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MK-801 Alters the $GABA_A$ Receptor Complex and Potentiates Flurazepam's Antiseizure Efficacy

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DEUTSCH, S. I., C. H. PARK, L. G. LUKACS, C. MORN, L. KOETZNER AND J. MASTROPAOLO. *MK-801 alters the $GABA_A$* receptor complex and potentiates flurazepam's antiseizure efficacy. PHARMACOL BIOCHEM BEHAV **51**(4) 909–915, 1995.—MK-801 is an uncompetitive allosteric antagonist that interferes with glutamate-gated calcium ion conductance through the NMDA receptor-associated ionophore. In an outbred strain of mouse, MK-801 elicits episodes of explosive "popping" behaviors that may serve as a preclinical screening paradigm for novel antipsychotic medications. This investigation examined the effects of MK-801, at doses associated with the elicitation of popping, on the $GABA_A$ receptor complex in cerebral cortex, and flurazepam's ability to antagonize electrically precipitated seizures. Twenty four hours after MK-801 administration, there was an increased density of the radiolabeled antagonist-preferring conformation of the central benzodiazepine binding site and a potentiation of flurazepam's antiseizure efficacy. The data show that interference with NMDA receptor-mediated calcium ion conductance is associated with a relatively selective change in the $GABA_A$ receptor complex in cerebral cortex, and has functional behavioral consequences. Moreover, the data provide additional evidence for a delicate balance between GABAergic and glutamatergic transmission. Disturbance of this balance can have behavioral consequences for the animal.

NMDA receptor $GABA_A$ receptor MK-801 Flurazepam

PHENCYCLIDINE (PCP) is a dissociative anesthetic agent related to ketamine and approved for use in veterinary medicine. PCP is a substance of abuse that precipitates a schizophreniform psychosis in susceptible individuals with both "positive" and "negative" features (3,9). The positive or productive features include hallucinations and delusions, whereas the negative or deficit features include lack of motivation, social withdrawal, anergy, and flattening of facial affect (3,9). The close resemblance of the PCP-induced psychosis to naturally occurring schizophrenia has stimulated intensive investigation of PCP's behavioral and pharmacologic actions, and their possible relevance to the pathophysiology of schizophrenia.

PCP binds to a hydrophobic domain within the *N*-methyl-D-aspartic acid (NMDA) receptor-associated ionophore and is an uncompetitive allosteric antagonist of glutamate-stimulated calcium ion conductance (2,6,7). The ability of this "open-channel" blocker to interfere with glutamatergic transmission has focused attention on the possibility that "hypoglutamatergia" may be an endogenous pathophysiologic mechanism involved in the expression of schizophrenic symptomatology (3).

The "PCP model of schizophrenia" stimulated us to examine and characterize "popping," a behavior in mice elicited by MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptane-5,10-imine maleate; dizolcipine], a high-affinity PCP analogue (5). MK-801 causes an outbred strain of NIH Swiss mice to display discrete episodes of explosive jumping behavior. Moreover, episodes of this MK-801-elicited popping behavior were reduced in a dose-dependent fashion by haloperidol, a conventional antipsychotic medication, and clozapine an atypical antipsychotic medication. The latter data are con-

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sistent with the development of "popping" as a preclinical screening paradigm for potential antipsychotic medications, and the potential relevance of this behavior to endogenous pathophysiologic processes occurring in schizophrenia.

GABAergic deficits have been described in postmortem brain samples obtained from patients with schizophrenia (1,16, 17). These deficits include a reduction in the binding of ^{3}H-nipecotic acid, a biochemical marker of the sodium-dependent GABA-reuptake site, in medial temporal lobe structures (i.e., left polar temporal cortex, and amygdala and hippocampus bilaterally) and basal ganglia, and an upregulation in the binding of bicuculline-sensitive ^{3}H-muscimol in the anterior cingulate cortex. An upregulation of ^{3}H-muscimol binding is consistent with the loss of inhibitory basket cells in this cortical structure. Moreover, the cingulate cortex is sensitive to the induction of toxic intraneuronal vacuole formation by MK-801 (13). The possession by alprazolam, a GABA-positive benzodiazepine, of adjuvant therapeutic properties in the treatment of schizophrenia is consistent with the existence of GABAergic abnormalities in this disorder (19). There is also very compelling evidence in primary cultures of rat cerebellar granule cells that the regulation of mRNA expression of various $GABA_A$ receptor polypeptide subunits is controlled by activation of the NMDA receptor complex (12). Thus, glutamatergic activity may be an important regulator of GABAergic tone in situ.

