Br. J. Pharmacol. (1988), **94**, 413–422

Effects of the calcium channel activator Bay K 8644 on general anaesthetic potency in mice

S.J. Dolin, *M.J. Halsey & [1]**H.J. Little

Department of Anaesthesia and Clinical Pharmacology Unit, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, *Division of Anaesthesia, Clinical Research Centre, Watford Road, Harrow, Middlesex and **Department of Pharmacology, The Medical School, University Walk, Bristol, BS8 1TD

1 The effects of the calcium channel activator, Bay K 8644, on the anaesthetic potencies of ethanol, argon and pentobarbitone were examined in mice.

2 Bay K 8644, at 1 mg kg^{-1} i.p., significantly antagonized the general anaesthetic potencies of ethanol and argon, but at 5 and 10 mg kg^{-1} this compound increased the general anaesthetic potency of these drugs.

3 At doses of 1, 5 and 10 mg kg^{-1} Bay K 8644 antagonized the anaesthetic effects of pentobarbitone.

4 Bay K 8644, at the highest dose used, did not alter the brain concentrations of pentobarbitone or the blood concentrations of ethanol.

5 The effects of the different doses of Bay K 8644 on the actions of ethanol and of argon are compatible with the known partial agonist properties of this compound on calcium channels *in vitro*.

6 The actions of Bay K 8644 on the anaesthetic effects of pentobarbitone suggests that specific interactions may be involved in the anaesthetic actions of this compound.

Introduction

There has been considerable interest recently in the effects on the central nervous system of drugs that affect calcium channels (Spedding & Middlemiss, 1985; Ramkumar & El-Fakahany, 1986), although under normal circumstances few neuronal effects of these drugs are seen *in vitro*, except at very high concentrations. High affinity radioligand binding sites have been found in the central nervous system (Yamamura *et al.*, 1982; Gould *et al.*, 1982) and dihydropyridine structures have particularly high affinities for these sites. The latter include compounds such as nitrendipine and nimodipine which decrease calcium channel opening and the calcium channel activator, Bay K 8644, which increases calcium flux (Schramm *et al.*, 1983).

We have shown previously that calcium channel antagonists, chosen from three different subgroups (Spedding, 1985), increased the general anaesthetic potency of a variety of compounds, including ethanol, pentobarbitone and argon (Dolin & Little, 1986). In addition, the dihydropyridine calcium channel antagonists were very effective in preventing the ethanol withdrawal syndrome, although they did not have sedative effects when given alone (Dolin & Little, 1986; Little *et al.*, 1986). We now show that the calcium channel activator, Bay K 8644, at a low dose (1 mg kg^{-1}) decreased the general anaesthetic actions of ethanol, argon and pentobarbitone, while higher doses (5 and 10 mg kg^{-1}) increased the potencies of ethanol and argon and decreased those of pentobarbitone.

These three general anaesthetics were chosen because of their diversity of chemical structures. Pentobarbitone has been widely used for small animal anaesthesia. Although the pharmacokinetics and ratio of lethal to anaesthetic doses make ethanol unsuitable for clinical anaesthetic use, it has similar actions to the more common anaesthetics, in producing general anaesthesia. Whilst argon is not normally considered to be an anaesthetic, all the inert gases will produce anaesthesia if given at pressures greater than atmospheric (Miller & Smith, 1973). The mechanism by which general anaesthesia is produced is not known (Paton, 1984), although many theories have been put forward. Most general anaesthetics are not thought to act through binding to

[1] Author for correspondence.

specific receptor sites, although binding sites for barbiturates have been demonstrated on the GABA ionophore complex, a protein molecule mediating the actions of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Ticku & Olsen, 1978). The contribution of this barbiturate binding site, which mediates potentiation of GABA, to the anaesthetic actions of pentobarbitone is unresolved. Evidence has been put forward that the general depressant actions, but not the anticonvulsant properties, of these compounds involves GABA potentiation (Allan & Harris, 1986), but it has also been reported that their effects on calcium action potentials correlate better with their anaesthetic actions than with their anticonvulsant actions (Heyer & Macdonald, 1982).

The effect of anaesthetics on neuronal ion channels is, however, an important stage in the production of general anaesthesia. Calcium has long been thought to be involved in general anaesthesia, but its exact role is not clear. Many general anaesthetics, including ethanol and the barbiturates, reduce movement of calcium into the nerve terminal (Blaustein & Ector, 1975; Stokes & Harris, 1982; Lynch & Littleton, 1983). On the other hand Baker & Shapiro (1980) suggested that anaesthetics might increase the sensitivity of endogenous calcium binding proteins, and Krnjevic considered that a rise in intracellular calcium might underlie the deceased neuronal excitability of anaesthesia (Krnjevic & Lisiewicz, 1972).

Parts of this work have been communicated to the British Pharmacological Society and the Physiological Society.

Methods

Loss of righting reflex was used in the present experiments to measure general anaesthetic potency as this endpoint, although not ideal, most closely approximates the 'loss of consciousness' which defines general anaesthesia in man. Effects of anaesthetics on other endpoints, such as responses to noxious stimuli, do not correlate with general anaesthesia potency (Paton, 1974). Dose-response curves to the anaesthetic actions were plotted so that ED_{50} values could be calculated. Blood concentrations of ethanol and pentobarbitone, and brain concentrations of pentobarbitone, were measured after administration of Bay K 8644, to determine whether or not there was a pharmacokinetic component to the interactions. As different doses of Bay K 8644 had qualitatively different effects with ethanol and argon, the brain concentrations of Bay K 8644 were also measured for comparison with the reported *in vitro* actions of Bay K 8644.

