General anaesthetics and field currents in unclamped, unmyelinated axons of rat olfactory cortex

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1 The effects of seven general anaesthetics and one local anaesthetic having a wide range of physical and chemical properties were studied on nerve terminal Na- and K-mediated currents in slices of olfactory cortex. These currents were measured from the groups of fine unmyelinated axons traversing the surface of the olfactory cortex and which give off synapses *en passant*. The amplitude of the K-current was visualized by depolarizing the axons via an electrode polarization.

2 The anaesthetics tested were ketamine (0.1–2 mM), pentobarbitone (0.1–5 mM), urethane (5–200 mM), halothane (0.5–5 mM), ether (10–200 mM), alphaxalone (0.001–0.05 mM), diisopropylphenol (0.05–0.5 mM) and lignocaine (0.01–0.5 mM). All had depressant effects on the axonal Na-current (at the higher concentrations tested) and on the K-current (at slightly lower concentrations). The apparent lower potency on the Na-current was considered to be due to a masking of effect as a consequence of the reduction in the K-mediated membrane rectification rather than any real difference in the susceptibilities of the Na and K-currents.

3 Some of the depressant effect of pentobarbitone and alphaxalone was γ -aminobutyric acid (GABA)-mediated as indicated by the reduced potency in the presence of bicuculline. The actions of ketamine and halothane were unaffected by bicuculline.

4 For some anaesthetics these axonal depressant effects might contribute to general anaesthesia, while for other substances the relatively high concentrations necessary would suggest that this mode of action does not produce effective anaesthesia *in vivo*.

Introduction

A wide variety of preparations and tissues have been used to study the mechanisms of general anaesthetic action. A substantial body of evidence indicates that many anaesthetics enhance central inhibitory synaptic transmission at low concentrations (Nicoll, 1972; 1975; Nicoll *et al.*, 1975; Barker & Ransom, 1978; Pickles & Simmonds, 1975; Scholfield, 1978; 1980; Harrison & Simmonds, 1984). On the other hand, some authors have examined the effects of these substances on excitatory synaptic transmission of various types and have drawn other conclusions: anaesthetics act at synapses either to interfere with the action of the transmitter substance with its receptor (Barker & Grainer, 1973; Adams, 1976; Richards & Smaje, 1976) or they have a presynaptic depressant action (Larrabee & Posternak, 1952; Weakly, 1969; Richards, 1972; Goldring & Blaustein, 1982). In previous studies, pentobarbitone was found to have a depressant effect on conduction along the lateral olfactory tract (LOT) (Pickles & Simmonds, 1976; Scholfield & Steel, 1985). However, pentobarbitone was an effective depressant only at concentrations which in plasma are considered lethal in whole animals. The possibility arises that the unmyelinated axons which make *en passant* synaptic connections are more anaesthetic-sensitive than the myelinated axons of the LOT from which they derive. This might explain the apparent greater anaesthetic susceptibility of synaptic transmission. We have therefore studied the actions of some general anaesthetics on these fine presynaptic axons using an extracellular recording method to distinguish the Na- and K-currents (Scholfield, 1987). The present experiments show that a variety of general anaesthetics non-selectively block both the Na- and K-currents, albeit at concentrations which are generally considered as somewhat higher than those existing in body fluids during anaesthesia.

Methods

Rats of either sex weighing 150–200 g were decapitated and the brains removed. Slices of olfactory cortex were cut to have

