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Subanesthetic Doses of Ketamine Transiently Decrease Serotonin Transporter Activity: A PET Study in Conscious Monkeys

Shigeyuki Yamamoto[1], Hiroyuki Ohba[1], Shingo Nishiyama[1], Norihiro Harada[1], Takeharu Kakiuchi[1], Hideo Tsukada[1] and Edward F Domino*[,2]

[1]Central Research Laboratory, Hamamatsu Photonics KK, Hamakita, Japan; [2]Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA

Subanesthetic doses of ketamine, an *N*-methyl-D-aspartic acid (NMDA) antagonist, have a rapid antidepressant effect which lasts for up to 2 weeks. However, the neurobiological mechanism regarding this effect remains unclear. In the present study, the effects of subanesthetic doses of ketamine on serotonergic systems in conscious monkey brain were investigated. Five young monkeys underwent four positron emission tomography measurements with [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile ([^{11}C]DASB) for the serotonin transporter (SERT), during and after intravenous infusion of vehicle or ketamine hydrochloride in a dose of 0.5 or 1.5 mg/kg for 40 min, and 24 h post infusion. Global reduction of [^{11}C]DASB binding to SERT was observed during ketamine infusion in a dose-dependent manner, but not 24 h later. The effect of ketamine on the serotonin 1A receptor (5-HT$_{1A}$-R) and dopamine transporter (DAT) was also investigated in the same subjects studied with [^{11}C]DASB. No significant changes were observed in either 5-HT$_{1A}$-R or DAT binding after ketamine infusion. Microdialysis analysis indicated that ketamine infusion transiently increased serotonin levels in the extracellular fluid of the prefrontal cortex. The present study demonstrates that subanesthetic ketamine selectively enhanced serotonergic transmission by inhibition of SERT activity. This action coexists with the rapid antidepressant effect of subanesthetic doses of ketamine. Further studies are needed to investigate whether the transient combination of SERT and NMDA reception inhibition enhances each other's antidepressant actions.
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INTRODUCTION

Prevalence estimates for major depressive disorder in an American population is ca. 16% (Kessler *et al*, 2003). Pharmacological therapy of depression was revolutionized in the late 1950s. Two classes of drugs were serendipitously found to be effective antidepressants. The first antidepressant was iproniazid, originally developed as an antitubercular drug, and the second the tricyclic antidepressant imipramine arose from antihistamine research (for review, see Nestler *et al*, 2002). The acute mechanism of action of both antidepressant medications had been identified. Iproniazid is a monoamine oxidase inhibitor, and imipramine inhibits serotonin and/or norepinephrine reuptake transporters (Frazer, 1997). Today, there is a wide range of available antidepressants. Selective serotonin reuptake inhibitors (SSRIs) and selective serotonin/noradrenalin inhibitors are the most common treatments for patients with depression (Celada *et al*, 2004). Because the serotonin transporter (SERT) located on the presynaptic nerve terminal has a key role in the regulation of the serotonin levels in the synaptic cleft, inhibition of SERT would be expected to result in enhanced serotonergic neurotransmission (Salomon *et al*, 1993). Especially, increased serotonin level in prefrontal cortex is thought to be a key step in the therapeutic mechanism of SSRIs (Bel and Artigas, 1992; Invernizzi *et al*, 1992, 1996). Positron emission tomography (PET) studies with [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile ([^{11}C]DASB; Wilson *et al*, 2000) have shown that 80% occupancy of SERT by SSRIs is needed for the improvement of depression symptom (Meyer *et al*, 2001, 2004; Suhara *et al*, 2003). However, only one-third of patients show significant mood improvement in response to an initial antidepressant treatment (Trivedi *et al*, 2006). Moreover, there is a time lag of several weeks before a therapeutic effect is observed (Krystal., 2010). This lengthy time to achieve remission is thought to be caused by indirect activation of the serotonin

*Correspondence: Dr Edward F Domino, Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-05632, USA, Tel: +1 734 764 9115, Fax: +1 734 763 4450, E-mail: efdabcde@umich.edu
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1A receptor (5-HT_{1A}-R) (Chaput *et al*, 1986; Invernizzi *et al*, 1996). Elevated extracellular serotonin levels in response to acute blockade of SERT engaged inhibition of the 5-HT_{1A}-R in presynaptic neurons (autoreceptor) of the dorsal raphe. This inhibits serotonergic neural activity, resulting in reduced subsequent serotonin release in terminal brain areas such as a frontal cortex (Bel and Artigas, 1992; Gartside *et al*, 1995; Invernizzi *et al*, 1992, 1996). In contrast, after chronic administrations of SSRIs, these 5-HT_{1A} autoreceptors are desensitized, resulting in a pronounced increase in serotonin levels in the prefrontal cortex (Invernizzi *et al*, 1994).