Male mice of the T.O. strain, 25–35 g, were used throughout. In any one experiment the weight range was less than 5 g. Animals were treated with Bay K 8644, 1, 5 or 10 mg kg^{-1} dissolved in water and Tween 80 (0.5%) or vehicle, by the intraperitoneal route, immediately before the administration of the anaesthetic. Tests on Bay K 8644-treated and vehicle-treated animals were carried out concurrently. All animals were placed in cages on heated mats, with overhead heating where necessary. Rectal temperatures were monitored throughout the experiments and maintained in the range 36.5–37.5°C.

General anaesthetic potency

General anaesthetic potency was determined by construction of dose-response curves from measurements of loss of righting reflex. Assessments were made by an observer who did not know the pretreatment (i.e. Bay K 8644 or vehicle) given to each animal. For pentobarbitone and ethanol, the anaesthetic was given i.p. and then each animal was assessed for righting reflex every 5 min by placing it on its back. Failure to regain the upright posture (on all four feet) within 60 s was taken as the criterion for anaesthesia. A minimum of four doses of each anaesthetic was studied, each animal being used once only. Ten mice were used in each group, i.e. at each dose of each general anaesthetic.

For argon anaesthesia the mice were placed in a rotating cage, with four divisions, inside a 20 litre steel pressure chamber. Oxygen was supplied to 0.4 atm and then argon added at a rate of 3 atm min^{-1} to the predetermined pressure. An equilibrium time of 15 min was used for argon, based on preliminary experiments. At the end of the equilibration time the righting reflexes were assessed by rotating the cage by remote control, thus rolling the animals on to their backs, in turn. A time of 60 s was allowed to regain the upright posture. A second pressure was then chosen and a second assessment made in the same way after a further 15 min equilibration. each mouse was used for only one experiment; eight mice were used in each group. One half were assessed at a lower pressure followed by a higher pressure and the other half conversely. A minimum of four different pressures was used for construction of each dose-response line. A separate control and a separate Bay K 8644-treated mouse were used for temperature monitoring in every experiment, kept in another cage in the chamber, adjacent to the rotating one. Rectal temperatures were maintained at 37 ± 0.5°C by alteration of ambient temperature.

As a check on the possibility that the hypertensive effects of Bay K 8644 were responsible for the changes in anaesthetic potency found after this com-

pound, the effects of methoxamine, a sympathomimetic with mainly α-agonist properties, were assessed on the anaesthetic potencies of pentobarbitone and ethanol. On the basis of other work (Bolger *et al.*, 1985), Bay K 8644 10 mg kg^{-1} and methoxamine 8 mg kg^{-1} were considered to be approximately equipotent in raising blood pressure, so 8 mg kg^{-1} methoxamine was selected for study.

Blood and brain concentrations of anaesthetics

In order to determine whether changes in anaesthetic potency were due to alterations in metabolism or distribution, the blood concentrations of the anaesthetics were measured after the highest dose of Bay K 8644, compared with vehicle treatment. Blood ethanol measurements were made 30 min after the administration of ethanol, 4 g kg^{-1}, i.p. with Bay K 8644 10 mg kg^{-1} or vehicle, and 2 h after injection of ethanol, 2 g kg^{-1}, i.p., with Bay K 8644, 1 mg kg^{-1} or vehicle. At these times after injection, in mice, the blood ethanol concentrations are declining, and so changes here would reflect altered metabolism; blood ethanol concentrations have been shown to parallel brain levels at these times (Goldstein, 1983). These two concentrations of Bay K 8644 had opposite effects on ethanol anaesthetic potency (Table 1).

Pentobarbitone plasma concentrations were measured 30 min after a dose of 60 mg kg^{-1}, when the anaesthetic effects were beginning to decline. Samples were taken after treatment with either Bay K 8644, 10 mg kg^{-1}, or vehicle, given at the same time as the anaesthetic, as in the anaesthesia experiments. In addition, in order to ensure that the central concentrations of anaesthetic were not altered, despite the lack of change in blood concentrations, the brain concentrations of pentobarbitone were measured 10 min after a dose of 30 mg kg^{-1} (when anaesthetic effects were at their peak). Bay K 8644, 10 mg kg^{-1}, i.p., (the highest dose used) was given immediately before the anaesthetic. Lower doses of Bay K 8644 were not tested as they had qualitatively the same effects on anaesthetic potency as the highest dose.

Blood samples, for the ethanol and pentobarbitone concentration measurements, were obtained from the carotid artery immediately after death, by cervical dislocation. The blood samples were centrifuged at 2,000 r.p.m. for 10 min. One microlitre of supernatant was taken from each and ethanol concentrations measured by gas chromatography and a flame ionisation detector, with isopropanol as the internal standard. The areas under the ethanol and internal standard curves were compared. Pentobarbitone concentrations were measured by gas-liquid chromatography (Flanagan & Withers, 1972), using chloroform extraction and tetraphenylethylene as an internal standard and comparing peak heights. Recoveries were 100% for ethanol and 94% for pentobarbitone.

For the brain pentobarbitone measurements, mouse whole brain was homogenized, in an Ultraturrax, for 30 s in normal saline, with a 1 : 4 dilution. The pentobarbitone was extracted by addition of chloroform, at a 1 : 1 ratio, to the brain homogenate. The extraction ratio was 0.56 over the drug concentration-range of 10–20 μg mg^{-1} wet weight. This ratio was consistent within 0.01 when repeat estimates were made. The internal standard was tetraphenylethylene, 10 μg ml^{-1}. A sample (4 μl) from each mouse was analysed by gas-liquid chromatography and peak heights of pentobarbitone and the internal standard were compared.

