ACTIONS AND INTERACTIONS OF HIGH PRESSURE AND GENERAL ANAESTHETICS IN RAT HIPPOCAMPAL CA1 NEURONES.

BY
ANDREW PAUL SOUTHAN (BSc Hons)
CLINICAL RESEARCH CENTRE
WATFORD ROAD
HARROW.

A THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY OF
UNIVERSITY COLLEGE
LONDON.

ABSTRACT.

The thesis is divided into two experimental sections: the first concerns the actions of general anaesthetics on rat hippocampal CA1 neurones at atmospheric pressure. The second investigates some properties of CA1 neurones under high helium pressure.

The general anaesthetics studied at one atmosphere were enflurane, isoflurane, halothane, ketamine and methohexitone. Antidromic field potential measurements were taken in the absence and presence of the anaesthetics in order to assess changes in axonal/somatic excitability. Accommodation behaviour of CA1 neurones was also investigated in intracellular experiments with the above anaesthetics.

The principal findings were that the anaesthetics studied decreased the amplitude of the antidromic field potential and induced hyperpolarization, with the exception of ketamine which enhanced antidromic responses at low concentration and had mixed effects upon the resting potential. Halothane also induced a second antidromic population spike. The inhalation anaesthetics (enflurane, isoflurane, halothane) all blocked accommodation. Ketamine was found to slightly compromise accommodation, whilst methohexitone had mixed effects. A voltage-clamp study indicated that enflurane reduced the M-current of CA1 neurones.

CA1 neurone responses to helium pressure (up to 13.3MPa) were investigated using a purpose built pressure chamber designed to facilitate intracellular recording. In field potential experiments antidromic and orthodromic responses (to both single and paired pulses) were studied at one atmosphere and following compression. Responses were found to be mixed at elevated pressure. Some preparations were found to be unaffected by pressure whilst others became more excitable. Ketamine and methohexitone were found to have similar actions at 10MPa to their actions at 0.1MPa.

Intracellular measurements were made at pressure (5MPa and 10MPa). Resting membrane potential, input resistance and threshold potential were found to be unaffected by pressure. High pressure was found to block accommodation and reduce the associated AHP in CA1 neurones.

ACKNOWLEDGEMENTS.

I would like to thank my supervisors Dr. K.T.Wann and Professor D.A.Brown for their invaluable assistance and input during the time it took to complete this thesis.

I wish to acknowledge Dr. C.D.Richards who managed to squeeze me into his already crowded lab and enabled me to finish the experimental work necessary for the thesis. His time and efforts are greatly appreciated.

Thanks to Dr. J.F.Nunn for accommodating me within the Division of Anaesthesia at the CRC and his assistance with measurement of anaesthetic concentrations.

Thanks also to Dr. P.C.Pearce, Catherine MacLean, Malcolm Ward, Bridget Wardley-Smith, Brenda Dobson and Gordon McPhie of the CRC Division of Anaesthesia for their friendship during my stay there.

The guidance and efforts of John Baker and the staff of the Bioengineering at the CRC are very much appreciated.

I am grateful to Dr. M.Landon for his help with the anaesthetic concentration measurements.

Dr. J.V.Halliwell is gratefully acknowledged for his loan of equipment and assistance with the M-current experiments.

Thanks to Professor K.M.Spyer and everyone within the Department of Physiology at the Royal Free Hospital School of Medicine.

I wish to thank Dr. L.D.Leake for encouraging me to take an interest in a scientific career.

Thanks to Alison Bradley for her typing skills and support during the completion of the thesis.

Finally, and by no means least, I wish to acknowledge my family for their unfailing support and encouragement.

CONTENTS 4

LIST OF FIGURES AND TABLES 8

GENERAL INTRODUCTION 12

Molecular aspects of general anaesthetics 12

Classification of general anaesthetics 12

Lipid theories of general anaesthesia 13

Membrane expansion theories 13

Cellular actions of general anaesthetics 15

Anaesthetics and excitatory synaptic transmission 15

Postsynaptic actions of general anaesthetics 16

Effects on ion movements 16

Effects upon receptor systems 16

Anaesthetics and the inhibitory synapse 19

High pressure and the nervous system 20

Synaptic effects of high pressure 20

Pressure effects on excitatory synaptic transmission 20

Pressure effects on inhibitory synaptic transmission 21

Pressure effects on synaptic receptors 21

Postsynaptic effects of pressure 22

Pressure effects on resting membrane potential 22

Voltage gated ion channels 22

Anaesthetic protection against the HPNS 23

Pressure reversal of anaesthesia 24

METHODS 26

A.Slice preparation 26

B.Incubation 26

C.Recording arrangements 26

Anaesthetic work at one atmosphere 26

High pressure experimental apparatus 27

D.Stimulation and recording 30

Both atmospheric and high pressure recording 30

Field potential data 30

Intracellular recording 31

Voltage-clamp studies 32

E.Drugs used and anaesthetic concentrations 32

F.Statistics 33

FIGURES 34-59

EXPERIMENTAL RATIONALE AND AIMS 60

PART I INTRODUCTION 63

Voltage-activated K^+ currents 63

Delayed rectifier 63

Transient K^+ current 63

Slowly inactivating outward current 64

Inward anomalous rectifier 65

Q-current 65

M-current 65

Calcium-activated potassium conductances 66

I_C 66

I_{AHP} 67

Anaesthetics and potassium channels 68

PART I RESULTS 71

Antidromic field potential studies 71

Inhalation agents 71

Intravenous agents 72

Intracellular experiments 72

Effects on passive membrane properties 72

Inhalation agents 72

Intravenous agents 73

Effects on accommodation 74

Inhalation agents 74

Intravenous agents 75

Effects on threshold behaviour 76

Inhalation agents 76

Intravenous agents 76

Voltage-clamp measurements 77

FIGURES 78-127

PART I DISCUSSION 128

PART II INTRODUCTION 136

PART II RESULTS 139

Field potential recordings 139

Controls 139

Antidromic field potentials at pressure 139

Orthodromic field potentials at pressure 140

Anaesthetic field potential controls 141

Ketamine 141

Methohexitone 141

Anaesthetic studies at pressure 142

Ketamine 142

Methohexitone 142

Intracellular studies at high pressure 143

Resting membrane potential 143

Input resistance 143

Effects on threshold behaviour 143

Effects on spike characteristics 144

Pressure effects on accommodation behaviour 144

FIGURES 145-208

PART II DISCUSSION 209

GENERAL CONCLUSIONS 216

REFERENCES 218-240

APPENDIX 241

Work published/in press whilst completing the thesis 241

Sample papers 242-262

LIST OF FIGURES AND TABLES.

Fig.1	Diagram of a hippocampal slice.	35
Fig.2	Photograph of the pressure chamber.	37
Fig.3	Artists impression of the pressure chamber.	39
Fig.4	Photograph of the pressure chamber recording assembly.	41
Fig.5	Line drawing of the pressure chamber recording assembly.	43
Fig.6	Photograph of the regulator assembly panel.	45
Fig.7	Photograph of a pressure chamber door.	47
Fig.8	Orthodromic field potential measurements recorded within the sealed pressure chamber at a Po_2 of 0.05MPa.	49
Fig.9.1	Points at which measurements were taken from orthodromic field potentials.	51
Fig.9.2	Points at which measurements were taken from antidromic field potentials.	51
Fig.10	Graph of halothane concentration in equilibration chamber and tissue chamber.	53
Fig.11	Calibration graph for the halothane vaporizer.	55
Table 1.0	Temperature correction factors to be applied to the calculation of partial pressure of halothane in solution.	57
Fig.12	Graph used for the correction for water vapour pressure at the temperature of the equilibration chamber.	59
Fig.13	Antidromic field potentials in the absence and presence of 5% delivered enflurane.	79
Fig.14	The effects of 5% delivered isoflurane on antidromic field potential responses.	81
Fig.15	The effects of 5% delivered halothane on the antidromic field potential.	83
Fig.16	The effects of 20μmol.litre^{-1} ketamine on the antidromic field potential.	85
Fig.17	Effect of ketamine 20μmol.litre^{-1} on the positive after-potential of the antidromic field potential.	87
Fig.18	Effects of methohexitone 100μmol.litre^{-1} on the antidromic field potential.	89

Table 2.0	Mean resting potential and input resistance of CA1 neurones in the absence and presence of anaesthetic agents.	91
Fig.19	Accommodation in the presence of 5% delivered enflurane.	93
Fig.20	Accommodation and AHP responses in the presence of 5% delivered isoflurane.	95
Fig.21	Accommodation and AHP responses in the presence of 5% delivered halothane.	97
Table 3.0	Tabulation of number of spikes during an 800ms current pulse and AHP amplitude during an 80ms current pulse in the absence and presence of anaesthetic agents.	99
Fig.22A	Accommodation in response to current pulses of 0.1-0.7nA in the presence and absence of 5% enflurane.	101
Fig.22B	Graph of pooled data for the type of experiment illustrated in Fig.22A.	103
Fig.23	Effects of enflurane on accommodation and AHP amplitude in the presence of 20μmol.litre^{-1} propranolol.	105
Fig.24A	Accommodation in response to 5% delivered enflurane in the presence of cimetidine, atropine and BRL 24924.	107
Fig.24B	Graph of pooled data for the type of experiment illustrated in Fig.24A.	109
Fig.24C	Calcium spike in the absence and presence of 5% delivered enflurane.	111
Fig.25	Accommodation and AHP characteristics in the absence and presence of 200μmol.litre^{-1} ketamine.	113
Fig.26	Responses of a CA1 neurone to depolarizing current in the presence of 50μmol.litre^{-1} methohexitone.	115
Fig.27	Responses of a CA1 pyramidal neurone to 80ms depolarizing current pulses during a series of drug applications.	117
Fig.28	Intracellular responses of a CA1 neurone to orthodromic stimulation in the absence and presence of methohexitone 50μmol.litre^{-1}.	119
Fig.29	Action of methohexitone and bicuculline (both 50μmol.litre^{-1}) on intracellular orthodromic responses.	121

Fig.30	Action of methohexitone and bicuculline (both 50μmol.litre^{-1}) on intracellular responses to direct stimulation.	123
Fig.31	The effect of carbachol on M-current relaxations.	125
Fig.32	The effects of enflurane upon M-current relaxations.	127
Fig.33A	Orthodromic field potential responses within the sealed pressure chamber at 0.1MPa.	146
Fig.33B	Paired pulse potentiation within the sealed pressure chamber at 0.1MPa.	148
Fig.33C	Histogram of pooled data from orthodromic control experiments within the sealed pressure chamber.	150
Fig.33D	Histogram illustrating the variation in enhancement of the potentiated population spike within the sealed pressure chamber.	152
Fig.34A	Antidromic field potential responses at 0.1, 0.5 and 10MPa.	154
Fig.34B	Histogram illustrating the change in amplitude of the antidromic population spike at different pressures.	156
Fig.34C	Enhancement of the antidromic field potential response at elevated pressure.	158
Fig.35A	Depression of near threshold orthodromic field potential responses at elevated pressure.	160
Fig.35B	Histogram of the change in amplitude of orthodromic responses at normal and elevated pressure.	162
Fig.35C	Orthodromic field potential responses at elevated pressure.	164
Fig.35D	Enhancement of excitability of the orthodromic field potential response at high pressure.	166
Fig.36	Paired pulse potentiation at high pressure.	168
Fig.37	Example of input-output relationship for field potential responses at high pressure.	170
Fig.38A	Histogram of the amplitude of orthodromic responses in the presence of ketamine (20-1000μmol.litre^{-1}).	172
Fig.38B	Histogram illustrating the effect of ketamine on paired pulse potentiation.	174
Fig.38C	Effects of ketamine on the relationship between EPSP slope and population spike amplitude.	176

Fig.39A	Histogram showing how 50μmol.litre^{-1} methohexitone affects the amplitude of orthodromic field potential responses.	178
Fig.39B	Action of 50μmol.litre^{-1} methohexitone on paired pulse potentiation.	180
Fig.39C	Histogram illustrating how methohexitone (50μmol.litre^{-1}) alters the amplitude of the potentiated population spike.	182
Fig.39D	Effects of 50μmol.litre^{-1} methohexitone on the relationship between the EPSP slope and population spike amplitude.	184
Fig.40A	Effects of ketamine 20μmol.litre^{-1} on orthodromic field potential responses at pressure.	186
Fig.40B	Paired pulse potentiation at high pressure in the presence of 20μmol.litre^{-1} ketamine.	188
Table 4.0	Tabulation of resting membrane potential of CA1 neurones at 0.1, 0.5 and 10MPa.	190
Fig.41A	Effects of 50μmol.litre^{-1} methohexitone on orthodromic responses at high pressure.	192
Fig.41B	Paired pulse potentiation at pressure in the presence of 50μmol.litre^{-1} methohexitone.	194
Fig.42	Spontaneous depolarizing membrane potential swings at high pressure.	196
Fig.43	Intracellular responses to de- and hyperpolarizing current pulses at 5 and 10MPa.	198
Fig.44	Action potentials at 5 and 10MPa.	200
Fig.45	Graph of mean spike number in response to a depolarizing current pulse at 5 and 10MPa.	202
Fig.46	Intracellular responses of a CA1 pyramidal neurone at 5MPa.	204
Fig.47	Accommodation responses at 10MPa.	206
Fig.48	Graph showing the effect of pressure (5 and 10MPa) on AHP amplitude.	208

MOLECULAR ASPECTS OF GENERAL ANAESTHETICS.

Anaesthesia may be defined as a reversible loss of sensation (and awareness in the case of general anaesthetics). This state may be induced in animals and man by a wide variety of agents having striking structural diversity. Since the functioning of the nervous system is governed by the passage of ions through channels in the cell membrane, it seems reasonable to assume that anaesthetic molecules may act by altering the ionic permeability of some of the channels that govern nerve cell excitability. This interference may be at the level of a direct interaction with the protein molecule forming the ion channel; or an indirect action through perturbation of the surrounding lipid structure, which in turn would affect the function of ion channels embedded within it.

Classification of General Anaesthetics.

Any neuronal effects of general anaesthetic agents occur in the absence of chemical reactions and must therefore be the result of intermolecular interaction(s). A classification system has been proposed according to the type of intermolecular interaction that an anesthetic may enter into (Sandorfy, 1978; Trudeau, Dumas, Guérin & Sandorfy, 1980). Here the following categories were distinguished:-

1/ Anaesthetic molecules which are nonpolar and may only associate by dispersion forces or by dispersion forces and ion/induced dipole or dipole/induced dipole forces. For example: N_2O, Xenon, ethylene, paraffin hydrocarbons SF_6 & C_2F_6. The interactions involved here depend mainly on polarizibilities, these molecules interact preferentially with the hydrophobic parts of the membranes lipids and proteins.

2/ Molecules having appreciable dipole moments or charges can, in addition to dispersion or induction forces interact with the electrostatic forces of the ion/dipole or dipole/dipole types. Certain halocarbons like chloroform, halothane and methoxyflurane fall into this category. These molecules would be expected to interact with the polar sites in lipids or proteins. Hydrogen bonds may be formed by these molecules.

Considering the above information, and the fact that all attempts to classify anaesthetics according to their chemical structure have failed, it does not seem likely that a unitary theory (see below) can encompass all general anaesthetic agents. Such theories imply the same or similar sites of action for all anaesthetics, whether polar or non polar. It would be safer to restrict the meaning of the word "unitary" to the statement that anaesthetics exert their effects in every case by a change in intermolecular interactions (not

necessarily or exclusively hydrophobic in nature).

Lipid Theories of Anaesthesia.

The lipid theories of anaesthesia propose that general anaesthetics dissolve in the lipid portions of nerve cell membrane and alter their properties by acting at the same molecular site with the same mechanism in all cases. The lipid-hydrophobic-unitary hypothesis originated from the Meyer-Overton rule which establishes a good correlation between lipid solubility and anaesthetic potency (Meyer 1937). Here anaesthetic action would take place in the hydrophobic parts of the lipid bilayers of neurones and the anaesthetizing effect would occur when the substance attains a particular molar volume in the lipids of the cell (see Richards, 1980; Halsey, 1984).

Increased membrane fluidity has been proposed as one lipid theory (Trudell & Cohen, 1975). This view however is not universally accepted. Boggs, Young & Hsia (1976) proposed that during general anaesthesia the amount of anaesthetic in the membrane lipid is small (≈5mmoles per mole of lipid) and at such low concentrations there are no detectable changes in the fluidity of phospholipids. Studies using synaptosomal membranes have found that concentrations of halothane and barbiturates comparable to those found during anaesthesia do not increase membrane fluidity, but decrease it (Rosenberg, Eibl & Steir, 1975; Rosenberg, Jansson & Gripenberg, 1977). Also the n-alkanols (ethanol, propanol etc.) show an increasing capacity to fluidize membranes up to hexanol; then the fluidizing action decreases, until at decanol it is no longer present (Richards, Martin, Gregory, Keighley, Hesketh, Warren & Metcalfe, 1978). The effects of temperature on anaesthetic potency also show inconsistencies with the lipid solubility theory (Metcalfe & Richards, 1978; Cherkin & Catchpool, 1964). Hence increased membrane fluidity cannot by regarded as a general mechanism of anaesthetic action.

Membrane Expansion Theories.

Mullins (1954) proposed that anaesthetics, when they dissolve in the cell membrane lipids, occupy an amount of space that depends upon the concentration and size of the individual anaesthetic molecules. This led to what has been termed the critical volume hypothesis, which postulates that anaesthesia occurs when a critical volume fraction of anaesthetic agent is achieved in the cell membranes. Furthermore, it was suggested that a membrane expansion occurs, in addition to that caused by the anaesthetic molecules, which would lead to occlusion of the ion channels in the membrane thereby preventing the passage of ions. Since the underlying idea of the critical volume hypothesis is that the further membrane

expansion causes the anaesthetic action, it is predictable on thermodynamic grounds that at higher pressure the anaesthetizing effect would disappear. This therefore would accommodate the pressure reversal of anaesthesia originally observed in tadpoles and bacteria (Johnson, Brown & Marsland, 1942; Johnson and Flager, 1950) and subsequently with newts and mice (Lever, Paton, Smith & Smith, 1971). The pressure experiments of Miller, Paton, Smith and Smith (1973) yielded results that agreed more with the critical volume hypothesis than the simple lipid solubility rule (Miller, Paton, Smith & Smith, 1972). Halsey, Wardley-Smith and Green (1978), though, obtained results contrary to the critical volume hypothesis as it was proposed. Here they studied the pressure reversal produced in rats by ketamine, methohexitone, propanidid and thiopentone. The hypothesis as it stood predicted that the percentage change in anaesthetic potency should have a linear dependence on total pressure, with the same slope for all anaesthetics (Miller, Paton, Smith & Smith, 1973). Experimentally this did not occur, the degree of pressure reversal was found to vary for different agents and the slopes also varied. The critical volume hypothesis was therefore modified by Halsey, Wardley-Smith and Green (1978) and the "multi-site expansion hypothesis", involving more than one molecular site with different physical properties, was proposed. This hypothesis helped Halsey, Wardley-Smith and Wood (1986) to interpret the pressure reversal of alphaxalone/alphadolone (Althesin) and methohexitone anaesthesia in tadpoles. The potencies of these agents were decreased at high pressure, but to different extents, which was interpreted as evidence for the two agents acting at different molecular sites with different compressibilities, as predicted by the multi-site expansion hypothesis. Arguments to this effect were also voiced by Richards & White (1981). They pointed out that if the lipid-hydrophobic-unitary hypothesis of anaesthesia were true then the effects of anaesthetics in a mixture should be additive. This did apply in several cases (for example: alphaxalone and propanidid, alphaxalone and alphadolone, propanidid and methohexitone), but in other cases (alphaxalone and etomidate, alphaxalone and methohexitone) a greater potency than would be suggested by strict additivity was exhibited. These synergic effects were interpreted as evidence against the lipid-hydrophobic-unitary hypothesis and were in favour of the assumption that the two components act at different sites in the neuronal membrane. This would appear to favour a multi site hypothesis like the "degenerative perturbation hypothesis" which Richards, Martin, Gregory, Keighley, Hesketh, Smith, Warren & Metcalfe (1978) had suggested earlier.

The Meyer-Overton relationship suggests that uptake in the lipid phase of the membrane is a condition for the production of anaesthesia. This does

not necessarily mean that it is the mechanism of anaesthesia. The weight of experimental evidence suggests that anaesthetic agents may act at disparate sites with the net effect of inducing an anaesthetized state. Therefore, at the present time, the question of the molecular mechanism(s) underlying anaesthesia still remains to be resolved.

CELLULAR ACTIONS OF GENERAL ANAESTHETICS.

Turning to the cellular actions of anaesthetic agents we must consider the level at which agents interfere with the function of neurones within the central nervous system. Do anaesthetics act by impeding the propagation of action potentials along axons or by directly altering the process of synaptic transmission?

Anaesthetics and Excitatory Synaptic Transmission.

There can be little doubt that general anaesthetics perturb synaptic function and that this effect contributes towards the state of anaesthesia. Following on from the early observations of Sowton & Sherrington (1905) that chloroform inhibits monosynaptic reflexes at lower concentrations than those required for blockade of action potential propagation in motor nerves, a wealth of data has evolved regarding anaesthetic depression of synaptic activity. Both in vivo and in vitro experiments have determined that anaesthetics depress excitatory synaptic transmission (Larabee & Posternack, 1952; Løyning, Oshima & Yokota, 1964; Matthews & Quilliam, 1964; Richards 1972, 1973, 1978, 1982, 1985; MacIver & Roth, 1989; el-Beheiry & Puil, 1989; Pearce, Stringer & Lothman, 1989). This depression has been shown in many experiments to be due to the depression of EPSPs by the anaesthetic (for example, Richards, 1972, 1973, Løyning, Oshima & Yokota, 1964).

Experiments have also been performed to determine whether the depression of excitatory synaptic transmission is due to decreased impulse conduction. Small diameter fibres of peripheral nerves have been shown to be more readily blocked by general anaesthetics than larger fibres (Uehara, 1960), and small myelinated fibres of the rat hippocampus are more sensitive to blockade than larger myelinated fibres (Berg-Johnsen & Langmoen, 1986). Should the effects of general anaesthetics on synaptic transmission be due to impairment of conduction in the presynaptic fibres it would be expected that a decrease in amplitude of the synaptic potential be accompanied by an increase in its latency. No such increase has been found experimentally (Berg-Johnson & Langmoen, 1986; Oshima & Richards, 1988; Richards & White, 1975; Richards, 1980). These agents cause a progressive decline in the EPSP amplitude before any discernable effect upon the action

potential. So it may appear that general anaesthetic action is mainly at the level of the synapse and not by disturbing impulse conduction in nerve axons. Because the net result is depressed excitatory transmission, the action of the general anaesthetics could be to decrease the release of excitatory neurotransmitter from presynaptic nerves and/or to desensitize postsynaptic receptors to released neurotransmitter. Presynaptically it is clear that general anaesthetics disturb the evoked release of neurotransmitter. A number of general anaesthetics depress the acetylecholine output evoked from in vitro preparations of mammalian superior-cervical ganglion, the ileum, neuromuscular junction and rat brain (Matthews and Quilliam, 1964; Speden, 1965; Richter & Waller, 1977). General anaesthetics, though, do not exclusively depress synaptic transmission, but have also been found to enhance the release of neurotransmitter: for example the endogenous release of acetylecholine from the guinea pig ileum is increased by some anaesthetic gases (Halliday, Little and Paton, 1979) and the convulsant barbiturate 5-(2-cyclohexylidene-ethyl)-5-ethyl barbituric acid (CHEB) enhances acetylecholine release from rat brain (Holtman & Richter, 1983), although this may be an indirect effect due to the excitatory nature of this agent. The weight of electrophysiological evidence suggests that evoked excitatory synaptic transmission in the central nervous system is sensitive to depression by general anaesthetic agents (Somjen, 1963; Somjen & Gill, 1963; Weakly, 1969; Richards, 1972, 1982, 1985; & see above).

Postsynaptic Actions of General Anaesthetics.

Effects on Ion Movements

The mechanism of anaesthetic induced synaptic depression has been the focus of several studies. Anaesthetics may interfere with the role of Ca^{2+}. Barbiturates and high concentrations of ethanol depress voltage-dependent $^{45}Ca^{2+}$ influx (Blaustein & Ector, 1975; Elrod & Leslie, 1980; Harris & Hood, 1980). Also Na^{+}-dependent Ca^{2+}-ATPase is stimulated by ethanol and pentobarbitone (Yamamoto & Harris, 1983) and the Ca^{2+} buffering capacity of mitochondria is sensitive to pentobarbitone (Pincus & Hsiao, 1981). There are at least three types of voltage gated Ca^{2+} channel; denoted L, N and T (Nowycky, Fox & Tsien, 1985), any of which could be a target for anaesthetic action. It is quite likely that in presynaptic nerve terminals the combined actions of anaesthetics on Ca^{2+} movements could modify Ca^{2+} levels which in turn would lead to the depressed release of neurotransmitter.

The action of anaesthetics upon voltage-gated ion channels has attracted surprisingly little interest. The early inward sodium current has been shown

to be depressed (Blaustein, 1968; Haydon & Urban, 1982 a,b,c; Armstrong & Binstock, 1964; Johnson, Yang, Zormumski & Kleinhaus, 1989) by alcohols and a range of anaesthetic agents, as has the late outward K^+ current (Harper, MacDonald & Wann, 1983; Moore, Ulbricht & Takata, 1964; see Elliot & Haydon, 1989 for review). These data though are derived mostly from the squid giant axon preparation and mainly involved the use of high concentrations of anaesthetic agents.

It seems unlikely that anaesthetics exert their effects by depleting vesicular transmitter stores. Akeson & Deamer (1989) demonstrated that anaesthetics do not cause a major depletion of catecholamines from isolated chromaffin granules. Also there was no discernable leakage from bovine chromaffin cells (whether intact or permeabilized) (Pocock & Richards, 1987, 1988). These experiments examined the effects of pentobarbitone, minoxalone and methoxyflurane on the relationship between intracellular free Ca^{2+} and catecholamine secretion. This was to distinguish whether the depression was due to direct effects on a voltage-gated Ca^{2+} channel(s) or on the intracellular events leading to exocytosis. The anaesthetic induced depression of catecholamine secretion was parallelled by an inhibition of Ca^{2+} influx and found not to be due to an effect on the relationship between intracellular free Ca^{2+} and secretion.

A similar depression of K^+ evoked Ca^{2+} influx has been reported in rat brain synaptosomes exposed to barbiturates (Blaustein and Ector, 1975), and barbiturates have been found to decrease the voltage-dependent Ca^{2+} conductance of cultured neurones (Werz & Macdonald, 1985). From the above evidence it may be concluded that most general anaesthetics inhibit depolarization induced secretion of transmitters by inhibiting Ca^{2+} influx through voltage gated Ca^{2+} channels. Exceptions are halothane and ketamine which have been found to have little depressant effect on K^+ evoked Ca^{2+} influx or neurosecretion (Pocock & Richards, 1988; Oshima & Richards, 1988; Takara, Wada, Arita, Sumikawa & Izumi, 1986).

Effects Upon Receptor Systems.

The postsynaptic effects of general anaesthetics have been the focus of numerous studies. As mentioned earlier, anaesthetics must disturb the movement of ions through ion channels in the cell membrane. Ligand operated ion channels may have their function compromised by anaesthetics acting to promote desensitization of the receptor, thereby reducing the number of active channels. They may also reduce the specific conductance of the channels or reduce the channel open time. The effect of general anaesthetics on the response to microiontophoretically applied aspartate and

glutamate in the CNS has received some attention. Studies by Crawford and Curtis (1966, 1970) illustrated that the barbiturates reduced the sensitivity of cerebral cortex neurones to glutamate. This study was confirmed and extended to show that this was true of certain inhalational anaesthetics also (Richards, Russel & Smaje, 1975; Richards & Smaje, 1976). Halothane, however, was one exception (Richards & Smaje, 1986; Crawford, 1970); responses were resistant at anaesthetic concentrations of this agent. Enhancement of glutamate induced firing has also been reported by Catchglove, Krnjević and Maretic (1972). Subsequent to these studies, the glutamate receptor has been divided into three subtypes named: N-methyl D-aspartate (NMDA), kainate and quisqualate (Watkins, 1984). Ketamine and phencyclidine have been shown to depress responses of the NMDA subclass (Lodge & Anis 1982, 1984; Anis, Berry, Burton & Lodge, 1983; Lodge, Anis & Burton 1982). Pentobarbitone has been shown to depress the sensitivity of hippocampal neurones to all three agonists with quisqualate showing greatest sensitivity (Sawada & Yamomoto, 1985). Anaesthetics have also been shown to have a direct effect upon the nicotinic receptor or the processes associated with its activation (Gothert, Dorn & Loewenstein, 1976; Gothert & Wendt, 1977; Holmes, 1973; Pocock & Richards, 1987, 1988). Pentobarbitone, halothane and methoxyflurane all inhibit carbachol-induced catecholamine secretion in a non-competitive manner (Pocock & Richards, 1987, 1988). These anaesthetics also inhibit the influx of both Na^+ and Ca^{2+} ions evoked by carbachol. The inhibition of Ca^{2+} influx closely parallelled the inhibition of catecholamine release. This, when the failure of agents such as halothane to inhibit either the catecholamine secretion or Ca^{2+} influx evoked by direct depolarization is taken into account, may suggest that anaesthetics could inhibit Ca^{2+} movement mediated by activation of the nicotinic receptor in addition to their effects upon voltage-gated Ca^{2+} channels. Certain anaesthetics have also been shown to promote desensitization of the nicotinic receptor of Torpedo electroplax to acetylcholine (Boyd & Cohen, 1984; Firestone, Sauter, Braswell & Miller, 1986; Heidmann, Oswald, Changeaux, 1983; Miller, Firestone, & Forman, 1987). For barbiturates this appears to result from an increase in high affinity binding of acetylcholine to the receptor from which it is slow to dissociate. This effectively reduces the number of active receptors and could contribute to the depression of excitatory synaptic transmission. Non-competitive reduction of the rate of decay of nerve evoked endplate currents has been described for the barbiturates (Adams, 1976), this observation being consistent with anaesthetic molecules blocking the active receptor-channel complex. The view that the open state is made less stable by anaesthetics (Gage & McKinnon, 1985) is supported by recent patch clamp work (Jacobson, Pocock & Richards, 1989). Here pentobarbitone

reduced the open time of nicotinic channels of adrenal chromaffin cells without altering their specific conductance. Similar actions of ketamine have been reported (Wachtel, 1988).

Anaesthetics and the Inhibitory Synapse.

Anaesthetics have been shown to both enhance (Eccles, Schmidt & Willis, 1963; Gage & Robertson, 1985; Nicoll, Eccles, Oshima & Rubia, 1975; Scholfield, 1980) and depress (Eccles, Schmidt & Willis, 1963; el-Beheiry & Puil, 1989) inhibitory processes. The barbiturates have been found to mainly enhance inhibitory mechanisms, whereas ether and isoflurane were depressant. A variety of responses have also been reported for the action of other volatile anaesthetics (Yoshimura, Higashi, Fujita & Shimoji, 1985; Shimoji, Fujikoa & Ebata, 1984). An explanation for the differences in anaesthetic action at excitatory and inhibitory synapses may lie in the differing nature of their neurotransmitters and the characteristics of their postsynaptic receptors. Possibly anaesthetics depress neurotransmitter release at both types of synapse, but interactions at the postsynaptic receptor-channel complex may be responsible for their different actions.

The actions of anaesthetic agents on GABAergic inhibitory transmission has received a considerable amount of attention. The barbiturates enhance synaptic inhibition mediated by GABA (Nicoll, 1972; Nicoll, Eccles, Oshima & Rubia, 1985; Barker & Ransom, 1978; Brown & Constanti, 1978). This action is also shared by ethanol (Banna, 1969; Nestoros, 1980), etomidate (Evans & Hill, 1978) and ketamine (Little & Atkinson, 1984). The increase in the effect of GABA is apparently not the result of either a decrease in its uptake or an increase in affinity of the receptor for GABA (Jessel & Richards, 1977). Cl^- current measurements support this view (Peters, Kirkness, Callachan, Lambert & Turner, 1988) and this study also showed that barbiturates could directly activate Cl^- conductances in some cells (see also Jacobson, Pocock & Richards, 1989). Considering that metabolism (Nicoll, 1972) or release (usually depressed: Jessel & Richards, 1977; Cutler, Markowitz & Dudzinski, 1974) cannot explain the effect of anaesthetics on GABAergic transmission, it is reasonable to suggest that the effect may principally be explained by an effect upon the postsynaptic $GABA_A$ receptor channel complex. Measurements of Cl^- currents activated by GABA in chromaffin cells support this view (Peters, Kirkness, Callachan, Lambert & Turner, 1988) and results from cultured adrenal medulla cells provide further evidence (Jacobson, Pocock & Richards, 1989). Therefore the GABA receptor Cl^- channel complex would appear to be another target for anaesthetic molecules.

In addition to their depressant effects at the synapse, anaesthetics have been shown to have other subtle effects, some of which may contribute towards anaesthesia and others toward the "excitant" effects seen with some anaesthetics. The introduction to part I of the thesis will deal with reviewing the relevant data with particular reference to anaesthetic induced disturbance of K^+ channel function.

HIGH PRESSURE AND THE NERVOUS SYSTEM.

When unanaesthetized animals and man are exposed to high pressures of helium, various neurological symptoms occur which contribute to what has been termed the high pressure neurological syndrome (HPNS) (Brauer, 1975; Bennet, 1975; Rostain, 1980; Halsey, 1982; Brauer, 1984). The symptoms, principally described by Hunter & Bennett (1974), consist of EEG changes (Bennett, 1975; Rostain, 1980), muscle tremors (Hunter & Bennett, 1974) and, at higher pressures, two types of seizure; type I or clonic and type II or tonic (Brauer, Mansfield, Beaver & Gillen, 1979). Associated with these disturbances are other effects such as an interference with co-ordination, disorientation, nausea and deficits in attention and memory. The symptoms reflect neuronal excitability and/or hyperactivity of various structures of the brain (Rostain, 1980; Brauer, Mansfield, Beaver & Gillen, 1979) and spinal cord (Fagini, Weiss, Pellet & Hugon, 1982). Traditional explanations for this enhanced neuronal excitability have included:

1.Facilitated excitatory synaptic transmission.

2.Depressed inhibitory synaptic transmission.

In addition to these traditional ideas one further possibility must be considered:

3.Abnormal behaviour of ion channel systems, particulary those carrying K^+ currents (pressure may intervene at this level and directly alter neuronal excitability. This in turn, would affect synaptic efficiency).

Synaptic Effects of High Pressure.

Pressure Effects on Excitatory Synaptic Transmission

In vitro electrophysiological analysis of various peripheral nerves and synapses has illustrated that high pressure invariably depresses excitatory synaptic transmission, regardless of the neurotransmitter or species involved. Pressures of around 10MPa (hydrostatic) reversibly depress excitatory synaptic transmission in the crustacean and amphibian neuromuscular junction (Campenot, 1975; Ashford, Macdonald & Wann, 1982) and in Helix and Aplysia neurones (Wann, Macdonald & Harper, 1979; Parmentier, Shistrav & Bennet, 1981). Helium pressure ($\leq$13.7MPa) severely depressed fast and slow excitatory synaptic transmission in the isolated rat superior cervical ganglion (Kendig, Trudell & Cohen, 1975).

Neuromuscular transmission was similarly depressed in the isolated rat diaphragm (Kendig & Cohen, 1976). More recently, experiments using the rat hippocampal slice preparation, the only mammalian central nervous structure studied electrophysiologically at pressure in vitro thus far, have suggested that excitatory synapses are similarly depressed by pressure (helium ≤9MPa) (Fagni, Zinebi & Hugon, 1987).

Pressure Effects on Inhibitory Synaptic Transmission

The action of pressure on inhibitory synapses still has to be investigated in depth. From the data available it seems that synaptic transmission is also depressed. Hydrostatic pressure (6.8MPa) has been shown to suppress depolarization induced Ca^{2+}-dependent release of ^{3H}GABA and ^{3H}glycine in guinea pig spinal cord preparations (Gilman, Colton & Dukta, 1988) and ^{3H}GABA release in cerebrocortical synaptosomal preparations (Gilman, Colton & Hallenbeck, 1986). The amplitude of the postsynaptic spontaneous IPSP in Helix neurones seems to be reduced by pressure (hydrostatic 5MPa), (Wann, Macdonald & Harper, 1979) and the release of GABA at the crustacean neuromuscular junction is depressed by pressure (helium, 0.4MPa) (Colton & Colton, 1982). In the rat hippocampus, pressure (helium, 5 or 7MPa) reduces the efficiency of GABAergic inhibitory transmission (Zinebi, Fagni & Hugon, 1988).

The available data for both excitatory and inhibitory synaptic transmission suggests that synaptic depression is a universal effect of pressure on excitable cells. The exocytosic process in both mast cells and chromaffin cells is very sensitive to pressure, and it is thought that pressure inhibits release at one of the later stages of the reaction chain (Heinemann, Conti, Stühmer & Neher, 1987). If such presynaptic depression does occur in the CNS then it seems difficult to ascribe any excitant actions of pressure to action at the synapse (unless excitatory and inhibitory synapse function are depressed to differing extents giving rise to net excitation).

Pressure Effects on Synaptic Receptors

The acetylcholine (ACh) receptor channel system has been studied both biochemically and electrophysiologically. Here it was reported that high pressure (helium, ≤30MPa) affects conformational transitions within the system (Sauter, Braswell, Wankowicz & Miller, 1981). This results in a reduction in ACh binding which could explain the observed reduction in amplitude of the miniature endplate current in the frog neuromuscular junction (Ashford, Macdonald & Wann, 1982a; 1982b). The time constant of decay is increased by 33% at 15MPa suggesting that channel lifetime is increased. Indeed subsequent patch clamp studies using embryonic rat

muscle illustrated that pressure (hydrostatic, ≤40MPa) does not affect single channel conductance but increases mean open and closed times of the ACh receptor channel (Heinemann, Stühmer & Conti, 1987): (Considering the extremely high pressures attained here, one must question the relevance of this data to the HPNS).

Postsynaptic Effects of High Pressure.

Neuronal excitability could be modified by high pressure at the level of the ion channels in the cell membrane. The excitability of neurones is dependent upon the differential distribution and movement of ions across the cell membrane. Therefore any modification of the permeability of the membrane to various ions will alter neuronal excitability.

Pressure Effects on Resting Membrane Potential

The available data for resting membrane potential changes at pressure shows that there may be no change, as for squid axon (helium 20.4MPa, Henderson & Gilbert, 1975; hydrostatic 34MPa, Spyropolous, 1957a), or that cells depolarize, as for Helix neurones (hydrostatic 5-35MPa, Wann, Harper, Wilcock & Macdonald, 1977; Wann, Macdonald & Harper, 1979) and lobster muscle fibres (hydrostatic 20MPa, Campenot, 1975).

Voltage Gated Ion Channels

High pressure has been shown to increase the duration of the action potential in amphibian nerve (hydrostatic, 66.6MPa) (Spyropolous, 1957b) and muscle (helium, <20MPa), squid giant axon (hydrostatic, 20 MPa; 34 MPa) (Wann, Macdonald, Harper & Wilcock, 1979; Spyropolous, 1957), mammalian preganglionic sympathetic neurones (helium, 3.5-10.3MPa) (Kending, Trudell & Cohen, 1975) and molluscan neurones (hydrostatic, 5 - 36 MPa) (Wann, Macdonald & Harper, 1979). The basis of this change was investigated in squid axon using the voltage-clamp technique and it was found that the lengthening of the action potential in some preparations was due to a decrease in both peak depolarization and repolarization rates. The peak inward Na^+ current is invariably reduced (Conti, Fioravanti, Segal & Stühmer, 1982a) and the Hodgkin-Huxley outward K^+ current is either reduced, as in Helix neurones (hydrostatic,20.8MPa) (Harper, Macdonald & Wann, 1981) and amphibian myelinated nerve (helium, ≤10MPa) (Kendig, 1984), or unaffected as in the case of the squid giant axon (helium, 20.4MPa) (Henderson & Gilbert, 1975; Conti, Fioravanti, Segal & Stühmer, 1982a). Whole cell Na^+ and Ca^{2+} currents in bovine adrenal chromaffin cells have been measured up to 40MPa (hydrostatic) (Heineman, Conti, Stühmer & Neher, 1987). Na^+ currents were mildly affected with mean amplitude and gating kinetics depressed by 20% at 10MPa.

Of particular interest to any excitatory effects of pressure must be any abnormal behaviour of the K^+ currents that control the firing rates of excitable cells. The M-current (Segal, Rogawski & Barker, 1984; Adams, Brown & Constanti, 1982; Halliwell & Adams, 1982; Brown, 1988), A-current (Connor & Stevens, 1971; Neher, 1971) and Ca^{2+}-dependent K^+ currents (Madison & Nicoll, 1984) must be strongly considered as potential targets for high pressure. Indeed high hydrostatic pressure has been shown to reversibly decrease the A-current in Helix neurones, contributing to increased spontaneous nerve discharge (Harper, Macdonald & Wann, 1981). This effect of pressure is consistent with its ability to induce hyperexcitability. Although high pressures were needed in the Helix preparation (20.8MPa), K^+ currents at high pressure merit study in the mammalian CNS.

Anaesthetic Protection Against the HPNS.

Another facet to the HPNS is that some, but by no means all, anaesthetics can reduce the severity and delay the onset of the motor disturbances in whole animals (Halsey, 1982). It must be noted however that certain anaesthetics may actually worsen some aspects of the HPNS. For example the intravenous agent methohexitone facilitates pressure-induced convulsions in rats and mice (Green, Halsey & Wardley-Smith, 1977; Shearer, Ross & Manson, 1981). The intravenous agents ketamine and Althesin (mixture of alphaxalone/alphadolone) protect against the onset of tremor and delay convulsions at sub-anaesthetic doses in the rat (Green, Halsey & Wardley-Smith, 1977; Bailey, Green, Halsey & Wardley-Smith, 1977). The basis of this protection is at present unknown. One possibility is the enhancement by anaesthetics of GABAergic inhibitory transmission (see above). There are drawbacks to taking this simplistic view of protection, for example both methohexitone and ketamine prolong the inhibitory conductance change due to GABA in the guinea pig olfactory cortex in vitro (Scholfield, 1980), yet as noted earlier, ketamine protects against, and methohexitone facilitates pressure symptoms (Green, Halsey & Wardley-Smith, 1977). Exactly how anaesthetics afford protection against the HPNS remains unclear.

An explaination for anaesthetic protection against pressure induced symptoms must take into account certain observations. Firstly it is unclear how the ability of an anaesthetic to reverse pressure effects, and the ability of pressure to reverse the anesthetic effect, correspond. One would expect, that, if common molecular events were involved, an agent that was effectively reversed by high pressure would give little protection against the HPNS (i.e. high pressure is the more potent influence at the molecular

target). This would appear to be the case for the example of ketamine and methohexitone in rats. Thus methohexitone offers little protection against the HPNS and its action is relatively easily reversed by pressure (Halsey, Wardley-Smith & Green, 1978). The opposite being true for ketamine. But it must also be stressed that in mice the ability of the barbiturates to protect against HPNS convulsions is unrelated to their behaviour as pressure reversed anaesthetics (Beaver, Brauer & Lahser, 1977). Secondly, anaesthetic potency and effectiveness in protecting against the HPNS do not always correlate (Brauer, Goldman, Beaver & Sheehan, 1974; Green, Halsey & Wardley-Smith, 1977). The gases N_2, H_2 and N_2O illustrate a reasonable correlation, yet certain intravenous agents including ketamine do not. Thirdly, particular phases of the HPNS differ in their sensitivity to anaesthetic agents (Brauer, Goldman, Beaver & Sheehan, 1974; Rowland-James, Wilson & Miller, 1981).

Pressure Reversal of Anaesthesia.

The pressure reversal of anaesthesia remains perhaps one of the most striking of all of the pressure-anaesthetic interactions in whole animals. The original observation linking a high pressure-anaesthetic interaction was made using a luminescent bacterium (Johnson, Brown & Marsland, 1942). This observation stimulated interest using whole animals and pressure (hydrostatic, 20.4MPa) reversal was demonstrated for tadpoles anaesthetized with a solution of ethanol and urethane (Johnson & Flager, 1950). These observations have subsequently been expanded and substantiated to include pressure reversal of anaesthesia in mammals (Brauer, Goldman, Beaver & Sheehan, 1974; Rowland-James, Wilson & Miller, 1981; Beaver, Brauer & Lahser, 1977; Green, Halsey & Wardley-Smith, 1977; Bailey, Green, Halsey & Wardley-Smith, 1974; Halsey, Wardley-Smith & Green, 1978; Dundas, 1979). The opposing effect of pressure is usually revealed by the appearance of spontaneous motor activity or of motor response to electric shock. In addressing the question of how this phenomenon occurs we must again consider the site of action of anaesthetics (see above for a more detailed account). Excitatory synaptic transmission has been shown to be very sensitive to anaesthetics, being depressed at concentrations lower than those required to block impulse conduction. However, the effects of anaesthetics (for example: halothane, chloroform, methoxyflurane) and helium pressure have been found to be additive at the synapse (Kendig, Trudell & Cohen, 1975; Kendig & Cohen, 1976). Helium pressure has also been shown to reverse the depression of the frog sciatic nerve compound action potential amplitude produced by nitrous oxide (Roth, Smith & Paton, 1976) and the compound action potential of rat preganglionic sympathetic fibres exposed to general

anaesthetics (Kendig, Trudell & Cohen, 1975: Kendig & Cohen, 1977). One cannot take a simplistic view of this phenomenon and argue that there is a direct antagonism between pressure and the anaesthetic agent at a single site (for example the synapse). It is more probable that there will be action at disparate sites to give the net result of reversal of anaesthesia. Indirect antagonism of the anaesthetic effect of pentobarbitone has been demonstrated at the lobster neuromuscular junction (Kending, Grossman & MacIver, 1988). Here both pressure and the anaesthetic depressed singly evoked excitatory junction potentials, but both pressure and pentobarbitone antagonized their own depressant actions by enhancing tetanic potentiation. This enhancement antagonized the anaesthetic induced depression of high pressure (helium, 10.1MPa). Here it is argued that the relief of depression was largely due to presynaptic actions, with additivity of anaesthetic and pressure responses being responsible for the "pressure reversal" effect. Clearly, from the data available, the pressure reversal of anaesthesia is a poorly understood phenomenon which warrants further study.

METHODS

A.Slice Preparation

All experiments were carried out on CA1 pyramidal neurones of hippocampal slices obtained from 5 to 7 week old Sprague-Dawley rats of either sex. Animals were stunned by a sudden blow to the upper body and the head removed. The scalp was opened along the midline and pulled down laterally, the skull then being cut along the central suture with a pair of sharp scissors. Bone forceps were then employed to reflect the bone, and the dura carefully removed with a small spatula. Insertion of a small spatula behind the cerebellum and under the brain facilitated its removal from the skull cavity. The brain was then placed on filter paper moistened with artificial cerebrospinal fluid (ACSF), and washed with cold (<4°C), oxygenated ACSF. The cerebellum and brainstem were then removed and the cerebral hemispheres separated using a razor blade. The right hand hemisphere was glued, medial aspect down, to the slicing stage with cyanoacrylate adhesive (Permabond DP-10 superglue). Chilled oxygenated ASCF was added to the slicing chamber and a Vibroslice (Campden Instruments U.K. Ltd.) fitted with a Gillette "Valet" blade was used to slice the tissue. The usual protocol was to discard the first 3.7mm of tissue (by slicing sequentially 2mm, 1mm and 700μm from the uppermost surface). 3 slices of 400μm were then cut and transferred, using an angled glass shovel, to a Petri dish. The Petri dish had been lined previously with black Sylgard and was filled with cold, oxygenated ACSF. The hippocampal area was then dissected free with a scalpel and the slices (Fig.1) transferred to the incubation chamber using a broken back Pasteur pipette.

B.Incubation

The slices were held in a Perspex incubation chamber of ≈15ml volume. The chamber was perforated with small holes and contained a nylon mesh platform, upon which the slices rest. The chamber was suspended in a Buchner funnel (volume≈150ml) filled with ACSF continually bubbled with 95% O_2/5% CO_2. The slices were incubated here for at least 1 hour (range 1-12 hrs) at room temperature (20-23°C) before selection and transference of a slice to the recording chamber.

C.Recording Arrangements

Anaesthetic Work at 1 Atmosphere

For the anaesthetic work at atmospheric pressure a perspex recording chamber similar in principle to the chamber used by Brown & Halliwell (1981) held the brain slice. It was fitted with 4 coarse manipulators, each providing downward ("Z") movement. The slice lay on a stainless steel mesh gently restrained by nylon strands fixed to a stainless steel "bat" attached to one of the

manipulators. The slice was totally submerged in the recording chamber and perfused with oxygenated artificial cerebrospinal fluid (ACSF) at a rate of 3ml.min^{-1}. The solution flowed under gravity from a water jacketed reservoir (volume≈40ml, heated to 40°-45°C) into the chamber at 28°-30°C and was aspirated via a bent syringe needle in the chamber side channel. Solutions were recirculated by means of a peristaltic pump (Gilson Minipuls 2). Stimulating and voltage recording electrodes were carried in mechanical (Prior) and hydraulic (Narishige, M0103N) manipulators respectively and could be placed in structures identified under visual control with the aid of a dissection microscope (Nikon Stereozoom, SM2-2B). Slices were illuminated with a fibre optic light source (Schott, KL1500). All of the manipulators and the tissue chamber were mounted on an air table (Wentworth Laboratories, AUT 701) to isolate the recording apparatus from external vibration.

High Pressure Experimental Apparatus

The pressure chamber is illustrated in Figs.2 & 3. It was horizontal and manufactured by Magpie (Fraserburgh, Scotland) from a steel cylinder (ST-52-3) to which flanges were welded at either end. It was 85cm long having internal diameter of 33cm. The cylinder wall thickness was 36mm and the internal capacity 58 litres. The flanges accepted 2 doors, the undercarriages of which were mounted on 4 wheels which ran on V-shaped rails. The doors were secured by 16 bolts (45mm diameter) and were pressure sealed by neoprene (70 SK) O rings. The working and hydrostatic test pressures were 20 and 28.5 MPa (Mega Pascals) respectively. A fan (Papst Multifan, 8312)/radiator assembly was mounted on one door and a cradle mounted on the other (see Fig.2 & 3). The fan/radiator assembly both mixed gases and allowed fine temperature control of the chamber interior, this being normally kept at 28°C by circulating hot water through rubber tubing coiled around the chamber body. This temperature alongside the heated tissue chamber assembly (see below) minimised heat loss from the preparation due to the high thermal conductivity of helium. If the temperature rose or fell to unacceptable levels, water (10°-60°C) was circulated through the radiator assembly to adjust it to acceptable levels. Temperature measurement was made with a YSI precision thermistor (4403) and a telethermometer (Yellow Springs Instrument Co. Ltd.). The thermistor was immersed in the ACSF flowing through the tissue bath. The cradle supported the apparatus that allowed recordings to be made from brain slices (See Figs.4 & 5). This duplicated as closely as possible the apparatus used at atmospheric pressure. It consisted of a recording chamber differing only by being manufactured partly from aluminium, having a perspex insert holding the tissue chamber. Embedded in the aluminium block was a heating element allowing control of the tissue chamber temperature (between 30°-34°C, at 20°C ambient). 3 coarse manipulators providing "Z" movement were mounted on the

aluminium block. Again the slice lay on a stainless steel mesh gently restrained by the nylon strands fixed to a stainless steel bat. The slice was totally submerged and perfused with ACSF from a small "header" tank (volume 15ml, Fig.4). The total ACSF volume was 150ml, it was continuously bubbled (prior to door closure) with 95% O_2/5% CO_2 and circulated via a 4 channel peristaltic pump (M4V, Schuco Scientific Ltd.) mounted on a small platform attached to the cradle assembly. The recording chamber was mounted on a microscope stage providing movement in 2 directions (X & Y). The microscope stage being mounted on the cradle, consisting of 2 perspex discs and 3 Duralumin (15.9 mm) rods, mounted on the inner face of one of the chamber doors. A horizontal rod clamped at a right angle between the Duralumin supporting rods held a coarse manipulator (Prior) which was used to position a stimulating electrode on the slice (Stratum radiatum or alveus; Fig.1). The headstage amplifier was held by an electrically driven micromanipulator (DC3-K, Micro Instruments Ltd.) clamped to one of the horizontal Duralumin rods. A remote control unit (MS-316) for the micromanipulator allowing steps as small as 1μm was mounted on a shelf below the large view port (Fig.2). 2 view ports allowed viewing and illumination of the slice within the chamber. A conical viewport (136 x 50mm) provided a view of the length of the chamber interior and was used to view the preparation with the aid of a Nikon Stererozoom (SM2-2B) microscope (working distance 20cm, x12 magnification). A flat viewport set at a 20° angle to the conical viewport was used to facilitate illumination via the end of the swan neck fibre optic pipes of a Schott KL 1500 light source.

Gases (Helium and 95% O_2/5% CO_2) were supplied from cylinders via a control panel on which were mounted high pressure self venting regulators (44-1100, Tescon or PR 56, Go Inc. Products; Fig.6) and associated ball and needle valves (Whitey Co.). All connections were of copper tubing (4mm i.d., wall thickness 1mm). A total of 5 gas inlet/outlets were available on the chamber; 4 were used: 1 each for helium, oxygen, decompression and the pressure gauges (0-30 and 0-2000 psi, Nagretti and Zambra).

Electrical connections were made via Conax electrical feed-throughs (Tg 2016 Hellerman Electronic Components Ltd.) mounted on the door holding the cradle assembly. A total of 64 connections through 4 glands were possible, although all of these were not utilized (Fig.7).

For all experiments involving the pressure chamber, viability of the slice was tested with the cradle door open. Once satisfied with the responses from the slice, the recording electrode was usually withdrawn from the slice (sometimes this was left in situ for field potential recordings) and the door slowly and gently closed. Bubbling of the ACSF would stop for a short period (1-1.5 mins) during

bolt tightening and the chamber would then be pressurized to 0.13MPa with 95% O_2/ 5% CO_2 (total chamber Po_2 0.05MPa). Under these conditions stable intracellular and field potential recordings could be obtained from slices at 0.05MPa O_2 within the chamber for periods exceeding 2 hours (Fig.8), the characteristics of neurones at 0.13MPa being indistinguishable from those at 0.1MPa with the door open. The gas tensions and pH of liquid samples from the tissue chamber (at 0.13MPa) were measured by connecting fine tubing to a stainless steel pipe penetrating the door. A needle valve on the outside of the door allowed ACSF to be drawn off. At 36°-37°C (typical sealed chamber temperature) representative values after 1 hour were Po_2=0.048MPa, Pco_2=0.0036MPa and pH 7.54 (measured by blood gas analyzer, Instrumentation Laboratory System, 1302). These values lie within the range of values obtained when making viable recordings from slices outside the chamber. Sampling the gas in the chamber via the decompression line with a Servomex O_2 analyzer (OA 272) gave Po_2 values between 0.05-0.075MPa.

In 5 independent experiments (conducted using the cradle assembly with the door open) the Po_2 of the ACSF was allowed to fall by switching off the delivery gas (95%O_2/5%CO_2) to the reservoir of ACSF. Cutting off the supply of O_2/CO_2 leads to a drop in Po_2 and rise in pH (due to low Pco_2) of the ACSF. Under these conditions the neurones depolarize markedly (up to 15mV) within 10 min. The effect is usually reversible if oxygenation is resumed within 10 min. Neurones were still found to accommodate even when markedly depolarized. These experiments indicate some of the consequences of reducing the O_2 supply in intracellular experiments. In field potential experiments low Po_2 leads to much diminished orthodromic responses (usually total depression at 0.02MPa O_2) which is normally reversible upon return to 0.05MPa O_2 (See Lipton & Whittingham, 1979). The fact that stable orthodromic responses could be elicited from field potentials over a period of time (Fig.8) and that recorded resting potentials were within the range considered normal, served as an independent guide to the adequacy of oxygenation within the pressure chamber.

For the orthodromic field potential measurements involving high pressure and the actions of anaesthetic agents at elevated pressure a high pressure liquid chromatography (HPLC) pump (Kontron 414) was used to deliver solutions (Fig.7). Solutions were bubbled with 95% O_2/5% CO_2 outside the chamber and then pumped through the chamber door to a small header tank (volume≈5ml) within the chamber. In this case solutions were not recirculated, but aspirated and then pumped into a large waste ACSF beaker within the chamber. This method allowed fast switching of solutions at pressure and provided a means of studying water-soluble anaesthetic effects at high pressure. Using this method the experiment approaches a purely hydrostatic pressure experiment and allows

us to be more certain that any described effects are due to elevated pressure and not helium. Supplementary oxygen was not added to the chamber for these experiments.

D.Stimulation and Recording

Both Atmospheric and High Pressure Recording

Orthodromic responses were evoked by placing a unipolar tungsten wire electrode of 50μm diameter (WT-2T, Clarke Electromedical) in the stratum radiatum. Antidromic responses were elicited by stimulation of the alveus using an identical electrode (Figs.1 & 4). Digitimer DS2 constant current stimulators were used in both cases.