In view of the delicate interaction between glutamatergic and GABAergic neural transmission, we wondered whether MK-801-elicited "popping" and the associated perturbation of NMDA-mediated glutamatergic transmission affected the $GABA_A$ receptor complex, the peripheral benzodiazepine binding site and a functional measure of GABAergic transmission. Thus, mice treated with MK-801 were separated into two groups displaying either "high" or "low" episodes of popping. Components of the $GABA_A$ receptor complex and the peripheral benzodiazepine binding site were measured in cerebral cortical tissue obtained from these animals 30 min and 24 h after MK-801 administration. We explored possible MK-801-induced changes in both the $GABA_A$ receptor complex and peripheral benzodiazepine binding in cerebral cortical tissue because of a report that stereotaxic infusion of 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), a competitive NMDA antagonist, into the medial prefrontal cortex of rats stimulated "episodic darting and occasional jumping" (14). Thus, the cerebral cortex might be an anatomic region involved in the mediation of MK-801-elicited popping behavior. Moreover, to demonstrate that MK-801-induced biochemical changes of the $GABA_A$ receptor complex have functional behavioral relevance for the animal, the ability of flurazepam to antagonize electrically precipitated tonic hindlimb extension was studied in these mice 24 h after MK-801 administration.

METHOD

Subjects

Experimentally naive, male outbred NIH Swiss mice weighing 25–30 g were used throughout the experiments. Mice were housed in groups of five per cage, and maintained in a temperature controlled room on a 12L : 12D cycle with free access to food and water.

Drugs

MK-801 was obtained from Research Biochemicals International (Natick, MA), and flurazepam HCl was obtained from Hoffmann-La Roche (Nutley, NJ). All drugs were injected intraperitoneally (IP) in a volume of 0.01 ml/g body wt. Flurazepam was always injected 20 min before the incremental electroconvulsive shock (IECS) procedure (see below).

Observations for Episodes of Popping

Mice were injected IP with MK-801 at a dose of 1.0 mg/kg body wt. MK-801 solution was freshly prepared in distilled water and administered IP. Immediately after injection, individual mice were placed in clear plastic cages (28 × 20 × 15 cm) with crushed corn cobs as bedding for behavioral observations. Discrete episodes of explosive "popping" behavior were counted for the entire 30-min interval following IP injection of MK-801 (5). Episodes of popping were counted because of the prominence of this behavior. The episodes often differed from each other with respect to duration and intensity. Groups of five animals were observed simultaneously; thus, the number of "pops" shown by an individual mouse during a discrete episode of "high-frequency" popping could not be quantified by visual observation alone. "Low popping" and "high popping" groups of mice were defined as those displaying fewer than 10 and more than 25 discrete episodes of popping during the 30-min observation interval, respectively. Mice from both groups were decapitated immediately after 30 min of observation, and 24 h after injection of MK-801. Cerebral cortices were dissected on ice and stored at −85°C until the time of assay. This procedure was approved by the Animal Studies Subcommittee and Research Committee of this Department of Veterans Affairs Medical Center.

