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Spinal effects of four injectable anaesthetics on nociceptive reflexes in rats: a comparison of electrophysiological and behavioural measurements

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1 To assess the direct spinal contributions to the depression of reflexes caused by general anaesthetics, the intravenous potency of four injectable anaesthetics has been compared in two preparations: in decerebrate, spinalised rats, using a novel preparation requiring little surgical intervention, and in intact rats with chronically implanted i.v. cannulae.

2 Methohexitone (1–8 mg kg^{-1} i.v.), alphaxalone/alphadolone (0.5–8 mg kg^{-1} i.v.), alpha-chloralose (20–80 mg kg^{-1} i.v.) and ketamine (0.5–16 mg kg^{-1} i.v.) all produced a dose-dependent depression of single motor unit activity evoked by controlled noxious mechanical stimuli in decerebrate, spinalised animals.

3 The sedative and motor effects brought about by equivalent doses to those used in the electrophysiological experiments were assessed in intact rats. Methohexitone, alphaxalone/alphadolone and alpha-chloralose all caused similar levels of behavioural sedation at the doses that caused depression of spinal reflexes. Ketamine required relatively much higher doses to cause sedation.

4 To determine whether background anaesthesia modulated the potency with which these compounds affected spinal reflex activity, depressant effects in decerebrate, unanaesthetized rats were compared with those in animals maintained under anaesthesia with either alpha-chloralose or the steroid mixture of alphaxalone/alphadolone. The presence of either of these two agents as maintenance anaesthetics did not influence the effectiveness with which other compounds depressed nociceptive responses. However, additional doses of the maintenance anaesthetics were less effective than the same doses tested in decerebrate animals.

5 All the anaesthetics tested produced a significant depression of spinal reflex responses to noxious stimuli at doses well below those required for anaesthesia. Whilst the presence of maintenance anaesthetics appears not to distort pharmacological tests of other agents, there may nonetheless be a biasing of the samples of cells recorded.

Introduction

The group of compounds collectively known as general anaesthetics act to produce a widespread depression of the central nervous system involving hypnosis, analgesia, reflex suppression and relaxation of voluntary muscle. For each agent, the relative magnitudes of these effects are unique and vary with the level of anaesthesia.

It has been shown that anaesthetics exert direct influences on the spinal cord, on both dorsal and ventral horn neurones, so as to modulate spinal function in response to sensory stimuli (for reviews see Heavner, 1975; Davidoff & Hackman, 1983; see also Lodge & Anis, 1984). Although some similarities in modulation of transmitter action may be observed between compounds (e.g. alphaxalone/alphadolone, chloralose and barbiturates have all been shown to modulate the actions of γ-aminobutyric acid (Davidoff & Hackman, 1983; Lambert *et al.*, 1987)), clinical and behavioural observations of anaesthesia indicate that there are marked differences even within such groupings.

Despite the potential influences these compounds have on the central nervous system, the majority of electrophysiological studies concerned with spinal processing of sensory stimuli *in vivo* have been undertaken in the presence of one or several of a wide variety of gaseous and injectable anaesthetics. In view of this, it was the intention in this study to generate information relevant to three questions. Firstly, what are the relative potencies of various injectable anaesthetics within the spinal cord in depressing spinal nociceptive reflexes? Secondly, what is the relationship between behavioural sedation induced by injectable anaesthetics of various classes and spinal depression of nociceptive reflexes? Thirdly, to what extent are different maintenance anaesthetic regimes likely to affect the results obtained in unitary pharmacological tests of spinal function when performed *in vivo*?

A model was developed which requires little surgical intervention compared with more standard electrophysiological measurements of spinal function and which allowed continuous, stable recording of the reflex activity of single motoneurones to controlled noxious stimuli. This preparation reduces the influence that surgery has on the potencies with which anaesthetics depress spinal reflexes (Hartell *et al.*, 1990) and enabled comparisons to be made between different compounds in the same animal under the same conditions. Such results were compared with the behavioural effects produced by equivalent doses administered to conscious animals chronically prepared with intravenous cannulae.

