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Effect of CGS 19755, a Competitive N-Methyl-D-Aspartate Antagonist, on General Anesthetic Potency

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DANIELL, L. C. *Effect of CGS 19755, a competitive N-methyl-D-aspartate antagonist, on general anesthetic potency.* PHARMACOL BIOCHEM BEHAV **40**(4) 767–769, 1991.—CGS 19755 is a competitive N-methyl-D-aspartate (NMDA) receptor antagonist which penetrates the blood-brain barrier. The effect of pretreatment with subanesthetic doses of CGS 19755 on general anesthetic potency was determined in mice. Mice were pretreated with saline or CGS 19755 by intraperitoneal (IP) administration 30 min before IP administration of an anesthetic dose of ethanol or pentobarbital or measurement of the volatile anesthetic minimum alveolar concentration (MAC). CGS 19755 increased the duration of ethanol- and pentobarbital-induced loss of righting reflex in a dose-dependent manner. The highest dose of CGS 19755 tested, 50 mg/kg, increased duration of loss of righting reflex by about four- and twofold for ethanol and pentobarbital, respectively. CGS 19755 also decreased the MAC for halothane. However, CGS 19755 pretreatment had no effect on the MAC for diethyl ether. These results suggest that the potency of certain general anesthetic agents can be increased by antagonism of brain NMDA receptors.

N-Methyl-D-aspartate L-Glutamate CGS 19755
5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) Phencyclidine Ethanol Pentobarbital
Halothane Diethyl ether Anesthesia Brain Mice

N-METHYL-D-ASPARTATE (NMDA) receptors are a subtype of the various excitatory brain glutamate receptors. Activation of NMDA receptors has been implicated in a wide range of physiological actions including seizure production (13), long-term potentiation (4), and anoxia-induced brain damage (3). Recent work also showed that alcohols, at concentrations relevant to intoxication and anesthesia in vivo, inhibit NMDA responses in central neurons (5, 7, 8, 11). These studies suggest that block of NMDA receptors may be mechanistically related to general anesthesia.

My recent work showed that pretreatment with subanesthetic doses of a series of noncompetitive NMDA receptor antagonists, 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), phencyclidine (PCP) and ketamine, increase the duration of loss of righting in response to ethanol and pentobarbital (6). These agents also decreased the MAC for halothane and diethyl ether. The ability of these NMDA antagonists to increase the potency of general anesthetic agents was paralleled by their potency in inhibiting NMDA responses in vivo (16,17) and their ability to displace MK-801 binding in brain membranes (14). This work provided in vivo evidence suggestive of the role of NMDA receptors in the mechanism of action of general anesthetic agents.

Until recently, the use of competitive NMDA antagonists in in vivo studies was limited by the inability of these agents to reach the brain after peripheral administration. But the recent availability of the orally active competitive NMDA antagonist, CGS 19755 (1, 2, 9), has provided the means for determination of in vivo effects of a competitive NMDA receptor antagonist. Recent work showed that CGS 19755 is a potent and selective competitive antagonist at NMDA receptors (9). In this study the ability of CGS 19755 to alter the potency of a variety of general anesthetics in mice was determined to further investigate the role of NMDA receptors in general anesthesia.

Abbreviations used: N-methyl-D-aspartate (NMDA); 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801); phencyclidine (PCP); intraperitoneal (IP); minimum alveolar concentration (MAC); Protected Least Significant Difference (PLSD).

METHOD

Materials

Male ICR mice (Harlan) 55 to 90 days old were used in all experiments. CGS 19755 was obtained from Research Biochemicals, Inc. Pentobarbital was obtained from Sigma Chemical Co. Halothane was from Ayerst Laboratories, Inc. and diethyl ether was obtained from Fisher. All other chemicals used were of the highest grade. With the exception of halothane and diethyl ether, all drugs were dissolved in saline.

Determination of Duration of Ethanol and Pentobarbital-Induced Anesthesia

Hypnotic doses of ethanol (4.2 g/kg, 25% w/v solution) or pentobarbital (50 mg/kg) were administered IP 30 min after IP administration of saline or CGS 19755. Duration of anesthesia was defined as the time from ethanol or pentobarbital administration to regaining of righting reflex. In control animals, loss of righting reflex typically occurred within 2 to 3 min after ethanol administration and 7 min after pentobarbital administration.