a thickness of 100–200 μ m and were maintained in solution bubbled with 95% O₂:5% CO₂ for at least 60 min before use. Each slice was bisected parallel with the axons radiating from the LOT and then, immediately before recording, another vertical cut was made perpendicular to the axonal orientation (Scholfield, 1988). The slice was placed on a nylon mesh suspended within a recording bath of effective volume 0.5 ml. A glass electrode having an angled tip and a fire-polished orifice 40 μ m in diameter was offered up to the freshly cut face of the slice. Gentle suction was applied to draw in Krebs solution followed by a 50 μ m length of the tissue containing the unmyelinated axons (Scholfield, 1988). The electrode was connected to a current recording amplifier of input resistance 10 k Ω and capacitively coupled to subsequent stages (time constant 3 s). The current amplifier also contained the facility for applying various polarizations to the recording electrode between +200 and –200 mV. These polarizations were applied as square wave pulses of 600 ms duration beginning 400 ms before application of the stimulus. This is a long period relative to the latency of the response and was necessary because the onset of the polarization caused a large and slow capacitive current. With a 400 ms prepolarization, the recording baseline had settled down to an acceptable level. The axons were stimulated by a pair of 100 μ m diameter lacquered tungsten wires placed close to the area where the axons leave the LOT. Stimuli of 20–40 V, 0.2 ms duration were delivered at 0.1 Hz. Statistical comparisons were made by the two-tailed *t* test, paired or unpaired as appropriate. Results were expressed as a mean $\pm$ standard error of mean.

Solutions

The normal Krebs solution had the following composition (mM): Na 144, K 6.1, Ca 2.5, Mg 1.3, Cl 132, HCO₃ 25 and (+)-glucose 11. Experiments were conducted at 23–25°C and anaesthetics were dissolved directly into the solution perfusing the recording bath. In the case of the volatile anaesthetics (halothane and ether), particular precautions were exercised to avoid loss of the substance through evaporation and partitioning into the silicon rubber tubing connecting the reservoir to the recording bath. All the long lengths of rubber tubing were replaced by glass or stainless steel. The substances

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were dissolved, by gentle agitation, directly into solution freshly equilibrated with 95% O₂:5% CO₂ and with the air space over the solution replaced by 95% O₂:5% CO₂. No further gassing of the solution was performed and the solution made only a single passage through the recording chamber at 5 ml min⁻¹; the normal practice is to recirculate solutions from a gassed reservoir once the previous solution has been flushed out. Increasing the bath perfusion rate to 10 ml min⁻¹ produced no further effect of the anaesthetic. If ether solution was recirculated after an effect had equilibrated, the preparation recovered at the same rate as it did when the ether was washed out with normal solution. The dissolving of alphaxalone was aided by an ultrasonic cleaning bath. All anaesthetics were applied for 15 min periods in sequentially increasing concentrations. Some experiments were also conducted in the presence of 10 μ M bicuculline which was added 15 min before the anaesthetic. A stock solution of bicuculline in HCl (10 mM) was diluted into the bathing solution immediately before use.

Results

Figure 1 shows typical recordings obtained from the olfactory cortex with a current amplifier. Without electrode polarization, application of a single electrical stimulus to the axons derived from the LOT resulted in a positive current transient (of 19.3 ± 1.3 nA in 56 slices) followed by a small negative current (of 2.8 ± 0.4 nA). Stimulation during the application of a +100 mV electrode polarization caused the negative current to become greatly enlarged (to 13.6 ± 1.0 nA) and the positive current reduced a little (to 15.9 ± 1.4 nA).

Figure 1 Current records generated by stimulation of unclamped, unmyelinated axons in the rat olfactory cortex. The traces in the left column were obtained by using a recording electrode without any polarization and those on the right during application of a +100 mV polarization. In the upper pair of traces the preparation was in normal solution and then the preparation was bathed in ketamine (0.5 mM) for 15 min (middle traces). In the lower traces, the ketamine had been washed out for 20 min. The dashed line shows the extrapolated recording base-line. These are single responses recorded with 5 kHz filtering and were replayed from a computer on to a digital plotter. The arrows show the point of stimulus application and stimulus artefact. The latter tend to be large and variable because of the closeness of the stimulating electrodes to the recording electrode.

In previous experiments, the negative current was shown to have a pharmacological profile compatible with a K-mediated event and the earlier short positive current a Na-event (Scholfield, 1988; 1989). By this recording technique the K-current depends upon the generation of the propagated Na-current. Thus any synchronous effect on both the Na- and K-currents might be the result of a selective depression of either the Na channels or both Na and K channels. Therefore concentration-effect curves were constructed to agents known to block specifically Na- and K-currents (tetrodotoxin (TTX), and 3,4-diaminopyridine (3,4-DAP) respectively) to provide a base to compare the effects of other drugs which may have specific actions on one or other current.

