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DISSIMILAR INFLUENCES OF SOME INJECTABLE ANAESTHETICS ON THE RESPONSES OF RETICULO-SPINAL NEURONES TO INHIBITORY TRANSMITTERS IN THE LAMPREY

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1 Intracellular recordings were made from identified bulbar reticulo-spinal neurones in the medulla of lamprey ammocoetes. Responses to iontophoretically applied inhibitory transmitters were measured as changes in membrane potential and input resistance.

2 Dose-dependent alterations in the responses to γ-aminobutyric acid (GABA) and glycine during bath application of injectable anaesthetic drugs were measured; the compounds used were pentobarbitone, ketamine, metomidate and the steroid mixture alphaxalone/alphadolone (Saffan).

3 GABA responses were potentiated by pentobarbitone ($1-3 \times 10^{-4}$ M) and prolonged by ketamine (3.7×10^{-5} M); but depressed by high concentrations (10^{-3} M) of all drugs, as well as by anaesthetic concentrations of alphaxalone ($1-3 \times 10^{-5}$ M).

4 Glycine responses were depressed by alphaxalone ($1-3 \times 10^{-5}$ M) and by supra-anaesthetic concentrations of ketamine (3.7×10^{-4} M) and metomidate (1.8×10^{-3} M). No drug potentiated the glycine responses.

5 In the absence of an effect common to the 4 anaesthetics, it is concluded that neither potentiation nor inhibition of all GABA or glycine responses is an essential feature of anaesthesia. However, effects comparable to those described here may contribute to the overall clinical picture during anaesthesia of higher vertebrates. The findings do not support the notion that all anaesthetic agents act on biological membranes by a single mechanism.

Introduction

Anaesthesia is thought to result from a reversible drug-induced disruption of brain function. Since Sherrington (1906) drew attention to the susceptibility of reflex arcs, attention has focussed on the synapse as the site of action of anaesthetic drugs. It has become apparent that anaesthetics differ in their actions at individual synapses (Larrabee & Posternak, 1952) and that different central nervous pathways have unequal sensitivities (Mark & Steiner, 1958). Indeed some such differential action is required to explain the characteristic clinical states which follow administration of the various drugs (Vickers, Wood-Smith & Stweart, 1978).

In this study we have compared the abilities of four injectable anaesthetic drugs to interfere with the actions of the inhibitory transmitters γ-aminobutyric acid (GABA) and glycine. The drugs were applied to bulbar reticulospinal neurones in the medulla of the lamprey ammocoete, cells readily identifiable and known to possess distinct GABA and glycine receptors (Martin, 1978; Matthews & Wickelgren, 1979). Anatomical and pharmacological analogies have been drawn between these cells and cells in the central nervous systems of higher vertebrates (Shapovalov, 1975; Martin, 1979a).

The anaesthetics, chosen to represent four classes of compound used in clinical anaesthetic practice, were bath-applied to the preparation in concentrations similar to those likely to obtain in the brain during clinical anaesthesia (Richards, 1972; Minchin, 1981). Although anaesthetics could equally interfere with synaptic transmission by a presynaptic action, we have concentrated on postsynaptic mechanisms, applying the putative transmitters directly to the cells and taking steps to preclude transsynaptic effects.

Methods

The preparation

Ammocoetes 8–15 cm long of the species *Lampetra fluviatilis* and *Lampetra planeri* were anaesthetized with neutralized tricaine methane sulphonate (100 μg/l) and then transected caudal to the gills. The rostral portion was divided in the ventral midline and, after tissues covering the ventral notochord had been removed, was pinned out on Sylgard in the experimental chamber, dorsal surface uppermost. A simple

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dissection exposed the bulbar reticulo-spinal cells, clustered on either side of the midline near the centre of the 4th ventricle (Figure 1). The preparation was continuously perfused with a cooled Ringer solution at 6 ml/min, and the recorded temperature in the chamber (capacity, 1 ml) maintained at 8–10°C by the circulation of cooled fluid through an outer jacket.

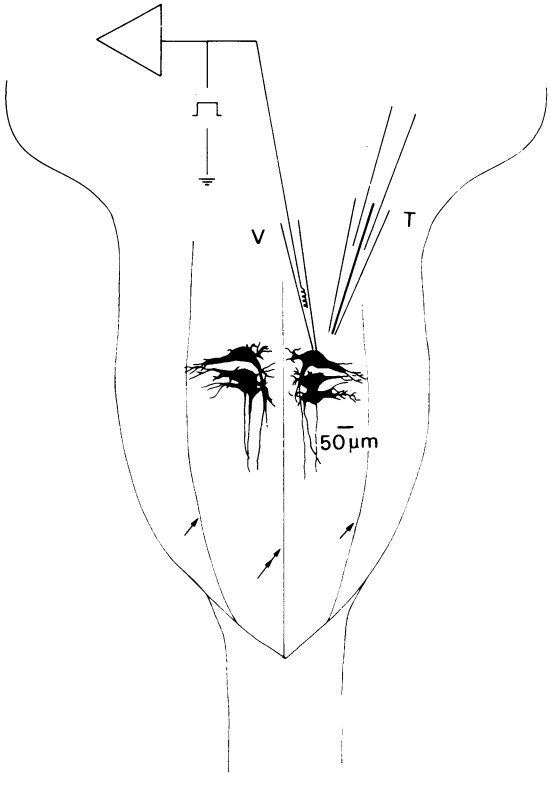

Figure 1 Diagram of the preparation showing the location of micropipettes. Bulbar reticulo-spinal cells lie in the floor of the 4th ventricle between the sulcus limitans of His (arrows) and the median longitudinal sulcus (double arrow) of the ammocoete larva. Positions of recording (V) and iontophoresis (T) electrodes are indicated.

