Ketamine Has Equal Affinity for NMDA Receptors and the High-Affinity State of the Dopamine D_2 Receptor

To the Editor:

The important study by Kegeles et al (2000) finds that ketamine enhances the amphetamine-induced release of endogenous dopamine in healthy volunteers. These types of experiments should be helpful in determining a basis for the dopamine overactivity hypothesis of schizophrenia (van Rossum 1967) currently supported by imaging data that relate to heightened dopamine release in patients (Laruelle et al 1999), and the fact that all antipsychotic drugs block dopamine D_2 receptors in relation to their clinical potency (Seeman and Tallerico 1998; Seeman et al 1975).

In principle, because ketamine blocks N-methyl-D-aspartate (NMDA) receptors, ketamine could be useful in modeling a possible basis for dopamine hyperactivity in schizophrenia, under the assumption that it arises from an abnormal glutamate/dopamine interaction in schizophrenia. However, the use of ketamine is premised on its selectivity for the NMDA receptor.

Recent data from our laboratory causes us to question this selectivity, because we find that ketamine has an equal affinity for NMDA receptors and the high-affinity state of dopamine D_2 receptors, as shown in Figure 1. As previously described (George et al 1985; Seeman et al 1985), the high-affinity states of dopamine D_2 receptors are more readily revealed in the presence of 10 mM NaCl. Under these conditions, we find that ketamine shows equal affinity for the NMDA receptor and the high-affinity state of the dopamine D_2 receptor (as measured using the antagonist [^{3}H]-raclopride or the agonist [^{3}H]-dopamine).

As presented in Figure 1, it can be readily observed that ketamine inhibits the high-affinity state of dopamine D_2 receptors (labeled by 2 nM [^{3}H]raclopride) with a dissociation constant of 1 ± 0.15 μM (using [^{3}H]raclopride), while inhibiting NMDA receptors (labeled by 2–6 nM [3H]MK801) with a dissociation constant of 0.5 ± 0.15 μM (Figure 1, bottom). Even though the density of high-affinity states for cloned dopamine D_2 receptors was low because of the low concentration of G protein in these tissue culture cells, ketamine also inhibited these high-affinity states with a dissociation constant of 0.5 ± 0.2 μM (Figure 1, middle). Dopamine itself has a dissociation constant of 1.5 ± 0.2 nM for the high-affinity state of dopamine D_2 receptors in rat striatal homogenates and on cloned dopamine D_2short(human) receptors (in GH4Cl cells). These high-affinity sites for the dopamine D_2 receptors were eliminated in the presence of 200 μM guanilylimiodiphosphate, and the affinity of ketamine was much lower for the low-affinity sites for the dopamine D_2 receptors in the presence of the guanine nucleotide, as illustrated in Figure 1 (top and middle).

Thus, because the high-affinity states of the dopamine D_2 receptors are physiologically functional (George et al 1985), ketamine would be expected to inhibit the binding of dopamine at these high-affinity states at presynaptic dopamine receptors. In addition, we found that ketamine (in the concentration range of