Whole brain concentrations of Bay K 8644

Whole brains were removed from rodents which had been injected 20 min previously with Bay K 8644 1, 5 or 10 mg kg^{-1}, i.p. At this time clear effects of Bay K 8644 were seen on anaesthetic potency, in the studies described above. Whole brain tissue was ground to a uniform consistency; 30 mg was mixed with 800 μl of distilled water and 150 μl of 1 M NaOH, and extracted into toluene already containing the internal standard, nifedipine. Quantitation was by peak height using a RSL-150 capillary column with electron capture detection, on a Perkin Elmer Sigma 2B gas chromatograph.

Drugs

Bay K 8644 (Bayer U.K.) was suspended in Tween 80 (polyoxyethylene sorbitan mono-oleate, Sigma) 0.5% and sonicated. Pentobarbitone (May & Baker Ltd) was dissolved in saline, and ethanol (Analar quality, James Burrough) was mixed with distilled water, final concentration 20% v/v. Argon was obtained from the British Oxygen Company.

Statistical analysis

For the measurements of anaesthetic potency, the data, as the number of mice out of each group of ten (eight for argon experiments) which lost their righting reflex, at each anaesthetic dose, were analysed by probit analysis (Ross, 1980) and dose-response curves constructed. Lines of best fit were compared by Chi-squared analysis, with results expressed as

Table 1 (a) The effects of Bay K 8644 on the anaesthetic potency of ethanol, 20 min after drug injections

Treatment	*Dose of Bay K 8644* ($mg\,kg^{-1}$)	*Ethanol ED_{50} values* ($g\,kg^{-1}$)	*s.e. mean* ($g\,kg^{-1}$)
Vehicle		3.2	0.2
Bay K 8644	1	3.5*	0.2
Vehicle		3.1	0.1
Bay K 8644	5	2.6**	0.1
Bay K 8644	10	2.8*	0.1

(b) The effects of Bay K 8644 on the anaesthetic potency of argon, using 15 min equilibration times

Treatment	*Dose of Bay K 8644* ($mg\,kg^{-1}$)	*Argon ED_{50} values* (atmospheres)	*s.e. mean* (atmospheres)
Vehicle		16.6	0.1
Bay K 8644	1	17.5*	0.1
Vehicle		17.2	0.1
Bay K 8644	5	13.8***	0.1
Bay K 8644	10	15.8**	0.1

(c) The effects of Bay K 8644 on the anaesthetic potency of pentobarbitone, 20 min after drug injections

Treatment	*Dose of Bay K 8644* ($mg\,kg^{-1}$)	*Pentobarbitone ED_{50} values* ($mg\,kg^{-1}$)	*s.e. mean* ($mg\,kg^{-1}$)
Vehicle		27.2	0.9
Bay K 8644	1	31.2	1.2
Vehicle		30.9	1.2
Bay K 8644	5	55.0***	1.3
Bay K 8644	10	55.7***	1.3

(d) The effects of methoxamine, $8\,mg\,kg^{-1}$, on the anaesthetic potencies of ethanol and of pentobarbitone

Anaesthetic	*Control ED_{50} values*	*ED_{50} values with methoxamine*
Ethanol	$2.09 \pm 0.06, g\,kg^{-1}$	$2.97 \pm 0.06, g\,kg^{-1}$
Pentobarbitone	$25.4 \pm 0.9, mg\,kg^{-1}$	$27.8 \pm 1.2, mg\,kg^{-1}$

The results are expressed as ED_{50} values, with standard errors of the mean, derived from the dose-response curves for loss of righting reflex. Ten animals were used for each point on the dose-response curves (except for the argon experiments where eight were used, at two pressures each). At least four anaesthetic doses were tested for each curve. Either Bay K 8644 or Tween vehicle were given immediately before each anaesthetic. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with concurrently tested controls, Chi squared analysis.

ED_{50} values and tests were made for line fit, parallelism and heterogeneity. The line fit tested each set of control and Bay K 8644-treatment results and determined whether one or two lines provided the best fit of the data. This indicated whether or not there were significant differences between the dose-response lines.

The blood concentrations of ethanol and the brain concentrations of pentobarbitone in the presence and absence of Bay K 8644 were compared by the Mann-Whitney U test.

Results

General anaesthetic potency

The effects of Bay K 8644 on general anaesthetic potency followed two patterns. The first was seen with ethanol (Figures 1a and 2a and Table 1a) and argon (Figure 1b and Table 1b). At the lowest dose of Bay K 8644, $1\,mg\,kg^{-1}$, the general anaesthetic potencies of both drugs were significantly decreased. The dose-response curves in each case showed a

parallel shift to the right. These curves were obtained to each anaesthetic (except argon) every 5 min for 30 min, but for clarity only the results at 20 min after anaesthetic administration are given in the Tables and Figure 1. At this time interval the ED_{50} value of ethanol was increased by 6% by Bay K 8644, 1 mg kg^{-1} and that of argon, at the 15 min equilibration time, was increased by 7%; these differences were significant ($P < 0.05$). However, at higher doses of Bay K 8644, 5 and 10 mg kg^{-1}, anaesthesia was increased, the potencies of both general anaesthetics being significantly greater than controls. The percentage changes in the ED_{50} value for ethanol, at 20 min were −22% and −15% and those for the ED_{50} value for argon at 15 min were −16% and −9%, for 5 and 10 mg kg^{-1} Bay K 8644, respectively (Table 1). Again the dose-response curves after Bay K 8644 were parallel to those of controls and the data showed no heterogeneity, i.e. any residual variation was consistent with binomial sampling.