Ringer solutions

The usual composition of the Ringer solution was (mM): NaCl 71, KCl 2.1, $CaCl_2$ 2.6, $MgCl_2$ 15, $NaHCO_3$ 20, NaH_2PO_4 0.18 and glucose 4.0. The pH was adjusted to 7.1–7.2 with HCl and the solution gassed with a mixture of CO_2 5% and O_2 95%. The high concentration of magnesium was used to block the release of endogenous transmitter.

Recording system

Standard intracellular recording techniques were used, with direct visual control of cell penetration. The recording electrode was connected by an Ag/AgCl wire to a high impedance pre-amplifier which incorporated a bridge balance circuit enabling current pulses to be injected for the measurement of input impedance. When filled with 2 M potassium methyl sulphate the glass micropipettes used had d.c. resistances in the range 20–30 MΩ. An Ag/AgCl reference electrode was connected to the bath.

Membrane potentials were displayed on a cathode ray oscilloscope (Tektronix 5113 Dual Beam Storage Oscilloscope) and a permanent record made by a high speed pen recorder (Lectromed MX212). A thermistor probe connected to the pen recorder provided a continuous record of the temperature in the experimental chamber.

Iontophoresis

Three-barrelled assemblies were used for iontophoresis. The micropipettes were back-filled with GABA (0.5 M, pH 2.5), glycine (0.5 M, pH 5.0), or NaCl (0.5 M). GABA and glycine were ejected as cations. NaCl was incorporated as a control in some experiments; in others, application of current pulses of reversed polarity served instead. In all experiments an Ag/AgCl indifferent electrode was placed in the bath for the return of iontophoretic current. Retaining currents were not routinely employed, any leakage of drug was insufficient to cause detectable changes in the membrane potential or resistance of the cells.

The ejection cycle was controlled by a microiontophoresis programmer (Model 160, W-P Instruments Inc.) driven by a Digitimer. The transmitters were applied alternately by currents of the same strength, usually in the range 50 to 90 nA. Pulses lasting between 0.3 and 7.0 s were applied at intervals of 1 or 3 min. The pulse duration was determined for each transmitter individually and then kept constant for the duration of an experiment.

In experiments testing the influence of transmitter concentration on the anaesthetic effect only one transmitter was used. To vary the agonist dose a cycle of iontophoretic pulses of differing duration but the same amplitude was employed. The quantity of drug ejected by an iontophoretic pulse is affected by the immediately preceding pulse, but the use of a fixed pulse sequence ensured that this factor was unchanged during an experiment. Differences between the amounts of the agonist delivered by the test pulses were therefore constant although unknown, so measurements of the responses are comparable but only within an experiment.

The effects of anaesthetics on transmitter responses

Anaesthetics were dissolved in the perfusing Ringer solution and applied to the preparation. No application was continued after 25 min; this limit allowed the responses to recover before cell deterioration supervened. The sequence of iontophoretic pulses continued during the perfusion with anaesthetic. Changes in input resistance associated with the transmitter actions were monitored by the injection of constant hyperpolarizing current pulses (strength 0.5–1.0 nA, frequency 1 Hz) whose duration was long (30 ms) in relation to the time constant of the cell (< 10 ms). Because of the possibility that anaesthetic drugs may interact (Richards & White, 1981), only one was tested on any individual preparation. Most experiments were terminated within 10 h of dissection.

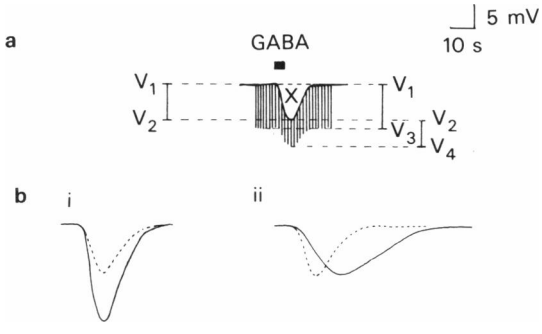

Figure 2 (a) Tracing of a typical response to an iontophoretic pulse of γ-aminobutyric acid (GABA). Vertical deflections (downward) from the resting membrane potential are in response to repeated injections of hyperpolarizing current pulses (1 nA, 30 ms). GABA was applied during the period indicated by the horizontal bar. Features measured: (1) peak change in membrane potential ($V_2 - V_1$), (2) membrane potential change in response to hyperpolarizing current pulses before ($V_3 - V_1$) and during ($V_4 - V_2$) action of GABA. The height of each vertical deflection is directly proportional to the input resistance. Because constant current pulses were used, the fractional change in input resistance at peak GABA effect is

$$\frac{(V_3 - V_1) - (V_4 - V_2)}{(V_3 - V_1)}.$$

(3) area (X) enclosed by the envelope of the membrane potential response. (b) Diagram to illustrate use of measurement of ratio area:peak. Control (- - -) and test (———) responses are superimposed. (i) Simple potentiation of membrane potential response. (ii) Alteration of response profile showing both delayed peak response and slowed recovery. In both (i) and (ii) the area of the response is increased from 9 mV.s (control) to 19 mV.s (test); but whereas in (i) the ratio remains 3.5 s, that in (ii) is increased from 3.5 s (control) to 7.2 s (test).

Drugs

The drugs used were GABA (Sigma), glycine (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.

Analysis of results

The responses to GABA and glycine were essentially similar. Numerical values were obtained for the peak resistance changes as indicated in Figure 2a. A videoplan (Reichert-Jung) was used to measure the time integral of the membrane potential response (area 'X'). Figure 2b shows how the ratio of this area to the peak hyperpolarization distinguishes between an increase in area due solely to an altered peak amplitude and one effected by prolongation of the response. The ratio was calculated in experiments in which pentobarbitone or ketamine were tested, because these drugs altered the GABA response profile.

