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Investigations into pharmacological antagonism of general anaesthesia

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1 The effects of convulsant drugs, and of thyrotropin releasing hormone (TRH), were examined on the general anaesthetic actions of ketamine, ethanol, pentobarbitone and propofol in mice. The aim was to investigate the possibility of selective antagonism, which, if seen, would provide information about the mechanism of the anaesthesia.

2 The general anaesthetic effects of ketamine were unaffected by bicuculline; antagonism was seen with 4-aminopyridine and significant potentiation with 300 mg kg^{-1} NMDLA (N-methyl-DL-aspartate). The calcium agonist, Bay K 8644, potentiated the anaesthesia produced by ketamine and antagonism of such anaesthesia was seen with TRH.

3 A small, but significant, antagonism of the general anaesthesia produced by ethanol was seen with bicuculline, and a small, significant, potentiation with 4-aminopyridine. There was an antagonist effect of TRH, but no effect of NMDLA.

4 Potentiation of the anaesthetic effects of pentobarbitone was seen with NMDLA and with 4-aminopyridine and the lower dose of bicuculline (2.7 mg kg^{-1}) also caused potentiation. There was no significant change in the ED_{50} value for pentobarbitone anaesthesia with TRH.

5 Bicuculline did not alter the anaesthetic actions of propofol, while potentiation was seen with NMDLA and 4-aminopyridine. TRH had no significant effect on propofol anaesthetic, but Bay K 8644 at 1 mg kg^{-1} significantly potentiated the anaesthesia.

6 These results suggest that potentiation of $GABA_A$ transmission or inhibition of NMDA receptor-mediated transmission do not appear to play a major role in the production of general anaesthesia by the agents used.

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Abbreviations: 4-AP; 4-aminopyridine; GABA, γ-aminobutyric acid; NMDA, N-methyl-D-aspartate; NMDLA, N-methyl-DL-aspartate; TRH; thyrotropin releasing hormone

Introduction

The mechanism(s) by which drugs produce general anaesthesia are not well understood (Halsey, 1992; Angel, 1993; Little, 1996). Many different effects of general anaesthetic drugs on synapses have been demonstrated, but the most important aspect, often neglected, is the possible relationship between these synaptic actions and the production of general anaesthesia. One problem in the investigation of such a relationship has been the lack of specific antagonists for general anaesthesia. There have been a small number of studies reporting antagonism of general anaesthesia with various drugs but no systematic investigation has been made and usually only one or two anaesthetics have been studied. Compounds which have been reported to antagonize anaesthesia are 4-aminopyridine (4-AP), against ethanol and ketamine (Agostin *et al*., 1980; Sellin & Laakso, 1987), and thyrotropin releasing hormone (TRH) against ethanol and pentobarbitone (Breese *et al*., 1974; French *et al*., 1993; Matsushita *et al*., 1995). Anticholinesterase agents have also been shown to have some effects (Livingston & Waterman, 1978; Leeuwin *et al*., 1984). Antagonism of the general anaesthetic effects of ethanol, pentobarbitone and argon was reported with the calcium channel agonist, Bay K 8644 (Dolin *et al*., 1988).

The only reproducible and consistent way so far demonstrated in which general anaesthesia can be antagonized is by the application of high pressure (Lever *et al*., 1971). Increasing the ambient pressure to approximately 80–100 atmospheres reverses the anaesthetic effects of a variety of types of compound, including inhalational anaesthetics, barbiturates and steroids (Halsey & Wardley-Smith, 1975; Halsey *et al*., 1978; Miller & Wilson, 1978). In these studies, after the application of pressure following administration of anaesthetic, the animals recovered their righting reflex and appeared to be able to move normally. Pressure alone produces hyperexcitability and convulsions, and this can be demonstrated using helium gas, which has little general anaesthetic action itself, or by hydrostatic pressure (Lever *et al*., 1971). Evidence has been presented that the effects of pressure which prevent general anaesthesia are exerted at different neuronal sites than those at which the hyperexcitability is produced (Miller, 1977) but pressure reversal of general anaesthesia was demonstrated in recordings of evoked somatosensory responses in rats (Angel *et al*., 1980). The mechanism of the changes produced by pressure are not fully understood. High pressure does not appear to decrease the effects of GABA at $GABA_A$ receptors (Little, 1982) but the effects of NMDA on hippocampal CA1 cells was found to be increased (Zinebi *et al*., 1988). Daniels *et al*. (1991) found that depression by pentobarbitone of kainate responses in receptors expressed in oocytes was not affected by pressure.

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The main synaptic actions which have been suggested to cause general anaesthesia are increases in the effects of GABA (at $GABA_A$ receptors), decreases in excitatory amino acid transmission (Sawada & Yamamoto, 1985; Anis *et al*., 1983) and alterations in various ion channels, such as calcium (Kress *et al*., 1987; Llinas, 1988; Twombly *et al*., 1990) and potassium (Nicoll & Madison, 1982). Potentiation of the effects of GABA is seen with many different types of compound, such as barbiturates (Nicoll *et al*., 1975), steroids (Harrison & Simmonds, 1984), inhalational agents (Longon *et al*., 1993) and propofol (Collins, 1988). However such potentiation is also seen with benzodiazepines, which do not produce the state which we know as general anaesthesia until near lethal doses (Little & Bichard, 1984), although these drugs potentiate the general anaesthetic actions of other drugs.