Incremental Electroconvulsive Shock (IECS) Procedure

The influence of treating mice with MK-801 (0.5, 1.0, and 1.5 mg/kg) on flurazepam's antiseizure efficacy was tested 24 h after its administration. On day 2, all animals were injected with vehicle or one of several doses of flurazepam (5.6, 10, 18, 32, or 56 mg/kg, IP) 20 min before the IECS procedure. In the IECS procedure, a Hittman electroconvulsive shock generator (Medcraft model B24-II) was used to administer 0.3 s of voltage via earclip electrodes. To determine the threshold voltage for the precipitation of tonic hindlimb extension, the procedure began with 70 V and was increased in 10 V increments every 2 s until maximal tonic hindlimb extension occurred or 170 V was reached. A voltage of 180 was recorded for animals that did not show tonic hindlimb extension.

Tissue Preparation

Crude membranes for ^{3}H-flunitrazepam, ^{3}H-Ro15-1788, and 35S-TBPS binding assays were prepared according to the description of Park et al. (15). A slight modification of the method described by Weissman et al. (18) was used to prepare crude membranes to measure the specific binding of ^{3}H-PK-11195 and ^{3}H-Ro5-4864. Briefly, for measurement of the peripheral benzodiazepine binding site, cerebral cortices were homogenized in 50 vol of 50 mM Tris-HCl (pH 7.4) buffer with a Brinkmann Polytron (10 s at maximal speed), and centrifuged at 20,000 × g for 20 min at 4°C. The resulting pellets were resuspended in 100 vol of the same Tris buffer and used as the final homogenate. The concentration of protein in all of the final tissue suspensions was determined by the method of Lowry et al. (10).

^{3}H-Flunitrazepam and ^{3}H-Ro15-1788 Binding

The specific binding of ^{3}H-flunitrazepam (spec. act. 91 Ci/mmol; New England Nuclear, Boston, MA) and ^{3}H-Ro15-

1788 (83.2 Ci/mmol; New England Nuclear) was performed as described by Park et al. (15). Briefly, binding was studied in an incubation volume of 1.0 ml using 100 μl of either a ^{3}H-flunitrazepam or ^{3}H-Ro15-1788 solution, 100 μl of tissue homogenate (approximately 0.1 mg protein), and 50 mM Tris-citrate buffer (pH 7.4) to the final volume. Nonspecific binding was determined with the addition of 100 μl midazolam (final concentration 10 μM) and represented < 10% of the total binding. The assay was initiated with the addition of tissue homogenate and proceeded for 60 min at 4°C. In the experiments examining the chloride ion dependence of ^{3}H-flunitrazepam binding, tissue homogenate was incubated in the presence of 200 mM NaCl, the concentration shown to have the greatest effect on ^{3}H-flunitrazepam binding in a previous study (15). The reaction was terminated by addition of 5.0 ml ice-cold Tris-citrate buffer and immediate vacuum filtration through Whatman GF/B glass fiber filters (Whatman, Clifton, NJ). The filters were then rinsed twice with 5.0 ml ice-cold buffer and counted using liquid scintillation spectrometry.

^{35}S-t-Butylbicyclophosphorothionate (TBPS) Binding:

The binding of 2 nM ^{35}S-TBPS (spec. act. 110–130 Ci/mmol; New England Nuclear) was performed according to the modifications described by Park et al. (15). Briefly, binding was studied in an incubation volume of 1.0 ml consisting of 100 μl ^{35}S-TBPS solution, 500 μl tissue homogenate (approximately 0.4–0.5 mg protein), 100 μl 2 M NaCl, 100 μl of various concentrations of GABA solution (1 μM to 50 μM), and buffer to final volume. Nonspecific binding was determined using 10 μM picrotoxinin and represented < 20% of the total binding. The reaction was initiated with the addition of ^{35}S-TBPS and proceeded at 25°C for 100 min. The reaction was terminated by filtration through Whatman GF/B glass fiber filters; the filters were presoaked in ice-cold 0.05% polyethylenimine to reduce nonspecific binding. The filters were washed twice with 5 ml ice-cold Tris-citrate buffer (pH 7.4) and counted using liquid scintillation spectrometry.