So that the results of different experiments could be compared, the changes in GABA and glycine responses measured during exposure to anaesthetics were expressed as percentages. Student's *t* test was applied to these standardized data to assess the statistical significance of the anaesthetic effects.

Results

Responses to γ-aminobutyric acid and glycine

Most cells had resting potentials in the range −60 to −65 mV and input resistances > 3 MΩ. The usual response to application of either GABA or glycine was a transient hyperpolarization accompanied by reduction in the input resistance to the cell. A small minority of cells (4 out of 92) showed depolarizing responses to both amino acids. High concentrations of either agonist elicited biphasic hyperpolarizing/depolarizing responses and were therefore avoided.

Effect of anaesthetics on the responses to γ-aminobutyric acid and glycine

Sixty-three ammocoetes were exposed to the anaesthetics: pentobarbitone (19 ammocoetes), ketamine (15), alphaxalone (19), metomidate (10); 10 ammocoetes were tested with the Saffan vehicle alone. No anaesthetic consistently altered the mem-

Table 1 The effects of anaesthetics on the responses to γ-aminobutyric acid (GABA) and glycine

Anaesthetic	*Agonist*					
	GABA			*Glycine*		
	Resistance	*Area*	*Area/Peak*	*Resistance*	*Area*	*Area/Peak*
Pentobarbitone (M)						
10^{-3}	−63 ± 11 (2)	−90 ± 11 (2)		+8 (1)	+3 (1)	
$3-5 \times 10^{-4}$	+13 ± 5 (13)*	+25 ± 10 (11)*	+25 ± 7 (8)**	−15 ± 9 (9)	+4 ± 16 (8)	
10^{-4}	+9 ± 11 (6)	+36 ± 17 (8)	+71 ± 30 (8)*	+27 ± 34 (5)	−4 ± 26 (6)	
Ketamine (M)						
3.7×10^{-3}	−4 ± 15 (2)	+108 ± 49 (2)	+233 ± 0 (2)***	−82 ± 1 (2)**	−86 ± 2.5 (2)*	
3.7×10^{-4}	+16 ± 14 (9)	+124 ± 44 (10)*	+78 ± 17 (10)**	−33 ± 9 (9)**	−23 ± 13 (10)	−8 ± 12 (9)
3.7×10^{-5}	+10 ± 15 (8)	+55 ± 43 (8)	+29 ± 17 (8)	−2 ± 9 (10)	−5 ± 11 (10)	+10 ± 8 (10)
Alphaxalone (M)						
$1-3 \times 10^{-4}$	−58 ± 17 (6)*	−69 ± 30 (4)		−39 ± 9 (4)*	−45 ± 20 (5)	
$1-3 \times 10^{-5}$	−56 ± 10 (8)***	−74 ± 5 (8)***		−36 ± 8 (7)**	−49 ± 11 (7)**	
$1-3 \times 10^{-6}$	−15 ± 4 (3)	−22 ± 11 (2)		−5 ± 5 (6)	+22 ± 18 (3)	
Saffan vehicle	−23 ± 6 (10)**	−21 ± 9 (10)*		−19 ± 13 (6)	0 ± 7 (6)	
Metomidate (M)						
1.8×10^{-3}	−100 ± 0 (3)***	−100 ± 0 (3)***		−73 ± 15 (6)**	−77 ± 17 (6)**	
1.8×10^{-4}	−27 ± 12 (5)	−1 ± 17 (5)		−6 ± 10 (4)	+28 ± 23 (4)	
1.8×10^{-5}	−6 ± 5 (6)	+6 ± 7 (6)		+5 ± 9 (7)	+11 ± 12 (7)	

Saffan vehicle dilution equivalent to 10^{-5}–10^{-4} M alphaxalone.
Tabulated figures are percentage changes in response during application of anaesthetic: mean ± s.e. Numbers of observations in parentheses.
Significant effects indicated (*t* test): *$P<0.05$; **$P<0.01$; *** $P<0.001$.

brane potential and only the highest concentrations of pentobarbitone and metomidate (10^{-3} M) affected the input resistance, causing a small reduction. The alterations in the GABA and glycine responses measured during exposure to the four anaesthetics are displayed in Table 1.

Most experiments were carried out at 8–10°C but the results were qualitatively similar at room temperature and at 2°C (not illustrated).

Pentobarbitone

The effects of pentobarbitone were complex and dose-dependent. Records from typical experiments are displayed in Figure 3. Low concentrations ($1-5 \times 10^{-4}$ M) potentiated the GABA responses in some cells, but only in 8 out of 19 tests did the potentiation exceed 10%. The remaining 11 tests showed little change. Potentiated responses were increased both in amplitude and duration (Table 1). In two preparations the concentration was raised to 10^{-3} M: this caused a reduction in amplitude of the GABA response.

No concentration of pentobarbitone significantly affected the glycine responses (Table 1, and see Figure 3).