TTX was added for 15 min periods at progressively increasing concentrations between 10 and 100 nM and the initial positive current was depressed in a concentration related manner (Figure 2a). The after negative current was also depressed but

Figure 2 Concentration-effect curves for tetrodotoxin (TTX) and 3,4-diaminopyridine (3,4-DAP) on the field currents generated in unmyelinated axons of rat olfactory cortex. (a) In 6 slices, TTX was added in four sequential 15 min applications of increasing concentration in normal solution. The negative after-current is plotted as (■) and the initial positive current as (□). The *P* values aligned at each concentration denote the level of the significance between these two currents. The continuous lines were interpolated by 'eye'. In another set of 6 slices, TTX was added in the same concentrations in the presence of 100 μ M 3,4-DAP and 5 mM TEA (Δ). There was no significant difference between the initial positive current in normal solution and 3,4-DAP/TEA. (b) A similar curve for 3,4-DAP in the presence of 5 mM TEA and 100 μ M Cd with another 6 slices. The Na current is plotted as (□) and the K current as (■). The *P* value for the elevation of the Na current at various concentrations was 0.07 to 0.2. Ordinates: Maximum amplitude of the appropriate current as a percentage of that before addition of the first concentration. Abscissae: concentration of TTX (a) or 3,4-DAP (b) in the bathing solution. When appropriate, the s.e.means at each point are shown in one direction to reduce cluttering. The absence of a s.e.mean indicates it is smaller than the point.

at the lower concentrations, consistently less than the positive current, while at higher concentrations, the negative after-current was relatively more depressed (Figure 2a). To exclude secondary effects of the K-current on the Na-current, TTX was also tested in the presence of 3,4-DAP and TEA (Figure 2a).

A concentration-effect curve to K-channel blockade was also constructed. In this preparation, several pharmacologically distinct K-conductances are present (Scholfield, 1989). Of these, the aminopyridine-sensitive current is principally responsible for repolarizing the axonal membrane after the action potential. Therefore a concentration-effect curve to 3,4-DAP in the presence of 5 mM TEA and 100 mM Cd (to block slower and Ca-activated K-currents) was constructed. The negative after-current was depressed while the initial positive current was enhanced (Figure 2b). Since the aminopyridines are regarded as specific for the 'A' type K-conductance at the concentrations used in the present experiments, then the elevation of the Na-current was an indirect consequence of this blockade of the K-current. In the absence of TEA and Cd, the elevation is more consistent (Scholfield, 1988).

Since the recording electrode does not voltage clamp the axons, a large part of these responses were currents resulting from changing potentials across the axonal membrane capacitance (Scholfield, 1989). Therefore this procedure should be seen solely as a way of distinguishing the two pharmacologically characterised currents rather than a study of their voltage dependence and kinetics.

Effect of ketamine

Figure 1 shows a depression produced by ketamine on the currents recorded by this technique. The onset of the depressant effect occurred when the ketamine reached the slice and was complete within 15 min of continuous application (Figure 3). On washing out the ketamine both the Na and K-currents recovered over a 30 min period. In a further 8 slices, ketamine was applied in a serially increasing range of concentrations between 0.1 and 2 mM. The depressant effect of ketamine was concentration-dependent (Figure 4a) and ketamine appeared to have a slightly more selective effect on the K-current (Figures 1, 3 and 4a). In another 6 slices, ketamine was found to be significantly more effective in the presence of 3,4-DAP and TEA (Figure 4a). In the presence of bicuculline (10 μ M) the depressant action of ketamine was indistinguishable from that without bicuculline.