^{3}H-Ro5-4864 and ^{3}H-PK-11195 Binding

The specific binding of ^{3}H-Ro5-4864 (spec. act. 86.3 Ci/mmol) and ^{3}H-PK-11195 (spec. act. 83.3 Ci/mmol) was performed according to a slight modification of the procedure described by Weissman et al. (18). The binding assay was done in a total incubation volume of 1.0 ml containing 100 μl of radioactive ligand, 600 μl of tissue homogenate (approximately 0.15 mg protein), and Tris-HCl buffer to the final volume. Nonspecific binding was determined with the addition of 20 μl of either an unlabelled solution of Ro5-4864 or PK-11195 (final concentration 5 μM); unlabelled Ro5-4864 and PK-11195 were dissolved in 100% ethyl alcohol to a final concentration of 250 μM. Nonspecific binding ranged between approximately 3 and 30% of the total binding depending on the concentration of the radiolabeled ligands. The assay was initiated with the addition of tissue homogenate and continued for 60 min at 4°C. The incubation was terminated with 5 ml of ice-cold Tris-HCl buffer and immediate vacuum filtration through Whatman GF/B glass fiber filters that were presoaked in ice-cold 0.05% polyethylenimine solution. The filters were rinsed two times with 5 ml ice-cold buffer and counted using liquid scintillation spectrometry.

Data Analysis and Statistics

The equilibrium binding constants were determined by Scatchard analysis. The raw data, expressed as disintegrations per minute (DPM), were subjected to equilibrium binding data analysis (EBDA), using the Collection of Radioligand Binding Analysis Programs (11). The program provided a mean and SEM for replicate determinations at each point. For each point, the program calculated the amount of bound (total, specific) and unbound (free) ligand concentration. The initial estimates of the dissociation constant (K_d), and maximum number of binding sites (B_{max}) were obtained from Scatchard plots using EBDA; for the final estimate of the constants the LIGAND program was used. All statistical analyses were carried out on an IBM PC AT computer using the GB-STAT Professional Statistics and Graphics version 3.0 (8).

In the IECS behavioral procedure, groups of at least 12 mice were tested in each of the experimental conditions. Data from the experiments were analyzed with a two-way analysis of variance (ANOVA) and subsequent post hoc tests when appropriate. All reports of statistical significance were based upon a p value of < 0.05.

RESULTS

Specific ^{3}H-flunitrazepam and ^{3}H-Ro15-1788 binding in cerebral cortex did not change 30 min after MK-801 injection in either mice displaying low or high episodes of popping, compared with controls (Table 1). In contrast to the results obtained 30 min after injection, the maximal density of specific ^{3}H-Ro15-1788 binding sites was increased significantly 24 h after MK-801 injection in mice displaying high episodes of popping behavior relative to control animals; the binding in mice displaying low episodes of this behavior did not differ from control animals (Table 2). There were no changes in the parameters of specific ^{3}H-flunitrazepam binding 24 h after MK-801 injection in either the low or high popping groups. Moreover, MK-801 injection or the intensity of popping was not associated with alterations of specific ^{3}H-Ro5-4864 or ^{3}H-PK-11195 binding to the peripheral or mitochondrial benzodiazepine binding site when assessed 30 min and 24 h after injection (Tables 3 and 4). As expected, there was a potentiation of the specific binding of ^{3}H-flunitrazepam by chloride ions (200 mM) in cerebral cortical membranes prepared from mice dis-

TABLE 1

^{3}H-FLUNITRAZEPAM AND ^{3}H-Ro15-1788 BINDING TO MOUSE CEREBRAL CORTEX 30 MIN AFTER MK-801 (1.0 mg/kg) INJECTION

	Control	Low Popping	High Popping
^{3}H-Flunitrazepam			
K_d (nM)	1.58 (0.15)	1.58 (0.14)	1.61 (0.23)
B_{max} (pmol/mg protein)	3.24 (0.58)	3.49 (0.21)	3.50 (0.33)
n	5	4	4
^{3}H-Ro15-1788			
K_d (nM)	0.83 (0.04)	0.79 (0.04)	0.77 (0.01)
B_{max} (pmol/mg protein)	4.19 (0.55)	4.66 (0.33)	4.68 (0.48)
n	5	5	5

All assays were performed in duplicate on individual cortices and values represent mean (sem). A one-way ANOVA showed that there was no significant difference in K_d ($F = 0.009$, $p = 0.991$) and B_{max} ($F = 0.117$, $p = 891$) for ^{3}H-flunitrazepam binding among the different groups. Also, ^{3}H-Ro15-1788 binding did not differ significantly among the three groups with respect to K_d ($F = 0.722$, $p = 0.505$) and B_{max} ($F = 0.016$, $p = 0.984$).