Methods

Preparation of animals for electrophysiological recording

Surgery was performed, under halothane anaesthesia, on 62 male Wistar rats weighing between 250 and 400 g; respiration was spontaneous throughout and inspired air was supplemented with oxygen. Cannulae were inserted into the trachea,

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carotid artery and jugular vein. A small incision was made in the low thoracic region and the musculature covering the dorsal surface of the spinal cord between the thoracic 9–11 vertebrae retracted. One or two laminae were removed and the spinal cord transected at the level of thoracic 9–10 vertebrae. All wound surfaces were covered throughout with lignocaine (1%) plus adrenaline (1:200,000) and the skin incisions repaired with sutures.

Rectal temperature was monitored continuously and maintained at 37°C with the aid of a feedback controlled blanket and a dorsally positioned lamp. Blood pressure was also monitored and used as an indication of technical acceptability. Experiments were terminated if the systolic pressure fell below 100 mmHg for any sustained period.

Animals were then prepared according to one of three experimental protocols. One group was decerebrated under halothane anaesthesia by aspiration of all cranial contents rostral to a mid collicular level, and anaesthesia discontinued. A second group was given a $50\,mg\,kg^{-1}$ i.v. dose of alpha chloralose (dissolved in either a borax buffered solution or in isotonic saline) and the halothane discontinued. Supplementary doses of $20\,mg\,kg^{-1}$ i.v. were given at intervals of approximately one hour. Rats in the third group were anaesthetized with a continuous infusion of alphaxalone/alphadolone (6–$12\,mg\,kg^{-1}$ i.v.) and the halothane discontinued. The right hind limb was immobilised in a plaster of Paris cast and a period of at least one hour allowed before recording, so as to allow recovery from the effects of halothane and to permit recovery from the spinalisation. Fluid, either isotonic saline or Haemaccel (Hoechst), was given over the course of the experiment at a rate of approximately $80\,ml\,kg^{-1}\,24\,h^{-1}$. In some cases, particularly with decerebrate animals, 1–2 ml of whole blood was given postoperatively.

Electrical activity was recorded from hind limb flexor muscles by tungsten microwires embedded in a needle. The wires were 50 μm in diameter with a Teflon coating making the total diameter 75 μm. Electrodes were inserted through a small skin incision into either biceps femoris or, more usually, into flexor digitorum longus or flexor hallucis longus. Single motor unit responses were evoked by application of noxious pinch stimuli to the receptive field on the ipsilateral hind limb. Receptive fields were similar between animals and invariably encompassed the first and second most lateral toes and extended towards the hock. Stimuli were delivered by means of a pair of electronically controlled pneumatically driven Allis tissue forceps (Brown *et al.*, 1984) and were applied for a period of fifteen seconds at intervals of three minutes. Although the force of the stimulus applied to the limb was not measured directly, it was applied at similar pneumatic pressures between animals. Units were selected which responded with minimal adaptation both over the course of each stimulus and between successive stimuli.

Single motor units were discriminated according to spike height; each spike was delayed and displayed on an oscilloscope so that spike configuration could be monitored, thereby ensuring single unit recording over the entire experiment. Chart records were made of the number of spikes per second and of counts of the number of spikes during various parts of the stimuli. Spike counts over a period of 'early pinch', the first five seconds, were separated from those over the subsequent ten seconds ('late pinch'). It is presumed that the early phase of the pinch contains a considerably greater rapidly adapting, low threshold component. Most cells showed adaptation over this period and drugs affected this to a lesser extent. Therefore, quantitative analysis was restricted to the period of late pinch. A microcomputer was used for the online calculations of drug effects (Headley *et al.*, 1985); these were expressed as percentages of the mean of three stable control responses before drug administration. Results were only considered to be technically acceptable if the recovery from the maximum drug effect exceeded at least 50% of the reduction and the time course of recovery matched that expected for the known kinetics of the compound under study. The only exception to this was for alpha-chloralose which has too long-lasting an action for this criterion to be feasible.

Behavioural methods

Indwelling intravenous cannulae were implanted in sixteen male rats under halothane anaesthesia. Polythene tubing (i.d. 0.58, o.d. 0.96 mm), prefilled with sterile isotonic saline, was inserted aseptically through a small ventral incision, into the right jugular vein via a small side branch. The free end was passed under the skin and out through a dorsal incision at the back of the neck. Animals were allowed to recover from the surgery for at least 96 h before experimentation. They were housed individually and were given food and water *ad libitum*. To reduce the number of animals required, several drugs were tested on each animal. To minimize possible interactions between compounds tested, different drugs were tested on different days and the order of testing was rotated. All experiments were carried out in a quiet room free from interruption.