Ketamine

Ketamine selectively potentiated the GABA re-

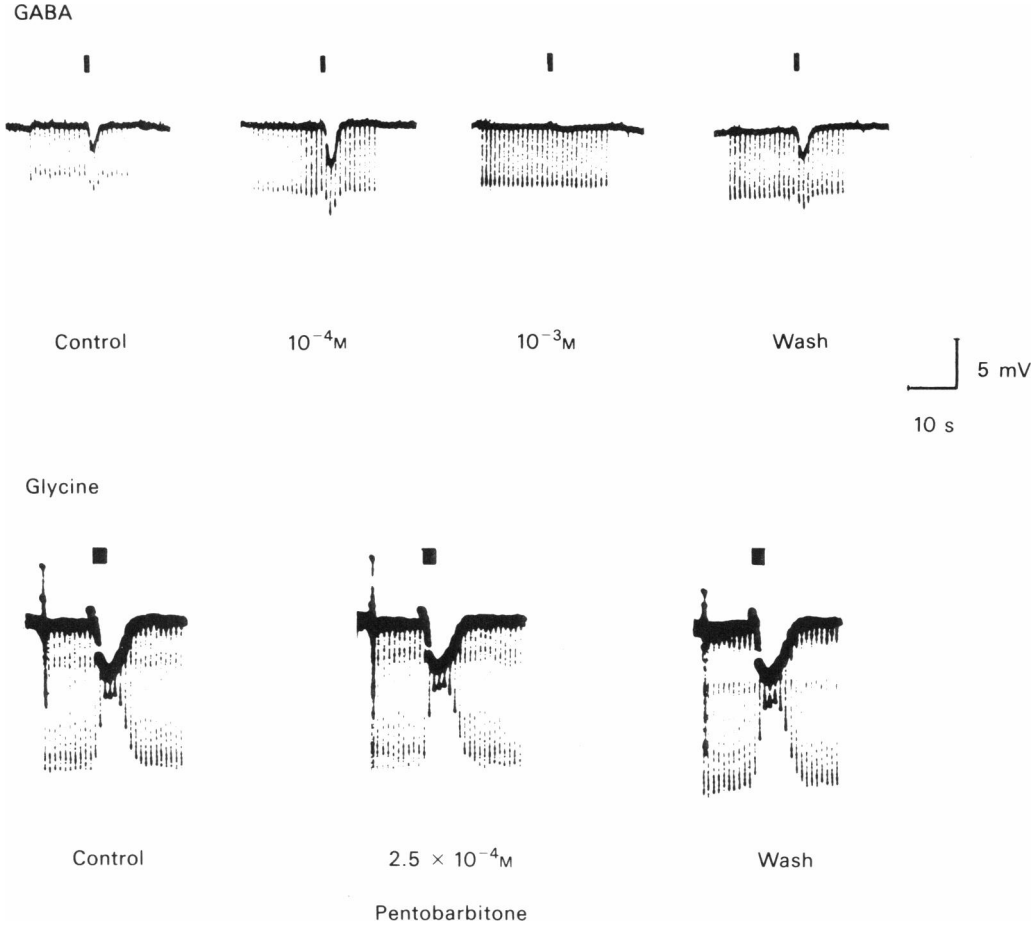

Figure 3 Effects of pentobarbitone on γ-aminobutyric acid (GABA) and glycine responses. Records from 2 cells showing: (upper traces), GABA potentiation by 10^{-4} M pentobarbitone and antagonism by 10^{-3} M pentobarbitone (no intervening wash); (lower traces) lack of effect of 2.5×10^{-4} M pentobarbitone on glycine responses. Durations of exposures to pentobarbitone: (upper trace) 13 min (10^{-4} M), 6 min (10^{-3} M); (lower trace) 14 min. Preparations washed for 8 min (upper trace), 14 min (lower trace). Iontophoretic pulses: GABA 0.5 s, 60 nA; glycine 3 s, 200 nA. Hyperpolarizing current pulses: 1 nA (upper traces), 0.7 nA (lower traces).