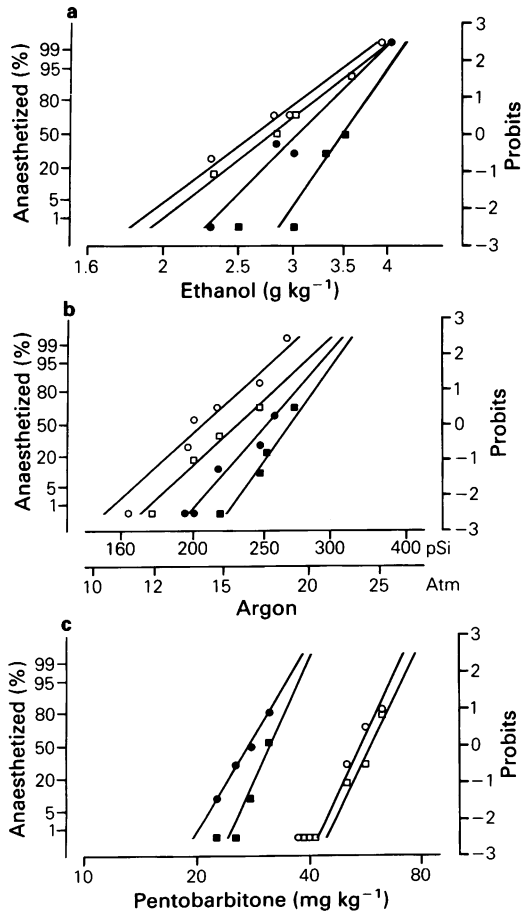

Figure 1 The effects of Bay K 8644 on the general anaesthetic effects of (a) ethanol (20 min), (b) argon (15 min) and (c) pentobarbitone (20 min). The left hand axis gives the percentages of mice anaesthetized at each dose of anaesthetic, calculated using $n = 10$ for each dose of ethanol and of pentobarbitone and $n = 8$ for argon. On the right hand axis these values have been converted to probits. Times given are from the injection of anaesthetic and Bay K 8644 or vehicle.
(●) Vehicle; Bay K 8644: (■) 1 mg kg^{-1}, (○) 5 mg kg^{-1}, (□) 10 mg kg^{-1}.

The second pattern of change with Bay K 8644 was seen with pentobarbitone (Figure 2a and Table 1c). At 1 mg kg^{-1} Bay K 8644 the ED_{50} of pentobarbitone was slightly lower than the control value (−5% change in ED_{50}) but this was not a significant change. However, at 5 and 10 mg kg^{-1}, Bay K 8644 caused marked antagonism of the general anaesthetic effects of pentobarbitone. This was in contrast to the effects of these doses on the anaesthesia produced by ethanol or argon (see above). At 20 min, the changes in the ED_{50} value for pentobarbitone were +78% and +80%, after 5 and 10 mg kg^{-1} Bay K 8644 respectively (Table 1). Comparison of the dose-response curves in each set of experiments showed no significant deviation from parallelism and the data showed no heterogeneity.

Figure 2 shows the time course of the effects of Bay K 8644 on ethanol and on pentobarbitone general anaesthesia. Table 1d shows the ED_{50} values for ethanol and for pentobarbitone after administration of methoxamine, 8 mg kg^{-1}. There were no differences between methoxamine treated animals and controls.

Blood and brain concentrations

The presence of Bay K 8644 did not significantly alter the blood concentrations of ethanol or the brain concentrations of pentobarbitone ($P > 0.05$, Table 2).

The brain concentrations of Bay K 8644 are given in Table 3. The brain concentrations in these tables were calculated assuming an equal distribution of the drugs throughout the brain. It is appreciated that this calculation is approximate, as the presence of Bay K 8644 in blood vessels and extracellular fluid will contribute. The results are of interest in the case of Bay K 8644, for comparison with results obtained by other workers *in vitro* (see below).

Discussion

Bay K 8644 showed some antagonist actions against the general anaesthetic effects of all three drugs studied, but the pattern of changes varied. With ethanol and argon a small shift to the right of the

