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# Mode of binding of [$^3$H]dibenzocycloalkenimine (MK-801) to the *N*-methyl-D-aspartate (NMDA) receptor and its therapeutic implication

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Binding of the labeled anticonvulsant drug [$^3$H]dibenzocycloalkenimine ([$^3$H]MK-801) to the *N*-methyl-D-aspartate (NMDA) receptor and its dissociation from the receptor at 25°C are slow processes, both of which follow first order kinetics ($t_{1/2} \simeq 70$ and 180 min, respectively). Both reactions are markedly accelerated by glutamate and glycine ($t_{1/2} \simeq 5-8$ and 4 min, respectively), which allow bimolecular association kinetics of the labeled drug with the receptors whereas equilibrium binding of [$^3$H]MK-801 ($K_d$ 2–4 nM) is hardly affected by glutamate and glycine. The data suggest that MK-801 acts as a steric blocker of the NMDA receptor channel. The competitive antagonist D-(−)-2-amino-5-phosphovaleric acid (AP-5) freezes the receptor in a state which precludes either binding of [$^3$H]MK-801 to the receptor channel or its dissociation from it. These findings have therapeutic implications.

Phencyclidine; NMDA receptor; Dibenzocycloalkenimine

## 1. INTRODUCTION

PCP and related drugs, including the anticonvulsant MK-801, are potent noncompetitive blockers of glutamate receptors of the NMDA subtype [1,2]. Several authors have demonstrated a glutamate-induced enhancement of the binding of [$^3$H]MK-801 as well as of [$^3$H]TCP, a potent PCP analog [2–5]. We recently showed that the enhanced binding of [$^3$H]TCP is due not to a change in the equilibrium binding but rather to an acceleration in the rates of both association and dissociation of the receptor-ligand complex [5]. The mechanism of interaction between [$^3$H]MK-801 and the NMDA receptor is however not known. In this paper we describe kinetic and equilibrium binding experiments with [$^3$H]MK-801, which point to a nonallosteric interaction between NMDA receptor against binding sites and the [$^3$H]MK-801 binding site.

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*Abbreviations:* PCP, phencyclidine; TCP, *N*-[1-(2-thienyl)-cyclohexyl]piperidine; NMDA, *N*-methyl-D-aspartate; AP-5, D-(−)2-amino-5-phosphovaleric acid; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate

## 2. MATERIALS AND METHODS

[$^3$H]MK-801 (29.4 Ci/mmol) was purchased from New England Nuclear. All other chemicals were as detailed [5]. Binding assays were performed in triplicate at 25°C with repeatedly washed rat cerebral cortex membranes (80 μg protein) as described elsewhere [5]. Assays were carried out in the absence or in the presence of $10^{-4}$ M AP-5, or in the presence of 10 μM $MgCl_2$ together with 1 μM L-glutamate and 1 μM glycine. Nonspecific binding was determined in samples containing $10^{-4}$ M PCP. Data were expressed as the specific binding of [$^3$H]MK-801 and analyzed according to the two-step binding model of interactions between noncompetitive blockers and the NMDA receptor channel [5]. The model assumes a first order diffusion of the ligand from the outside into the interior of the channel, followed by association of the ligand with its receptor sites. Under the two extreme conditions employed

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here, viz., binding of [$^3$H]MK-801 in the presence of agonists (unlimited diffusion) and in the absence of agonists (limited diffusion) the time courses of receptor occupation should respectively follow pseudo-first order (eqn 1) and first order (eqn 2) kinetics [5]:

$$([RL]_{eq} - [RL])/([RL]_{eq} \equiv \exp. - (k_1k_2[L]/k_b - k_{-1})t \quad (1)$$

$$1 - \{K_d[RL]/[L]([R_T] - [RL]\} = \exp. - k_bt \quad (2)$$

where L, $R_T$, $RL_{eq}$ and RL are respectively the ligand, the total number of binding sites, the bound ligand at equilibrium and at time $t$. $k_a$ and $k_b$ are the forward and backward diffusion constants; $k_1$ and $k_{-1}$ are the second order on-rate and the first order off-rate constants for the binding process. The overall equilibrium binding constant ($K_d$) is given by $k_{-1} \cdot k_b/k_1 \cdot k_2$.