The present study is a systematic investigation of the effects of convulsants and thyrotropin releasing hormone (TRH) on general anaesthesia, to determine the extent of any antagonism and to see what information could be obtained about the mechanism by which the anaesthesia was produced. The anaesthetics were ketamine, ethanol, pentobarbitone and propofol, chosen because they represent different chemical classes of compound and because their synaptic actions have been widely studied. The convulsants were chosen on the basis of the extent of knowledge of their mechanism of action and previous reports of antagonism of general anaesthesia. Bicuculline is a specific $GABA_A$ receptor antagonist; NMDLA (N-methyl-DL-aspartate) is an excitatory amino acid agonist with selective actions at the NMDA subtype of receptor; 4-aminopyridine (4-AP) is a potassium channel blocker, previously reported to have antagonist actions against general anaesthesia (Agostin *et al*., 1980; Sellin & Laakso, 1987), which causes a non-selective blockade of voltage-dependent potassium channels (Griffith & Sim, 1990), with increased intracellular calcium concentrations (Heemskerk *et al*., 1991), and increased release of many transmitters. Bay K 8644 is a dihydropyridine which increases the conductance of the 'L'-subtype of voltage-sensitive calcium channels (Freedman & Miller, 1984). The effects of TRH were also examined in view of the previous reports of antagonism of anaesthesia by TRH (Breese *et al*., 1974; French *et al*., 1993; Matsushita *et al*., 1995).

Methods

Male mice of the TO strain were used in all experiments, bred in house. The weight range was 30–40 g, with no more than 5 g difference in one experiment. They were kept in a 12 h light/dark cycle (lights on 0800 h to 2000 h with access *ad libitum* to tap water and laboratory rodent chow (CRM).

Measurement of general anaesthesia

The general anaesthetic actions of the drugs were determined using loss of the righting reflex as an index of anaesthesia. After injection, the mice were gently turned on to their backs at 5 min intervals; if they did not regain their posture within 10 s they were recorded as having lost their righting reflex. The estimation of loss of righting reflex were made throughout by observers who did not know what prior drug treatment the animals had received. The body temperatures of the mice were maintained throughout the measurements of general anaesthesia at $37 \pm 1^{\circ}C$, by the use of heating mats.

Drug administration

All drugs were given by the intraperitoneal route; the volume used was 10 ml kg^1, except in the case of propofol, where 20 ml kg^{-1} was used, and ethanol, that was given consistently at a concentration of 20% v v^{-1}. Each treatment group contained 9–15 mice. The anaesthetic doses were chosen to cause loss of righting reflex in between 0 and 100% of mice, excluding those extreme values; a minimum of three anaesthetic doses was used to construct each dose response curve.

The times of administration for the convulsants were chosen so that the maximum convulsant action coincided with the maximum general anaesthetic effects; this resulted in the convulsants being given at the same time as the ketamine and ethanol and 5 min before the pentobarbitone and propofol. The time of administration and doses of TRH were chosen from published studies; the pretreatment time was 5 min and the doses were 10 and 20 mg kg TRH.

All results which were compared were obtained from measurements made concurrently. If it was necessary (owing to the supply of animals) for different doses of a convulsant to be tested on different days, the control dose response curves were established each time, to ensure valid comparison of results.

Drugs used

Ketamine, 4-aminopyridine, NMDLA and TRH-acetate (all obtained from Sigma) were dissolved in saline. Ethanol was given in a 20% v v^{-1} mixture with distilled water. Pentobarbitone (Sigma) and racemic Bay K 8644 (a gift from Bayer AG, Wuppertal, Germany) were suspended in Tween 80, 0.5% and sonicated before use. Propofol ('Diprovan') was diluted in Tween 80, 0.5% and sonicated. The vehicles used for the convulsant drugs and TRH were administered to the corresponding control mice, which then provided data for the control dose-response curves for each general anaesthetic.

Bay K 8644 = $\pm$methyl 1.4 dihydro-2,6-dimethyl-3-nitro-4-(trifluoromethylphenyl)-pyridine-5-carboxylate.

Statistical analysis

Dose-response curves were constructed for each anaesthetic, for each 5 min interval, until the animals were awake. The results were expressed as percentage anaesthetized, on a probit scale *versus* log dose of anaesthetic. ED_{50} values and 95% confidence limits were calculated and compared by the probit analysis method of Wilcoxon & Litchfield (1949), as in our earlier study (Dolin et al., 1988). The Maximum Likelihood Programme, (Numerical Algorithms Group) was used to check the heterogeneity of the data; i.e. whether any residual variation was consistent with binomial sampling. None of the dose response lines for each anaesthetic and convulsant or TRH combination showed significant differences from parallelism. These calculations were made for the results obtained every 5 min after injection of the anaesthetics.

Results

Presentation of results

The duration of anaesthesia was approximately 15–20 min for each compound. During this time, dose response lines were obtained for the effects of the anaesthetics every 5 min from

administration, with and without each convulsant or TRH. The pattern of results and the direction of effects of the convulsants and TRH were found to be the same at the different time points within each experiment (one experiment being the study of one dose of convulsant or TRH against one anaesthetic). For clarity therefore, results from all the time intervals have not been presented. The 10 min time interval demonstrated the maximal interactions, so the ED_{50} values, 95% confidence limits and dose-response lines are given for this time interval, with additional information on the time course of the effects where relevant.