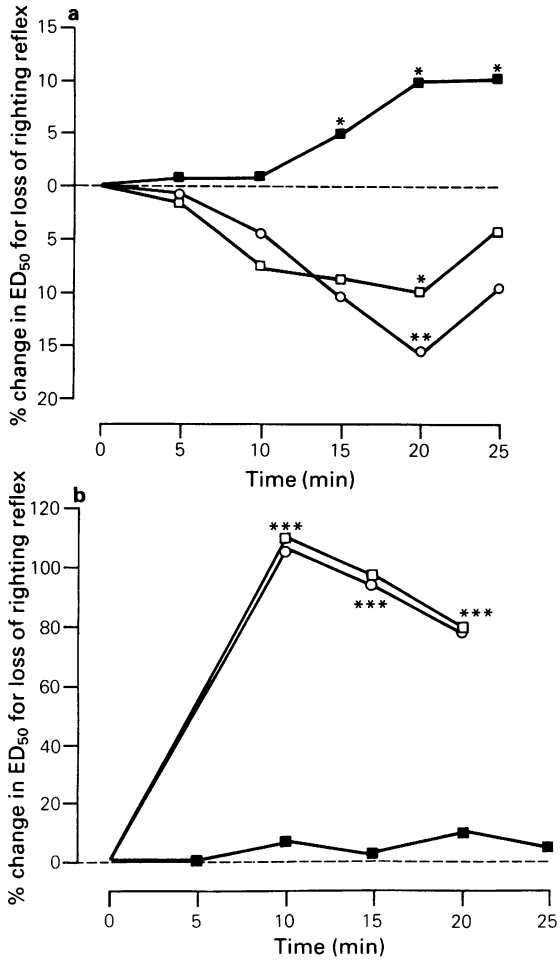

Figure 2 The time courses of the actions of Bay K 8644 on the anaesthetic actions of (a) ethanol and of (b) pentobarbitone. The ED_{50} values were obtained from the dose-response lines, such as those illustrated in Figure 1, which were obtained every 5 min from the start of the experiments to waking. The percentages were calculated using the control ED_{50} values at each time point (i.e., the ED_{50} values in control animals given vehicle injections rather than Bay K 8644). (In (b) the numbers of animals with loss of righting reflex 5 min after injections of Bay K 8644, 5 and 10 mg kg^{-1}, with pentobarbitone, were insufficient to enable calculation of ED_{50} values.)
Bay K 8644: 1 mg kg^{-1} (■), 5 mg kg^{-1} (○), 10 mg kg^{-1} (□).

dose-response curve was seen with 1 mg kg^{-1} Bay K 8644, but the higher doses of the calcium channel activator (5 and 10 mg kg^{-1}) potentiated the general anaesthesia. The effects of Bay K 8644 were dose-dependent to an extent, in that the two higher doses produced greater or substantially different responses to the lowest dose, 1 mg kg^{-1}. However, the effects of the highest dose 10 mg kg^{-1} were not significantly different from 5 mg kg^{-1}, suggesting that the effects of Bay K 8644 on anaesthesia may be via a saturable mechanism. Previous experiments (unpublished results) showed that loss of righting reflex did not occur when high doses of Bay K 8644, up to 100 mg kg^{-1}, were given alone. This was in agreement with previous observations (Bolger *et al.*, 1985). Bay K 8644 has behavioural effects when given alone including ataxia and limb clonus (Bolger *et al.*, 1985). However, these did not preclude the use of loss of righting reflex as an endpoint in the present experiments as the clonus was not seen after the anaesthetic administration.

Consideration was given to possible pharmacokinetic interactions in explaining the effects of Bay K 8644 on anaesthetic potency. Although it was conceivable that Bay K 8644 might have altered the amount of anaesthetic in the brain, it is difficult to postulate a mechanism by which this could occur, in view of the different effects on different anaesthetic agents. Bay K 8644 did not significantly alter blood ethanol or pentobarbitone concentrations, or brain pentobarbitone concentrations, after any of the doses tested (Table 2). These results exclude a pharmacokinetic explanation of the effects of Bay K 8644 on the general anaesthetic actions of pentobarbitone and ethanol.

As Bay K 8644 has been shown to produce a rapid rise in systemic blood pressure (Schramm *et al.*, 1983), the question was addressed whether hypertension *per se* is capable of altering anaesthetic potency. Steffey & Eger (1975) examined the effects of inotropic and vasopressor drugs on halothane MAC in dogs (MAC is the minimum alveolar concentration of an anaesthetic that will prevent response to a noxious stimulus). Although the MAC value for halothane was increased by infusions of ephedrine and mephentermine (which increase central catecholamine release), other vasoconstrictors without central effects, e.g. phenylephrine, metaraminol, methoxamine, at doses which increased blood pressure by 50–100%, did not alter the MAC value. These results indicated that arterial hypertension *per se* did not affect the general anaesthetic potency. In the present experiments, methoxamine, 8 mg kg^{-1}, did not affect significantly ethanol or pentobarbitone anaesthetic potencies (Table 1d). It therefore seemed unlikely that the hypertensive action of Bay K 8644 was responsible for the changes seen in general anaesthetic potency. The effects of Bay K 8644 on regional cerebral blood flow were not assessed, and have not been reported in the literature. It would be difficult to propose a mechanism by which altered regional cerebral blood flow

Table 2 (a) Blood ethanol concentrations, in mg $100\,ml^{-1}$ whole blood, (i) 30 min after injection of ethanol, $4\,mg\,kg^{-1}$; (ii) 2 h after injection of ethanol, $2\,g\,kg^{-1}$

Treatment	*Ethanol* ($mg\,ml^{-1}$)	n
(i) Vehicle	290 ± 12	9
Bay K 8644, $10\,mg\,kg^{-1}$	275 ± 12	9
(ii) Vehicle	51 ± 3	8
Bay K 8644, $1\,mg\,kg^{-1}$	65 ± 8	8

(b) Plasma concentrations of pentobarbitone in $\mu g\,ml^{-1}$ plasma, 30 min after injection of pentobarbitone, $60\,mg\,kg^{-1}$

Treatment	*Pentobarbitone* ($\mu g\,ml^{-1}$)	n
Vehicle	15 ± 1	8
Bay K 8644, $10\,mg\,kg^{-1}$	19 ± 2	8

(c) Whole brain pentobarbitone concentrations, in $\mu g\,mg^{-1}$ wet weight of tissue. Brains removed 10 min after injection of pentobarbitone, ($30\,mg\,kg^{-1}$)

Treatment	*Pentobarbitone* ($\mu g\,g^{-1}$)	n
Vehicle	18 ± 2	6
Bay K 8644, $10\,mg\,kg^{-1}$	17 ± 1	6

Samples taken after administration of either Bay K 8644 or vehicle. The latter injections were made at the same time as those of the anaesthetics; all injections were by the intraperitoneal route.
Values are mean ± s.e. mean.

could affect the distribution of different anaesthetics in such a variety of ways.

