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Effects of injectable anaesthetics on responses to L-glutamate and on spontaneous synaptic activity in lamprey reticulo-spinal neurones

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1 Intracellular recordings were made from reticulo-spinal cells in the medulla of lamprey ammocoetes; potential changes in response to iontophoretically applied L-glutamate were measured before, during and after the preparation was superfused with anaesthetic solutions.

2 Of the anaesthetics pentobarbitone, ketamine, alphaxalone/alphadolone (Saffan) and metomidate, only pentobarbitone ($> 10\,\mu$M) had a consistent dose-related depressant effect on glutamate responses.

3 Spontaneous excitatory postsynaptic potentials (e.p.s.ps) and inhibitory postsynaptic potentials (i.p.s.ps) were diminished in frequency by high concentrations (1 mM) of all anaesthetics. Anaesthetic concentrations of all drugs also reduced i.p.s.ps; for e.p.s.ps this was true of pentobarbitone ($100\,\mu$M) immediately, and of ketamine ($370\,\mu$M) and alphaxalone ($10-30\,\mu$M) after a transitory increase in activity.

4 Consideration of the results in the light of previous observations on inhibitory responses suggests a basis for some of the excitatory side effects of these compounds, assuming that the equivalent mammalian cells are similarly affected.

Introduction

The possibility and potential importance of interactions between anaesthetic drugs and neuronal responses to transmitters was recognized early in the study of CNS transmitters (e.g. Krnjević & Phillis, 1963). These early experiments frequently involved the iontophoretic application of transmitters to the spinal roots of anaesthetized animals; and it was common practice, when inhibitory transmitters were investigated, to employ an excitatory amino acid such as L-glutamate to activate neurones that were otherwise silent.

We have shown previously that the bulbar reticulospinal cells of the lamprey ammocoete respond to iontophoretic application of the inhibitory amino acids γ-aminobutyric acid (GABA) and glycine, and that these responses are differentially modified by bath applied anaesthetics (Cullen & Martin, 1982). We present here the results of complementary experiments in which responses to L-glutamate have been tested with the same four injectable anaesthetics. L-Glutamate has been shown to excite these cells, causing a small decrease in input resistance associated with a membrane depolarization and bursts of synaptic potentials (Wickelgren, 1977); and has been proposed as the probable natural excitatory transmitter (Matthews & Wickelgren, 1979). Application of glutamate in higher concentrations and the injection of depolarizing current pulses into the cells both elicit bursts of action potentials.

As in the previous publication, observations have in the main been confined to postsynaptic effects: raised magnesium concentrations have been used to suppress synaptic transmission and hence any indirect effects. However, examination of a cell's response to exogenous transmitters gives only a partial insight into the effects of anaesthetics on that cell's activity. Therefore, we have also investigated the effects of the anaesthetics on the spontaneous synaptic potentials that can be recorded from the reticulospinal cells in the presence of more physiological concentrations of magnesium. We have not examined the effects of the anaesthetics on the excitability of cells.

Methods

The experiments were carried out using 12–15 mm ammocoetes of the species *Lampetra fluviatilis* and *Lampetra planeri*, and methods substantially the same as those described in detail previously (Cullen & Martin, 1982). These are briefly restated here.

An ammocoete was anaesthetized using 100 μM tricaine methane sulphonate (MS-222: Sandoz) and transected in the gill region. The rostral portion was transferred to the experimental chamber (liquid capacity 1 ml) where it was pinned to Sylgard and continuously perfused with cooled Ringer at a flow rate of 6 ml min^{-1}. This flow flushed tricaine methane sulphonate from the preparation before the experiment was started (1–2 h after anaesthetization). A coolant, circulated through an outer jacket, controlled the temperature in the chamber. Dissection of the brain stem exposed the bulbar reticulo-spinal cells in the floor of the 4th ventricle. These large cells are arranged symmetrically in 8 pairs on either side of the midline. Although most experiments were conducted at 10°C, the effect on spontaneous synaptic activity of temperature fluctuations within the range 2 to 18°C was also noted.