Following intravenous administration of the various compounds, several simple behavioural activities were assessed. These included the presence or absence of a righting reflex, and arbitrary scales of the degree of ataxia produced. From these observations, several estimations of drug-induced motor impairment were made. These were firstly, the dose required to produce a minimal ataxia in at least 75% of the animals (defined as the inability of the animal to walk on the rim of its cage), secondly the dose required to produce maximal ataxia (seen as an inability to walk on a flat surface), thirdly, the dose required to eliminate the righting reflex in at least 75% of the animals and, finally, the loss of spontaneous movements such as blinking and whisker twitch. Complete recovery of motor coordination was monitored.

Drugs and administration protocol

In order to allow comparisons between electrophysiological and behavioural experiments, the same drugs and administration procedures were used. The following compounds were selected for study: methohexitone (Eli Lilly), a short acting barbiturate; ketamine hydrochloride (Parke-Davis), a dissociative anaesthetic; the steroid mixture of alphaxalone/alphadolone (Saffan; Pitman-Moore) and alpha-chloralose (Sigma). All doses were administered in logarithmic (base 2) increments starting with doses found to be near threshold for effects on most cells in the electrophysiological tests. Those compounds with a short time course (methohexitone and alphaxalone/alphadolone) were administered as discrete doses at intervals appropriate for complete recovery judged according to both observation and their reported elimination kinetics. Ketamine and alpha-chloralose produce longer-lasting effects and so were given in a cumulative regime at intervals of 6 and 15 min, respectively (chloralose has been shown to have delayed onset: Collins *et al.*, 1983); this regime was halted once the evoked responses had been reduced to about 25% of control or less. The effects of alpha-chloralose last for hours, so in the acute experiments cumulative doses were given only as the last drug to be tested, and recovery was not followed. This regime of drug administration was necessary in the electrophysiological experiments to allow several drugs to be tested on each animal, thereby allowing both direct comparisons to be made between compounds and enabling numbers of experiments to be kept to a minimum. Since complete dose-response regimes were not performed on all units tested, the traditional mean dose-response analysis was not possible. As higher doses of drug were only tested on those units which were more resistant, the apparent dose-response curves for the collected data are more shallow than expected (see Parsons & Headley, 1989).

As it was the aim of the behavioural experiments to study the effects of doses which caused known degrees of depression

of spinal reflexes in the electrophysiological experiments, the same doses were used. As can be seen in the results section, this led to rather large steps in behavioural effects; in some cases a single dose increment caused effects that spanned 2–3 of the behaviourally defined states.

Results

Comparison of the depression of spinal reflexes caused by anaesthetics under decerebrate and different baseline anaesthetic protocols

The potencies of the compounds used in this investigation in suppressing spinal reflexes were compared in three groups of animals, namely those anaesthetized with either alphaxalone/alphadolone (23 rats) or with alpha-chloralose (21 rats), and a group of unanaesthetized decerebrate rats ($n = 18$). Dose-response relationships were obtained for alpha-chloralose (20–80 mg kg^{-1} i.v.), methohexitone (1–8 mg kg^{-1} i.v.), alphaxalone/alphadolone (0.5–8 mg kg^{-1} i.v.) and ketamine (0.5–16 mg kg^{-1} i.v.) in each of the experimental groups.

Figure 1 provides examples of the effects of two different anaesthetics on single motor unit responses to noxious pinch stimuli, recorded from two decerebrate, unanaesthetized animals. The top trace reveals that methohexitone depressed this 'naturally'-evoked response in a dose-dependent and rapidly reversible manner; the recovery seen between doses corresponds to that expected for this rapidly metabolised barbiturate. Alpha-chloralose also produced a dose-dependent depression of spinal reflex activity, but recovery was not followed because of the long acting nature of the drug.

In order to allow quantification of data and subsequent comparative analysis, the effects of each dose of drug were measured and the data subsequently pooled for the units in each of the three experimental groups: the calculation of reductions was the percentage of the control number of spikes evoked during late pinch. Wherever practicable, increasing doses of the drugs were administered until a dose was reached at which evoked activity was reduced to below 25% of control. However, as is often the case with this kind of *in vivo* pharmacological study, there was much variation in drug potency even between animals prepared in the same way. In order to illustrate the collected data between the three groups, the results are displayed in bar chart form. Figure 2 is an example showing the relative effectiveness of methohexitone and ketamine. Neither of these compounds displayed any preferential potency between the three experimental groups; this was confirmed statistically by use of both non-parametric (Mann-Whitney U-test) and parametric (Student's t) tests).