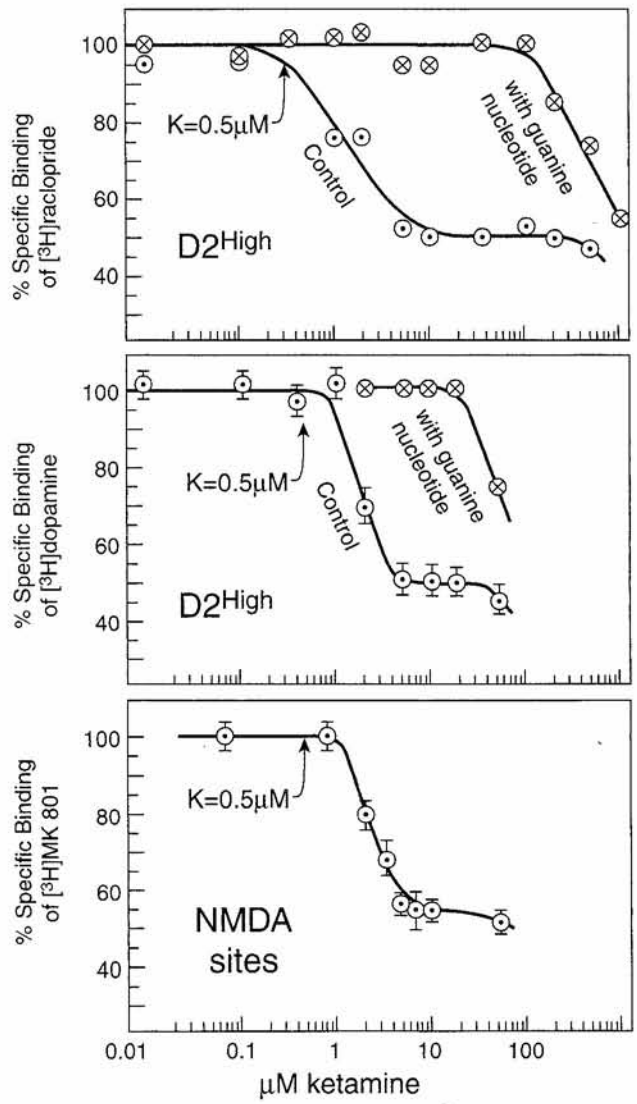

Figure 1. Ketamine had similar affinity for the NMDA receptors (bottom; rat striatal homogenate; labeled by 2 nM [^{3}H]MK 801) and the high-affinity state of dopamine D_2 receptors, as labeled by either 4 nM [^{3}H]dopamine (middle; cloned D_2short (human) receptors in GH_4Cl cells) or 2 nM [^{3}H]raclopride (top; rat striatal homogenate), indicating that ketamine is not selective for NMDA receptors. The addition of 200 μM guanilylimidodiphosphate converted the high-affinity states of D_2 into low-affinity states, which were not sensitive to ketamine in the μM range (top). Shown here are representative experiments. For a series of experiments, the dissociation constant (Ki) for ketamine was 0.5 ± 0.2 μM at the cloned dopamine D_2high receptor, 1 ± 0.2 μM (n = 5) at the dopamine D_2high receptor in rat striatal homogenate, and 0.5 ± 0.15 μM (n = 4) at the NMDA receptor [where the respective dissociation constants (Kd) were [^{3}H]raclopride (1.9 nM), [^{3}H]dopamine (1.5 nM), or [^{3}H]MK 801 (2 nM), as separately determined by Scatchard-type experiments]. Nonspecific binding was that which occurred in the presence of 10 μM S-sulpiride for [^{3}H]raclopride, 200 nM haloperidol for [^{3}H]dopamine, and 10 μM phencyclidine for [^{3}H]MK 801. The buffer contained 50 mM Tris-HCl, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl2, 4 mM MgCl2 and 10 mM NaCl.

0006-3223/01/$20.00
PII S0006-3223(01)01110-6

0.3 to 1 μM) behaved as a partial agonist/antagonist on the dopamine D_2 receptor. For example, using the incorporation of [^{35}S]GTP-gamma-S as a measure of agonist action, ketamine stimulated the incorporation of [^{35}S]GTP-gamma-S into tissue cell membranes containing cloned dopamine D_2 receptors in GH_4Cl cells. At the same time, however, ketamine diminished the ability of dopamine to stimulate the incorporation of [^{35}S]GTP-gamma-S in these same tissue culture cells (Kapur and Seeman, in preparation).

If ketamine exerts similar effects on the high-affinity states of the dopamine D_2 receptors in vivo, it could contribute to the main finding in the Kegeles et al study through nonglutamatergic mechanisms. For example, through an effect on the presynaptic autoreceptors, it may enhance the dopamine release (Starke et al 1978). It is true that Kegeles et al did not see a change in their V3″ measure with ketamine alone—and this would argue against a direct effect of ketamine on dopamine receptors. But several other PET findings would be consistent with our present data. First, three other PET studies, using [^{11}C]-raclopride, noted a decrease in binding (Breier et al 1998; Smith et al 1998; Vollenweider et al 2000), and we are not aware of any other negative finding in this regard. Secondly, a recent PET study combined with microdialysis and PET with [^{11}C]-raclopride (Tsukada et al 2000) observed a significant decrease in the [^{11}C]raclopride binding under the influence of ketamine, without an increase in dopamine in the extracellular fluid—compatible with the idea that ketamine may have a direct effect on the high-affinity states of the dopamine receptors.

In summary, we question the use of ketamine as a proof or as a model for a hypoglutamatergic state. In vitro data suggests that ketamine has multiple sites of action in the concentration range of 0.3 to 1 μM, and, therefore, a more NMDA-selective medication needs to be found to examine glutamate-dopamine interactions in schizophrenia.