Solutions

For iontophoresis experiments a Ringer solution of the following composition was used (mM): NaCl 71, KCl 2.1, $CaCl_2$ 2.6, $MgCl_2$ 15, $NaHCO_3$ 20; NaH_2PO_4 0·18, glucose 4.0. The pH was adjusted to 7.1–7.2 with HCl and the solution aerated with a gas mixture of 5% CO_2 and 95% O_2 at a rate which did not alter the pH by more than 0.1. Observations of spontaneous synaptic potentials were made when the $[Mg^{2+}]$ of the perfusate was 1.8 mM.

Recording and iontophoresis

Standard electrophysiological techniques were employed. The resistance of the recording micropipettes, when filled with 2 M potassium methyl sulphate, was in the range 20–30 MΩ. After a cell had been penetrated, a continuous record of its membrane potential was made using a pen recorder (Lectromed: MX212). A bridge balance circuit incorporated in the high input impedance pre-amplifier allowed the injection of square hyperpolarizing current pulses through the recording electrode, and thus the measurement of the input resistance of cells. Spontaneous synaptic potential transients were recorded at high gain and with a slow paper speed. Because the membrane potential was sensitive to temperature changes, a continuous record was made also of the temperature in the bath. The shapes of individual transients were displayed on an oscilloscope screen (Tektronix 5113 Dual Beam Storage Oscilloscope).

Glutamate was applied by iontophoresis: ejected as an anion from a micropipette filled with 2 M glutamate at pH 8·7. Ejection was controlled by an iontophoresis programmer (Model 160: W–P Instruments Inc.) driven by a Digitimer (D100). Retaining currents were not routinely employed. Controls were provided by application of pulses of reversed polarity or by the incorporation of 0.5 M NaCl in one barrel of the electrode assembly. Two ejection procedures were used: (i) fixed test pulses repeated at intervals of 1 or 3 min, (ii) a repeated sequence of test pulses in order of increasing length – in case the anaesthetic effect was related to the dose of transmitter. The pulses used (70 to 400 nA) had durations between 1 s and 7 s. Since there was no direct measure of the drug concentration achieved at the cells' receptors, and since the pulse length bears a non-linear relation to the quantity of drug ejected from an electrode, the only valid comparisons are those between the responses of an individual cell to pulses of different length.

Anaesthetics were applied dissolved in the perfusing Ringer. No osmotic compensation was made because the anaesthetic concentration never exceeded 1 mM. When repeated anaesthetic applications were made to a preparation, at least twenty minutes was allowed as a recovery period between tests. More usually this period was between thirty and forty minutes; but when successive solutions were applied in order of increasing anaesthetic concentration there was sometimes no intervening wash.

In many cells the glutamate-evoked change in input resistance could not be measured accurately, so measurements were confined to the peak changes in membrane potential and in the area enclosed by the envelope of the response (see Figure 2 in Cullen & Martin, 1982). The results of different experiments were combined after the proportional change in glutamate response amplitude at the peak of the drug effect had been calculated. An unpaired t test was used to assess the statistical significance of anaesthetic effects.

Records of spontaneous synaptic activity are presented as segments of the chart records. Vertical transients represent membrane potential events. The lateral separation of spontaneous transients is indicative of their frequency; their length a measure of the amplitude of the potential displacements, within the limits imposed by the frequency response of the chart recorder. Possible distortions introduced by the recorder do not invalidate a qualitative assessment of changes in the level of synaptic activity. The duration of a typical small excitatory postsynaptic potential (e.p.s.p.) was in the range 30–40 ms; the frequency-response of the recorder was flat ±0.5dB at 100 Hz (manufacturer's specification).

Drugs

The drugs used were L-glutamate (BDH), tricaine methane sulphonate (MS-222, Sandoz), pentobarbitone (May & Baker), ketamine (Vetalar, Parke-Davis), alphaxalone/alphadolone (Saffan, Glaxovet) and metomidate (Hypnodil, Janssen). The vehicle present in Saffan was kindly donated by Glaxovet. Although the product Saffan was used in the experiments, it will be referred to henceforth as alphaxalone because concentrations are expressed in terms of this constituent.