Throughout the results, the *P* values are for comparisons with control values obtained in concurrent tests on mice given the appropriate vehicle plus the anaesthetic. The figures below illustrate the lines fitted to the results by the method described above; for clarity the 95% confidence limits have been omitted from the figures. However, it is important to emphasise that the tests for significant differences between ED_{50} values, and the tests for significant deviations from parallelism were carried out on the original data. In some instances where the lines on the figures do not appear to be parallel there was no significant difference from parallelism in the statistical analysis carried out on the original data.

Effects of the convulsants

The initial doses of the convulsants used in the present study were the ED_{50} values for production of clonic seizures, i.e. 2.9 mg kg^{-1} for bicuculline, 300 mg kg^{-1} for NMDLA and 8 mg kg^{-1} for 4-aminopyridine. These values were calculated from the data presented in Table 1. Further, lower, doses of the convulsants (2.7 mg kg^{-1} for bicuculline, 250 mg kg^{-1} for NMDLA and 7 mg kg^{-1} for 4-aminopyridine) were also used, in order to determine the consistency of the effects. All the lower doses were effective in producing either full clonic convulsions (as indicated in Table 1) or myoclonic jerks in naïve animals of this mouse strain, so that their effectiveness on central synapses was established.

An important aspect of this study was whether or not the convulsant drugs affected the ability of the animals to regain their righting reflex. Had their ability to regain loss of righting reflex been directly affected by the convulsant drugs, this would have interfered with the measurement of general anaesthesia by this end-point. Careful observation on the effects of the convulsants when administered alone showed that none of the four convulsant drugs altered the posture of the mice or their ability to move around the cages, and no loss of righting reflex was seen, unless a full convulsion was produced. Convulsions were not seen when the general anaesthetics were given with the convulsant drug, and none of the doses of convulsants used exceeded the ED_{50} values for production of convulsions in the absence of anaesthetic. It is very unlikely therefore that any changes seen would have been due to interference by the convulsant drugs with the maintenance of posture by the mice.

The results presented are for the 10 min interval after administration of the anaesthetics, but the same patterns were seen at the other times of measurement. As the choices of times of administration for the convulsants were made so that the maximum convulsant action, determined in earlier studies, coincided with the maximum general anaesthetic effects, it is unlikely that the variation in the effects of the convulsants was due to differences in time courses of action. No significant changes in the slope of the anaesthetic dose response curve were seen when the anaesthetics were given with any of the convulsants or TRH.

Table 1 Production of clonic convulsions in absence of anaesthetic

Convulsant	*Dose* (mg kg)	*Proportion convulsing*	*ED_{50} mg kg* (95% confidence limits)
Bicuculline	2.5	1/10	2.9 (2.7, 3.2)
	3.0	6/10	
	3.5	9/10	
NMDLA	225	1/10	303 (271, 340)
	275	5/10	
	325	5/10	
	375	8/10	
4-AP	8	4/10	8.1 (7.2, 9.0)
	9	8/10	
	11	9/10	

Ketamine

Administration of bicuculline, 2.7 or 2.9 mg kg^{-1} had no effect on the anaesthetic actions of ketamine (Table 2). The 4-AP had a large and significant antagonist effect ($P<0.05$), when given at either 7 or 8 mg kg^{-1}, with a parallel shift to the right of the dose-response curve for ketamine (Table 2 and Figure 1a).

NMDLA caused a significant potentiation of the anaesthetic actions of ketamine, when given at 300 mg kg^{-1}, but there was no significant change with the lower dose of 250 mg kg^{-1} NMDLA (Table 2 and Figure 1b). Bay K 8644, at doses of 1 and 5 mg kg^{-1}, caused a shift to the left of the dose response line for ketamine, and the differences in ED_{50} values were significant ($P<0.05$ for both doses of Bay K 8644). TRH caused a small, but significant antagonism of the anaesthesia produced by ketamine (Table 2).

Table 2 Effects of convulsant drugs and TRH on the ED_{50} values for loss of righting reflex produced by ketamine

Treatment	*ED_{50}* (mg kg^{-1})	*95% Confidence limits* (mg kg)	P *values, for comparison with vehicle control*
Vehicle	79	67–93	
Bicuculline 2.7 mg kg^{-1}	79	63–98	NS
Vehicle	91	78–104	
Bicuculline 2.9 mg kg^{-1}	80	70–92	NS
Vehicle	79	67–93	
4-AP 7 mg kg^{-1}	125	105–150	$P<0.05$
Vehicle	91	78–104	
4-AP 8 mg kg^{-1}	149	139–167	$P<0.05$
Vehicle	79	67–93	
NMDLA 250 mg kg^{-1}	71	61–85	
Vehicle	91	78–104	
NMDLA 300 mg kg^{-1}	66	55–78	$P<0.05$
Vehicle	101	87–118	
Bay K 8644 1 mg kg^{-1}	91	79–105	$P<0.05$
Bay K 8644 5 mg kg^{-1}	84	68–104	$P<0.05$
Vehicle	79	67–93	
TRH 10 mg kg^{-1}	99	83–117	$P<0.05$
TRH 20 mg kg^{-1}	109	95–124	$P<0.05$

The ED_{50} values presented are for 10 min after the administration of ketamine. Comparisons were made between the ED_{50} values after administration of the convulsants or TRH and the ED_{50} values after injection of the corresponding vehicle. NS = not significantly different from concurrently measured value after vehicle injections.

Ethanol

Bicuculline caused a small, but significant, antagonism of the general anaesthesia produced by ethanol (Table 3 and Figure 2a). The 4-AP had a small, significant, potentiating effect on the anaesthetic action of ethanol (Table 3 and Figure 2b). With NMDLA there was no significant change in the ED_{50} value for ethanol, but TRH caused a small, significant antagonism (Table 3).