TABLE 2

^{3}H-FLUNITRAZEPAM AND ^{3}H-Ro15-1788 BINDING TO MOUSE CEREBRAL CORTEX 24 h AFTER MK-801 (1.0 mg/kg) INJECTION

	Control	Low Popping	High Popping
^{3}H-Flunitazepam			
K_d (nM)	2.19 (0.18)	2.19 (0.18)	2.00 (0.19)
B_{max} (pmol/mg protein)	3.06 (0.18)	3.05 (0.11)	3.06 (0.24)
n	6	5	5
^{3}H-Ro15-1788			
K_d (nM)	0.74 (0.01)	0.76 (0.02)	0.74 (0.03)
B_{max} (pmol/mg protein)	3.83 (0.19)	4.42 (0.18)	4.72 (0.20)
n	5	5	5

All assays were performed in duplicate on individual cortices and values represent mean (sem). A one-way ANOVA showed that there was no significant difference in K_d ($F = 0.314$, $p = 0.736$) and B_{max} ($F = 0.00039$, $p = 0.999$) for ^{3}H-flunitrazepam binding among different groups. A one-way ANOVA ($F = 5.61$, $p = 0.019$) followed by post hoc Scheffé comparisons showed that the B_{max} for ^{3}H-Ro15-1788 binding differed significantly between the high popping and control groups ($p = 0.05$), whereas the other comparisons were not significant. K_d values for ^{3}H-Ro15-1788 ($F = 0.297$, $p = 0.748$) did not differ significantly among the three groups.

playing low and high episodes of popping and control animals at both 30 min and 24 h after injection (Table 5). The potentiation of binding by 200 mM chloride ions was due to both statistically significant reductions of K_d and increases of B_{max} values. The chloride-dependent potentiation of ^{3}H-flunitrazepam binding did not differ between mice injected with MK-801 and control animals at either time point. Furthermore, the dose-dependent ability of GABA to inhibit the binding of ^{35}S-TBPS was not altered by MK-801 or the intensity of popping at either 30 min or 24 h after injection (Figs. 1 and 2).

TABLE 3

^{3}H-Ro5-4864 and ^{3}H-PK-11195 BINDING TO MOUSE CEREBRAL CORTEX 30 MIN AFTER MK-801 (1.0 mg/kg) INJECTION

	Control	Low Popping	High Popping
^{3}H-Ro5-4864			
K_d (nM)	2.43 (0.29)	2.52 (0.30)	3.20 (0.59)
B_{max} (pmol/mg protein)	1.09 (0.08)	1.15 (0.14)	1.28 (0.08)
n	4	4	4
^{3}H-PK-11195			
K_d (nM)	0.69 (0.04)	0.74 (0.07)	0.63 (0.04)
B_{max} (pmol/mg protein)	2.09 (0.14)	1.95 (0.05)	1.83 (0.13)
n	6	5	5

All assays were performed in duplicate on individual cortices and values represent mean (sem). A one-way ANOVA showed that there was no significant difference in K_d ($F = 1.000$, $p = 0.405$) and B_{max} ($F = 0.839$, $p = 0.463$) for ^{3}H-Ro5-4864 binding among the different groups. Also, ^{3}H-PK-11195 binding did not differ significantly among the three groups with respect to K_d ($F = 0.839$, and $p = 0.463$) and B_{max} ($F = 0.839$, $p = 0.463$).