Results

Responses of bulbar reticulo-spinal cells to glutamate

Most cells had resting membrane potentials in the range −60 to −70 mV. The response to glutamate, measured in a total of 45 cells, was always depolarizing, the magnitude and speed of onset/offset varying between cells. Small amplitude responses were seen in cells with high resting potentials as well as in those that were relatively depolarized. In 4 cells there was a fast low amplitude response which was not increased appreciably when the strength or duration of the iontophoretic pulses were increased; yet the depolarization was sustained and there was no sign of desensitization. These 4 cells showed a minimal change in input resistance. In other cells, input resistance decreases ranged from 65% to undetectable (< 15% in 28 cells; between 20% and 30% in 11 cells); their extent was not correlated with the change in membrane potential.

In the absence of anaesthetic effects, the glutamate response remained stable over periods of an hour or more. The different types of response were not distinguished when the effects of the anaesthetics were assessed.

Elevation of the $[Mg^{2+}]$ reduced the tendency for the glutamate-induced depolarization to be interrupted by synaptic potentials and intermittent firing of action potentials. However, in some experiments such potentials occurred even though there was no spontaneous activity between applications of glutamate. Response amplitudes were not measured in these experiments because the full extent of the depolarization was masked. There was no evidence that magnesium acted as an antagonist of the glutamate responses.

General effects of the anaesthetics

No anaesthetic consistently altered the membrane potential. Input resistance was only affected by the highest concentrations of pentobarbitone and metomidate (1 mM); which caused a small reduction (10–15%). Responses were measured in cells from a total of 33 ammocoetes. Numbers of ammocoetes exposed to the anaesthetics were: pentobarbitone 10, ketamine 7, alphaxalone 10, metomidate 4. In addition two ammocoetes were exposed only to the Saffan vehicle. Sample records are displayed in Figures 1 and 2, and the collected results for each drug are presented in Table 1.

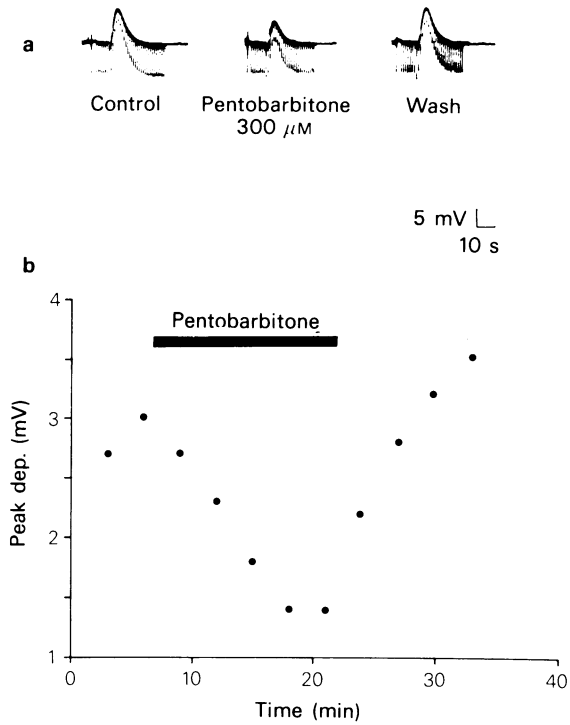

Figure 1 The effect of pentobarbitone on glutamate responses. (a) Pentobarbitone (300 μM) reduced both the depolarization and the change in input resistance produced by standard pulses of glutamate (exposure time: 3 min; wash time: 8 min; iontophoretic pulse: 600 nA, 2 s; hyperpolarizing pulses: 1 nA). (b) Serial measurements made during a different experiment when 300 μM pentobarbitone was applied for 18 min. The depolarizing responses to standard pulses of glutamate (200 nA) were depressed.

Pentobarbitone

A clear dose-related depression of the glutamate response was observed, and only in 3 out of 16 tests in which measurements were made did depression not exceed 10%. Figure 1 shows sample records from a cell exposed to 300 μM pentobarbitone, and serial measurements made during a different experiment.