Conventional intra- and extracellular recording techniques were employed. For extracellular recording broken back microelectrodes (5μm tip) typically had resistances in the range 2-10MΩ when filled with 4M NaCl. For intracellular recording, microelectrodes had resistance in the range 70-150MΩ and were filled with 4M CH_3COOK. The microelectrodes were manufactured using a Flaming Brown puller (P801PC, Sutter Instrument Co.) using 1mm (o.d.) Omega dot glass tubing (GC100F-15, Clark Electromedical). Microelectrodes were held in silver/silver chloride half-cells (EH 1S 1.0, Clark Electromedical) and mounted directly in the headstage of the amplifier (Axoclamp-2A, Axon Instruments Ltd.) which was set to bridge mode. All experiments involving field potential recordings followed a similar protocol.

Field Potential Data

A recording electrode was lowered into the pyramidal cell body layer of the CA1 field (Fig.1). Field potential responses were initially evoked by stimulations at a low voltage (1-3V) at 0.2Hz. The recording electrode was positioned at a point where the spike of greatest amplitude occurred at this voltage. The voltage would then by increased to check for recruitment of a second spike. If there was no recruitment at the maximum amplitude of the spike the slice was judged to be healthy and the control measurements were taken (Fig.8). Fig.9 indicates precisely where measurements of amplitude and width were taken.

Control recordings were made over at least 20 min to ensure that stable responses could be obtained from the slice. Where there was a marked variation in amplitude (±10% at intermediate and maximal stimulation) or recruitment of a second population spike, the slices were discarded. For all pressure chamber experiments 3 levels of stimulation were used. These are termed threshold, intermediate and maximal stimulation in the text. Threshold intensity was the stimulus intensity required to elicit a population spike of 0.5-1mV amplitude, maximal intensity was the stimulus required to evoke a spike of

maximum amplitude and intermediate intensity, that which produced a spike 50% of the maximal amplitude. These stimulus values were established during the control period. For anaesthetic work at atmospheric pressure stimulation is expressed as multiples of the threshold stimulus.

For all paired pulse potentiation experiments stimulus intensity was set to that which evoked a half maximal population spike in response to a single pulse. Following drug application or pressurization of the chamber, if required the stimulus intensity was adjusted to reset the first population spike to its control amplitude. Stimuli were typically 0.1Hz, 1-20V, 40-100μs and a 22ms interval was adopted for the paired pulse experiments. Hard copies of potentials illustrated in figures are averages of 4 responses at a particular stimulus intensity.

Intracellular Recording

For intracellular measurements neurones were impaled using conventional methods. Before penetration: a/ the "offset" potential on the input circuit was cancelled out with the DC offset potentiometer, b/ the voltage drop across the microelectrode during current passage was nulled by the adjustment of a counterbalancing circuit (bridge balance), thereby providing an estimate of the microelectrode resistance. (The bridge balance was monitored throughout the experiment and adjusted if necessary, by examining the onset and offset of hyperpolarizing current pulses.); and c/ the capacity compensation was optimally adjusted. For impalement, negative (0.5A) current pulses (80ms, 1Hz) were passed through the recording electrode. Changes in microelectrode resistance (R_e) were continuously monitored as the electrode was advanced through the slice. A sudden increase in R_e was followed by a brief oscillation of the capacity compensation (1-5 ms). Following an indicated increase in potential (ie. impalement of a cell), negative holding current (0.7nA) would be applied to aid the formation of a "good seal". If successful, the holding current was then slowly decreased to zero in order to observe the true resting potential of the cell.

Resting membrane potential (Em) was estimated as the change in indicated potential on withdrawal of the electrode from the cell.

Amplified responses were displayed on a digital oscilloscope (1425, Gould Instrument Co.) which allowed capturing of single events or averaging of several transients. "Hard copies" of data were obtained using either a Colourwriter (6120, Gould Instrument Co.) or a chart recorder (222OS, Gould Instrument Co.). Data was stored, in digital form, on video tape (JVC-E-180PRO), and analyzed off line at a later stage. Analogue to digital conversion was performed

by a digital audio processor (Sony, PCM 701 ES) modified to give 4 one bit channels and 2 analog channels (DC-20KHz band width, 16 bit resolution). Signals were recorded using a standard video cassette recorder (Panasonic NW G7). Accurate triggering of equipment was made possible using a Digitimer (D100, Digitimer Ltd.).

Voltage-Clamp Studies

Single electrode voltage-clamp measurements were made in CA1 pyramidal neurones. The recording system was an Axoclamp-2A (Axon Instruments Ltd.) set to discontinuous single electrode voltage-clamp (dSEVC) with a switching frequency of 3KHz (50% duty cycle). Output bandwidth was set to 3KHz and the phase shift and anti-alias facilities were set to give maximum gain with optimal clamp settling (Finkel & Redman, 1985). Capacity compensation was optimally set so that no voltage deflection could be seen when passing current through an extracellularly positioned electrode in "switched current clamp" mode. Solution level was set as low as possible (≈300μm).

M-currents were recorded following the protocol of Halliwell & Adams (1982). Cells were held at their natural resting potential and then clamped to a potential 14mV more negative for 1 second. Apart from the tails of the capacity transients, the resting inward current flow was approximately square. The cell was then depolarized to -40mV for several seconds and the same hyperpolarizing command applied. An additional time-dependent current became visible due to a time dependent change in the M-conductance. M-currents were stored on magnetic tape (Ampex, 704 151B11) using a Racal Store-4 DS recorder. Chart records were made using a TDM Par100B recorder (TDM Tape Services Ltd.).

E.Drugs Used and Anaesthetic Concentrations

All drugs were bath applied. Enflurane (Ethrane) and isoflurane (Forane) were obtained from Abbot and halothane (Fluothane) from ICI. They were delivered in 95%O_2/5%CO_2 (flow rate 0.5-1litre.min^{-1}) using commercial vaporizers (for example Enfluratec) to the reservoir containing ACSF (40ml) at 40°- 42°C. With this method of applying the anaesthetic certain losses must be taken into consideration before the tissue bath concentration may be quoted. (Barometric pressure is assumed to be 760mmHg for the calculation).

(i) The concentration of halothane in the tissue chamber was measured by high pressure liquid chromatography. This was found to be consistent after 15 min of the start of bubbling and reached 70% of the concentration in the equilibration bath. The difference (see Fig.10) was the result of loss from the tissue bath and through the tubing. The value of 30% loss was arrived at with a flow rate of 2.9 ml.min^{-1}, reservoir temperature of 40°C, tissue bath temperature

of 30°C and vaporizer setting of 5%. Therefore a correction factor of 0.7 must be applied to the calculation.

3 other factors had to be taken into account before the effective partial pressure of halothane (as a percentage of a standard atmosphere) in the tissue bath could be known:-

(ii) Calibration of the vaporizer: the halothane vaporizer was calibrated by infra-red analysis at a carrier gas flow rate of 600 ml.min^{-1} (Fig.11). At a dial setting of 5% the vaporizer was found to deliver 5.6% halothane.

(iii) A correction for water vapour pressure at the temperature of the equilibrium chamber must be applied (see Fig.12).

(iv) Temperature difference between the equilibration chamber and the tissue bath must be taken into account.

Hence:- $[\text{Halothane}] = Pt_1 \times \lambda t_1 = Pt_2 \times \lambda t_2$

$\therefore Pt_2/Pt_1 = \lambda t_1/\lambda t_2$

P = partial pressure of halothane in solution, at temperature (t).

λ = solubility coefficient.

To derive $P_{(halothane)}$ in the lower bath multiply by the correction factor (calculated by dividing the solubility coefficient at 40°C by the coefficient at 30°C) shown in the chart (Table 1). This factor does not allow for loss of halothane [Factor (i)]

Taking the above factors into account for experiments involving 5% halothane the effective $P_{(halothane)}$ in the tissue bath =

5.6 x 0.7 x 0.925 x 0.665 = 2.41% of barometric pressure.

This would be the partial pressure difference driving the anaesthetic into the brain slices. The same considerations would apply to enflurane and isoflurane.

DL-Propranolol hydrochloride, tetraethylammonium hydrochloride (TEA), tetrodotoxin (TTX), atropine and bicuculline were obtained from Sigma. Ketamine (Ketalar) was obtained from Parke-Davies, Methohexitone (Brietal sodium) from Eli-Lilly. Cimetidine was a gift from Smith, Kline & French and BRL 24924 was obtained from Dr. J.V. Halliwell. The ACSF had the following composition (in mmol.litre^{-1}):- NaCl 124; $NaHCO_3$ 26; KCl 3; $MgSO_4$ 2 or 0.5; NaH_2PO_4 2.5; $CaCl_2$ 2 or 4; glucose 10 and had a pH of 7.4 when bubbled with 95% O_2/CO_2.

F.Statistics

Averaged values are mean ± standard error of the mean ($\bar{x} \pm$ SEM). Significance was determined with the aid of a microcomputer (Macintosh IIx) software package (StatView II) using non-parametric calculation (Wilcoxon Signed Rank test).

Fig.1

Fig.1

Diagram of a hippocampal slice showing placement of the stimulating and recording electrodes.

CA1-3 = cornu Ammonis fields 1 & 3 (Hippocampal pyramidal neurones).

AD = area dentata (dentate granule neurones).

mf = mossy fibres.

pp = perforant path.

SchC = Schaffer collaterals.

Str = Stratum.

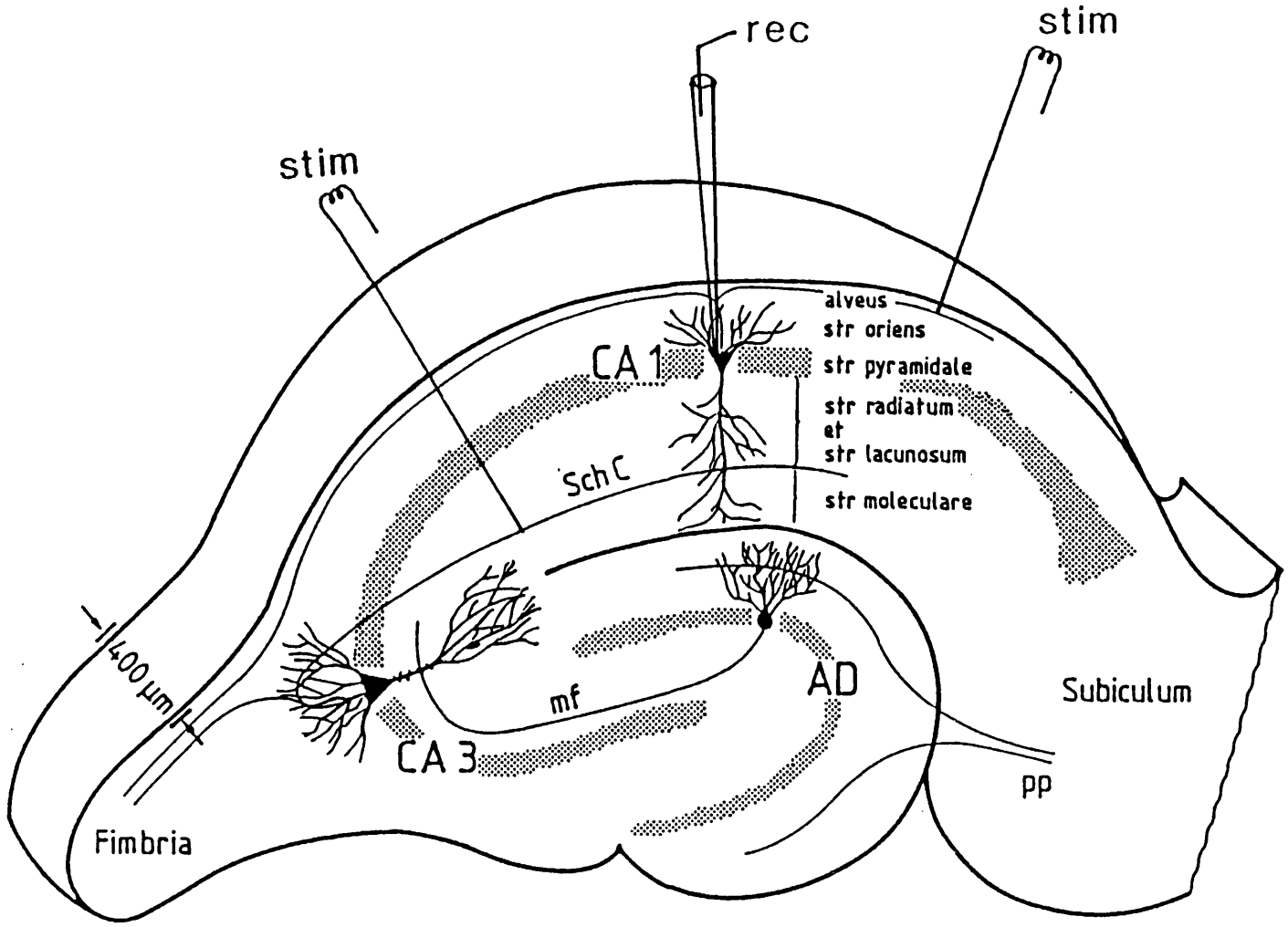

rec
stim
stim
alveus
str oriens
str pyramidale
str radiatum
et
str lacunosum
str moleculare
CA 1
Sch C
400 µm
mf
CA 3
AD
Subiculum
pp
Fimbria

Fig.2

Fig.2

Photograph of the pressure chamber.

A. High pressure gauge.

B. Low pressure gauge.

C. Gas lines and pressure relief valves.

D. Fibre optic light source.

E. Binocular microscope for viewing preparation inside the pressure chamber.

F. Remote control unit for the microelectrode micromanipulator.

G. Removable binocular microscope used for visualizing the slice preparation during the initial setting up procedure.

H. Peristaltic pump and waste ACSF platform.

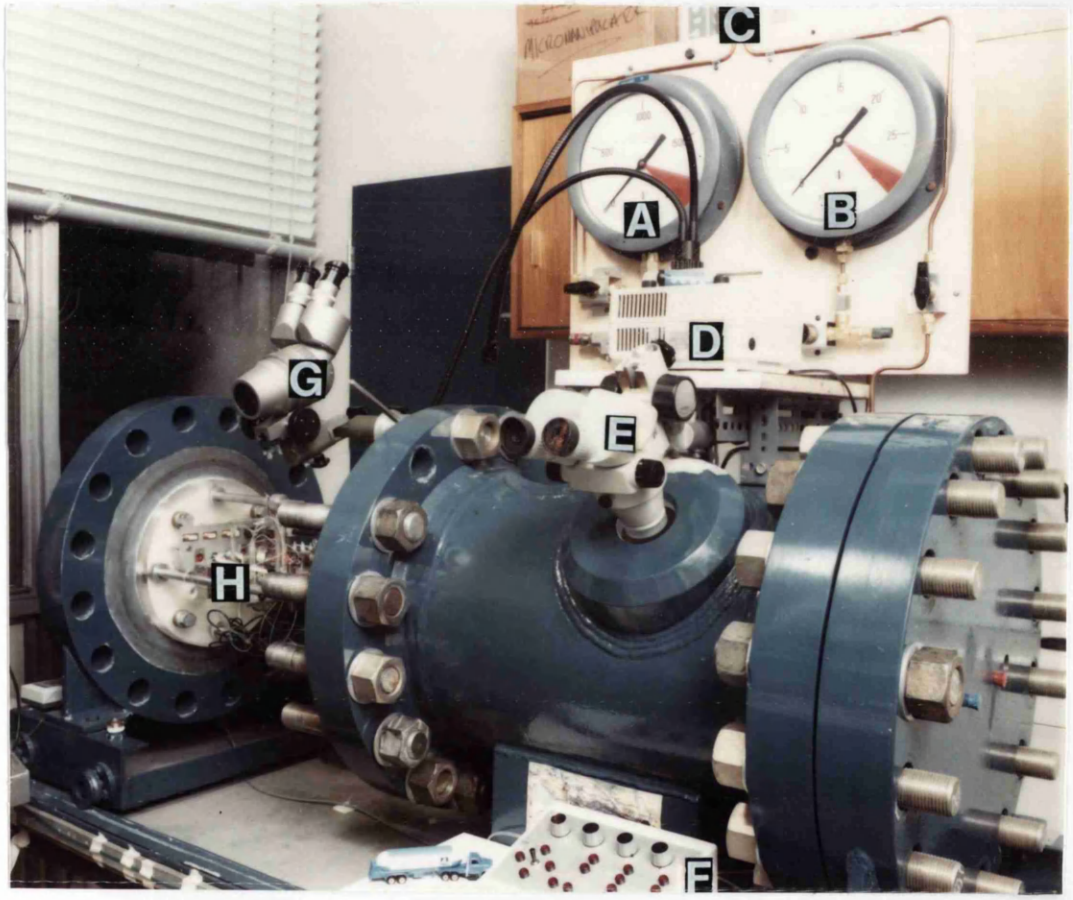

C
A
B
D
G
E
H
F

Fig.3

Fig.3

Artist's impression of the high pressure chamber illustrating its main features. The high pressure gas lines which penetrate the chamber body and the electrical connections which penetrate the cradle door are not illustrated.

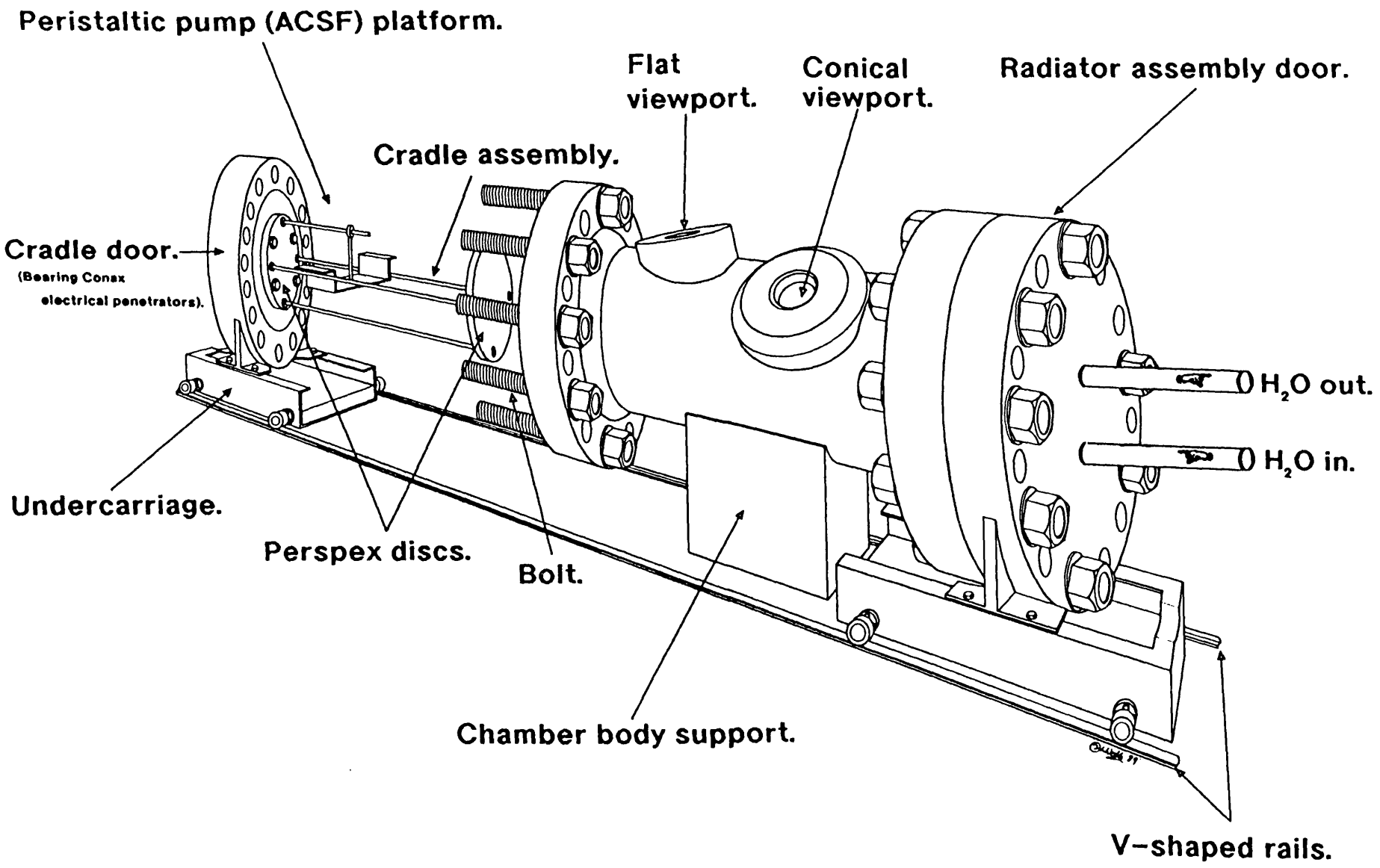

Peristaltic pump (ACSF) platform.
Flat viewport.
Conical viewport.
Radiator assembly door.
Cradle assembly.
Cradle door.
(Bearing Conax electrical penetrators).
H_2O out.
H_2O in.
Undercarriage.
Perspex discs.
Bolt.
Chamber body support.
V-shaped rails.

Fig.4

Fig.4

Photograph of the high pressure chamber recording assembly.

A. Electrically driven micromanipulator.

B. Axoclamp headstage amplifier.

C. Header tank.

D. Electrically heated recording chamber.

E. "Bat" for holding slice in position.

F. Stimulating electrode.

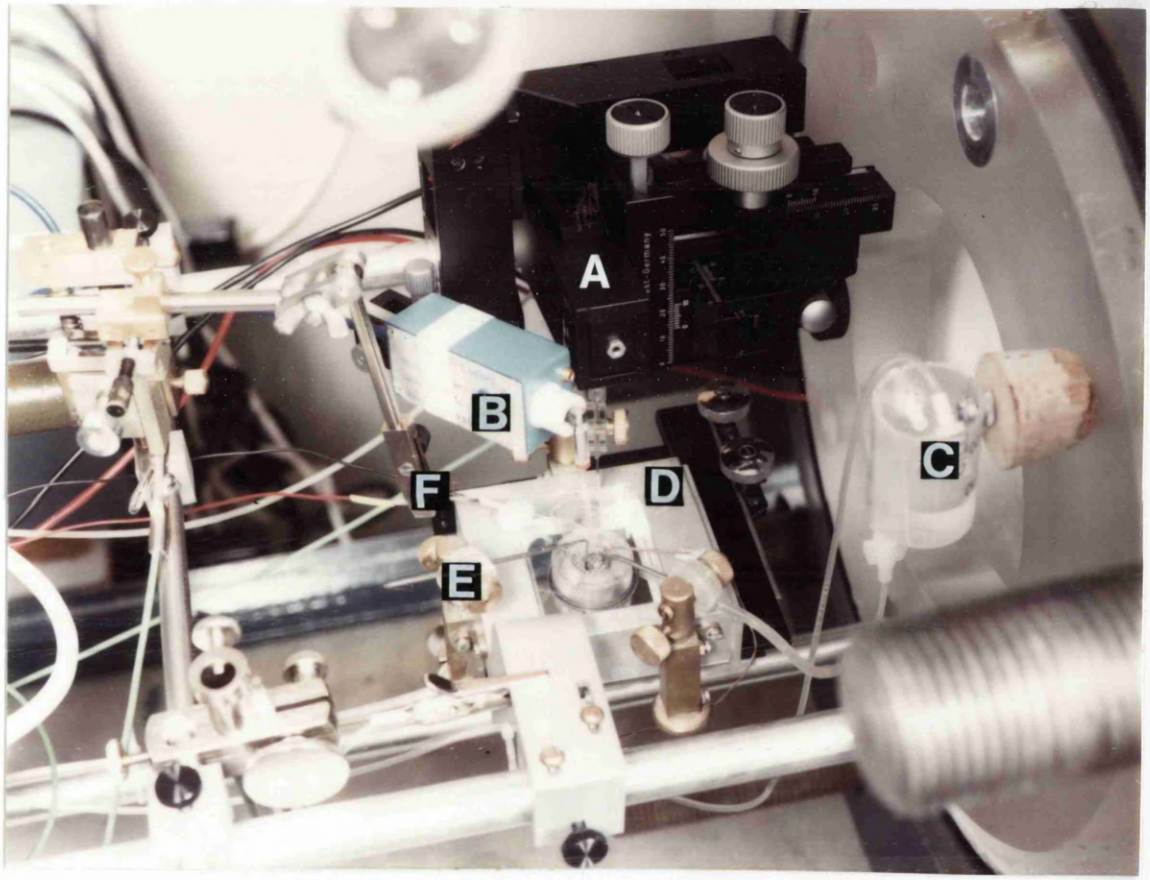

A
B
C
D
E
F

Fig.5

Fig.5

Schematic view of the pressure chamber recording apparatus illustrating its main features.

Note; for the field potential experiments involving the addition of anaesthetic agents at pressure the reservoir was replaced by a HPLC pump perfusing liquid through the pressure chamber door. Solutions were not recirculated during these experiments.

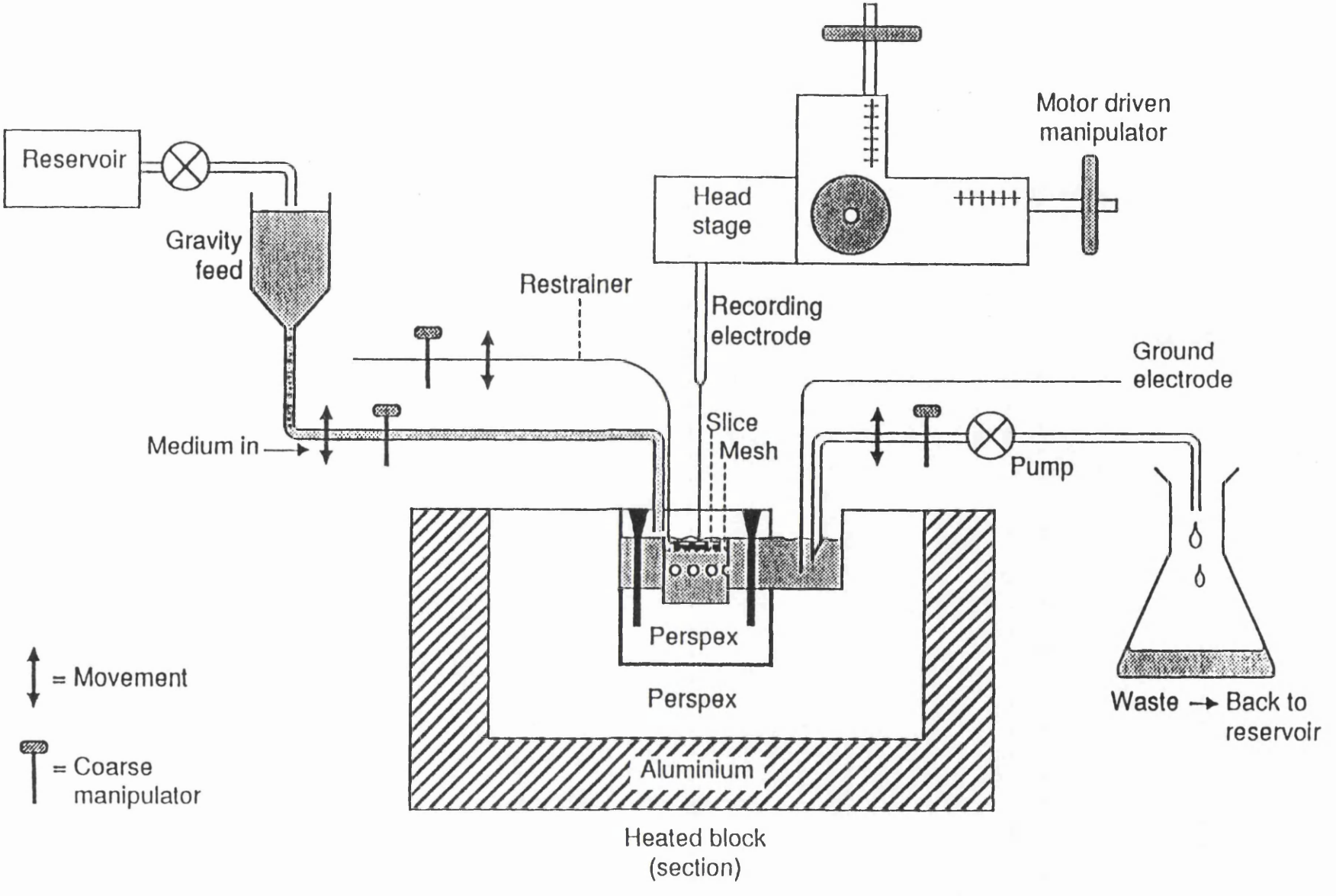

Reservoir
Gravity feed
Motor driven manipulator
Head stage
Restrainer
Recording electrode
Ground electrode
Medium in
Slice
Mesh
Pump
Perspex
Perspex
Aluminium
Waste
Back to reservoir
= Movement
= Coarse manipulator
Heated block (section)

Fig.6

Fig.6

Photograph of the regulator assembly panel.

A. High pressure regulator.

B. Inlet (helium cylinder pressure) gauge.

C. Outlet gauge.

Note; the oxygen regulator is not illustrated. This was added subsequent to the photograph being taken.

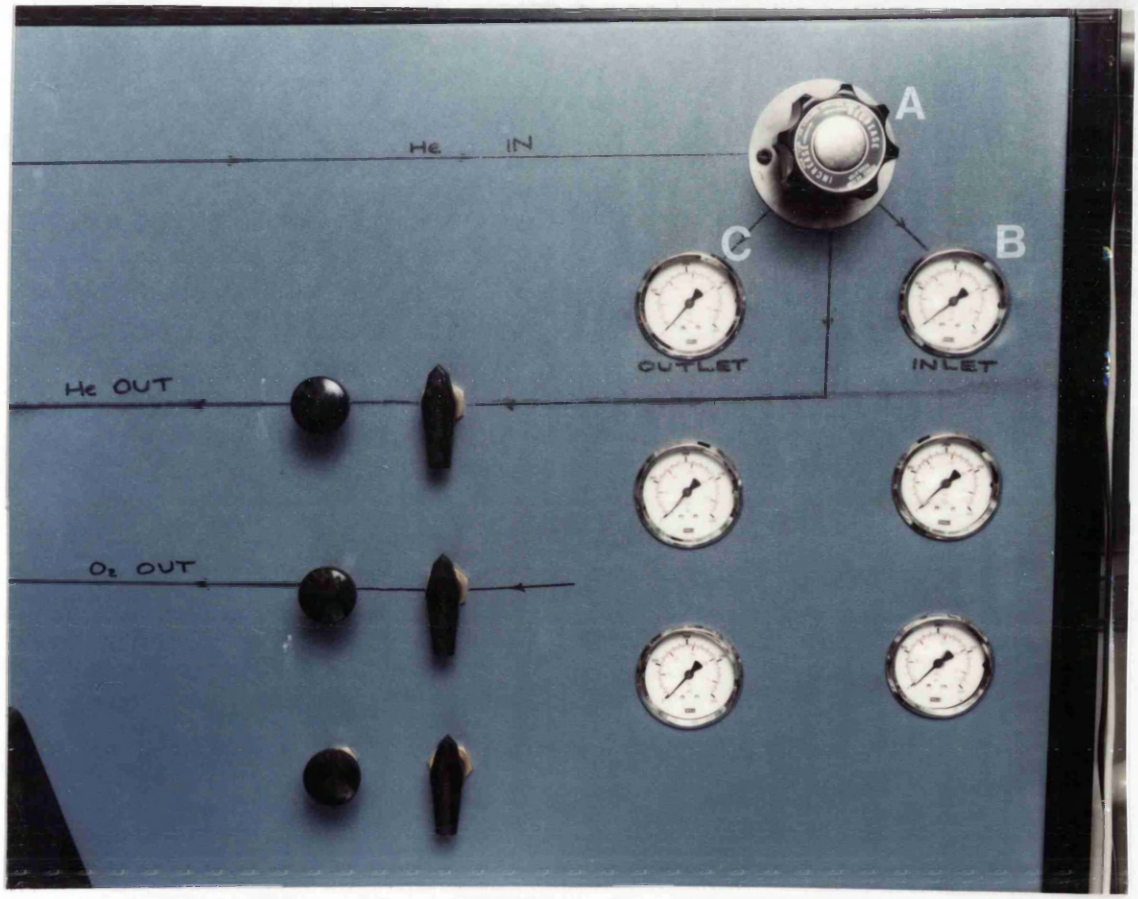

He IN
A
C
B
OUTLET
INLET
He OUT
O2 OUT

Fig.7

Fig.7

Photograph of the chamber door to which the cradle assembly was attached.

A. Conax electrical penetrators. Note: the lower penetrator was used for the ACSF perfusion line.

B. DC power supplies.

C. Recording chamber temperature regulator.

D. High pressure liquid chromatography pump.

Note; the illustrated pump was found unsuitable for perfusion above 2MPa.

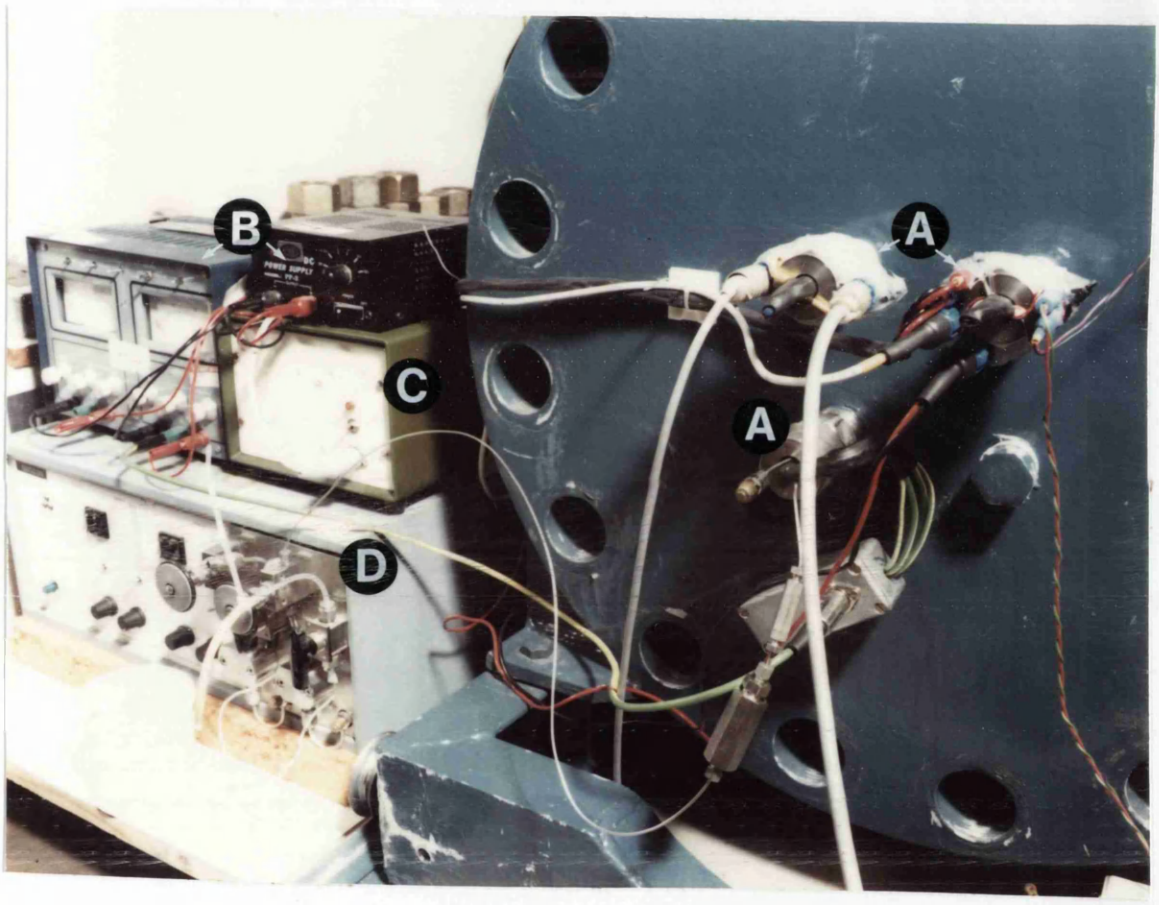

B
A
C
A
D

Fig.8

Fig.8

Orthodromic field potential measurements taken from the sealed pressure chamber at a chamber Po_2 of 0.05MPa.

A. Responses to stimulation (0.1Hz/80μs) at varying intensity after 10 min within the sealed chamber.

B. Responses to identical stimuli 2 hours later.

Temperature = 35°C. (Note; stimulus artifacts have been removed for clarity).

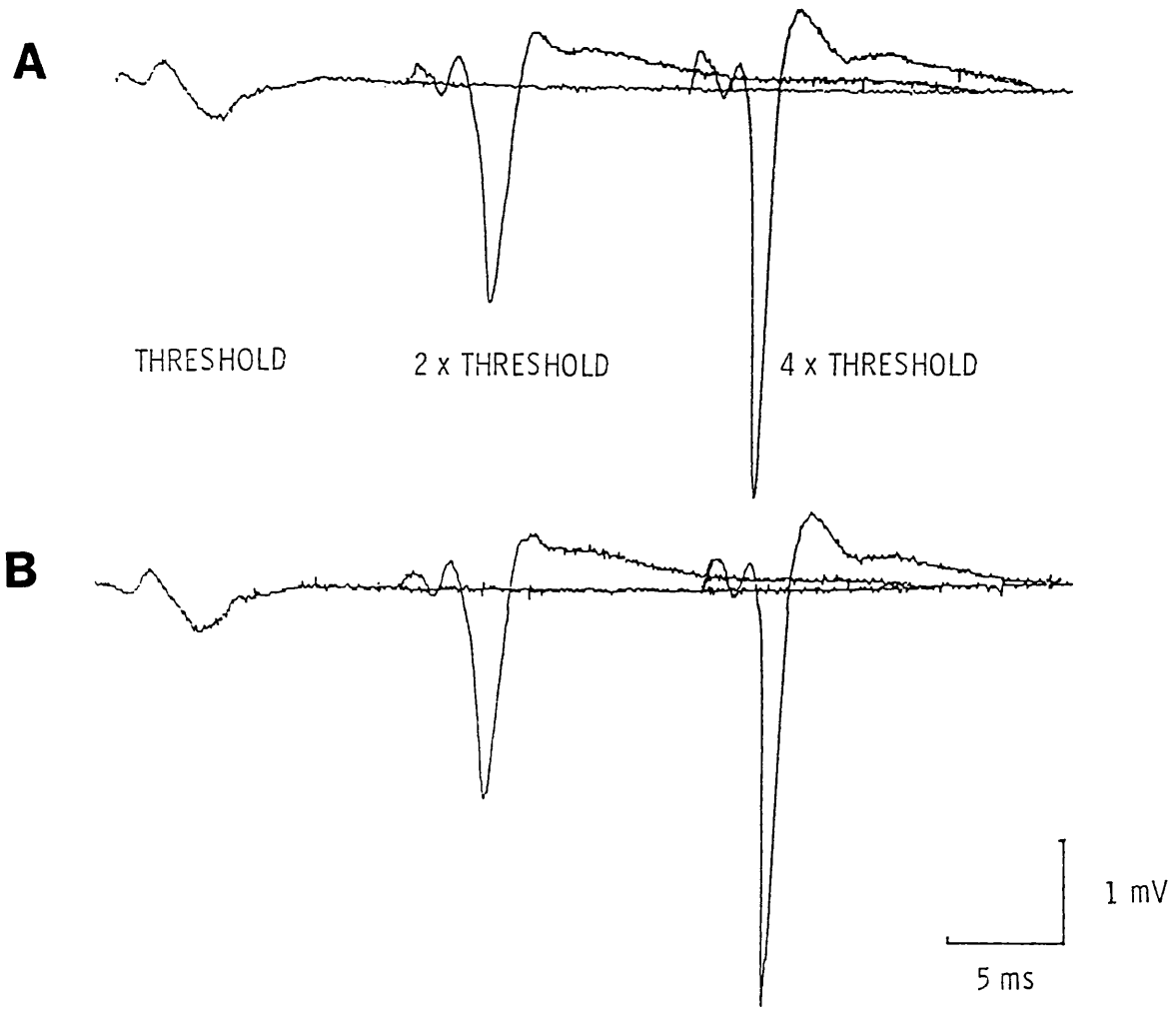

A
THRESHOLD
2 x THRESHOLD
4 x THRESHOLD
B
1 mV
5 ms

Fig.9.1
Fig.9.2

Fig.9.1

Averaged orthodromic field potential responses illustrating the points at which measurements were taken.

A. Spike amplitude.

B. Latency.

C. Width at half amplitude.

Rate of rise of the EPSP was calculated from the line of best fit to the rising phase of the EPSP disregarding the initial and final 20%.

Fig.9.2

Antidromic field potential illustrating the points at which measurements were taken.

D. Spike amplitude.

E. Width at half amplitude.

F. Latency.

Temperature = 32°C (Stimuli 0.1Hz; 80μs).

Note; stimulus artifacts have been removed for clarity.

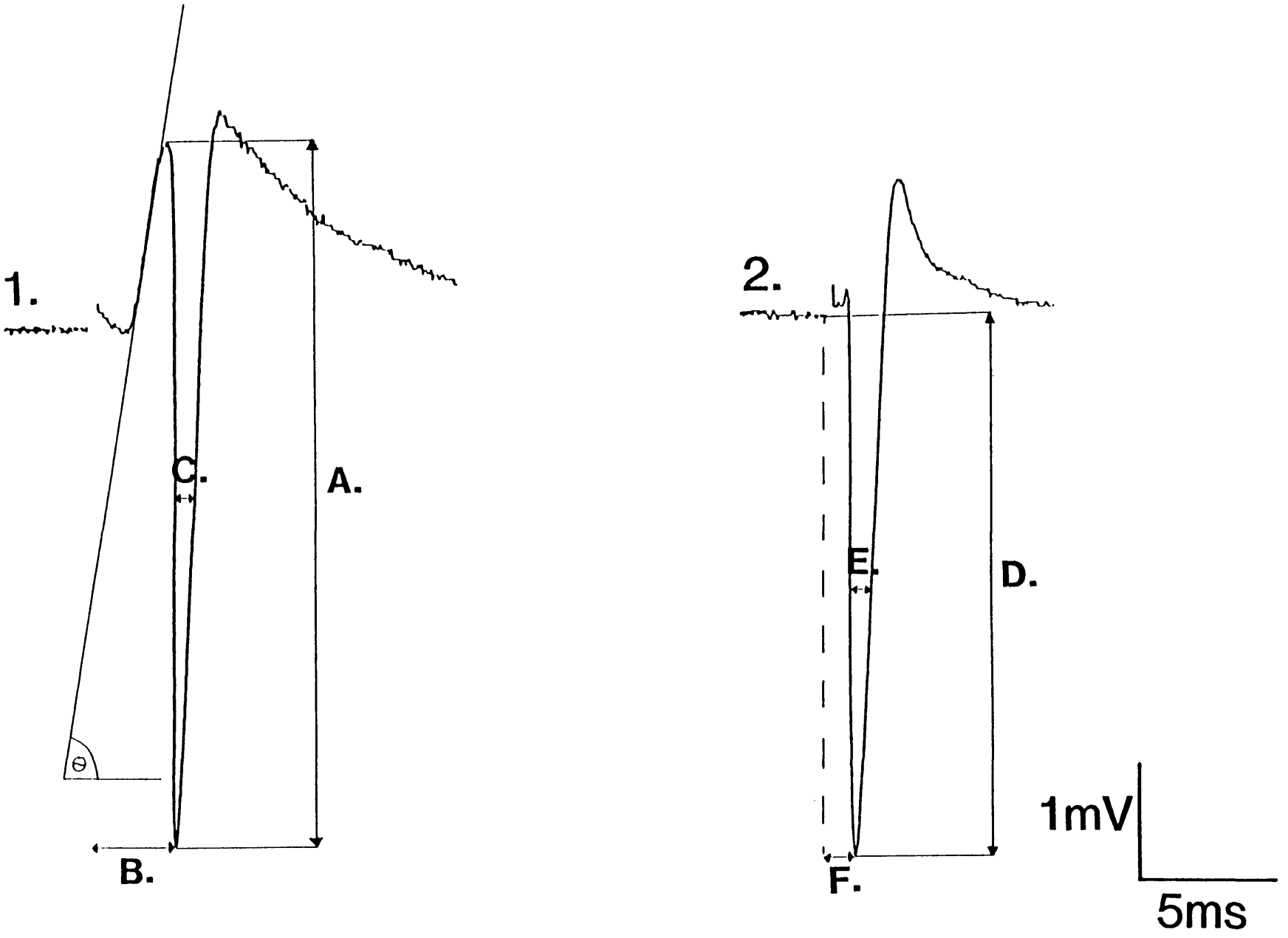

1.
C.
A.
θ
B.
2.
E.
D.
F.
1mV
5ms

Fig.10

Fig.10

Graph showing how the concentration of halothane in the bubbling bath and tissue chamber changed with time. 5% halothane was bubbled through the equilbration chamber commencing t=0, the points represent concentrations measured by HPLC analysis as time progressed.

- O :- concentration in tissue chamber.
- ● :- concentration in equilibration chamber.

The number below each data point is the temperature at which the measurement was taken (in °C).

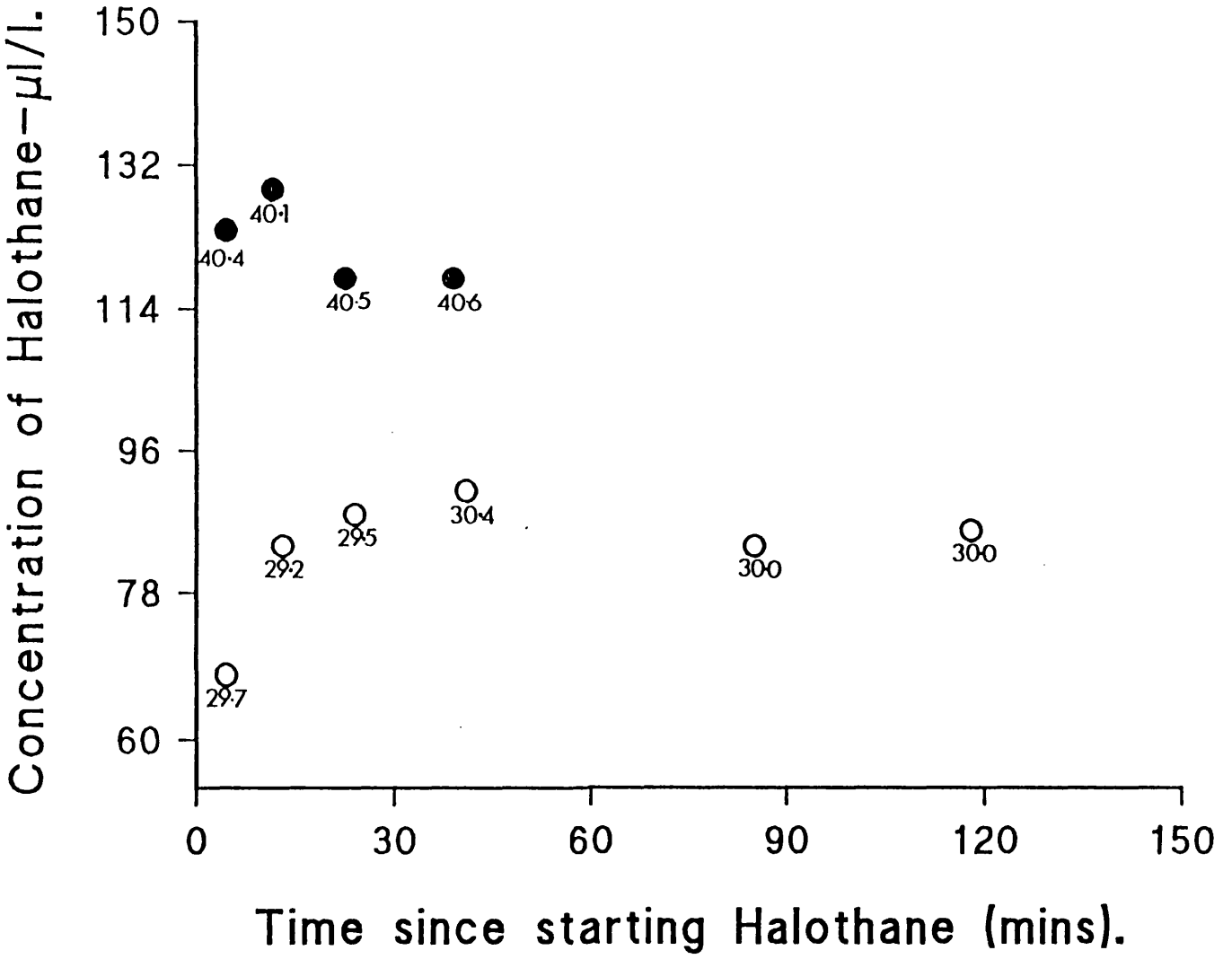

Concentration of Halothane–µl/l.
150
132
114
96
78
60
0
30
60
90
120
150
Time since starting Halothane (mins).
40·1
40·4
40·5
40·6
30·4
29·5
29·2
30·0
30·0
29·7

Fig.11

Fig.11

Calibration graph for the halothane vaporizer. Gas samples were measured by infra-red analysis at a carrier gas flow rate of 600ml.min^{-1} at room temperature (23°C).

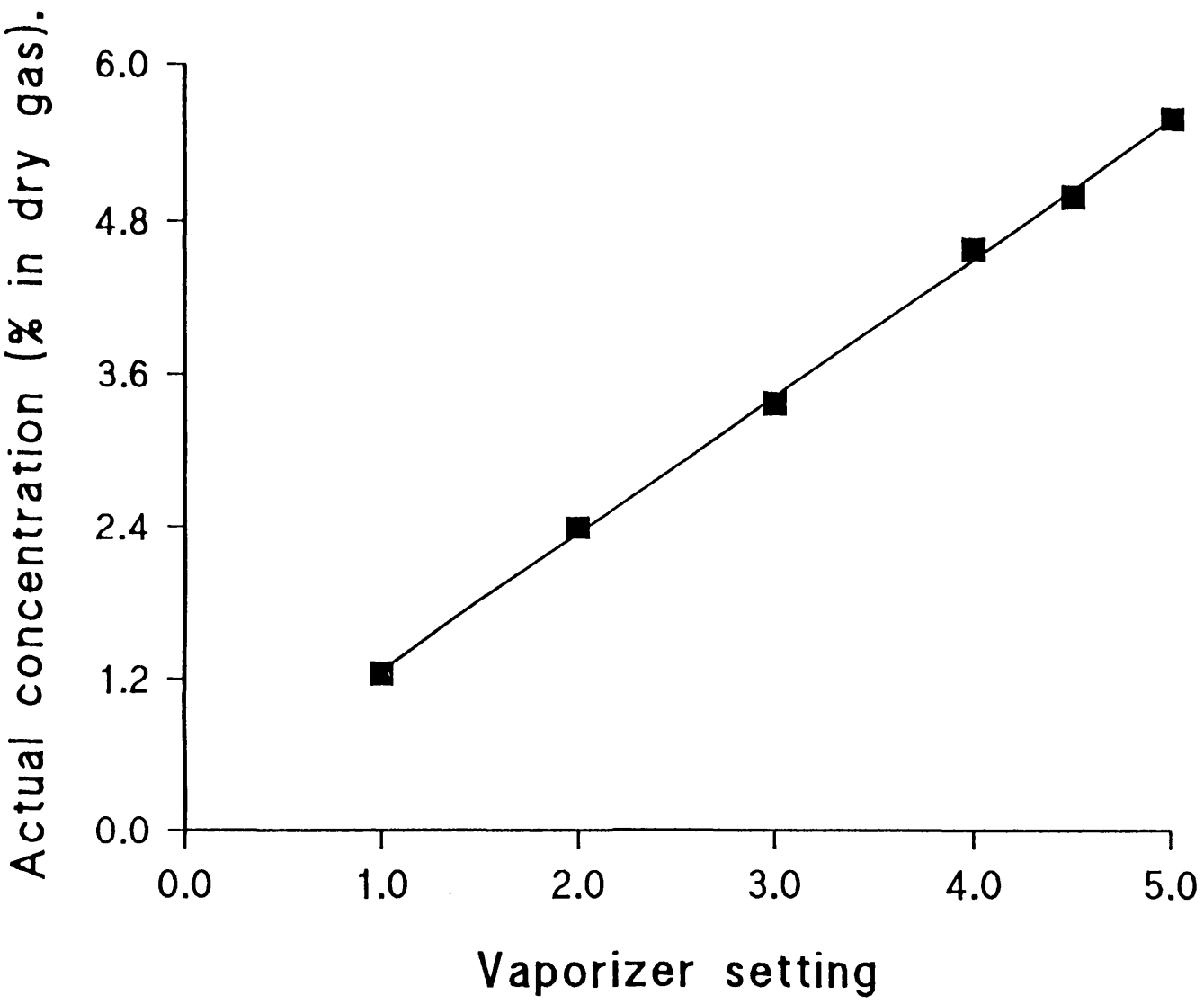

Actual concentration (% in dry gas).
6.0
4.8
3.6
2.4
1.2
0.0
0.0
1.0
2.0
3.0
4.0
5.0
Vaporizer setting

Fig.12

Fig.12

Curve used for the correction for water vapour pressure at the temperature of the equilibration chamber. The value for halothane at the temperature of 40°C is 0.925 (source Dr. J.F. Nunn personal communication).

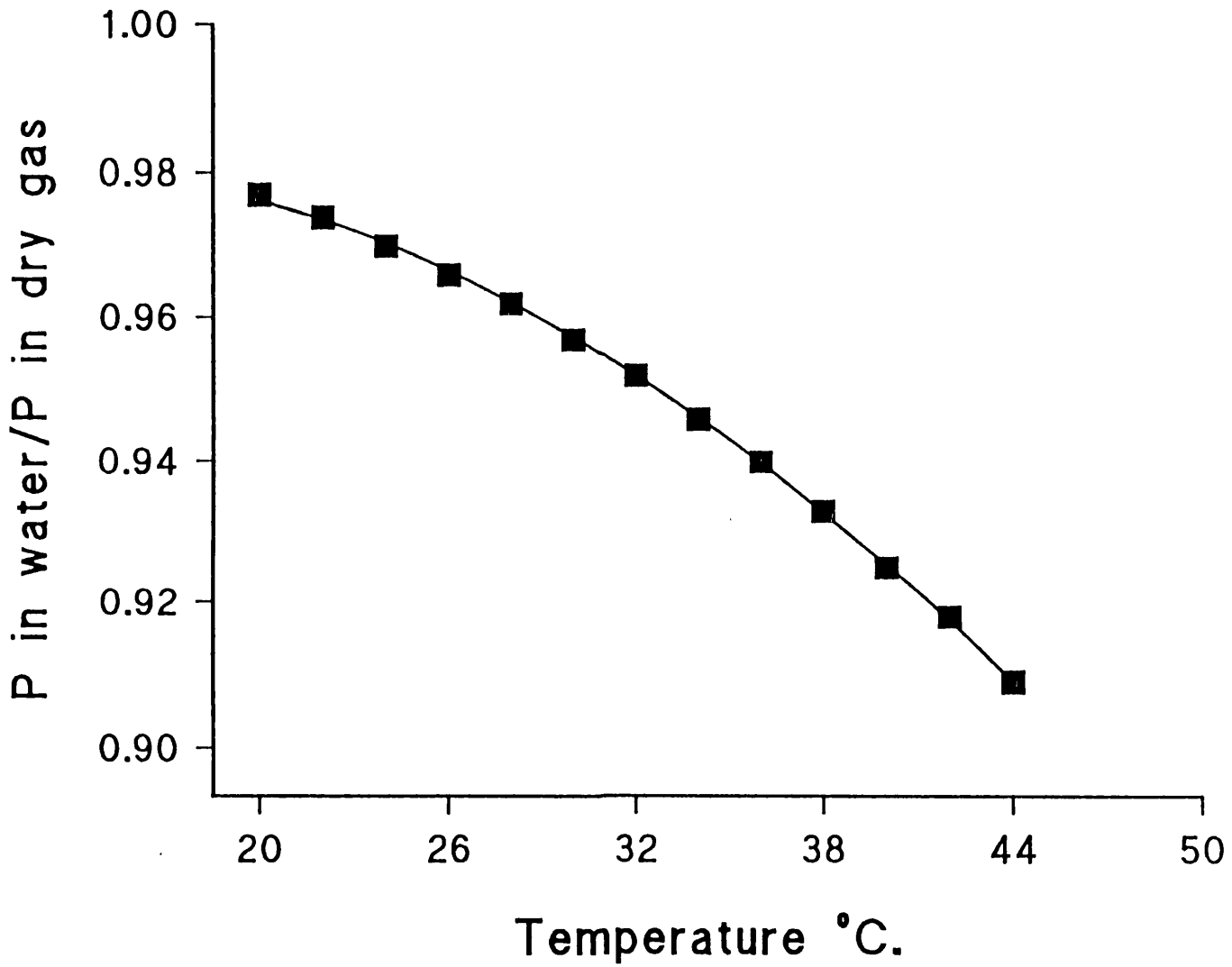

1.00
0.98
0.96
0.94
0.92
0.90
P in water/P in dry gas
20
26
32
38
44
50
Temperature °C.