We previously investigated the clinical pharmacological effects of CI-581, now known as ketamine, a noncompetitive *N*-methyl-D-aspartic acid (NMDA) glutamate receptor antagonist (Domino *et al*, 1965). Ketamine has analgesic and dissociative anesthetic properties (Reich and Silvay, 1989). The brain distribution and kinetics of ketamine have already been studied with PET. The uptake of [^{11}C]ketamine reflects the distribution of NMDA receptors in the brain with a rapid brain-plasma exchange rate (Hartvig *et al*, 1994; Kumlien *et al*, 1999). Ketamine in anesthetic doses affects several monoaminergic neuronal systems. In conscious monkeys and animal, PET anesthetic doses of ketamine significantly alter the synthesis rate of dopamine (Tsukada *et al*, 2000). Although dopamine D_2 receptor binding was decreased (Ohba *et al*, 2009; Onoe *et al*, 1994; Tsukada *et al*, 2000), dopamine transporter (DAT) availability is increased in living monkey brains (Harada *et al*, 2004; Tsukada *et al*, 2001). Although an inhibitory effect of ketamine on SERT was reported *in vitro* (Martin *et al*, 1990; Nishimura *et al*, 1998; Zhao and Sun, 2008), no one has evaluated the effects of subanesthetic doses of ketamine on SERT *in vivo*.

The rapid antidepressant effect of ketamine at subanesthetic dose for treatment-resistant depressed patients suggests a possible new approach for its therapy, compared with the standard medications required for several weeks (Krystal, 2010). In addition to robust and rapid antidepressant effects after a single dose, ketamine has sustained antidepressant effects in depressed patients for 1–2 weeks (Berman *et al*, 2000; Diazgranados *et al*, 2010; Price *et al*, 2009; Zarate *et al*, 2006). This is surprising because of an approximate 3-h half-life of ketamine in plasma (Clements *et al*, 1982) and its absence in brain in 24 h. These findings suggest that the neurobiological mechanism underlying the antidepressant effects of ketamine is far more complex than simple antagonism of the NMDA receptor.

The aim of present study was to determine the effects of subanesthetic doses of ketamine on serotonergic activity in the conscious monkey brain. First, PET studies were performed with [^{11}C]DASB for the SERT and 4-[^{18}F] fluoro-N-[2-[1-(2-methoxyphenyl)-1 piperazinyl]ethyl-N-2-pyridinyl-benzamide ([^{18}F]MPPF) for the 5-HT_{1A}-R, respectively, after the administration of subanesthetic doses of ketamine. We also performed PET studies with [^{11}C]2-β-carbomethoxy-3β-(4-flurophenyldopamine ([^{11}C]β-CFT) for the DAT, to verify the specificity of subanesthetic ketamine effects on serotonergic systems. Next, it was determined whether subanesthetic doses of ketamine increased serotonin levels in the extracellular fluid (ECF) of prefrontal cortex by microdialysis.

MATERIALS AND METHODS

Subjects and Drug

Experiments were conducted in accordance with the recommendations of the US National Institutes of Health and the guidelines of the Central Research Laboratory, Hamamatsu Photonics. Eight male rhesus monkeys (*Macaca mulatta*; 7.8 ± 0.8 years old, weighing 6.4 ± 1.4 kg) were studied. Five monkeys participated in the PET experiments, and another three were used for the microdialysis experiment. The doses of ketamine hydrochloride were based on human clinical studies (Diazgranados *et al*, 2010). Saline or each dose of ketamine was infused intravenously for 40 min. PET scans were started after the end of ketamine infusion. Vital signs including heart rate, respiration rate, systolic and diastolic blood pressure, and body temperature were monitored throughout the ketamine infusion.

PET Experiments

A high-resolution animal PET scanner (SHR-7700; Hamamatsu Photonics, Hamamatsu, Japan) with a transaxial resolution of 2.6 mm full-width half-maximum in the enhanced 2D mode and a center-to-center distance of 3.6 mm (Watanabe *et al*, 1997) was used. PET images were reconstructed by a filtered backprojection method with a 4.5-mm Hanning filter, resulting in an in-plane reconstructed resolution of 4.5 mm. PET scans with [^{11}C]DASB and [^{18}F]MPPF were performed with arterial blood sampling. To avoid excessive arterial blood sampling, PET scans with [^{11}C]β-CFT were performed without sampling. A saphenous venous cannula in an inferior limb and another cannula in the femoral artery of the other leg were inserted. The trained animal's head was rigidly fixed to the upper frame of a monkey chair using an acrylic head-restraining device. The animal sitting in a restraining chair was placed at a fixed position in the PET gantry with stereotactic coordinates aligned parallel to the orbitomeatal line. Transmission data with a ^{68}Ge–^{68}Ga pin source were obtained for an attenuation correction. After i.v. bolus injection of each radiotracer, PET scans were acquired for 91 min. The injected dose of [^{11}C]DASB, [^{18}F]MPPF, and [^{11}C]β-CFT was 212.1 ± 37.0, 98.3 ± 13.2, and 199.4 ± 65.3 MBq/kg (mean ± SD, $n = 5$), respectively. A summation image from 28–40 min postinjection was obtained. The brain MRI was automatically coregistered to the PET images by pixel-wise kinetic modeling (PMOD) software (PMOD Technologies, Zurich, Switzerland). For each monkey, the following regions of interest (ROIs) were obtained on the basis of the registered MRI: midbrain, thalamus, striatum, prefrontal cortex, and cerebellum.