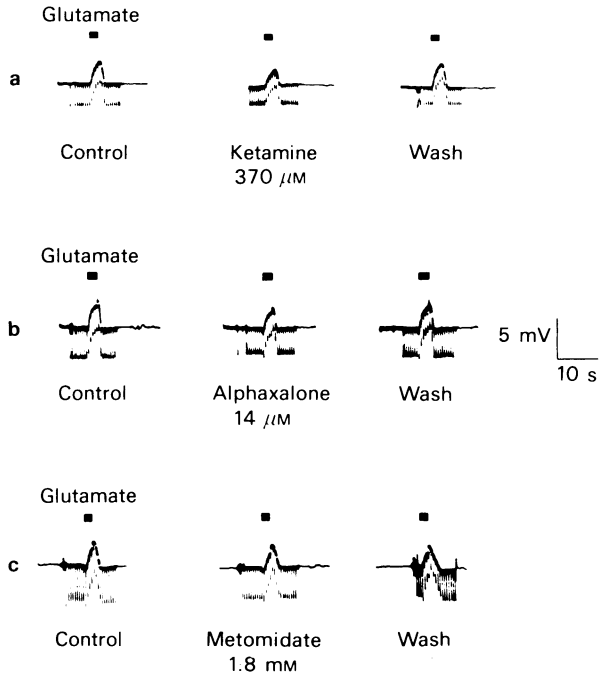

Figure 2 Effects of ketamine, alphaxalone and metomidate on glutamate responses. Ketamine (a) and alphaxalone (b) depressed the glutamate responses slightly but metomidate (c) had little effect. (a) Sample responses: control; after 8 min exposure to ketamine; after 10 min wash (iontophoretic pulse: 20 nA, 5 s; hyperpolarizing pulses: 1 nA). (b) Sample responses: control; after 11 min exposure to alphaxalone; after 27 min wash (iontophoretic pulses: 500 nA, 5 s; hyperpolarizing pulses: 0.9 nA). (c) Sample responses: control; after 7 min exposure to metomidate; after 16 min wash (iontophoretic pulses: 400 nA, 4 s; hyperpolarizing pulses: 1 nA).

The latter illustrate the reversible depression of glutamate responses during exposure to a relatively high concentration of pentobarbitone (300 μM).

Ketamine

Sample records from a single experiment are displayed in Figure 2a. The accumulated results (Table 1) suggest a dose-related depression detectable at 37 μM (the clinically relevant dose), but the effect did not reach statistical significance.

Alphaxalone

Alphaxalone 100–300 μM depressed the glutamate responses ($P<0.05$, using a 1 tailed test) but no other concentration had a statistically significant effect. However, more cells were depressed, and to a greater extent, in the presence of alphaxalone than when the commercial vehicle was presented alone. In the experiment illustrated in Figure 2b there was slight depression induced by 30 μM alphaxalone.

Table 1 The effects of anaesthetics on the responses to glutamate

Anaesthetic	*Peak glutamate response % change*
Pentobarbitone (μM)	
200–500	−39 ± 10(6)**
100	−17 ± 8(8)†
30	−17 ± 1(2)*††
Ketamine (μM)	
3,700	−50(1)
370	−12 ± 10(7)
37	−13 ± 13(6)
3.7	−4 ± 7(4)
Alphaxalone (μM)	
100–300	−20 ± 7(4)†
10–30	−4 ± 6(12)
1–3	−17 ± 10(7)
Saffan vehicle	−16 ± 9(4)
Metomidate (μM)	
1,800	−21 ± 13(3)
180	−18 ± 8(4)
18	+22 ± 17(3)

Each value represents the percentage change in response during application of an anaesthetic: mean ± s.e. mean. Numbers of observations in parentheses. Significant effects indicated (unpaired t tests): * $P<0.05$; ** $P<0.01$–2-tailed test; † $P<0.05$; †† $P0.01$–1-tailed test. The 1-tailed test assumes that none of the anaesthetics potentiates the effect of an excitatory transmitter. Saffan vehicle dilution equivalent to 10 to 100 μM.