TABLE 4

^{3}H-Ro5-4864 and ^{3}H-PK-11195 BINDING TO MOUSE CEREBRAL CORTEX 24 h AFTER MK-801 (1.0 mg/kg) INJECTION

	Control	Low Popping	High Popping
^{3}H-Ro5-4864			
K_d (nM)	2.25 (0.39)	1.87 (0.39)	2.26 (0.45)
B_{max} (pmol/mg protein)	1.06 (0.10)	1.23 (0.16)	1.17 (0.07)
n	6	4	5
^{3}H-PK-11195			
K_d (nM)	0.61 (0.03)	0.80 (0.09)	0.93 (0.17)
B_{max} (pmol/mg protein)	1.82 (0.10)	2.06 (0.09)	1.82 (0.15)
n	5	5	3

All assays were performed in duplicate on individual cortices and values represent mean (sem). A one-way ANOVA showed that there was no significant difference in K_d ($F = 0.257$, $p = 0.777$) and B_{max} ($F = 0.662$, $p = 0.531$) for ^{3}H-Ro5-4864 binding among the different groups. Also, ^{3}H-PK-11195 binding did not differ significantly among the three groups with respect to K_d ($F = 2.03$, $p = 0.182$) and B_{max} ($F = 1.54$, $p = 0.259$).

Twenty-four hours after mice were treated with MK-801 (0.5, 1.0, and 1.5 mg/kg), flurazepam's ability to antagonize electrically precipitated tonic hindlimb extension was increased (Fig. 3). The potentiation of flurazepam's efficacy did not appear to result simply from a failure to metabolize or clear MK-801 from brain as the "antiseizure" efficacy of the flurazepam-vehicle did not differ between groups. A two-way ANOVA revealed, as expected, a significant main effect for flurazepam dose [$F(2, 5) = 62.3$, $p < 0.01$], indicating that flurazepam raised the threshold voltage for seizure production in a dose-dependent manner. In addition, the analysis also revealed a significant main effect for MK-801 [$F(1, 3) = 5.5$, $p < 0.01$], indicating that MK-801 potentiated flurazepam in a dose-dependent manner. Thus, treatment of mice with MK-801 increased their sensitivity to flurazepam's antiseizure action when tested 24 h later in the IECS procedure.

DISCUSSION

Interference with glutamate-gated calcium ion conduction by MK-801 resulted in a delayed emergence (i.e., 24 h later) of an increased density of the antagonist-preferring conformation of benzodiazepine binding site in cerebral cortices obtained from the high popping group. The emergence of the increased density of antagonist-preferring binding site was not seen 30 min after injection, suggesting involvement of genomic mechanisms (i.e., synthesis of $GABA_A$ receptor subunits whose combination results in the benzodiazepine antagonist-preferring conformation). Interestingly, in prior studies, we showed that cold water swim stress was also associated with relatively specific changes in the radiolabeled antagonist-preferring site; however, these stress-related changes were primarily in hippocampus and cerebellum (15). MK-801 treatment was not associated with changes in the radiolabeled benzodiazepine agonist-preferring site or biochemical measures thought to reflect allosteric interactions between modulatory sites on the $GABA_A$ receptor complex and the channel domain (i.e., potentiation of ^{3}H-flunitrazepam binding by chloride ions and dose-dependent inhibition of ^{35}S-TBPS bind-

TABLE 5

EFFECT OF CL ION ON ^{3}H-FLUNITRAZEPAM BINDING TO MOUSE CEREBRAL CORTEX 30 MIN AND 24 h AFTER MK-801 (1.0 mg/kg) INJECTION