The biphasic effects of Bay K 8644 on ethanol and argon anaesthetic potencies are interesting in view of recent reports that Bay K 8644 can possess both Ca^{2+} agonist and antagonist properties (Hess *et al.*, 1984; Garcia *et al.*, 1984). The positive inotropic and vasoconstrictor actions of Bay K 8644 showed biphasic effects. In heart and smooth muscle preparations the dose-response curves are bell-shaped. At concentrations greater than 10^{-7} to 10^{-6} M the agonist effects of Bay K 8644 declined (Schramm *et al.*, 1983; Dube *et al.*, 1985). In isolated heart preparations Bay K 8644 showed a negative inotropic effect at greater than 3 μM, which could be reversed with additional Ca^{2+} (Thomas *et al.*, 1984). The situation is further complicated by the fact that differences have been found between the stereoisomers of Bay K 8644; the (+)-isomer was shown to possess calcium channel antagonist properties (Schramm *et al.*, 1985). The isomers of Bay K 8644 were not available at the time of this study, so we were unable to compare their effects.

Table 3 Whole brain concentrations of Bay K 8644, in $ng\,g^{-1}$ net weight of tissue, and calculated molar concentrations

Dose of Bay K 8644 ($mg\,kg^{-1}$)	*Bay K 8644* ($ng\,g^{-1}$)	*Bay K 8644* (μM)	n
1	358 ± 21	1.0 ± 0.1	8
5	1663 ± 65	4.6 ± 0.2	8
10	4216 ± 295	11.8 ± 0.8	8

Values are mean ± s.e. mean. Brains removed 20 minutes after i.p. injection.

In the present experiments, brain concentrations of Bay K 8644 were measured in mice at the time and doses to correspond to the observed effects on anaesthetic potency. Bay K 8644 1, 5 and $10\,mg\,kg^{-1}$ produced whole brain concentrations of approximately 1.0, 5 and 12 μM respectively, so the concentration at which Bay K 8644 changed from antagonism to potentiation of anaesthesia occurred between 1.0 and 5 μM, which agreed very closely with the concentrations at which changes between Ca^{2+} agonism and antagonism occurred in cardiac and smooth muscle preparations (Schramm *et al.*, 1983; Thomas *et al.*, 1984), as well as inositol phospholipid hydrolysis in brain (Hudspith & Littleton, 1986). The biphasic effects of Bay K 8644 on ethanol and argon anaesthetic potency could therefore be explained by

this compound acting as a partial agonist at neuronal Ca^{2+} channels.

As an agonist, at the lowest dose, $1\,mg\,kg^{-1}$, Bay K 8644 would increase neuronal Ca^{2+} entry, in the opposite direction to anaesthetic drugs (Carmichael & Israel, 1975). As an antagonist, at the higher doses, 5 and $10\,mg\,kg^{-1}$, Bay K 8644 may affect anaesthetic potency in a similar manner to other Ca^{2+} antagonists (Dolin & Little, 1986) by reducing Ca^{2+} entry. These effects may be mediated through changes in the release of neurotransmitters. A wide variety of transmitters have been shown to be released by Bay K 8644, including dopamine (Woodward & Leslie, 1985), 5-hydroxytryptamine (Middlemiss & Spedding, 1985), acetylcholine (Middlemiss, 1985) and catecholamines (Garcia *et al.*, 1984). However, postsynaptic effects may also be important, and release from cell bodies rather than nerve terminals may be involved (Littleton & Little, 1987).

Bay K 8644 produced a consistent antagonism of pentobarbitone anaesthesia, and did not display the biphasic effects seen with ethanol and argon. Ethanol and argon are relatively simple chemical structures that have not been shown to undergo any specific receptor-mediated interactions. On the other hand, barbiturates have specific structure-activity relationships with binding sites in the central nervous system (Ticku & Olsen, 1978). The binding site for barbiturates has been shown to exist on the GABA chloride ionophore complex. This site has not been shown to be associated with a Ca^{2+} conductance, although clear effects of barbiturates in decreasing calcium influx into neurones have been demonstrated (e.g. Elrod & Leslie, 1980). There is a certain amount of doubt that the specific interactions of barbiturates are responsible for their anaesthetic properties (see Introduction), and these drugs still obey the relationship between lipid solubility and potency (Hansch & Anderson, 1967). The specific interactions may account for the different patterns seen with Bay K 8644. Barbiturates have been shown to displace [^{3}H]-nitrendipine binding, although for a series of barbiturates there was a poor correlation between general anaesthetic and displacement potencies (Harris *et al.*, 1985). However, the actions of barbiturates on calcium influx need not be mediated through this receptor site; Harris & Stokes (1982) suggested that the actions of barbiturates in decreasing calcium influx correlated with their sedative actions. These authors also showed that, unlike ethanol, pentobarbitone increased the efflux of calcium from synaptosomes. This action may be involved in the different patterns of interaction of these two compounds with Bay K 8644. It is also possible that the interaction between Bay K 8644 and pentobarbitone is mediated through the dihydropyridine-sensitive calcium channel if the barbiturate could alter the conformation of the receptor site enabling Bay K 8644 to act as a full agonist.