Metomidate

Metomidate depressed glutamate responses only at supra-anaesthetic concentrations, and no concentration had a statistically significant effect. It is notable that in two out of three tests the depression by the highest concentration used (1.8 mM) did not exceed 15%. Records taken from an experiment in which the effects of metomidate were only slight are displayed in Figure 2c.

The effect of agonist dose on the anaesthetic effects

In no case did variation in the glutamate pulse length have any detectable effect on the influence of the anaesthetics (not illustrated).

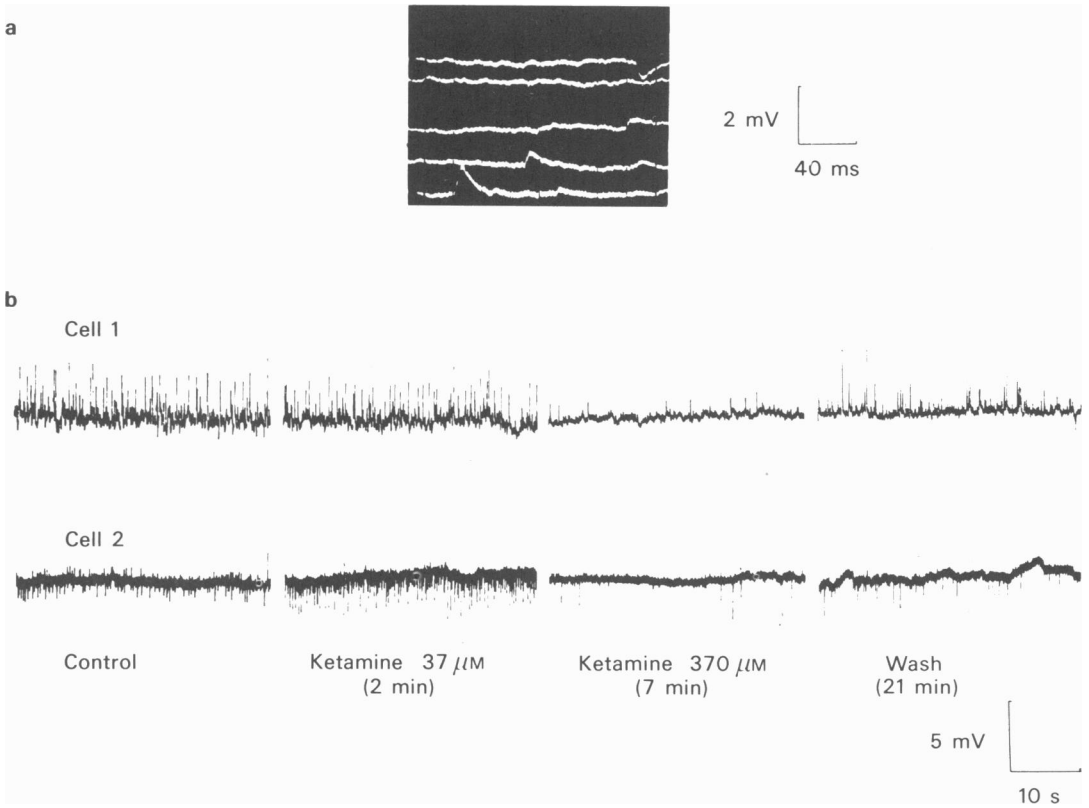

Figure 3 (a) Spontaneous membrane potential transients recorded in 'normal' Ringer (1.8 mM Mg^{2+}). Successive sweeps on oscilloscope stored and photographed. Upward transients (depolarizing) have been interpreted as e.p.s.ps; downward transients (hyperpolarizing) as i.p.s.ps. The larger amplitude transient in row 5 was produced by superimposition of e.p.s.ps. (b) The effects of ketamine on spontaneous synaptic activity. Sample records taken from two cells showing predominantly e.p.s.ps and i.p.s.ps respectively. Frequency of e.p.s.ps (cell1) and i.p.s.ps (cell2) initially increased by 37 μM ketamine. This increase was sustained for 3 min only and in cell 1 was barely detectable. A partially reversible loss of both e.p.s.ps and i.p.s.ps occurred during exposure to 370 μM ketamine (both samples taken after 7 min exposure). Records during wash period retouched.