Table 1.0

Table 1.0

Tabulation of correction factors for solubility to be applied to the calculation of halothane partial pressure in solution for a range of temperatures. The value highlighted is the correct value for a fall in temperature of 10°C, from 40°C to 30°C,

TABLE 1.0

TISSUE CHAMBER TEMPERATURE	EQUILIBRATION CHAMBER TEMPERATURE					
	38°C	39°C	40°C	41°C	42°C	43°C
28°C	.6648	.6382	.6126	.5882	.5647	.5421
29°C	.6923	.6646	.6328	.6125	.5881	.5645
30°C	.7214	.6926	**.6648**	.6383	.6128	.5883
31°C	.7515	.7214	.6925	.6648	.6383	.6128
32°C	.7828	.7515	.7214	.6925	.6699	.6383
33°C	.8153	.7827	.7513	.7213	.6926	.6648

EXPERIMENTAL RATIONALE & AIMS.

The hippocampal slice was chosen as the preparation to be used in this investigation for the following main reasons:-

I.Hippocampal cellular physiology is relatively well understood as a result of both in vivo and in vitro experiments performed over the past 20 years.

II.The hippocampal slice preparation; due to its mechanical stability, controllable environment and ability to produce reproducible potentials over a period of some hours; is an ideal tool for the study of the effects of drugs or environmental variables upon the function of CNS neurones.

III.Its laminated structure facilitates intracellular recording in identifiable neurones and enables characteristic extracellular orthodromic and antidromic potentials to be recorded.

IV.The hippocampus is reputed to have the lowest seizure threshold of all the brain structures. Its unusually high density and regular alignment of nerve cell bodies and dendrites, as well as the scarcity of glia in the cellular layer, make it exceptionally prone to generating synchronized neuronal discharges (Krinjević, Dalkara & Yim, 1988). This property was considered desirable for this study which aimed mostly to uncover anaesthetic- or pressure-induced excitatory events.

Part I of the thesis concentrates upon the effects of the general anaesthetic agents enflurane, isoflurane, halothane, ketamine and methohexitone upon CA1 neurone function at atmospheric pressure. The majority of experimental work for this section of the thesis was carried out in parallel with the construction and installation of the high pressure chamber.

The investigation aimed to provide information that could be relevant to both the production of anaesthesia in vivo and the "excitant" effects observed both in vivo and in vitro, with some anaesthetic agents. The experimental approach was as follows:-

I.Antidromic field potential recordings were taken in the absence and presence of the anaesthetics to test if the agents were significantly active postsynaptically.

II.To complement the field potentials, basic intracellular measurements were taken in order to investigate postsynaptic effects in more detail. This approach made possible the recording of properties such as resting membrane potential, threshold potential and input resistance which could be compared to previously published work.

III.In addition the accommodation properties of CA1 neurones in the presence of anaesthetic agents was examined. The potassium currents underlying this neuronal response to long depolarizing inputs exert a

powerful "braking" action on the discharge of action potentials in these cells. An investigation of possible anaesthetic induced modification of this behaviour with either "excitant" or purely depressant anaesthetics present was planned with the aim of trying to uncover excitability changes that one could relate to the observed in vivo effects.

IV.Finally, if the previous section uncovered promising data, a detailed voltage-clamp analysis of possible anaesthetic induced modification of at least two of the K^+ currents underlying accommodation (M- and I_{AHP}) was planned. This experimental section was unfortunately not fully realized due to a combination of unavailability of equipment and lack of time.

Part II of the thesis concentrates upon the effects of high pressure on CA1 neurones and upon the actions of the anaesthetic agents ketamine and methohexitone at high pressure. The main experimental sections are outlined below:-

I.The initial experimental approach aimed to verify that the pressure chamber was a suitable environment to record stable field potential responses at atmospheric pressure.

II.Subsequent to being satisfied with the series of control experiments, field potentials (both antidromic and orthodromic) were recorded at elevated pressure. This series of experiments was designed with the aim of both reproducing (and hopefully verifying some of the work of Fagni and co-workers) and then extending the study to include a range of stimulus strengths at pressure and to see whether responses to paired orthodromic stimuli at pressure were altered. By extending the range of stimulus strengths it was hoped that consistent excitability changes that could be attributed to high pressure would be in evidence when the preparation was stimulated to give a maximum/near maximum response following compression of the chamber. Paired pulse orthodromic stimulation was studied in order to check whether changes in synaptic efficiency emerge during pressurisation that may contribute toward the high pressure CNS disturbance.

III.Intracellular experiments were then planned which would provide basic information about the properties of CA1 neurones at high pressure. The original aim was to impale individual neurones at atmospheric pressure and subsequently maintain recording during compression to 10MPa. This approach proved to be impractical as the cell was invariably lost during the early stages of compression. Populations of neurones were therefore sampled at differing pressures and compared statistically. Resting membrane potential, input resistance, threshold potential and action potential characteristics were the basic neuronal properties to be recorded that were regarded as essential data to this investigation.

IV.Accommodative behaviour of the neurones at pressure was another neuronal variable chosen to be investigated.

Disturbance of potassium channel function by elevated pressure has been a possibility that has been mostly ignored in the literature and this experimental approach would therefore generate novel data that would also be complementary to the rest of the study.

PART I INTRODUCTION

This section of the thesis involved taking antidromic field potential measurements to assess the effect of anaesthetics upon axonal/somatic excitability, and intracellular measurements to investigate anaesthetic induced changes in accommodation behaviour, resting membrane potential and somatic excitability in CA1 neurones. Particular reference will be made to anaesthetic/K^+ channel interactions, therefore a brief review of K^+ channel types reported in hippocampal neurones will now follow. (Halliwell, 1990 provides a more in depth review of K^+ channel currents; see also Cook, 1988; Castle, Haylett & Jenkinson, 1989; Rudy, 1988).

A single hippocampal neurone may possess a variety of distinct K^+ conductances that influence excitability. Some of the currents are activated by depolarization (eg. delayed rectifier, A-current, slowly inactivating outward current and M-current) and Ca^{2+} entry (fast and slow Ca^{2+}-activated K^+) and others by hyperpolarization (Q-current and inward anomalous rectifier). All are important in governing the postsynaptic excitability of hippocampal neurones.

Voltage-Activated K^+ Currents.

Delayed rectifier ($I_{K(DR)}$)

Voltage-clamp studies in the mammalian CNS have shown that upon depolarization a delayed outward current is produced (Segal & Barker, 1984). When Ca^{2+} and Na^+ influx have been eliminated with specific blockers, several discrete K^+ conductances may be observed above -40mV, that produce a maintained and slowly declining outward current. The result of these conductances is delayed outward rectification. The delayed rectifier current is very slow to activate (50-200ms at 0mV) and even slower to inactivate (2-4s) at depolarized potentials. It may be distinguished from other K^+ conductances (Ca^{2+} activated, $I_{K(A)}$ & $I_{K(D)}$) by its insensitivity to Ca^{2+} channel block and sensitivity to TEA (10mM). It is relatively insensitive to 4AP. Rat hippocampal pyramidal cells have been shown to have K^+ conductances with similar properties to those outlined above both in cell culture (Segal & Barker, 1984) and when freshly dissociated (Numann, Wadmann & Wong, 1987).

Transient K^+ current($I_{K(A)}$)

This K^+ current activates much more rapidly upon depolarization than $I_{K(DR)}$ (activation time constant >5ms). It also inactivates rapidly (time constant 20-30ms). It was originally discovered in invertebrate neurones (Connor & Stevens, 1971) and was termed the A-current. Its counterpart has been

recorded in mammalian hippocampal neurones in vitro (Gustaffson, Galvan, Grafe & Wigstrom, 1982; Sah, French & Gage, 1985). Inactivation of this conductance is complete at membrane potentials positive to -55mV and it is insensitive to TEA (Halliwell, Othman, Pelchen-Matthews & Dolly, 1986). The current does not seem to be Ca^{2+}-activated, although a faster Ca^{2+}-sensitive component has been noted (Zbicz & Wright, 1986; Doerner & Alger, 1988). 4AP (>100μmol.litre^{-1}), DTX (<50-300nmol.litre^{-1}) (Halliwell et al, 1986) and Toxin I (Harvey & Adams, 1985) all readily block I_A with differing potencies (Toxin I>DTX>>4AP). Although none of the blockers above have actions confined to I_A alone functional changes which do lie within the time span of I_A, such as the decrease in threshold seen when I_A is blocked, may reasonably indicate the role of I_A within the CNS. Connor & Stevens (1971) suggested that I_A in invertebrates may be involved in setting the frequency of neuronal discharge during repetitive firing influencing the interspike voltage trajectory (see also Storm, 1987). Also I_A (alongside the other voltage- and Ca^{2+}-activated K^+ conductances) might contribute towards generating the hyperpolarizing spike afterpotential following spike activation. Noradrenaline acting at ß receptors (Sah, French & Gage, 1985) and acetylcholine acting at muscarinic receptors (Nakajima, Nakajima, Leonard & Yamaguchi, 1986) have been reported to depress I_A in hippocampal dissociated pyramidal neurones. Hence control of I_A via this mechanism may influence both firing patterns and threshold for firing, thus modulating the behaviour of central neurones.

Slowly inactivating outward current($I_{K(D)}$)

This current exhibits rapid activation (milliseconds) and complete inactivation (≈1s) throughout its activation range. This current is distinguishable from $I_{K(DR)}$ by its insensitivity to TEA, sensitivity to 4AP (blocked at 30μmol.litre^{-1}; Storm, 1988) and activation at membrane potentials negative to -60mV. It contrasts with I_A in having a 10-20mV more negative activation range and contributes a smaller (one sixth) overall maximal conductance. It also recovers from inactivation with a prolonged time course (τ=4.7s). Functionally $I_{K(D)}$ introduces a long delay in the firing induced by a just-suprarheobasic current injection from the resting potential. Firing is only initiated upon inactivation of $I_{K(D)}$ (Storm, 1988). $I_{K(D)}$ also enables a cell to integrate separate depolarizing episodes and makes the encoding properties of a cell vary with resting potential. Depolarizing pulses of around 200ms duration which are too small to activate an action potential may initiate inactivation of $I_{K(D)}$. When these pulses are presented repetitively inactivation of $I_{K(D)}$ may summate to such a point that an action potential is generated with the previously sub threshold stimulus. This might allow temporal summation of inputs by the cell. It also makes spike discharge frequency

sensitive to E_m thereby changing the input/output relationship for the neurone.

Inward anomalous rectifier($I_{K(IR)}$)

The term anomalous rectifier was first used to describe the passage of inward but not outward K^+ currents in skeletal muscle membrane under conditions of symmetrical K^+ (Katz, 1949). The criteria for "classical" anomalous rectification are: activation by hyperpolarization negative to the reversal potential for K^+ (E_{K+}); activation potential shifting with E_{K+}; blockade by extracellular Cs^+ and Ba^{2+}; inactivation or blockade by extracellular cations at very negative potentials (>-120mV) and inward conductance impermeable to Rb^+ (Stanfield, 1988). A rapidly activating (<10ms) K^+ current satisfying these criteria has been reported in some hippocampal neurones (Owen, 1987). Various neurotransmitters including GABA (via $GABA_B$ receptors), 5-hydroxytryptamine and adenosine can induce an inwardly rectifying K^+ current in hippocampal neurones (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Colino & Halliwell, 1987; Andrade & Nicoll, 1987) mediated via a GTP binding protein (Adrade, Malenka & Nicoll, 1986).

Q current (I_Q)

This is a time dependent mixed cation (Na^+ and K^+) current, which is also activated by hyperpolarization. It may be recorded in hippocampal cells hyperpolarized to -80mV and more (Adams & Halliwell, 1982; Halliwell & Adams, 1982). It is responsible for the depolarizing sag seen in the electrotonic potential of cells hyperpolarized negative to -85mV. Its reversal potential lies between -53 and -69mV (Halliwell & Adams, 1982). It is blocked by 1mM external Cs^+ but unlike $I_{K(DR)}$ remains unaffected by Ba^{2+} ions. It activates over the range -75 to -120mV with voltage-dependent kinetics that speed up with increasing hyperpolarization (τ=100ms at -82mV; τ=37ms at -130mV) (Halliwell & Adams, 1982). Functionally I_Q does not appear to contribute to the normal resting potential, but its activation seems to resist hyperpolarizing deviations from the resting potential, inducing a characteristic rebound depolarization during a hyperpolarizing current step which often leads to an anode break spike following termination of the pulse.

M-current $I_{K(M)}$

This current originally observed in sympathetic neurones (Brown & Adams, 1980) may be recorded in hippocampal cells (Halliwell & Adams, 1982). The M-current is non-inactivating but turns on at potentials positive to -60mV with slow activation (τ=90ms at -45mV, 30°C; Halliwell & Adams, 1982). It probably does not contribute significantly to the steady membrane current seen at resting potentials of -70mV or more, but does form a component of

outward rectification seen in cells which are depolarized, and may contribute to an appreciable fraction of the membrane conductance between -70 and -40mV. It may be envisaged that I_M opposes steady state depolarizing inputs to a neurone. If I_M is reduced, depolarizing stimuli are enhanced due to the removal of its "braking" influence. Thus I_M plays a role notably in post-spike hyperpolarization, but also summation during spike trains, and contributes to the accommodation behaviour seen in CA1 neurones (Madison & Nicoll, 1984). I_M may be blocked by muscarinic receptor agonists (hence its name) (Halliwell & Adams, 1982; Gahwiler & Brown, 1985; Madison, Lancaster & Nicoll, 1987) and is inhibited also by Ba^{2+} (Halliwell & Adams, 1982). Modulation of the M-current may be seen with acetylcholine (Brown & Adams, 1980), 5-hydroxytryptamine (Colino & Halliwell, 1987) and somatostatin (activation) (Moore, Madamba, Joels & Siggins, 1988). The actual method of acetylcholine-induced modulation is as yet not well established, but preliminary experiments have pointed towards the generation and action of inositol-1,4,5 trisphosphate (IP3) (Dutar & Nicoll, 1988).

Calcium-Activated Potassium Conductance

Hippocampal neurones have been shown to possess two K^+ currents gated by intracellular Ca^{2+}. They may be indirectly blocked by Ca^{2+} channel antagonists and have been separated with regard to their kinetics and pharmacological sensitivity.

$I_C (I_{K(Ca)(fast)})$.

This large, time- and voltage-dependent current was termed I_C (Brown & Griffith, 1983) by analogy to comparable currents in molluscan neurones (Thompson, 1977). It is activated rapidly ($\approx$ 1-2ms) when a Ca^{2+} charge flows through voltage-gated Ca^{2+} channels. Following repolarization of the cell the current is deactivated within 50-150ms, depending upon the voltage (Brown & Griffith, 1983). The current is evidenced, in CNS neurones, by the hyperpolarization lasting $\approx$10ms following an action potential. These AHPs are blocked by 1-10mmol.litre^{-1} TEA and this action is accompanied by broadening of the spike. This originally suggested the delayed rectifier but it is now clear that Ca^{2+} channel blockers (30nmol.litre^{-1} CTX, 0.3mmol.litre^{-1} Cd^{2+}) have similar effects (Storm, 1987; Schwindt, Spain, Foehring, Stafstrom, Chubb & Gill, 1988). The current is probably carried by high conductance (150-270pS) "BK" channels which have been detected in membrane patches from hippocampal neurones (Brett & Lancaster, 1985; Ikemoto, Ono, Yoshida & Akaike, 1989). These channels require a relatively high concentration of Ca^{2+} for their activation and show strong voltage sensitivity (Francolini, 1988). Concentration jump experiments imply that the

distribution of "BK" channels must be in close proximity to those mediating Ca^{2+} entry in order for the channel to see a sufficiently high concentration of Ca^{2+} for them to open within the 1-2ms necessary during an action potential (Ikemoto, Ono, Yoshida & Akaike, 1989). The current is also not affected by intracellular injection of EGTA, although the "fast" buffer BAPTA is effective (Storm, 1987; Lancaster & Nicoll, 1987). I_C seems to participate in spike repolarization generating the early phase of the spike AHP (Storm, 1987; Lancaster & Nicoll, 1987). Also in conjunction with I_M, I_C contributes towards the AHP (50-100ms) following action potentials (Storm, 1988). There is no information available currently to indicate any transmitter modulation of this current.

I_{AHP} ($I_{K(Ca)(slow)}$).

Hippocampal neurones that are subjected to long depolarizing current injections (>300ms) display marked accommodation of spike firing (Madison & Nicoll, 1984). The cessation of firing occurs before the termination of current injection and is due to a hyperpolarization of several seconds duration (AHP) (Hotson & Prince, 1980). Both the accommodation of spike firing and the AHP are reduced by Ca^{2+} channel antagonists (Madison & Nicoll, 1984) with the slow part of the AHP being particularly sensitive. It has been shown (Lancaster & Adams, 1984) that the current underlying the AHP is a TEA resistant K^+ conductance that can be activated by aliquots of Ca^{2+} entering for each action potential fired. Therefore the size and duration of the AHP is proportional to the number of action potentials fired. The current that underlies this behaviour has been termed I_{AHP}. It is smaller in amplitude, rises more slowly following Ca^{2+} entry (suggesting that the channels may be remote from the site of Ca^{2+} entry) and declines more slowly on repolarization than I_C (Lancaster & Adams, 1986). I_{AHP} is voltage insensitive (Lancaster & Adams, 1986); the decline of the AHP has an identical τ decay to that for the I_{AHP} measured at different membrane potentials between -40 and -80mV. Much lower Ca^{2+} concentrations are needed than those needed to activate I_C, AHPs have been recorded in slice preparations with Ca^{2+} levels of 30-60nmol.litre^{-1} (Knöpfel, Vransec, Gähwiler & Brown, 1990). I_{AHP} is also insensitive to TEA or CTX (Lancaster & Adams, 1986; Storm, 1987; Lancaster & Adams, 1987). This current may be mediated by the low conductance "SK" channels and appropriate channels of about 19pS conductance have been found in hippocampal patches (Lancaster, Nicoll & Perkel, 1991). I_{AHP} may also be inhibited by a variety of neurotransmitters which include; acetylcholine (Cole & Nicoll, 1983), noradrenaline (Madison & Nicoll, 1982, 1986; Lancaster & Adams, 1986), histamine (Haas & Konnerth, 1983; Haas & Greene, 1986), 5-hydroxytryptamine (Colino & Halliwell, 1987; Baskys, Niesen & Carlen, 1987; Chaput, Araneda & Andrade, 1989; 1990)

and dopamine (Malenka & Nicoll, 1986). It has also been reported that dopamine can increase accommodation (Bernardo & Prince, 1982) by potentiating the AHP, and similar actions have been seen with adenosine (Greene & Haas, 1985). cAMP appears to be involved in modulation of the AHP. Noradrenaline and adenosine have opposing effects on stimulation of cAMP and have opposite effects on accommodation. It is presumed that either the channels involved or the mechanism coupling raised $(Ca^{2+})_i$ with channel activation are a target for cAMP-dependent protein kinase, since transmembrane Ca^{2+} flux appears not to be reduced as a result of noradrenaline action (Madison & Nicoll, 1982, 1986). Histamine's action via H_2 receptors is probably also mediated by stimulating adenylate cyclase (Haas, 1985). In the case of ACh it seems that protein kinase C is involved (Baraban, Snyder & Alger, 1985; Madison & Nicoll,1986). For 5-hydroxytryptamine the mechanism is yet to be identified (Colino & Halliwell, 1987), although it is thought to be a novel receptor which is not $5\text{-}HT_3$ (Chaput, Areneda & Andrade, 1990).

K^+ channels are therefore important in governing postsynaptic excitability: they control resting membrane potential, threshold potential and action potential discharge. Anaesthetic action at the level of the K^+ channel could have important implications for explaining the proconvulsant actions of anaesthetics such as enflurane and ketamine (MacIver & Roth, 1987; MacIver & Kendig, 1989; Mori, Kawamata, Mitani, Yamazaki & Fujita, 1972) and could additionally provide information relevant to the mechanism(s) of anaesthesia.

Anaesthetics and Potassium Channels.

Anaesthetic-K^+ channel interaction is not a new idea. Thus, general anaesthetic-induced increases in the permeability of cell membranes to K^+ ions were first reported over twenty years ago (Sato, 1967; Chalazonitis, 1967; Krnjević, 1972). More recently studies have reported that a variety of general anaesthetics hyperpolarize vertebrate neurones (Nicoll & Madison, 1982; Berg Johnson & Langmoen, 1987; Carlen, Nataly & Durand, 1982), although several studies have reported no change in resting potential (Richards & Strapinski, 1986; Sawada & Yamomoto, 1985; Somgen & Gill, 1963; Yoshimura, Higashi, Fujita & Shimoji, 1985). Membrane hyperpolarization would therefore not appear to be a universal feature of anaesthetic action upon CNS neurones.

Augmentation by ethanol of the afterhyperpolarization following repetitive firing in hippocampal neurones has been reported (Carlen, Gurevich & Durand, 1982). This was not due to increased Ca^{2+} entry and so may possibly be due to a direct anaesthetic-channel interaction, although the

delay time to onset of the effects of ethanol may suggest second messenger involvement. Significant inhibition of the bullfrog atrial cell delayed rectifier current by 2% halothane has been demonstrated (Hirota, Momosa Takeda & Nakanishi, 1986). Here selective activation of a K^+ current was highlighted, with $I_{K(DR)}$ being depressed whilst the resting potential remained unchanged. The delayed rectifier current of squid giant axon has been shown to be sensitive to n-alkanols, non-polar and inhalational anaesthetics (Haydon & Urban, 1986). The reduction of current originated from more than one type of interaction, suggesting that, in this preparation, certain anaesthetics, as well as interacting with the adjacent lipid, may have an additional site of action in or on the channel itself. A novel K^+ current, which is not I_A, $I_{K(DR)}$ or $I_{K(Ca)}$, displaying sensitivity to surgical levels of general anaesthetics (halothane, isoflurane, chloroform and ether) has been reported in an identified molluscan neurone (Franks & Lieb, 1988). Activation of this current, which is not appreciably voltage gated, leads to hyperpolarization and cessation of spontaneous firing. It is particularly interesting regarding anaesthesia due to its selectivity and presence in only one cell in the ganglion studied. Methohexitone (100-1000μmol.litre^{-1}) has been shown to reduce both $I_{K(DR)}$and $I_{K(A)}$ in the leech Retzius cell and $I_{K(DR)}$ in chick dorsal root ganglion cells (Johansen, Yang, Zormumski & Kleinhaus, 1989). K^+ currents of guinea pig myocytes are impaired by the n-alkanols and high concentrations of halothane (Niggli, Rudishuli, Maurer & Weingart, 1989). Both the delayed rectifier and inward rectifier exhibited sensitivity. Anaesthetic concentrations of halothane depress the delayed rectifier current in guinea pig atrial cells (Hiroto, Ito, Masuda & Momose, 1989) whilst not affecting the inwardly rectifying K^+ current. Here the resting potential remained unchanged. Inhibition of a Ca^{2+}-activated K^+ conductance by 2.4mmol.litre^{-1} halothane has been shown in intact erythrocytes (Scharff & Foder, 1989). Here increasing the level of intracellular Ca^{2+} causes the opening of Ca^{2+} sensitive K^+ channels and leads to hyperpolarization. A23187, a Ca^{2+} ionophore, promotes hyperpolarization by increasing Ca^{2+} entry; addition of halothane before A23187 inhibits this hyperpolarization. Halothane was not found to alter Ca^{2+} permeability and must act subsequent to this to inhibit the K^+ current.

Some K^+ channels therefore would appear to exhibit sensitivity to anaesthetic agents. Anaesthetics acting at K^+ channels may lead to small changes in the membrane potential of certain cells which will modulate the activity of low threshold voltage-gated ion channels which in turn would have important consequences for the pattern of neural activity. This section of the thesis will attempt to ascertain whether anaesthetics modify K^+ channel function in hippocampal neurones. Particular attention is paid to the

accommodative behaviour of CA1 neurones with reference to the excitant actions observed with some anaesthetics (Joas & Eger, 1971; Mori, Kawamata, Mitani & Yamazaki, 1972; Po, Watson & Hansen, 1968).

PART I RESULTS

Antidromic Field Potential Studies.

The amplitude and characteristics of antidromically evoked population spikes were measured in the absence and presence of anaesthetic agents in order to highlight changes in axonal/somatic excitability. A control period of 20 min, during which deviations of no more than 10% of the initial amplitude were allowed, verified the stability of each preparation. Preparations which exhibited deviations above this level were discarded. Anaesthetic agents were applied for 30 min and any effects assessed by constructing spike amplitude vs. stimulus strength curves for a range of stimulus voltages from threshold to maximum. Changes in conduction velocity (assessed from shifts in the antidromic field potential peak) and appearance of multiple population spikes were also noted.

Inhalation Agents

All concentrations of inhalation agents quoted are vaporizer dial settings. (For effective concentrations, see Methods.)

Enflurane (Fig.13): 5% enflurane depressed the amplitude of the antidromic population spike over the whole range of voltages tested (n=5). The depression became noticeable after approximately 5 min and had reached steady state after 30 min exposure to enflurane. The depression (to 74% of the control value) was significant ($P<0.05$) for the 5x threshold voltage . 3% delivered enflurane was either ineffective or produced insignificant ($P>0.05$) shifts to the right of the spike amplitude vs. stimulus strength curve. There was no evidence of multiple population spikes at either concentration. The latency increased by up to 12% and spike width increased by up to 10% during exposure to 5% enflurane. After returning to the control media for 30 min, reversal of the observed depression occurred.

Isoflurane (Fig.14): 5% isoflurane (n=5) depressed antidromic field potential responses in a similar fashion to 5% delivered enflurane. This depression (to 85% of the control value at 5x threshold) was significant ($P<0.05$) over the whole range of voltages tested. 3% delivered isoflurane produced only very small shifts to the right in the antidromic field potential vs. stimulus strength curve (n=3). Spike width increased by up to 9% and latency increased by up to 8% in the presence of 5% isoflurane.

Halothane (Fig.15): 5% halothane induced multiple changes in the antidromic field potential (n=3). In addition to the depression ($P<0.05$) observed in the amplitude of the spike (to 70% of the control value at 5x threshold) halothane

induced a second population spike (amplitude 0.3 to 0.7mV). In parallel with the appearance of the second spike, appreciable widening of the first spike occurred. Analyzing spikes of amplitude midway between threshold and maximal (roughly 3.5mV amplitude) the average width at half amplitude was 0.75 ± 0.021msec in control conditions. This increased to 1.00 ± 0.08msec in the presence of 5% halothane, washing out to give 0.77 ± 0.0msec (i.e. mean increase of 33.91 ± 7.83%; n=3). All effects of halothane were completely reversible upon return to control medium.

Intravenous Anaesthetics

Ketamine (Fig.16): Low concentrations of ketamine were found to increase the amplitude of the antidromic field potential. 20μmol.litre^{-1} ketamine produced a significant ($P<0.05$) shift to the left in the relationship between the antidromic field potential and the stimulus voltage. After 30 min the mean increase in amplitude of the spike at 5x threshold was 33% (n=6) (Fig.16). Comparing potentials of the same amplitude from the control conditions and following exposure to 20μmol.litre^{-1} ketamine, ketamine was found to increase the amplitude of the positive wave following the spike (Fig.17). Spike width and latency were found to deviate by no more than 7% of the control value at 5x threshold. Effects of ketamine were only poorly reversed by prolonged washing (up to 1 hour): in only 2 of 6 experiments were effects reversed completely. Higher concentrations of ketamine produced no effects (200μmol.litre^{-1}, n=4) or irreversible depression (1000μmol.litre^{-1}, n=1).

Methohexitone (Fig.18): Methohexitone, 10 and 100μmol.litre^{-1} (n=4 for each concentration)(Fig.18), induced no significant ($P>0.05$) changes in field potential responses after a 30 min exposure period.

Intracellular Experiments.

Effects on Passive Membrane Properties. (Table 2.0)

Inhalation Agents

Neurones exposed to 5% enflurane hyperpolarized slightly. Hyperpolarizations lay within the range 2-8mV, with mean control, test and wash values being -62.2 ± 2.16mV, -67.3 ± 2.12mV and -64.05 ± 1.15mV respectively. The effect was significant ($P<0.001$). Input resistance was not significantly affected ($P>0.1$), with corresponding values being 48.75 ± 3.92MΩ, 47.86 ± 3.8MΩ and 41.8 ± 3.52MΩ in control, test and washout respectively (n=11).

The tendency to hyperpolarize in the presence of enflurane was also noted in 6 experiments performed with simultaneous application of 20μmol.litre^{-1} propranolol. Hyperpolarizations lay within the range 2-5mV ($P<0.001$), with mean control, test and wash values being -67.3 ± 1.96mV, -69.7 ± 2.38mV

and -66.0 ± 1.86mV respectively. Corresponding input resistance values were 39.5 ± 2.87MΩ, 41.5 ± 7.22MΩ and 38.0 ± 3.46MΩ in control, test and washout.

In 4 experiments in the presence of 30μmol.litre^{-1} BRL 24924, 20μmol.litre^{-1} cimetidine and 1μmol.litre^{-1} atropine similar resting potential shifts were seen. Exposure to 5% enflurane induced hyperpolarizations within the range 2-9mV ($P<0.002$). Mean control, test and washout values were -65.5 ± 2.74mV, -71.4 ± 3.28mV and -66.05 ± 6.0mV respectively. No detectable shifts in input resistance were noted (n=2).

In 5 experiments with 5% halothane a similar tendency to hyperpolarize was noted ($P<0.05$). Resting potential was either unaffected (n=1) or hyperpolarized (n=4) by up to 10mV with mean control, test and washout values being -72.60 ± 1.79mV, -75.8 ± 1.82mV and -73.2 ± 1.7mV respectively. Mean input resistance values were 32.0 ± 2.87MΩ, 34.67 ± 1.90MΩ and 30.0 ± 0.0MΩ in control, test and washout ($P>0.1$).

5% isoflurane (n=4) also hyperpolarized CA1 pyramidal neurones. Resting potential shifts were within the range 0 to -8mV ($P<0.05$), with mean control test and washout values being -66.25 ± 1.6mV, -72.25 ± 2.65mV and -63.3 ± 3.4mV respectively. The corresponding mean input resistance values were 50.25 ± 3.28MΩ, 48.25 ± 5.0MΩ and 49.3 ± 5.81MΩ ($P>0.5$).

Intravenous Agents

Methohexitone (50μmol.litre^{-1}) caused small reversible steady state hyperpolarizations in 9/10 neurones. In 10 neurones mean control, test and washout values were -66.08 ± 2.51mV, -71.93 ± 2.51mV and -68.37 ± 3.04mV respectively ($P<0.001$). In 2 neurones exposed to 100μmol.litre^{-1} methohexitone a similar tending to hyperpolarize was noted (hyperpolarizations of 7mV and 4mV were recorded). Input resistance decreased in the presence of 50μmol.litre^{-1} methohexitone ($P<0.002$), mean resistance being 49.4 ± 4.4MΩ, 30.2 ± 2.5MΩ and 53.8 ± 3.63MΩ in control, test and washout. In addition to the increase in steady state resting potential, transient spontaneous depolarizations were often seen in the presence of methohexitone: depolarizations of up to 5mV and 2 seconds duration were observed (See Fig.27).

The action of ketamine (20, 50, 200 and 400μmol.litre^{-1}) was tested in 14 neurones. Variable effects were recorded. Of 3 neurones exposed to 20μmol.litre^{-1} ketamine 1 depolarized by 7mV, and of 9 neurones exposed to 200μmol.litre^{-1} ketamine, 3 depolarized by up to 7mV and 2 hyperpolarized by 1mV and 5mV. At 200μmol.litre^{-1} ketamine mean control, test and washout

values were -67.33 ± 1.63mV, -67.66 ± 2.51mV and -67.22 ± 2.04mV respectively (n=11) (P>0.5). Corresponding input resistance values were 38.25 ± 2.79MΩ, 39.0 ± 4.33MΩ and 37.0 ± 4.59MΩ in control, test and washout (n=8) (P>0.5).

Effects on Accommodation.

Inhalation Agents. (See table 3.0 for pooled data and significance values) Enflurane (n=9) (Fig.19), isoflurane (n=4) (Fig.20) and halothane (n=4) (Fig.21) [concentration range 3-5% delivered] all significantly reduced accommodation of spike discharge to 800ms (600ms in the case of some early enflurane experiments) depolarizing current pulses (usually 0.5nA). They also reduced the compound AHP following 80ms depolarizing current pulses (measurements were taken at or near to -70mV in all cases). In 4 experiments a range of depolarizing current pulses(0.1-1.0nA) were employed. In this instance spike numbers increased with escalating current intensity up to a maximum of 6 spikes with an 800ms/0.7nA depolarizing current in control conditions. After 15 min exposure to 5% enflurane responses to weak depolarizing currents were slightly reduced whilst responses to strong depolarizing inputs were markedly enhanced (for example; 13.25 ± 1.7 spikes average compared to 6.0 ± 0.91 in control) (Fig.22A&B).

In 6 other neurones 5% enflurane, in the presence of 20μmol.litre^{-1} propranolol (Fig. 23), reduced the accommodation of spike discharge to 0.5nA depolarizing current pulses in a manner similar to that seen in experiments in the absence of propranolol. Propranolol 20μmol.litre^{-1} was present for a control period of 20 min before testing the anaesthetic.

4 experiments in the presence of cimetidine (20μmol.litre^{-1}), atropine (1μmol.litre^{-1}) and BRL 24924 (30μmol.litre^{-1}) were performed to test whether the neurotransmitters histamine, acetylcholine and 5-hydroxytryptamine, which are known to reduce accommodation in CA1 neurones, are involved in the previously observed behaviour in the presence of enflurane. Slices were pretreated with the antagonists for 30 min and a range of depolarizing currents (800ms, 0.1-0.7nA) were used to test accommodation. In the presence of the antagonists similar changes were seen following application of 5% enflurane, as with 5% enflurane alone. The maximum control spike number at 0.7nA was 5.75 ± 0.75 increasing to 13.5 ± 1.55 after 15 min exposure to enflurane (Fig.24A&B). In all experiments involving enflurane the compound AHP following an 80ms current pulse (see Storm, 1989 and Introduction) was reduced (See table 3.0). Returning to anaesthetic-free ACSF led to a gradual recovery of the compound AHP and the accommodative properties of most neurones tested: although complete recovery of the characteristic "sag" in

the voltage trace was rarely seen (Fig.24).

In 4 experiments the characteristics of calcium spikes recorded in the presence of TTX (1μmol.litre^{-1}) and TEA (5mmol.litre^{-1}) remained unchanged by addition of 5% delivered enflurane (Fig. 24C).

The above effects observed with the inhalational agents occured in the absence of changes in threshold current required to elicit a single spike; in the 2 early enflurane experiments, where the cells hyperpolarized and were not returned to their control resting potential, the threshold current actually increased.

Intravenous Anaesthetics

The effects of ketamine (20, 50, 70 and 400μmol.litre^{-1}) on the accommodative properties of 13 CA1 neurones were investigated. Of the 8 neurones exposed to 200μmol.litre^{-1} 4 had their accommodative properties compromised (Fig.25). The mean number of action potentials following an 800ms/0.5nA pulse rose from 6.5 ± 0.57 in control to 12.2 ± 1.5 in the presence of ketamine. In 1 of 2 neurones exposed to 20μmol.litre^{-1} ketamine accommodation was also reduced, the number of spikes rising to 11 from a control value of 6. Surprisingly, both for neurones that kept their accommodation properties and those in which it was compromised, the amplitude of the compound AHP following an 80ms (and 800ms) depolarizing current pulse remained unchanged (i.e. 6.0 ± 1.38 in the presence of ketamine, 5.8 ± 1.16 in control, 5.4 ± 1.02 following washout). The characteristic sag in the voltage response usually seen in accommodating neurones was abolished with ketamine and this effect was poorly reversed on prolonged washing (as were any other effects of ketamine).

Methohexitone 50μmol.litre^{-1} had mixed effects on the accommodative properties of 4 neurones. 2 neurones had their spike responses totally abolished, 1 neurone had responses reduced and 1 neurone became slightly more excitable. Throughout the range of currents used (0.1-0.7nA) mean spike responses were not significantly altered, but a significant reduction in AHP amplitude did occur (Table 3.0). AHPs were either completely abolished or sometimes reduced so that the AHP became slightly depolarizing (Fig.26). In neurones that did not have the depolarizing after potential, some of the medium AHP (50-100ms) appeared to persist even when the slow AHP (>1s) had been blocked. Mean control AHP amplitude following a 0.7nA current pulse was 8.76 ± 1.78mV (n=6); this reduced to 3.41 ± 1.54mV after 15 min exposure to 50μmol.litre^{-1} methohexitone. The effect was reversible, the AHP returning to 7.5 ± 1.25mV after 30 min washout of anaesthetic. In 2 other neurones 100μmol.litre^{-1} methohexitone exerted similar actions to those observed with 50μmol.litre^{-1}. Following washout of methohexitone recovery of the AHP was

achieved and spike responses were enhanced. Thus from a value of 3.6 ± 1.85 spikes in methohexitone, 30 min washout led to an increase in spike number to 9.5 ± 0.41. This was the case for all depolarizing currents tested (Fig.26). In 3 neurones the abolition of the AHP following an 80ms current pulse was partially reversed by application of 50μmol.litre^{-1} bicuculline (Fig.27). The medium AHP component recovering more completely than the slow AHP component.

Table 3.0 summarizes the data presented in the above section.

Effects on Threshold Behaviour.

Inhalation Anaesthetics

Direct Stimulation: Thresholds for single spike generation were assessed with an 8ms depolarizing current pulse through the intracellular electrode. No reduction in threshold current was observed for any of the inhalation agents.

Orthodromic Stimulation: The voltage required to elicit a single orthodromic spike was not systematically monitored during all experiments. However, a tendency for an increase in threshold (up to 20%) in the presence of anaesthetic was noted.

Intravenous Anaesthetics

Direct Stimulation: 8 of the neurones exposed to ketamine became more sensitive to direct stimulation (8ms pulse width). Thus in the 3 neurones exposed to 20μmol.litre^{-1} ketamine and in 5 of the 8 exposed to 200μmol.litre^{-1} there was a decrease in direct threshold current. In a few cases the current required to elicit a single spike decreased by up to 40% of the control value. Spontaneous firing was observed in 1 neurone exposed to 200μmol.litre^{-1}. Prolonged washing of up to 1 hour only partially reversed any excitability changes. Methohexitone (50 or 100μmol.litre^{-1}) produced variable changes in the current required to stimulate the cell directly. Cells were either unaffected, or threshold was increased by up to 70%.

Orthodromic Stimulation: Orthodromic threshold responses remained unchanged in the 2 neurones exposed to 20μmol.litre^{-1} ketamine. 200μmol.litre^{-1} however usually reduced markedly the voltage required to elicit an orthodromic action potential. In 4 of 5 cells monitored at 200μmol.litre^{-1}, the mean percentage reduction was 75 ± 7.20%.

Methohexitone produced variable changes in orthodromic threshold current. 1 preparation exhibited no change in orthodromic threshold, whilst the others varied from 10% increase in threshold to 1 preparation in which a spike could not be evoked by up to 100V stimulation (control stimulation being 18V).

Whilst monitoring orthodromic thresholds it was noted that methohexitone consistently (within 10 min) induced a large amplitude (up to 26mV, $\bar{x}$=10.64 ± 7.06mV, n=11), long lasting (up to 16 seconds, $\bar{x}$=7.5 ± 1.34 seconds, n=11) depolarization which followed the AP-IPSP or EPSP-IPSP sequence (Fig.28). The amplitude of the response increased with escalating stimulus voltage and occasionally gave rise to action potentials at higher voltages. A large decrease in input resistance occurred during the depolarization, as assessed by 0.5nA, 80ms, 2Hz current pulses (Fig.29). The decrease was maximal at the peak of the depolarization and progressively recovered as the membrane potential returned to the resting state. Bicuculline 50μmol.litre^{-1} either greatly reduced (Fig.29) or totally abolished (Fig.30) the large amplitude after depolarization. Bicuculline also fully reversed the decrease in input resistance seen in methohexitone. These effects of methohexitone were reversible upon return to anaesthetic free ACSF.

Voltage-Clamp Measurements.

Single microelectrode voltage-clamp measurements were made in 7 hippocampal CA1 neurones. The mean resting potential upon insertion of the electrode was -67.0 ± 1.70mV. In order to avoid the problem of synaptic activity and unclamped action potentials distorting the clamp current records, analysis was carried out in 1μmol.litre^{-1} TTX. This has the advantage of suppressing virtually all synaptic noise so that the observed currents could be ascribed to postsynaptic effects. M-currents were recorded from the 7 neurones according to the protocol of Halliwell & Adams, 1982; (A complete description of the rationale and protocol appears in the Methods section). Inward relaxations occurring for negative steps between -40mV and -70mV were recorded in the 7 neurones that were voltage-clamped. Application of 50μmol.litre^{-1} carbachol reduced these time dependent currents and elicited an inward shift in the holding current when the cell was held at -40mV, but not when the cell was held at -70mV (Fig.31). These observations agree with previous clamp studies testing for the presence of the M-current. 5% enflurane consistently reduced the M-current in all neurones tested. The reduction was progressive and failed to washout completely. Fig.32 illustrates a typical voltage-clamp experiment with 5% enflurane. After 10 min exposure to enflurane the current relaxation was much reduced. Some recovery is evident following a 20 min wash period but is not complete. This lack of reversibility may not be surprising considering the lack of reversibility of the reduction of the "sag" in the voltage trace noted earlier.

Fig.13

Fig.13

Antidromic field potentials in the absence (A) and presence (B) of 5% delivered enflurane at the stimulus intensities of threshold (Thr) 2x, 4x & 6x threshold.

A. Control.

B. 30 min 5% delivered enflurane.

C. Pooled data for five experiments. The effects of 5% delivered enflurane on the hippocampal CA1 neurone antidromic population spike amplitude vs stimulus strength curve (data points are mean ± SEM).

Control = open circles, filled circles = 5% delivered enflurane.

Temperature A & B =32°C; C = 28-32°C. (Stimuli 0.1Hz; 80μs).

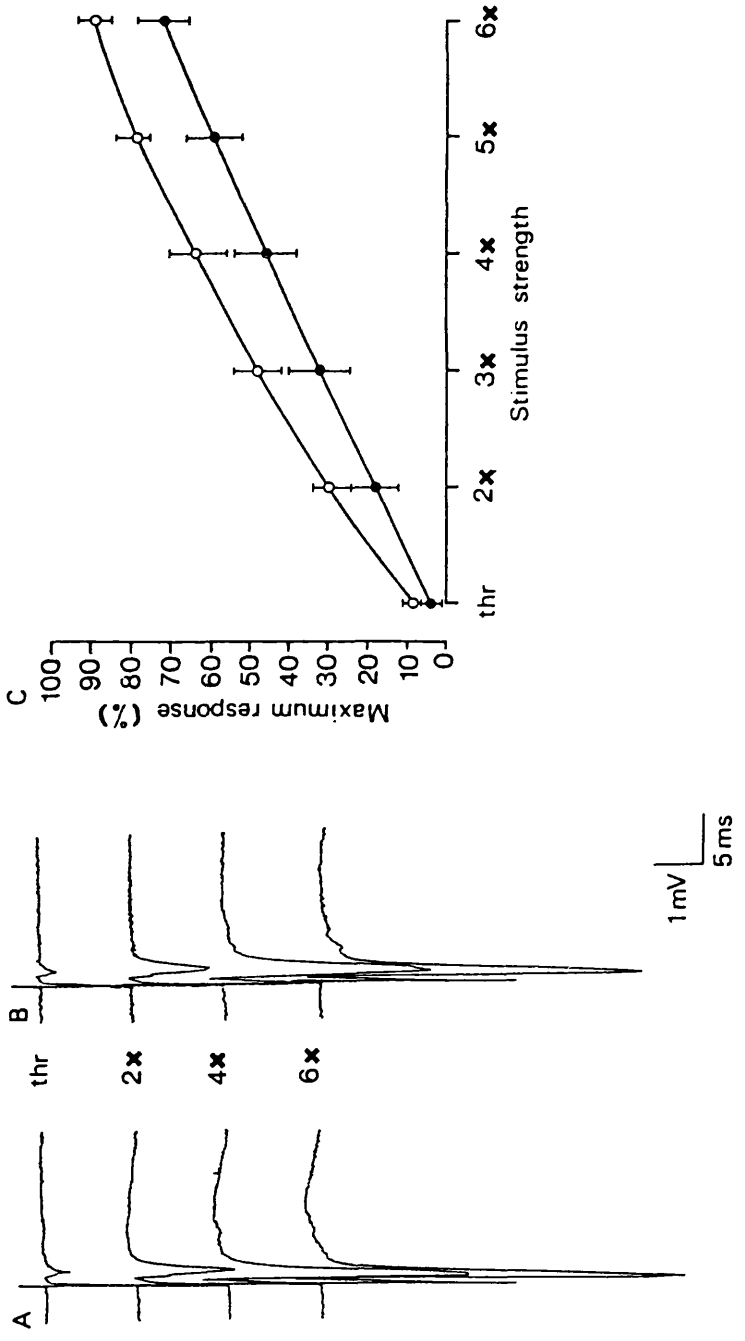

A
B
C
thr
2×
4×
6×
1mV
5ms
Maximum response (%)
100
90
80
70
60
50
40
30
20
10
0
thr
2×
3×
4×
5×
6×
Stimulus strength

Fig.14

Fig.14

The effects of 5% delivered isoflurane on the antidromic field potential of hippocampal CA1 neurones at differing stimulus intensities.

A. Control.

B. 5% delivered isoflurane (30 min exposure).

C. 30 minute washout.

Graph shows the pooled data (n=5) illustrated as antidromic field potential amplitude vs. stimulus strength curve (mean ± SEM).

For clarity no SEM are included for wash values.

Temperature A,B,C = 30°C, graph = 28-32°C. (Stimuli, 0.1Hz, 80μs).

Stimulus artifacts have been removed for clarity.

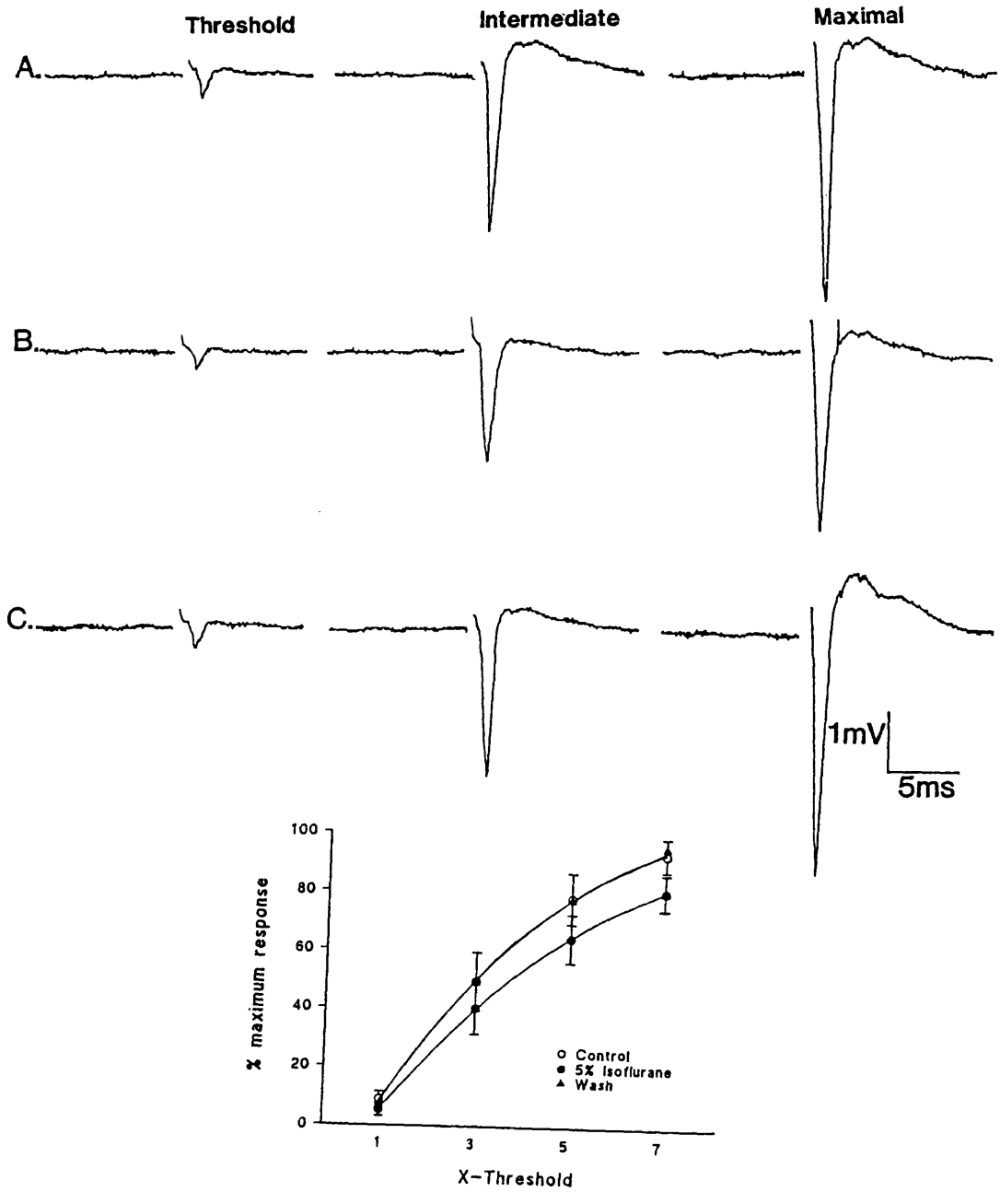

Threshold
Intermediate
Maximal
A.
B.
C.
1mV
5ms
100
80
60
40
20
0
% maximum response
1
3
5
7
X-Threshold
Control
5% Isoflurane
Wash

Fig.15

Fig.15

The effects of 5% delivered halothane on the antidromic field potential.

A. Control.

B. 5% delivered halothane (30 min).

C. Wash (30 min).

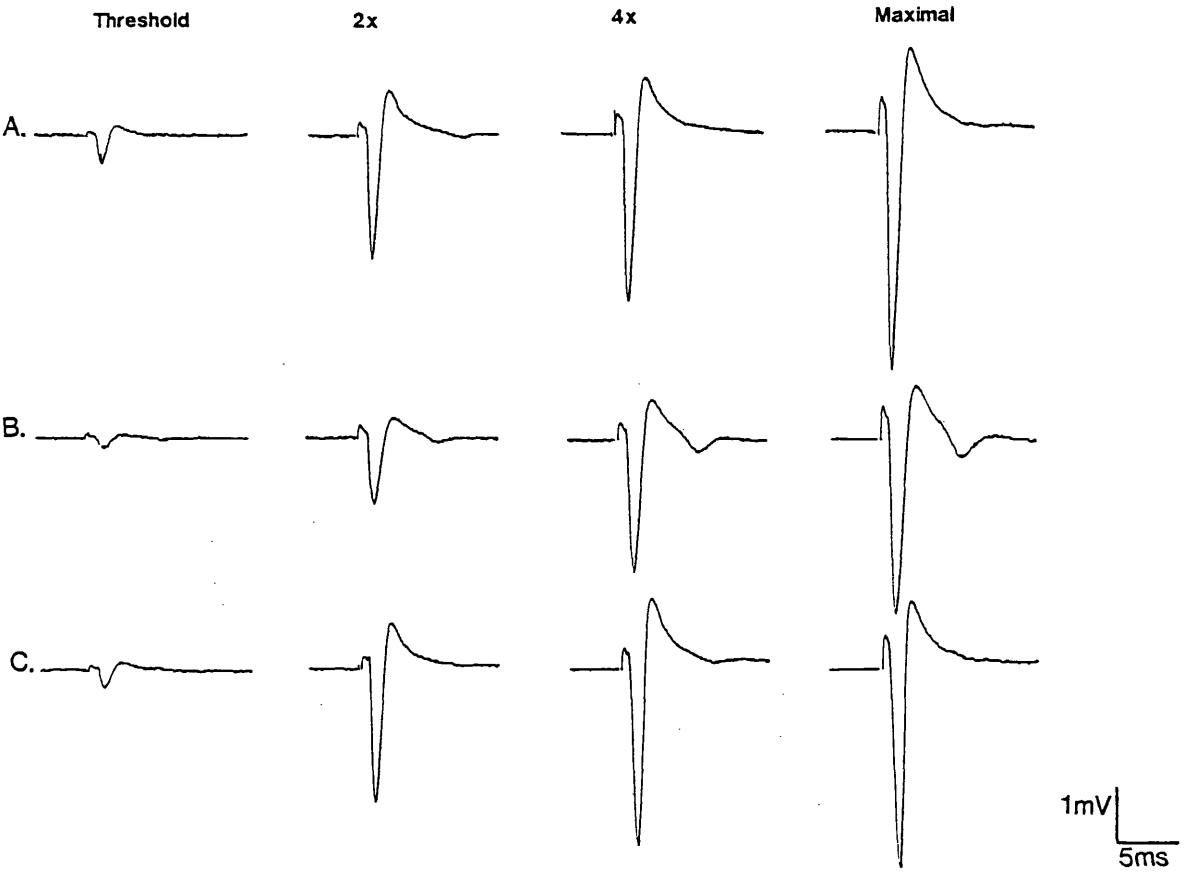
Note the appearance of the second population spike in B.

Graph shows relationship between the antidromic field potential (percentage of maximum response) and the stimulus voltage (Means ± SEM, n=3).

For clarity no SEM are shown for wash values.

Temperature:- A,B,C = 30°C; Graph = 28-32°C. (Stimuli 0.1Hz; 80μs).

Stimulus artifacts have been removed for clarity.


Threshold
2x
4x
Maximal
A.
B.
C.
1mV
5ms

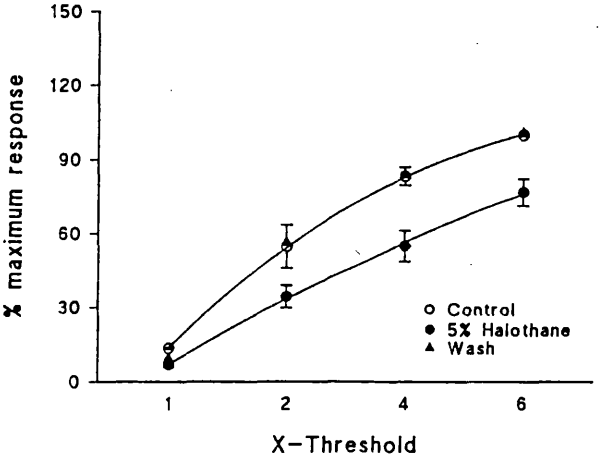

% maximum response
150
120
90
60
30
0
1
2
4
6
X-Threshold
Control
5% Halothane
Wash

Fig.16

Fig.16

The effect of 20μmol.litre^{-1} ketamine (30 min) on the amplitude of the antidromic field potential recorded from the CA1 pyramidal neurone field.

A. Control.

B. Ketamine.

C. Wash.

Responses were recorded at 1V (threshold), 2V, 3V, 4V & 5V.

D. Relationship between the amplitude of the antidromic field potential (% max control response) and the stimulus voltage (means ± SEM, n=6).

For clarity no SEM are shown for wash values.

Temperature = A,B,C 30°C; D 28-32°C. (Stimuli 0.1Hz, 80μs).

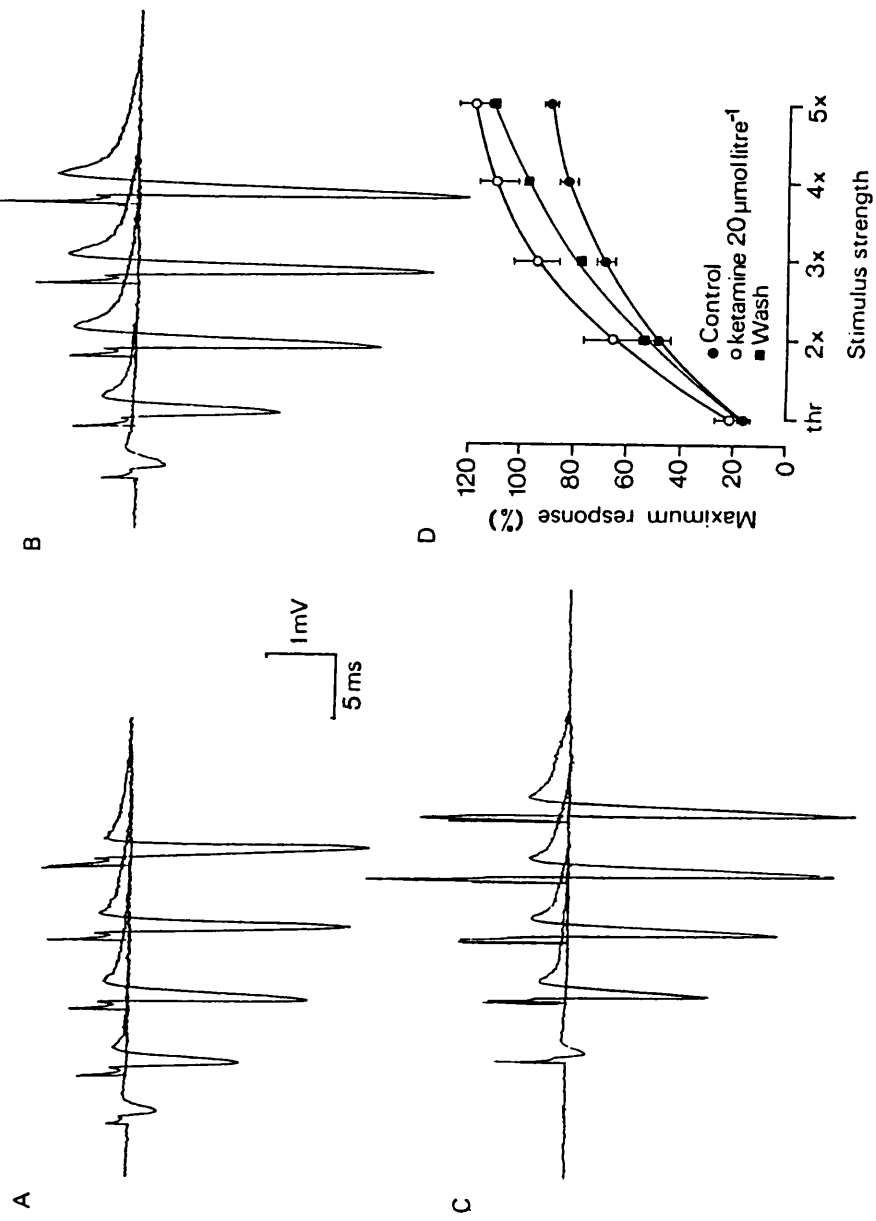

A
1mV
5ms
B
C
D
Maximum response (%)
120
100
80
60
40
20
0
● Control
○ ketamine 20 μmol litre⁻¹
■ Wash
thr
2x
3x
4x
5x
Stimulus strength

Fig.17

Fig.17

Spikes of identical amplitude in the absence and presence of 20μmol.litre^{-1} ketamine (30 min) have been superimposed.