Pentobarbitone

Bicuculline had a potentiating effect on the anaesthetic actions of pentobarbitone, with a significant decrease in the ED_{50} value after 2.7 mg kg^{-1}, but not 2.9 mg kg^{-1}, bicuculline (Table 4 and Figure 3a). NMDLA also potentiated the anaesthetic effects of pentobarbitone, when given at 250 or 300 mg kg^{-1} (Table 4 and Figure 3b); with significant differences in the ED_{50} values ($P<0.05$). A significant potentiating effect was also seen when 4-AP was given with the pentobarbitone (Table 4), at either 7 or 8 mg kg^{-1}. TRH did not alter the ED_{50} value for pentobarbitone, when given at either 10 or 20 mg kg^{-1} (Table 4).

Propofol

Bicuculline, at 2.7 or 2.9 mg kg^{-1}, did not significantly alter the anaesthetic actions of propofol (Table 5 and Figure 4a). NMDLA, in contrast, had a considerable potentiating action on the production of anaesthesia by propofol, at both the 250 or 300 mg kg^{-1} doses (Figure 4b). A significant potentiation of the anaesthetic effect of propofol was also seen when 4-AP was administered at 8 mg kg^{-1}, but not at 7 mg kg^{-1} (Table 4). TRH did not significantly alter the ED_{50} value for

Table 3 Effects of convulsant drugs, and TRH on the ED_{50} values for loss of righting reflex produced by ethanol

Treatment	ED_{50} (g kg^{1})	*95% Confidence limits* (mg kg^{-1})	P *values, for comparison with vehicle control*
Vehicle	3.6	3.43–3.78	
Bicuculline 2.7 mg kg^{-1}	3.86	3.6–4.15	$P<0.05$
Bicuculline 2.9 mg kg^{-1}	3.85	3.71–3.99	$P<0.05$
Vehicle	3.42	3.27–3.58	
4-AP 7 mg kg^{-1}	3.32	3.18–3.45	$P<0.05$
4-AP 8 mg kg^{-1}	3.24	3.02–3.46	$P<0.05$
Vehicle	3.6	3.43–3.78	
NMDLA 250 mg kg^{-1}	3.59	3.46–3.73	NS
NMDLA 300 mg kg^{-1}	3.51	3.3–3.74	NS
Vehicle	3.43	3.27–3.6	
TRH 10 mg kg^{-1}	3.54	3.44–3.64	$P<0.05$
TRH 20 mg kg^{-1}	3.61	3.46–3.77	$P<0.05$

The ED_{50} values presented are for 10 min after the administration of ethanol. Comparisons were made between the ED_{50} values after administration of the convulsants or TRH and the ED_{50} values after injections of the corresponding vehicles. NS = not significantly different from concurrently measured value after vehicle injections.

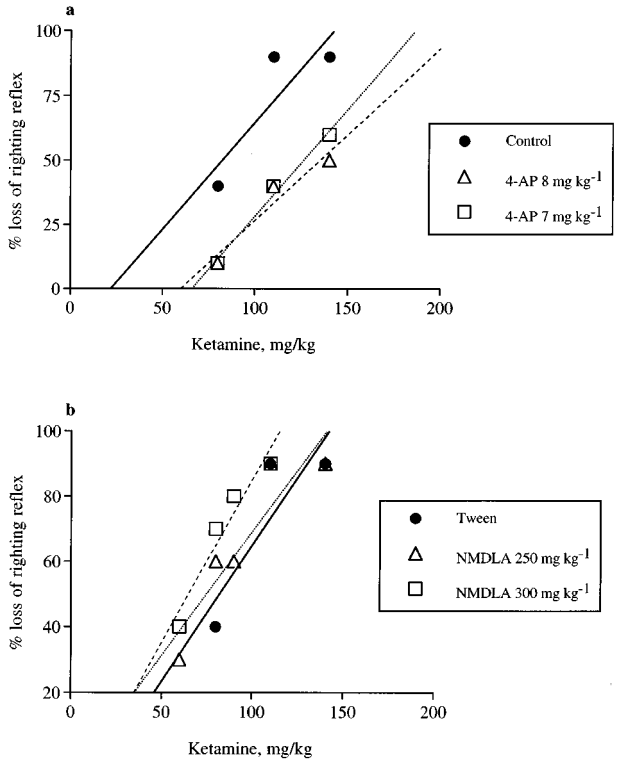

Figure 1 Measurements of loss of righting reflex produced by ketamine. (a) illustrates results after administration of 7 or 8 mg kg^{-1} dose of 4-AP and (b) shows the results obtained after vehicle or 250 mg kg^{-1} or 300 mg kg^{-1} NMDA. All measurements were made 10 min after administration of ketamine. The general anaesthetic potency, calculated by the ED_{50} value for ketamine, was significantly decreased by 4-AP ($P<0.05$) and significantly increased by NMDA 300 mg kg^{-1} ($P<0.05$).

