study was that sabcomeline affected the kinetics of both [$^3$H]raclopride and [$^3$H]NMSP binding. *In vivo* dopamine receptor binding has been reported to be altered by various pharmacological perturbations in both animal and human brain. In previous studies, PET was used to assess the effects of acetylcholine,[14] serotonin,[22,23] GABA[24] and NMDA/glutamate[25] on striatal [$^{11}$C]raclopride binding. Dewey et al.[26] proposed the hypothesis that competitive inhibition by endogenous dopamine is the main mechanism for alteration in [$^{11}$C]raclopride binding, and several reports using micro-dialysis and an *in vivo* raclopride binding study have supported this hypothesis.[27] Assessment of modification of cholinergic systems revealed a decrease in [$^{11}$C]raclopride binding by scopolamine in human volunteers,[14] and benztropine, an mACh receptor antagonist with slight dopamine transporter inhibitory activity, induced a reduction of [$^{11}$C]raclopride binding as measured by PET in the conscious monkey brain.[28] However, other findings cannot be fully explained on the basis of competitive inhibition by endogenous dopamine. For example, although isoflurane has been reported to increase synaptic dopamine levels,[29] it decreased the *in vivo* binding of [$^{11}$C]raclopride to a much lesser extent than that of [$^{11}$C]NMSP.[30] In the animal experiment in conscious monkeys, scopolamine did not alter the apparent static dopamine concentration in the striatum, but it reduced *in vivo* [$^{11}$C]raclopride binding and altered the apparent affinity ($1/K_d$) of dopamine $D_2$ receptors.[31] In

our laboratory, opposite effects of oxotremorine, an mACh receptor agonist, were observed on [$^3$H]raclopride and [$^3$H]NMSP binding in mouse striatum.[32] Apparent [$^3$H]raclopride binding was increased relative to control animals, whereas [$^3$H]NMSP binding appeared to decrease by pretreatment with oxotremorine. As shown in Figures 3–4, similar changes in the binding kinetics of [$^3$H]raclopride and [$^3$H]NMSP in mouse striatum were observed with sabcomeline. A previous report showed sabcomeline had no affinity for dopamine $D_2$ receptors in an *in vitro* binding study.[7] There remains some possibility that blood flow may alter via peripheral muscarinic effects by sabcomeline. However, in this kinetics analysis, in which cerebellum was used as a reference region, the effects of alterations in cerebral blood flow induced via peripheral muscarinic system might be negligible. Sabcomeline significantly decreased $k_3$ and $k_4$, resulting in an increase in the binding potential ($BP = k_3/k_4 = B_{max}/K_d$) of [$^3$H]raclopride binding, whereas there was an approximately 15% decrease in the $k_3$ of [$^3$H]NMSP binding in the striatum (Table 4). If the decrease in [$^3$H]NMSP binding was caused by an increase in endogenous dopamine concentration, a more pronounced decrease in [$^3$H]raclopride binding potential would be expected, because [$^3$H]raclopride binding is much more sensitive to competitive inhibition than [$^3$H]NMSP binding both *in vitro* and *in vivo*.[33–36] Consequently, these observations cannot be explained simply by competitive inhibition by endogenous dopamine, and another factor, for example, alteration of the association or dissociation rate constants of [$^3$H]raclopride and [$^3$H]NMSP, should be considered. Although the mechanism is still unclear, measurement of changes in dopamine receptor binding induced by sabcomeline in human brain would be of some interest.

In conclusion, sabcomeline was found to be a non $M_1$ selective ligand by an *in vivo* binding method. It occupied muscarinic acetylcholine receptors in the cerebral cortex, striatum and hippocampus in a dose-dependent manner, and considerably rapid dissociation of sabcomeline from mACh receptor in mouse brain was observed. In addition, sabcomeline affected both [$^3$H]raclopride and [$^3$H]NMSP binding to dopamine $D_2$ receptor in mouse striatum.

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