Such equipotency of drug action between decerebrate and anaesthetized animals was characteristic, except for those instances in which the drug under test was also being used as the maintenance anaesthetic. Figure 3 is a firing rate record of a single motor unit responding to pinch stimuli which were uniform throughout, and shows the depression of reflex activity caused by alphaxalone/alphadolone under two different baseline anaesthetic conditions. The same dose of alphaxalone/alphadolone had less effect on the reflex whilst the animal was maintained on alphaxalone/alphadolone than when the background anaesthetic was subsequently switched to alpha-chloralose. Note that the firing rates evoked during the stimuli before testing were similar in the two cases.

This phenomenon was also seen when the pooled data between the groups were compared. Figure 4 shows bar charts of the dose-dependent effects of alphaxalone/alphadolone and alpha-chloralose. Figure 4a shows that the same doses of alphaxalone/alphadolone (0.5–8 mg kg^{-1} i.v.) were significantly less effective when given to animals already anaesthetized with alphaxalone/alphadolone than when administered to chloralose anaesthetized or decerebrate animals. This suggests that the presence of chloralose as a

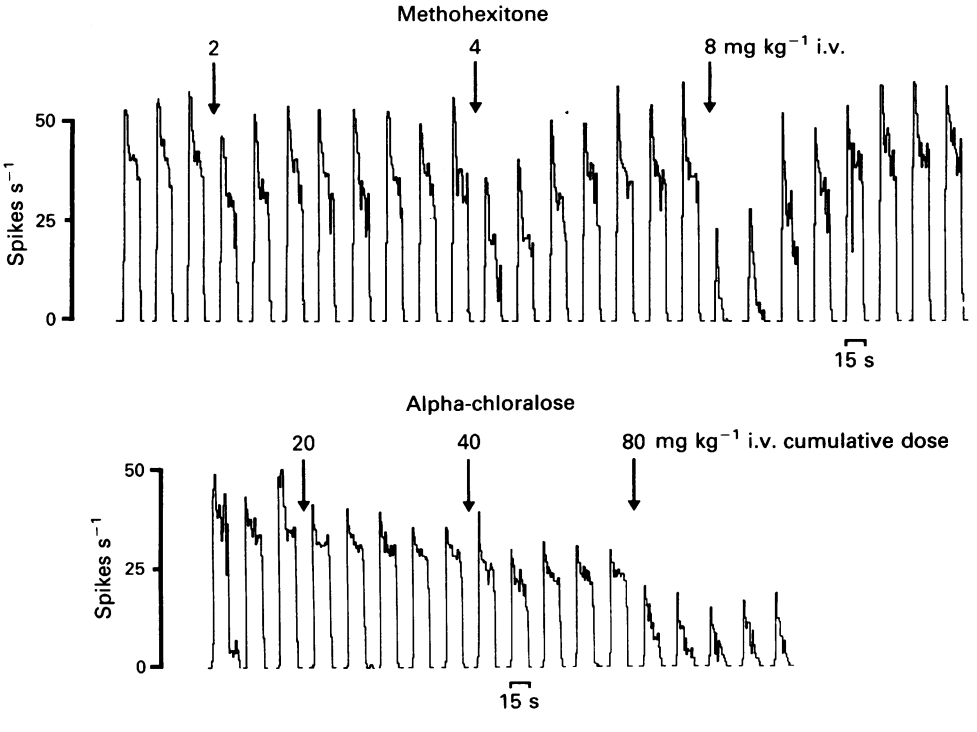

Figure 1 Chart records displaying the effects of two anaesthetics on stimulus-evoked firing of single motor units recorded from the flexor digitorum longus muscle in decerebrate, spinalised rats. Fifteen second pinch stimuli were appled to a single toe of the ipsilateral hind paw of the rats and were repeated at three minute intervals. The chart was halted between pinch stimuli. Methohexitone (top trace) caused a dose-dependent and rapidly reversible reflex depression over the range of 2–8 mg kg^{-1} i.v. In view of the short acting nature of this barbiturate, additional doses were given only following recovery from the previous dose to control levels. The lower trace displays the effect of alpha-chloralose (20–80 mg kg^{-1} i.v.) administered in a cumulative dose regime. The depressant effect of alpha-chloralose on these spinal reflexes is maintained for several hours, as is expected for this long lasting anaesthetic, and so recovery was not followed. The peak effect of alpha-chloralose did not occur, in some cases, for up to 15 min after administration.