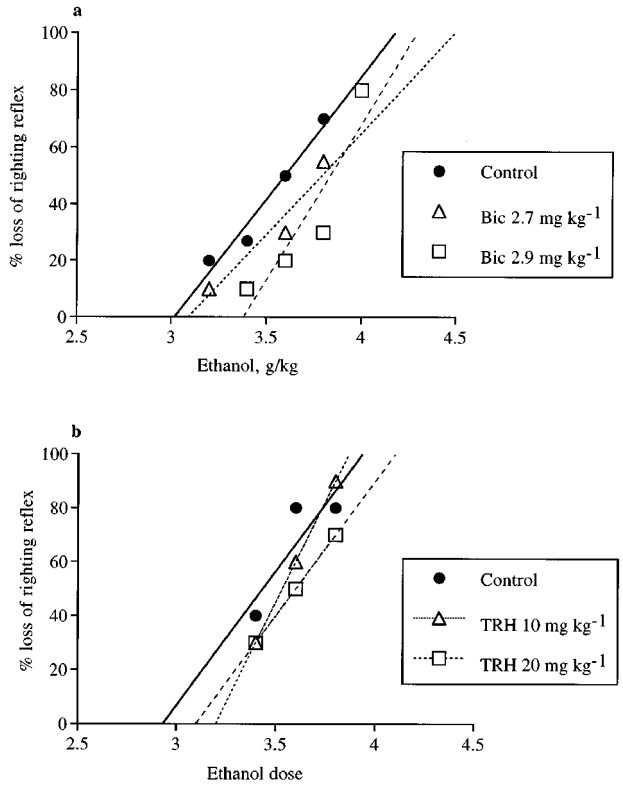

Figure 2 Measurements of loss of righting reflex produced by ethanol. (a) shows the results after administration of vehicle, or bicuculline (Bic) 2.7 or 2.9 mg kg^{-1} and (b) the results after vehicle or 10 mg^{-1} or 20 mg kg^{-1} of TRH. All measurements were made 10 min after administration of ethanol. The general anaesthetic potency, calculated by the ED_{50} value for ethanol, was significantly decreased by bicuculline ($P<0.05$) and there was also a significant antagonism of ethanol anaesthesia with both doses of TRH ($P<0.05$).

propofol or the slope of the dose response curves. The calcium channel agonist, Bay K 8466, cause a significant potentiation of the general anaesthetic action of propofol when given at 1 mg kg^{-1}, but no change was seen when the calcium channel antagonist was administered at 5 mg kg^{-1}.

Table 4 Effects of convulsant drugs, and TRH on the ED_{50} values for loss of righting reflex produced by pentobarbitone

Treatment	*ED_{50}* (g kg^{1})	*95% Confidence limits* (mg kg^{-1})	P *values, for comparison with vehicle control*
Vehicle	31.6	27.3–36.2	
Bicuculline 2.7 mg kg^{-1}	27.7	25.6–29.9	$P<0.05$
Bicuculline 2.9 mg kg^{-1}	30.4	28.1–33.0	NS
Vehicle	32.6	27.5–38.6	
4-AP 7 mg kg^{-1}	27.9	24.3–32.0	$P<0.05$
4-AP 8 mg kg^{-1}	27.0	24.3–30.1	$P<0.05$
Vehicle	31.6	27.3–36.5	
NMDLA 250 mg kg^{-1}	23.9	21.2–26.9	$P<0.05$
NMDLA 300 mg kg^{-1}	27.8	25.6–30.1	$P<0.05$
Vehicle	34.7	31.9–37.6	
TRH 10 mg kg^{-1}	35.6	32.8–38.6	NS
TRH 20 mg kg^{-1}	36.1	33.5–38.9	NS

The ED_{50} values presented are for 10 min after the administration of pentobarbitone. Comparisons were made between the ED_{50} values after administration of the convulsants or TRH and the ED_{50} values after injections of the corresponding vehicles. NS = not significantly different from concurrently measured value after vehicle injections.

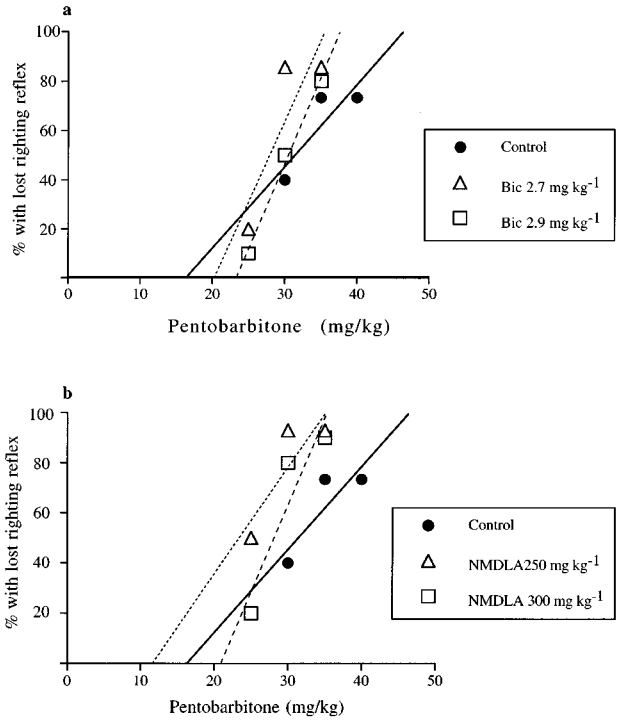

Figure 3 Measurements of loss of righting reflex produced by pentobarbitone. (a) illustrates the results after administration of vehicle, bicuculline (Bic) 2.7 or 2.9 mg kg^{-1} and (b) shows results after administration of vehicle or NMDA 250 or 300 mg kg^{-1}. All measurements were made 10 min after administration of pentobarbitone. The general anaesthetic potency, calculated by the ED_{50} value for pentobarbitone, was significantly increased by bicuculline at 2.7 mg kg^{-1-1} ($P<0.05$), but not by 2.9 mg kg^{-1} bicuculline and by both doses of NMDLA ($P<0.05$).