Arterial blood samples were obtained every 8 s from 10 to 66 s, followed by 96, 156, 246, and 336 s, then 20, 30, 45, 60, 75, and 90 min after tracer injection. Blood samples were centrifuged to separate plasma, weighed, and radioactivity was measured. For metabolite analysis, methanol was added to some plasma samples (sample/methanol = 1/1), centrifuged, and the supernatants were developed with a thin-layer chromatography plate (AL SIL G/UV; Whatman, Kent, UK) using a mobile phase of dichloromethane: diethyl ether:triethylamine = 20 : 15 : 1 for [^{11}C]DASB and chloroform:methanol:triethylamine = 38 : 2 : 1 for [^{18}F]

MPPF. At each sampling time point for analysis, the ratio of radioactivity in the unmetabolized fraction to that in total plasma was determined using a phosphoimaging plate (FLA-7000; Fuji Film, Tokyo, Japan). Time-activity curves of radioactivity in metabolite-corrected arterial plasma were used as the arterial input function.

PET Data Analysis

For [^{11}C]DASB and [^{18}F]MPPF analysis, the Logan plot with an arterial input function was performed in PMOD software (PMOD Technologies). The Logan arterial input method determines the total distribution volume (Vt) using the following equation (Logan *et al*, 1990):

$$\int_0^T \mathrm{ROI}(t)dt/\mathrm{ROI}(T) = V\mathrm{t}\int_0^T Cp(t)dt/\mathrm{ROI}(T) + C$$

where ROI(T) and Cp(T) represent tissue and arterial plasma radioactivities, respectively, at time $t = T$, Vt is the slope, and C is the intercept on the y-axis. In reversibly labeled compounds, the Logan plot becomes linear after some time with a slope that is equal to Vt. The ratio of Vt in target ROI ($V\mathrm{t}_{\mathrm{tar}}$) to Vt in the reference ($V\mathrm{t}_{\mathrm{ref}}$) minus one ($V\mathrm{t}_{\mathrm{tar}}/V\mathrm{t}_{\mathrm{ref}} - 1$) was calculated as binding potential nondisplaceable ($\mathrm{BP}_{\mathrm{ND}}$), which is the ratio at equilibrium of specifically bound radioligand to that of nondisplaceble (ND) radioligand in tissue (Innis *et al*, 2007). In Logan arterial input method, t^* was fixed at 15 min, and Vt value of the cerebellum was used as $V\mathrm{t}_{\mathrm{ref}}$. Parametric images were also generated of the $\mathrm{BP}_{\mathrm{ND}}$ by the Logan plot method with an arterial input function based on PXMOD. [^{11}C]β-CFT binding was quantified by the Simplified Reference Tissue Model with the cerebellum as a reference region (Sasaki *et al*, 2012).

Microdialysis Experiments

Three monkeys were used for the microdialysis experiment. Determination of serotonin and dopamine in the ECF of prefrontal cortex was done by reverse-phase high-performance liquid chromatography with electrochemical detection as described previously (Tsukada *et al*, 2004; Yamamoto *et al*, 2007). The guide cannula was implanted into the prefrontal cortex (anterior, 35 mm; lateral, 10 mm; and depth, 1 mm) according to the MRI with reference to the stereotactic brain atlas during the procedure for the attachment of the acrylic plate. Each monkey had two microdialysis sessions (saline and ketamine at 1.5 mg/kg). A microdialysis probe with a membrane region 250 μm in diameter and 3 mm in length (Eicom A-I-8-03; Eicom, Tokyo, Japan) was inserted into the prefrontal cortex via the guide cannula. The probe was initially perfused with Ringer's solution at a rate of 2 μl/min for 120 min to remove serotonin and dopamine overflow from the damaged tissues. Then, 30 μl samples were collected every 15 min. The averaged data obtained from 0 to 120 min before the administration of ketamine were used as baseline data. Saline or ketamine at dose of 1.5 mg/kg in 10 ml saline was i.v. infused in 40 min, and the changes in serotonin and dopamine levels in the ECF of prefrontal cortex were measured. The serotonin and dopamine levels were expressed as percentages (%) of corresponding baselines. To verify the exact positioning of the inserted probe, 5 μl of China ink was injected via the guide cannula at the end of measurements. Animals were anesthetized with sodium pentobarbital and decapitated. The brains were quickly removed, coronal sections were cut on a cryostat, and the location of probe implantation site was determined visually.