Anaesthetic modulation of spontaneous synaptic activity

When intracellular recordings were made at high gain and the Ringer contained 1.8 mM Mg^{2+}, small brief perturbations of the membrane potential (amplitude 0.5–3.0 mV) could be distinguished. Such spontaneous potential changes occurred in both hyperpolarizing and depolarizing directions, and have been interpreted as inhibitory postsynaptic potentials (i.p.s.ps) and e.p.s.ps, respectively (see Gold & Martin, 1983). The rise and decay times varied with the amplitudes of the potentials, but small events commonly lasted 30–40 ms and had time constants close to 10 ms (rise) and 15 ms (decay). When displayed on an expanded time scale it was evident that some of the larger transients were composed of superimposed smaller events (Figure 3a). When the spontaneous activity was greatly increased, as during the application of glutamate, action potentials were elicited. In a few cells periodic depolarizations of 6–7 mV were each accompanied by a burst of action potentials. Other cells showed intermittent hyperactivity.

It was of interest to see whether the effects on transmitter responses would be reflected in anaesthetic effects on natural transmission. To this end 26 preparations were exposed to the anaesthetics: pentobarbitone 5, ketamine 13, alphaxalone 4 and metomidate 4 under conditions (1.8 mM Mg^{2+}) permitting the recording of spontaneous e.p.s.ps and i.p.s.ps from the reticulo-spinal cells.

The frequency of occurrence of spontaneous potentials showed strong temperature-dependence; individual events could not be resolved at 15°C, where-

Table 2 The effect of anaesthetics on spontaneous synaptic activity

	I.p.s.ps				*E.p.s.ps*			
	inc.	*dec.*	*n.c.*	*(dec.)*	*inc.*	*dec.*	*n.c.*	*(dec.)*
Pentobarbitone (μM)								
100	0	1	0	0	0	1	0	0
300–500	0	2	0	0	0	2	0	0
1,000	0	1	0	0	0	1	0	0
Ketamine (μM)								
0.37	1	0	0	0	1	0	0	0
3.7	0	0	1	0	1	0	0	0
37	1	2	4	0	3	1	3	0
370	0	6	0	0	0	6	0	0
Alphaxalone (μM)								
2.7	0	1	1	0	1	0	1	0
27	1	1	4	1	4	0	2	2
Metomidate (μM)								
18	0	2	5	0	1	0	3	2
180	1	2	1	0	1	2	1	0
1,800	1	2	0	1	1	3	0	1

Tabulated values are the numbers of tests in which an increased (inc.), decreased (dec.) or unchanged (n.c.) level of activity (frequency of potential transients) was observed. In a few cases there was a biphasic anaesthetic effect consisting of initial stimulation followed by suppression. These tests are recorded in the columns headed (dec.) in addition to that headed inc.

as at 4°C the transients were widely spaced in time (not illustrated). Elevation of the $[Mg^{2+}]$ in the Ringer to 15 mM abolished all these potentials. Sample traces are displayed in Figure 3 and the collected results from experiments at 10°C, when individual potential transients could usually be resolved, are shown in qualitative form in Table 2. The anaesthetic effects were time-dependent: there was no steady-state effect other than the quiescence seen during exposure to high concentrations of anaesthetics.

Apart from the depression caused by high concentrations of all anaesthetics, the principal finding was that i.p.s.ps were the more labile: they were reduced in frequency by all drugs at anaesthetic or supra-anaesthetic concentrations and this reduction was longer lasting than that of e.p.s.ps. When activity was enhanced it was predominantly excitatory; although when the increase was pronounced, as in the presence of 0.37 μM ketamine, it was impossible to resolve individual events and to ascribe a polarity to them.