There are no antagonists of general anaesthetics corresponding to those seen with compounds acting at specific receptor sites, i.e. which do not have any effects when given in the absence of agonist. Some antagonism has been reported with the convulsants picrotoxin (Frank & Jhamandras, 1970; Liljequist & Engel, 1982) and 4-aminopyridine (Agoston *et al.*, 1980). Increased ambient pressure antagonizes the anaesthetic actions of a wide variety of compounds (Lever *et al.*, 1971), but high pressure also causes hyperexcitability and convulsions, the mechanism of which is not known (Halsey, 1982). General anaesthesia is not antagonized by all convulsants, however, strychnine for example had no effect. (Frank & Jhamandras, 1970). The results of this paper suggest that, while Bay K 8644 is capable of producing a substantial antagonism of pentobarbitone anaesthesia, this property did not extend fully to other anaesthetics such as ethanol and argon. A drug with full agonist properties at the dihydropyridine binding site may possess wider anaesthetic antagonist properties.

S.J.D. was supported by the W.A. and M.G. Saw Medical Research Fellowship from the University of Western Australia. H.J.L. is a Wellcome Trust Lecturer in Mental Health.

References

AGOSTON, S., SALT, P.J., ERDMANN, W., HILKEMEIJER, T., BENCINI, A. & LANGEHR, D. (1980). Antagonism of ketamine-diazepam anaesthesia by 4-aminopyridine in human volunteers. *Br. J. Anaesth.*, **52**, 367–370.

ALLAN, A.M. & HARRIS, R.A. (1986). Anaesthetic and convulsant barbiturates alter γ-aminobutyric acid-stimulated chloride flux across brain membrane. *J. Pharmacol. Exp. Ther.*, **238**, 763–768.

BAKER, P.F. & SHAPIRO, A.H.V. (1980). Anaesthetics increase light emission from aequorin at constant ionised calcium. *Nature*, **204**, 168–169.

BLAUSTEIN, M.P. & ECTOR, A. (1975). Barbiturate inhibition of calcium uptake by depolarised nerve terminals in vitro. *Mol. Pharmacol.*, **11**, 369–378.

BOLGER, G.T., WEISSMAN, B.A. & SKOLNICK, P. (1985). The behavioural effects of the calcium agonist Bay K

8644 in the mouse: antagonism by the calcium antagonist nifedipine. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **328**, 373–377.

CARMICHAEL, F.J. & ISRAEL, Y. (1975). Effects of ethanol on neurotransmitter release by rat brain cortical slices. *J. Pharmacol. Exp. Ther.*, **193**, 824–833.

DOLIN, S.J. & LITTLE, H.J. (1986). Augmentation by calciun channel antagonists of general anaesthetic potency in mice. *Br. J. Pharmacol.*, **88**, 909–914.

DUBÉ G.P., BAIK, Y.H. & SCHWARTZ, A. (1985). Effects of a novel calcium agonist dihydropyridine analogue Bay K 8644 on pig coronary artery: biphasic mechanical response and paradoxical potentiation of contraction by diltiazem and nimodipine. *J. Cardiovasc. Pharmacol.*, **7**, 377–389.

ELROD, S.V. & LESLIE, S.W. (1980). Acute and chronic effects of barbiturates on depolarisation-induced calcium influx into synaptosomes from rat brain regions. *J. Pharmacol. Exp. Ther.*, **212**, 131–136.

FLANAGAN, R.J. & WITHERS, G. (1972). A rapid micromethod for the screening and measurement of barbiturates and related compounds in plasma by gas-liquid chromatography. *J. Clin. Pathol.*, **25**, 899–904.

FRANK, G.B. & JHAMANDRAS, K. (1970). Effects of drugs acting alone and in combination on the motor activity of intact mice. *Br. J. Pharmacol.*, **39**, 696–706.

GARCIA, A.G., SALA, F., REIG, J.A., VINIEGRA, S., FRIAS, J., FONTERIZ, R. & GANDIA, L. (1984). Dihydropyridine Bay K 8644 activates chromaffin cell calcium channels. *Nature*, **309**, 69–71.

GOLDSTEIN, D. (1983). *Pharmacology of Alcohol.* New York: Oxford University Press. p. 7.

GOULD, R.J., MURPHY, K.M.M. & SNYDER, S.H. (1982). [^{3}H]-nitrendipine labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc. Natl. Acad. Sci., U.S.A.*, **79**, 3656–3660.

HALSEY, M.J. (1982). Effects of high pressure on the central nervous system. *J. Physiol.*, **62**, 1341–1377.

HANSCH, C. & ANDERSON, S.M. (1967). The structure-activity relationship in barbiturates and its similarity to that in other narcotics. *J. Med. Chem.*, **10**, 745–753.

HARRIS, R.A., JONES, S.B., BRUNO, P. & BYLUND, D.B. (1985). Effects of dihydropyridine derivatives and anticonvulsant drugs on [^{3}H]-nitrendipine binding and calcium and sodium fluxes in brain. *Biochem. Pharmacol.*, **34**, 2187–2191.

HARRIS, R.A. & STOKES, J.A. (1982). Effects of a sedative and an anticonvulsant barbiturate on synaptic calcium transport. *Brain Res.*, **242**, 157–163.

HESS, P., LANSMAN, J.B. & TSIEN, R.W. (1984). Differential modes of Ca^{2+} channel gating behaviour favoured by dihydropyridine Ca^{2+} agonists and antagonists. *Nature*, **311**, 538–544.