Table 1 Vital Signs During PET Measurements

Variable	Vehicle	0.5 mg/kg, 40 min	1.5 mg/kg, 40 min	1.5 mg/kg, 24 h
Heart rate, mean ± SD	177.5 ± 17.1	167.5 ± 39.5	155.0 ± 34.2	185.0 ± 17.3
Systolic blood pressure	127.5 ± 9.6	115.0 ± 19.1	117.5 ± 12.6	125.0 ± 26.5
Diastolic blood pressure	85.0 ± 12.9	70.0 ± 14.1	72.5 ± 9.6	87.5 ± 27.5

Data are show as mean ± SD for five animals.

Statistics

The regional $\mathrm{BP}_{\mathrm{ND}}$ values obtained with vehicle, 0.5 mg/kg, and 1.5 mg/kg were compared with one-way repeated-measures ANOVA, followed by Bonferroni correction. The regional $\mathrm{BP}_{\mathrm{ND}}$ values between baseline and 24 h were compared with paired t-tests. The level of significance was $P < 0.05$.

RESULTS

Although no significant changes of heart rate, systolic, and diastolic blood pressure were observed between baseline and ketamine or saline challenge, there was a slight tendency to be decreased after ketamine administration (Table 1).

Representative $\mathrm{BP}_{\mathrm{ND}}$ images of [^{11}C]DASB to the SERT are illustrated in Figure 1a. Consistent with the known distribution of the SERT in monkey, as previously reported (Yamamoto *et al*, 2007; Yokoyama *et al*, 2010), the $\mathrm{BP}_{\mathrm{ND}}$ of [^{11}C]DASB in the baseline scan was the highest in the midbrain and thalamus, followed by the striatum, and lowest in the cortices. Although [^{11}C]DASB binding in the prefrontal cortex was lower than that in midbrain, [^{11}C]DASB can be used for the evaluation of SERT-specific binding in the prefrontal cortex (Szabo *et al*, 2002). The mean $\mathrm{BP}_{\mathrm{ND}}$ values of [^{11}C]DASB from all five animals are illustrated in Figure 2a. The $\mathrm{BP}_{\mathrm{ND}}$ values of [^{11}C]DASB 40 min post ketamine at 1.5 mg/kg in the midbrain, thalamus, striatum, and prefrontal cortex were significantly lower than those in baseline (Figure 2a). The reduction in $\mathrm{BP}_{\mathrm{ND}}$ from baseline to 40 min post ketamine at 1.5 mg/kg was 24% in the midbrain, 28% in the thalamus, 22% in the striatum, and 34% in the prefrontal cortex. There were no significant differences in the $\mathrm{BP}_{\mathrm{ND}}$ of [^{11}C]DASB between baseline and 24 h post ketamine at 1.5 mg/kg in any brain regions. The cerebellar Vt values of each experimental condition (vehicle, 40 min post ketamine at 0.5 mg/kg, 40 min post ketamine at 1.5 mg/kg, and 24 h post ketamine at 1.5 mg/kg) for [^{11}C]DASB were 14.7 ± 3.5, 15.4 ± 2.7, 13.3 ± 2.3, and 14.1 ± 1.4, respectively, showing no significant differences among conditions ($P = 0.62$).