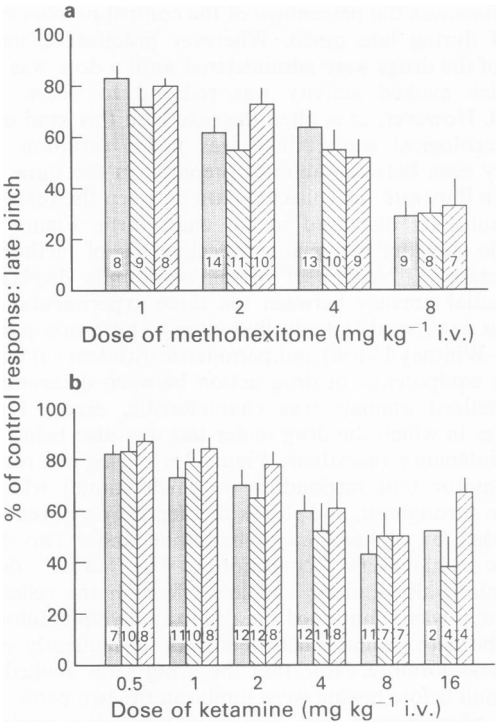

Figure 2 Dose-dependent effects of methohexitone (a) and ketamine (b) on noxious pinch evoked single motor unit activity. Responses, expressed as a percentage of the mean pre-test control values are compared at each dose between animals anaesthetized with alphaxalone/alphadolone (▩), those anaesthetized with alpha-chloralose (▧) and decerebrated, unanaesthetized rats (▨). Analysis was restricted here to counts of the spikes evoked during the late part of the pinch stimulus. Bars show s.e. and numbers within columns are the numbers of units recorded.

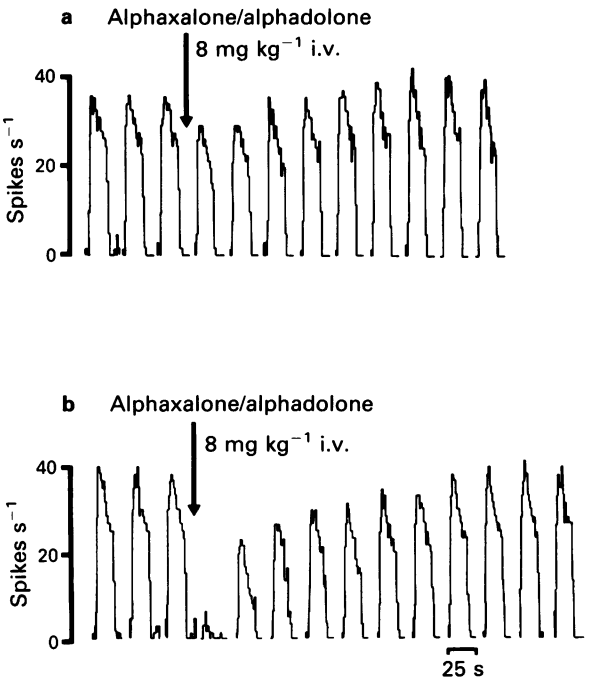

Figure 3 Chart record of single motor unit firing rate recorded from flexor digitorum longus, illustrating the different effects of a single dose of alphaxalone/alphadolone (8 mg kg^{-1} i.v.) when given under different maintenance anaesthetic protocols. The pinch stimuli were delivered at the same site (toe 5) with the same force for 15 s, repeated once every 3 min throughout the experiment. (a) Recorded whilst the animal was anaesthetized with alphaxalone/alphadolone infused at a rate of 12 mg kg^{-1}h^{-1}. Over a subsequent two hour period, the maintenance anaesthetic was changed to alpha-chloralose (60 mg kg^{-1} i.v. initially with 20 mg kg^{-1} i.v. supplementary) and the effects of the steroid infusion allowed to wear off. The same drug test was then repeated (b). The effects were completely reversible.

maintenance anaesthetic does not affect the potency with which alphaxalone/alphadolone depresses single motor unit response. The results shown in Figure 4b indicate that chloralose behaved in an equivalent manner.