Table 5 Effects of convulsant drugs, and TRH on the ED_{50} values for loss of righting reflex produced by propofol

Treatment	*ED_{50}* (mg kg^{1})	*95% Confidence limits* (mg kg^{-1})	P *values, for comparison with vehicle control*
Vehicle	94	81–109	
Bicuculline 2.7 mg kg^{-1}	92	84–101	NS
Bicuculline 2.9 mg kg^{-1}	86	76–98	NS
Vehicle	94	81–109	
4-AP 7 mg kg^{-1}	82	66–103	NS
4-AP 8 mg kg^{-1}	60	47–76	$P<0.05$
Vehicle	97	86–109	
NMDLA 250 mg kg^{-1}	56	49–65	$P<0.05$
NMDLA 300 mg kg^{-1}	52	43–62	$P<0.05$
Vehicle	113	86–147	
Bay K 8644 1 mg kg^{-1}	81	57–114	$P<0.05$
Bay K 8644 5 mg kg^{-1}	108	91–128	NS
Vehicle	97	86–109	
TRH 10 mg kg^{-1}	100	91–107	NS
TRH 20 mg kg^{-1}	99	91–108	NS

The ED_{50} values presented are for 10 min after the administration of propofol. Comparisons were made between the ED_{50} values after administration of the convulsants or TRH and the ED_{50} values after injections of the corresponding vehicles. NS = not significantly different from concurrently measured value after vehicle injections.

Discussion

The primary conclusions from these results were, firstly, that the four convulsant drugs had different effects, producing either antagonism or potentiation or no change in the general anaesthetic potency of the compounds, and secondly that the effects varied with the general anaesthetic agent. The interactions demonstrated in these experiments are summarized in Table 6. A shift to the right of the dose response curve was interpreted as antagonism of the anaesthesia and a shift to the left as potentiation, but such changes are not necessarily suggested to involve competitive interactions at the receptors. Antagonism was seen in the effects of 4-AP against ketamine anaesthesia and with TRH on the actions of ethanol and ketamine, but for several of the drug combinations the result was actually potentiation of anaesthesia, and for the interaction between propofol and NMDLA, the increase in the anaesthetic potency was considerable.

The general anaesthetic effects of ketamine were antagonized by 4-AP, and by TRH, while NMDLA at the higher dose of 300 mg kg^{-1} caused potentiation and no effect was seen with bicuculline. Ketamine is well established to have a non-competitive antagonist effect against responses at NMDA receptors (Anis *et al*., 1983), as well as having interactions with opiate transmission. Its effects on GABA transmission are less clear; no effect was seen on the actions of GABA on spinal neurones (Anis *et al*., 1983) but potentiation of inhibitory currents has been reported in olfactory cortex (Scholfield, 1980), on ganglionic neurones (Little, 1982) and, at higher concentrations, in hippocampal preparations (Gage & Robertson, 1985).

In contrast to its effects on ketamine anaesthesia, 4-AP potentiated the general anaesthetic effects of ethanol. No significant change was seen when NMDLA was combined with ethanol, but small effects were seen with bicuculline and with TRH, in the antagonist direction. Ethanol has been shown in some instances to potentiate GABA transmission but this

Table 6 Summary of drug interactions seen in the present study

Convulsant or TRH	*Ketamine*	*Ethanol*	*Pentobarbitone*	*Propofol*
Bicuculline	No change	Antagonism	Potentiation (at 2.7 mg kg^{-1} bicuculline only)	No change
4-AP	Antagonism	Potentiation	Potentiation	Potentiation (at 8 mg kg^{-1} 4-AP only)
NMDLA	Potentiation (at 300 mg kg NMDLA only)	No change	Potentiation	Potentiation
TRH	Antagonism	Antagonism	No change	No change
Bay K 8644	Potentiation	(Antagonism, Dolin *et al*., 1988)	(Antagonism, Dolin *et al*., 1988)	Potentiation (with 1 mg kg^{-1} Bay K 8644)

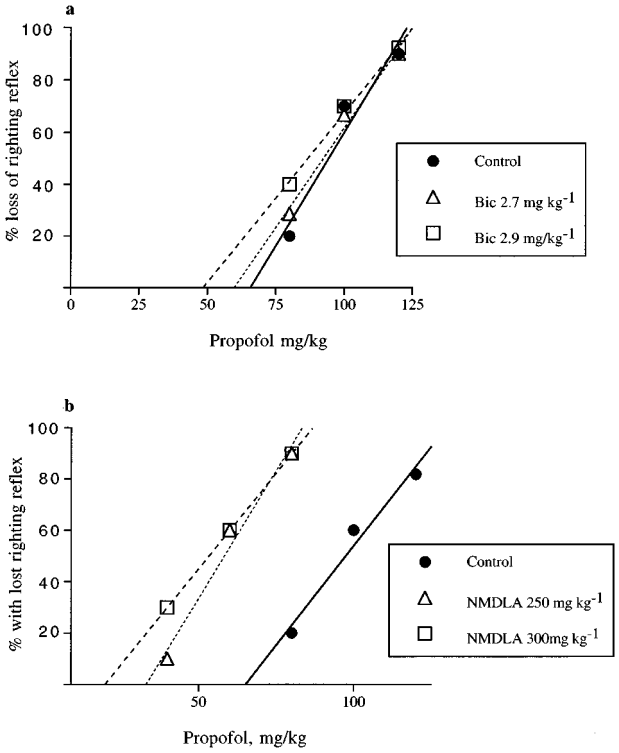

Figure 4 Measurements of loss of righting reflex produced by propofol. (a) Illustrates the results after administration of vehicle, bicuculline (Bic) 2.7 mg kg^{-1} or 2.9 mg kg^{-1}, and Figure 4b shows results after administration of vehicle or NMDLA 250 or 300 mg kg^{-1}. All measurements were made 10 min after administration of propofol. The general anaesthetic potency, calculated by the ED_{50} value for propofol, was not significantly affected by bicuculline and was significantly increased by NMDLA ($P<0.05$).