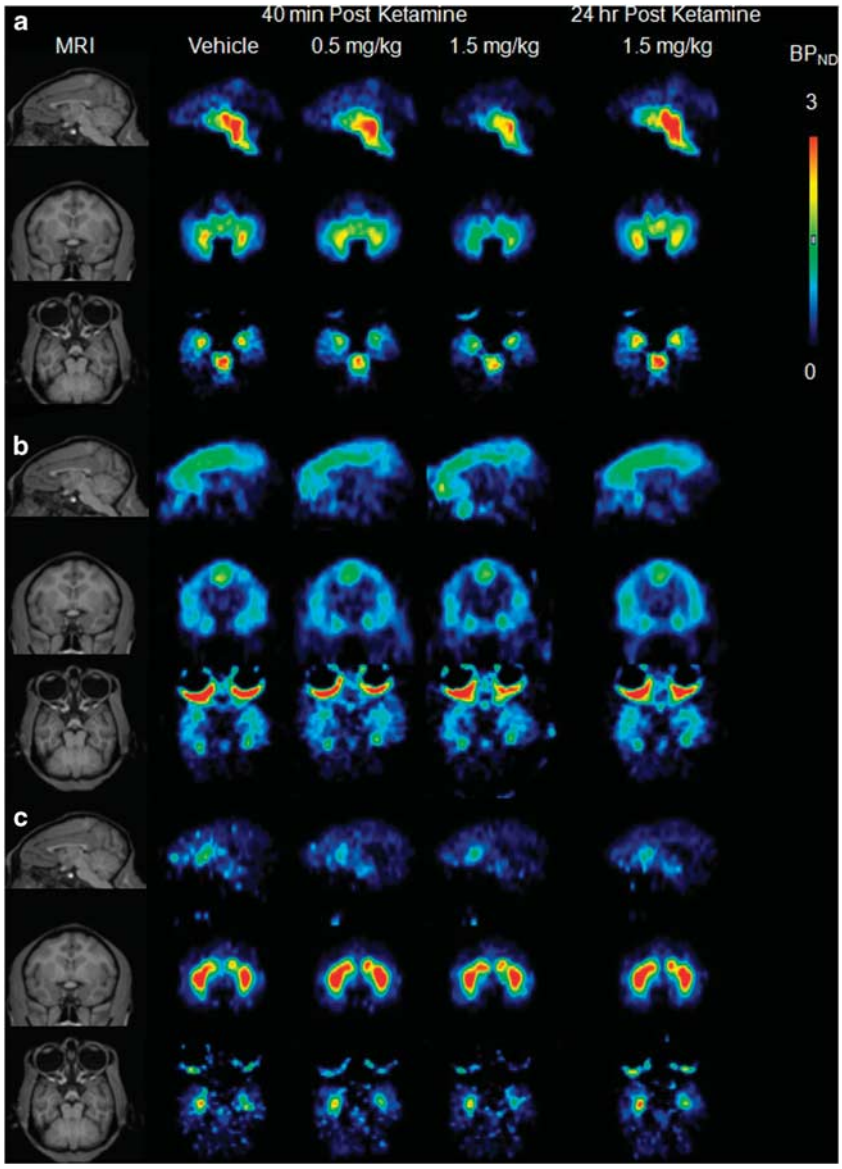

Figure 1 Typical MRI (left) and parametric PET images of [^{11}C]DASB (a), [^{18}F]MPPF (b), and [^{11}C]β-CFT (c) binding in conscious monkey brain. Parametric maps of BP_{ND} of [^{11}C]DASB and [^{18}F]MPPF were calculated by Logan plot with an arterial input method. Parametric maps of the BP_{ND} of [^{11}C]β-CFT were calculated by the Simplified Reference Tissue Model.

Representative BP_{ND} images of [^{18}F]MPPF to the 5-HT_{1A}-R are illustrated in Figure 1b. The BP_{ND} of [^{18}F]MPPF in the baseline scan was the highest in the hippocampus, followed by the cortices and midbrain. The BP_{ND} pattern of [^{11}C]MPPF was consistent with the previously reported 5-HT_{1A}-R distribution determined in monkey brain with PET (Udo de Haes *et al*, 2006). There were no significant differences in the BP_{ND} values of [^{11}C]MPPF between baseline and ketamine administration in any brain region (Figure 2b). The cerebellar VT values of each experimental condition for [^{18}F]MPPF were 2.1 ± 0.6, 1.8 ± 0.4, 1.7 ± 0.2, and 1.7 ± 0.2, respectively, indicating no significant differences among conditions ($P = 0.45$).

Representative BP_{ND} images of [^{11}C]β-CFT to DAT are presented in Figure 1c. As expected, the BP_{ND} of [^{11}C]β-CFT in the baseline scan was the highest in the striatum, followed by the hippocampus, and lowest in the cortices. The distribution pattern of [^{11}C]β-CFT was consistent with the previously reported DAT distribution determined in monkey brain with PET (Harada *et al*, 2004; Tsukada *et al*, 2001). There were no significant differences in the BP_{ND} values of [^{11}C]β-CFT between baseline and ketamine administration in any brain regions (Figure 2c).

As described in the Materials and methods, the microdialysis probes were inserted with coordinates relevant to the prefrontal cortex. Just after the start of the intravenous infusion of ketamine in a dose of 1.5 mg/kg, the serotonin level in the ECF of prefrontal cortex transiently increased up to 2.4 times greater than baseline, followed by a decrease to control (Figure 3a). In contrast, no significant changes were observed after saline infusion in the ECF serotonin release. Dopamine levels in the ECF of prefrontal cortex remained at constant level without any significant changes after ketamine or saline infusion (Figure 3b).