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Reply

To the Editor:

Kapur and Seeman report the new observation that the in vitro affinity of ketamine for the high affinity state of the D_2 receptor is similar in magnitude to that for the NMDA receptor (Kapur and Seeman 2001). This situation contrasts with the affinities of ketamine for several other receptors known to play a role in dopamine release (mu opioid, sigma, dopamine transporter), which are one to two orders of magnitude lower than that for the NMDA receptor (Kegeles et al 2000b). Based on their new finding, Kapur and Seeman conclude that "ketamine has multiple sites of action in the concentration range of 0.3–1 μmol/L, and, therefore, a more NMDA-selective medication needs to be found to examine glutamate-dopamine interactions in schizophrenia."

We agree that a more selective noncompetitive NMDA receptor antagonist would be desirable for human investigation; however, the observation reported by Kapur and Seeman does not invalidate the conclusion of our study (Kegeles et al 2000b),

which is that ketamine administration increases amphetamine-induced DA release in humans, and that this effect is mediated by NMDA receptor blockade.

Based on the findings that ketamine displays comparable affinity in vitro for the high affinity state of the D_2 receptor and for the NMDA receptors, Kapur and Seeman raised the possibility that the increased amphetamine-induced displacement of [^{123}I]IBZM observed in the presence of ketamine (0.2 mg/kg bolus followed by a constant infusion of 0.4 mg/kg/hour for 4 hours) could be due to a direct effect of ketamine on [^{123}I]IBZM binding rather than an augmentation of amphetamine-induced dopamine release; however, this possibility can be excluded because, in our study, no effect of ketamine alone was detectable on [^{123}I]IBZM binding (Kegeles et al 2000b). This observation provides evidence of an absence of significant occupancy of the D_2 receptor by ketamine in vivo at the dose used in our study. In addition, MK-801, another noncompetitive NMDA antagonist, has been shown to increase amphetamine-induced DA release in rodents (Miller and Abercrombie 1996). Thus, the most reasonable interpretation of our study is that the effect of ketamine on amphetamine-induced [^{123}I]IBZM displacement was mediated by NMDA receptor blockade, and that extrapolation of in vitro data such as those reported by Kapur and Seeman to human in vivo conditions can sometimes be hazardous.

Aware that the data from our study contradicts their hypothesis, Seeman and Kapur cited the results of three PET studies in humans (Breier et al 1998; Smith et al 1998; Vollenweider et al 2000) and one in nonhuman primates (Tsukada et al 2000) that found a significant decrease in [^{11}C]raclopride in vivo binding produced by ketamine. They pointed out, as we had in our article, that the lack of ketamine effect on [^{123}I]IBZM binding observed in our study conflicts with the results of these [^{11}C]raclopride studies.

In the article, we postulated that this contradiction might be due to a lower vulnerability to endogenous competition of [^{123}I]IBZM binding compared to [^{11}C]raclopride binding. To test this hypothesis, we have recently gathered new data with [^{11}C]raclopride, but failed to observe any effects of ketamine on [^{11}C]raclopride binding potential (Kegeles et al 2000a).

The effect of ketamine was studied twice in five healthy volunteers (n = 10 experiments). A 2-hour PET scan was performed with a bolus plus constant infusion of [^{11}C]raclopride (bolus to infusion ratio of 105 min), and ketamine was administered beginning at 50 min (0.12 mg/kg IV bolus followed by 0.65 mg/kg/hour IV infusion for 70 min). Striatal specific to nonspecific binding ratios were quantified pre (30–50 min) and during ketamine infusion (75–100 min). We observed no significant differences in striatal [^{11}C]raclopride specific binding (V_3'') before or during ketamine infusion.