It is possible that a significant difference in neuronal responsiveness, evident as disparate levels of evoked activity, might account for the difference between anaesthetic states. Although we were unable to measure directly the force with which the stimuli were applied, the same pinching device, operated at consistent pneumatic pressures, and always applied to one toe, was used in all animals. We were therefore able to estimate relative excitability by comparing the mean firing rates evoked by each stimulus during control periods (prior to drug administration) between the decerebrate and anaesthetized groups of animals. The following results were obtained and are expressed as mean firing rate during late pinch ± s.e.mean for n cells tested: decerebrate rats, 30.4 ± 1.8 spikes per second ($n = 9$); chloralose anaesthetized, 21.3 ± 3.1 ($n = 11$); alphaxalone/alphadolone anaesthetized 24.2 ± 1.8 ($n = 12$). The mean firing rates obtained in decerebrate animals were significantly higher ($P < 0.02$; unpaired Student's t test) than in chloralose or alphaxalone/alphadolone anaesthetized animals, but there was no significant difference between the two groups of anaesthetized animals.

Comparison of the depression of reflex activity caused by behaviourally similar doses of four anaesthetics

Sixteen conscious intact rats, previously implanted with intravenous cannulae, were used for the behavioural studies. Each anaesthetic was examined at the same doses as those tested in the electrophysiological experiments, and the behavioural effects (see Methods for criteria) and the time courses of recovery noted. The time courses of recovery from doses of

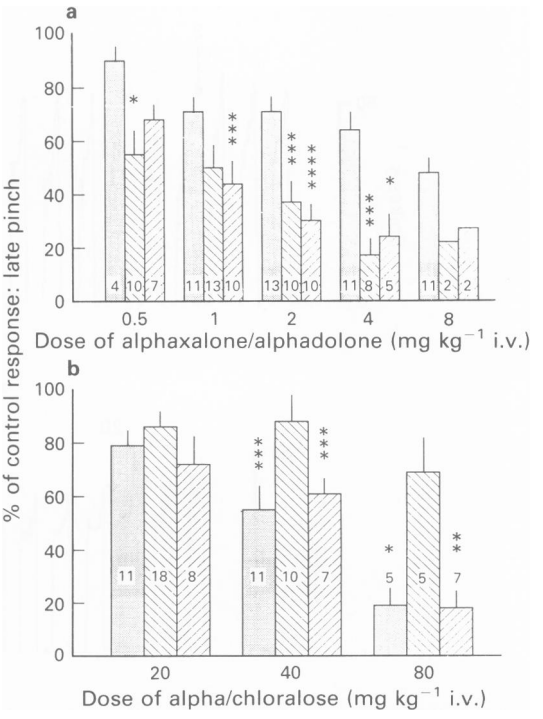

Figure 4 Dose-dependent effects of alphaxalone/alphadolone (a) and alpha-chloralose (b); presented in the same format as Figure 2, but with the addition of statistical data. Where indicated, significance was reached (Mann-Whitney U-test, * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$ and **** $P < 0.001$, 1 tailed). Data were compared between those obtained from animals anaesthetized with alphaxalone/alphadolone (a) and alpha-chloralose (b). For key to shading of columns see Figure 2 legend.

methohexitone and alphaxalone/alphadolone were similar to those observed in the electrophysiological experiments, having approximate half recovery times of 3–4 and 6 min respectively. The effects of ketamine and alpha-chloralose were more long lasting and these compounds were found to have full recovery times of about 2.5–3.5 h (from a total dose of ketamine of 128 mg kg^{-1} i.v.) and 5–6.5 h (from a total dose of alpha-chloralose of 80 mg kg^{-1} i.v.). Table 1 shows the behavioural effects produced by the four anaesthetics. Alongside these data are shown the effects on nociceptive spinal reflexes measured in spinalised, decerebrated animals.