	Control			Low Popping			High Popping		
	−CL	+CL	%Change	−CL	+CL	%Change	−CL	+CL	%Change
30 min									
K_d (nM)	1.92 (0.012)	1.12 (0.012)	−41.5% (5.59)	1.73 (0.022)	0.95 (0.006)	−42.4% (5.59)	1.65 (0.011)	1.09 (0.008)	−33.9% (4.00)
B_{max} (pmol/mg protein)	3.75 (0.024)	4.21 (0.019)	+13.0% (3.06)	3.44 (0.015)	4.08 (0.025)	+18.8% (3.02)	3.41 (0.030)	4.10 (0.027)	+21.8% (4.94)
n		6			6				6
24 h									
K_d (nM)	2.18 (0.019)	1.10 (0.005)	−48.8% (2.90)	2.19 (0.019)	1.28 (0.012)	−40.0% (8.53)	2.12 (0.021)	1.08 (0.005)	−47.2% (6.78)
B_{max} (pmol/mg protein)	3.06 (0.020)	3.67 (0.021)	+21.2% (7.86)	3.06 (0.012)	3.32 (0.016)	+12.8% (2.81)	3.10 (0.034)	3.44 (0.032)	+11.8% (4.50)
n		5			5				4

All assays were performed in duplicate on individual cortices and values represent mean (sem). The assays were performed in the presence or absence of 200 mM NaCL. There was a significant change in affinity (30 min $F = 46.5$, $p < 0.0001$; 24 h $F = 78.1$, $p < 0.0001$) and B_{max} (30 min $F = 9.3$, $p = 0.0047$; 24 h $F = 10.4$, $p = 0.003$) as a function of chloride ion. At both 30 min and 24 h, there was no significant difference in either K_d or B_{max} between control and treatment groups in the effect of chloride.

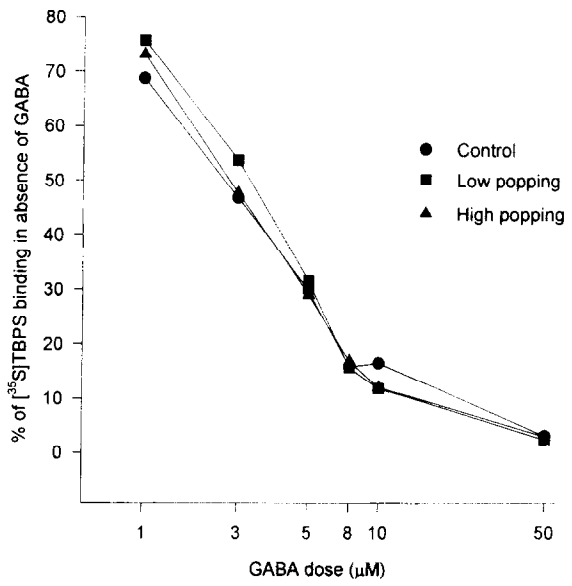

FIG. 1. GABA displacement of ^{35}S-TBPS binding to mouse cerebral cortex 30 min after injection of 1.0 mg/kg MK-801. All assays were performed in duplicate on individual cortices, and each point represents the mean of four to five values. The IC_{50}, inhibition constant (1×10^{-6} M), from the 30-min group were 2.16, 2.61, and 2.31 for control, low popping, and high popping, respectively. A one-way ANOVA revealed no significant difference ($F = 0.31$, $p = 0.74$).

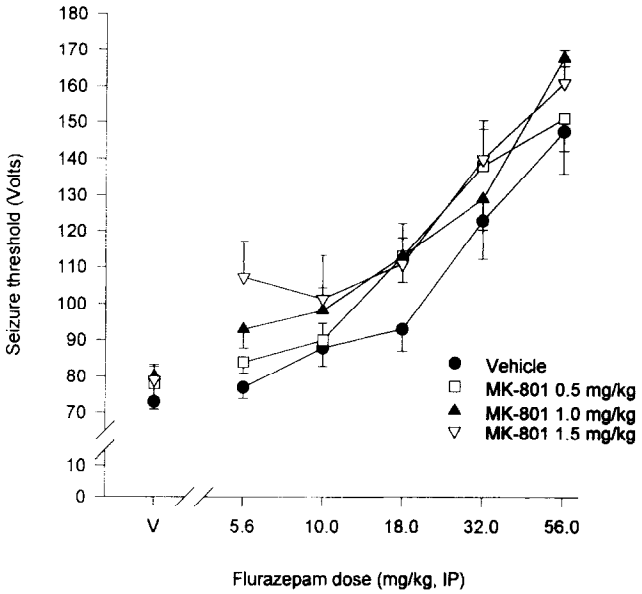

FIG. 3. Mean (points) and SEM (vertical bars) for groups of mice (n = 12–14/group) injected with saline (points above V) or various doses of flurazepam 24 h after being injected with saline (●), 0.5 mg/kg (□), 1.0 mg/kg (▲), or 1.5 mg/kg (△) MK-801.