E.p.s.ps were decreased in frequency by pentobarbitone (100 μM) immediately, and by alphaxalone (10–30 μM) and ketamine (370 μM) after a transitory increase. Sub-anaesthetic concentrations of ketamine (< 10 μM) stimulated activity in the one preparation tested. Below the high concentration (1 mM) at which all the drugs suppressed all spontaneous activity, metomidate had little effect.

Discussion

The most striking feature of these experiments was the lesser effect of anaesthetics on glutamate than on GABA and glycine responses (compare Cullen & Martin, 1982), despite the fact that small changes in membrane conductance would exert a more readily detectable effect on the glutamate response, whose reversal potential (about −35 mV: Matthews & Wickelgren, 1979) is far from the resting potential. This contrast, which was particularly dramatic with metomidate, indicates that the depressant effects of high concentrations of all the anaesthetics on the inhibitory responses cannot be the result of an entirely non-specific neuronal depression. None of the drugs caused potentiation of the glutamate responses, and only two had any consistent depressant effect.

Depression of glutamate responses by pentobarbitone is consistent with earlier findings that barbiturates depress responses to excitatory amino acids (e.g. Krnjević & Phillis, 1963; Crawford & Curtis, 1966; Johnson *et al.*, 1969; Richards & Smaje, 1976; Nicoll & Wojtowicz, 1980). A presynaptic effect may have contributed to the abolition of spontaneous potentials (Table 2) since pentobarbitone (100 μM) has not been shown to depress responses to GABA or glycine (Cullen & Martin, 1982). Pentobarbitone

had no effect likely to increase activity in the reticulospinal axons: all effects described would be expected to reduce motor responses to stimulation and so promote muscle relaxation.

The small, statistically insignificant inhibitory effect of ketamine on glutamate responses is consistent with the observation (Anis *et al.*, 1983) that ketamine selectively inhibits NMDA responses and has a lesser depressant effect on responses to glutamate, quisqualate and kainate. The pharmacology of the glutamate receptor present on lamprey reticulo-spinal cells has not been studied in detail but any NMDA receptors present should be inhibited by the 15 mM Mg^{2+} used (Evans & Watkins, 1978; Ault *et al.*, 1980).

Anaesthetic concentrations of ketamine depressed glycine responses more than those to glutamate, and i.p.s.ps were more susceptible to block than were e.p.s.ps. Both results predict a resultant disinhibition of these motor cells and so, potentially, exaggerated motor responses to stimulation. The increased frequency of spontaneous potentials seen during exposure to low concentrations of ketamine was not matched by an equivalent change in the cells' responses to exogenous amino acid transmitters.

The small depressant effect of alphaxalone, apparent only at high concentrations (100 μM), is consistent with the increased frequency of e.p.s.ps recorded from reticulo-spinal cells during exposure to low concentrations of alphaxalone, as is the depression of GABA and glycine responses shown previously. When the anaesthetic concentration was higher the presynaptic depressant effect implied by the abolition of e.p.s.ps and i.p.s.ps probably offset the disinhibition. However, the result contrasts with the 60–100% depression of glutamate-evoked firing by lower concentrations of alphaxalone (c. 10 μM) reported in the prepyriform cortex (Richards & Smaje, 1976). Alphaxalone's and metomidate's failure to depress glutamate responses markedly at low concentrations should reinforce the block of responses to inhibitory amino acids that we have described previously (Cullen & Martin, 1982).

It was a general finding that the i.p.s.ps were more susceptible to anaesthetic blockade. If i.p.s.ps are in part concerned with discriminative inhibition, as has been suggested by Andersen *et al.*, (1980), this may contribute to the reduction in the fine control of motor responses commonly found during induction and recovery from anaesthesia (e.g. Cullen & Martin, 1982); as also may the depression of the lamprey's reticulo-spinal cells, part of a pathway which has inhibitory as well as excitatory components (Buchanan, 1982; Buchanan & Cohen, 1982). Greater sensitivity of inhibitory than of excitatory mechanisms to anaesthetics has previously been described by Scholfield (1980) in the olfactory cortex; but Barker & Ransom (1978) in studies on cultured mouse neurones found the sensitivities of the GABA and glutamate responses to pentobarbitone to be about equal.

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