By comparing our electrophysiological data with the behavioural observations, it is apparent that spinal reflex activity in decerebrate spinalised animals was significantly depressed by the doses of these compounds which produced ataxia in intact animals. However, if one compares different compounds, the ataxic effects occurred at very different proportions of the doses needed to cause immobility (i.e. the doses which approach those needed for adequate experimental anaesthesia). For example, while alphaxalone/alphadolone produced depression of reflexes and ataxic effects only at doses approaching those causing loss of consciousness, ketamine depressed motor coordination and spinal reflexes at a dose much smaller than that required to produce some degree of dissociative anaesthesia. These differences are highlighted by the ratio of the dose producing loss of spontaneous motor activity to that producing maximal ataxic effects. Within the limits of the dose regime utilised the following ratios were found, ketamine = 16; alphaxalone/alphadolone = 1; methohexitone = 1; chloralose = 2.

Discussion

The first question posed in this study, the relative potency within the spinal cord of these anaesthetics in depressing nociceptive reflex responses of spinal flexor motoneurones, is answered by the experiments on decerebrate spinalised rats, and is summarized in Figures 2 and 4.

In order to assess the relevance of these spinal actions of anaesthetics to those experiments in which anaesthetics need to be used, it is necessary to relate the doses causing depression of spinal reflexes to those causing the various stages of anaesthesia. For this reason the same intravenous doses as those examined in our current electrophysiological experiments were tested in chronically prepared conscious animals of the same strain.

One major problem of such comparisons is that because these compounds act in such different ways, it is difficult to select the criteria for determining equivalent levels of anaesthesia. We have approached the problem by observing arbitrarily defined dose-related degrees of behavioural sedation and by comparing these with the reflex depression caused by equivalent doses in spinalised decerebrate animals. Whilst the behavioural states we recorded do not necessarily equate to anaesthesia *per se*, they are, according to text book definitions, phases through which animals pass during induction and may be used to help define various planes of anaesthesia.

The spinal flexion reflex was depressed in decerebrate, spinalised rats at doses of each drug that produced a minimal ataxia in intact rats. The relative depression of the reflex from control levels was, however, different between drugs, methohexitone having the greatest effect (Table 1). Those doses that prevented the animals from walking ('maximal ataxia') in all cases had a greater direct effect on the spinal cord, causing a significantly greater depression of nociceptive reflexes. These data therefore indicate that anaesthetics have considerable depressant actions on the spinal cord at sub-anaesthetic doses. For alphaxalone/alphadolone and methohexitone, the dose-response relationship was sufficiently steep for maximal ataxia and loss of spontaneous activity to be encompassed by one dose step within our logarithmic administration protocol. The depression of reflexes caused by equivalent doses was significant.

Alpha-chloralose is often considered to protect reflex responses (Shimamura *et al.*, 1968) and alphaxalone/alphadolone has been shown to preserve spinal reflexes at doses which cause hypnosis (Lovick, 1986). In contrast, barbiturates are often considered to depress spinal activity relatively more. The present data, however, suggest little difference in the direct depressant effect of these three anaesthetics on spinal reflexes at the lowest dose required to cause loss of spontaneous activity.

The third aim of this set of experiments was to determine whether anaesthetics, when used to maintain animals in an anaesthetized condition suitable for experimental recording, are likely to affect the potency with which other drugs depress nociceptive spinal reflex transmission. Over the dose ranges indicated, neither ketamine nor methohexitone displayed any differential potency between tests performed in spinalised animals that were either unanaesthetized and decerebrate, or anaesthetized with either alpha-chloralose or alphaxalone/alphadolone. This suggests not only that there is no differential effect between these two maintenance anaesthetics, but also that the presence of either of these two anaesthetics does not interfere with the spinal reflex depressant actions of ketamine or methohexitone.

Work already carried out in this laboratory (Parsons & Headley, 1989) has shown that if two intensities of pinch are given so that the same motoneurone responds alternately to strong and weak pinch stimuli with high and low firing rates respectively, then the weaker of the responses is invariably reduced to a greater degree by the same dose of drug, whether opiate or anaesthetic. This highlights the importance of matching stimulus intensity if one is to compare the potencies of compounds. Although we have made a direct comparison between the three experimental groups, the question still arises as to whether the noxious stimuli administered in the present study may have been different between groups. Whilst we cannot state that the intensity of stimuli given to each of the groups was identical, we did use constant pneumatic pressures to drive the stimulator as well as similar placements of the pincher device on the toes, so that it is unlikely that there

Table 1 Comparison of the behavioural and the reflex depressant effects of four injectable anaesthetics