ing by GABA). Moreover, MK-801 treatment did not alter the binding of either ^{3}H-Ro5-4864 or ^{3}H-PK-11195 to the peripheral or mitochondrial benzodiazepine binding site. Stimulation of the mitochondrial benzodiazepine binding site results in cholesterol translocation and the initiation of "neurosteroid" biosynthesis (4). Neurosteroids are 3-α-hydroxy naturally occurring ring-A reduced steroid metabolites that allosterically modulate GABA-gated chloride ion conduction subsequent to their binding to a hydrophobic domain of the $GABA_A$ receptor-associated chloride ionophore. The mitochondrial benzodiazepine binding site-mediated regulation of neurosteroid biosynthesis represents an important potential link between the peripheral and central benzodiazepine binding sites (4). In any event, the MK-801-induced alterations in GABA-gated chloride ion conduction do not appear to result from a persistent alteration in the peripheral benzodiazepine binding site.

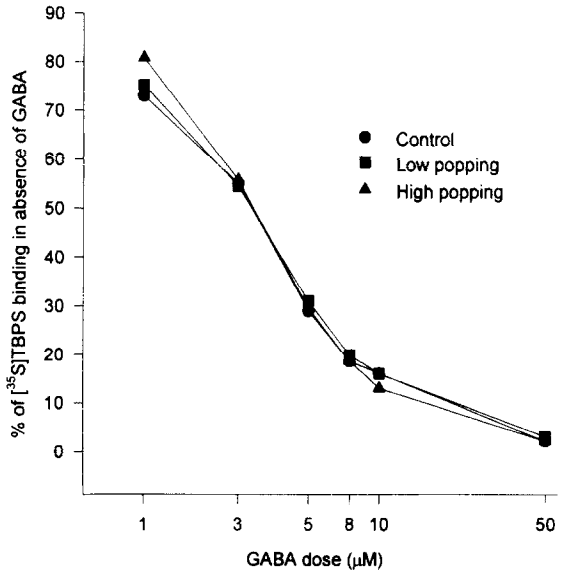

FIG. 2. GABA displacement of ^{35}S-TBPS binding to mouse cerebral cortex 24 h after injection of 1.0 mg/kg MK-801. All assays were performed in duplicate on individual cortices, and each point represents the mean of four to five values. The IC_{50} from the 24-h group were 2.55, 2.65, and 2.81 for control, low popping, and high popping, respectively. A one-way ANOVA revealed no significant difference ($F = 0.46$, $p = 0.64$).

The effect of MK-801 treatment on the $GABA_A$ receptor complex appears to be remarkably specific to the benzodiazepine antagonist-preferring site in cerebral cortex. A major consideration of this study was to investigate whether interference with NMDA-mediated neural transmission resulted in behaviorally relevant changes in GABAergic transmission. MK-801 treatment was associated with a potentiation of flurazepam's antiseizure efficacy. The latter data are consistent with an MK-801-induced increase in the sensitivity of the population of $GABA_A$ receptor complexes mediating flurazepam's antiseizure effect to this action of flurazepam. Clearly, the basis for this potentiation of flurazepam's antiseizure efficacy is not known; it seems unlikely that it would be related to the increased density of the benzodiazepine radiolabeled antagonist-preferring site in the cerebral cortex. The results provide unambiguous evidence for the maintenance of a delicate balance between GABAergic and glutamatergic transmission in the intact animal. A disruption of this balance is likely to have behaviorally relevant consequences.

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