Determination of MAC of Diethyl Ether and Halothane

MAC was determined by measurement of the responsiveness to an aversive stimulus of fractions of small groups of mice at successively increased anesthetic concentrations. Groups of five mice were placed in an airtight Plexiglas box of dimensions 66 cm wide × 29 cm deep × 30 cm high. Volatile anesthetics mixed with air entered the box at one end and were vented at the other end. MAC determination was begun 15 min after IP administration of saline or CGS 19755. After equilibration with each inspired concentration of anesthetic for 15 min, animals were tested for responsiveness to a stimulus. The stimulus consisted of a hemostat clamp (5-inch Halstead mosquito forceps, Fisher) placed 1 cm from the distal end of the tail adjusted to the first notch for 30 s. Responsiveness was defined as purposeful movement and did not include reflexive increases in respiration. Each group of five animals was treated as a single experiment. The MAC value for each experiment was obtained by least squares linear regression analysis of fractional responsiveness data for the different volatile anesthetic concentrations tested.

Statistics

The data were analyzed by one-way analysis of variance (ANOVA) and the Fisher Protected Least Significant Difference (PLSD) post hoc test.

RESULTS AND DISCUSSION

Various doses of CGS 19755 were administered 30 min before administration of an anesthetic dose of ethanol (4.2 g/kg IP) or pentobarbital (50 mg/kg IP). Simple visual observation showed that CGS 19755 produces dose-dependent increases in sedation in male ICR mice administered 10 and and 50 mg/kg doses but not 1 mg/kg dose. Therefore, the threshold for sedative effects of CGS 19755 in male ICR mice is between 1 and 10 mg/kg. Typical PCP-like effects, such as head-weaving and jumping, were not observed at any of the doses of CGS 19755 used in this study (up to 50 mg/kg). These findings are consistent with a previous study in mice which found mild ataxic effects of CGS 19755 at 10 and 30 mg/kg (2). PCP-like effects are noted with CGS 19755 in mice only at doses higher than those required to produce ataxia (1).

CGS 19755 increased the duration of loss of righting reflex induced by both ethanol and pentobarbital in a concentration-dependent manner (Fig. 1). The ability of CGS 19755 to increase duration of loss of righting reflex appeared to be larger in the ethanol- than in the pentobarbital-treated animals. Similarly, my previous work demonstrated that the noncompetitive NMDA receptor antagonist, MK-801, is more potent in increasing the duration of loss of righting reflex induced by ethanol than pentobarbital (6). This differential enhancement of ethanol anesthesia over pentobarbital anesthesia was also evidenced by PCP (6), a less potent compound which acts at the same site of the NMDA receptor as MK-801 (14).

The effect of CGS 19755 pretreatment on volatile anesthetic

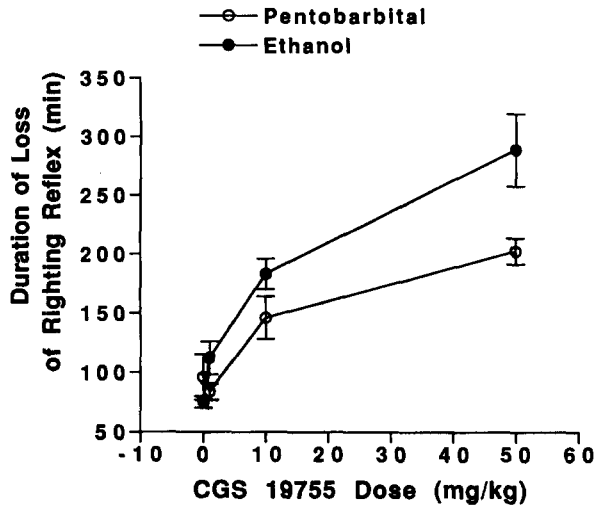

FIG. 1. Mice were treated with saline or various doses of CGS 19755 administered IP 30 min before administration of pentobarbital (50 mg/kg IP) or ethanol (4.2 g/kg IP). Data are the means ± SE of 7 (CGS 19755 10 and 50 mg/kg, ethanol) or 8 (all others) animals. The data were analyzed by one-way ANOVA with these results: for ethanol data, $F(3,26) = 28.7$, $p = 0.0001$; for pentobarbital data, $F(3,28) = 13.8$, $p = 0.0001$. By Fisher PLSD test, $^*p < 0.05$ for comparison with appropriate saline control.