Halothane

Ten preparations were used to examine the effects of halothane over a concentration-range of 0.5 to 5 mM. Halothane showed depressant effects in a concentration-related manner between 1 and 5 mM (Figure 4b). The K-current appeared a little more sensitive to the action of halothane than the Na-current. Halothane was retested in a further 5 slices at one concentration (3 mM) alone. Then after washing out and recovery, halothane was reapplied in the presence of 10 μ M bicuculline. In 3 mM halothane, the amplitude of the Na-current was $69 \pm 7\%$ of that in normal solution and $64 \pm 8\%$ in bicuculline and the respective amplitudes for the K-current were 78 ± 6 and $84 \pm 10\%$. The responses in bicuculline were not significantly different by paired *t*-test. In another 10 slices, halothane had a significantly more potent depressive effect on the Na-current in the presence of 3,4-DAP and TEA than in normal solution (Figure 4b).

Ether

In six experiments, ether was tested at concentrations between 1 and 200 mM. It had little effect up to 20 mM but at concentrations higher than this, both the Na and K-currents were depressed in a concentration-dependent manner (Figure 4c). On washing out ether, the response fully recovered within 3 min. This contrasts with all the other agents tested where full recovery required 15–40 min of washing out.

Diisopropylphenol

This substance was tested in the concentration range 0.05–0.5 mM and again the K-current was more depressed than the Na-current at each concentration (Figure 4d). In the presence of 3,4-DAP and TEA, diisopropylphenol was more effective on the Na-current (Figure 4b).

Lignocaine

For comparison, a local anaesthetic was also tested and this depressed the Na-current and also, more potently, the K-current (Figure 4e) with a time-course and manner similar to the depression produced by general anaesthetics. Lignocaine was more effective on the Na-current in the presence of 3,4-DAP and TEA (Figure 4d).

Figure 3 Continuous time-courses for the maximum amplitude of the Na-current (upper trace) and the K-current (lower trace). The preparation was stimulated at 0.05 Hz and the current waveforms stored by computer. The peak height of the positive current for each waveform was measured by computer and plotted out on an X-Y plotter. The lower trace ('K-current') was obtained by measuring the amplitude of the current at a constant latency (15 ms after the stimulus). During the experiment, 0.2 mM ketamine and then 1 mM ketamine were added to the solution superfusing the slice at the points arrowed followed by their washout (washout). Records were obtained throughout this experiment during an electrode polarization of +100 mV. In the presence of 1 mM ketamine, the K-current decreased and was replaced by a positive tail.

Figure 4 Dose-response curves for the maximum amplitude of the Na-current (unpolarized electrode) and maximum K-current ($+100$ mV polarization) obtained by sequential increases in concentration of ketamine (a), halothane (b), ether (c), diisopropylphenol (d), lignocaine (e) and urethane (f): ($\square$) Na-current; ($\blacksquare$) K-current (continuous lines); ($\blacktriangle$) Na-current in the presence of $100 \mu\text{M}$ 3,4-diaminopyridine (3,4-DAP) and 5 mM tetraethylammonium (TEA). The P values given at each point are for the differences between the Na-current in normal solution and in the presence of 3,4-DAP and TEA. The asterisks denote the level of significance between the effects on the Na- and K-currents under the same conditions: * $P < 0.02$ and ** $P < 0.002$. Ordinate scales: The amplitude of the respective current is expressed as a percentage of that before application of anaesthetic. For some points the s.e. mean bar is shown in one direction only to reduce cluttering. Points not showing bars have errors smaller than the symbol. The lines are drawn 'by eye'.

Urethane

Six slices were used to study the effect of 5 – 200 mM urethane. At all concentrations above 10 mM there was a depression of the K-current while the Na-current was only depressed at 150 and 200 mM (Figure 4f). In the preceding experiments, the anaesthetics were also more effective on the K-current but this was particularly marked with urethane (Figure 4f): in some experiments, the Na-current was a little elevated in 100 mM urethane. This might have resulted from a selective depressant action on the K-current such as is observed with K-channel blockers (Scholfield, 1987). Therefore 10 slices were pre-equilibrated with $100 \mu\text{M}$ 3,4-diaminopyridine and 5 mM tetraethylammonium to block axonal K-currents (Scholfield & Steel, 1988). Under these conditions, urethane had a more potent effect on the Na-current than when the K-current was

intact (Figure 4f). The implication of this result is that the depressant action of urethane on the Na-current in normal solution is masked by a blockade of the K-current.