action varies with the tissue, species, the receptor subunit composition and the state of phosphorylation of the receptor (Little, 1991; 1999). An inhibitory action of ethanol on NMDA-receptor mediated responses has been reported, which in some, but not all, neurones is seen at lower concentrations of ethanol than corresponding effects on responses at the other subtypes of excitatory amino acid receptors (Lovinger *et al*., 1989; 1990; Neiber *et al*., 1998).

Previous studies have reported the sleeping time following a single large dose of ethanol to be reduced after administration of the GABA antagonists, bicuculline or picrotoxin (Liljequist & Engel, 1982; Martz *et al*., 1983), The former study demonstrated effects of bicuculline at 2–8 mg kg^{-1}, i.p. so the convulsant doses used in the present study were in the same range, albeit in the lower part of the range. In earlier studies, in our strain of mice, lethal effects of bicuculline were seen when this drug was given alone at 5 mg kg^{-1}, with full convulsions in all animals. The above quoted authors all used sleeping time, rather than ED_{50} values, as an index of the anaesthetic actions, so that only one dose of ethanol, 5 g kg^{1} i.p., higher than any of those required to measure ED_{50} values, was used. Ro 15-4513, a benzodiazepine receptor ligand with inverse agonist properties, has been reported to decrease the motor inco-ordinating effects of ethanol (Suzdak *et al*., 1986), but not the hypnotic effects of high ethanol doses (Bonetti *et al*., 1989; Suzdak *et al*., 1988). Ferko (1992; 1994) reported that, when given after recovery of loss of righting reflex, NMDA demonstrated potentiation of the anaesthetic actions of ethanol by reinstating the loss of righting reflex; bicuculline had no effect in that study.

Potentiation of the anaesthetic effects of pentobarbitone was seen with NMDLA and 4-AP and the lower dose of bicuculline. The *in vitro* effect of barbiturates in increasing $GABA_A$ transmission is well established and is seen at the concentrations which are found during anaesthesia (Nicoll *et al*., 1975). Barbiturates block excitatory amino acid transmission, but responses at AMPA and, in particular, kainate receptors were more susceptible than those mediated by NMDA receptors (Sawada & Yamamoto, 1985; Zeman & Lodge, 1991). Carlen *et al*. (1985) demonstrated an effect of pentobarbitone in blocking calcium-dependent potassium channels, but these were not the channels sensitive to 4-AP.

A different pattern again was seen when anaesthesia was produced by propofol. Bicuculline did not alter the anaesthetic action, while quite large potentiating effects were seen when 4-AP or NMDLA were given with propofol. *In vitro*, propofol increases the effects of GABA at $GABA_A$ receptors (Collins, 1988; Hales & Lambert, 1991). Less work has been carried out on the effects of propofol on excitatory amino acid transmission, but a slight blockade of NMDA-receptor mediated responses was suggested by the results of Yamakura *et al*. (1995). The clear potentiation of propofol anaesthesia by 4-AP and by NMDLA suggests that antagonist effects of propofol at the sites sensitive to these compounds are not part of the mechanism by which propofol produces general anaesthesia, and also suggests that this mechanism is more complex than previously thought. It also raises the question of the mechanism of the potentiation of propofol anaesthesia, which requires further study.

Potentiation of $GABA_A$ transmission has often been suggested, and assumed, to be the main mechanism responsible for the production of general anaesthesia by many types of compound. Such potentiation by barbiturates (Uchida *et al*., 1996) and by propofol (Albertson *et al*., 1996) is prevented *in vitro* by bicuculline. However, the effect of bicuculline in the present study in potentiating the general anaesthetic effects of pentobarbitone and the lack of effect of bicuculline on

propofol anaesthesia suggests that potentiation of $GABA_A$ transmission is not a major factor in the production of general anaesthesia by these compounds. The GABA receptor antagonist action of bicuculline is not dependent on the receptor subunit composition (Krishek *et al*. 1996), so it is unlikely that the differences between these results obtained *in vivo* and the previous studies on synaptic actions *in vitro* are due to the involvement of different subunits. A possibility is that the potentiation of $GABA_A$ transmission has the effect of increasing general anaesthesia while the primary mechanism of anaesthesia involves a different site. This possibility is suggested by the potentiation of general anaesthetic effects of many compounds by benzodiazepine agonist drugs, which have very specific effects in increasing $GABA_A$ transmission but which do not cause true general anaesthesia when given alone.

There is less information available on the effects of anaesthetics on potassium currents, although increases in afterhyperpolarizations, which involve calcium-dependent potassium conductances, were reported in hippocampal and cerebellar neurones with both ethanol and pentobarbitone (Carlen *et al*., 1985), at concentrations of the drugs which were lower than those required to cause potentiation of GABA. However, 4-AP has little effect on the calcium-dependent potassium channels involved in these afterhyperpolarizations, as it affects voltage-sensitive potassium channels, I_A, I_D, I_M, but not the delayed rectifier I_K, or calcium-dependent potassium conductances I_C, I_K, I_{AHP} (Storm, 1990). Intracellular calcium concentrations and phosphorylation by protein kinase C are increased by 4-AP (Heemskerk *et al*., 1991).