potency was assessed by determination of MAC values for each anesthetic. The MAC value is the concentration of volatile anesthetic (vol/vol) which is required to produce anesthesia in 50% of a group of animals. A reduction in MAC values indicates an increase in volatile anesthetic potency. The halothane MAC was reduced in a dose-dependent manner by pretreatment with CGS 19755 (Table 1). The halothane MAC was significantly decreased by 10 and 50 mg/kg doses of CGS 19755. The ability of CGS 19755 to decrease the halothane MAC appeared to plateau at CGS 19755 doses between 10 and 50 mg/kg. However, the diethyl ether MAC was unaltered by any dose of CGS 19755 tested, up to 50 mg/kg (Table 1).

These results are similar to my previous work which showed that MK-801 and PCP produced a larger reduction in the MAC of halothane than of diethyl ether (6). The noncompetitive an-

TABLE 1

CGS 19755-INDUCED DECREASE IN HALOTHANE AND DIETHYL ETHER MAC

CGS 19755 Dose (mg/kg)	MAC (% vol/vol) Halothane	n	Diethyl Ether	n
0	0.45 ± 0.01	5	2.94 ± 0.06	5
1	0.46 ± 0.02	2	N/D	
5	0.42 ± 0.01	2	N/D	
10	0.35 ± 0.01*	3	3.11 ± 0.22	5
50	0.33 ± 0.02*	3	3.09 ± 0.26	5

MAC values were determined in the indicated number of groups (n) of mice after pretreatment with saline or various doses of CGS 19755 administered IP. Values shown are the means ± SE. N/D = not determined. The data were analyzed by one-way ANOVA with these results: for halothane data, $F(4,10) = 22.9$, $p = 0.0001$; for diethyl ether data, $F(2,12) = 0.2$, $p = 0.81$. By Fisher PLSD post hoc test, $^*p < 0.05$.

tagonists, MK-801 and PCP, also had a greater ability to reduce volatile anesthetic MAC overall than did CGS 19755 in this study [27% maximal reduction of halothane MAC by CGS 19755 in this study compared to a 43% maximal reduction by MK-801 in my previous study (6)], suggesting that the noncompetitive NMDA receptor antagonists produce greater increases in general anesthetic potency than competitive antagonists.

In a previous study, the diethyl ether MAC was reduced by pretreatment with the noncompetitive NMDA antagonists, MK-801 and PCP (6). In contrast, the diethyl ether MAC was unaltered by CGS 19755 pretreatment, suggesting that the ability of NMDA antagonists to increase diethyl ether potency is dependent on the mode of NMDA receptor antagonism (competitive versus noncompetitive). This may indicate possible differences in the site of action of the various general anesthetics.

Recent binding studies indicate that brain NMDA receptors are heterogeneous and that some NMDA receptors are less sensitive to modulation by agents which allosterically modulate different sites of the NMDA receptor including MK-801 (15), glycine (12) or polyamines (18). The idea that brain NMDA receptors are heterogeneous is further supported by behavioral experiments which show that the effects of noncompetitive NMDA antagonists only partially generalize to those of competitive NMDA antagonists in drug discrimination studies (16). Also, unlike noncompetitive NMDA antagonists, competitive NMDA antagonists are not self-administered (16). These data and my findings of differences in the ability of competitive and noncompetitive antagonists of the NMDA receptor to increase potency of various general anesthetics are consistent with the idea that a subpopulation of brain NMDA receptors may be involved in anesthesia. Further work examining general anesthetic effects on in vitro NMDA receptor responses, currently in progress in this laboratory, may clarify the possible role of various subtypes of NMDA receptors in general anesthesia.

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