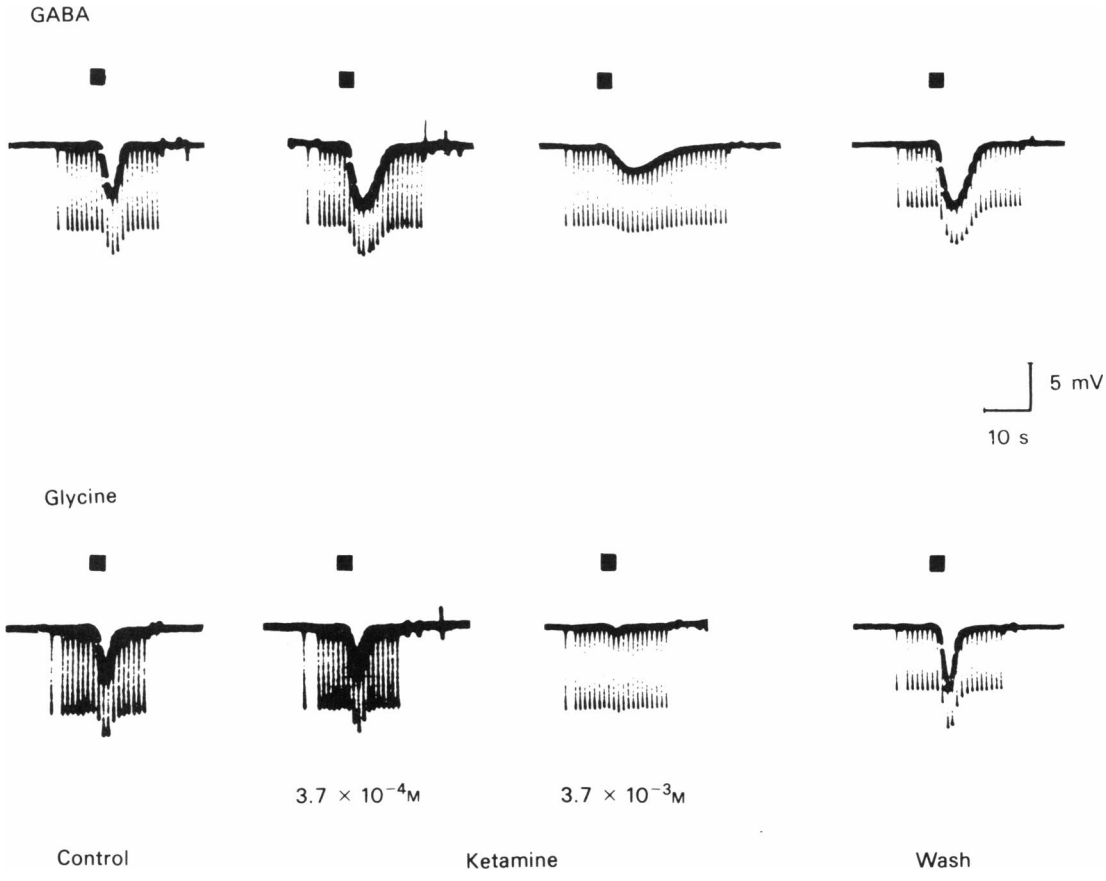

Figure 4 Effects of ketamine on γ-aminobutyric acid (GABA) and glycine responses. Records from 1 cell showing GABA potentiation by 3.7×10^{-4} M ketamine and complex effect of 3.7×10^{-3} M (no intervening wash). In this cell glycine responses were unaffected by 3.7×10^{-4} M ketamine but reversibly depressed by 3.7×10^{-3} M. Duration of exposure to ketamine: 8 min (3.7×10^{-4} M), 10 min (3.7×10^{-3} M). Preparation washed for 10 min (upper trace), 11 min (lower trace). Iontophoretic pulses: GABA and glycine 3 s, 60 nA. Hyperpolarizing current pulses: 1 nA.

sponses (Figure 4), an effect consistently detectable at 3.7×10^{-5} M and reaching statistical significance at 3.7×10^{-4} M Ketamine (Table 1). Unlike the potentiation by pentobarbitone, that due to ketamine was almost entirely attributable to prolongation of the response, as is shown by the respective area/peak ratios. Slowing of the onset and offset of the GABA response was most striking at high concentrations of ketamine (3.7×10^{-3} M).

In contrast, Table 1 records a dose-dependent antagonistic action of ketamine on glycine responses. This effect was statistically significant at 3.7×10^{-4} M when the response was measured as the peak change in resistance. Only two cells were tested with ketamine at 3.7×10^{-3} M but in both cases depression of the response was dramatic. No effect of ketamine on the response profile was detected for glycine (e.g. Figure 4).

Alphaxalone

The action of alphaxalone ($> 10^{-5}$ M) was consistently to depress responses to both GABA and glycine (Table 1, and see Figure 5). The commercial vehicle, which contains poly-oxyethylated castor oil, had an antagonistic action in some experiments (7 out of 10 tests using GABA and 3 out of 6 with glycine showed a reduction in response amplitude which exceeded 10%). This latter effect was small, and statistically significant only in the case of resistance measurements on GABA responses.

Metomidate

Metomidate, too, had a dramatic depressant action on both GABA and glycine responses (Figure 6), but only at high concentration (1.8×10^{-3} M). There was

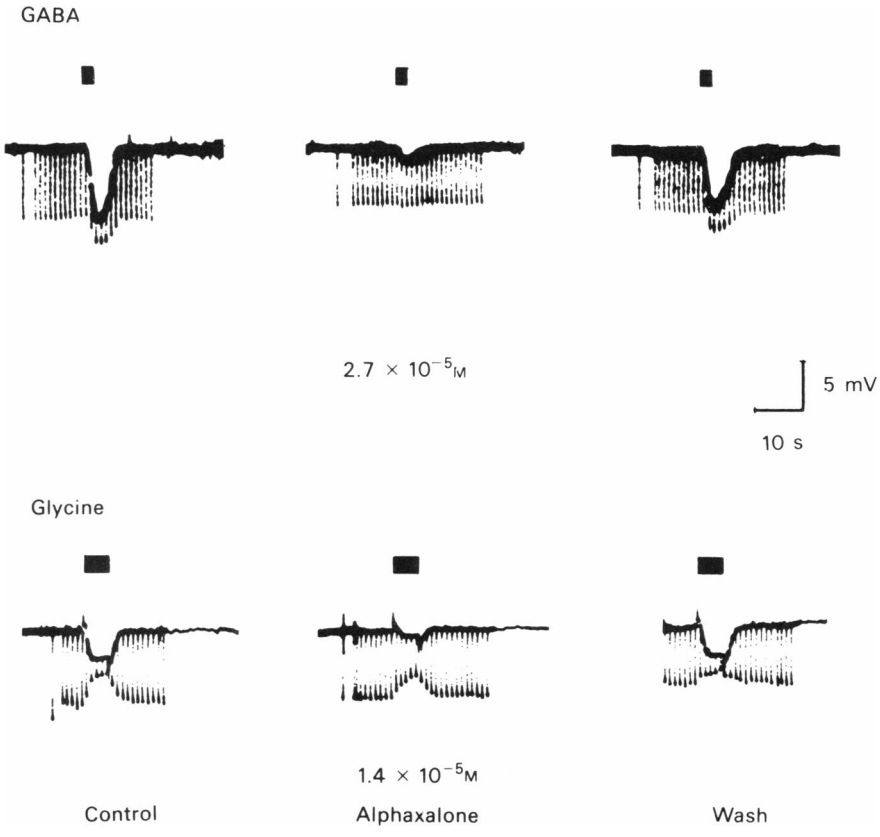

Figure 5 Effect of alphaxalone on γ-aminobutyric acid (GABA) and glycine responses. Records from 2 cells showing reversible depression of GABA (9 min exposure to 2.7×10^{-5} M alphaxalone) and of glycine responses (8 min exposure to 1.4×10^{-5} M). Preparations washed for 22 min (upper trace), 8 min (lower trace). Iontophoretic pulses: GABA 2 s, 30 nA; glycine 5 s, 500 nA. Hyperpolarizing current pulses: 1 nA in upper traces, 0.9 nA in lower traces.

no evidence of potentiation at any concentration (Table 1), nor of alteration in the response profile.