## 3. RESULTS AND DISCUSSION

Fig.1A demonstrates typical time courses for [$^3$H]MK-801-receptor association, with and without glutamate and glycine. The association rate of 5 nM [$^3$H]MK-801 with the receptor in the absence of exogenous agonists was very slow ($t_{1/2}$ $\simeq$ 70 min) and reached equilibrium only after a prolonged incubation time (>6 h). Upon addition of glutamate and glycine the rate of [$^3$H]MK-801 binding to the receptor was markedly increased ($t_{1/2}$ $\simeq$ 7 min) and the reaction approached

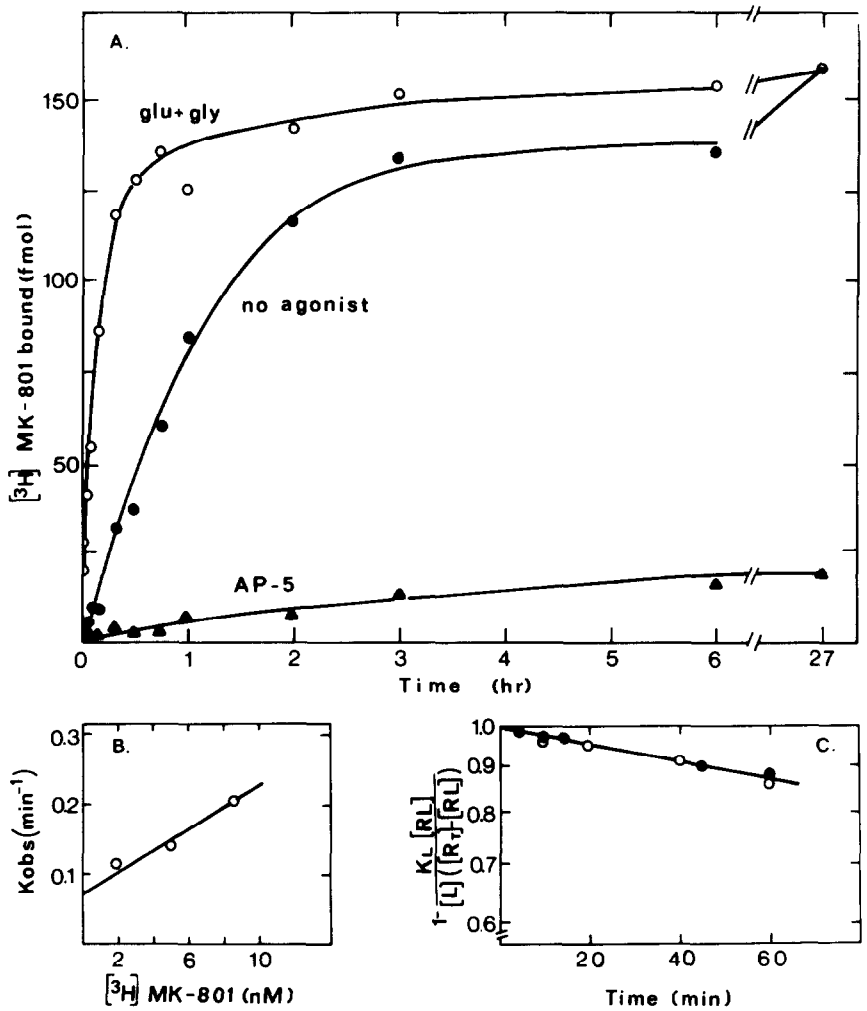


Fig.1. (A) Time course of association of [$^3$H]MK-801 (5 nM) with the NMDA receptor channel. Binding was determined at 25°C as a function of time in the absence (●) and in the presence of glutamate and glycine (○) or in the presence of AP-5 (▲). (B) The observed time constants ($K_{obs}$) are derived from the pseudo-first order plots of the association of 2, 5 and 8.6 nM [$^3$H]MK-801 in the presence of glutamate and glycine plotted as a function of the ligand concentration. (C) First order plot (eqn 2) of [$^3$H]MK-801-receptor association in the absence of agonists determined with ligand concentrations of 2 nM (○) and 8.6 nM (●).

equilibrium within 45–60 min. The increase in ligand-receptor binding was time-dependent; immediately after the onset of binding it was very high (~9 times that of the control) and then it declined (fig.1A). Similar time courses (not shown) were followed by 2 nM and 8.6 nM [$^3$H]MK-801. The kinetics of [$^3$H]MK-801 binding in the presence of glutamate and glycine followed a pseudo-first order scheme (eqn 1): the observed time constants ($K_{obs}$) varied linearly with [$^3$H]MK-801 concentrations (fig.1B), and the ratio between the apparent on-rate time constant ($1.55 \times 10^7$ $M^{-1} \cdot min^{-1}$) and the apparent off-rate time constant (0.075 $min^{-1}$) was 4.8 nM. This kinetically derived dissociation constant ($K_d$) was similar to the $K_d$ for [$^3$H]MK-801 determined at equilibrium in the presence (4.2 nM) and in the absence (2.1 nM) of glutamate and glycine (fig.2).