NMDA antagonists, like benzodiazepine agonists, potentiated the general anaesthetic effects of pentobarbitone, ethanol and halothane, but not the effect of diethyl ether (Daniell, 1990; 1991), but they do not appear to cause loss of righting reflex when given alone (unpublished observations). McFarlane *et al*. (1995) found an increase in the anaesthetic potency of halothane after administration of an antagonist at the glycine recognition site on the NMDA receptor complex. Duration of loss of righting reflex was the method used in these earlier studies to measure 'anaesthetic potency' for ethanol and pentobarbitone, while loss of response to a painful stimulus was used for the inhalational agents. As diethyl ether is known from clinical studies to have a pronounced analgesic action it is possible that the measurements made were of the analgesic, rather than the anaesthetic effects of the agent.

Antagonist effects of TRH were seen on anaesthesia produced by ketamine and by ethanol. Earlier reports have demonstrated antagonism of both ethanol and pentobarbitone (Breese *et al*., 1974; French *et al*., 1993; Matsushita *et al*., 1995), but no effect was seen with pentobarbitone in the present study. The earlier reports, however, all used 'sleeping time' to measure the potency of the effects of the anaesthetics so only high doses of anaesthetics, which produced loss of righting reflex in all animals, were used. It is possible that effects of TRH are evident only at the highest doses of the anaesthetics, but the evidence presented here suggests that TRH does have effects on lower doses of ethanol and ketamine. Although TRH does not pass the blood-brain barrier very well, it has been found to produce effects on the CNS when given systemically (Callaghan *et al*., 1997). This hormone has been reported to have a range of effects on neuronal function, including decreasing GABA transmission (Stocca & Nistri, 1996), potentiation of NMDA-receptor mediated excitatory amino acid transmission (Chizh & Headley, 1996), decreased potassium conductance (Livingston & Berger, 1993), and increased striatal dopamine release (Kreutz *et al*., 1990).

Consistent antagonism of the anaesthetic effects of ethanol, pentobarbitone and argon by the calcium channel activator, Bay K 8644 was demonstrated previously (Dolin *et al*., 1988). As this earlier work was carried out by the same research group, using the same method of measuring ED_{50} values for the anaesthetics as the present study, it was not considered justifiable to repeat these interactions. In the earlier study a parallel shift to the left of the dose-response curve for each anaesthetic was seen when Bay K 8644, 1 mg kg^{-1}, was given concurrently. This consistency suggested, at that stage, that there might be a common involvement of voltage-sensitive calcium channels in general anaesthesia. However, in the present study, both ketamine and propofol provided exceptions to this pattern, as a potentiation of the anaesthetic effects of ketamine was seen with both doses of Bay K 8644 and of propofol with the 1 mg kg^{-1} dose of Bay K 8644. Correlation between concentrations producing inhibition of dihydropyridine binding and general anaesthesia were demonstrated for several general anaesthetics, including pentobarbitone and propofol, but were not found for ketamine (Hiroto & Lambert, 1996). The strain of mice used by Dolin *et al*. (1988) was albino CD1 while the present studies used the albino TO strain, so it is possible that a strain difference may have been involved in the different pattern of results, but evidence is not yet available concerning this possibility.

One of the primary questions which remains unanswered in the field of anaesthesia is whether or not all the agents produce anaesthesia by the same mechanism (discussed in detail in Little, 1996). The different interactions demonstrated in the present study have two possible explanations: either the anaesthetics produce anaesthesia by different mechanisms, or a single mechanism is involved, that was not affected by the convulsant drugs or by TRH.

However, whether similar or different mechanisms are involved in the production of general anaesthesia by different compounds, the question remains as to how such anaesthesia is produced. With the concentration on the effects of anaesthetics on synaptic transmission in recent years, two important aspects have been relatively neglected. The neuroanatomical location of the production of anaesthesia, elegantly described by Angel (1993) has been almost submerged in the wealth of studies on the detailed synaptic effects of anaesthetic agents. Interference with the patterns of synaptic activity, as shown, for example, by Whittington *et al*. (1996), in one or more specific areas of the brain, such as demonstrated by Angel & Gratton (1982), may be more likely as a site of production of general anaesthesia than actions on single specific receptor-mediated transduction mechanisms. It is also notable that the inert gases can produce general anaesthesia, as illustrated for example in our earlier work (Dolin *et al*., 1988), and studied in detail in earlier investigations (Miller, 1974; Miller *et al*., 1978), but this has received little attention in the synaptic studies. If a mechanism could be identified by which these agents cause general anaesthesia, this could shed valuable light on the mechanism of action of structurally more complex agents.

In conclusion, the results presented here raise important questions about the involvement of certain transmitter systems, such as GABA, and NMDA-receptor mediated excitatory transmission, which have been suggested to play an important role in the production of general anaesthesia. Because, for example, potentiation of $GABA_A$ transmission and blockade of NMDA receptor-mediated responses are seen *in vitro* with a number of general anaesthetic agents, the assumption has been made that these effects are the basis mechanisms of general anaesthesia *in vivo*. The present results

indicate this assumption may not be justified and that further investigation of other possible mechanisms of general anaesthesia would be fruitful.

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