The effect of agonist dose on the modulating influence of anaesthetics

It was recognized that the agonist concentration at the receptors might affect the recorded influence of the anaesthetic drugs. Experiments were therefore performed in which different doses of a single transmitter were applied to a cell.

The same anaesthetic-transmitter combinations as previously described were tested in these experiments, each combination receiving 2–8 tests in between 2 and 4 preparations. Only the potentiation effects were consistently affected by transmitter dose, this being marked particularly with pentobarbitone, which only consistently potentiated GABA responses when short iontophoretic pulses were used. Measurements of peak change in input resistance obtained in one such experiment are shown in Figure 7. In this particular experiment 10^{-4} M pentobarbitone approximately doubled the response to a 0.5 s GABA pulse, whereas the response to a 2 s pulse was increased by only about a quarter. Higher concentrations of pentobarbitone caused a reversible loss of this effect.

Discussion

The lamprey as a model for the study of the effects of anaesthetics

The lamprey brainstem has several features that make it a suitable preparation in which to study the neuropharmacology of anaesthetic drugs. Intracellular recording from identified neurones is practicable and enables changes in input resistance and membrane potential to be measured. This is preferable to

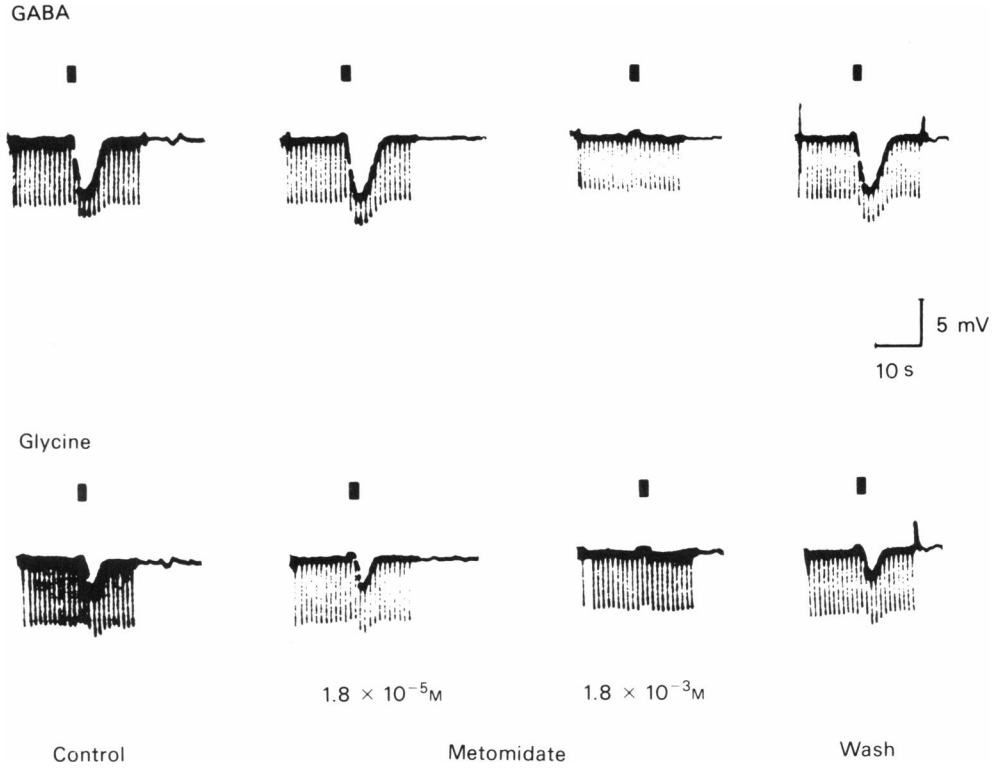

Figure 6 Effect of metomidate on γ-aminobutyric acid (GABA) and glycine responses. Records from 1 cell showing lack of effect of 1.8×10^{-5} M metomidate on GABA and glycine responses. Antagonism by 1.8×10^{-3} M metomidate is total in both cases. Duration of exposures to metomidate (no intervening wash): 10 min (1.8×10^{-5} M), 5 min (1.8×10^{-3} M). Preparation washed for 18 min (upper traces), 17 min (lower trace). Iontophoretic pulses: GABA and glycine 2 s, 300 nA. Hyperpolarizing current pulses: 1 nA.

the use of indirect measures of cell responses, such as changes in spike frequency. The bulbar reticulospinal cells lie close to the surface of the 4th ventricle, covered only by a single layer of ependymal cells; thus the diffusion path for drugs applied in the perfusing Ringer is short (Martin, 1979a, b). In the lamprey brain, blood vessels are confined to the periphery and so neither obscure nor impede access to the cells; visualization is also aided by the absence of myelin.

The neuro-biology of the lamprey has been studied in some detail (for review see Rovainen, 1979). Wickelgren (1977) described diverse sensory inputs to the bulbar neurones and reported that these cells constitute a major motor outflow to the somatic musculature. Any stimulation of the neurones by anaesthetics, effected either directly or via the removal of inhibitory influences, should therefore elicit movements. A similar result is predicted in higher vertebrates, where analogous cells are found (Shapovalov, 1975).