Control stimulus voltage = 5V, ketamine stimulus voltage = 3V. Note the enhancement of the positive wave following the antidromic spike in the presence of ketamine.

For clarity the stimulus artifacts have been removed.

Temperature = 30°C. (Stimuli 0.1Hz, 80μs).

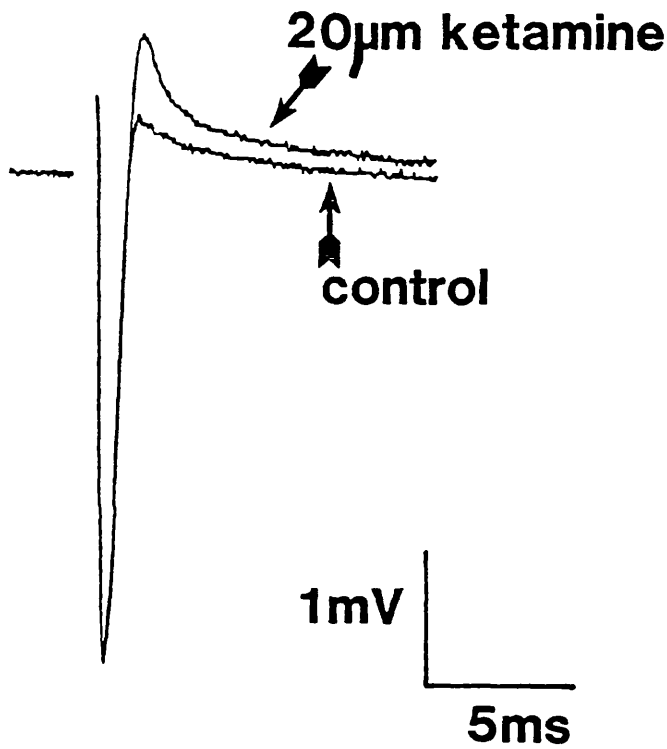

20μm ketamine
control
1mV
5ms

Fig.18

Fig.18

The effects of methohexitone 100μmol.litre^{-1} on the antidromic field potential evoked by stimulation of the alveus.

A. Control.

B. 30 min 100μmol.litre^{-1} methohexitone.

C. 30 min washout.

Graph shows pooled data (n=4) for the effects of 100μmol.litre^{-1} methohexitone on the antidromic population spike amplitude vs. stimulus strength curve

(Data points are mean ± SEM).

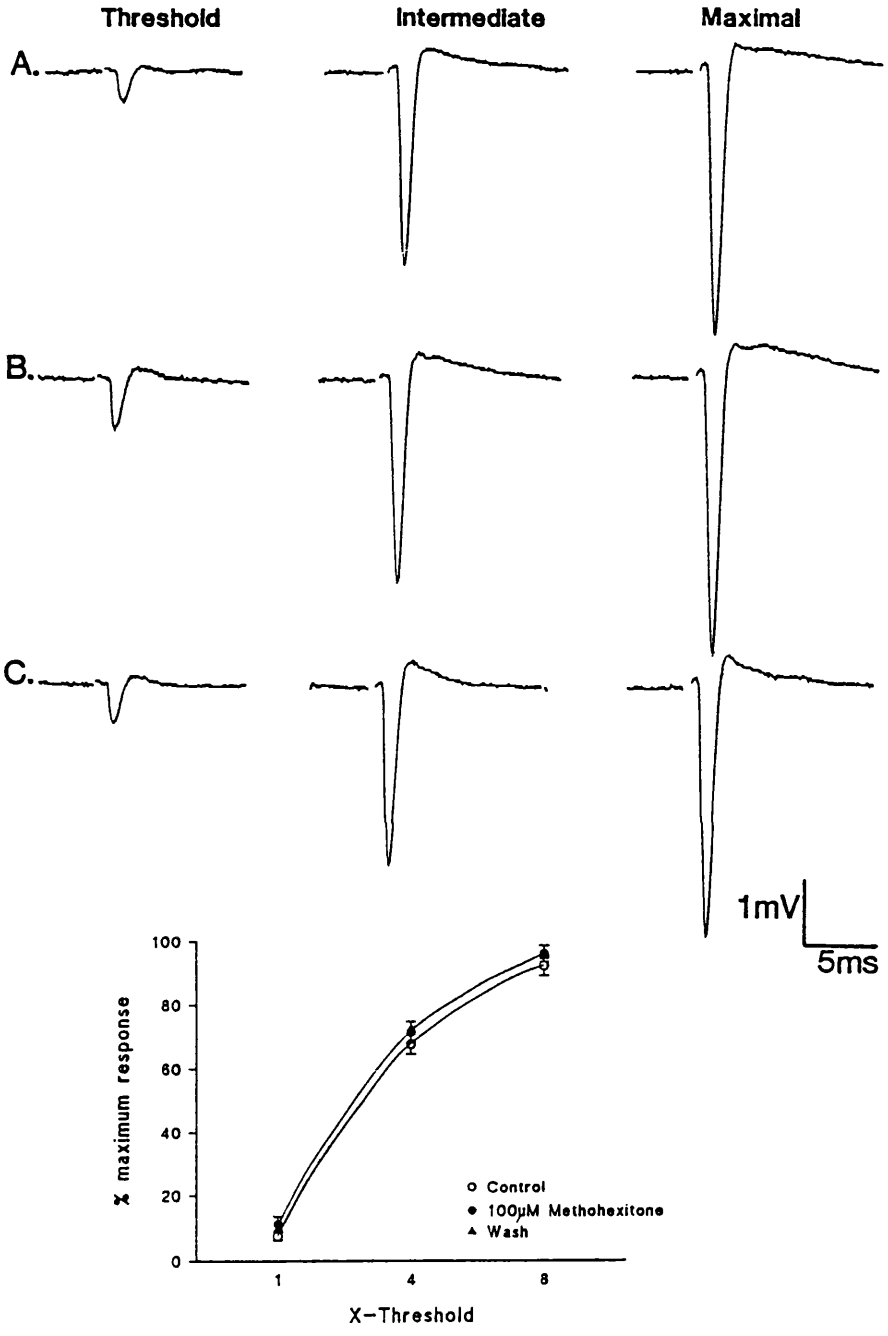

Threshold
Intermediate
Maximal
A.
B.
C.
1mV
5ms
% maximum response
100
80
60
40
20
0
1
4
8
X-Threshold
Control
100μM Methohexitone
Wash

Table 2.0

Table 2.0

Mean resting membrane potential (mV) and cell input resistance (MΩ) for neurones in the absence and presence of the inhalational and intravenous agents. Values are mean ± SEM.

* - Denotes value significantly different from the control value ($p<0.05$).

"Antagonists" were cimetidine (20μmol.litre^{-1}), atropine (1μmol.litre^{-1}) & BRL 24924 (30μmol.litre^{-1}).

Table 2.0		
Conditions	Resting Potential	Input Resistance
Control (n=11) 5% Enflurane Wash	-62.0±2.2 -67.0±2.1 * -64.1±1.2	48.8±3.9 47.9±3.8 41.8±3.5
Control (n=6) 5% Enflurane + 20μM Propranolol Wash	-67.3±2.0 -69.7±2.4 * -66.0±1.2	39.5±2.9 41.5±7.2 38.0±3.5
Control (n=4) 5% Enflurane + Antagonists Wash	-65.5±2.8 -71.4±3.3 * -66.1±6.0	no change (n=2)
Control (n=5) 5% Halothane Wash	-72.6±1.8 -75.8±1.8 * -73.2±1.7	34.7±1.9 32.0±2.9 30.0±0.0
Control (n=4) 5% Isoflurane Wash	-66.3±1.6 -69.3±2.7 * -65.3±3.4	50.2±3.3 48.3±5.1 49.3±5.8
Control (n=10) 50μM Methohexitone Wash	-66.1±2.5 -71.9±2.5 * -68.4±3.0	49.4±4.5 30.2±2.5 * 53.8±3.6
Control (n=11) 200μm Ketamine Wash	-67.3±1.6 -67.7±2.5 -67.2±2.0	38.3±2.8 39.0±4.3 37.0±4.6

Fig.19

Fig.19

Effect of 5% delivered enflurane on the accommodation of action potential discharge in a CA1 pyramidal neurone.

A. Control.

B. 15 min exposure to 5% delivered enflurane.

C. Current pulse (600ms/0.9nA).

Resting membrane potential was -71mV in A and -76mV in B. Note; action potentials are attenuated due to limited sampling frequency of the digital oscilloscope.

Temperature = 32°C.

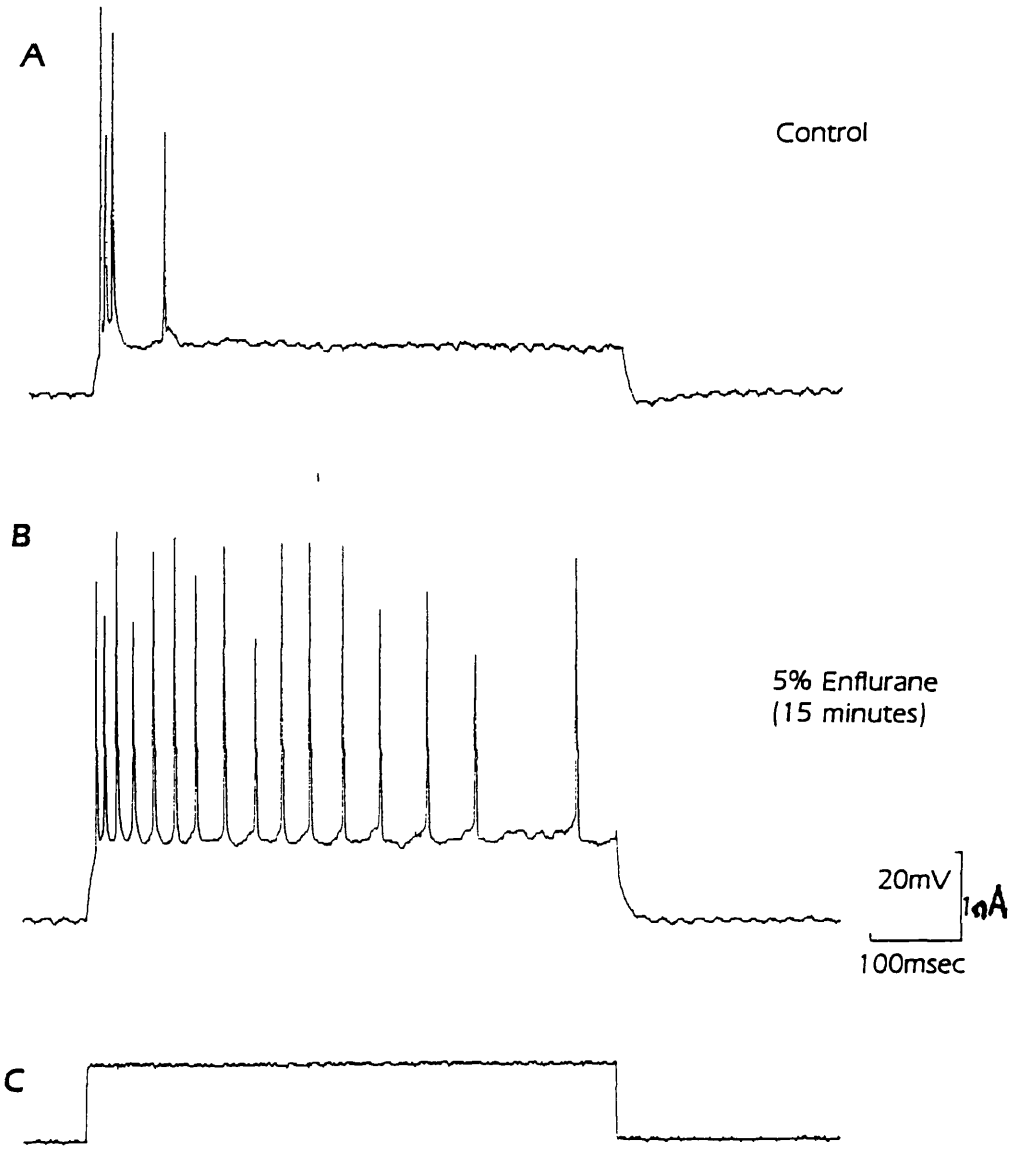

A
Control
B
5% Enflurane
(15 minutes)
20mV
1nA
100msec
C

Fig.20

Fig.20

Action of 5% delivered isoflurane on the accommodation of a CA1 neurone to a long depolarizing current pulse (800ms)(A_1, B_1, C_1) and the after-hyperpolarization (A_2, B_2, C_2) following an 80ms current pulse.

A_1 A_2 = Control.

B_1 B_2 = 5% delivered halothane.

C_1 C_2 = Wash.

Current pulse D_1 D_2 = 0.5nA. Temperature = 30°C.

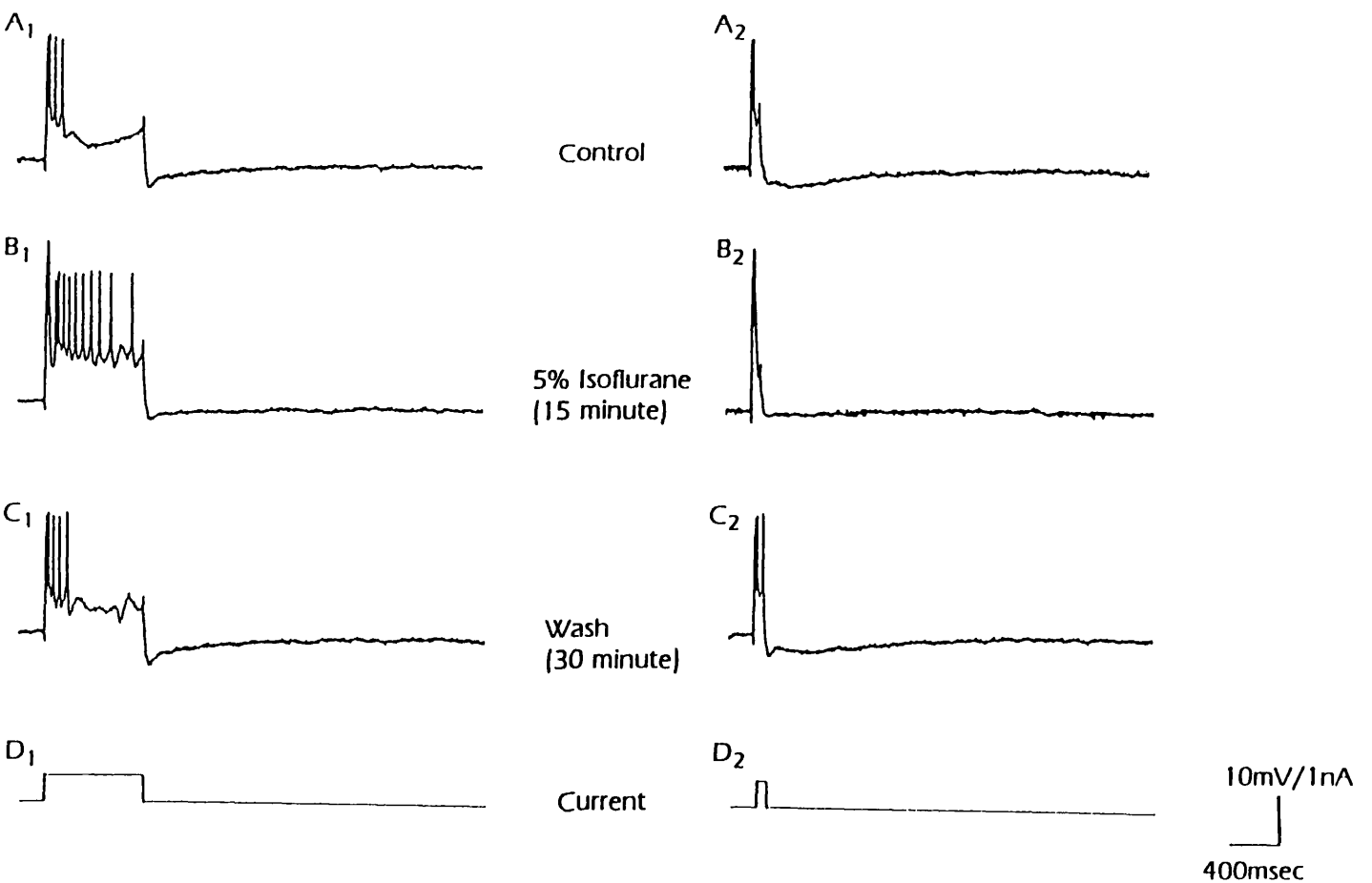

A1
A2
Control
B1
B2
5% Isoflurane
(15 minute)
C1
C2
Wash
(30 minute)
D1
D2
Current
10mV/1nA
400msec

Fig.21

Fig.21

5% delivered halothane was found to reduce accommodation to long depolarizing current pulses (800ms)(A_1, B_1, C_1) and the after-hyperpolarization following a shorter pulse (80ms) (A_2, B_2, C_2).

A_1, A_2 = Control.

B_1, B_2 = 5% delivered halothane.

C_1, C_2 = Washout.

Current pulse 0.5nA.

Temperature = 30°C.

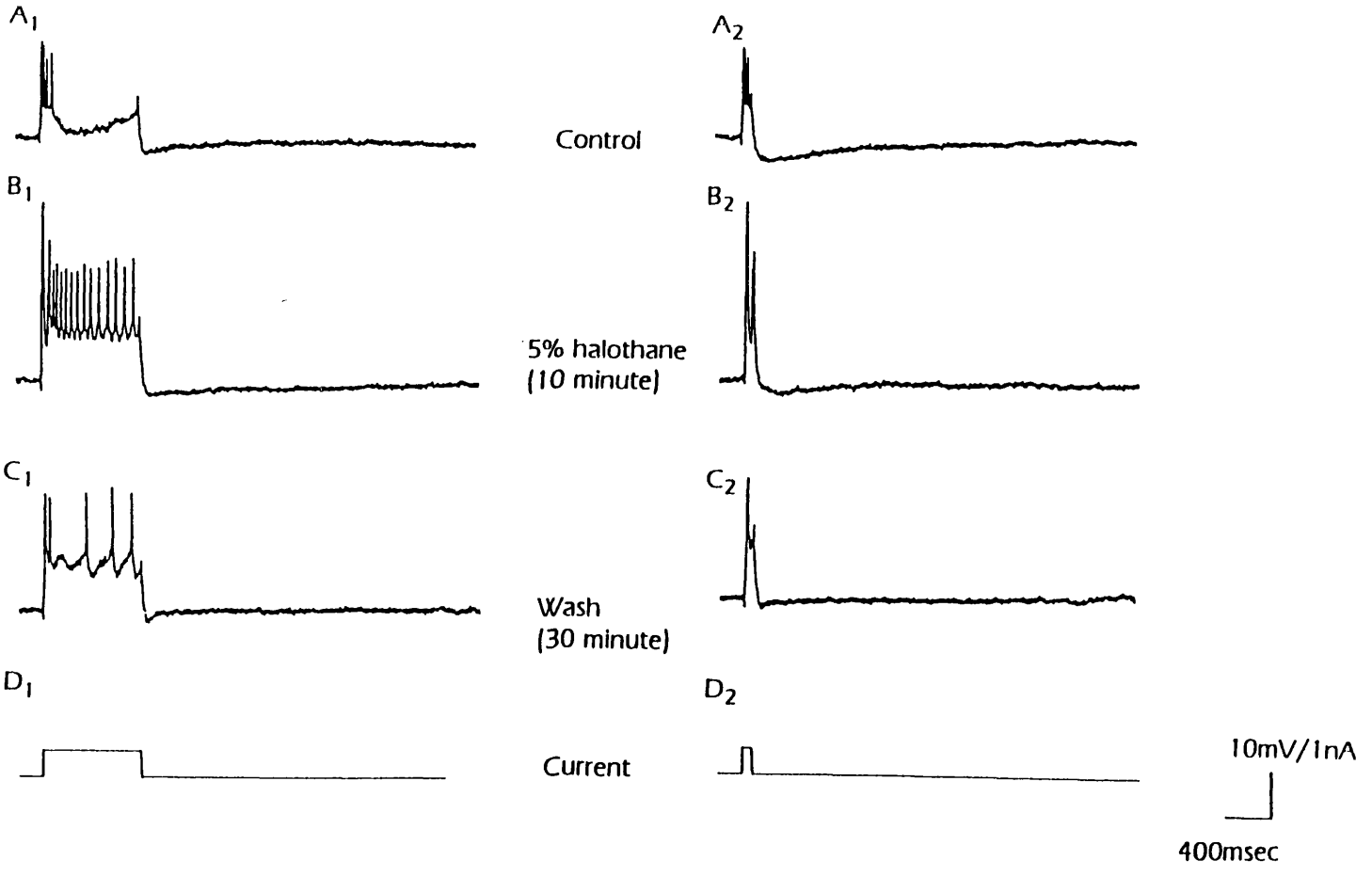

A1
A2
Control
B1
B2
5% halothane
(10 minute)
C1
C2
Wash
(30 minute)
D1
D2
Current
10mV/1nA
400msec

Table 3.0

Table 3.0

Summary of the effects of the anaesthetics studied upon the number of spikes evoked by an 800ms current pulse and the amplitude of the after-hyperpolarization (mV) following an 80ms current pulse. (Current pulses were 0.5 or 0.7nA). Values = mean ± S.E.M.

* - Denotes value significantly different from the control value.

"Antagonists" were cimetidine 20μmol.litre^{-1}, atropine 1μmol.litre^{-1} and BRL 24924 30μmol.litre^{-1}.

TABLE 3.0		
Conditions	Number of spikes (800ms)	AHP amplitude (80ms)
Control (n=5) 2.5% Enflurane Wash	5.4±0.5 14.4±1.2 * 6.2±0.7	6.1±0.8 3.8±0.6 * 4.2±0.7
Control (n=6) 2.5% Enflurane + 20μM Propranolol Wash	6.1±0.8 12.7±2.4 * 7.0±0.9	5.8±1.8 2.2±0.8 * 4.9±1.1
Control (n=4) 2.5% Enflurane + 3 Antagonists Wash	5.8±0.8 13.5±1.6 * 6.5±1.8	Not Measured
Control (n=5) 2.5% Halothane Wash	6.1±0.8 12.7±2.4 * 6.0±0.0	4.2±0.4 1.4±0.5 * 2.2±0.1
Control (n=4) 2.5% Isoflurane Wash	5.8±0.5 13.5±0.6 * 7.5±0.4	5.8±0.5 3.9±0.5 * 5.5±0.6
Control (n=9) 200μM Ketamine Wash	6.5±0.6 12.2±1.5 * -	5.8±1.2 6.0±1.4 5.4±1.0
Control (n=4) 50μM Methohexitone Wash	4.7±0.3 3.6±1.9 9.5±0.4	8.8±1.8 3.4±1.5 * 7.5±1.3

Fig.22A

Fig.22A

The dependence of number of spikes evoked in response to current pulses of differing intensities (0.1 - 0.7nA/800ms) in the absence and presence of 5% delivered enflurane.

A. Control.

B. 15 min 5% delivered enflurane.

C. Washout (30 min).

Temperature = 32°C. Resting membrane potential = -70mV, for all measurements. Numbers represent number of action potentials in response to the current pulse.

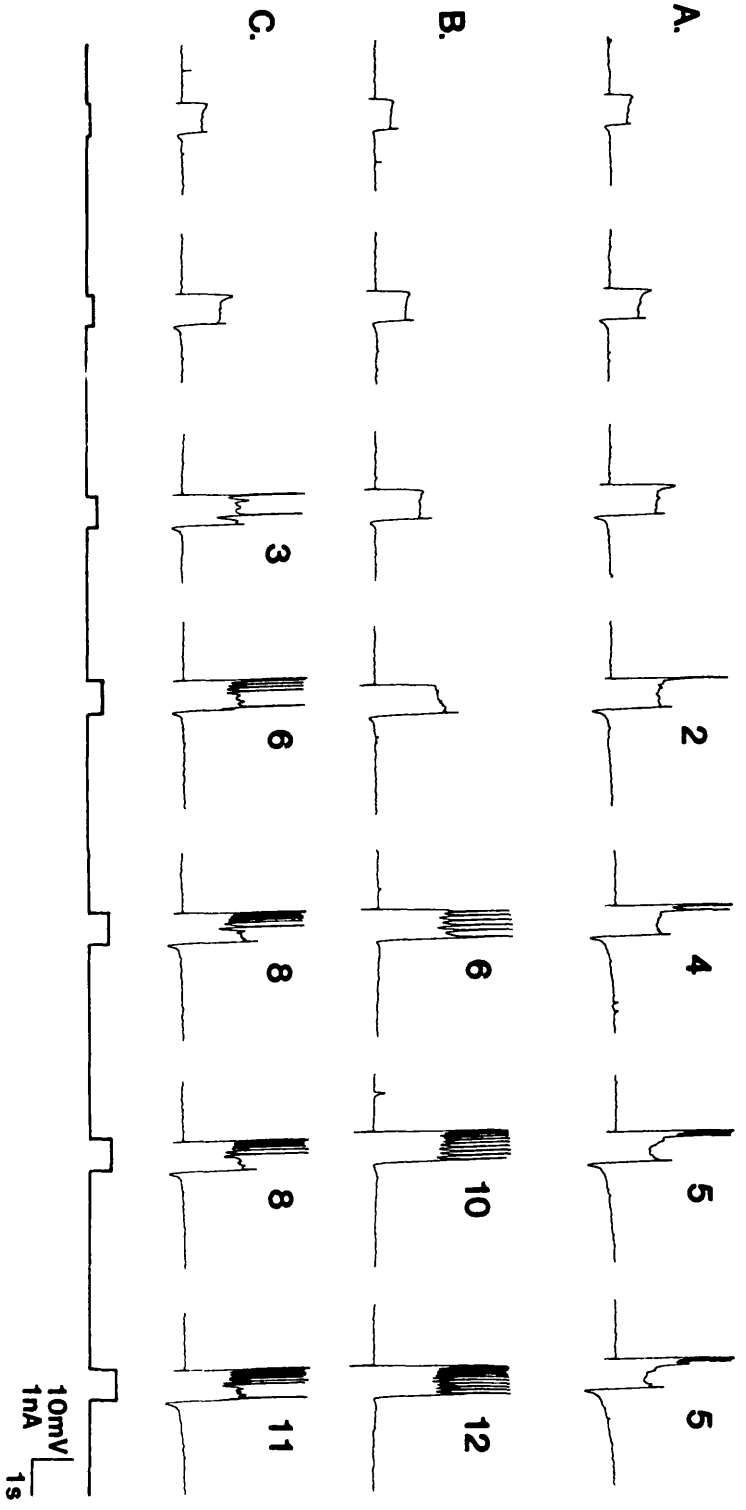

A.
B.
C.
2
4
5
5
6
6
10
12
3
6
8
8
11
10mV
1nA
1s

Fig.22B

Fig.22B

Graph showing pooled data (n=4) for the type of experiment illustrated in Fig.22A. Current pulses were 800ms duration. Data points = mean ± SEM.

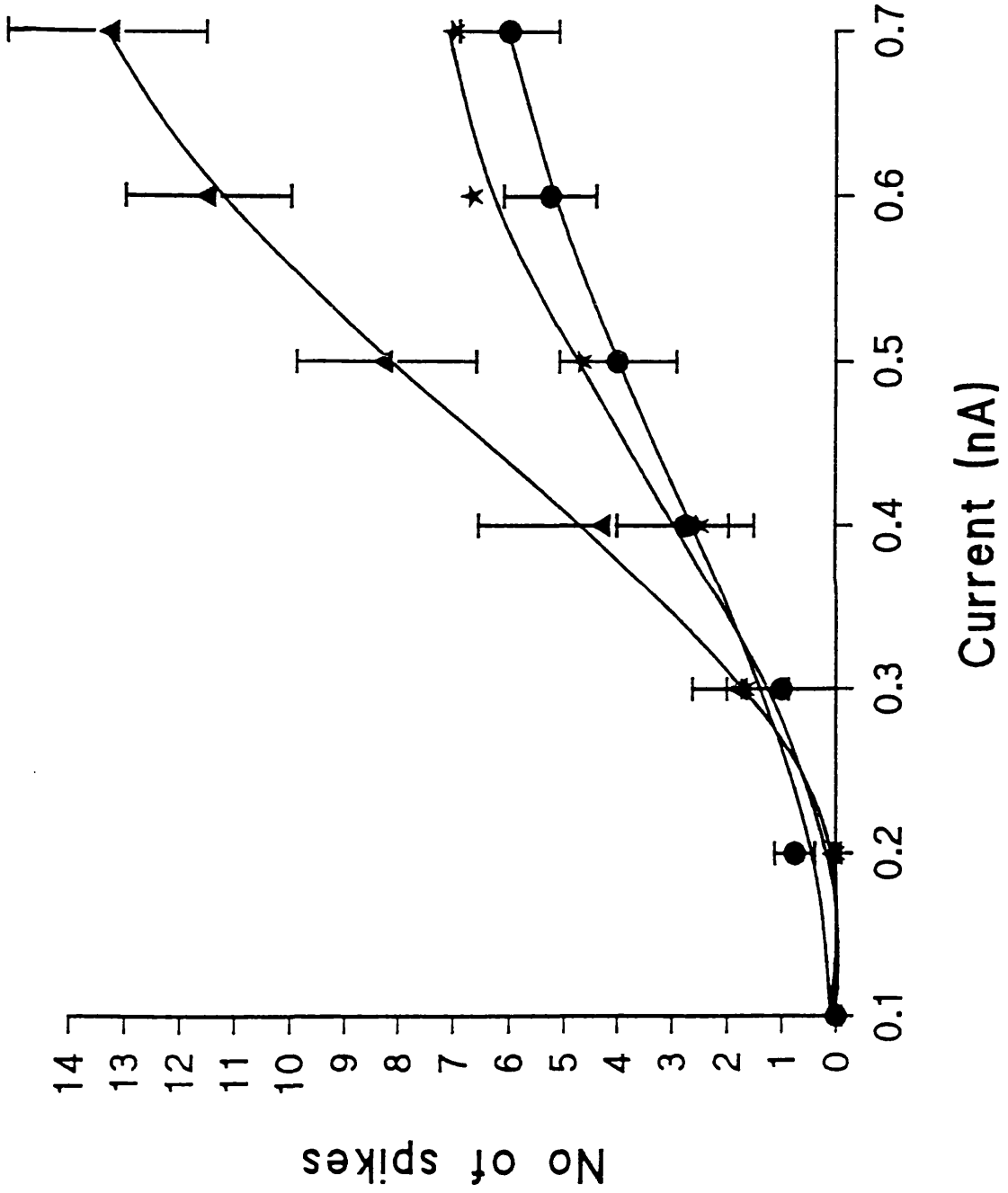

No of spikes
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Current (nA)

Fig.23

Fig.23

The effects of a 15min exposure to 5% delivered enflurane in the presence of propranolol 20μmol.litre^{-1} (throughout A, B, C) on the accommodation (A_1, B_1, C_1) of a CA1 neurone to depolarizations of long duration (800ms) and the after-hyperpolarization (A_2, B_2, C_2) following 80ms current pulses. The current pulse was 0.5nA in each case.

Temperature = 32°C. Resting membrane potential = -70mV.

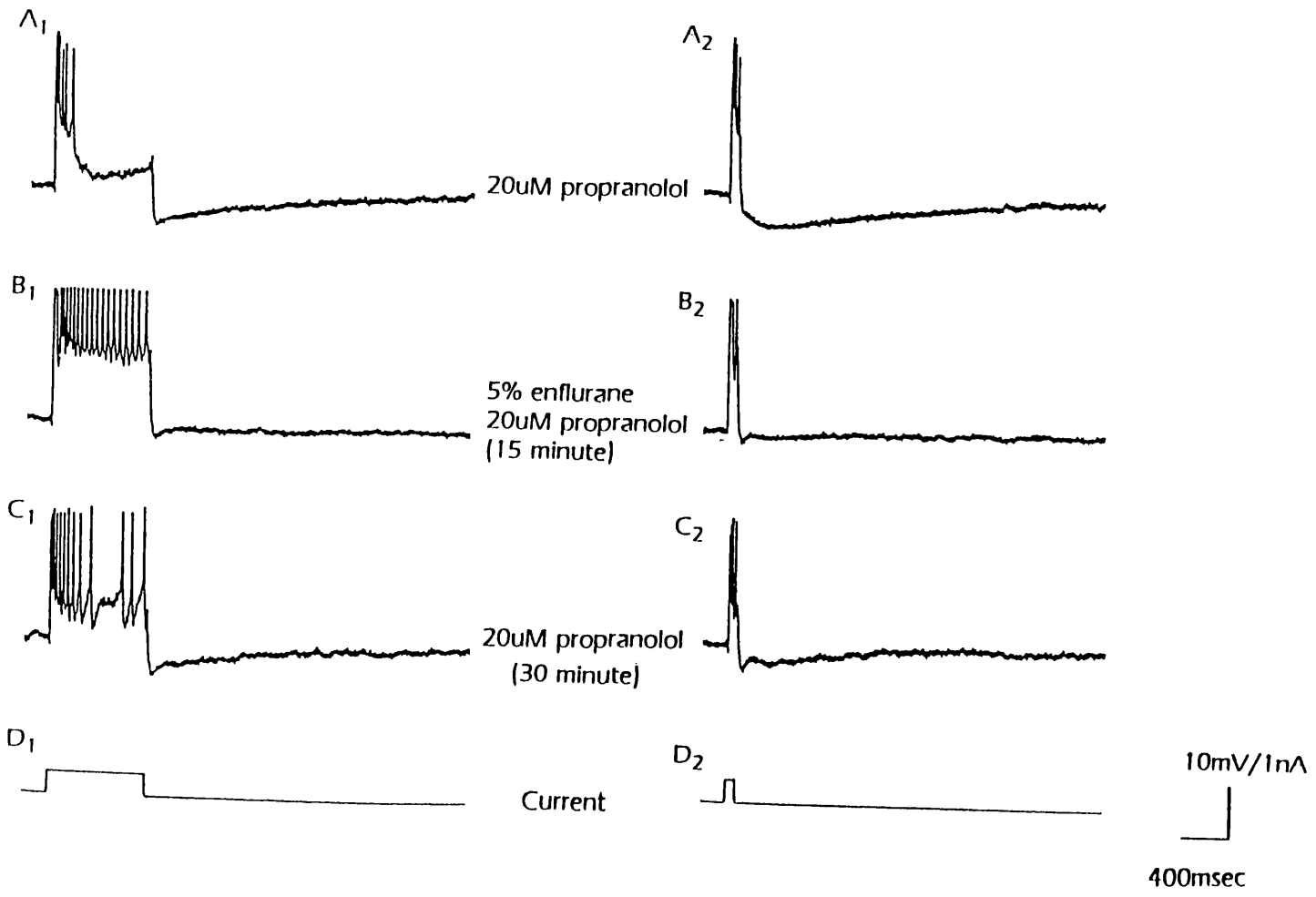

A1
A2
20uM propranolol
B1
B2
5% enflurane
20uM propranolol
(15 minute)
C1
C2
20uM propranolol
(30 minute)
D1
D2
Current
10mV/1nA
400msec

Fig.24A

Fig.24A

Accommodation of action potential discharge in the absence (A and C) and presence (B) of 5% delivered enflurane. 20μmol.litre^{-1} cimetidine, 30μmol.litre^{-1} BRL 24924 and 1μmol.litre^{-1} atropine were present at all times (A, B & C).

A. Control.

B. Enflurane.

C. 30 min washout of anaesthetic.

Numbers denote number of action potentials in response to the current pulse. Note the lack of recovery of the "sag" in the voltage trace (arrowed).

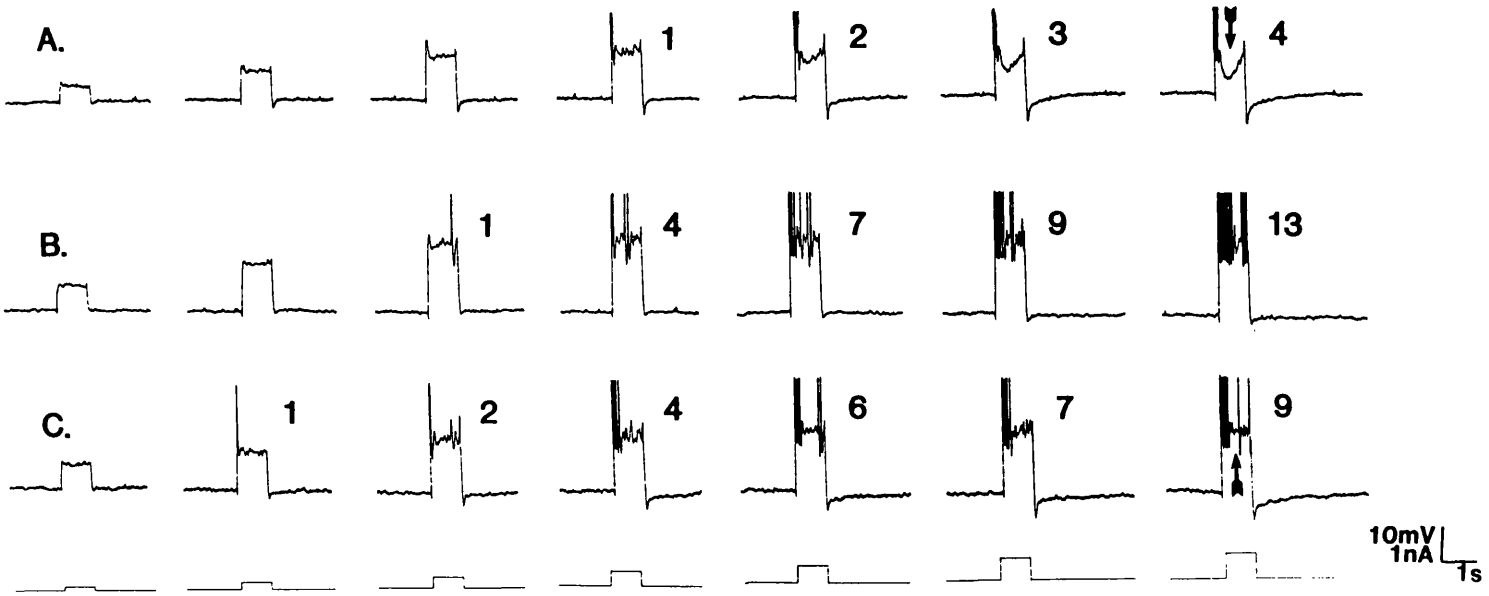

A.
1
2
3
4
B.
1
4
7
9
13
C.
1
2
4
6
7
9
10mV
1nA
1s

Fig.24B

Fig.24B

Graph of pooled data (n=4) for number of spikes in response to a range of current pulses.

Filled circles = control.

Filled triangles = 5% delivered enflurane.

1μmol.litre^{-1} atropine, 30μmol.litre^{-1} BRL 24924 and 20μmol.litre^{-1} cimetidine were present throughout the experiments.

Filled stars = 30 min washout.

Temperature = 30-32°C. Resting membrane potential = -70mV.

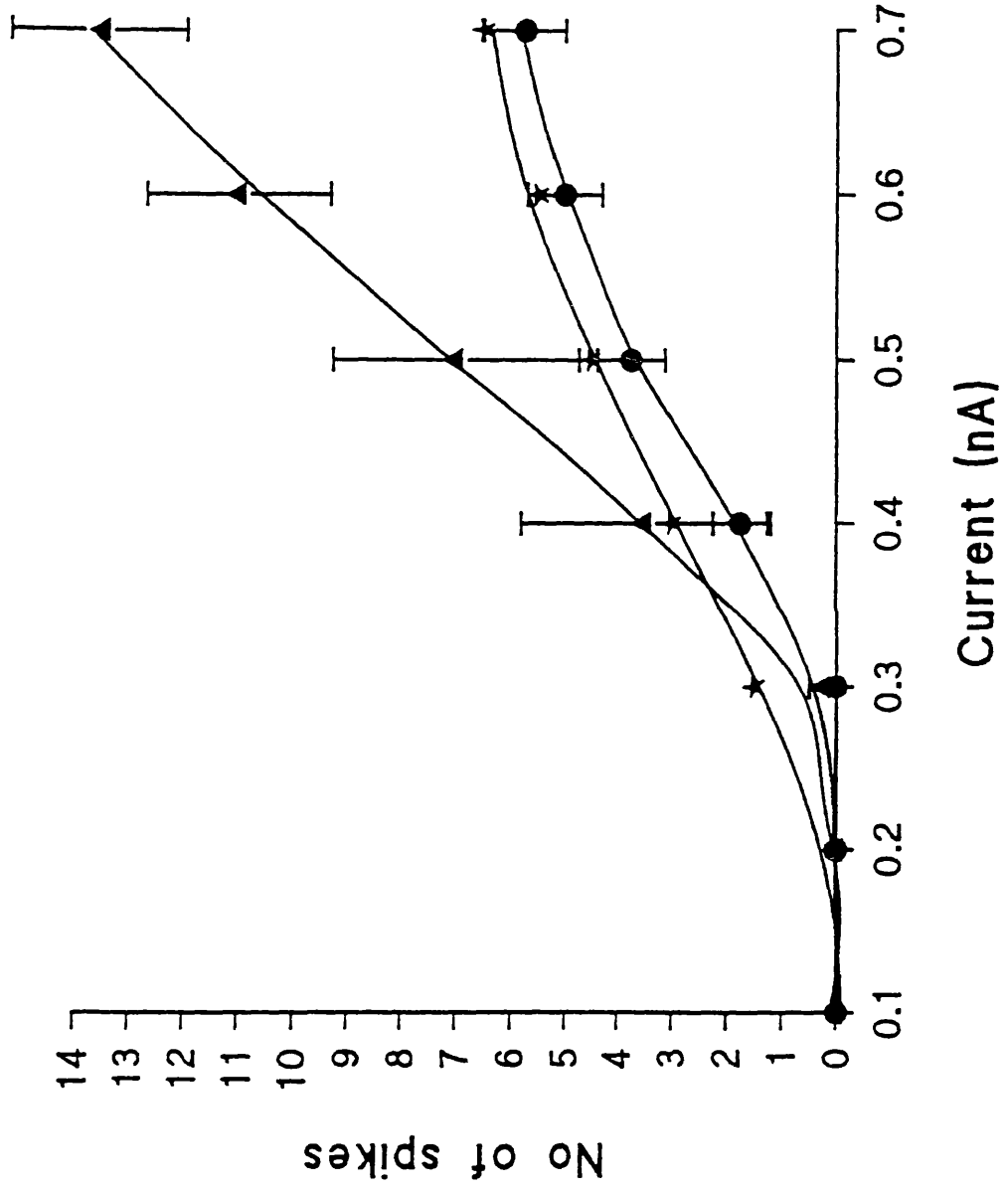

No of spikes
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Current (nA)

Fig.24C

Fig.24C

A, B, C = action potentials of a single pyramidal neurone under differing conditions.

A. Action potential in response to a 0.11nA current pulse in control conditions.

B. Calcium spike in response to a 0.6nA current pulse recorded in the presence of 1μmol.litre^{-1} TTX and 5mmol.litre^{-1} TEA.

C. Calcium spike in response to a 0.4nA current pulse after 15 min exposure to 5% delivered enflurane (TTX and TEA still present).

Resting membrane potential = -69mV. Temperature = 31°C.

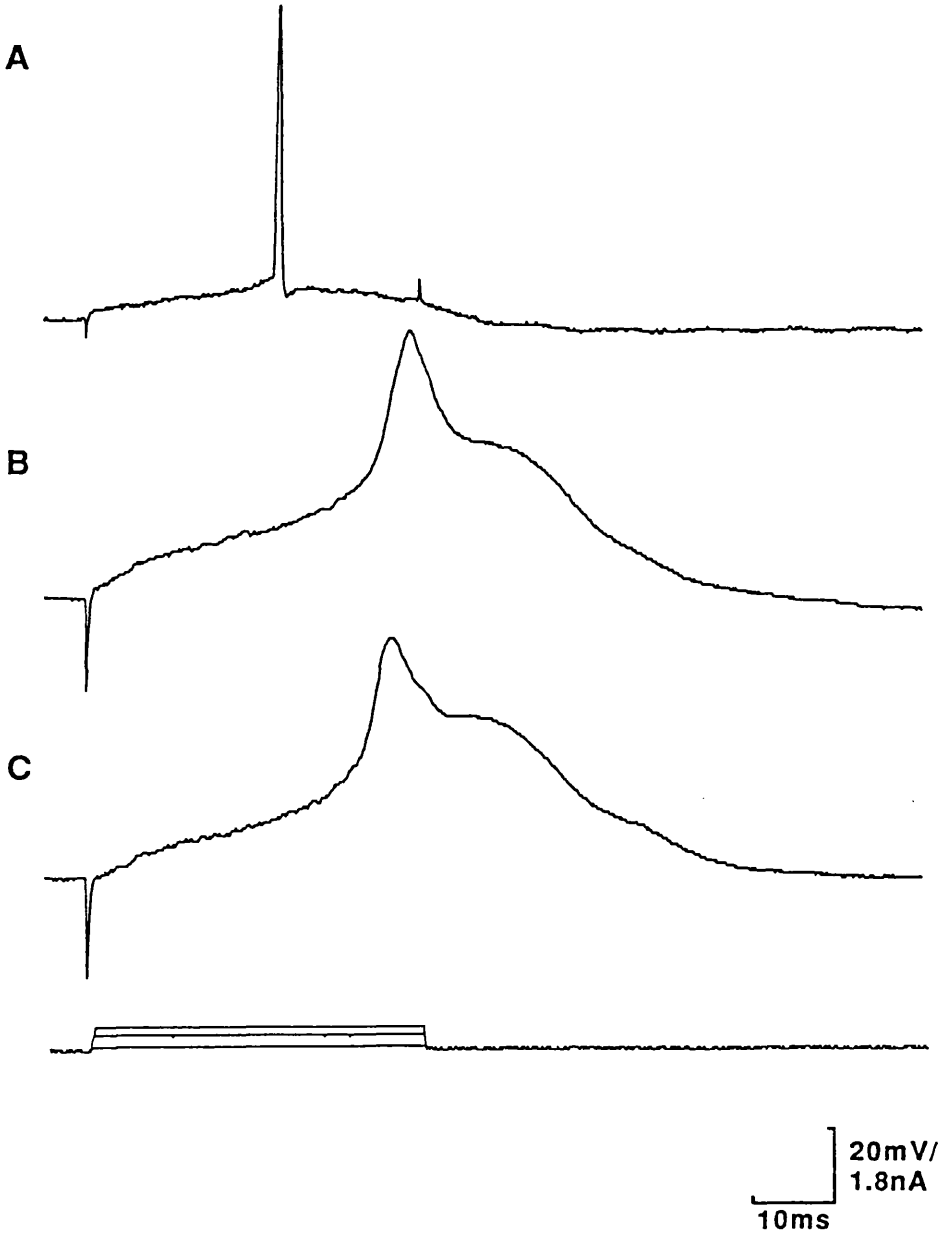

A
B
C
20mV/
1.8nA
10ms

Fig.25

Fig.25

Chart records of the effects of 200μmol.litre^{-1} ketamine (20min) on the accommodation of a CA1 neurone to current pulse of long duration (800ms/0.5nA) (A_1, B_1, C_1) and the after-hyperpolarization (A_2, B_2, C_2) following 80ms depolarizing current pulses.

A_1/A_2. Control.

B_1/B_2. Ketamine.

C_1/C_2. Wash 20 min.

Resting membrane potential = -66mV. Temperature = 29°C.

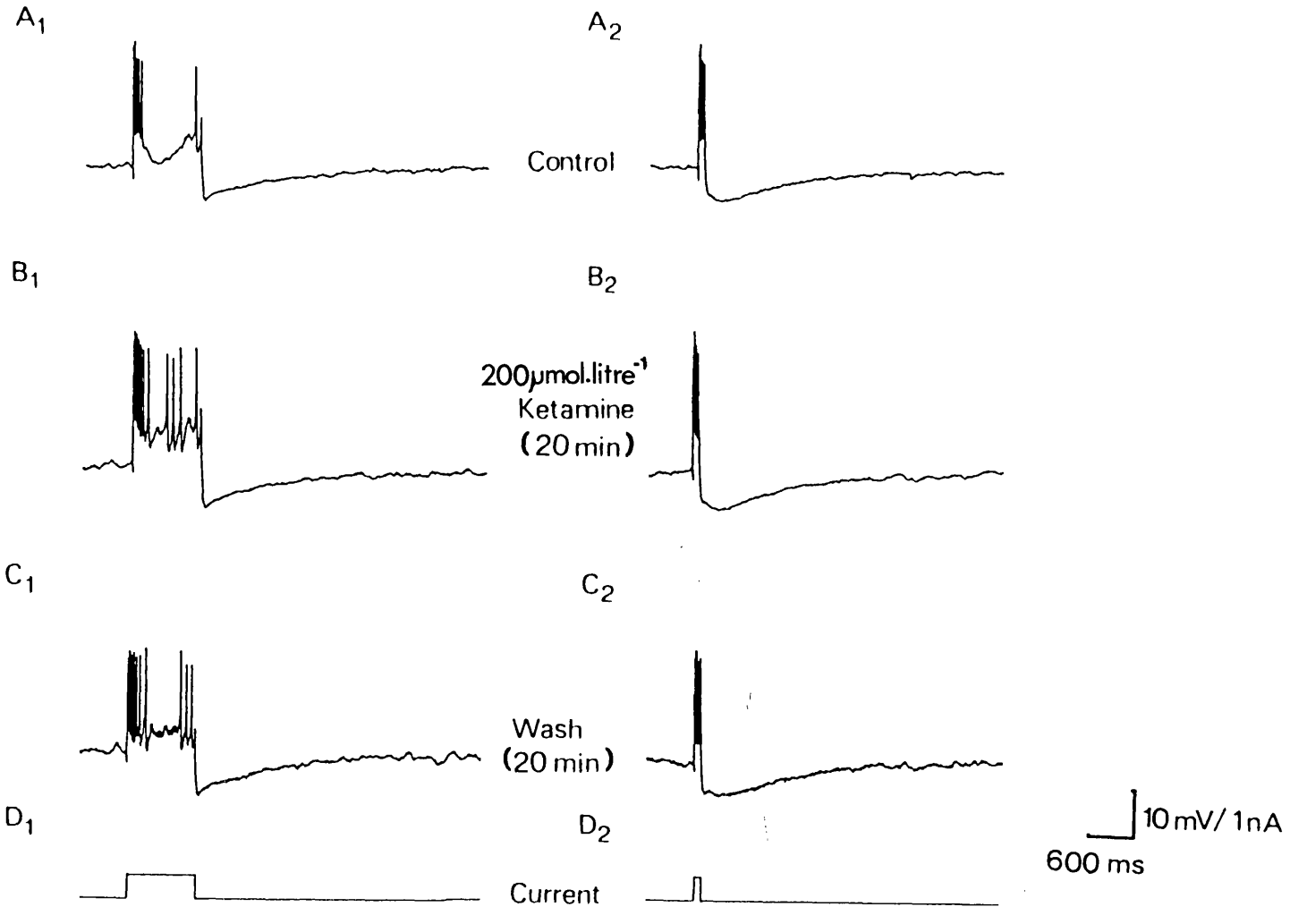

A1
A2
Control
B1
B2
200μmol.litre-1
Ketamine
(20 min)
C1
C2
Wash
(20 min)
D1
D2
Current
10 mV/ 1nA
600 ms

Fig.26

Fig.26

Chart records of the response of a CA1 pyramidal neurone to depolarizing current pulses of varying amplitude.

A. Control.

B. Methohexitone 50μmol.litre^{-1} (20 min).

C. Washout (30 min).

Temperature = 31°C. Resting membrane potential = -70mV.

Numbers represent the number of spikes in response to the current pulse.

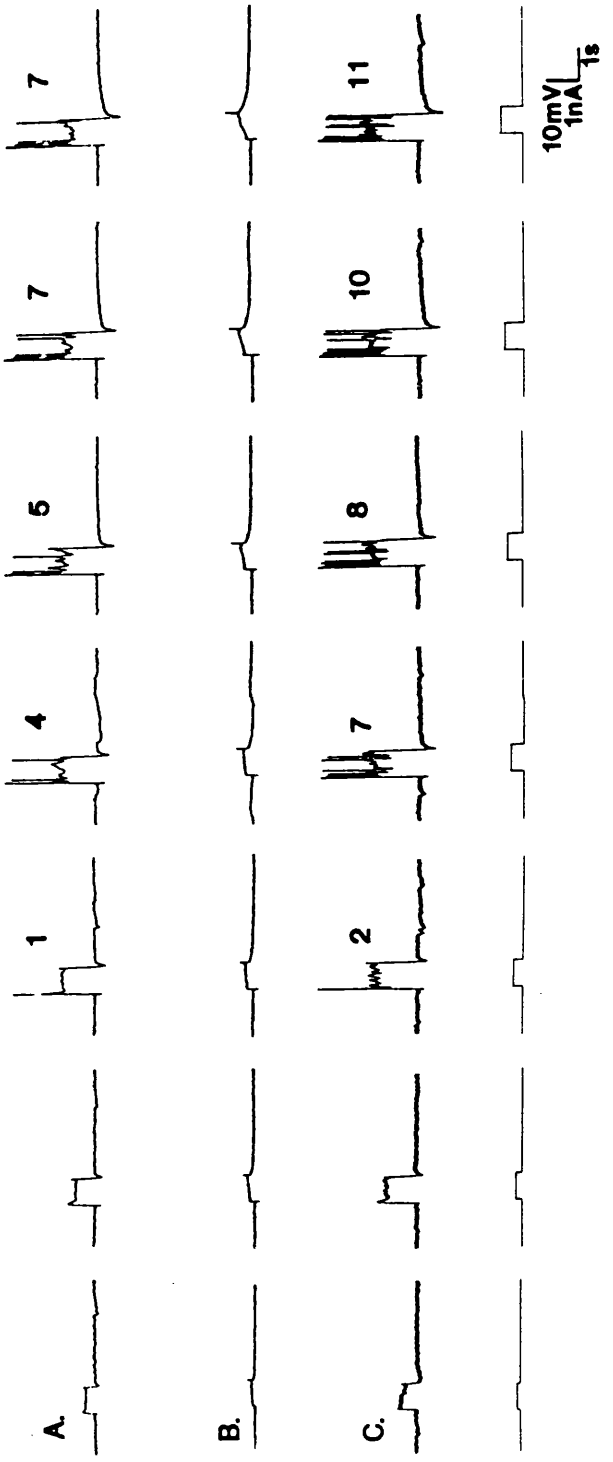

A.
B.
C.
1
2
4
7
5
8
7
10
7
11
10mV
1nA
1s

Fig.27

Fig.27

Chart records of the response of a pyramidal neurone to 80ms depolarizing current pulses (0.7, 0.6 & 0.5nA) during a sequence of drug applications.

A. Control.

B. Methohexitone 50μmol.litre^{-1} (20 min).

C. Methohexitone 50μmol.litre^{-1} plus bicuculline 50μmol.litre^{-1} (15 min).

D. Methohexitone 50μmol.litre^{-1} (15 min).

E. Washout (30 min).

Numbers represent the number of spikes in response to the current pulse.

Current trace is below voltage trace E.

Arrow denotes small spontaneous depolarization.

Temperature = 30°C. Resting membrane potential = -72mV.