The dose of ketamine used in our [^{11}C]raclopride study (0.12 mg/kg IV bolus followed by 0.65 mg/kg/hour IV infusion for 70 min) was selected to be identical to the dose used by Breier et al (1998), and the general imaging method (ketamine administered as a bolus plus infusion in the middle of a [^{11}C]raclopride bolus plus infusion study) was also similar to the method used by Breier et al (1998). Yet, these authors reported a significant decrease in [^{11}C]raclopride BP induced by ketamine ($-11.2 \pm 8.9\%$, $n = 9$). In our opinion, the discrepancy in the results might stem from a difference in a critical experimental parameter, the [^{11}C]raclopride bolus to infusion rate ratio (K_{bol}). Breier et al (1998) used a K_{bol} of 130 min (i.e., the amount of activity in the bolus was equal to the amount of activity infused over 130 min after decay correction). A subsequent report by the same group (Watabe et al 2000) indicated that this K_{bol} was associated with a risk of "overshoot" (i.e., too much activity in the bolus relative to the infusion rate), and suggested the use of a lower K_{bol} (i.e., 105 min). We followed this suggestion in our study, and used a K_{bol} of 105 min after confirming that this K_{bol} was appropriate (Martinez et al 2000). The use of a K_{bol} of 130 min in the Breier et al (1998) study might have been associated with an artifactual decrease in [^{11}C]raclopride BP in subjects who received ketamine. Experiments performed in our group confirmed that the use of a K_{bol} of 130 min was associated with an overshoot, and an artifactual decrease in striatum to cerebellum ratio from the 30–50 min to the 75–100 min interval (in the absence of any pharmacological challenge).

In the Smith et al (1998) study, two [^{11}C]raclopride scans were obtained before and after ketamine administration (0.5 mg/kg infused over 20 min). [^{11}C]raclopride was given as a single bolus. Sixty minutes of data were obtained and analyzed with the method of Logan et al (1990). These authors reported a significant decrease ($-13.7 \pm 9.3\%$) in [^{11}C]raclopride striatal specific distribution volume (DV) following ketamine. Yet, the results of this study are questionable for two reasons. First, for each subject, the authors took the unusual approach of averaging the cerebellum DV measured in pre- and postketamine conditions, and used this "average cerebellum DV" to calculate striatal specific DV. Second, the authors did not report the effect of the challenge on the distribution volume ratio (DVR, i.e., specific striatal DV/cerebellum DV, or V_3''). For a within-subject design, DVR is generally considered a more robust outcome measure than specific striatal DV. In the absence of DVR information and a more standard computation method for striatal specific DV, it is difficult to evaluate the robustness of the effect reported by Smith et al (1998).

The study of Vollenweider et al (2000) is the most suggestive that sufficiently high dosing of ketamine may produce a decrease in [^{11}C]raclopride BP in healthy humans. That study used (S)-ketamine, in contrast to the U.S. studies where the racemic mixture was used (Breier et al 1998; Kegeles et al 2000a, 2000b; Smith et al 1998). In addition, the dosing in Vollenweider et al (2000) (bolus of 15 mg, followed by 0.84 mg/kg/hour infusion rate) was higher than in all the U.S. studies, yielding an hourly rate of (S)-ketamine administration nearly triple that of the U.S. studies using an infusion paradigm (Breier et al 1998; Kegeles et al 2000a, 2000b). Thus, the Kapur and Seeman finding might be relevant to the robust effect of these high doses of (S)-ketamine on [^{11}C]raclopride BP reported by Vollenweider et al (2000).

In a similar way, the markedly higher ketamine dosing (3 or 10 mg/kg/hour) used in the primate study of Tsukada et al (2000) might have resulted in intracerebral levels of ketamine associated with potentially detectable occupancy of the D_2 receptor high affinity state.

On the whole, the variable effects of ketamine on the binding of dopamine-receptor ligands is not surprising in view of the findings in laboratory animals that the dopaminergic activity and

release of dopamine induced by the prototypic noncompetitive NMDA receptor antagonist MK-801 has been found to vary considerably in different experiments reported in the literature and even in different parts of the dopaminergic system (Miller and Abercrombie 1996; Svensson 2000). Thus, variations in the displacement of dopamine-receptor ligands by ketamine are not unexpected but may be induced by variations in the release of the endogenous transmitter. This in turn may be related to variations in the baseline conditions, resulting, for example, in variations in glutamatergic tone.

In conclusion, the finding of Kapur and Seeman could possibly place a limit on the selectivity of ketamine for the NMDA receptor and be relevant to the interpretation of the results reported by Vollenweider et al (2000) and Tsukada et al (2000); however, at the dose used in the human U.S. studies, ketamine does not seem to affect the in vivo binding potential of [^{123}I]IBZM or [^{11}C]raclopride. Therefore, NMDA receptor blockade remains the most likely mechanism underlying the increase in amphetamine-induced [^{123}I]IBZM displacement observed following ketamine in this study. This mechanism should be confirmed using other pharmacological strategies to impair NMDA transmission.

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PII S0006-3223PII S0006-3223(01)01112-X

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