Pentobarbitone

This substance was tested at 0.1 to 5 mM in 15 slices. Little effect was observed at the lowest concentration tested (0.1 mM), but between 0.1 and 2 mM , both the Na- and K-currents were progressively depressed with increasing concentration (Figure 5a). Pentobarbitone was also tested in the presence of bicuculline where its action was noticeably reduced (Figure 5a, 5 slices) such that little depression of the Na-current occurred until the concentration reached 1 mM . The depressant action of pentobarbitone on the K-current was less influenced by bicuculline (Figure 5a). The effect of 3,4-DAP

Figure 5 Dose-response curves for pentobarbitone (a and b) and alphaxalone (c and d). (a) and (c). The effect of bicuculline. The open symbols (Δ and $\square$) refer to the Na-current and filled symbols ($\blacktriangle$ and $\blacksquare$) to the K-current; ($\square$ and $\blacksquare$) connected by the continuous line are for the Na- and K-currents in normal solution and (Δ and $\blacktriangle$) and dashed lines refer to the currents obtained in the presence of $10\mu\text{M}$ bicuculline. The P values given at each point are for the differences between the Na-current in normal solution and in the presence of $10\mu\text{M}$ bicuculline. (b) and (d) The effect of K channel blockade. The Na-current is represented by ($\square$) and the K-current by ($\blacksquare$); ($\bullet$) Na-current in the presence of $100\mu\text{M}$ 3,4-diaminopyridine (3,4-DAP) and 5mM tetraethylammonium (TEA). Bicuculline ($10\mu\text{M}$) was present throughout all the experiments. The P values given at each point are for the differences between the Na-current in normal solution and 3,4-DAP. The asterisks refer to the differences in the percentage reduction between the Na- and K-currents: * $P < 0.02$; ** $P < 0.002$.

and TEA on the action of pentobarbitone was also tested in the presence of bicuculline in a further 12 slices (Figure 5b): the Na-current showed a sensitivity to pentobarbitone similar to that of the K-current in normal solution.

Alphaxalone

In six experiments, alphaxalone was tested at concentrations between 1 and $50\mu\text{M}$. The Na-current was weakly depressed and the K-current a little more depressed by alphaxalone (Figure 5c). In another 6 slices, the alphaxalone was tested in the presence of bicuculline ($10\mu\text{M}$). Under these conditions the depressant effect of alphaxalone on the Na-current was not observed and the effect on the K-current was weaker (Figure 5c). Alphaxalone was also tested in the presence of bicuculline, 3,4-DAP and TEA. In this solution, alphaxalone showed a slight depression but the effect was not significant.

Discussion

Seven general anaesthetics having a wide range of physical and chemical properties have been tested on the Na- and K-currents generated in unmyelinated axons of the rat olfactory cortex. All the substances showed some evidence of depressant effects on axonal currents. Do these effects have any relevance to the cellular mechanism of general anaesthesia *in vivo*?

Since the depressant action of the general anaesthetics was shared by low concentrations of lignocaine, a substance not regarded as a general anaesthetic, it would seem unlikely that the depression of axonal function by these substances could

entirely account for the cellular actions of general anaesthetics during anaesthesia *in vivo*. Several cellular mechanisms of action of general anaesthetics in mammals have been proposed. Among them are: (i) enhanced inhibition; (ii) depression of transmitter release or action and (iii) depression of axonal conduction.