In the absence of exogenous agonists the kinetics of ligand-receptor association did not follow a pseudo-first order scheme, as shown by the fact that the half-times of the reactions (60–70 min) did not vary as a function of [$^3$H]MK-801 concentration. These kinetics fitted well to the first order reaction scheme (eqn 2), and the observed time constant ($k_b$) derived from the kinetics of ligand receptor association ($2.28 \times 10^{-3}$ $min^{-1}$, fig.1C) was similar to the dissociation time constant obtained when receptor-ligand dissociation was measured in the absence of agonists ($5.5 \times 10^{-3}$ $min^{-1}$, fig.2). Also, the addition of 1 $\mu$M glutamate to [$^3$H]MK-801-receptor complexes resulted in a marked increase in the dissociation rate (half-time 18 min, compared to the control value of 180 min, fig.2) and the effect was enhanced by glycine (half-time 4 min, fig.2). Consistency with the two-step binding model (see section 2) was further shown by the fact that the dissociation rate constant determined in the presence of glutamate and glycine (0.1 $min^{-1}$, fig.2) was similar to $k_{-1}$ value obtained under the same conditions during ligand-receptor association (0.075 $min^{-1}$, fig.1B). Taken together, the association and dissociation kinetics of [$^3$H]MK-801 binding to the receptor suggest that in the absence of agonists the rate-limiting step is the diffusion of the outside ligand ($L_o$) into the interior ($L_c$) of the presumably closed NMDA receptor channel

$$L_o \underset{k_b}{\overset{k_a}{\rightleftharpoons}} L_c,$$

whereas in the presence of agonists this limitation is removed (presumably because the channel is

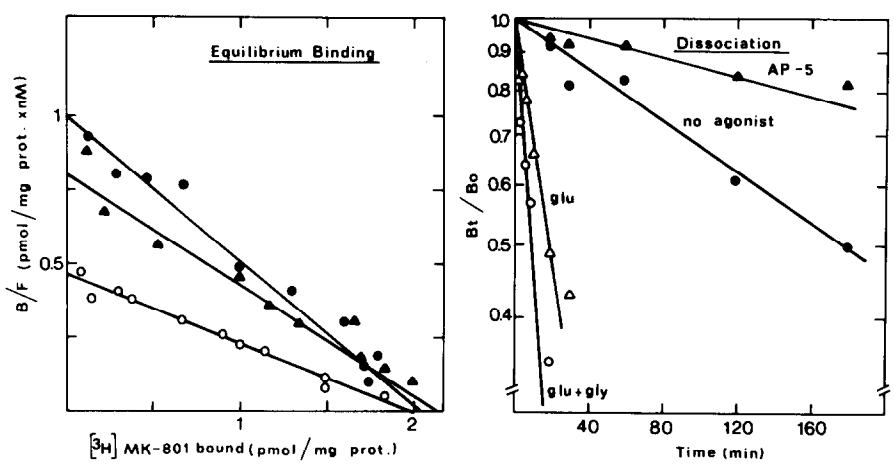


Fig.2. (Left) Equilibrium binding of [$^3$H]MK-801. Binding of [$^3$H]MK-801 as a function of its concentration was assayed after incubation for 4 h at 25°C in the absence (●) or in the presence (○) of glutamate and glycine. Also shown are data for [$^3$H]MK-801 binding to the receptor measured after their incubation for 3 h followed by an additional 1 h of incubation in the presence of 100 $\mu$M AP-5 (▲). Data are expressed in the form of Scatchard plot. (Right) First order plots of the dissociation of [$^3$H]MK-801-receptor complexes. Samples were incubated at 25°C with 8 nM [$^3$H]MK-801 for 2 h. The dissociation reaction was initiated by the addition of 100 $\mu$M unlabeled PCP. Data show the dissociation reaction without added agonists (●) and in the presence of 1 $\mu$M glutamate (△), 1 $\mu$M glutamate + 1 $\mu$M glycine (○), or 100 $\mu$M AP-5 (▲). Reactions were terminated either immediately (zero time) or at the indicated times. $B_o$, amount of [$^3$H]MK-801 bound at zero time. $B_t$, amount of [$^3$H]MK-801 bound at time $t$.

open) and the rate-limiting step is the binding process itself.

$$R + L_c \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RL.$$

It should be noted, however, that the competitive NMDA-receptor antagonist, AP-5, by itself decreases the rates of [$^3$H]MK-801-receptor association (fig.1A) and dissociation (fig.2) as compared to the control level, possibly indicating the presence of residual glutamate (or glycine) in the membrane preparation. It seems that AP-5 'freezes' the receptor in such a way that [$^3$H]MK-801 is prevented from penetrating into the channel (see fig.1A) or dissociating from it (fig.2). Indeed, the addition of AP-5 to preequilibrated [$^3$H]MK-801-receptor complexes did not alter the equilibrium binding (fig.2).

The kinetic results together with the equilibrium binding data indicate that glutamate and glycine affect mainly the rates of [$^3$H]MK-801-receptor complex formation and dissociation, and not the maximal number of binding sites (figs 1A and 2) or $K_d$ (fig.2). Thus, in agreement with the electrophysiological data [6], [$^3$H]MK-801 like [$^3$H]TCP [5] appears to act as a steric blocker of the NMDA-receptor channel and not as an allosteric effector.

The findings of this study have important therapeutic implications: the channel-ligand complexes formed following in vivo administration of MK-801 can be prevented from dissociating by a competitive antagonist (e.g. AP-5), which freezes the complex.

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