Anaesthetics differ in their postsynaptic effects

We set out to compare the abilities of four anaesthetic drugs to interfere with the responses of reticulospinal neurones to the inhibitory amino acid transmitters GABA and glycine. Many workers have stressed the GABA-potentiating properties of the barbiturates in a range of preparations (e.g. Barker & Ransom, 1978). In our study, as in that of Bowery & Dray (1978), the GABA-potentiating action of pentobarbitone was evident only in a proportion of cells, and showed marked dependence on the agonist dose, being detectable only if low amplitude or short iontophoretic currents were employed.

Ketamine also was found to potentiate GABA responses selectively, but the effect differed from that of pentobarbitone in that it was principally on the time course of the response; both the onset and offset of the GABA response were slowed by ketamine ($> 10^{-4}$ M). Other studies, e.g. Minchin (1981), suggest that this potentiation is not the re-

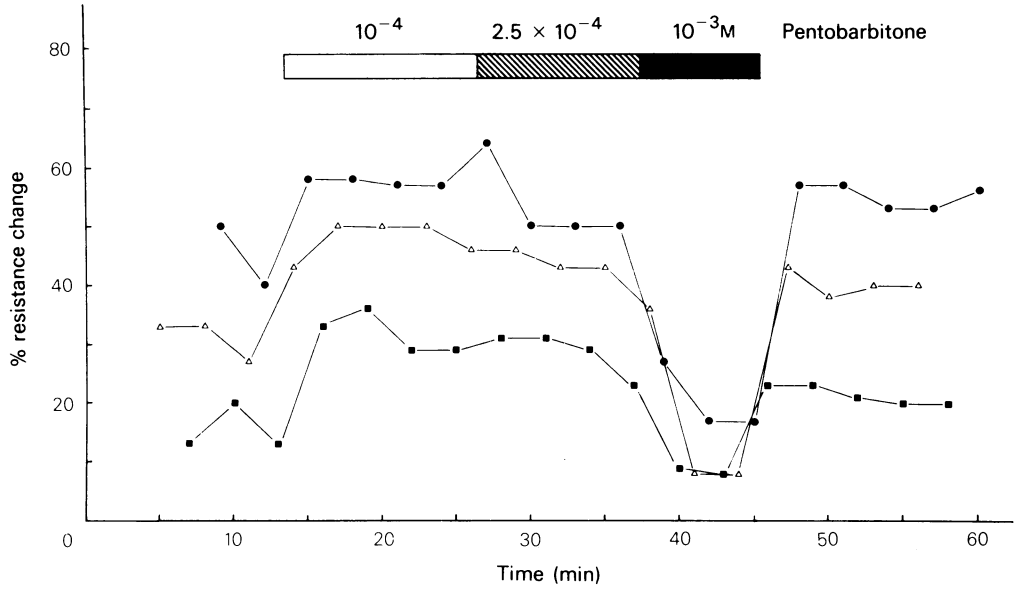

Figure 7 γ-Aminobutyric acid (GABA)-induced resistance changes measured in a single cell before, during and after application of increasing concentrations of pentobarbitone. Ordinate scale: GABA-induced resistance change (%); abscissa scale: time after pulse sequence started (min). GABA pulses (amplitude 60 nA) applied for 0.5 s (■), 1.0 s (△) and 2.0 s (●) in repeated sequence. Pentobarbitone 10^{-4} M approximately doubled the response to a 0.5 s GABA pulse whereas the response to a 2.0 s pulse increased by only about a quarter. Higher concentrations of pentobarbitone reversibly eliminated the potentiation.

sult of altered GABA uptake mechanisms. We are unaware of any other studies using ketamine that are directly comparable with ours.

In contrast, neither alphaxalone nor metomidate caused significant potentiation of GABA responses at any concentration tested; instead, both drugs produced parallel depressions of the responses to GABA and glycine. There are no previous reports of the effects of either anaesthetic on responses to the inhibitory transmitters; but etomidate, an imidazole derivative closely related to metomidate, has been studied: it is reported to be similar in action to pentobarbitone, selectively potentiating GABA responses at low doses (10^{-5} M) but having a depressant effect at high doses (10^{-3} M). As with the barbiturates, the potentiating action has been reported to show stereo-specificity (Hill & Taberner, 1975; Huang & Barker, 1980). We have not tested the optical isomers of metomidate separately, but those of etomidate have been shown to differ in their pharmacological actions (Hill & Taberner, 1975).

Although published information about the actions of alphaxalone/alphadolone on inhibitory mechanisms is lacking, Pennefather & Quastel (1980) described alterations in endplate currents recorded at the neuromuscular junction; and Smaje (1976) and Richards & Smaje (1976) reported depressed responses to acetylcholine and glutamate in the olfactory cortex.

Relevance to clinical anaesthesia

Although the reticulo-spinal cells are not concerned with consciousness, the effects we have described are likely to operate during anaesthesia and may be related to side effects produced by the drugs. Our results are hard to reconcile with a unitary biophysical hypothesis of anaesthetic action. Such a hypothesis predicts similarity of action of different anaesthetic compounds (Metcalfe, Hoult & Colley, 1974), however exerted, and this we have not observed. The concentrations at which the anaesthetics have been tested in this study include those likely to obtain in the brain during clinical anaesthesia (Table 3), and were themselves capable of inducing anaesthesia in lamprey ammocoetes (Table 2, and see appendix).