(i) In the olfactory cortex, pentobarbitone, alphaxalone, and to a lesser extent halothane and diisopropylphenol, enhance γ -aminobutyric acid (GABA)-mediated inhibition at concentrations lower than those used in the present study (Pickles & Simmonds, 1978; Scholfield, 1978; 1980; Harrison & Simmonds, 1984; Cottrell *et al.*, 1987; Collins, 1988). In the case of pentobarbitone and alphaxalone, the enhancement of inhibition occurs at relatively low concentrations. The effect is seen at the anaesthetic concentrations seen *in vivo* (Chenoweth *et al.*, 1962; Sear & Prys-Robert, 1979) and this is considered by several authors to be their principal mechanism of action. Furthermore, some of the depressant effect on axonal function can be accounted for by an action via GABA or axonal Cl-channels. In contrast, halothane and ketamine showed no such bicuculline-sensitivity although they have a weak potentiating effect on inhibition (Scholfield, 1980). In this present study, thin slices were used to avoid postsynaptic cells. Such slices would also exclude inhibitory neurones (Scholfield, 1978; Halliwell, 1982) which might give rise to GABA-mediated feedback inhibition on the presynaptic axons (Schmidt, 1963; Nicoll, 1975; Pickles & Simmonds, 1976; Cain & Simmonds, 1982). With larger slices or *in vivo*, the effect of alphaxalone and pentobarbitone may be more substantial, particularly with high stimulus rates.

(ii) Ether, halothane and diisopropylphenol are more potent depressors at some excitatory synapses (Richards, 1973;

Richards & Smaje, 1976; Collins, 1988) than they were depressors of axonal function in the present study. Thus the action at the excitatory synapse may make a major contribution to the action of these substances as general anaesthetics *in vivo*.

(iii) Urethane and ketamine had a similar potency in depressing excitatory transmission (Scholfield, 1980) and depressing axonal currents in the present study. Since excitatory transmission is dependent on axonal function, the possibility exists that a major cellular effect of these substances is via this axonal action. Notwithstanding this, enhancement of inhibition (Nicoll, 1972; Scholfield, 1980) or NMDA glutamate receptor antagonism (Johnson & Lodge, 1984; Harrison & Simmonds, 1985) are more likely to make major contributions to the *in vivo* actions.

Thus the depression of axonal currents should not be considered as being responsible for the cellular action of the general anaesthetics studied here. However, the effect may contribute in part or in whole to the lethal actions of these substances.

In a previous study (Scholfield & Harvey, 1975) lignocaine was found to be a slightly more potent depressor of the excitatory postsynaptic potential (e.p.s.p.) ($ED_{50} = 0.18$ mM) than of the action potential of the myelinated LOT ($ED_{50} = 0.26$ mM) in the olfactory cortex. These concentrations compare to an ED_{50} of 0.10 mM for the unmyelinated axons (Figure 4). This difference between potency values for the myelinated and unmyelinated axons is substantial and indicates that the LOT axons can be a poor indicator of presynaptic susceptibilities.

Judging from the effect of TTX in normal solution (without K-channel blockade), any substance which selectively blocks Na-channels will have a slightly more preferential effect on the

Na-current than the K-current. In all the experiments performed in the present study, the Na-current was consistently less sensitive than the K-current. Therefore, it seems unlikely that these substances had any selective effect on the Na channels in this preparation as is the case with most anaesthetics on squid axon (Haydon & Urban, 1986). This contrasts with the preferential effect of urethane on the invertebrate Na-current (Gierasimov & Janiszewski, 1967; Pichon, 1979).

The K-currents which normally aid the recovery of the membrane from the action potential appeared to be more sensitive to the depressant action of all these substances. This might be expected for two reasons: (i) with the present methodology, the generation of the K-current is dependent upon the prior activation of the Na-current and therefore any selective effect on the Na-current would be overlooked; (ii) as the K-current is depressed, there will be some enhancement of the Na-current as is the case for substances such as the aminopyridines which selectively block the 'A' type current (Scholfield & Steel, 1988; Scholfield, 1988). With 100 mM urethane there was a suggestion of augmentation of the Na-current for this reason. When the K-current was blocked, the Na-current had a similar sensitivity to the K-current. Thus, at the level of the independent channel currents, urethane at least shows little selectivity for Na or for K.

In conclusion, a wide range of anaesthetics have non-selective depressant effects on both the Na- and K-currents but with regard to the concentrations required, it would seem that most of the substances do not make a major contribution to anaesthesia *in vivo* via this mechanism.

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