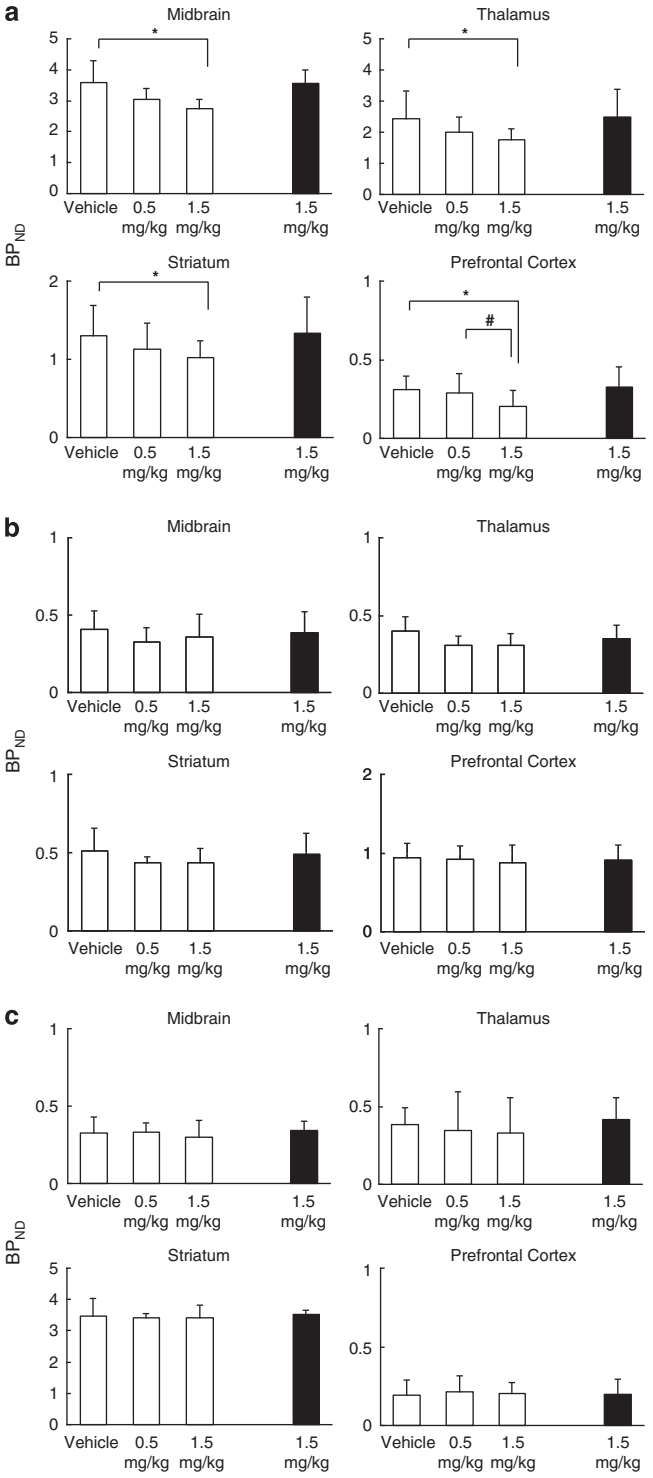

Figure 2 BP$_{ND}$ values of [^{11}C]DASB (a), [^{18}F]MPPF (b), and [^{11}C]β-CFT (c) in conscious monkey brain. Statistical differences were obtained using one-way repeated-measures ANOVA ($P<0.05$) with dose as factors. Bonferroni tests confirmed a significant difference at $^{*}P<0.05$ compared with baseline scan. Bonferroni tests also confirmed significant differences at each time point at $^{\#}P<0.05$ values, mean ± SD.

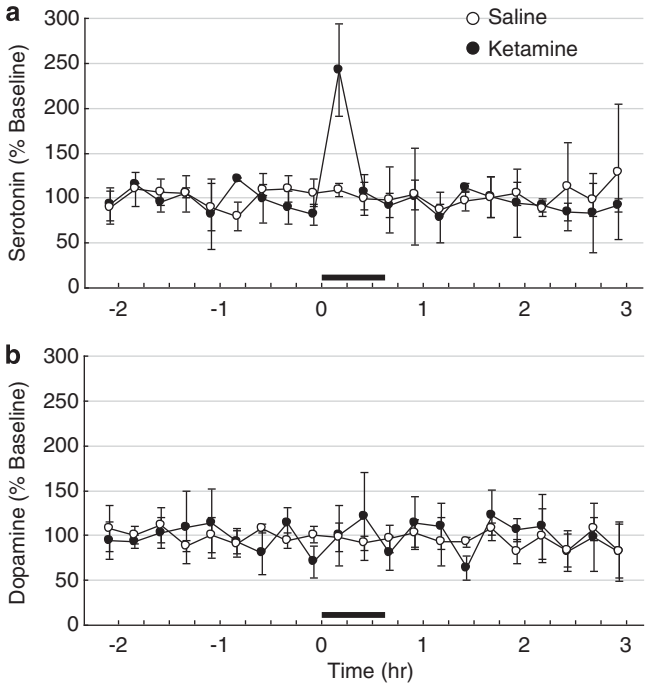

Figure 3 Effects of subanesthetic ketamine on extracellular serotonin (a) and dopamine (b) levels in the prefrontal cortex in the conscious monkey brain. Open and filled circles represent the time courses of neurotransmitter change, following saline and ketamine infusion, respectively. A microdialysis probe was inserted into the prefrontal cortex according to MRI via a guide cannula. Ketamine was administrated in a dose of 1.5 mg/kg at time 0. Samples were collected every 15 min. Serotonin- and dopamine-release concentrations were measured using high-performance liquid chromatography with electrochemical detection. The serotonin and dopamine levels were expressed as percentage of the baseline preketamine infusion.

DISCUSSION

To our knowledge, this is the first study to demonstrate the rapid effects of subanesthetic doses of ketamine on the SERT in the brain of conscious monkey. Infusion of ketamine at subanesthetic doses results in the decreased [^{11}C]DASB binding to the SERT in the midbrain and prefrontal cortex in a dose-dependent manner. About a 30% decrease in [^{11}C]DASB binding was observed during ketamine infusion, but the lowered [^{11}C]DASB binding was not present at 24 h. The 5-HT$_{1A}$-R binding measured with [^{18}F]MPPF was not affected by subanesthetic doses of ketamine in conscious monkey brain.