Our finding that neither GABA nor glycine responses were potentiated by alphaxalone and metomidate lends no support to the contention that inhibitory mechanisms are enhanced during anaesthesia. However, that idea derives largely from studies in which barbiturates or volatile anaesthetic agents have been used (e.g. Eccles, Schmidt & Willis,

Table 2 The effects of injectable anaesthetic drugs when bath-applied to intact lampreys

Drug	*Concentration* (M)	*Stage of anaesthesia reached after various time intervals*			
		2 min	5 min	10 min	30 min
Pentobarbitone	10^{-3}	—	—	I	I
Ketamine	3.7×10^{-5}	—	I	II–1	II–1
	3.7×10^{-4}	I	II–1	II–2	II–2
	3.7×10^{-3}	II–1	II–2	II–2	III/IV
Alphaxalone	2.7×10^{-6}	—	—	I	II–2
	2.7×10^{-5}	II–1	II–2	II–2	
	2.7×10^{-4}	II–2	III	III/IV	
Metomidate	1.8×10^{-5}	—	—	I–2	I–2
	1.8×10^{-4}	—	II–1	II–2	II–2
	1.8×10^{-3}	II–2	II–2	II–2	

Drugs dissolved in river water (unbuffered); times measured from moment of transfer of ammocoetes to anaesthetic solution. Classification of anaesthesia after McFarland (1959), tabulated stages are the averages for the group (3–8 ammocoetes).

1963), and those results are not contradicted by our experiments. There is no evidence that GABA and glycine receptors on the reticulo-spinal cells of the lamprey differ pharmacologically from those in higher vertebrates (Martin, 1979a; Nistri & Constanti, 1979).

Only with alphaxalone was depression of GABA and glycine responses seen at concentrations likely to be encountered during clinical anaesthesia in fish (Table 3) and higher vertebrates. Should a comparable depression of inhibitory mechanisms underlie the excitatory phenomena commonly observed during anaesthesia with alphaxalone/alphadolone in mammals, a logical preventive treatment would be premedication with a GABA-potentiating agent. It is interesting that there are clinical reports of a reduced incidence of tremors and purposeless movements when diazepam is used as a pre-medicant before anaesthesia with etomidate (Holdcroft, Morgan, Whitwam & Lumley, 1976) or alphaxalone/alphadolone (Vickers *et al.* 1978). The benzodiazepines as a group are known to potentiate the actions of GABA (Curtis, Lodge, Johnston & Brand, 1976).

This work was made possible by the generous support of the Wellcome Trust.

Appendix

Abilities of the drugs to anaesthetize lampreys (Table 2)

Experiments were carried out to ensure that the drugs used in the iontophoresis experiments were capable of inducing anaesthesia in intact lampreys. These experiments give an indication of the concentrations required but do not provide an accurate estimate of the concentration any anaesthetic reached in the brain. The behaviours of groups of 3 ammocoetes immersed in anaesthetic solutions were compared with that of an untreated group. Anaesthesia was assessed according to the criteria of McFarland

Table 3 Estimated brain concentrations of anaesthetics during clinical use in fish

Anaesthetic	*Dose*	*Brain Conc.* (M) (estimate)	*Effect on fish*	*Ref.*
Pentobarbitone	30 mg/kg i.p.	1.1×10^{-4}	Sedation	1
	48 mg/kg i.p.	1.8×10^{-4}	Apnoea	1
Alphaxalone	18 mg/kg i.p.	8.1×10^{-5}	Surgical anaesthesia	1
Ketamine	130 mg/kg i.m.	9.5×10^{-5}*	Surgical anaesthesia	1
Metomidate	7.5 mg/l (bath)	2.8×10^{-5}*	Surgical anaesthesia	2

*Upper limit. Ref. 1 = Oswald (1978), 2 = Stuart (1981).
Assumptions(Minchin, 1981): rapidly acting compounds (alphaxalone, metomidate) distributed in E.C.F. (= 30% body weight); slowly acting compounds (pentobarbitone, ketamine) distributed in total body water (= 66% body weight); protein binding 40% for alphaxalone and pentobarbitone.

(1959). The anaesthetics were dissolved in river water to the same concentrations as were employed in the iontophoresis experiments and each drug was tested on a minimum of 6 ammocoetes. Most of the experiments were conducted at room temperature (water temperature 15–17°C); but alphaxalone was also tested at 4°C, with similar results. It is recognized that both the rate of induction and the depth of anaesthesia in fishes are affected by temperature, the induction time commonly being shorter at higher temperature (discussed by McFarland, 1959).

Ketamine, alphaxalone and metomidate, when used at concentrations that affected the transmitter responses, induced similar behavioural states that were comparable to those McFarland (1959) reported in fish. A transient period of intense hyperactivity immediately after the introduction of high concentrations of metomidate or ketamine was attributed to the acid reaction of these solutions. A similar reaction of fish to the anaesthetic tricaine methane sulphonate has been described (e.g. Ohr, 1976). No such hyperactivity was noted following the introduction of pentobarbitone or alphaxalone.

The anaesthetic effect of alphaxalone ($> 10^{-5}$ M) stood out because of the rapidity of its onset coupled with the slow recovery; after the ammocoetes were removed from 2.7×10^{-4} M alphaxalone to fresh water it was an hour before there was slight movement in response to tail squeezing. During recovery there was a period when non-propulsive and rolling movements were observed, but frank convulsions were not elicited. In contrast, the lampreys were swimming within 30 min of their removal from 10^{-3} M metomidate. Recovery from high concentrations of ketamine was also slow, but was quiet in comparison to that of ammocoetes exposed to 3.7×10^{-5} M ketamine which was characterized by a period of apparent excitement.

The finding that bath application of even a high concentration of pentobarbitone induced only mild sedation, is consistent with reports that bath application of the barbiturates is an unsatisfactory method of anaesthetizing fis(see McFarland, 1959).

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