HEYER, E.J. & MACDONALD, R.L. (1982). Barbiturate reduction of calcium-dependent action potentials: correlation with anesthetic action. *Brain Res.*, **236**, 157–171.

HUDSPITH, M.J. & LITTLETON, J.M. (1986). Enhanced effect of Bay K 8644 on inositol phosphate breakdown in brain slices from ethanol-dependent rats. *Br. J. Pharmacol.*, **89**, 623P.

KRJNEVIC, K. & LISIEWICZ, A.J. (1972). Injection of calcium ions into spinal neurones. *J. Physiol.*, **225**, 363–390.

LEVER, M.J., MILLER, K.W., PATON, W.D.M. & SMITH, E.B. (1971). Pressure reversal of anaesthesia. *Nature*, **231**, 368–371.

LILJEQUIST, S. & ENGEL, J. (1982). Effects of GABAergic agonists and antagonists on various ethanol-induced behavioural changes. *Psychopharmacol.*, **78**, 71–75.

LITTLE, H.J., DOLIN, S.J. & HALSEY, M.J. (1986). Calcium channel antagonists decrease the ethanol withdrawal syndrome. *Life Sci.*, **39**, 2059–2065.

LITTLETON, J.M. & LITTLE, H.J. (1987). Adaptation in neuronal calcium channels as a common basis for physical dependence on central depressant drugs. In: *Tolerance and Sensitisation to Psychoactive Drugs.* ed. Emmett-Oglesby, M. & Goudie, A.J. New Jersey: Humana Press, (in press).

LYNCH, M.A. & LITTLETON, J.M. (1983). Possible association of alcohol tolerance with increased synaptic Ca^{2+}-sensitivity. *Nature*, **303**, 175–176.

MIDDLEMISS, D.N. (1985). The calcium channel activator, Bay K 8644, enhances K^+-evoked efflux of acetylcholine and noradrenaline from brain slices. *Naunyn-Schmeiderbergs Arch. Pharmacol.*, **331**, 114–116.

MIDDLEMISS, D.N. & SPEDDING, M. (1985). A functional correlate for the dihydropyridine binding site in rat brain. *Nature*, **314**, 94–96.

MILLER, K.W. & SMITH, E.B. (1973). Intermolecular forces and the pharmacology of simple molecules. In *A Guide to Molecular Pharmacology – Toxicology.* ed. Featherstone, R.M. pp. 1–13. New York: Marcel Dekker Inc.

PATON, W.D.M. (1974). Unconventional anaesthetic molecules. In *Molecular Mechanisms of Anaesthesia.* ed. Halsey, M.J., Millar, R.A. & Sutton, J.A. pp. 48–64. Edinburgh, London & New York: Churchill Livingston.

PATON, W.D.M. (1984). How far do we understand the mechanism of anaesthesia? *Eur. J. Anaesth.*, **1**, 93–103.

RAMKUMAR, V. & EL-FAKAHANY, E. (1986). The current status of the dihydropyridine calcium channel antagonist binding sites in the brain. *Trends Pharmacol. Sci.*, **7**, 171–172.

ROSS, G.J.S. (1980). *Maximum Likelihood Program.* Rothampstead Experimental Station, Harpenden, Herts, England.

SCHRAMM, M., THOMAS, G., TOWART, R. & FRANCKOWIAK, G. (1983). Activation of calcium channels by novel 1,4-dihydropyridines. *Arzneim. Forsch.*, **33**, 1268–1272.

SCHRAMM, M., TOWERT, R., LAMP, B. & THOMAS, G. (1985). Modulation of calcium ion influx by the 1,4-dihydropyridines, nifedipine and Bay K 8644. *J. Cardiovasc. Pharmacol.*, **7**, 493–496.

SPEDDING, M. (1985). Calcium antagonist subgroups. *Trends Pharmacol. Sci.*, **6**, 109–114.

SPEDDING, M. & MIDDLEMISS, D.N. (1985). Central effects of calcium antagonists. *Trends Pharmacol. Sci.*, **6**, 309–310.

STEFFEY, E.P. & EGER, E.I. (1975). The effects of seven vasopressors on halothane MAC in dogs. *Br. J. Anaesth.*, **47**, 435–438.

STOKES, J.A. & HARRIS, R.A. (1982). Alcohols and synaptosomal calcium transport. *Mol. Pharmacol.*, **22**, 99–104.

THOMAS, G., GROSS, R. & SCHRAMM, M. (1984). Calcium channel modulation: Ability to inhibit or promote

calcium influx resides in the same dihydropyridine molecule. *J. Cardiovasc. Pharmacol.*, **6**, 1170–1176.

TICKU, M.K. & OLSEN, R.W. (1978). Interaction of barbiturates with dihydropicrotoxin binding sites related to the GABA receptor-ionophore system. *Life Sci.*, **22**, 1643–1645.

WOODWARD, J.J. & LESLIE, S.W. (1985). Inhibition by nimodipine of Bay K 8644 and KCl stimulated Ca^{2+} entry into and endogenous dopamine release from striatal synaptosomes. *Soc. Neurosci. Abstr.*, **11**, 1002.

YAMAMURA, H.I., SCHOEMAKER, R.G., BOLES, R.G. & ROESKE, W.A. (1982). Diltiazem enhancement of [^{3}H]-nitrendipine binding to calcium channel associated drug receptor sites in rat brain synaptosomes. *Biochem. Biophys. Res. Comm.*, **108**, 640–646.

(Received August 28, 1987
Revised January 10, 1988
Accepted January 20, 1988)