One of the possible mechanisms of lowered [^{11}C]DASB binding by subanesthetic doses of ketamine was direct competitive inhibition on the SERT. *In vitro* studies have reported that ketamine in concentrations of $>10^{-6}$ M inhibited the uptake of [^{3}H]5-HT by the SERT transfected in human embryonic kidney 293 cells in a dose-dependent manner (Nishimura *et al*, 1998; Zhao and Sun, 2008). Another *in vitro* study also showed that ketamine in concentrations of $>10^{-5}$ M inhibited the uptake of [^{3}H]5-HT in the rat brain (Martin *et al*, 1990). It is unknown whether administration of ketamine in the doses of 0.5 and 1.5 mg/kg used in the present study reaches 10^{-6} M concentrations in the living brain. However, ketamine in concentrations of $>10^{-6}$ M inhibits DAT availability transfected in human embryonic kidney 293 cells (Nishimura *et al*, 1998). Our present data demonstrate that subanesthetic doses of ketamine affect SERT availability and the extracellular serotonin level in the prefrontal cortex, but not DAT availability and extracellular dopamine level.

The effect of extracellular serotonin concentration on the SERT expression is another concern. Our previous PET study showed that the changes in the extracellular serotonin level did not affect the [^{11}C]DASB binding to SERT, when the increase in extracellular serotonin was <10 times compared with baseline (Yamamoto *et al*, 2007). The microdialysis data indicate that the serotonin level in the ECF of the prefrontal cortex increased only 2.4 times greater than the baseline during ketamine infusion. This result was consistent with previous microdialysis studies showing that a noncompetitive NMDA antagonist, dizocilpine (MK-801), in doses of 0.25–1 mg/kg, transiently increased serotonin levels about two times in the rat brain (López-Gil *et al*, 2007; Whitton *et al*, 1992; Yan *et al*, 1997). There is little literature on the effects of subanesthetic or low doses of ketamine on serotonin release in the brain. Nikiforuk *et al* (2010) reported that 10 mg/kg of ketamine did not affect serotonin concentration in the rat prefrontal cortex. It is unlikely that the slightly increased serotonin level by ketamine infusion affected the [^{11}C]DASB binding in the present study. Milak *et al* (2005) reported that [^{11}C]DASB binding is decreased when the extracellular serotonin level was 'decreased' in the living brain of nonhuman primate. They explained that the decreased [^{11}C]DASB binding was due to an internalization of SERT, probably by protein kinase C (PKC)-dependent phosphorylation and sequestration (Ramamoorthy and Blakely, 1999). Serotonin prevents PKC-dependent phosphorylation and sequestration of SERT. A decreased synaptic serotonin level promotes the internalization of SERT. It is also unlikely that the decreased [^{11}C]DASB binding by ketamine infusion was induced by an internalization of the SERT in the present study, because ketamine infusion induced 'increased', not decreased, levels of serotonin in the ECF.

The present study indicates that subanesthetic doses of ketamine decreased SERT activity and increased prefrontal serotonin release for only a short time. In clinical settings, ketamine brings about both rapid and long-lasting antidepressant effect (Berman *et al*, 2000; Diazgranados *et al*, 2010; Price *et al*, 2009; Zarate *et al*, 2006). Probably, there is a mechanism to switch the initial transient effects to long-lasting antidepressant effect. Among the multiple serotonin receptor subtypes, 5-HT$_{1A}$-R is highly enriched in the prefrontal cortex (Feng *et al*, 2001; Kia *et al*, 1996a). Postsynaptic 5-HT$_{1A}$-Rs are found only in the dendritic compartment and associated with dendritic spines (Kia *et al*, 1996b) in which glutamate receptors are concentrated. Intriguingly, physical interactions between 5-HT$_{1A}$-R and NMDA receptors in the prefrontal cortex have been reported. Although some contradictory results are reported (Purkayastha *et al*, 2012; Yuen *et al*, 2005), activation of 5-HT$_{1A}$-R by enhanced serotonin transmission suppresses NMDA receptors in the prefrontal cortex (Yuen *et al*, 2005). Poleszak *et al* (2011) suggested a possible contribution of the serotonergic system to the antidepressive effect of glycine/NMDA receptor antagonists. When animals were pretreated with an inhibitor of serotonin synthesis, the antidepressant effects of glycine/NMDA receptor antagonists were abolished. Li *et al* (2010) reported that activation of mammalian target of rapamycin (mTOR) signaling by ketamine elevated the expression of synapse-associated proteins and spine numbers in the prefrontal cortex of rat. In addition, these effects resulted in enhanced serotonergic neurotransmission observed at 24 h post ketamine injection, which represented a mechanism for the rapid antidepressant actions of ketamine (Li *et al*, 2010). Recently, a basic study of liver disease showed that serotonin activated mTOR signaling in mice fed with a high fat and high fructose diet (Osawa *et al*, 2011). Enhanced serotonergic neurotransmission during ketamine infusion initiates an activation cascade of the mTOR pathway. This interaction between the serotonergic system and NMDA receptor might provide the switching mechanism of the transient effects to long-lasting antidepressant effects of subanesthetic doses of ketamine even after the rapid ketamine disappearance from the brain.