Anaesthetic	*Lowest dose causing minimal ataxia* (mg kg^{-1} i.v.)	*Effect on reflex responses at same dose* (% control)	*Lowest dose causing maximal ataxia* (mg kg^{-1} i.v.)	*Effect on reflex responses at same dose* (% control)	*Lowest dose causing loss of righting reflex* (mg kg^{-1} i.v.)	*Lowest dose causing loss of spontaneous activity* (mg kg^{-1} i.v.)
Ketamine	1 (n = 7)	84 ± 3.1 (n = 8)	8 (n = 7)	50 ± 12.3 (n = 7)	64 (n = 7)	128 (n = 7)
Alphaxalone/alphadolone	0.5 (n = 7)	68 ± 6.6 (n = 7)	4 (n = 8)	24 ± 10.4 (n = 5)	4 (n = 8)	4 (n = 8)
Methohexitone	4 (n = 8)	52 ± 7.5 (n = 9)	8 (n = 8)	33 ± 11.4 (n = 7)	8 (n = 8)	8 (n = 8)
Alpha-chloralose	20 (n = 16)	72 ± 9.8 (n = 8)	40 (n = 16)	61 ± 5.9 (n = 7)	80 (n = 16)	80 (n = 16)

See Methods for the interpretation of the behavioural terms used. The doses causing each level of behavioural depression were those having effects in >75% of the rats tested. For 'minimal ataxia' and 'maximal ataxia' the effect of this dose on the electrophysiologically recorded reflexes is also shown: values are % of pre-drug controls.

were consistent differences in stimulus intensity between the groups. Our examination of the firing rates of motor units prior to drug administration indicates that in the decerebrate preparations, the pinch stimuli elicited a slightly but significantly greater firing rate. This was not, however, associated with any corresponding decrease in the potency of methohexitone or ketamine, as might have been predicted from the previous study (Parsons & Headley, 1989).

Equipotency of drug action between the three experimental groups only occurred when the test compound differed from the maintenance anaesthetic. For example, alpha-chloralose displayed similar effects when administered to alphaxalone/alphadolone anaesthetized or decerebrate animals, but was significantly less potent when given as additional doses to chloralose maintained animals. Similar effects were seen for alphaxalone/alphadolone which was selectively less potent on animals already anaesthetized with this steroid anaesthetic. There are two possible explanations for these findings. If we consider a hypothetical dose-response curve for alpha-chloralose, for example, then an animal maintained on this compound would be expected to have a modified reflex response compared to a decerebrate, unanaesthetized animal. This is suggested by the data since the firing rates during pre-drug control responses were lower in anaesthetized than in decerebrate preparations. One might expect, therefore, that in anaesthetized animals, we are looking at drug effects over the upper part of the dose response curve and that, accordingly, the effects of subsequent additional doses are less than when administered to decerebrate animals. Scrutiny of the data suggests an alternative explanation, namely that there are different populations of units, some more resistant to particular drug action than others. Therefore, whilst searching for units under one particular maintenance anaesthetic, we automatically bias our sample of units to those which are resistant to that compound. This is suggested by the observation that the mean stimulus evoked firing rate in rats with an anaesthetic dose of alpha-chloralose (about $80\,mg\,kg^{-1}$ i.v. as indicated by the behavioural experiments) was 70% of that in decerebrate unanaesthetized animals. In contrast, Figure 4 suggests that such a dose would have reduced responses of the units recorded in decerebrate preparations to about 20% of control.

We can conclude, firstly that all the anaesthetics tested act in the spinal cord to produce a significant depression of spinal reflex responses to noxious stimuli at doses below those required for full anaesthesia. Methohexitone, alphaxalone/alphadolone and alpha-chloralose had similar direct actions on the cord to reduce spinal reflexes at doses causing similar levels of behavioural sedation. Secondly, it appears that neither the presence of the anaesthetic, nor the choice between alphaxalone/alphadolone and chloralose, influenced the effectiveness with which various other compounds depressed naturally evoked single motor unit responses evoked by noxious somatic stimuli. This is suggested both by the tests performed in this study with other anaesthetic agents, and by data with μ and κ opioids (Hartell & Headley, 1989 and unpublished observations). Whilst this finding implies that the use of anaesthetics may not be detrimental to pharmacological testing *in vivo*, the decreased potency of additional doses of the same anaesthetic may be explained by biasing the cells recorded.

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