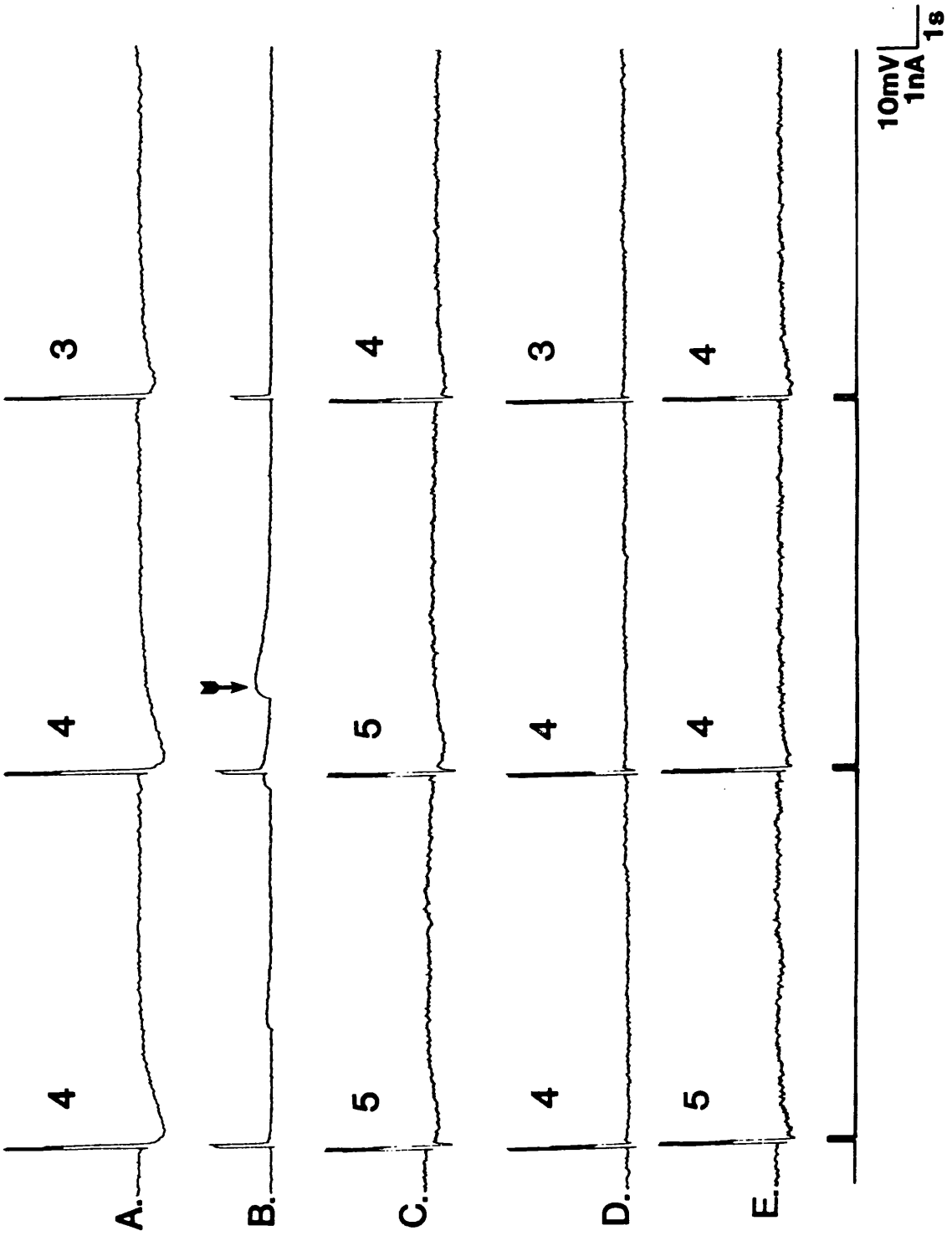

A.
B.
C.
D.
E.
4
4
3
5
4
5
4
4
3
5
4
4
10mV
1nA
1s

Fig.28

Fig.28

Chart records of responses of a CA1 pyramidal neurone elicited by stimulation of the Schaffer collaterals (5-15V, 80μs).

A. Control responses to a 50V stimulus.

B, C & D. Responses to differing stimulus voltages following a 15 min exposure to 50μmol.litre^{-1} methohexitone.

E. Recovery following a 10 min wash (same calibration as A, B, C).

Note the action potentials on the depolarizing wave are truncated in C because of the limited frequency response of the pen recorder. Also in D, the hard copy of an oscilloscope trace showing more detail of the arrowed section within C, the action potentials are truncated because of the limited resolution of the digital storage display.

Temperature = 29°C.

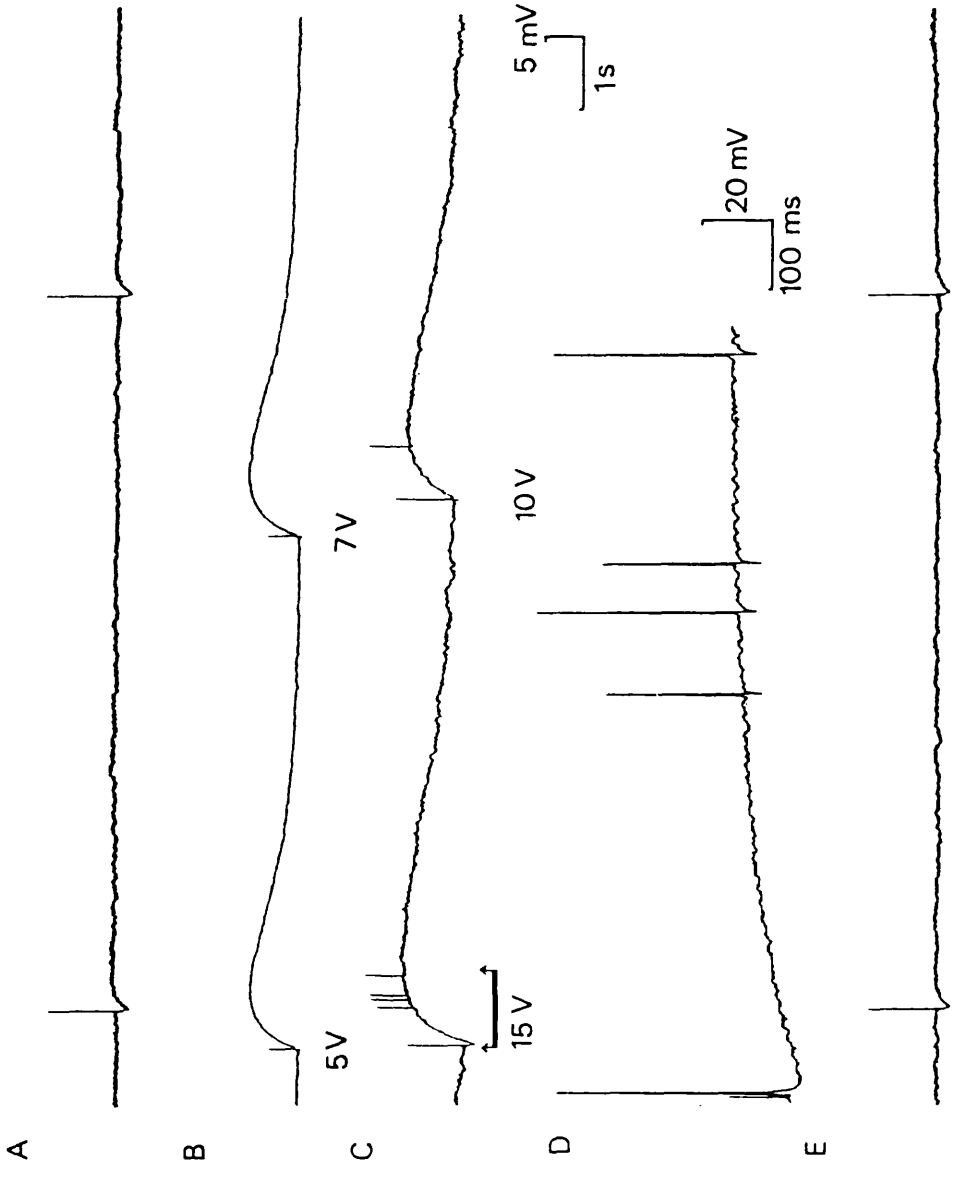

A
B
C
D
E
5V
7V
10V
15V
5 mV
1s
20 mV
100 ms

Fig.29

Fig.29

A. Methohexitone 50μmol.litre^{-1} (20 min exposure) induces a depolarizing after-potential with associated conductance increase following orthodromic stimulation.

B.The amplitude of the depolarizing after potential was reduced by the addition of 50μmol.litre^{-1} bicuculline (15 min).

Temperature = 30°C. Resting membrane potential = -61.5mV.

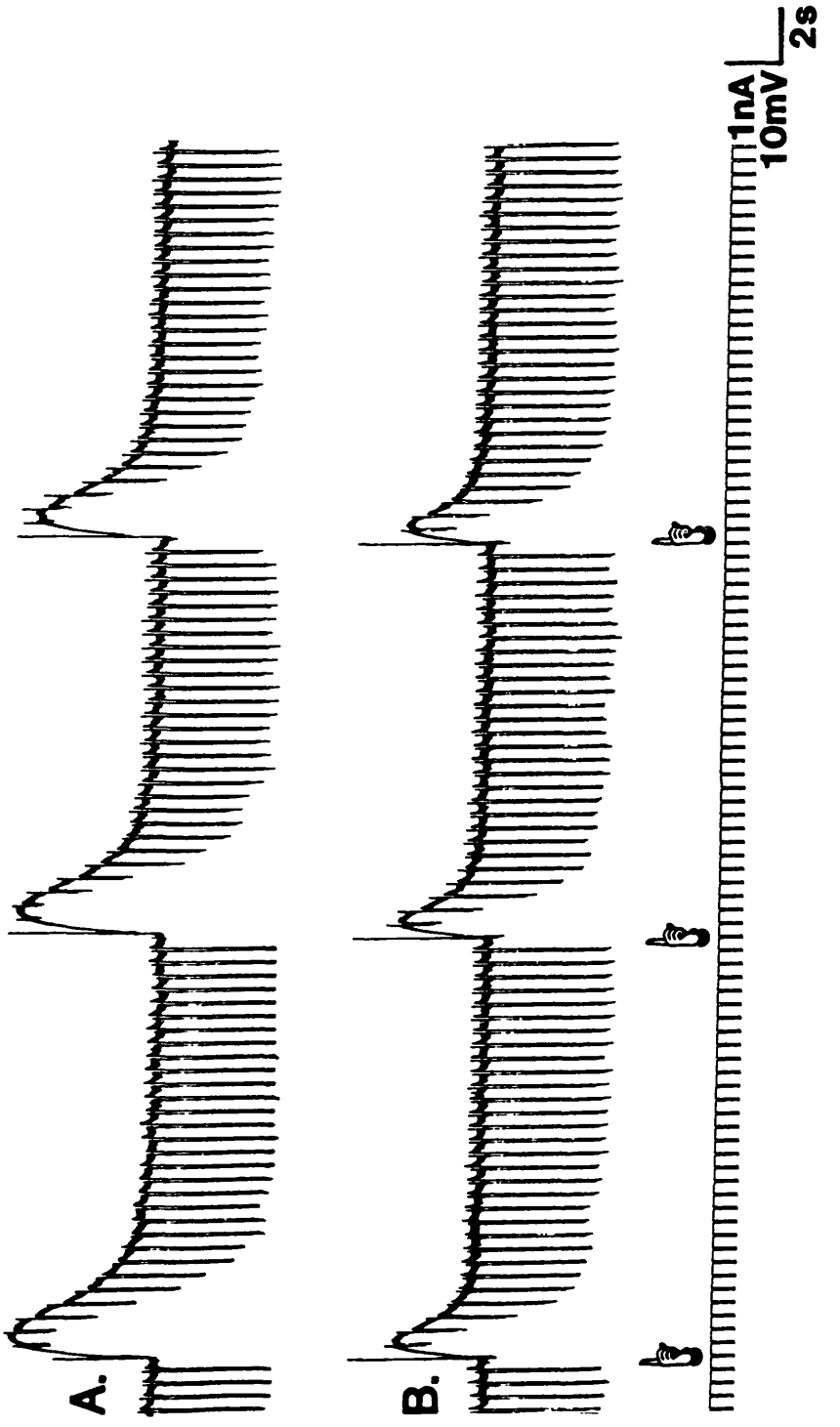

A.
B.
1nA
10mV
2s

Fig.30

Fig.30

Chart records of intracellular measurements taken from a CA1 pyramidal neurone. Throughout all traces (A - E) a 0.5nA/ 0.5Hz/ 80ms current pulse was injected through the intracellular electrode.

A. Control record showing response to stimulation of the Schaffer collaterals (5V).

B. Stimuli of 5V and 10V after 10 min exposure to 50μmol.litre^{-1} methohexitone.

C. Stimuli of 5V and 10V following the addition of 50μmol.litre^{-1} bicuculline (10 min) (50μmol.litre^{-1} methohexitone still present).

D. Stimuli of 5V and 10V following perfusion of 50μmol.litre^{-1} methohexitone (10 min).

E. Wash (20 min). Stimuli 10V in each case.

Temperature = 30°C. Resting membrane potential = -60mV.

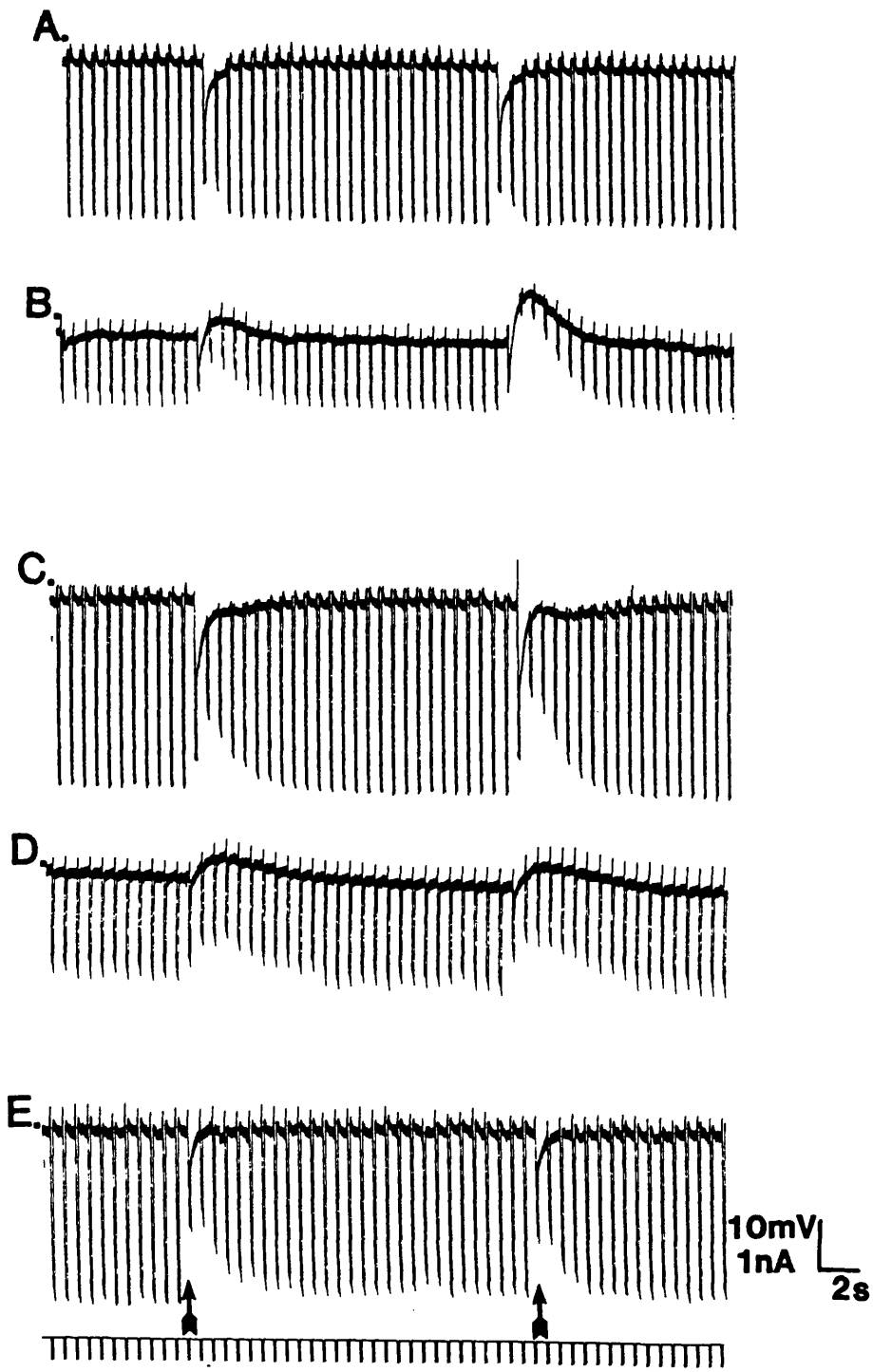

A.
B.
C.
D.
E.
10mV
1nA
2s

Fig.31

Fig.31

The effect of carbachol on current relaxations generated by a hippocampal CA1 neurone at rest and at a depolarized holding potential. The traces are currents initiated by a hyperpolarizing voltage clamp step of 14mV from a holding potential of -40mV (A_1, B_1, C_1) and from resting potential(-73mV)(A_2, B_2, C_2); before (A_1, A_2), during (B_1, B_2) and 20 min after (C_1, C_2) bath application (10 min) of carbachol (50μmol.litre^{-1}). During drug administration an inward current developed at the more positive holding potential (B_1) but not a rest (B_2). TTX (1μmol.litre^{-1}) was included in the bathing medium to eliminate Na^+ dependent action potentials.

Temperature = 30°C.

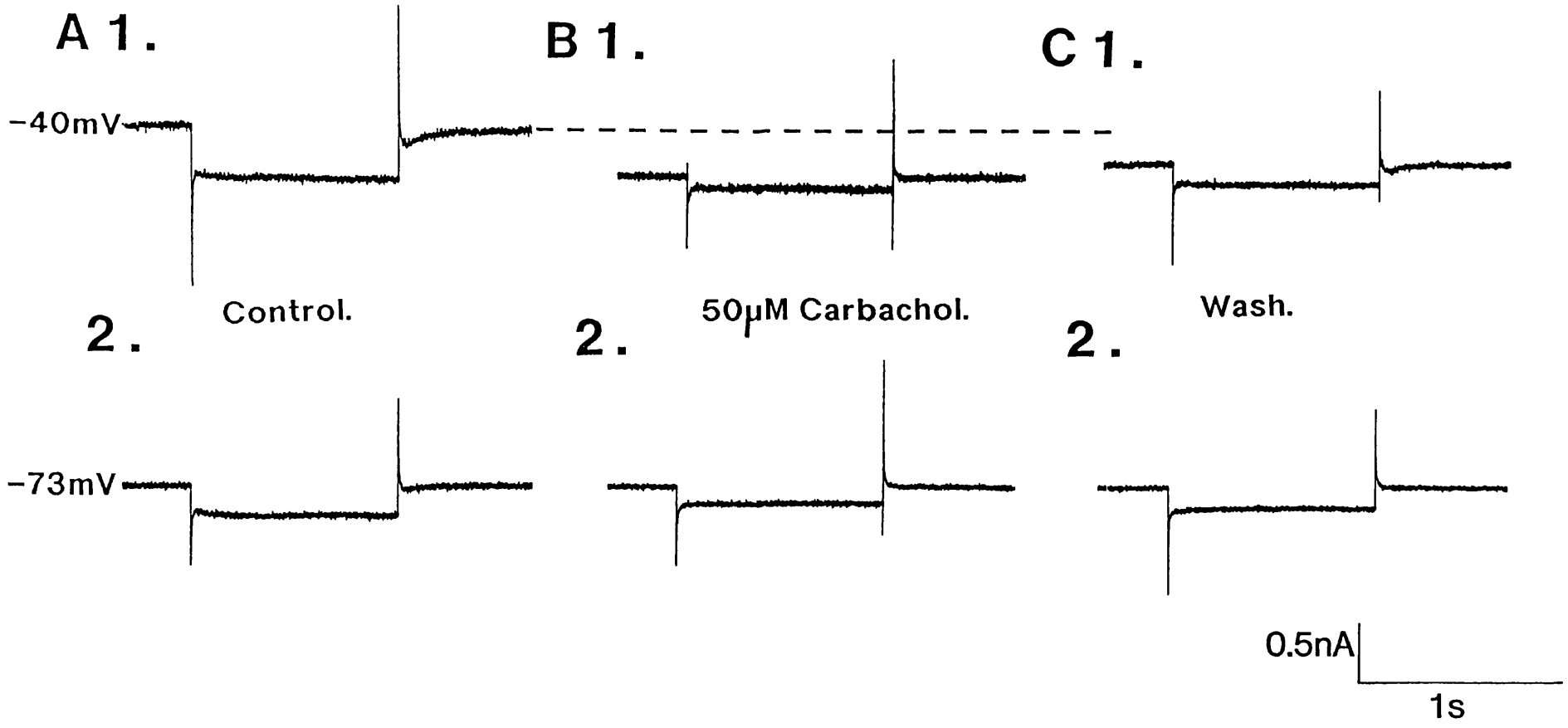
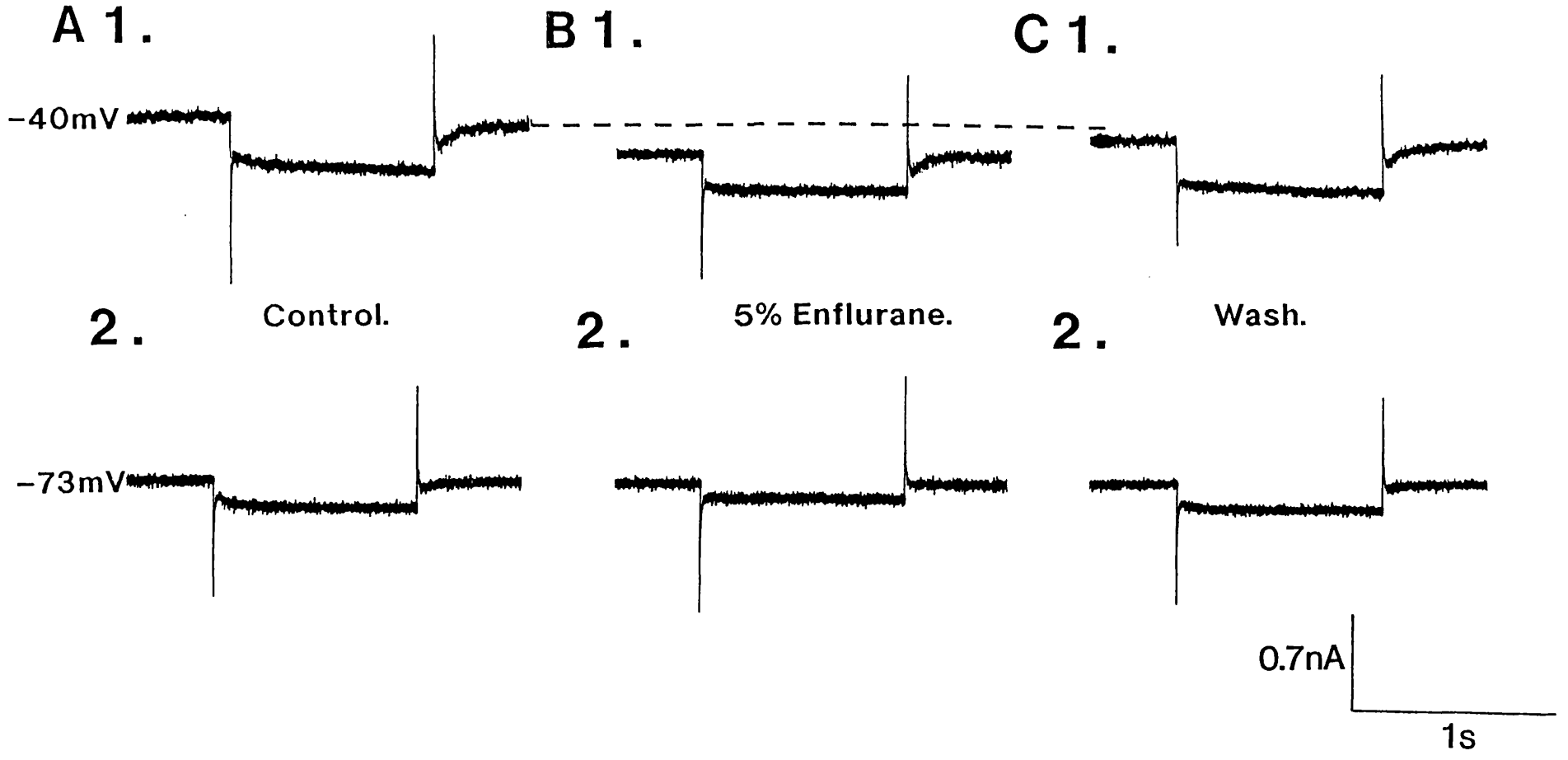

A 1.
B 1.
C 1.
-40mV
Control.
50μM Carbachol.
Wash.
2.
2.
2.
-73mV
0.5nA
1s

Fig.32

Fig.32

The effect of enflurane on the current relaxations generated by a hippocampal CA1 neurone at rest and at a depolarized holding potential. The voltage step was 14mV hyperpolarizing from holding potentials of -40mV (A_1, B_1, C_1) and resting potential (-70mV)(A_2, B_2, C_2). A_1 and A_2 are control records; B_1 and B_2 are records following 10 min exposure to 5% delivered enflurane and C_1 and C_2 are records following 30 min washout of anaesthetic. Note the appearance of an inward current during exposure to the anaesthetic. TTX (1μmol.litre^{-1}) was included in the bathing medium for the duration of the experiment. Temperature = 31°C.


A 1.
B 1.
C 1.
-40mV
2.
Control.
2.
5% Enflurane.
2.
Wash.
-73mV
0.7nA
1s

PART I DISCUSSION

This section of the thesis focused upon the postsynaptic effects of general anaesthetics with the aim of uncovering excitability changes that may reflect modification of the function of potassium channels. Anaesthetic induced modification of neuronal excitability at this level may be considered of possible relevance to contributing towards the depressant effects of anaesthetics, also to certain agents proconvulsant profile and the ability to ameliorate symptoms of the HPNS (see Part II Discussion).

The results demonstrate that the inhalation agents enflurane, isoflurane and halothane may modify both resting and evoked properties of CA1 pyramidal neurones. The principal discoveries are:-

I.All three agents tend to evoke slight hyperpolarization.

II.They reduce the amplitude of the antidromic population spike (and for halothane, induce repetitive firing).

III.A reduction of accommodation and the associated AHP was usually observed in the presence of the agents.

IV.Enflurane was shown to reduce the M-current.

At the effective concentration of approximately 2.5% (see Methods) all three agents tended to slightly hyperpolarize cells, with resting potential shifts of up to 10mV noted. It is possible that this observed hyperpolarization was due to anaesthetic-induced enhancement of a potassium conductance that contributes towards maintaining the resting potential of CA1 neurones. Anaesthetic induced hyperpolarizations have been reported previously (Nicoll & Madison, 1982; MacIver & Kendig, 1989; Berg-Johnsen & Langmoen, 1987) and it has been suggested that an increase in a potassium conductance may account for this response (Nicoll & Madison, 1982). The possibility of the hyperpolarization observed intracellularly being due to the anaesthetic agent "switching on" a potassium current in a manner similar to that observed for a discrete Lymnaea stagnalis neuron (Franks & Lieb, 1988) has to be considered. This a purely speculative view however, as the presence of such a current remains to be demonstrated in mammalian neurones, but if such anaesthetic activated potassium channels were to exist in certain neurones of higher animals then the decreased level of activity of such neurones could contribute towards generating and maintaining the state of general anaesthesia.

All three inhalation agents were found to produce shifts to the right in the relationship between amplitude of the antidromic field potential and the stimulus voltage. It seems reasonable to suggest that the small hyperpolarization observed intracellularly may contribute towards the decrease in amplitude of the

evoked antidromic field potentials. In addition the slight increases in latency and spike width observed (≈10% for isoflurane and enflurane) intimate that the anaesthetics have the effect of slightly reducing the conduction velocity of the fibres of the alveus. This would have the effect of making the population spike fatter and smaller in amplitude. This reduction in amplitude of the population spike agrees with the data presented by MacIver & Roth (1988), also Berg-Johnsen and Langmoen (1986) have reported slight decreases (≤8%) in the conduction velocity of rat hippocampal myelinated fibres in the presence of isoflurane.

Halothane induced unexpected and striking changes in the antidromic field potential. In the presence of 2.5% halothane appreciable reduction in amplitude alongside widening of the population spike accompanied the appearance of a second population spike. Previous in vitro work with halothane has not reported any CNS excitatory effects (Richards, 1973; Fujiwara, Higashi, Nishi, Shimoji, Sugita & Yoshimura 1988; Gage & Robertson, 1985) or presented evidence that may help explain the asynchronous discharge of antidromic population spikes. Synaptic transmission in the rat hippocampus is markedly depressed by lower concentrations (0.3-1.25%) of halothane (MacIver & Roth, 1988) than those used in this study. This depressant effect at the synapse, leading to a complete block of transmission at 3%, may mask any tendency to fire repetitively during orthodromic stimulation and explain why this activity has not been reported in the previous studies. However, as MacIver & Roth (1988) did not report observations of multiple peaks in their halothane depressed antidromic spikes, the observations reported in this study really need following up with complementary intracellular experiments in which neurones are antidromically activated, via the alveus, before any conclusions may be drawn.

Overall none of the inhalation anaesthetics tested had any significant effect upon the input resistance of CA1 pyramidal neurones. Only one out of eight neurones exposed to enflurane, one of the four neurones exposed to isoflurane and none of the neurones exposed to halothane exhibited any appreciable reversible change in input resistance. Previous studies have reported no change (Scholfield, 1980; Fujiwara, Higashi, Nishi, Shimoji, Sugita & Yoshimura, 1988), decreased (Gage & Robertson, 1985) or slightly increased (Yoshimura, Higashi, Fujita & Shimoji, 1985) input resistance. The bridge balance technique however, does not allow one to accurately measure small changes in input resistance (≈10%), therefore it is possible that in this study a slight decrease in input resistance [as one would expect during a hyperpolarizing membrane potential shift] has gone undetected.

The most dramatic change in CA1 neurone behaviour in the presence of inhalation anaesthetics was uncovered during the accommodation experiments. By studying the accommodative properties of CA1 neurones exposed to inhalation anaesthetics it was hoped that a postsynaptic excitatory effect of enflurane could be uncovered which may be important in explaining its proconvulsant profile [thus a relatively high concentration of anaesthetic was used]. All three inhalation agents, at approximately 2.5%, (lower concentrations of enflurane [1-2% delivered] also decreased accommodation) reversibly blocked the accommodative properties of pyramidal neurones and reduced the associated after-hyperpolarization. As the action of enflurane was not modified by propranolol, atropine, cimetidine and BRL 24924, and the shape of the calcium spikes recorded in the presence of TTX and TEA were not modified by the presence of enflurane, it would be reasonable to suggest that the effects on the Ca^{2+} activated K^+ current are direct rather than mediated via enflurane induced neurotransmitter release or suppression of calcium entry. Halothane and isoflurane presumably act via the same mechanism as enflurane.

I_{AHP} appears to be the main contributor toward accommodation: alongside the reinforcing action of the M-current (Madison & Nicoll, 1984; Jones & Heinemann, 1988). An investigation of M-current characteristics during exposure of neurones to anaesthetics was undertaken with the aim of describing the extent of the accommodation disturbance and providing further evidence for anaesthetic interaction with potassium channels. The reduction of the slow current relaxations associated with membrane potential jumps from -40mV to -54mV and the generation of an inward current in the presence of enflurane suggests a degree of M-current inhibition by the anaesthetic agent. Enflurane may decrease the available number of M-channels so that an inward current is developed at -40mV and not the cells natural resting potential. Associated with this is the reduction of the time dependant current relaxations. One might expect that alongside significant inhibition of the M-current, a depolarizing action upon the cell membrane potential would occur (Halliwell & Adams, 1982; Brown, 1988). Possibly the overriding tendency of enflurane to hyperpolarize neurones (presumably by activation of another potassium current) masked any depolarizing ionic shifts. Any inhibitory action upon I_M would have profound consequences for the excitability of a neurone. Release of part of its repolarizing drive during spike trains would decrease the rise in spike threshold normally present, thus increasing probability of a repetitive spike train occurring in response to depolarizing stimuli. Removal of part of this natural "braking" current would therefore contribute towards the accommodation disturbance in the presence of enflurane (and presumably isoflurane and halothane). The incomplete recovery of M-current relaxations upon washout of anaesthetic parallelled the incomplete recovery of the voltage "sag" normally seen in

accommodating neurones. Quite why this occurs is unclear. What does stand out from the results though, is that the reduction of the amplitude of the AHP is clearly more reversible than the M-current disturbance. It was disappointing that the constraints of time did not allow a thorough investigation of the M-current disturbance. Also voltage-clamp analysis of the AHP current in the presence of the inhalation agents could have yielded interesting results. From the available data though it does seem that the inhalation anaesthetics tested directly alter the function of the potassium channels underlying the M- and AHP currents.

Previously, in vivo experiments have demonstrated that enflurane-induced seizure activity is associated with concentration dependent excitatory and depressant actions on reticular neurone firing, cortical evoked responses and EEG activity (Stevens, Fuginaga, Oshima & Mori, 1984). In the cortical EEG, seizure activity was observed when reticular activity was depressed. Cortical evoked responses were however enhanced. These observations would suggest that the anaesthetic induced seizure activity could be associated with either a release of brain stem inhibition or a **direct** excitation of cortical neurones. In vitro evidence for increased postsynaptic excitability of CA1 neurones in the presence of enflurane has also been presented at the level of the field potential (MacIver & Roth, 1987). At first sight, the experiments presented in this thesis involving enflurane appear to support the view that enflurane-induced seizure activity in vivo is a result of a direct postsynaptic excitation. Furthermore, this excitation would appear to be mediated via a direct anaesthetic-potassium channel interaction. However, considering the similar (if not identical) actions of isoflurane and halothane, this explanation is not so convincing. All three agents effectively enhance strong depolarizing inputs by presumably inducing a reduction of the M- and AHP- currents. The significance of the accommodation block for the in vivo situation remains unclear, it is unlikely that it alone is responsible for the excitatory nature of enflurane considering the similar actions of isoflurane and halothane in vitro and their profile in vivo. One might speculate that the reduction of accommodation serves to render neurones more sensitive to excitation. Normally during exposure to inhalation anaesthetics excitatory synaptic input to CA1 neurones is reduced (MacIver & Roth, 1988), therefore an additional postsynaptic excitation must occur in the presence of enflurane, that combines with the reduced accommodative ability, and serves to create seizure activity. Further investigation is therefore required in order to understand fully the basis of seizure activity during enflurane anaesthesia.

The intravenous anaesthetic agents ketamine and methohexitone were chosen to be studied for two reasons. Firstly they both induce excitant side effects in vivo and secondly they are the most (ketamine) and the least (methohexitone)

desirable anaesthetics to use to ameliorate the HPNS in the rat (see General Introduction and also Part II Discussion).

The main findings are that ketamine and methohexitone have mixed effects upon the properties of CA1 pyramidal neurones. Ketamine had mixed effects upon resting membrane potential and antidromic field potential responses, increased both orthodromic and direct excitability and reduced accommodation. Methohexitone had no effect upon antidromic field potential responses, hyperpolarized CA1 neurones, had mixed effects upon accommodation and excitability in response to direct stimulation and induced a large slow after-depolarization following single orthodromic stimulation pulses.

Ketamine was found to alter membrane potential in the hyperpolarizing (n=2) or depolarizing (n=4) direction (n=14 total). Although at 200μmol.litre^{-1} the mean value of resting potential exhibits very little change over control as did the input resistance. This agrees with Oshima & Richards (1988) who reported no consistent change in the resting potential or input resistance of granule cells during application of ketamine (50μmol.litre^{-1}). Ketamine did however decrease the current required to stimulate CA1 neurones intracellularly (direct stimulation, n=8 of 13 neurones) decreasing by up to ≈40% of the control value in some neurones. This may be responsible for the reduction of accommodation seen in the presence of ketamine. This reduction was different to that observed for the inhalation agents. In this case the AHP remained intact whilst the spike accommodation properties were reduced. The characteristic "sag" in the voltage trace usually seen in accommodating neurones (see Figs.24A & 25) was abolished by the presence of ketamine. This sag is due to the "switching on" of hyperpolarizing currents in the cell to limit action potential discharge during prolonged depolarizing inputs. The reduction of the sag may have been due to the general excitant effect of ketamine, although the possibility of a specific effect on a current other than I_{AHP} contributing towards accommodation (possibly I_M) must be considered. Antidromic field potential responses were enhanced by the presence of 20μmol.litre^{-1} ketamine only, no excitatory actions were observed for any of the other concentrations used. This effect could be attributed to the increase in excitability uncovered by the intracellular experiments.

Another effect of ketamine noted during the field potential experiments was the enhancement of the positive wave following the antidromic spike (Fig.17). For spikes of identical amplitude the presence of ketamine (20μmol.litre^{-1}) was found to increase the amplitude of this wave. If we consider this wave to be due to activation of recurrent inhibition, the potentiating effect of ketamine on GABA responses (Little, 1982; Little & Atkinson, 1984) may be responsible for the

observed enhancement.

The excitant side effects of ketamine (and methohexitone) have been recognised for some time. The in vivo EEG and behaviourial effects of ketamine include CNS excitation coupled with catatonia, disorganized excitation characterized by a catatonic-anaesthetic state and finally electrograhic seizures without clinical correlates (Mori, Kawamata, Mitani, Yamazaki & Fujita, 1971). These changes are dose related, the higher the dose, the more severe the changes. Previous in vitro work with ketamine in the CNS has demonstrated an ability (>50μmol.litre^{-1}) to depress excitatory synaptic transmission in the dentate gyrus of the guinea pig hippocampus and to reduce the sensitivity of neurones to l-glutamate (5-200μmol.litre^{-1})(Oshima & Richards, 1988). Excitatory effects have been reported in the cuneate nucleus (Morris, 1978) and postsynaptic responses to NMDA are reduced (Anis, Berry, Burton & Lodge, 1983). Ketamine is also able to prolong the conductance change underlying the IPSP (Gage & Robertson, 1985; Scholfield, 1980). Ketamine (18-180μmol.litre^{-1}) also potentiates the effects of GABA in the rat superior cervical ganglion (Little, 1982; Little & Atkinson, 1984) and inhibits the uptake of 5-HT and catecholamines into synaptosmal fractions of rat brain (10μmol.litre^{-1} and above)(Azzaro & Smith, 1977). The results presented in this thesis show the main actions of ketamine to be excitatory in nature. At the near anaesthetic concentration of 20μmol.litre^{-1} (see Marietta, Way, Castagnioli & Trevor, 1972) the predominant actions of ketamine were excitant. To what extent these changes observed in vitro contribute towards the convulsant effects of ketamine remains to be established.

Methohexitone was found to have quite different actions to ketamine. In this study no change in antidromic field potential responses was uncovered (Fig.18), both the width and amplitude of responses were not significantly altered by 50 or 100μmol.litre^{-1} methohexitone. Previous depressions of orthodromic CA1 neurone field potential responses by methohexitone have been reported (MacIver & Roth, 1987) and were found in the control pressure experiments presented in Part II. This depression is therefore likely to be due to synaptic factors rather than changes in intrinsic excitability.

The lack of effect upon antidromic field potentials is a little surprising considering the hyperpolarizing effect of methohexitone. A hyperpolarizing action is quite well documented for the barbiturates (Sato, Austin & Yai, 1967; Nicoll & Madison, 1982) although variable changes and no change have also been reported (Brown & Constanti, 1978; Iwasaki, 1989). This action of methohexitone may be attributed, at least in part, to anaesthetic induced release of GABA. One type of GABA response is hyperpolarization with

accompanying decreased input resistance (the other being depolarization with decreased input resistance)(Andersen, Dingledine, Gjerstad, Langmoen & Laursen, 1980). It is likely that anaesthetic induced release of GABA from sites near to the CA1 neurones soma is responsible for the hyperpolarizing response and accompanying decrease in input resistance. This statement is based upon the results of the experiments performed in the presence of 50μmol.litre^{-1} bicuculline where recovery of the resting membrane and input resistance properties were observed (Fig.30). The small spontaneous transient depolarizations observed may be due to spontaneous release of GABA at the dendrites although a systematic study of this effect was not undertaken.

The slow after depolarization induced by orthodromic stimulation in the presence of methohexitone is of considerable interest and may also be attributed to GABA. Pentobarbitone (100-125μmol.litre^{-1}) has been shown to induce a late depolarizing IPSP mediated by GABA (Alger & Nicoll, 1979, 1982) and this response is very similar to, but smaller than, that observed for methohexitone. The response exhibits sensitivity to bicuculline (Figs.29 & 30) and at times led to discharge of action potentials. It is possible that this enhancement, by methohexitone, of what is normally an inhibitory process leads to the excitant effects seen in vivo (see above).

Accommodation in the presence of methohexitone was usually enhanced, sometimes up to the point where no spike responses could be evoked. AHP amplitudes were also reduced by methohexitone and sometimes reversed so that they became depolarizing. Again GABA seems to be partly or wholly responsible for these actions. Application of bicuculline to blocked AHPs (Fig.27) partially restored them, although the medium AHP component seemed more easily recoverable than the slow AHP (Fig.27C). It is possible therefore that methohexitone in addition to enhancing GABA actions disturbs calcium entry into the neurones. The variable changes in orthodromic threshold noted in the presence of methohexitone could also be due to GABA release. The extent to which GABAergic transmission was present in the slice may have determined the threshold increase. Thus slices in which GABA transmission remained were poor in control conditions and would yield cells with small increase in orthodromic threshold and vice versa. This could also account for the differences noted in amplitude of the after depolarization. Some preparations exhibiting very large after depolarization (up to 26mV) yet others being relatively small (5-6mV).

The frequent occurrence of muscle tremors during methohexitone anaesthesia (Po, Watson & Hansen, 1968) have limited its usefulness as a clinical anaesthetic agent. The experiments in the results section above were

performed with the aim of providing information related to this action. This was considered of value concerning this in vivo excitant effect and the interaction of methohexitone with the HPNS (see Part II). Methohexitone has been shown to depress the in vivo acetylcholine-induced excitation of cortical neurones (Catchglove, Krinjević & Maretic, 1972) and facilitate excitatory synaptic transmission within the cuneate nucleus (Morris, 1978). The barbiturates (which include methohexitone) usually have a depressant action at the excitatory synapse (Richards, 1972; Scholfield, 1980; Løyning, Oshima & Yokota, 1964; Barker & Gainer, 1973; Larabee & Posternack, 1952). They often hyperpolarize neurones (Sato, Austin & Yai, 1967; Nicoll & Madison, 1982) and enhance both pre- and post-synaptic inhibition (Nicoll, 1972) by modifying GABA-ergic function (Scholfield, 1980; Barker & Ransom, 1978; Collingridge, Gage & Robertson, 1984). The depression of excitatory transmission is usually ascribed to presynaptic factors (Weakly, 1969) although barbiturates often depress postsynaptic excitability (Somjen & Gill, 1963). Depression of hippocampal CA1 neurone field potential responses by barbiturates have been reported (MacIver & Roth, 1987).

PART II INTRODUCTION

Since the discovery that high pressure causes hyperexcitability in the central nervous system of higher animals (Brauer, Mansfield, Beaver & Gillen, 1979; Fagni, 1982; Fagni, 1985) various in vitro electrophysiological and biochemical studies have been attempted to discover the cellular basis of this high pressure disturbance. The studies thus far have concentrated on the effects of pressure (usually helium or hydrostatic) upon simple systems such as invertebrate neurones, invertebrate muscle, synaptosomal preparations, invertebrate and vertebrate peripheral axons and synapses (see General Introduction, also Wann & Macdonald, 1988). These have given little insight into the in vivo central effects of high pressure. In order to understand this more fully, the effects of pressure upon central neurones themselves must be investigated.

Experimental approaches to studying the central effects of pressure have included in vivo measurements involving electroencephalography (Brauer, 1979; Fagni, 1982; Pearce, Halsey, Ross, Luff, Bevilaqua & MacLean, 1989). Analysis of such data, although providing us with an important insight into patterns of brain activity at high pressure, does not really address the issue of the primary target for pressure upon central neurones. Fagni (1982), taking in vivo field potential readings, showed that a marked decrease in the responses of hippocampal CA1 pyramidal cells to single stimuli applied to commissural afferents accompanied pressurization (to 9.1 MPa) in the rat. This was accompanied by a reduction of paired pulse facilitation. The finding that hippocampal synaptic transmission is apparently depressed by high pressure agrees with the in vitro invertebrate and peripheral nervous system evidence reviewed in the General Introduction. In vitro field potential recordings from rat hippocampal neurones at pressure have also been reported by Fagni and co-workers (Fagni, Hugon, Folco & Imbert, 1987; Fagni, Zinebi & Hugon, 1987; Zinebi, Fagni & Hugon, 1988a;1988b). These data represent the first mammalian CNS preparation in vitro to be examined electrophysiologically at high pressure. The experiments employed conventional electrophysiological equipment located within a pressure chamber. Field potential measurements were made in the dendrites and soma of the CA1 area of the hippocampal slice following stimulation of Schaffer commissural afferents and the alveus. The data and conclusions drawn by the authors of these studies may be summarised as follows:-

I. Hippocampal excitatory synaptic function is depressed by helium pressure (≤90MPa). A decrease in the slope of the dendritically recorded population EPSP accompanied depression of the population spike recorded from the pyramidal cell body layer, which may be indicative of reduced

transmitter release or altered receptor activation.

II. From the dendritic recording no change in afferent fibre input volley was detected; this being interpreted as evidence for CA1 axon excitability remaining unchanged due to lack of effect on the Schaffer collateral axons.

III. A pressure induced depression in the amplitude of the antidromic field potential was also noted. This being ascribed to a possible pressure induced depolarization of the neurones.

IV. Whilst the transfer from afferent volleys to population EPSPs was inhibited, the transfer from population EPSP to population spike became facilitated at pressure. These responses were interpreted as being due to a pressure induced imbalance of GABA mediated inhibition and NMDA mediated excitation in the neurones. The authors proposed that pressure induces depolarization of the cells thereby reducing the voltage-dependent block of NMDA coupled channels and increasing the NMDA mediated excitability of CA1 neurones. The deficit in GABAergic inhibitory transmission was thought to involve $GABA_A$ synapses whose receptor sensitivity remained unchanged by pressure. It was suggested that the reduced inhibition was due to reduced GABA release.

V. The effects of quisqualate, l-glutamate, l-aspartate and kainate were not significantly affected by pressure, making it unlikely that the pressure induced depression of synaptic responses was due to reduced sensitivity of postsynaptic receptors. This was interpreted as evidence in favour of decreased transmitter release at pressure.

In the absence of complementary data from independent studies it is necessary that the work of Fagni and co workers be confirmed, and extended to the level of intracellular recording. The above studies failed to provide solid evidence regarding the effects of pressure at differing intensities of orthodromic stimulation. The potentials illustrated (Fagni, Zinebi & Hugon, 1987) are near threshold responses of less than 0.5mV and it would be interesting to see the effects of pressure throughout a range of stimulus intensities up to maximal response. This would give us a clearer picture of how, and to what extent, pressure affects a mammalian CNS synapse, possibly uncovering excitatory effects of pressure when the synaptic pathway is driven near to its maximal capacity. The experiments in the following section will address this question for orthodromic field potentials up to 10 MPa (antidromic potentials are also investigated up to 13.3MPa). In addition to the data derived from the single spike experiments, paired pulse orthodromic population spikes may provide data of relevance to the high pressure CNS disturbance. Should pressure modify paired pulse potentiation (possibly via disturbance of neurotransmitter release, reuptake or breakdown) one may be able to draw conclusions to the consequences of

such a modification on the pattern of neuronal activity in the intact CNS. This experimental approach was perceived to be the most useful approach to extending the work initiated by Fagni et al and promised to yeild genuinely novel field potential data. The effects of the anaesthetic agents ketamine and methohexitone are also investigated at pressure since these agents are the most and least effective anaesthetics respectively in ameliorating the HPNS (B. Wardley Smith, personal communication).

Also contained in this section are intracellular data from hippocampal CA1 neurones at pressure (up to 10MPa). This represents the first such data from mammalian central neurones at pressure and provides information concerning the effects of pressure upon properties such as resting membrane potential, input resistance, threshold potential and action potential characteristics. These observations were considered vitally important and would make it possible to verify\refute the suggestions put forward for the previous hippocampal pressure experiments. Particular attention is drawn to the accommodative properties of the neurones at pressure. K^+ channel dysfunction at pressure may have important implications for the genesis of the HPNS and it was therefore considered of interest to study a type of cellular behaviour known to be under the control of K^+ channels at high pressure.

PART II RESULTS

Field Potential Recordings.

Controls

In order to ensure that stable field potential responses could be obtained from slices within the pressure chamber environment, a series of control experiments (n=4) were performed. These experiments followed an identical protocol to a pressure experiment, but excluded pressurization of the chamber. Slices were exposed to the pressure chamber environment for at least 1 hour (Fig.33A,B) and in some cases 3.5 hours. Fig.33C illustrates the percentage change in amplitude of single population spike responses compared to control amplitude at 30 min and over 2 hours in the sealed pressure chamber. Effects on paired pulse potentiation were also investigated (Fig.33D). No significant ($P>0.05$) changes in amplitude of either the single or paired pulse responses occurred during the time period analyzed. It was therefore concluded that any significant deviations in response observed during the pressure experiments would be due to the pressurization procedure and not time dependent effects.

Slices which were to be used for pressure experiments were set up in the sealed pressure chamber at 1 atmosphere for a period of 30 min. During this time temperature stabilization (see Methods) was achieved (10 min) and control data were collected (20 min). If responses remained stable then pressurization of the chamber would commence. Slices were pressurized initially at 0.3MPa min^{-1} to 5MPa; this pressure was maintained for approximately 5 min for data collection. Pressurization was then recommenced at the same rate up to 10MPa. Data were collected upon reaching 10MPa and after a hold period of 20 min. For antidromic potentials pressurization was recommenced from 10MPa up to 13.3MPa following 5 min of data collection.

Antidromic Field Potentials at Pressure

The effects of pressure (up to 13.3MPa) on antidromically evoked population spikes were investigated in 5 experiments (n=6 up to 10MPa). Over the range of stimulus voltages and pressures used variable changes in excitability were recorded, the only significant ($P<0.05$) effects were slight increases in excitability of the half maximal spike and both the threshold and half maximal spikes at 5MPa and 10MPa respectively (Fig.35B). Spike width and latency remained unaffected by elevated pressure to 13.3MPa, the tendency for spikes to increase slightly in amplitude at the higher pressures occurred in 5/6 experiments. At 5MPa, with maximal stimulation, 2 of the 6 preparations exhibited increases between 4 and 14% whilst the other 4 exhibited depressions between 1 and 27%. At 10MPa, 5 of the 6 preparations exhibited increases between 7 and 38% at maximal stimulation, with the other preparation exhibiting

7% depression. At 13.3MPa, 4 of the 5 preparations exhibited increases between 15 and 32% the other being unaffected (1% change) (Fig.34A,B). 2 of the 6 preparations exhibited recruitment of a second population spike (amplitude ≈0.5mV) during compression at pressures above 5MPa, the second spike gradually increased in amplitude as the compression progressed (Fig.34C). The second population spike accompanied an increase in the amplitude of the first spike (15 and 29%).

Orthodromic Field Potentials at Pressure

During compression the preparation was stimulated at 0.03Hz at threshold intensity. In 5 out of 7 experiments this response became depressed during the early stages of compression. The spike would decline at around 0.4MPa until just the EPSP remained (see Fig.35A). The response then usually (6/7 expts.) recovered during the compression to 5MPa.

From the pooled data significant increases in amplitude of the population spike occurred at pressures of 5MPa for the half maximal spike and at 10MPa for the half maximal and maximal spikes ($P<0.05$) (Fig.35B,C). In fact responses were variable; up to 10MPa they were either unaffected (n=3) or were enhanced (n=4).

Another indication of an increase in excitability was the recruitment of a second population spike (3 out of 7 experiments to 10MPa). A spike of amplitude 1-2mV was recruited during the compression from 5MPa to 10MPa (Fig.35D).

Paired pulse potentiation was studied in 7 experiments (Fig.36). Pressure up to 10MPa slightly decreased the potentiated response in 6/7 preparations, by between 5% and 23%, whilst 1 preparation exhibited an 11% increase in potentiation. By combining the data from all 7 experiments mean potentiation was -7.9 ± 6.41% at 10MPa, this was not significantly different from the control potentiation ($P>0.05$).

Holding at 10MPa for 20 min in 5 experiments led to increases in potentiated spike amplitude of between 6% and 84%. Depressions of 8% and 15% were noted in 2 other experiments (comparison made between the 20 minute hold and 10MPa immediate spikes). The pooled data yielded a mean percentage change of 18.03 ± 12.4%. This was found to be insignificant ($p>0.05$).

Plotting the rate of rise of the EPSP vs. population spike amplitude gave variable results (6 experiments). In 2 experiments the curve was displaced slightly to the left, in 2 experiments the curve was displaced to the right and in 2 cases the curve remained unchanged (Fig.37 illustrates an example of the

curve being displaced to the right). There were therefore no consistent changes in these input-output relationships.

Anaesthetic field Potential Controls

In order to make a comparison between anaesthetic effects at atmospheric and elevated pressure, a series of control experiments were performed inside the pressure chamber at 1 atmosphere.

Ketamine

Fig.38A illustrates changes in orthodromic field potential excitability in the presence of ketamine (20 - 1000μmol.litre^{-1}). Slices were exposed to individual concentrations of ketamine for 20 min. Ketamine at 20μmol.litre^{-1} induced no consistent change in amplitude of the field potential response (n=3). Responses became variable at this concentration of ketamine, this being most pronounced at lower stimulus intensities. 200μmol.litre^{-1} (n=3) depressed field potential spike responses at threshold stimulation ($P<0.05$) but did not affect maximal or half maximal responses. 500μmol.litre^{-1} (n=1) was depressant throughout the range of stimulus intensities. 1000μmol.litre^{-1} (n=1) totally abolished responses to low and intermediate stimulation and markedly depressed the response to maximal stimulation. Any effects of ketamine were usually reversed by returning to control ACSF for 30 min (Fig.38A).

Ketamine 20μmol.litre^{-1} (n=3) and 200μmol.litre^{-1} (n=4) did not significantly alter paired pulse potentiation (Fig.38B). Control potentiation was 185 $\pm$ 14.4%, this changed to 205.1 $\pm$ 11.2% in the presence of 20μmol.litre^{-1} ketamine ($p>0.05$). 200μmol. litre^{-1} ketamine did not affect potentiation (182.0 $\pm$ 6.74%). Return to control ACSF led to potentiation of 186.7 $\pm$ 13.03%. 20μmol.litre^{-1} ketamine did not alter the relationship between rate of rise of the EPSP and amplitude of the population spike. 200μmol.litre^{-1} (n=2) and 500μmol litre^{-1} (n=1) reversibly shifted the curve to the right (Fig.38C).

Methohexitone

Overall methohexitone was depressant. After 20 min exposure to 50μmol.litre^{-1} methohexitone spike responses were reduced, the effect being significant at threshold and half maximal stimulation ($P<0.05$). Preparations tended to exhibit "rebound excitation" on washout of this concentration of methohexitone (Fig.39A).

Methohexitone (50μmol.litre^{-1}) also depressed paired pulse potentiation (Fig.39B,C). From a mean enhancement (n=3) of +38.9 $\pm$ 8.1% the second spike was reduced to half of the amplitude (49.41 $\pm$ 23.98%) of the first spike after 20 min exposure to methohexitone. This effect was significant ($P<0.05$)

and was reversible on returning to the control ACSF, potentiation returning to 138.07 ± 9.4% (Fig.39C).

Methohexitone 50μmol.litre^{-1} (n=3) also produced a slight shift to the right of the curve relating rate of rise of the EPSP to the amplitude of the population spike (Fig.39D).

Anaesthetic Studies at Pressure

The effects of the anaesthetic agents methohexitone and ketamine were tested on orthodromic responses at pressure. The agents were added after 10MPa of pressure had been maintained for 20 min. This maintenance period served as a control period to ensure that no marked variation of field potential responses occurred in these preparations due to holding at elevated pressure. Slices were perfused with anaesthetic containing ACSF for 20 min to ensure that responses were at steady state.

Ketamine

20μmol.litre^{-1} ketamine induced no significant (P>0.05) changes in orthodromic field potential responses maintained at 10MPa. Only threshold responses exhibited any variation, with 2 of 3 preparations having a 20% decrease in amplitude, the other showed 62% enhancement.

For intermediate and maximal stimulation no preparation exhibited more than a 4% change in amplitude whilst being exposed to 20μmol.litre ketamine (Fig.40A). Paired pulse potentiation was also not significantly affected (P>0.05) by the presence of 20μmol.litre^{-1} ketamine (n=3) at 10MPa. Potentiation being 154.5 ± 9.27% immediately upon reaching 10MPa, and 173.5 ± 13.5% after 20 min at 10MPa. Following addition of ketamine (20μmol.litre^{-1}) potentiation was 157.8± 17.1% (Fig.40B).