A major clinical limitation of SSRI treatment is the markedly delayed onset of therapeutic efficacy. This is thought to be caused by indirect activation of 5-HT$_{1A}$-R (Chaput *et al*, 1986; Invernizzi *et al*, 1996). [^{18}F]MPPF for the 5-HT$_{1A}$-R could be used for the evaluation of 5-HT$_{1A}$ autoreceptor internalization (Zimmer *et al*, 2004). It has been demonstrated that decreased [^{18}F]MPPF binding in the dorsal raphe occurs after an acute SSRI administration in both an animal study (Riad *et al*, 2004) and a clinical PET study (Sibon *et al*, 2008). Hence, the 5-HT$_{1A}$ autoreceptor internalization in response to acute blockade of SERT and a subsequent rise in extracellular 5-HT level in the dorsal raphe (Bel and Artigas, 1992; Gartside *et al*, 1995; Invernizzi *et al*, 1992) appears to be the most feasible interpretation for the decreased [^{18}F]MPPF binding in the dorsal raphe after SSRIs.

Synergies associated with combining NMDA antagonists and other treatments have been proposed by Krystal (2010). Artigas (1993) has proposed that the administration of 5-HT$_{1A}$-R antagonists could accelerate the clinical therapeutic efficacy of SSRIs. The 5-HT$_{1A}$-R antagonists could prevent the internalization of 5-HT$_{1A}$ autoreceptors in the dorsal raphe, and this mimic the 5-HT$_{1A}$-R desensitization produced by the prolonged administration of SSRIs. Animal studies (Beyer *et al*, 2002; Ceglia *et al*, 2004; Dawson and Nguyen, 1998; Gartside *et al*, 1995; Hjorth *et al*, 1997; Sharp *et al*, 1997), and a clinical trial (Artigas *et al*, 1994), partially support this hypothesis. However, one important concern is the lack of selectivity of 5-HT$_{1A}$-R antagonists for presynaptic *vs* postsynaptic 5-HT$_{1A}$-R (Rabiner *et al*, 2002). Full blockade of postsynaptic 5-HT$_{1A}$-Rs may cancel the increased serotonergic transmission. In the present study, the rapid inhibition of SERT by subanesthetic doses of ketamine, without affecting 5-HT$_{1A}$-R, may contribute to the quick antidepressant effect of ketamine. This interpretation is supported by the microdialysis results that extracellular serotonin levels in the prefrontal cortex increase rapidly after subanesthetic doses of ketamine.

It is known that ketamine at doses of 25–30 mg/kg induces dopamine release ca. 2–5 times in the rat prefrontal cortex (Lindefors *et al*, 1997; Verma and Moghaddam 1996). Ketamine at dose of 30 mg/kg also induced dopamine release in the striatum, although small amount of increase (ca. 25%) was observed (Moghaddam *et al*, 1997). In the several previous studies, [^{11}C]raclopride, a PET probe for dopamine D$_2$ receptor, has been used to monitor the synaptic dopamine level following administration

of subanesthetic ketamine, showing conflicting results. Thus, some reports demonstrated that the subanesthetic ketamine significantly decreased [^{11}C]raclopride binding in the striatum of human brain (Breier *et al*, 1998; Smith *et al*, 1998). Other reports, in contrast, showed no significant effect of ketamine on the striatal [^{11}C]raclopride binding in human brain (Aalto *et al*, 2002; Kegeles *et al*, 2002). At anesthetic doses of ketamine, we previously reported a dose-dependent decrease in [^{11}C]raclopride binding and increase in [^{11}C]β-CFT binding in the striatum of monkey brain (Tsukada *et al*, 2000). We interpreted that dynamic turnover of endogenous dopamine, accompanied by increased dopamine synthesis/release and facilitated DAT availability, resulted in the decreased [^{11}C]raclopride binding at the anesthetic doses of ketamine. As our present data showed no significant changes in DAT availability and extracellular dopamine level after subanesthetic dose of ketamine, we speculate that subanesthetic doses of ketamine might not affect [^{11}C]raclopride binding in the striatum of monkey brain.

A limitation in interpreting the results of the present study is that the changes in SERT availability, measured by PET, as well as the serotonin levels in the prefrontal cortex, as determined by microdialysis, were small. These alterations occurred using normal animals. Animal models of depression should be used with the same experimental protocol. It may be possible to detect greater changes in serotonergic transmission by low-dose ketamine more clearly, especially the mTOR signaling pathway, brain-derived neurotrophic factor release, and so on.

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