Methohexitone

50μmol.litre^{-1} methohexitone added after a 20 minute hold period at 10MPa induced a similar depression of spike responses to that seen at atmospheric pressure (n=3) (Fig.41A, 39A). Threshold responses were greatly reduced, 1 preparation having its spike response abolished whilst the other 2 were depressed to 5% and 45% of their initial values. Intermediate stimulus responses were depressed to between 49.2 and 96.3% of their initial values. The effect was significant in both cases (P<0.05). Maximal stimuli responses were recorded as being between 78.6 and 99.5% of their initial values.

Paired pulse potentiation was depressed by 50μmol.litre^{-1} methohexitone at 10MPa (n=3). This action was again significant (P<0.05) and similar to its

action at atmospheric pressure (Fig.39B,C). Control potentiation was 148.00 ± 12.20% immediately upon reaching 10MPa and 142.50 ± 6.50% after 20 min at 10MPa. Addition of 50μmol.litre^{-1} methohexitone resulted in a decrease in potentiation to 61.00 ± 14.08% (Fig.41B).

Intracellular Studies at High Pressure.

Resting Membrane Potential

Resting membrane potentials were monitored at 3 pressures; in cells at 0.1MPa (n=12), cells at 5MPa (n=23) and cells at 10MPa (n=5) (table 4.0). The mean resting potential of cells at 1 atmosphere was -62.75 ± 1.99mV. Cells sampled after reaching 5MPa had a mean resting potential of -61.2 ± 1.59mV (n=23) and were not significantly different ($P<0.05$) to cells impaled at 1 atmosphere. If the population of cells sampled at 5MPa was divided into cells impaled less than half an hour subsequent to reaching 5MPa (n=9, mean impalement time=18.55 ± 3.46mins) and cells impaled more than half an hour subsequent to reaching 5MPa (n=6, 38.5 ± 2.10mins) there was still no difference in mean resting potential noted. The values were -59.11 ± 3.07mV and -60.33 ± 2.56mV respectively. The mean resting potential of cells sampled at 10MPa was -63.17 ± 2.05mV (n=12). Again this was not significantly different from the control situation.

Although there was no tendency for cells to be either greatly de- or hyperpolarized at 5MPa or 10MPa of pressure, during compressions from 5MPa to 10MPa swings of resting potential (up to 20mV depolarizion) often occurred (Fig.42). The depolarizing swings which were often accompanied by bursts of spikes were also seen subsequent to pressurization of the chamber and after any slight fluctuation in temperature (≈+1°C) had settled down to control levels.

Input Resistance

Input resistance remained unaffected by elevated pressure. At 5MPa and 10MPa resistance values were 37.13 ± 1.89MΩ (n=11) and 35.9 ± 1.94MΩ (n=6) respectively, which compares favourably with the 0.1MPa value of 35.9 ± 2.37MΩ (Fig.43).

Effects on Threshold Behaviour

Thresholds for generation of a single spike were tested for with an 8ms depolarizing current pulse of varying amplitude passed through the intracellular microelectrode. Mean control (0.1MPa) threshold was 0.43 ± 0.05nA (n=13), cells sampled at 5MPa had a mean threshold of 0.42 ± 0.13nA (n=9). In the 4 cells that were able to be compressed from 5MPa to 10MPa mean threshold was, at 5MPa, 0.65 ± 0.2nA; this was not significantly different at 10MPa, threshold here being 0.6 ± 0.2nA. There was no significant change in threshold

at either pressure (P>0.05).

Effects on Spike Characteristics

Action potential characteristics were measured in the 4 cells that were compressed from 5MPa to 10MPa. Both spike width and amplitude appeared to be altered by pressurization. From a mean spike height of 62.22 ± 0.64mV at 5MPa, compression to 10MPa decreased mean spike height by 3mV, to 59.26 ± 1.61mV. Spike width at 5MPa was 0.75 ± 0.0ms which increased to 0.85 ± 0.02ms following compression to 10MPa (Fig.44).

Pressure Effects on Accommodation Behaviour

High pressure appeared to interfere with the accommodative properties of CA1 pyramidal neurones. A range of currents from 0.1 to 0.7nA were used to test accommodation at 0.1MPa, 5MPa and 10MPa. Fig.45 shows that at 1 atmosphere increasing current intensity led to a gradual increase in spike number up to a maximum of 8.64 ± 0.70 spikes (n=11) with 0.7nA current. In comparison, at 5MPa spike numbers tended to be increased. Responses were not significantly enhanced at low current intensities (0.1, 0.2 & 0.3nA), but enhanced responses were particularly evident at higher current intensities (0.4 - 0.7nA)($P<0.05$) at both 5 and 10 MPa. At 5MPa, 0.7nA current yielded 14.48 ± 1.58 spikes (n=21)(Fig.46). At 10MPa 16.25 ± 2.18 spikes were evoked with 0.7nA current (n=12). During any depolarizing swings that occurred during compression or at constant pressure, depolarizing inputs were markedly enhanced. Thus during a membrane potential fluctuation a tendency for a greater number of spikes to occur in response to an equivalent current pulse was apparent (Fig.47).

High pressure reduced the amplitude of the AHP following an 80ms current pulse in parallel to the disturbance of the accommodation properties. At 0.1MPa increasing current intensity from 0.1 to 0.7nA led to a gradual increase in the amplitude of the AHP up to a maximum of 6.57 ± 0.64mV at 0.7nA (n=13). At 5MPa the observed AHP was 4.9 ± 0.63mV (n=18). Again the situation persisted up to 10MPa (n=10), with the value here being 4.81 ± 0.66mV (Fig.48). The reduction at 5MPa was statistically significant ($P<0.05$) at all current intensities with the exception of the 0.1nA response. At 10MPa reductions were significant for all current intensities at $P<0.1$ and for the 0.2, 0.5 and 0.7nA responses at $P<0.05$.

Fig.33A

Fig.33A

Orthodromic field potential responses recorded from the CA1 pyramidal cell body layer, within the sealed pressure chamber at 0.1MPa.

A. Responses to 0.5, 2.0 and 5.0V stimulation after a 20 min acclimatization period within the chamber.

B. Responses to identical stimuli after 2 hours within the pressure chamber.

Temperature = 36°C. Stimuli = 80μs/0.1Hz.

Note; these potentials were recorded with the aid of a HPLC pump perfusing oxygenated solution through the pressure chamber door. The chamber Po_2 was 0.02MPa for these experiments.

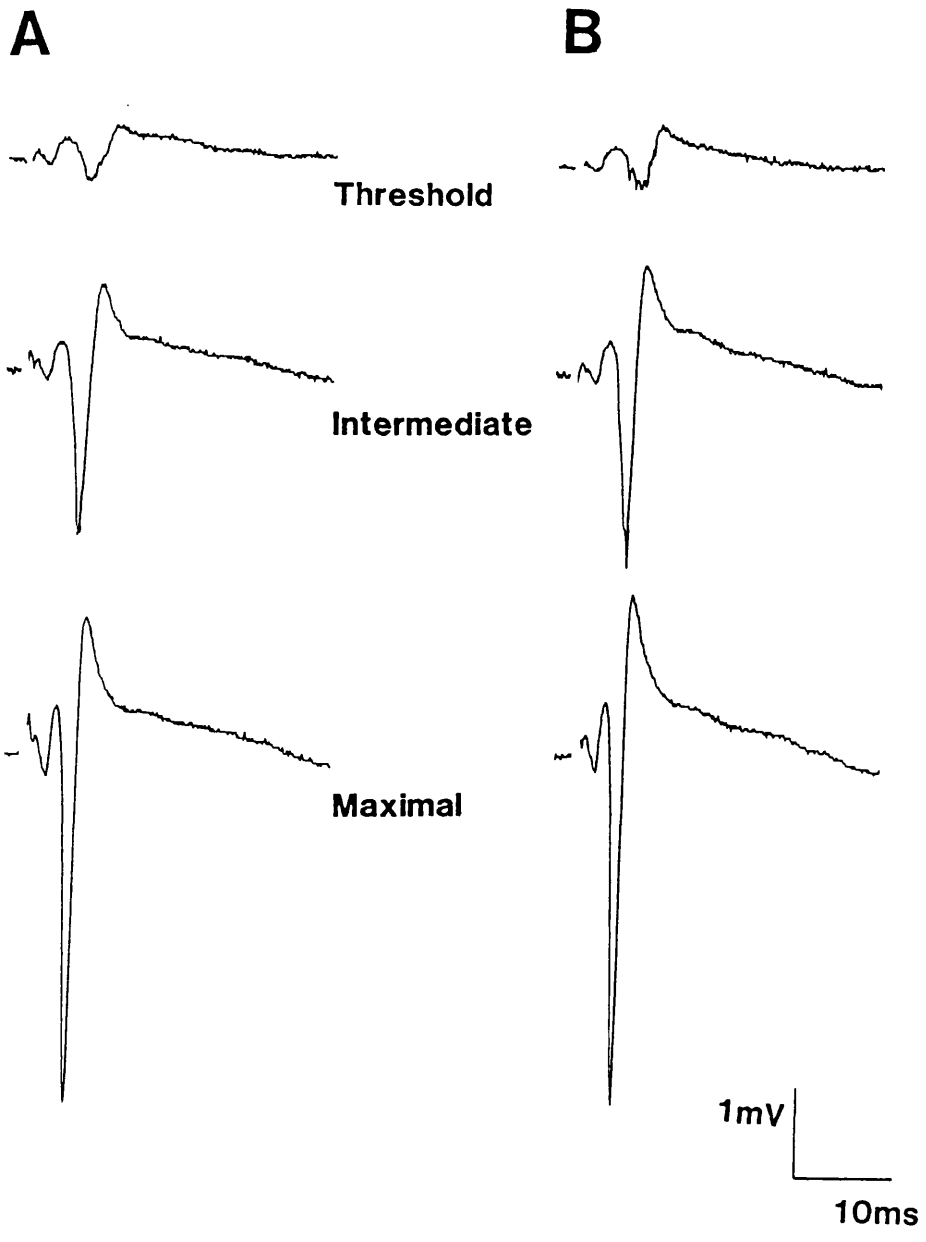

A
B
Threshold
Intermediate
Maximal
1mV
10ms

Fig.33B

Fig.33B

Paired pulse potentiation responses within the sealed pressure chamber at 0.1MPa. Potentials recorded from the CA1 neurone field were evoked by applying paired stimuli (22ms apart) to the Schaffer collaterals.

A. Response to 3V stimuli following a 20 min acclimatization period within the sealed chamber.

B. Response to identical stimuli after two hours within the pressure chamber.

Temperature = 36°C; Stimuli = 80μs/0.1Hz.

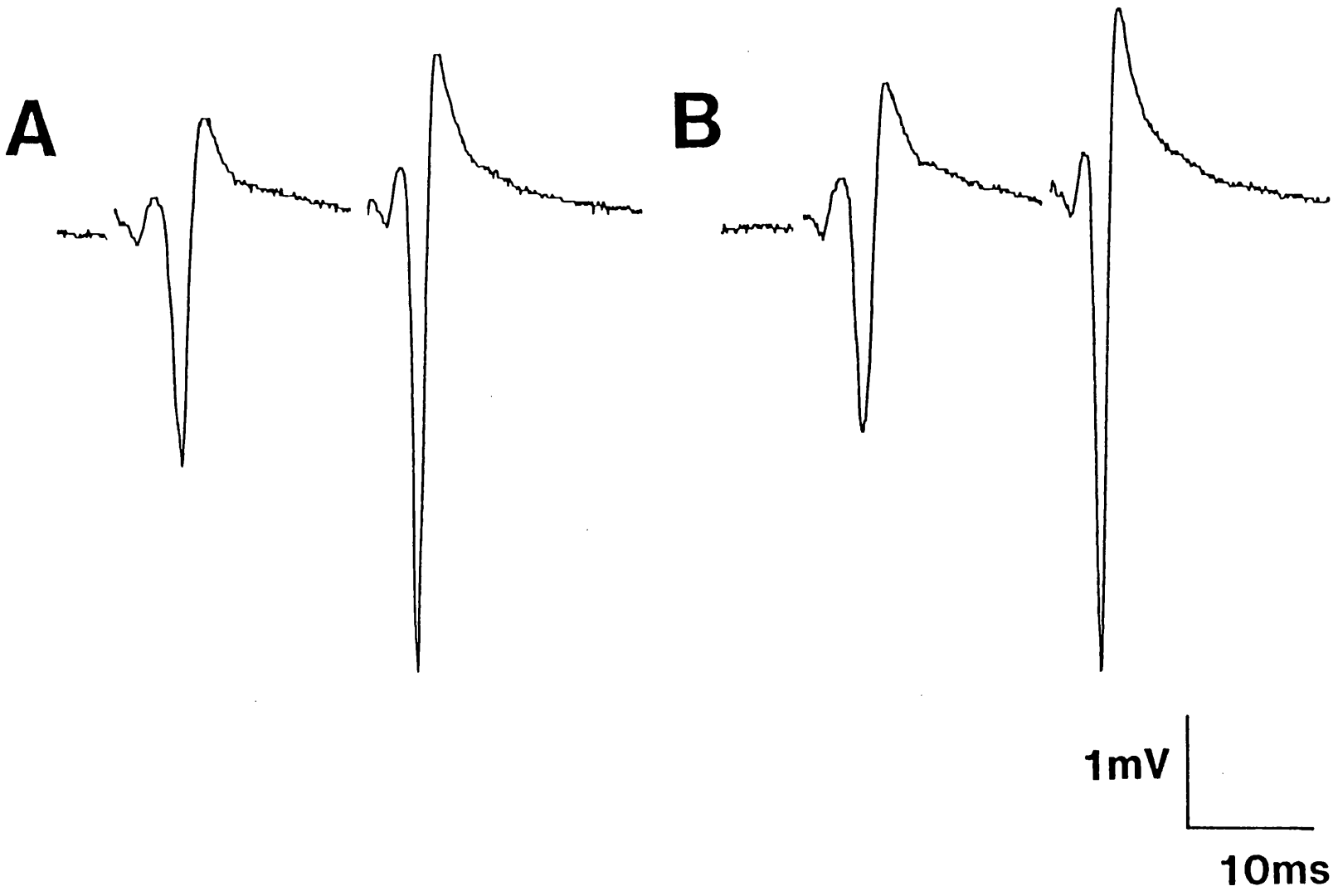

A
B
1mV
10ms

Fig.33C

Fig.33C

Histogram illustrating the pooled data from the series of control experiments at 0.1MPa. Slices had been exposed to the sealed pressure chamber environment for 30 min to allow for temperature stabilization and initial control readings to be taken. 30 min subsequent to this further control readings were taken (these values occur within the time period corresponding to a 10MPa pressure experiment).

Temperature = 35-37°C. Values are mean ± SEM.

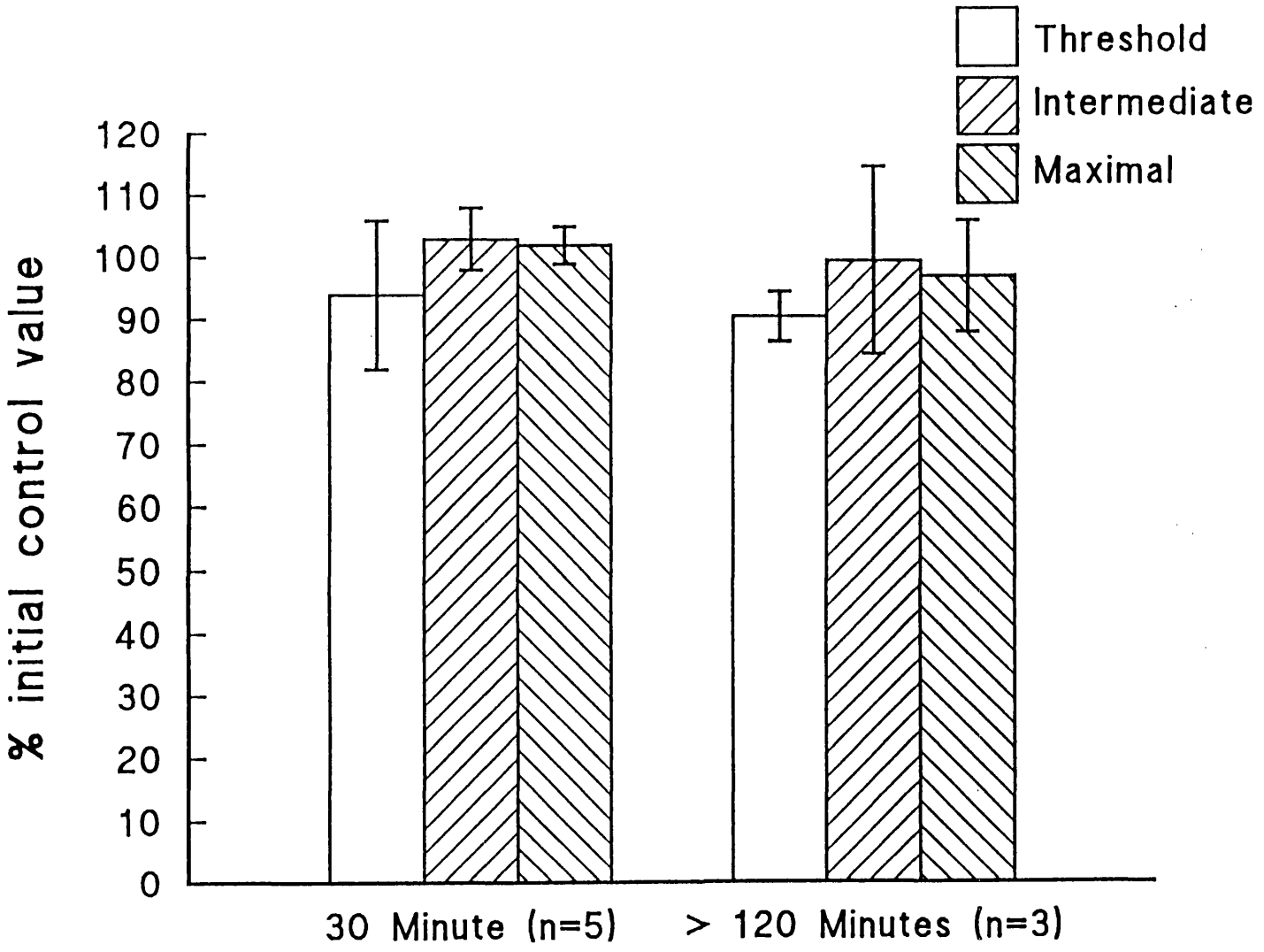

Threshold
Intermediate
Maximal
% initial control value
120
110
100
90
80
70
60
50
40
30
20
10
0
30 Minute (n=5)
> 120 Minutes (n=3)

Fig.33D

Fig.33D

Data obtained from paired pulse potentiation experiments which followed essentially the same protocol as that outlined in Fig.34C. The histogram illustrates the variation in enhancement of the second population spike with time within the sealed pressure chamber at 0.1MPa.

Temperature = 35-37°C. Values = mean ± SEM.

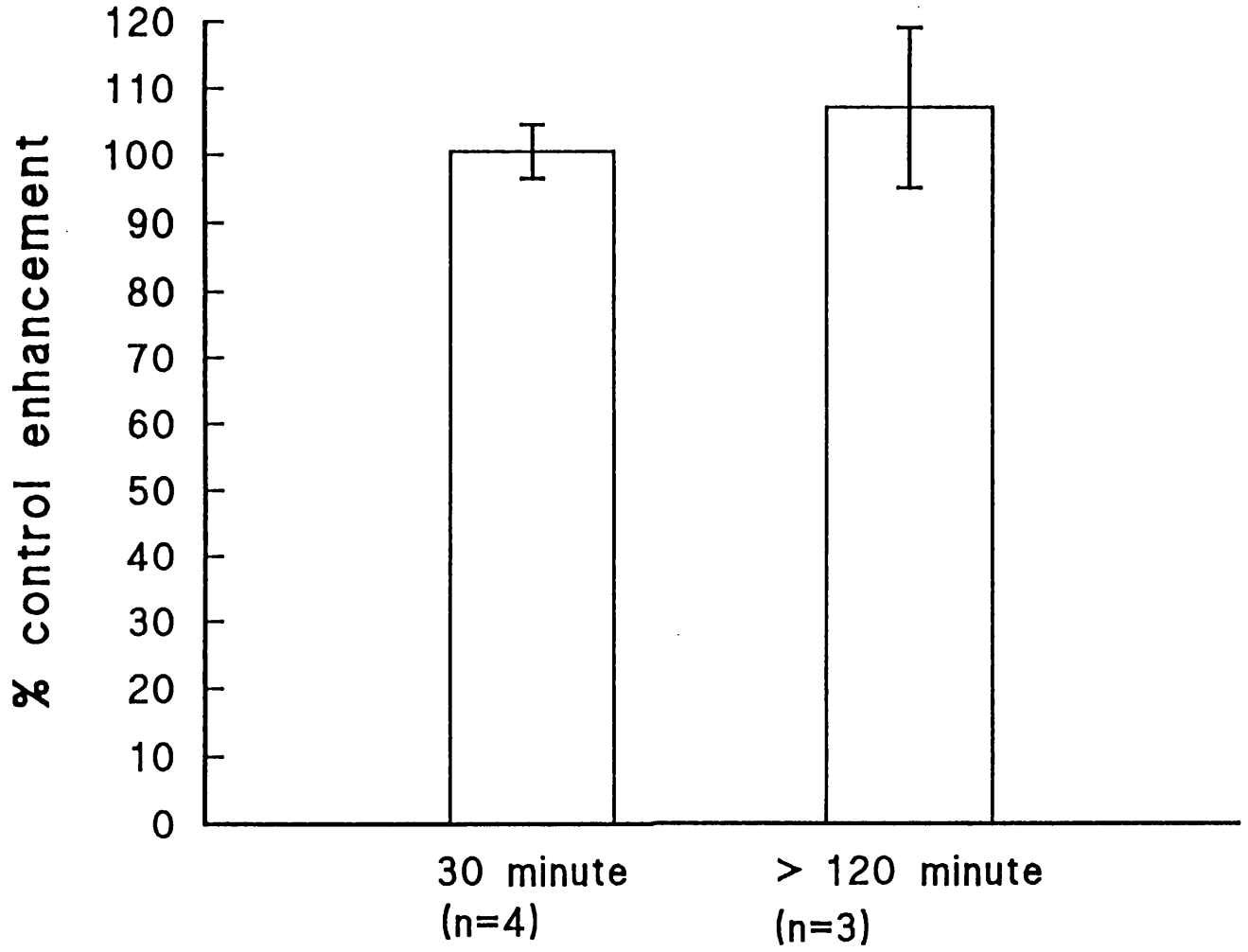

% control enhancement
120
110
100
90
80
70
60
50
40
30
20
10
0
30 minute
(n=4)
> 120 minute
(n=3)

Fig.34A

Fig.34A

Antidromic field potentials recorded within the pressure chamber in response to 0.3V, 0.9V, 1.8V, 3.6V stimulation (80μs/0.1Hz).

A. Initial measurements at 0.1MPa following a 10 min period for temperature stabilization.

B. Responses to identical stimuli at 0.1MPa 20 min later.

C. Responses after compression to 5MPa with helium gas.

D. Responses following further compression to 10MPa.

E. Maintenance of 10MPa pressure for 20 min.

Temperature = 36-37°C. Compression rate = 0.3MPa min^{-1}.

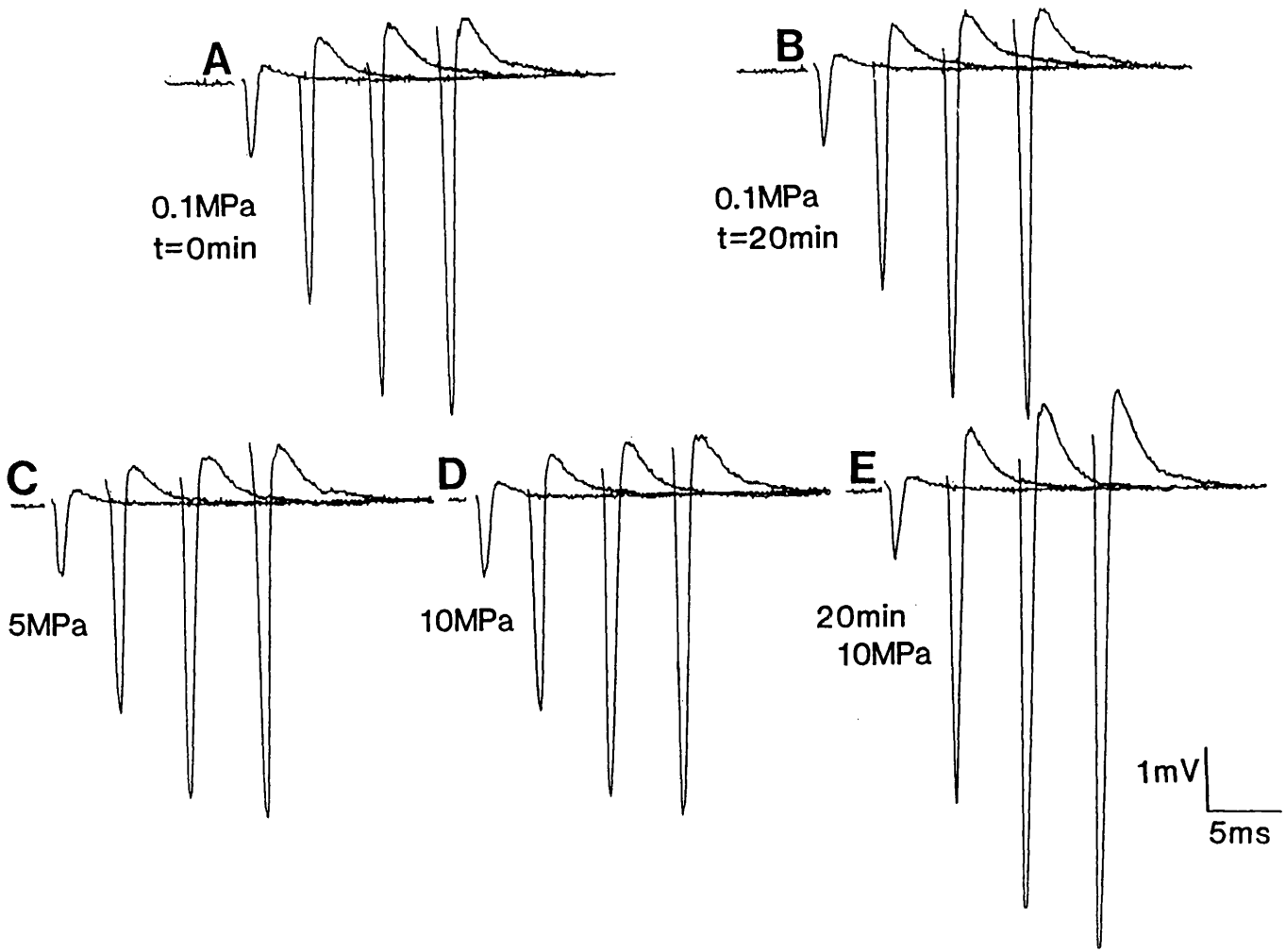

A
0.1MPa
t=0min
B
0.1MPa
t=20min
C
5MPa
D
10MPa
E
20min
10MPa
1mV
5ms

Fig.34B

Fig.34B

Histogram illustrating the change in amplitude of the antidromic population spike compared to initial control values (t=0), at 0.1MPa, 5MPa, 10MPa and 13.3MPa (n=6 to 10MPa, n=5 to 13.3MPa).

[Values are mean % change ± SEM].

▼ denotes significantly different from control ($p<0.05$).

Temperature = 35-37°C. Compression rate = 0.3MPa min^{-1}.

Stimuli 0.3-10V/180μs/0.1Hz.

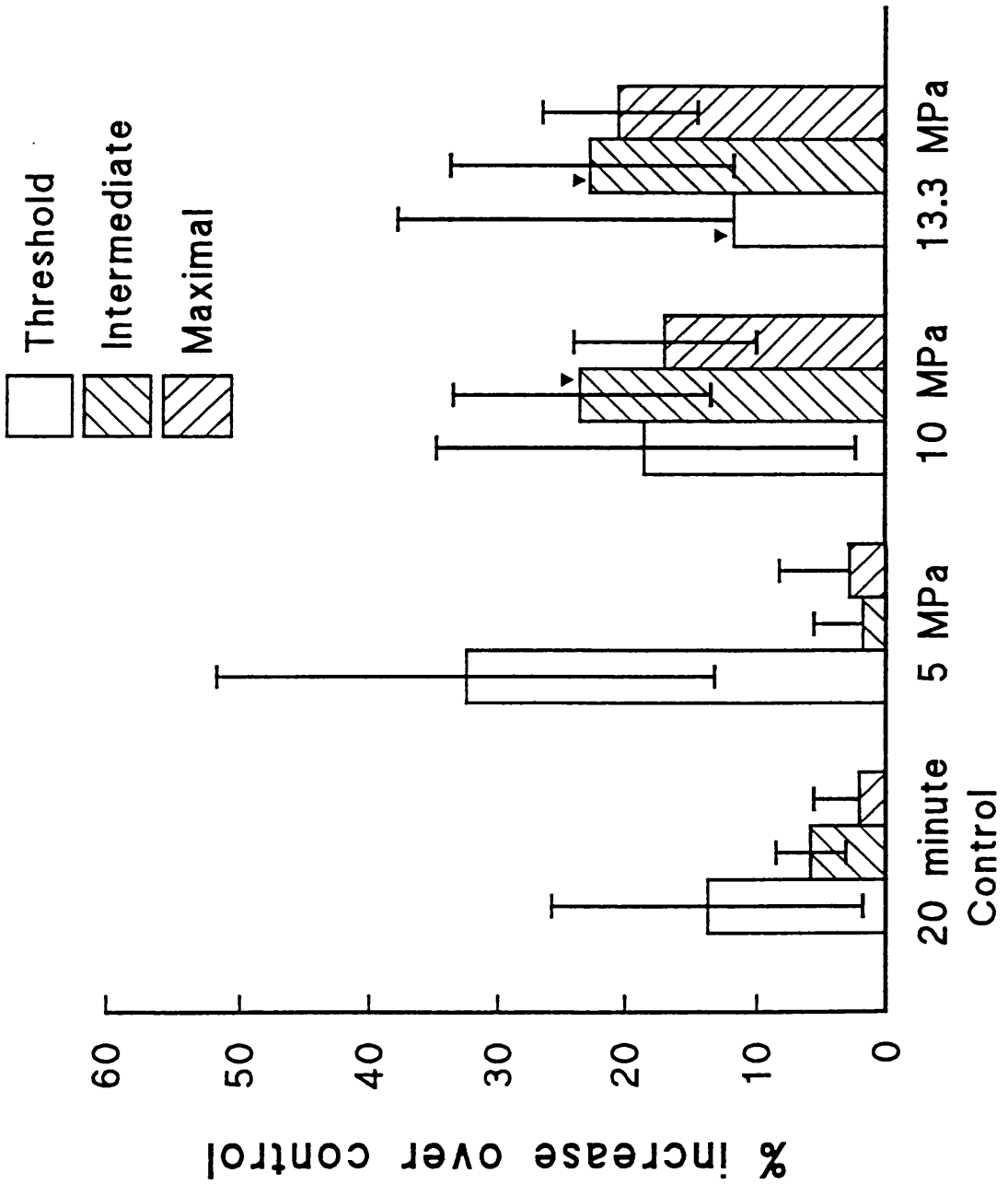

Threshold
Intermediate
Maximal
% increase over control
60
50
40
30
20
10
0
Control
20 minute
5 MPa
10 MPa
13.3 MPa

Fig.34C

Fig.34C

Antidromic field potentials recorded within the pressure chamber.

A. Control response at 0.1MPa. The amplitude and characteristics of this response remained stable for the 20 min control period.

B. Response following compression of the chamber to 5MPa.

C. Response after further compression to 10MPa.

D.& E. The response at 13.3MPa and after maintaining a pressure of 13.3MPa for a period of 20 min.

Stimuli = 10V/80μs/0.1Hz (the response was near maximum).

Temperature = 36-37°C.

Compression rate = 0.3MPa min^{-1}.

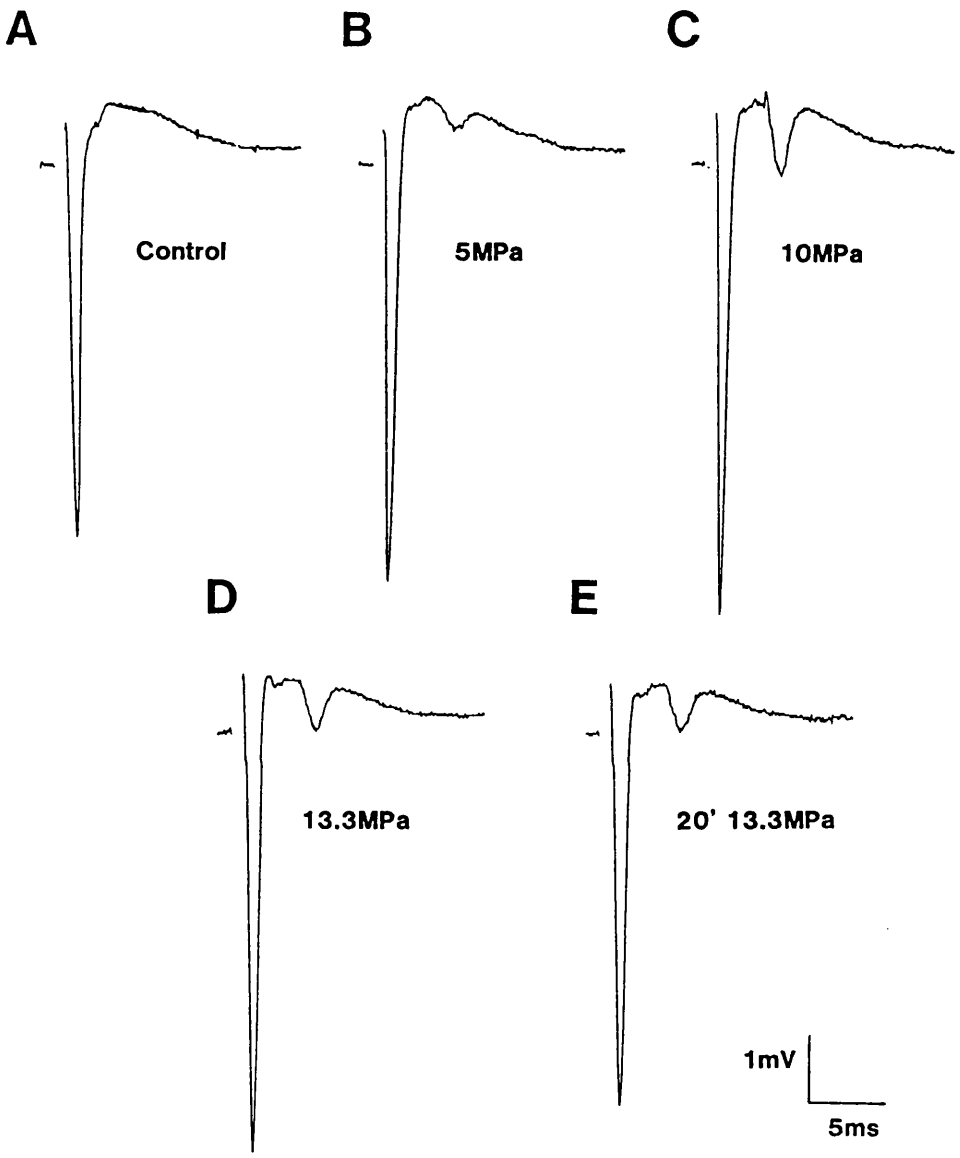

A
B
C
Control
5MPa
10MPa
D
E
13.3MPa
20' 13.3MPa
1mV
5ms

Fig.35A

Fig.35A

Orthodromic extracellular field potential responses to stimulation of the Schaffer collaterals.

A. EPSP with near threshold population spike at 0.1MPa.

B. Response to an identical stimulus following compression to 0.4MPa.

C. Response following further compression to 5MPa.

D. At 10MPa some recovery of the spike response is evident.

E. Response following decompression of the pressure chamber.

Stimuli = 0.5V/80μs/0.1Hz.

Temperature = 36°C.

Compression rate = 0.3MPa min^{-1}.

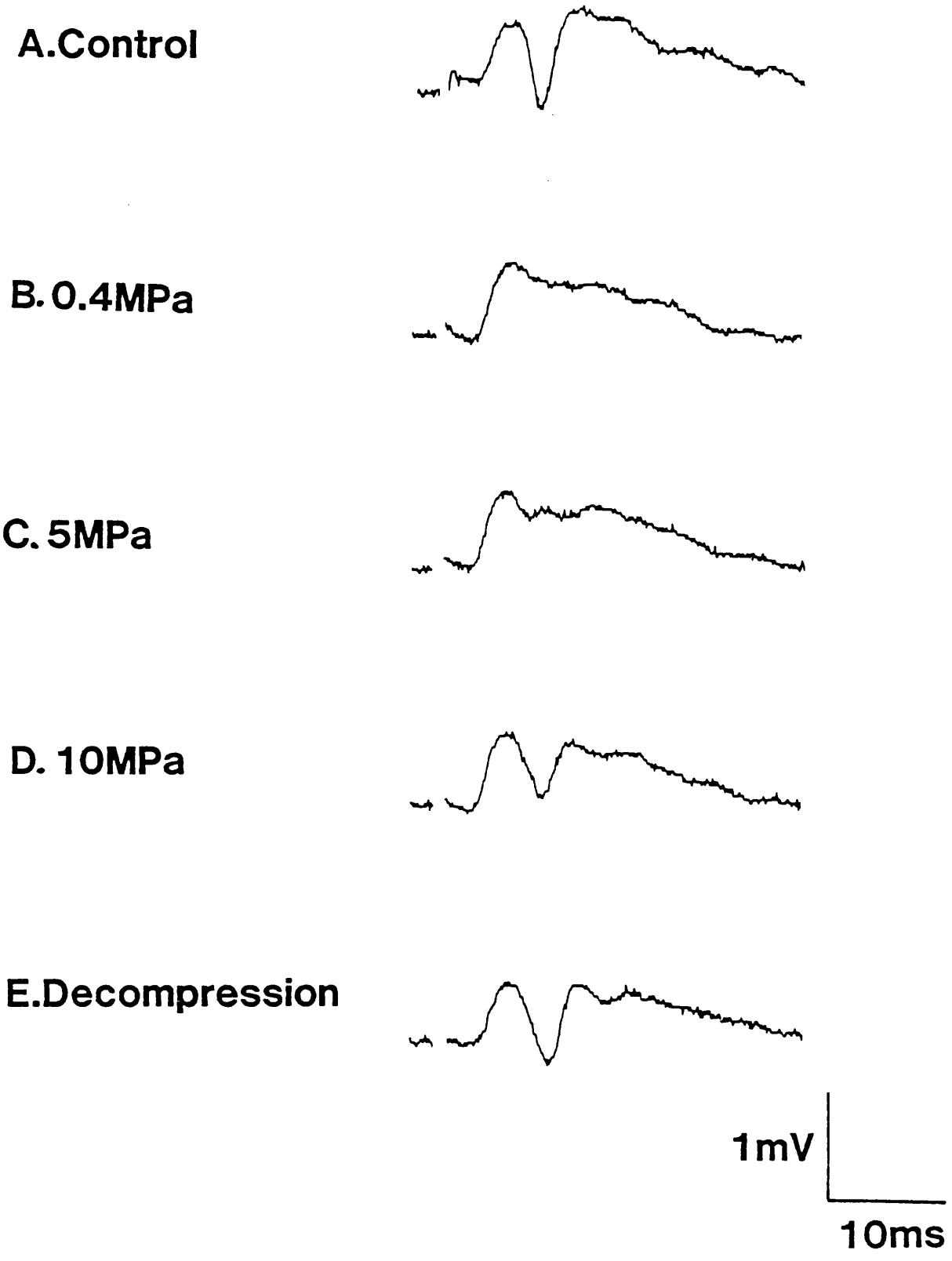

A.Control
B. 0.4MPa
C. 5MPa
D. 10MPa
E.Decompression
1mV
10ms

Fig.35B

Fig.35B

Histogram illustrating the change in amplitude of the orthodromic population spike, compared to initial control values (t=0), at 0.1MPa, 5MPa and 10MPa (n=7). [Values are mean % change ± SEM].

▼ denotes significantly different from control ($p<0.05$).

Temperature = 35-37°C. Stimuli = 0.5-12V/80μs/0.1Hz.

Compression rate = 0.3MPa min^{-1}.

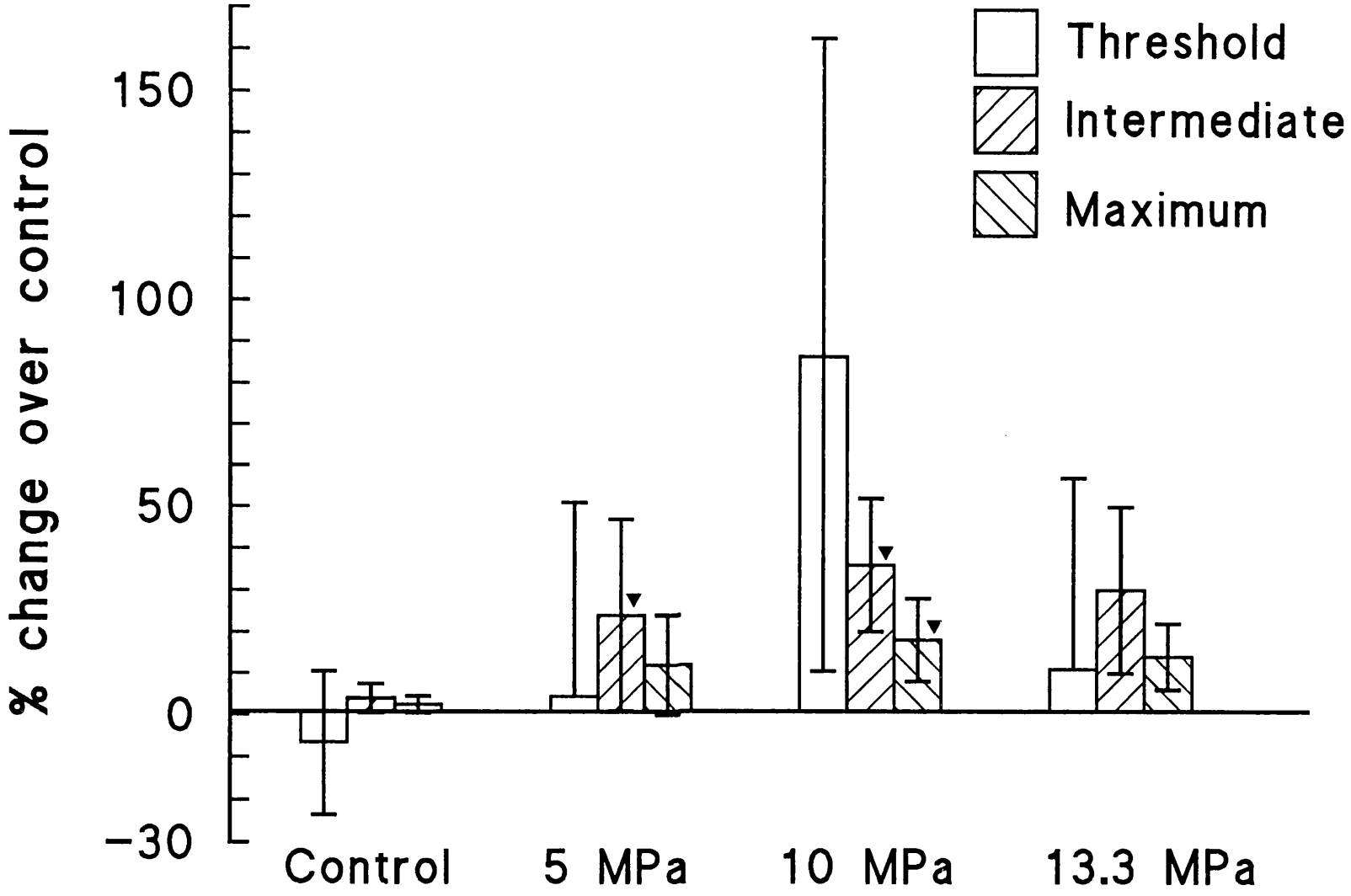

Threshold
Intermediate
Maximum
% change over control
150
100
50
0
−30
Control
5 MPa
10 MPa
13.3 MPa

Fig.35C

Fig.35C

Orthodromic field potential responses recorded in the pyramidal cell body layer of the CA1 neurone field within the high pressure chamber. Records show:

A. Control responses at 0.1MPa (the amplitude and characteristics of the responses remained stable over the control period of 20 min).

B. Responses following compression to 5MPa.

C. Responses after a further compression to 10MPa.

D. Maintenance of 10MPa pressure for 20 min.

Stimuli were 2.5, 5 and 10V /80μs/0.1Hz.

Temperature = 37°C.

Compression rate = 0.3MPa min^{-1}.

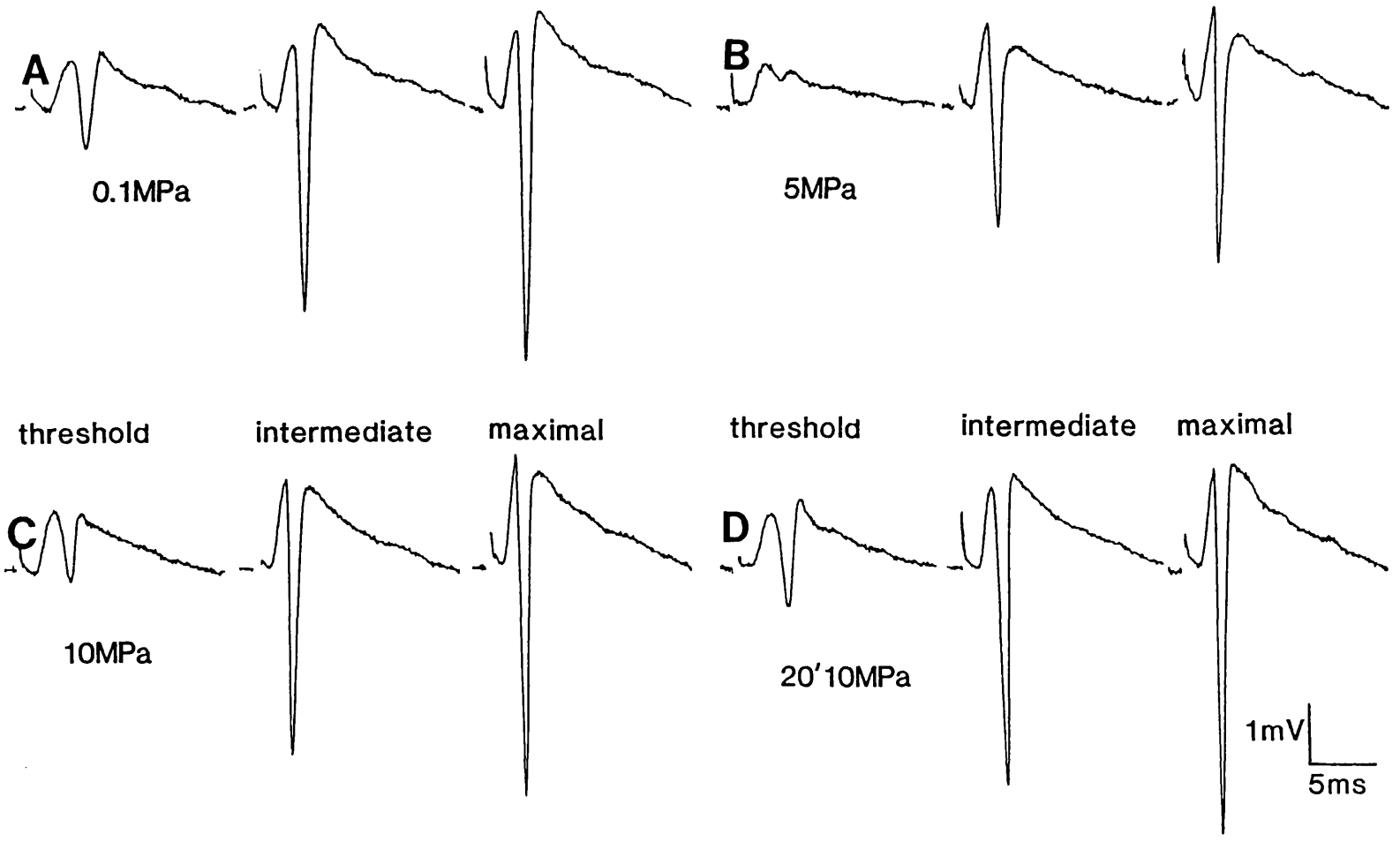

A
0.1MPa
B
5MPa
threshold
intermediate
maximal
C
10MPa
threshold
intermediate
maximal
D
20'10MPa
1mV
5ms

Fig.35D

Fig.35D

Single orthodromic population spikes evoked by stimulation of the Schaffer collaterals at three different pressures.

A. Control response. (The characteristics of this response remained stable for the 20 min control period at 0.1MPa).

B. Compression of the preparation to 5MPa.

C. Further compression to 10MPa introduces a second population spike in response to an identical stimulus.

Stimuli = 7V/80μs/0.1Hz.

Temperature = 36°C.

Compression rate = 0.3MPa min^{-1}.

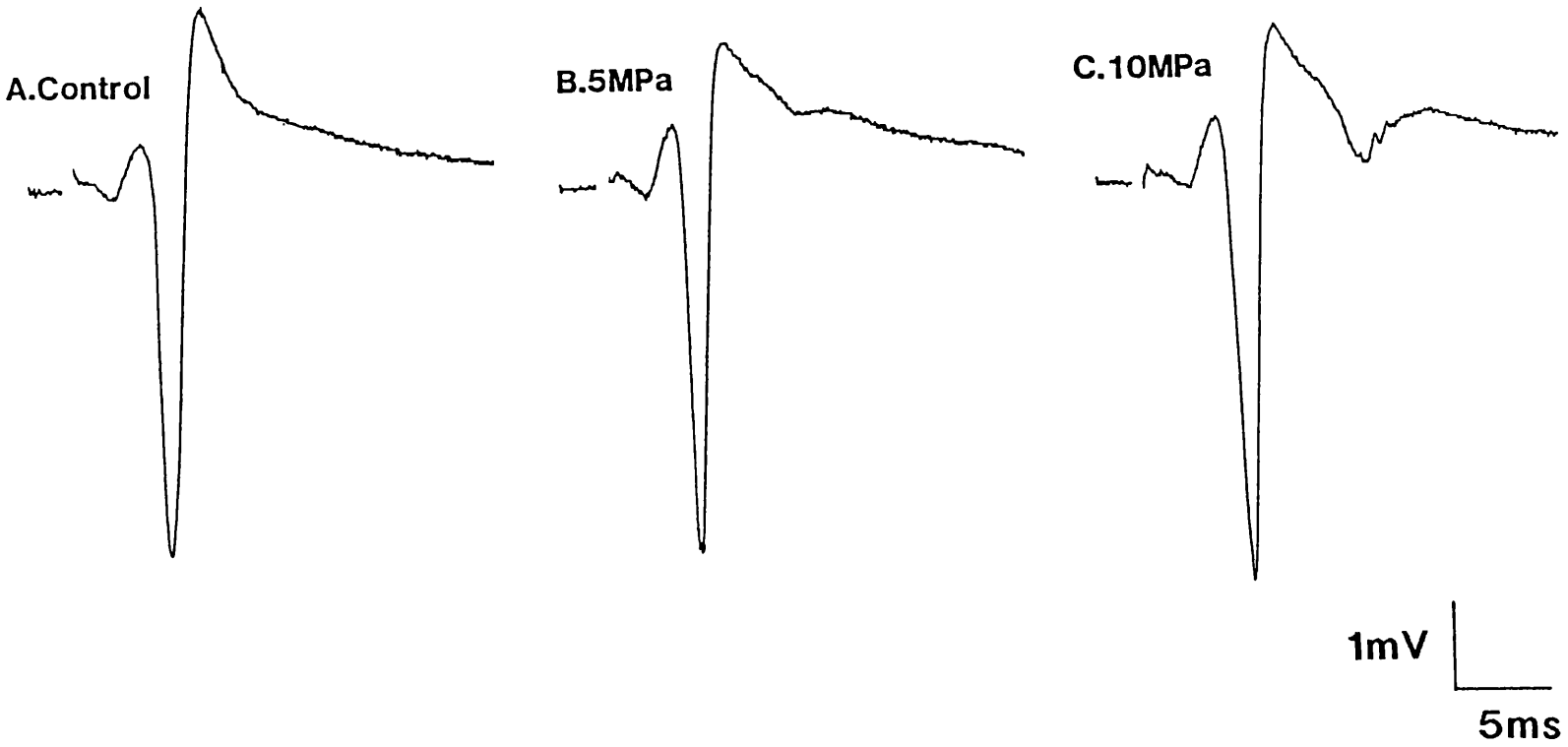

A.Control
B.5MPa
C.10MPa
1mV
5ms

Fig.36

Fig.36

Paired pulse potentiation in field potential recordings from the same preparation at different pressures.

A. Averaged responses to paired stimuli at atmospheric pressure (0.1MPa).

B. Potentiation subsequent to pressurization to 5MPa.

C. Responses following further pressurization to 10MPa.

D. Maintenance of 10MPa of pressure for 20 min.

Stimuli = 3V/80μs/22ms interstimulus interval/0.1Hz.

Temperature = 37°C.

Compression rate = 0.3MPa min^{-1}.

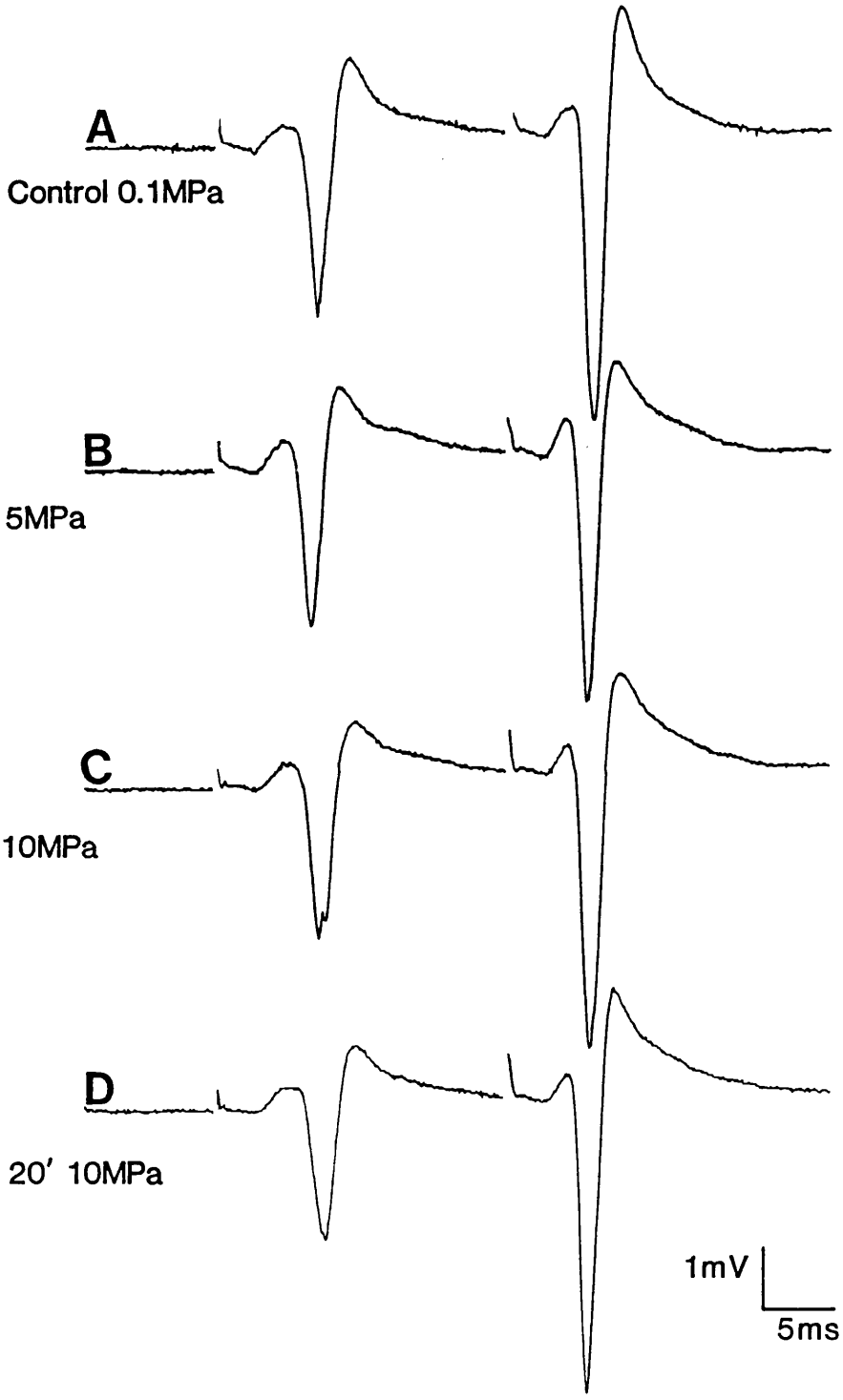

A
Control 0.1MPa
B
5MPa
C
10MPa
D
20' 10MPa
1mV
5ms

Fig.37

Fig.37

The relationship between the rise time (dV/dt) of the EPSP and the amplitude of the population spike describes the input-output relationship for a synaptic pathway. In this example, from a single experiment, the curve is displaced slightly to the right at 10MPa indicating a decrease in coupling between the EPSP and population spike.

Temperature = 37°C.

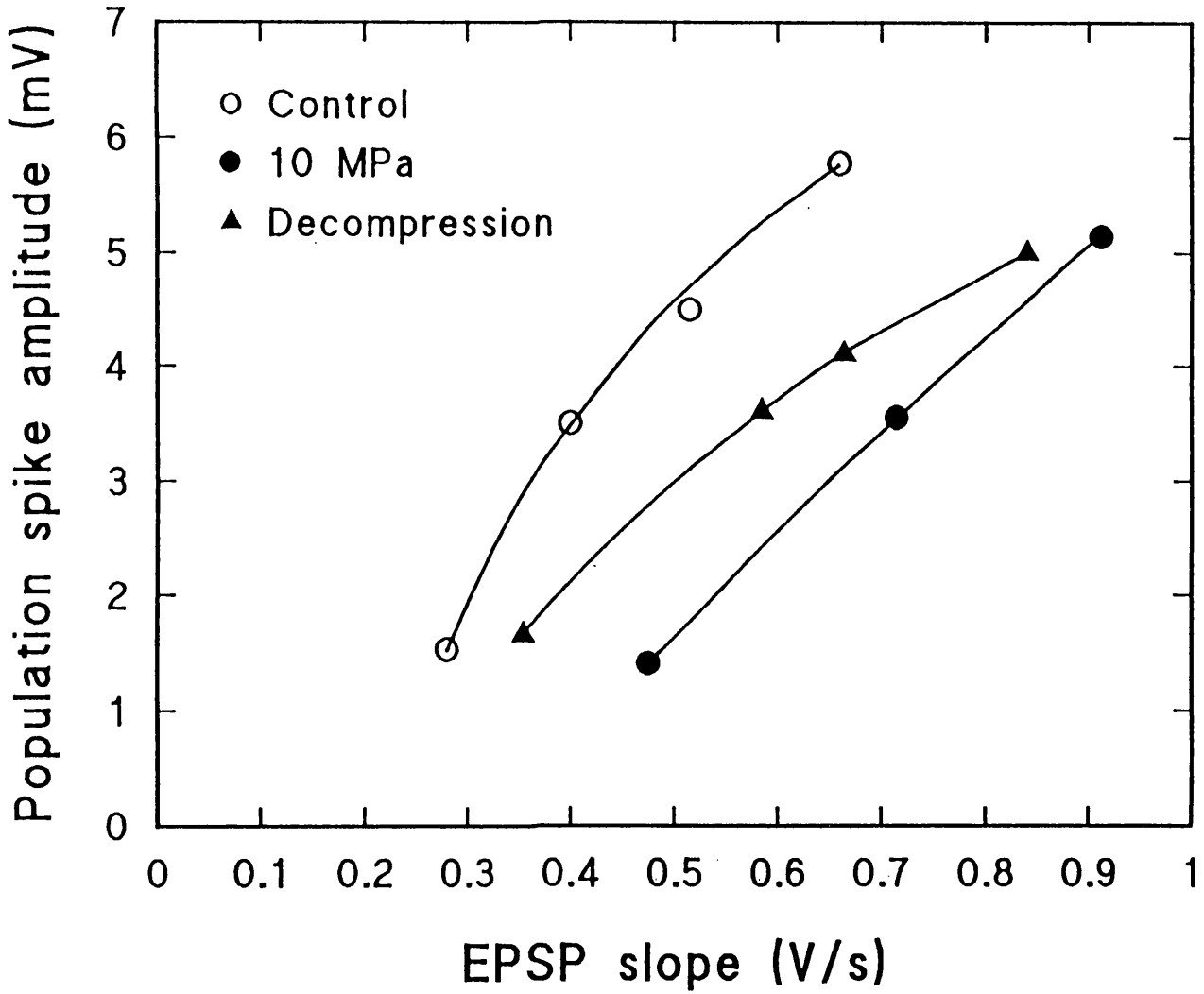

Population spike amplitude (mV)
EPSP slope (V/s)
Control
10 MPa
Decompression
0
1
2
3
4
5
6
7
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

Fig.38A

Fig.38A

Histogram showing amplitude of orthodromic field potential responses in the presence of the intravenous agent ketamine. 20, 200μmol.litre^{-1} and wash values are means of 3 experiments (bars represent ± SEM). 500 and 1000μmol.litre^{-1} are values taken from 2 individual experiments.

▼ denotes significantly different from control ($p<0.05$).

Temperature = 28-32°C.

Exposure time = 20 min at each concentration.

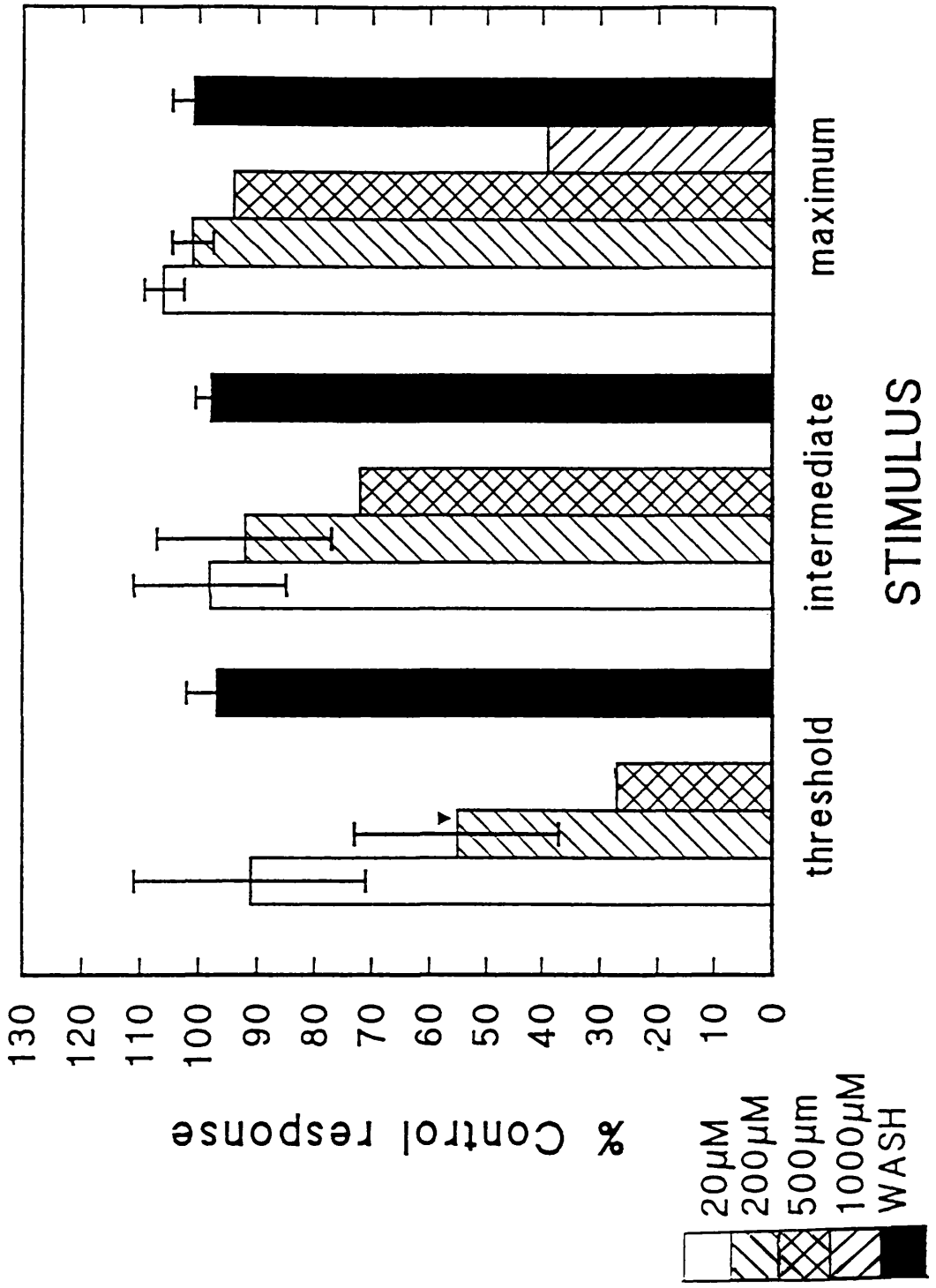

130
120
110
100
90
80
70
60
50
40
30
20
10
0
% Control response
threshold
intermediate
maximum
STIMULUS
20µM
200µM
500µm
1000µM
WASH

Fig.38B

Fig.38B

Histogram showing how ketamine (20 & 200μmol.litre^{-1}) affects paired pulse potentiation in the hippocampal slice preparation. Values are means of 4 experiments (3 in the case of 20μmol.litre^{-1}) where the potentiated spike is expressed as a percentage of the first spike; bars = ± SEM.

Temperature = 28-31°C.

Exposure time was 20min at each concentration.

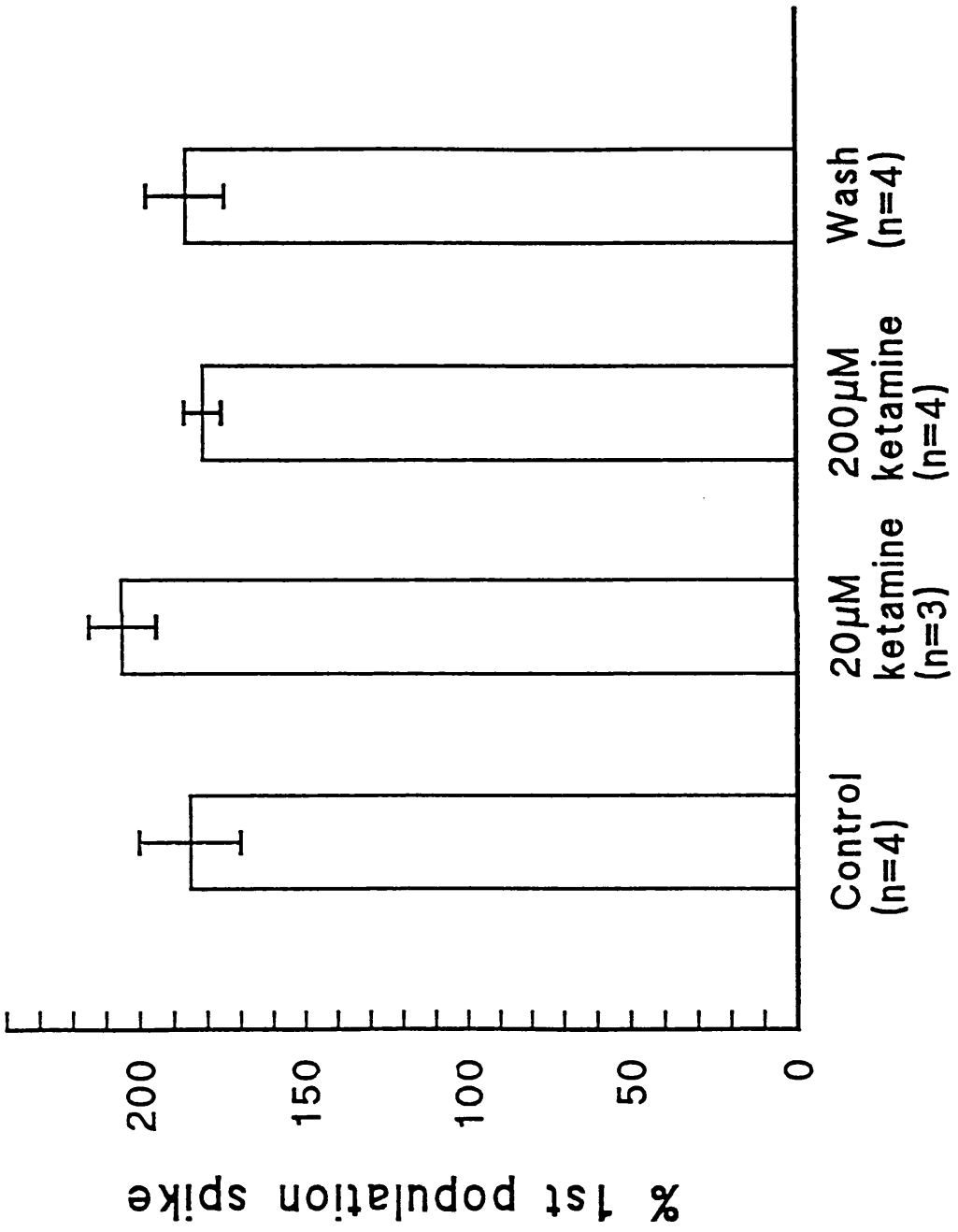

% 1st population spike
200
150
100
50
0
Control (n=4)
20μM ketamine (n=3)
200μM ketamine (n=4)
Wash (n=4)

Fig.38C

Fig.38C

Ketamine (500μmol.litre^{-1}) shifts the curve relating EPSP slope to population spike amplitude reversibly to the right. Graph shows data from a single experiment.

Temperature=30°C.

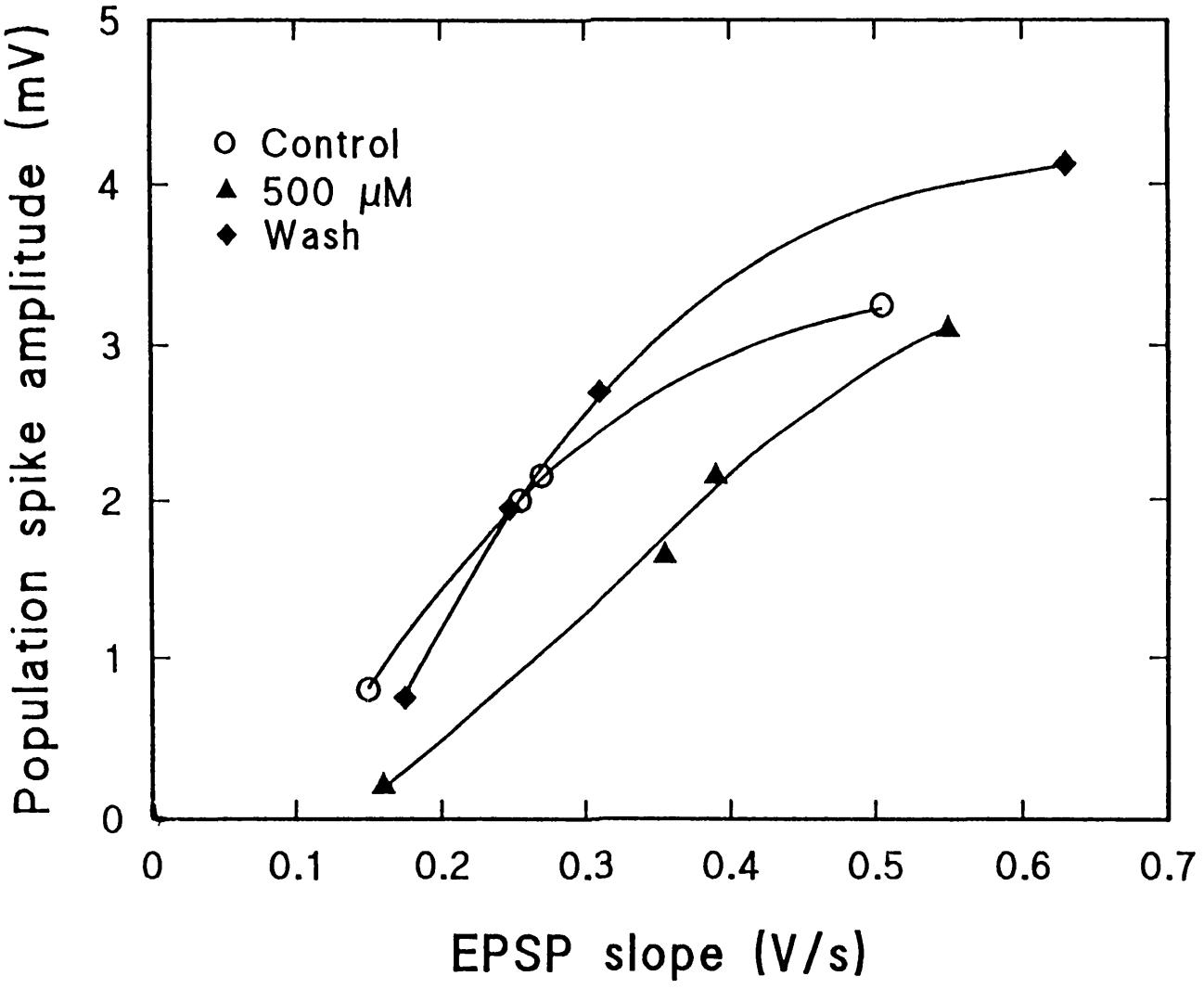

Population spike amplitude (mV)
EPSP slope (V/s)
Control
500 μM
Wash
0
1
2
3
4
5
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Fig.39A

Fig.39A

50μmol.litre^{-1} methohexitone depressed orthodromic field potential responses. Histogram shows how the amplitude of the population spike was affected by a 20 min exposure to methohexitone. Values are mean amplitudes [± SEM] (expressed as a percentage of the control response) for 3 experiments. Temperature = 29-30°C.

▼ denotes significantly different from control ($p<0.05$).

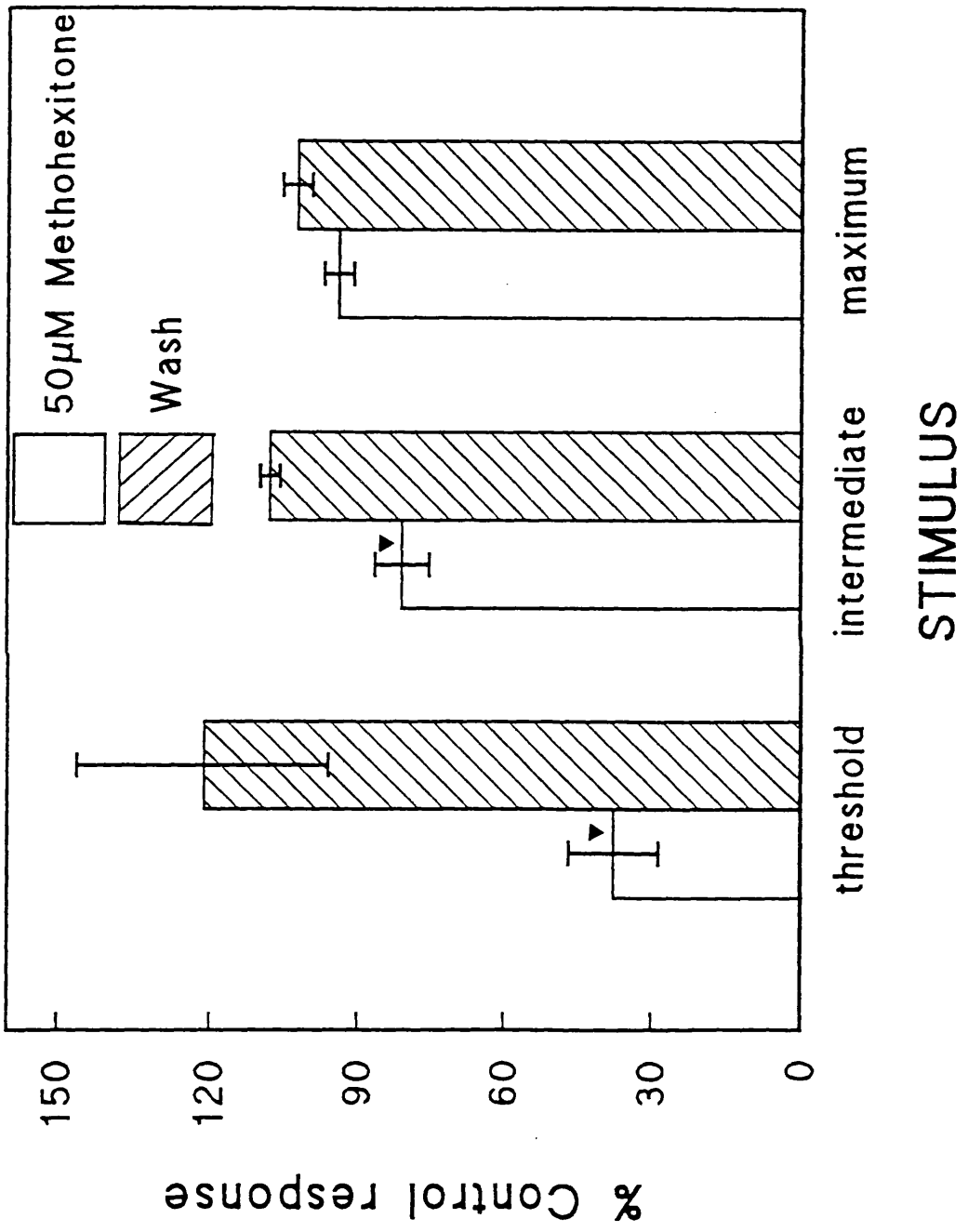

50μM Methohexitone
Wash
% Control response
150
120
90
60
30
0
threshold
intermediate
maximum
STIMULUS

Fig.39B

Fig.39B

Orthodromic field potential responses to paired stimuli.

A. Control.

B. Response in the presence of 50μmol.litre^{-1} methohexitone (20 min exposure). Stimulus had been increased to 6.5V to compensate for the depressant effect of methohexitone.

C. Response following a wash period of 30 min.

Stimului = 4.8V/0.1Hz/80μs/22ms apart. Temperature = 30°C.

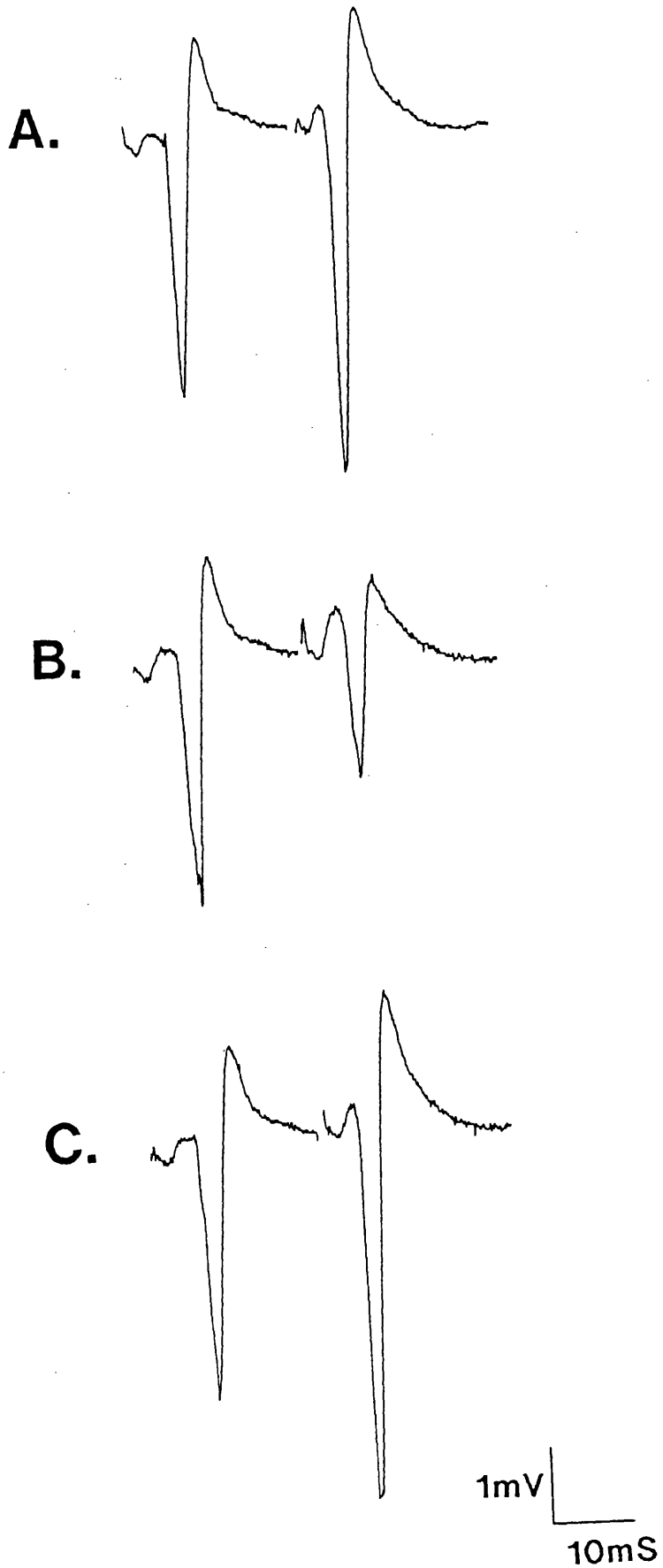

A.
B.
C.
1mV
10mS

Fig.39C

Fig.39C

Histogram illustrating how methohexitone alters the amplitude of the potentiated population spike. Values reflect mean values of 3 experiments [± SEM] in which slices were exposed to 50μmol.litre^{-1} methohexitone for 20 min.

Temperature = 29-30°C.

▼ denotes significantly different from control ($p<0.05$).

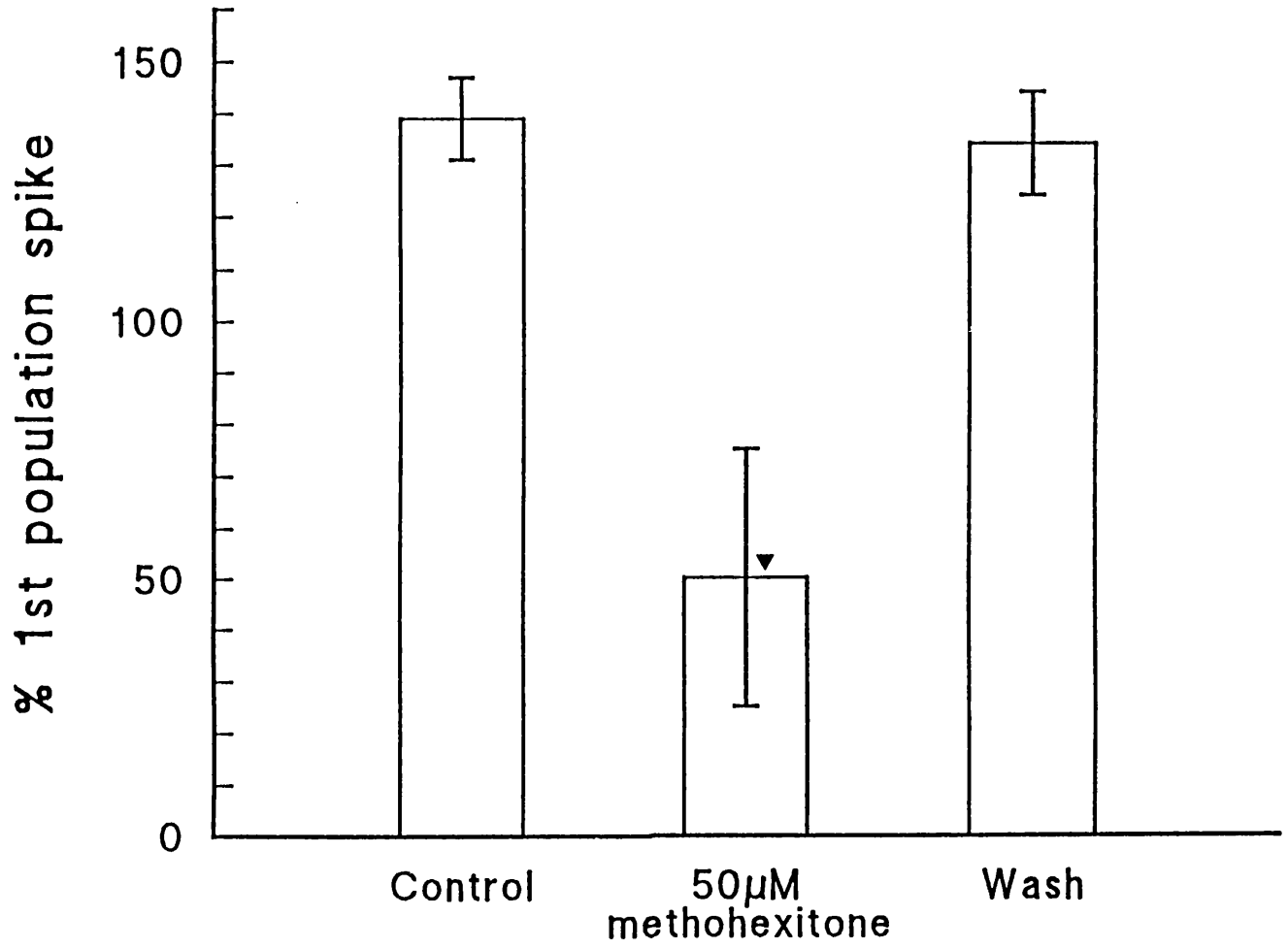

% 1st population spike
150
100
50
0
Control
50μM
methohexitone
Wash

Fig.39D

Fig.39D

Methohexitone (50μmol.litre^{-1}) reversibly shifts the curve relating EPSP slope to population spike amplitude to the right. Data points are values obtained from a single experiment. Temperature = 31°C.

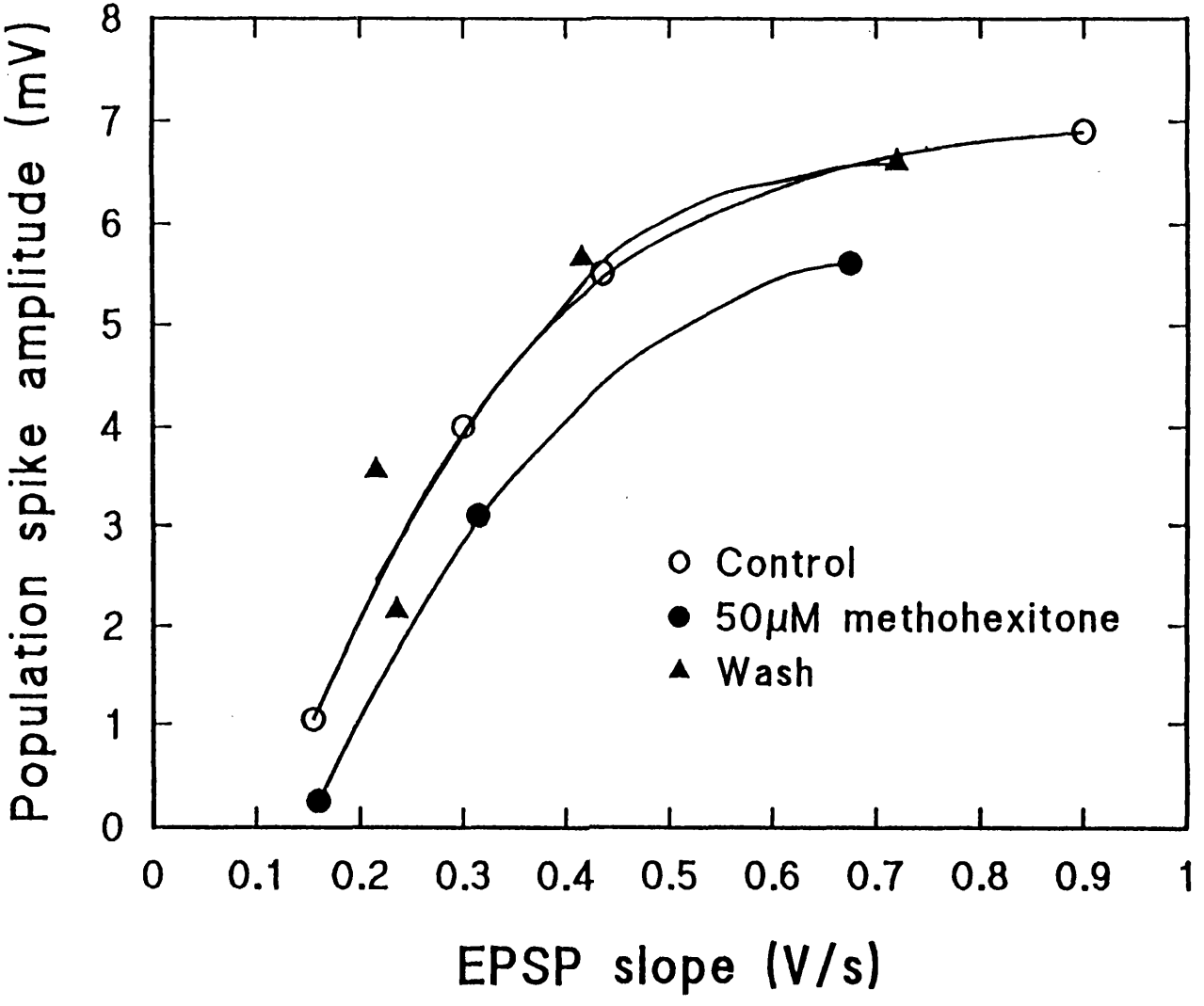

Population spike amplitude (mV)
8
7
6
5
4
3
2
1
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
EPSP slope (V/s)
Control
50μM methohexitone
Wash

Fig.40A

Fig.40A

Histogram showing how maintenance of 10MPa pressure for 20 min and the subsequent 20 min exposure to 20μmol.litre^{-1} ketamine at 10MPa affected the amplitude of the orthodromically evoked population spike. Values are means of 3 experiments [± SEM] expressed as a percentage of the spike amplitude upon attaining 10MPa. Temperature = 36-37°C.

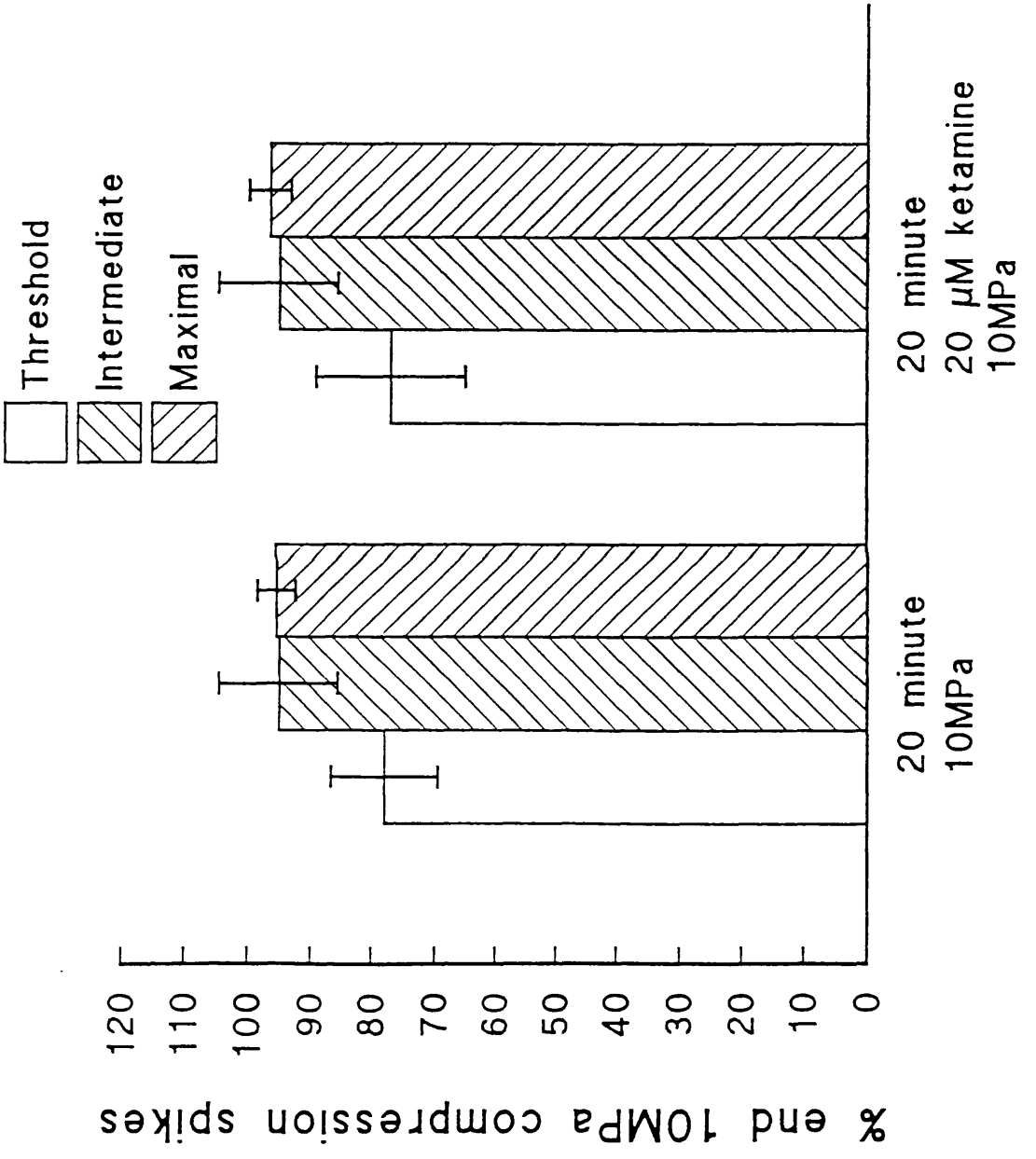

Threshold
Intermediate
Maximal
% end 10MPa compression spikes
120
110
100
90
80
70
60
50
40
30
20
10
0
20 minute
10MPa
20 minute
20 μM ketamine
10MPa

Fig.40B

Fig.40B

Paired pulse potentiation following a 20 min hold period at 10MPa and following perfusion of 20μmol.litre^{-1} ketamine at 10MPa.

Values are means of 3 experiments ± SEM.

Temperature = 36-37°C.

% 1st population spike
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10 MPa
20 minute
10 MPa
10 minute
20 μM
ketamine
10 MPa
20 minute
20 μM
ketamine
10 MPa

Table 4.0

Table 4.0

Tabulation of resting membrane potentials (mV) of cells sampled at 0.1MPa, 5MPa and 10MPa. "Time" is the period of time that elapsed between cessation of compression and impalement of the cell.

TABLE 4.0			
0.1MPa	5MPa		10MPa
Em	Em	Time	Em
-58	-74	4"	-73
-60	-55	7"	-52
-63	-56	10"	-72
-57	-52	14"	-54
-76	-50	18"	-59
-73	-55	24"	-65
-67	-52	30"	-70
-67	-73	30"	-60
-52	-65	32"	-68
-60	-58	35"	-65
-58	-52	37"	-55
-62	-64	38"	-65
	-55	43"	
	-65	46"	
	-68	Unknown	
	-70	"	
	-60	"	
	-70	"	
	-68	"	
	-70	"	
	-55	"	
	-56	"	
	-65	"	
$\bar{x}$=62.75±1.99	$\bar{x}$=-61.2±1.59		$\bar{x}$=63.17±2.05
	p>0.2		p>0.5

Fig.41A

Fig.41A

Histogram illustrating how maintenance of 10MPa pressure for 20 min and subsequent 20 min exposure to 50μmol.litre^{-1} methohexitone at 10MPa affected single spike orthodromic responses. Values are means of 3 experiments (± SEM) expressed as a percentage of the spike amplitude upon attaining 10MPa.

▼ denotes significantly different from control ($p<0.05$).

Temperature = 37°C.

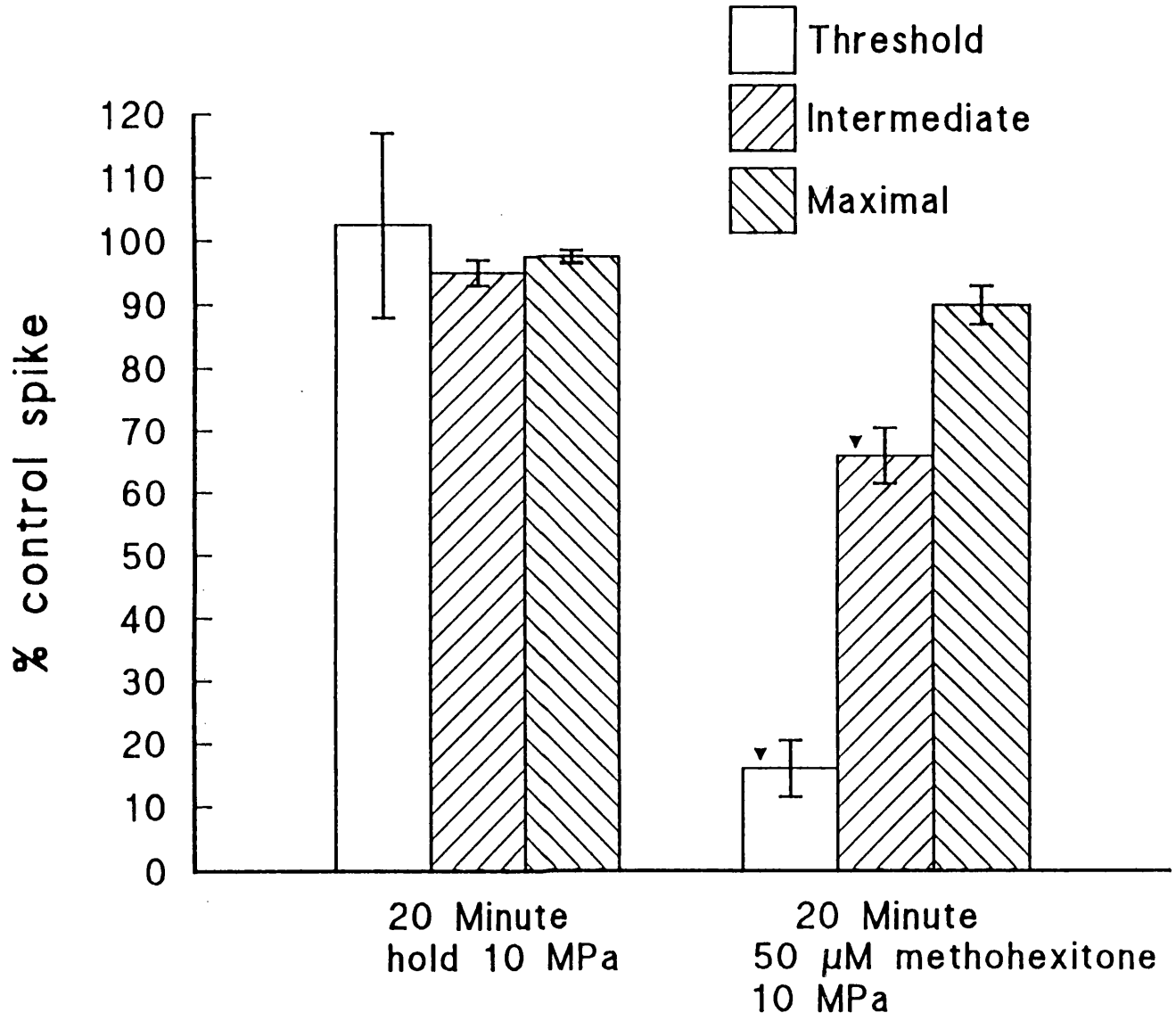

Threshold
Intermediate
Maximal
120
110
100
90
80
70
60
50
40
30
20
10
0
% control spike
20 Minute
hold 10 MPa
20 Minute
50 μM methohexitone
10 MPa

Fig.41B

Fig.41B

Paired pulse potentiation (the amplitude of the second spike being expressed as a percentage of the first) at 0.1MPa (control), 10MPa and following exposure to 50μmol.litre^{-1} methohexitone.

Temperature = 37°C.

Values are means of 3 experiments ± SEM.

▼ denotes significantly different from control ($p<0.05$).

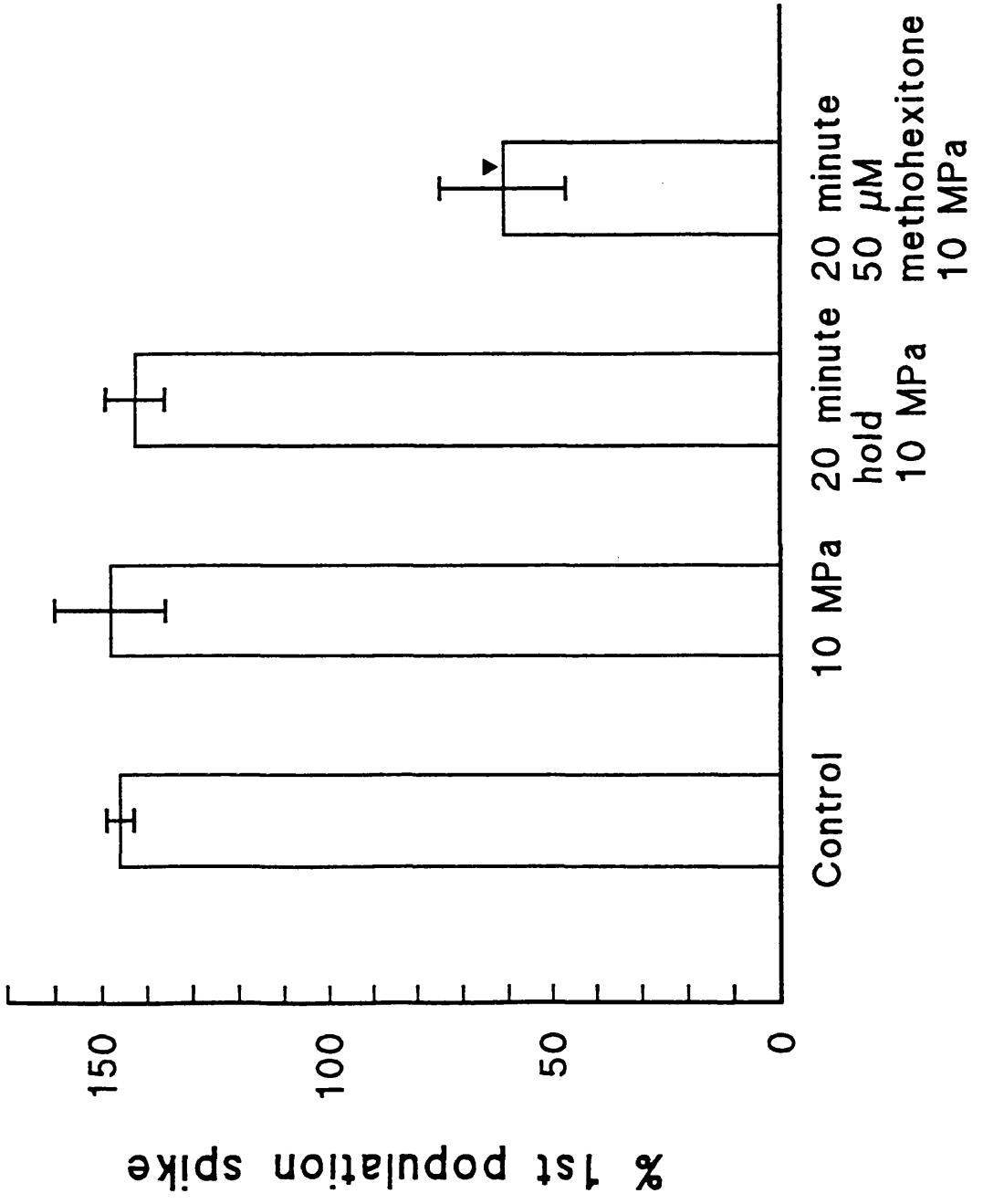

% 1st population spike
150
100
50
0
Control
10 MPa
20 minute hold 10 MPa
20 minute 50 μM methohexitone 10 MPa

Fig.42

Fig.42

A. Spontaneous membrane potential fluctuations of CA1 neurone during compression from 5MPa to 10MPa. Compression rate was constant at 0.3MPa min^{-1}. When quiescent the resting membrane potential of the neurone was -58mV, input resistance was 40MΩ. The spontaneous activity continued after holding at 10MPa.

B. Spontaneous membrane potential change of large amplitude seen during maintenance of 10MPa pressure. (Same calibration as A).

Temperature = 37°C.

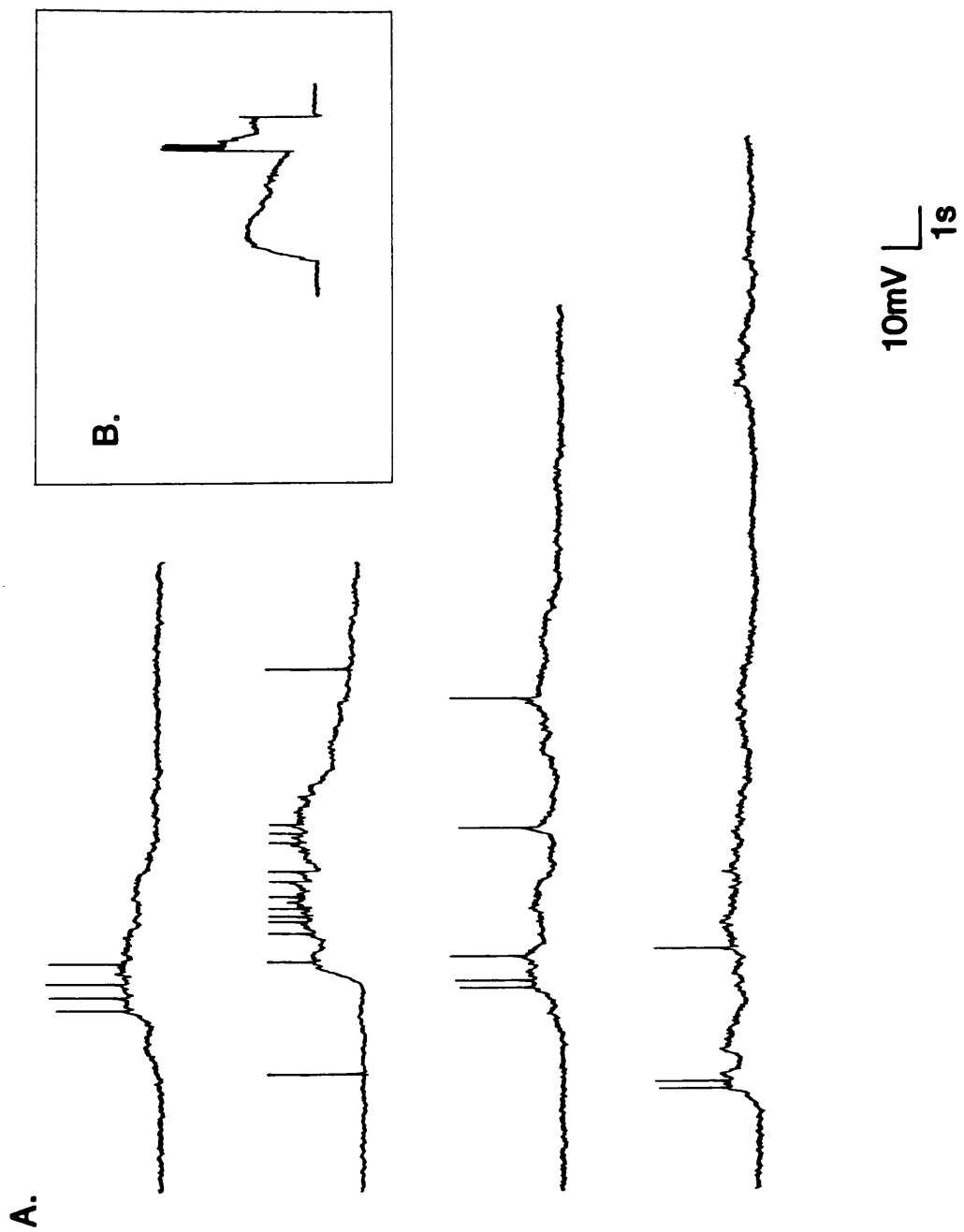

A.
B.
10mV
1s

Fig.43

Fig.43

Intracellular responses of a hippocampal pyramidal neurone to de- and hyperpolarizing current pulses at 2 different pressures.

A. Response of the cell following impalement at 5MPa.

B. Response of the same cell to identical current pulses following compression to 10MPa.

Temperature = 37°C.

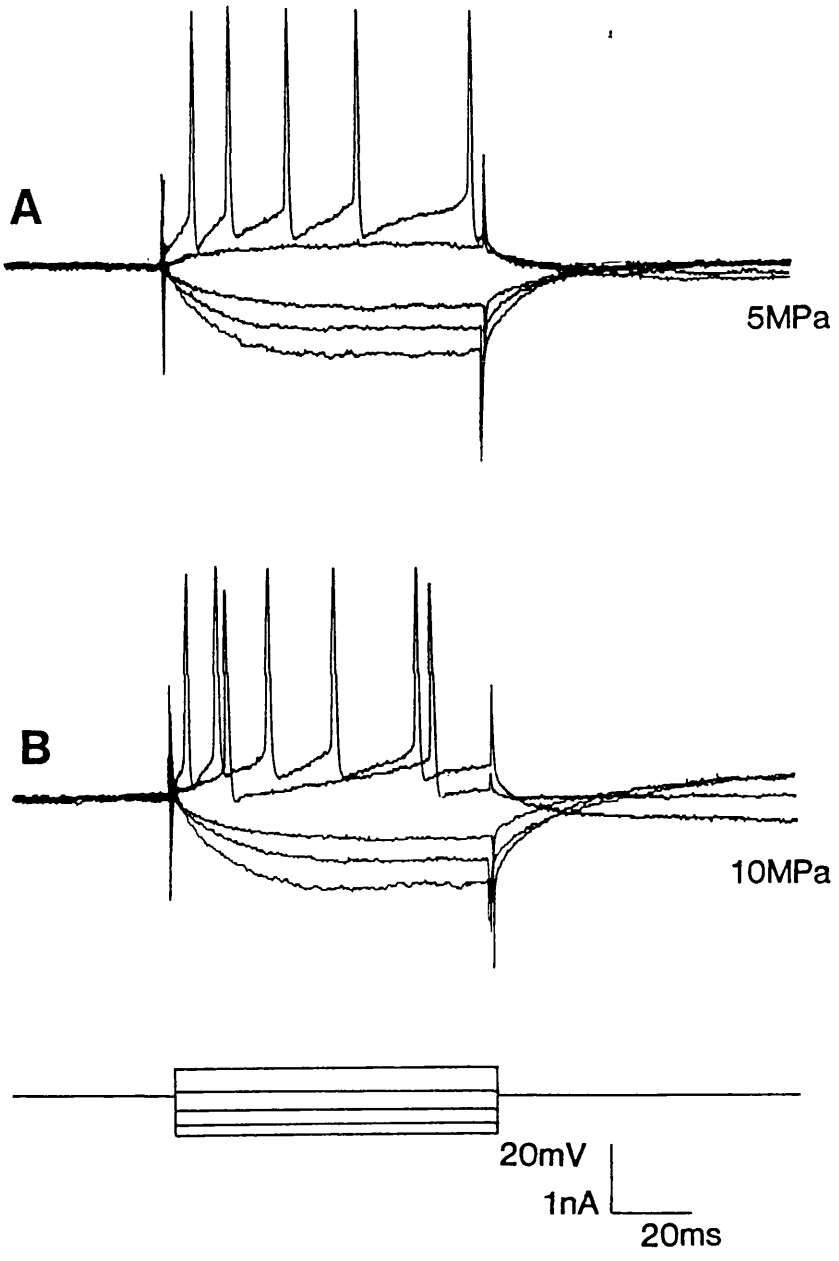

A
5MPa
B
10MPa
20mV
1nA
20ms

Fig.44

Fig.44

Action potentials evoked by direct intracellular stimulation of a single hippocampal CA1 neurone at pressure.

A. From left to right, spike responses are at 5MPa and following a 12 min hold at 5MPa. The potential on the right is the response from the same cell following compression to 10MPa.

B. Superimposed action potentials evoked at 5MPa and 10MPa.

Temperature = 37°C, Intracellular stimuli 0.1Hz/8ms/0.2-0.4nA.

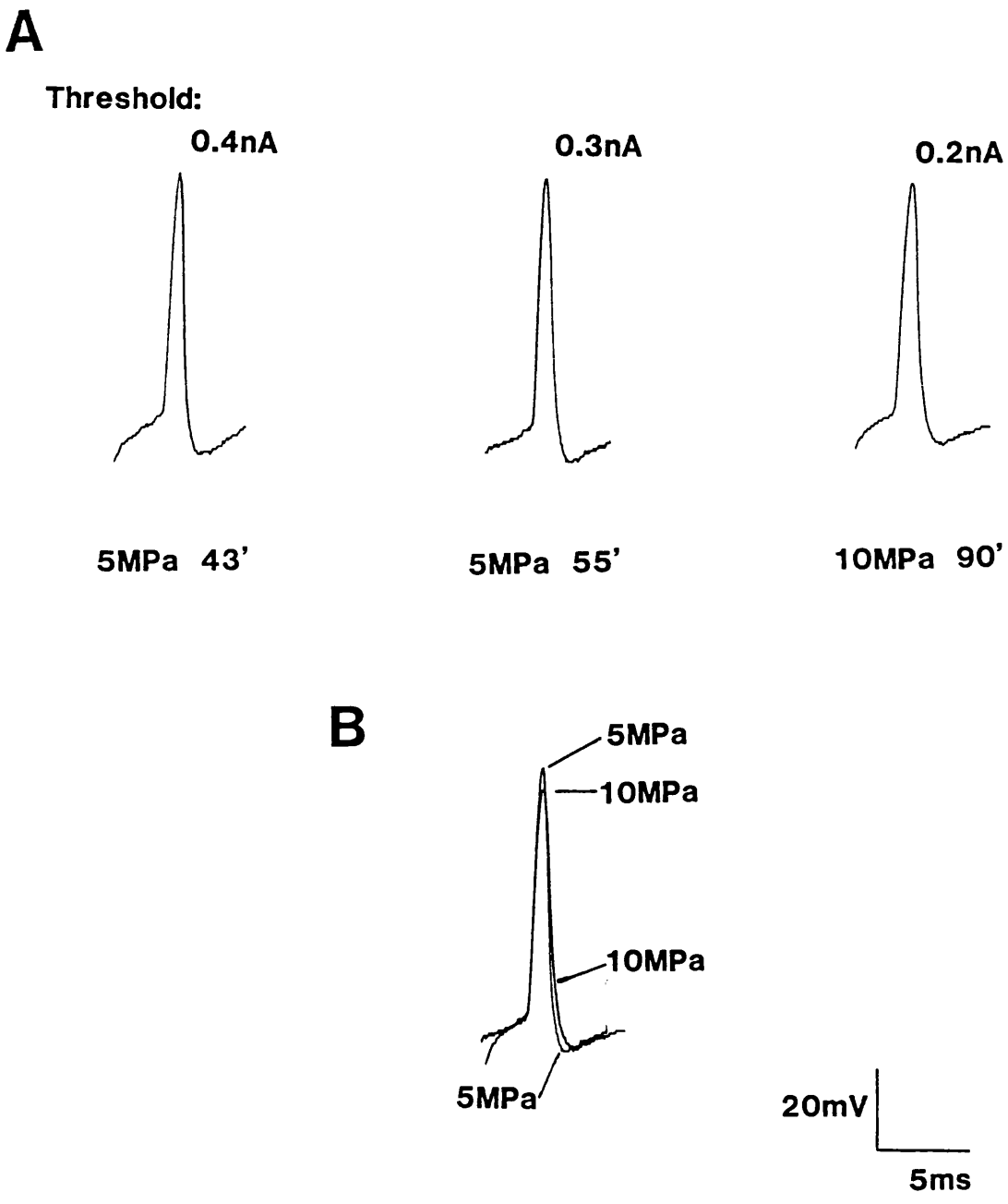

A
Threshold:
0.4nA
0.3nA
0.2nA
5MPa 43'
5MPa 55'
10MPa 90'
B
5MPa
10MPa
10MPa
5MPa
20mV
5ms

Fig.45

Fig.45

Graph showing data derived from individual cells at 0.1MPa (n=11), 5MPa (n=21) and 10MPa (n=12). Data points reflect mean number of spikes (± SEM) in response to an 800ms current pulse (0.1-0.7nA) passed through the intracellular electrode. For clarity no error bars are shown for the 10MPa data.

□ denotes significantly different from control ($p<0.05$).

Temperature = 37°C.

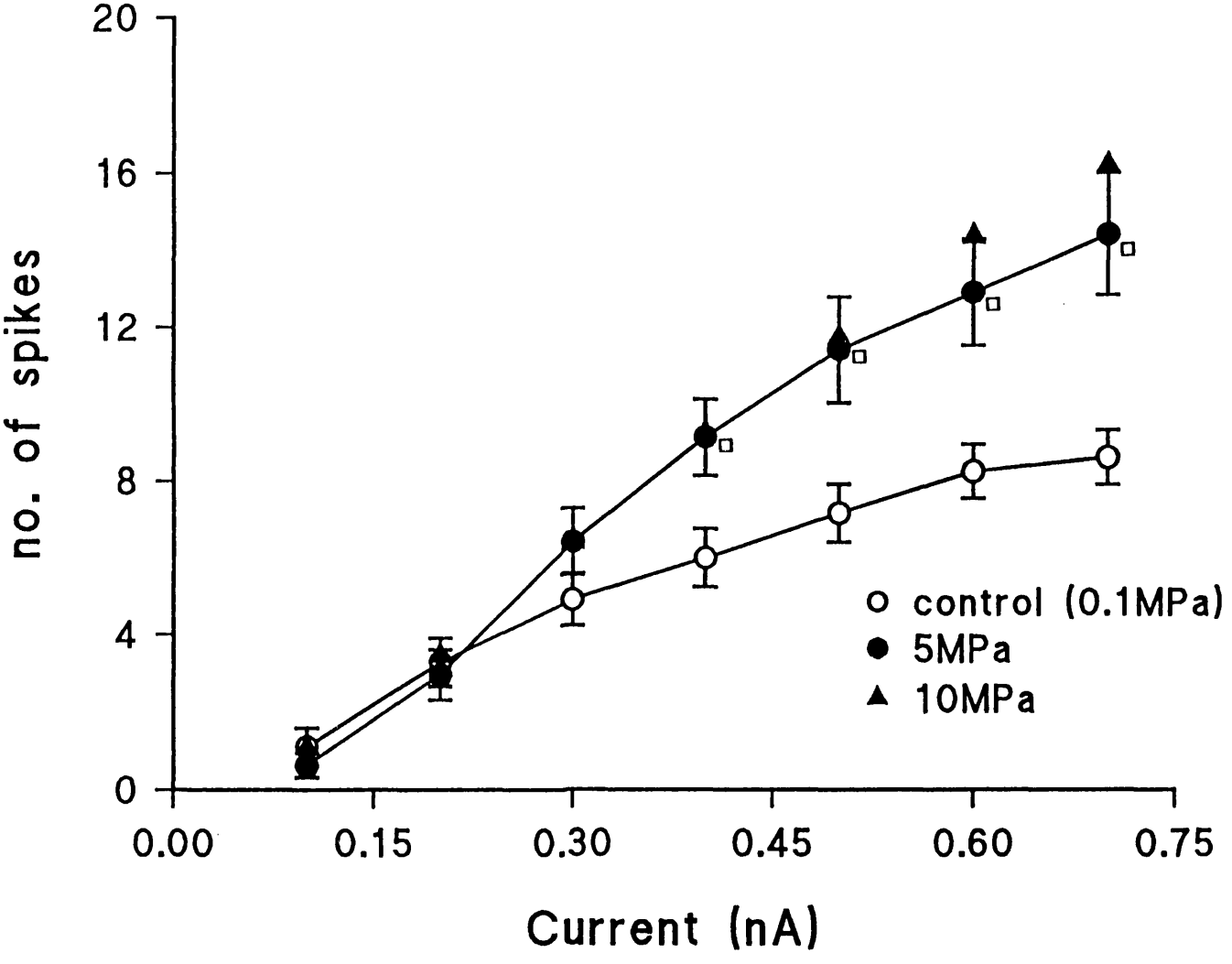

20
16
12
8
4
0
no. of spikes
0.00
0.15
0.30
0.45
0.60
0.75
Current (nA)
control (0.1MPa)
5MPa
10MPa

Fig.46

Fig.46

Responses of a CA1 pyramidal neurone to depolarizing current (0.1-0.7nA) passed through the intracellular electrode. The tendency to fire throughout the current pulse was noted for a significant number of cells impaled at high pressure (5-10MPa). Not all neurones however displayed disruption of accommodation behaviour.

A. Accommodation responses of a cell impaled and held at 5MPa.

B. AHP's evoked from the same cell at 5MPa.

Temperature = 37°C.

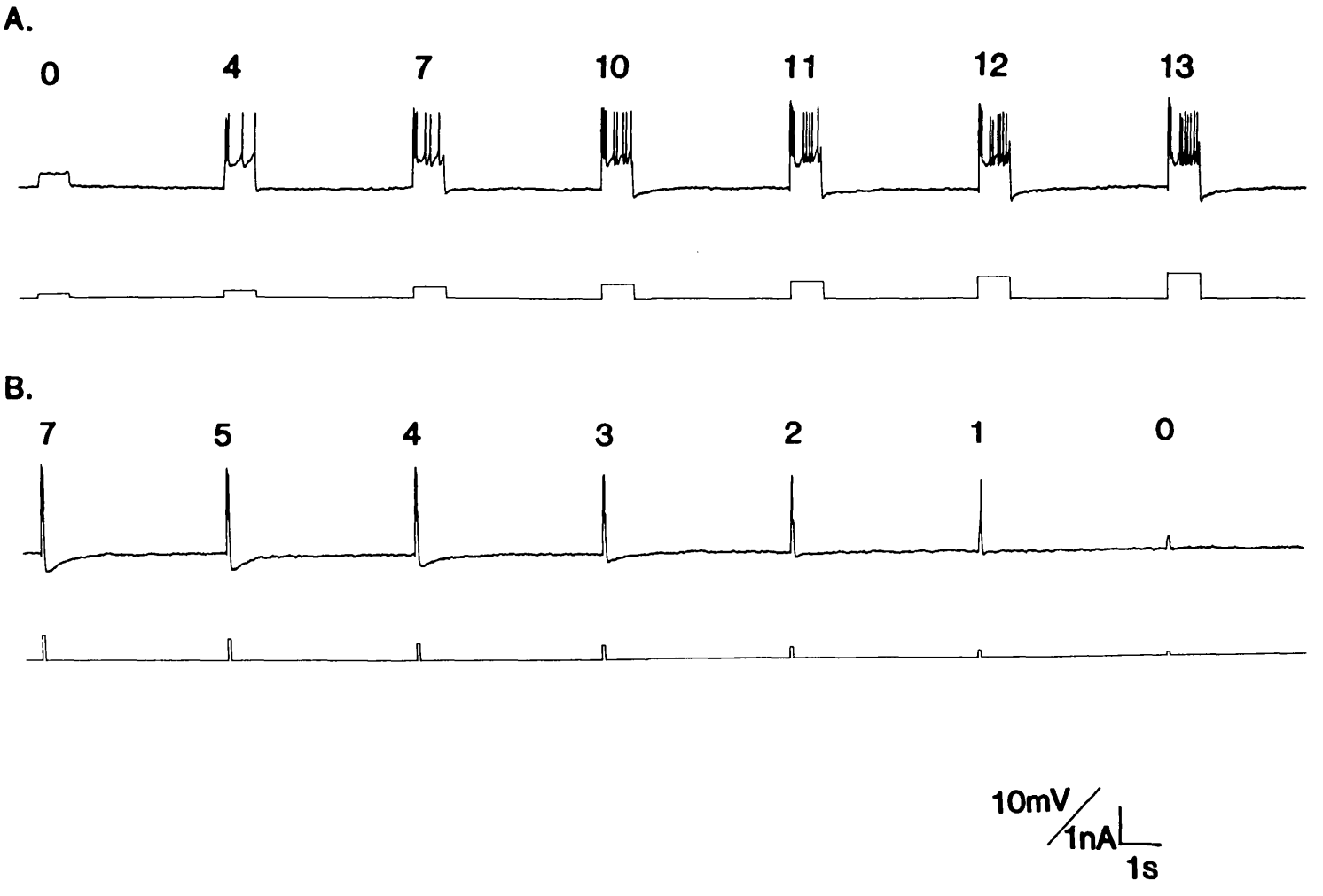

A.
0
4
7
10
11
12
13
B.
7
5
4
3
2
1
0
10mV
1nA
1s

Fig.47

Fig.47

Accommodation responses of a CA1 pyramidal neurone at 10MPa pressure. Note how accommodation is reduced during a spontaneous depolarizing membrane potential swing.

Temperature = 37°C.

Current pulses were 0.1-0.7nA/800ms.

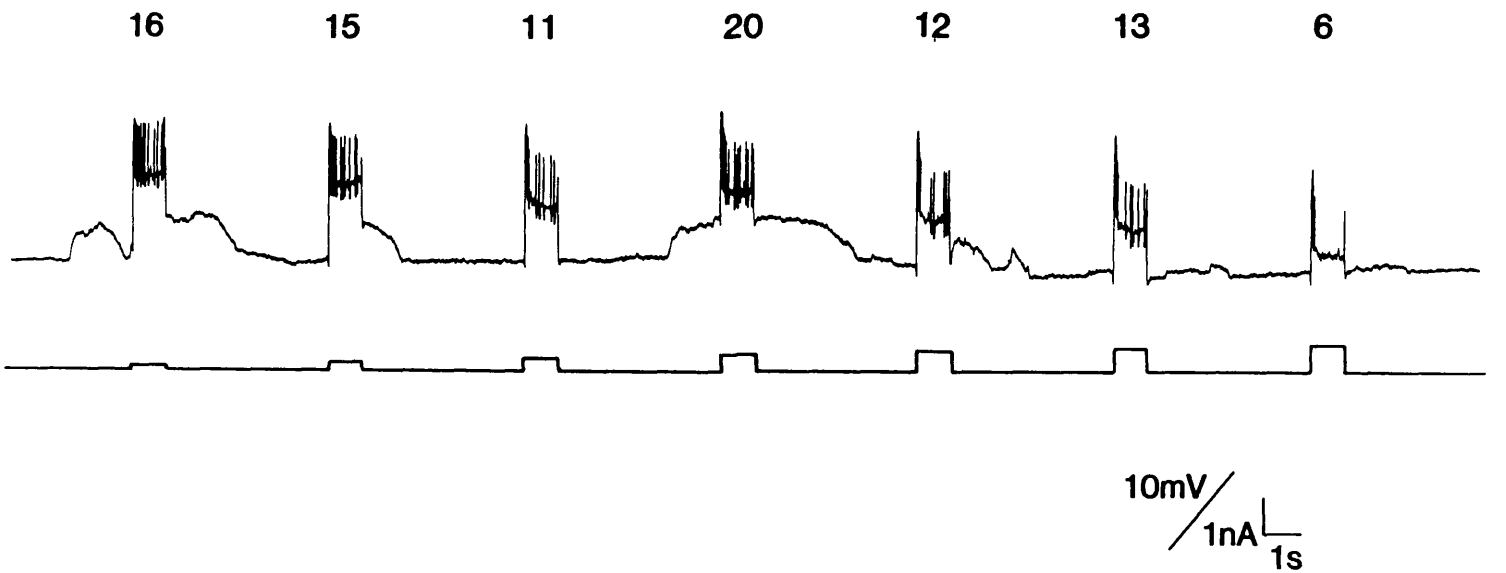

16
15
11
20
12
13
6
10mV
1nA
1s

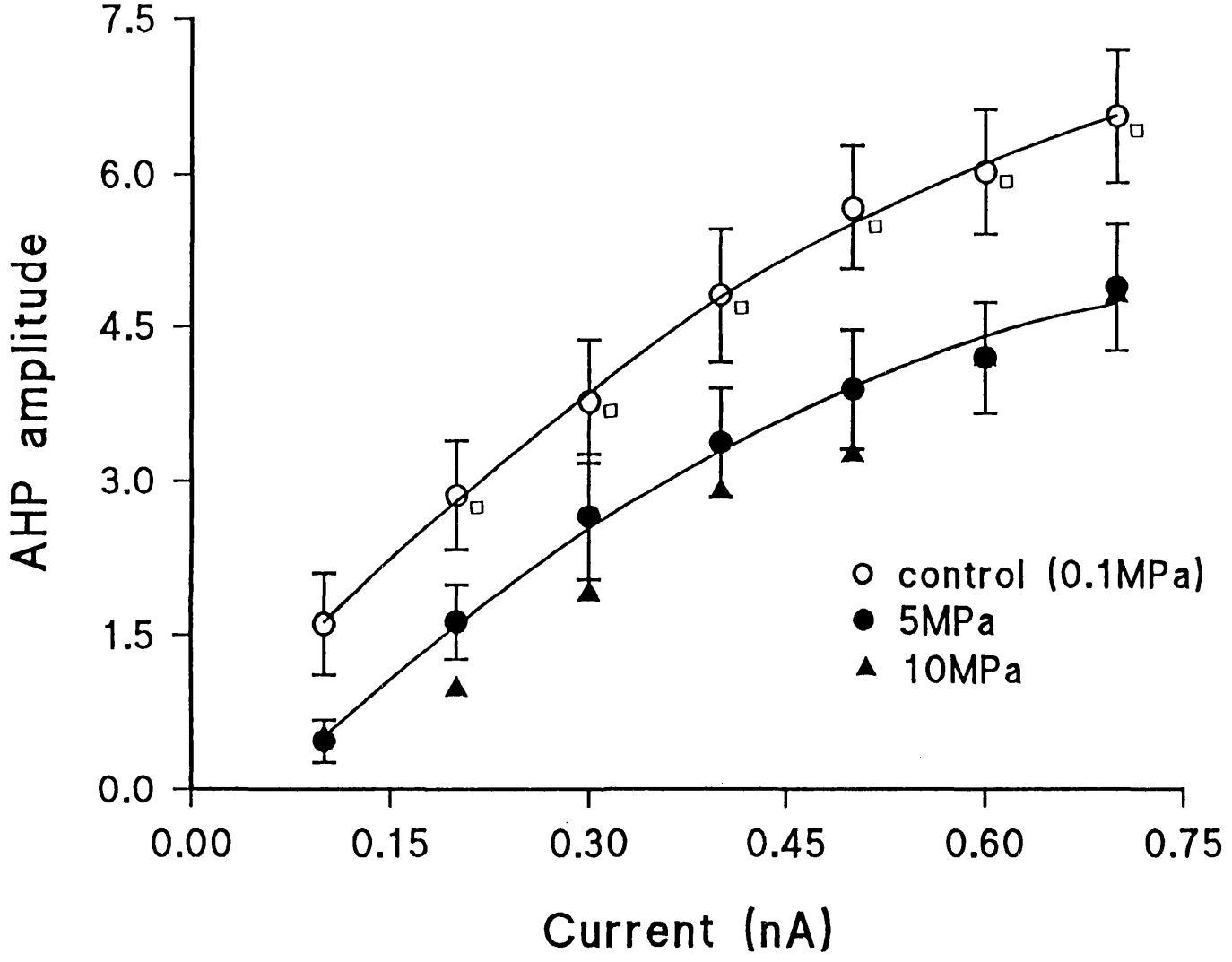
Fig.48

Fig.48

Graph showing effect of pressure on AHP amplitude derived from individual cells at 0.1MPa (n=11), 5MPa (n=21) and 10MPa (n=12). Data points show mean AHP amplitude (in mV) in response to 80ms depolarizing current pulses (0.1-0.7nA) passed through the intracellular electrode [bars, ± SEM]. For clarity no error bars are shown for the 10MPa data points.

□ denotes significantly different from control ($p<0.05$).

Temperature = 37°C.


7.5
6.0
4.5
3.0
1.5
0.0
AHP amplitude
0.00
0.15
0.30
0.45
0.60
0.75
Current (nA)
control (0.1MPa)
5MPa
10MPa

PART II DISCUSSION

This study was undertaken with the aim of uncovering and hopefully explaining neuronal excitability changes under high pressures of helium. From the results the main changes in excitability observed at pressure were:-

I.An increase in both orthodromic and antidromic excitability at pressures above 5MPa, as assessed by field potential experiments.

II.The induction of multiple population spikes in both orthodromic and antidromic field potentials at pressures above 5MPa.

III.A reduction of the accommodative ability of CA1 neurones above 5MPa.

IV.Depolarizing swings of resting potential and spontaneous firing above 5MPa, both during and after compression to 10MPa.

Before the above observations are considered the experimental apparatus deserves comment. It was considered of vital importance to verify that stable synaptic field potential responses could be recorded within the pressure chamber over a period of time corresponding to a pressure experiment. Although the electrophysiological recording equipment used was entirely conventional the lack of any facility to negate external vibrations may have made the recording apparatus susceptible to mechanical disturbance at any stage during an experiment thereby introducing artefacted responses. For example, the peristaltic pump initially caused major vibration problems with accompanying unstable responses until it was suspended in position by means of small pieces of elastic. The subsequent control experiments (Fig.34A,B,C,D) show that any variations that did occur over time at 0.1MPa, were of a magnitude experienced during a conventional experiment and confirmed the ability of the apparatus within the chamber to reliably record stable potentials.

It was initially hoped that the field potential recordings at high pressure would exhibit some repeatable enhancement of excitability that could be attributed to the applied pressure, which may then have been used as the basis of a study evaluating anaesthetic interactions at elevated pressure. The only comparable study to that which is presented here is, as mentioned earlier, that of Fagni and co-workers (see Introduction). A consistent feature of the results of their experiments has been depression of both orthodromic and antidromic field potential responses (Fagni, Zinebi & Hugon, 1987; Zinebi, Fagni & Hugon, 1988). Up to an 80% decrease (15-80% range, n=14) in the amplitude of antidromic potentials was found at a pressure of 9MPa in their study. This contrasts with the results presented here. Slight depression was noted in some preparations during the compression to 5MPa, but the main feature of the experiments to 10 and 13.5MPa were the increase in the amplitude of the response throughout the range of voltages tested. Hyperexcitability in two

slices was evidenced by the presence of a second population spike. The increase in population spike amplitude is probably not due to decreases in the steady state resting membrane potential of the neurones at elevated pressure, as the intracellular measurements do not suggest any great effect of pressure upon the resting potential (Table 4.0). Possibly increased antidromic invasion of the somato-dendritic region of the neurones could account for the tendency of antidromic spikes to increase in amplitude. Increased extracellular electrical interactions could be responsible for the observed increase. This could be due to an increase in the impedance of the extracellular space or an increase in the conductance of the apical dendrites. A somatic conductance decrease seems unlikely as the intracellular experiments indicated no change in input resistance (Fig.44). The finding that no significant change in afferent fibre input volley occurs at pressure (Fagni, Zinebi & Hugon, 1987) could suggest that the excitability of CA1 neurones axons remains unaffected. Obviously no conclusions may be drawn on this issue as complementary experiments were not performed in this study.

The recruitment of a second antidromic spike during two experiments certainly deserves comment. The antidromic field potential response is the most stable and robust measurement obtainable from hippocampal slices. If a maximal response is stable over a control period of twenty minutes, significant deviations from this response seldom occur even for extended periods of time (over 2 hours). Recruitment of a second antidromic population spike in a preparation, in the absence of a "convulsant" agent, such as 4-aminopyridine, very rarely occurs. Obviously the application of pressure enhanced the excitability of the populations of neurones in the experiments presented here.

The only evidence of significant synaptic depression seen in the field potential experiments was during the early stages of compression. One might have expected to find quite the opposite effect, namely significant depression of synaptic activity during the latter stages of compression (>3MPa). This view is based upon the wealth of data accumulated suggesting that pressure is a powerful synaptic depressant (see Introduction for references), the main insult being that of inhibition of neurotransmitter release (Campenot, 1975; Wann, Macdonald & Harper, 1979; Parmentier, Shrivastav & Bennet, 1981; Colton & Colton, 1982). The results show that the EPSP amplitude was not significantly altered during the early stages of compression (≤0.4MPa)(Fig.35A) yet the spike response became diminished. This might suggest that the initial pressurization procedure disrupts the coupling between dendritic depolarization and action potential induction in the soma. Here reduction of the extracellular space or disruptions to ionic conductances could again account for the observed action of pressure. Recovery of the spike response as pressurization progressed was

evident and suggests that the process responsible for the diminished response is sensitive to the rapid increase in pressure during the early phase of compression.

No other consistent evidence of significant synaptic depression was found. Near threshold responses became extremely variable although a trend towards increased amplitude is uncovered in the pooled results. The near threshold responses are comparable in amplitude to the published data (Fagni, Zinebi & Hugon, 1987) yet exhibit (apart from the early depression) no great trend towards depression even at the higher pressure (10MPa). Also the intermediate and maximal responses exhibited no tendency toward depression at 10MPa and preparations (4 of 7) became more excitable in terms of an amplitude increase. This perhaps seems a more reasonable effect in the CNS than a "blanket" depressant effect at excitatory synapses. Presumably significant CNS synaptic depression would manifest itself as a behaviourial depressant action rather than the well documented in vivo hyperexcitability and associated disrhythmias. Hyperexcitability was noted in three preparations, these preparations exhibited recruitment of a second population spike at pressures above 5MPa. It is possible that the post synaptic increase in excitability mentioned earlier contributed towards this effect.

The paired pulse experiment was considered important because it enables one to highlight use-dependent changes in synaptic transmission which remain hidden in the single pulse experiments. The summation of excitatory events occurring close together is thought to be due to presynaptic factors (Richards, 1972) and if pressure were to interfere with the process of neurotransmitter mobilization it might be expected that potentiation be affected. In the intact brain synaptic events will occur at much higher frequency than the events in the single stimulus in vitro work presented here (and reviewed in the Introduction), therefore the paired pulse experiment gives us a closer approximation of how excitatory synaptic events are disrupted at pressure in the whole animal (without the complicating factor of inducing long term potentiation). No statistically significant effect upon paired pulse potentiation was noted for the hippocampal slice preparation. A trend towards initial slight reduction of potentiation during pressurization to 10MPa was evident, this could reflect perturbation of mobility and subsequent release of neurotransmitter during repetitive inputs which may become more prominent during long trains of stimuli. From the results though, paired pulse potentiation at the Schaffer collateral-CA1 synapse is not significantly impaired. This contrasts with in vitro work at the crustacean neuromuscular junction. Here pressure has been shown to enhance the synaptic integrative properties of facilitation and tetanic potentiation (Grossman & Kendig, 1988; Grossman & Golan, 1990). This enhancement antagonized the

depressant effect on singly evoked responses.

It was hoped that by comparing the rate of rise of the EPSP-population spike relationships it would be possible to gain an insight into how the input-output relationship of field potentials is altered (if at all) by pressure. Fagni, Zinebi & Hugon (1987) reported a facilitation of transfer from EPSP to population spike at pressure (≈9MPa) and supported the view that the neuronal membrane is pressure sensitive, particularly the conductances involved in EPSP spike and generation. No consistent change in input-output relationship was uncovered in this study, responses being quite variable. With such variable results one obviously cannot confidently state that pressure has any consistent effect on the coupling between EPSP and population spike.

The intracellular measurements made at high pressure were performed primarily to investigate whether pressure modifies particular properties of CA1 pyramidal neurones that are governed by ion channels. As mentioned earlier potassium channels were thought to be likely candidates and thus the resting and accommodation behaviour of neurones was investigated. Unfortunately the pressurization procedure did not allow continuous recordings to be made from individual neurones between 0.1MPa and 5MPA. The intracellular electrode invariably became dislodged during the early stages (0.5 to 2MPa) of compression making this approach untenable. Therefore the analysis of groups of cells at differing pressures became necessary (with the exception of the four neurones compressed from 5MPa to 10MPa).

When one considers the pooled data (Table 4.0) pressure (up to 10MPa) does not appear to alter the processes associated with maintaining the steady state resting potential of pyramidal neurones at maintained pressures of 5 and 10MPa. Very little variation exists between populations even when factors such as time of impalement are taken into consideration. However during compression of neurones from 5MPa to 10MPa large swings of membrane potential often accompanied by action potentials were observed. Swings such as these occur extremely infrequently in pyramidal neurones at atmospheric pressure and lead one to the conclusion that the pressure step was responsible for this excitatory event. Earlier studies with squid giant axon (Spyropolous, 1957a), Helix neurones (Wann, Macdonald & Harper, 1979) and crayfish nerve fibres (Kendig, Schneider & Cohen, 1978) have reported the appearance of spontaneous firing at pressure (as low as 3.5MPa for crayfish). The origin of such activity is unclear at present but such excitatory events occurring in vivo could profoundly alter the behaviour of an animal.

No detectable changes in input resistance or somatic threshold potential

occurred in neurones at 5 or 10MPa although appreciable widening of the action potential occurred. This again has been observed in various other preparations for example amphibian nerve (Spyropolous, 1957b), squid giant axon (Spyropolous, 1957a, Henderson & Gilbert, 1975; Wann, Macdonald, Harper & Wilcock, 1979) mammalian preganglionic sympathetic nerves (Kendig, Trudell & Cohen, 1975) and molluscan neurones (Wann, Macdonald & Harper, 1979). The lengthening of the action potential has been attributed to a decrease in both the peak depolarization and repolarization rates of some preparations (eg. Wann, Macdonald & Harper, 1979; see also Wann & MacDonald, 1988). One might suggest that some decrease in peak rate of de- and/or repolarization occurs in the pyramidal cells at pressure, although in the absence of the appropriate measurements one cannot state this conclusively. It would be interesting to pursue this point further in order to clarify the action of pressure upon peak de- and repolarization and to ascertain the relative contribution towards the change in duration. Prolongation of the action potential at pressure may help counterbalance any depressant effect upon exocytosis thus helping to maintain synaptic transmission.

One very striking effect of pressure upon the hippocampal neurones was the decrease in accommodation observed at 5MPa and 10MPa. The increase in spike number with associated decrease in AHP at pressure suggests that the potassium channels underlying I_M and I_{AHP} had their function compromised by the pressurization procedure. As with the accommodation experiments using inhalation anaesthetics reduction of the normally pronounced "sag" in the voltage trace occurred suggesting interference with I_M. The pressure range over which the accommodation disturbances occur deserves comment. Motor disturbances begin to occur in the rat at pressures above 3MPa. Therefore the reduction of accommodation observed at 5MPa may contribute towards motor disturbances induced by pressure. Unfortunately a systematic study of the pressure of onset of accommodation reduction was not possible in the time available, making it difficult to correlate exactly the onset of accommodation disturbance with onset of motor disturbance. The reduction of accommodation associated reduction in AHP amplitude cannot conclusively be attributed to high pressure action upon potassium channels although the idea remains attractive. As discussed previously (see Discussion I) accommodation may be reduced by a variety of neurotransmitters and the chance that pressure induces release of one or more of these neurotransmitters remains a possibility (although this may be unlikely in view of the documented pressure induced depression of exocytosis [see General Introduction]).

The aim of the series of experiments in which the actions of ketamine and methohexitone were tested on orthodromic field potential responses at 0.1MPa

and 10MPa was to investigate whether any effect of the agents upon excitability at pressure could help explain their in vivo effects of aggravating or ameliorating the HPNS (see General Introduction). Also by comparing the effect of the agents at atmospheric and then at high pressure it was hoped that the data may have yielded clues to help explain the pressure reversal of anaesthesia.

20μmol.litre^{-1} ketamine was chosen as the test concentration at pressure because it is close to that found to be effective for amelioration of the HPNS in the rat (Bridget Wardley-Smith, personal communication). Also it is close to the effective blood plasma concentration found during anaesthesia in the rat (Marietta, Way, Castagnoli & Trevor, 1977). At 0.1MPa ketamine was not found to be particularly depressant at the synapse until quite high concentrations had been reached (>200μmol.litre^{-1}) (Fig.38A). Lower concentrations (20μmol.litre^{-1}) induced no consistent change in excitability of orthodromic field potential responses. Oshima & Richards (1988) reported dose dependent depression of field potential responses in the dentate gyrus by ≥50μmol.litre^{-1} ketamine which would appear to agree with the data presented in Fig.38A. Excitatory synaptic depression would not then seem to make a major contribution towards the anaesthetic effect of ketamine (unless of course synapses of discrete brain structures express greater sensitivity). Paired pulse potentiation (Fig.38B) was also not significantly altered by 20 or 200μmol.litre^{-1} ketamine suggesting that synaptic integrative properties are not affected. At pressure (10MPa), the action of ketamine (Figs.40A&B) did not appear to differ from its action at 0.1MPa. Overall 20μmol.litre^{-1} ketamine did not significantly modify either the response to single or paired stimuli at 10MPa shedding very little light upon the mode of action of the agent upon ameliorating the HPNS. From this data in the hippocampal slice one cannot really draw any conclusions as to the point of interaction by ketamine during the development of the HPNS. Also the results from the intracellular study highlighted only excitatory effects of the agent, which one would not expect to delay the onset of the excitatory phases of the HPNS. With regard to the pressure reversal of anaesthesia, as no events which might equate with "anaesthesia" in an in vitro preparation occurred at relevant concentration (for example, synaptic depression), it is not possible to address the mechanism of this event from the data obtained for ketamine.

At 0.1MPa, 50μmol.litre^{-1} methohexitone was found to be depressant at the excitatory synapse (Fig.39A) with a more profound effect at lower stimulus intensities. This depressant effect has been observed for a range of barbiturates (see Part I Discussion). At 10MPa methohexitone depressed field potential responses to an extent comparable with the 0.1MPa data. There was no evidence of any excitant action in the field potential experiments. In fact paired pulse potentiation was markedly depressed both at 0.1MPa and 10MPa

by methohexitone, with no discernable difference between the action at either pressure. The disturbance of paired pulse potentiation by methohexitone is difficult to rationalize. The hyperpolarizing IPSP was not markedly enhanced by methohexitone in the intracellular experiments and the late depolarizing phase occurs over too long a time course to be relevant to the paired stimuli experiment. There is obviously some inhibitory process at work decreasing the ability of the synapse to transmit excitatory events occurring close together. Possibilities include presynaptic depression of excitatory amino acid release and an effect upon calcium influx or mobilization.

With reference to the excitant action of methohexitone during pressurization of whole animals, the field potential data does not highlight anything that may explain how methohexitone facilitates the excitatory events of the HPNS. The intracellular experiments performed in the presence of methohexitone in the preceding section revealed a large enhancement of feed-forward inhibition that manifests itself as a depolarizing wave following orthodromic stimulation. It is possible that this effect, when combined with the excitant property of pressurization, is responsible for the observed enhancement of pressure symptoms.

The results provided no evidence of pressure reversal of the depressant effect of methohexitone at the synapse. Therefore it is not possible to comment upon possible mechanisms of pressure reversal of anaesthesia.

GENERAL CONCLUSIONS

The data presented in this thesis illustrates that mammalian central neurones are sensitive to both general anaesthetics and high pressure in vitro.

It is evident that general anaesthetics modify a number of neuronal properties (eg. induce hyperpolarization, modify accommodation) and that different agents evoke different responses. The anaesthetic-induced reduction of the accommodative properties of neurones, which persisted in the presence of various neurotransmitter antagonists, and the reduction of the M-current makes anaesthetic-potassium channel interaction a possibility worthy of further in depth study.

It is unlikely that the interference with the accommodative ability of CA1 neurones is soley responsible for the "convulsant" effects of some anaesthetics. It may however serve to sensitize certain neurones to additional excitatory stimuli which presumably occur in the presence of the "convulsant" agents.

Considering the lack of consistent neuronal responses to anaesthetics it is difficult to comment regarding the extent of the contribution of the observed in vitro modifications towards the production of anaesthesia in vivo. When the profile of an agent such as ketamine, which at a concentration relevant to anaesthesia had predominantly excitatory actions, is compared to an agent like methohexitone, the relative importance of any observed in vitro depressant effects towards generating an anaesthetized state in vivo becomes questionable. The induction of anaesthesia may therefore be dependent upon the disturbance of a number of neuronal properties which could, but not exclusively, include the observed hyperpolarization, synaptic depression and slowing of conduction velocity and the net anaesthetizing effect arising via the agent interfereing with these properties in a number of combinations. The experimental evidence does not support the hypothesis of a single neuronal target for anesthetic molecules.

Turning to the high pressure data, the results show that high pressure, over a range relevant to the HPNS, may produce excitatory effects in CA1 pyramidal neurones in vitro. The induction of a second population spike in both orthodromic and antidromic field potentials was an encouraging indication of high pressure increasing the excitability of a population of neurones. Although it did not occur in all preparations (this could have reflected the well documented variation in sensitivity to pressure between individuals) the pharmacological basis of the enhanced responses deserves further investigation.

The reduction of accommodative ability of CA1 neurones at pressures of 5MPa and above was particularly interesting. Direct action of pressure at the level of neuronal membrane potassium channels (likely candidates being SK- and M-channels) cannot be ruled out, although before a firm statement can be made indirect action on neurotransmitter release or Ca^{2+} flux has to be investigated.

Should the excitatory events recorded above 5MPa in vitro occur at pressure in vivo there are clear implications for disturbance to neuronal signalling contributing towards the induction and maintenance of the HPNS.

It is not possible at this stage to comment extensively on how high pressure and general anaesthetics interact to either alleviate or accelerate the HPNS. The addition of anaesthetic agents whilst holding at 10MPa failed to provide evidence of any interaction that could explain the in vivo observations. The large after-depolarization following orthodromic responses in the presence of methohexitone at 0.1MPa may be responsible for the facilitation of excitatory events during pressurization.

Suggested further experiments include:-

I.Further analysis of anaesthetic-induced modification of K^+ channel function. Detailed voltage-clamp investigation of M- and AHP- currents in the presence of both inhalation and intravenous agents is required.

II.Determine the lowest effective anaesthetic concentration for reducing accommodation. This may highlight differences in potency between the anaesthetic agents.

III.Investigate the mechanism of anaesthetic-induced hyperpolarization.

IV.Impale neurones at 0.1MPa and hold during compression to 10MPa. Determine the pressure of onset of accommodation disturbance and relate to published in vivo observations.

V.Ascertain the origin of the spontaneous depolarizations observed at pressure.

VI.An intracellular exploration of anaesthetic alleviation/aggravation of the HPNS. Anaesthetic agents should be added during pressurization for the purpose of this study.

REFERENCES

Adams, P.R. (1976). Drug blockade of open end-plate channels. J. Physiol., 260, 531-552.

Adams, P.R., Brown, D.A. and Constanti, A. (1982). Pharmacological inhibition of the M-current. J. Physiol., 332, 223-262.

Adams, P.R. & Halliwell, J.V. (1982). A hyperpolarization-induced inward current in hippocampal pyramidal cells. J. Physiol., 324, 62-63P.

Akeson, M.A. & Deamer, D.W. (1989). Steady-state catecholamine distribution in chromaffin granules. Biochemistry, 28, 5120-5127.

Alger, B.E. & Nicoll, R.A. (1980). Epileptiform burst after hyperpolarizations: A calcium-dependent potassium potential in hippocampal pyramidal CA1 cells. Science, 210, 1122-1124.

Alger, B.E. & Nicoll, R.A. (1982). Feed forward dendritic inhibition in rat hippocampal pyramidal cells studied in vitro. J. Physiol., 328, 105-123.

Andersen, P., Dingledine, R., Gjerstad, L., Langmoen, I.A. & Laursen, A.M. (1980). Two different responses of hippocampal pyramidal cells to application of gamma-amino butyric acid. J. Physiol., 305, 279-296.

Andrade, R., Malenka, R.C. & Nicoll, R.A. (1986). A G-protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. Science, 234, 1261-1265.

Andrade, R. & Nicoll, R.A. (1987). Pharmacologically distinct actions of serotonin on single pyramidal neurones of the rat hippocampus recorded in vitro. J. Physiol., 394, 99-124.

Anis, N.A., Berry, S.C., Burton, N.R. & Lodge, D. (1983). The dissociative anaesthetics, ketamine & phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br. J. Pharmac., 79, 565-575.

Armstrong, C.M. & Binstock, L. (1964). The effects of several alcohols on the properties of the squid giant axon. J. Gen. Physiol., 48, 265-277.

Ashford, M.L.J., MacDonald, A.G. & Wann, K.T. (1982a). The effects of high hydrostatic pressure on the spontaneous release of transmitter at the frog neuromuscular junction. J. Physiol., 333, 531-543.

Ashford, M.L.J., MacDonald, A.G. & Wann, K.T. (1982b). Hydrostatic pressure and octanol on the frog neuromuscular junction: Actions and interactions. Proceedings of the Eighth Annual Congress of European Undersea Biomedical Society. Report of the Proceedings. pp. 184-204. Lubeck-Travermunde.

Ashford, M.L.J., MacDonald, A.G. & Wann, K.T. (1984). Hydrostatic pressure modifies the action of octanol and atropine on frog endplate conductance. Br. J. Pharmac., 83, 477-484.

Azzaro, A.J. & Smith, D.J. (1977). The inhibitory action of ketamine HCl on ^{3}H-5-hydroxytryptamine accumulation by rat brain synaptasomal rich fractions: comparison with ^{3}H-catecholamine and ^{3}H-GABA uptake. Neuropharmacology, 16, 349-355.

Bailey, C.P., Green, C.J., Halsey, M.J. & Wardley-Smith, B. (1977). High pressure and intravenous steroid anaesthesia in rats. J. Appl. Physiol: Respirat. Environ. Exercise physiol., 43(2), 183-188.

Banna, N.R. (1969). Potentiation of cutaneous inhibition of alcohol. Experientia, 25, 619-621.

Baraban, J.M., Snyder, S.H. & Alger, B.E. (1985). Protein kinase C regulates ionic conductance in hippocampal pyramidal neurons: Electrophysiological effects of phorbol esters. Proc. Natl. Acad. Sci. USA., 82, 2538-2542.

Barker, J.L. & Gainer, H. (1973). Pentobarbital: Selective depression of excitatory postsynaptic potentials. Science, 182, 720-722.

Barker, J.L. & McBurney, R.N. (1979). Phenobarbitone modulation of postsynaptic GABA receptor function on cultured mammalian neurones. Proc. R. Soc. [Biol]., 206, 319-327.

Barker, J.L. & Ransom, B.R. (1978). Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. J. Physiol., 280, 355-372.

Baskys, A., Niesen, C.E. & Carlen, P.L. (1987). Altered modulatory actions of serotonin on dentate granule cells of aged rats. Brain Res., 419(1-2), 112-118.

el-Beheiry, H. & Puil, E. (1989). Anaesthetic depression of excitatory synaptic transmission in neocortex. Experimental Brain Res., 77, 87-93.

Beaver, R.W., Brauer, R.W. & Lahser, S. (1977). Interaction of central nervous system effects of high pressures with barbiturates. J. Appl. Physiol: Respirat. Environ. Exercise Physiol., 43(2), 221-229.

Benardo, L.S. & Prince, D.A. (1982). Dopamine modulates a Ca^{2+}-activated potassium conductance in mammalian hippocampal pyramidal cells. Nature, 297, 76-79.

Bennet, P.B. (1975). The high pressure nervous syndrome: Animals. In: The Physiology and Medicine of Diving and Compressed Air Work, pp. 248-263. Ed. P.B. Bennet & D.H. Elliot. Bailliere Tindall: London.

Berg-Johnsen, J. & Langmoen, I.A. (1986). The effect of isoflurane on unmyelinated and myelinated fibres in the rat brain. Acta Physiologica Scandanavica, 127, 87-93.

Berg-Johnsen, J. & Langmoen, I.A. (1987) Isoflurane hyperpolarizes neurones in rat and human cerebral cortex. Acta Physiologica Scandanavica, 130, 679-685.

Blaustein, M.P. (1968). Barbiturates block sodium and potassium conductance increases in voltage clamped lobster axons. J. Gen. Physiol., 51, 293-378.

Blaustein, M.P. & Ector, A.C. (1975). Barbiturate inhibition of calcium uptake by depolarised nerve terminals in vitro. Mol. Pharmacol., 11, 369-378.

Boggs. J.M., Yoong. T. & Hsia, J.C. (1976). Site and mechansim of anaesthetic action. II. Effect of anaesthetics and pressure on fluidity of spin labelled lipid vesicles. Mol. Pharmacol., 12, 127-135.

Boyd, N.D. & Cohen, J.B. (1984). Desensitisation of membrane bound Torpedo acetylcholine receptor by amine noncompetitive antagonist and aliphatic alcohols: studies of [^{3}H]acetylecholine binding and $^{22}Na^{+}$ ion fluxes. Biochemistry, 23(18), 4023-4033.

Brauer, R.W. (1975). The high pressure nervous syndrome: Animals. In: The physiology and medicine of diving and compressed air work, pp. 231-247. Ed. P.B. Bennet & D.H. Elliot. Bailliere Tindall: London.

Brauer, R.W. (1984). Hydrostatic pressure effects on the central nervous system: perspectives and outlook. Phil. Trans. R. Soc. Lond., B. 304, 17-30.

Brauer, R.W., Goldman, S.M., Beaver, R.W. & Sheehan, M.E. (1974). N_2, H_2 and N_2O antagonism of high pressure neurological syndrome in mice. Undersea Biomed. Res., 1(1), 59-72.

Brauer, R.W., Mansfield, W.M., Beaver, R.W. & Gillen, H.W. (1979). Stages in development of high pressure neurological syndrome in the mouse. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol., 46(4), 756-765.

Brett, R.S. & Lancaster, B. (1985). Activation of potassium channels by rapid application of calcium to inside-out patches of cultured hippocampal neurons. Soc. Neurocsi. Abstr., 10, 241.

Brown, D.A. (1988). M-currents: an update. Trends. Neurosci., 11(7), 294-299.

Brown, D.A. & Adams, P.R. (1980). Muscarinic suppression of a novel voltage-sensitive K^+-current in a vertebrate neurone. Nature, 283, 673-676.

Brown, D.A. & Constanti, A. (1978). Interaction of pentobarbitone and gamma aminobutyric acid on mammalian sympathetic ganglion cells. Br. J. Pharmacol., 63, 217-224.

Brown, D.A. & Griffith, W.H. (1983). Persistent slow inward calcium current in voltage-clamped hippocampal neurones of the guinea-pig. J. Physiol., 337, 303-320.

Brown, D.A. & Halliwell, J.V. (1981). Diencephalic IPN preparations. In: Electrophysiology of isolated mammalian CNS preparations, pp283-306. Ed. Kerkut, G.A. & Wheal, H.V. Academic Press: London.

Campenot, R.B. (1975). The effects of high hydrostatic pressure on transmission at the crustacean neuromuscular junction. Comp. Biochem. Physiol., 52B, 133-140.

Carlen, P.L., Gurevich, N. & Durand, D. (1982). Ethanol in low doses augments calcium mediated mechanisms intracellularly in hippocampal neurones. Science, 215, 306-309.

Castle, N.A., Haylett, D.G. & Jenkinson, D.H. (1989). Toxins in the classification of potassium channels. Trends. Neurosci., 12, 59-65.

Catchlove , R.F.H., Krnjevic, K. & Maretic, Z. (1972). Similarity between effects of general anaesthetics and dinitrophenol on cortical neurones. J. Physiol. Pharmacol., 50, 1111-1114.

Chalazonitis, N. (1967). Selective action of volatile anaesthetics on synaptic transmission and autorhythmicity in single identifiable neurones. Anesthesiology, 28, 111-123.

Chaput, Y., Areneda, R.C. & Andrade, K.C. (1989). Blockade of an atypical central 5-HT receptor by BRL 24924 in the rat hippocampus. Soc. Neurosci. Abs., 15, 93.10.

Chaput, Y., Areneda, R.C. & Andrade, R. (1990). Pharmocological and functional analysis of a novel serotonin receptor in the rat hippocampus. Euro. J. Pharmacol., 182, 441-450.

Cherkin, A. & Catchpool, J.F. (1964). Temperature dependence of anaesthesia in goldfish. Science, 144, 1460-1462.

Cole, A.E. & Nicoll, R.A. (1983). Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. Science, 221, 1299-1301.

Colino, A. & Halliwell, J.V. (1987). Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. Nature, 328, 73-77.

Colton, J.S. & Colton, C.A. (1982). An electrophysiological analysis of oxygen and pressure on synaptic transmission. Brain Res., 251, 221-227.

Cook, N.S. (1988). The pharmacology of K^+ channels and their therapeutic potential. Trends. Pharmacol., 9, 21-28.

Connor, J.A. & Stevens, C.F. (1971). Voltage-clamp studies of a transient outward membrane current in gastropod neural somata. J. Physiol., 213, 21-30.

Conti, F., Fioravanti, R., Segal, J.R. & Stühmer, W. (1982a). Pressure dependence of the potassium currents of squid giant axon. J. Membrane Biol., 69, 35-40.

Conti, F., Fioravanti, R., Segal, J.R. & Stühmer, W. (1982b). Pressure dependence of the sodium currents of squid giant axon. J. Membrane Biol., 69, 23-34.

Craig, N.C., Bryant, G.J. & Levin, I.W. (1987) Effects of halothane on dipalmitoylphosphatidylcholine liposomes: A Raman Spectroscopic Study. Biochemistry 26, 2449-2458.

Crawford, J.M. (1970). Anaesthetic agents and the chemical sensitivity of cortical neurones. Neuropharmacology, 9, 31-46.

Crawford, J.M. & Curtis, D.R. (1966). Pharmacological studies on feline Betz cells. J. Physiol., 186, 121-138.

Cutler, R.W.P., Markowitz, D. & Dudzinski, D. S. (1974). The effect of barbiturates on [^{3}H] GABA transport in rat cerebral cortex slices. Brain Res., 81, 189-197.

Doerner D. & Alger B.E. (1988) Cyclic GMP depresses hippocampal calcium current through a mechanism independent of cyclic GMP-dependent protein kinase. Neuron, 1(8), 693-699.

Dutar, P. & Nicoll, R.A. (1988). Classification of muscarinic responses in hippocampus in terms of receptor subtypes and second-messenger systems: Electrophysiological studies in vitro. J. Neurosci., 8, 4214-4224.

Dundas, C.R. (1979). Alphaxalone/alphadolone in diving chamber anaesthesia. Lancet, 1, 378.

Eccles, J.C., Schmidt, R.F. & Willis, W.D. (1963). Pharmacological studies on presynaptic inhibition. J. Physiol., 168, 500-530.

Elliot, J.R. & Haydon, D.A. (1989). The actions of neutral anaesthetics on ion conductances of nerve membranes. Biochem. et Biophys. Acta., 988, 257-286.

Elrod, S.V. & Leslie, S.W. (1980). Acute and chronic effects of barbiturates on depolarization-induced calcium influx into synaptosomes from rat brain regions. J. Pharmac. Exp. Ther., 212, 131-136.

Evans, R.H. & Hill, R.G. (1978). GABA-mimetic action of etomidate. Experientia, 34, 1325-1327.

Fagni, L., Hugon, M., Folco, A. & Imbert, G. (1987). A versatile chamber for microphysiological studies with gas mixtures under high pressure. Undersea Biomed. Res., 14(2), 161-168.

Fagni, L., Soumirel-Mourat, B., Cartier, E. & Hugon, M. (1985). A study of spontaneous and evoked activity in the rat hippocampus under helium-oxygen high pressure. Electroenceph. & Clin. Neurol., 60, 267-275.

Fagni, L., Weiss, M., Pellet, J. & Hugon, M. (1982). The possible mechanisms of high pressure induced motor disturbances in the cat. Electroenceph. Clin. Neurophysiol., 53, 590-601.

Fagni, L., Zinebi, F. & Hugon, M. (1987a). Evoked potential changes in rat hippocampal slices under helium pressure. Exp. Brain Res., 65, 513-519.

Finkel, A.S. & Redman, S.J. (1985) Optimal voltage-clamping with a single microelectrode. In: Voltage-clamping with microelectrodes. Ed. T.G. Smith et al. Williams & Watkins: Baltimore.

Firestone, L.L., Sauter, J.F., Braswell, L.M. & Miller, K.W. (1986). Actions of general anaesthetics on acetylcholine receptor-rich membranes from Torpedo california. Anesthesiology, 64, 694-702.

Franciolini, F. (1988). Calcium and voltage dependence of single Ca^{2+}-activated K^+ channels from cultured hippocampal neurones of rat. Biochem. Biophys. Acta., 943, 419-427.

Franks, N.P. & Lieb, W.R. (1988). Volatile general anaesthetics activate a novel neuronal potassium current. Nature, 333, 662-664.

Fujiwara, N., Higashi, H., Nishi, S., Shimoji, K., Sugita, S. & Yoshimura, M. (1988). Changes in spontaneous firing patterns of rat hippocampal neurones induced by volatile anaesthetics. J. Physiol., 402, 155-175.

Gage, P.W. & McKinnon, D. (1985). Effects of pentobarbitone on acetylcholine-activated channels in mammalian muscle. Br. J. Pharm., 85, 229-235.

Gage, P.W. & Robertson, B. (1985). Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. Br. J. Pharm., 85, 675-681.

Gahwiler, B.H. & Brown, D.A. (1985). $GABA_B$-receptor-activated K^+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. Proc. Natl. Acad. Sci. USA., 82, 1558-1562.

Galla, H.J. & Trudell, J.R. (1980). Asymmetric antagonistic effects of an inhalational anesthetic and high pressure on the phase transition temperature of dipalmitoyl phosphatidic acid bilayers. Biochim. Biophys. Acta. 599, 336-340.

Gilman, S.C., Colton, J.S. & Dutka, A.J. (1987). Effect of pressure on the release of radioactive glycine and GABA from spinal cord synaptosomes. J. Neurochem., 49, 1571-1578.

Gilman, S.C., Colton, J.S. & Hallenbeck, J.M. (1986). Effects of pressure on ^{3}H-GABA release by synaptosomes isolated from cerebral cortex. J. Appl. Physiol., 61(6), 2067-2073.

Gothert, M. & Wendt, J. (1977). Inhibition of adrenal medullary catecholamine secretion by enflurane. I. Investigations in vitro. Anesthesiology, 46, 400-403.

Gothert, M., Dorn, W. & Loewenstein, I. (1976). Inhibition of catecholamine release from the adrenal medulla by halothane. Site and mechanism of action. Naunyn-Schmiederbergs Archiv fur expeimentellen Pathologie u. Pharmacologie, 294, 239-249.

Green, C.J., Halsey, M.J. & Wardley-Smith, B. (1977). Possible protection against some of the physiological effects of high pressure. J. Physiol., 267, 46P.

Green, R.W. & Haas, H.L. (1985). Adenosine actions on CA1 pyramidal neurones in rat hippocampal slices. J. Physiol., 366, 119-127.

Grossman, Y. & Golan, H. (1990). Synaptic transmission under hyperbaric conditions. Proceedings of the 2nd international meeting on high pressure biology. EASSM-CERB, Toulon, France, 19-22 August 1990.

Grossman, Y. & Kendig, J.J. (1988). Synaptic integrative properties at hyperbaric pressure. J. Neurophysiol, 52, 692-708.

Gustafsson, B., Galvan, M., Grafe, P. & Wigstrom, H. (1982). A transient outward current in a mammalian central neurone blocked by 4-aminopyridine. Nature, 299, 252-254.

Gustafsson, B. & Wigstrom, H. (1981). Evidence for two types of after hyperpolarization in CA1 pyramidal cells in the hippocampus. Brain Res., 206, 462-468.

Haas, H.L. (1985). Histamine may act through cyclic AMP on hippocampal neurones. Agents and Actions, 16, 234-235.

Haas, H.L & Greene, R.W. (1986). Effects of histamine on hippocampal pyramidal cells of the rat in vitro. Exp. Brain Res., 62, 123-130.

Haas, H.L. & Konnerth, A. (1983). Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. Nature, 302, 432-434.

Halliday, D.J.X., Little, H.J. & Paton, W.D.M. (1979). The effects of inert gases and other general anaesthetics on the release of acetylcholine from the guinea pig ileum. Br. J. Pharmac., 67, 229-237.

Halliwell, J.V. (1990). K^+ channels in the CNS. In: Potassium channels structure, classification, function and therapeutic potential. Ed. Cook, N.S. pp 348-381. Ellis Horwood, Ltd., Chichester.

Halliwell, J.V., Othman, I.B., Pelchen-Mathews, A. & Dolly, J.O. (1986). Central action of dendrotoxin: selective reduction of a transient K conductance in hippocampus and binding to localized acceptors. Proc. Natl. Acad. Sci. USA., 83, 493-497.

Halsey, M.J. (1982). Effects of high pressure on the central nervous system. Physiol. Rev., 62(4), 1341-1377.

Halsey, M.J. (1984). A reassessment of the molecular structure-functional relationships of the inhaled general anaesthetics. Br. J. Anaesth., 56(9S), 95-255.

Halsey, M.J., Wardley-Smith, B. & Green, C.J. (1978). Pressure reversal of general anaesthesia. A multi-site expansion hypothesis. Br. J. Anaesth., 50, 1091-1097.

Halsey, M.J., Wardley-Smith, B. & Wood, S. (1986). Pressure reversal of alphaxalone/alphadolone and methohexitone in tadpoles: evidence for different molecular sites for general anaesthesia. Br. J. Pharmac., 89, 299-305.

Harper, A.A., Macdonald, A.G. & Wann, K.T. (1981). The action of high hydrostatic pressure on the membrane currents of Helix neurones. J. Physiol., 311, 325-339.

Harper, A.A., Macdonald, A.G. & Wann, K.T. (1983). The effect of temperature on the nerve blocking action of benzyl alcohol on the squid giant axon. J. Physiol., 338, 51-60.

Harris, R.A. & Hood, W.F. (1980). Inhibition of synaptosomal calcium uptake by ethanol. J. Pharmac. exp. Ther., 213, 562-568.

Harvey, A.L. & Anderson, A.J. (1975). Dendrotoxins: snake toxins that block potassium channels and facilitate neurotransmitter release. Pharmac. Ther., 31, 33-35.

Haydon, D.A. & Urban, B.W. (1983a). The action of hydrocarbons and carbon tetrachloride on the sodium current of the squid giant axon. J. Physiol., 338, 435-450.

Haydon, D.A. & Urban, B.W. (1983b). The action of alcohols and other non-ionic surface active substances on the sodium current of the squid giant axon. J. Physiol., 341, 411-427.

Haydon, D.A. & Urban, B.W. (1983c). The effects of some inhalation anaesthetics on the sodium current of the squid giant axon. J. Physiol., 341, 429-439.

Haydon, D.A. & Urban, B.W. (1986). The actions of some general anaesthetics on the potassium current of the squid giant axon. J. Physiol. 338, 435-450.

Heidmann, T., Oswald. R.E. & Changeaux, J-P. (1983). Multiple sites of action for noncompetitive blockers on acetylcholine receptor rich membrane fragments from Torpedo marmorata. Biochemistry, 22, 3112-3127.

Heinemann, S.H., Conti, F., Stühmer, W. & Neher, E. (1987). Effects of hydrostatic pressure on membrane processes: sodium channels, calcium channels and exocytosis. J. Gen. Physiol. 90(6), 765-768.

Heinemann, S.H., Stühmer, W. & Conti, F. (1987). Single acetylcholine receptor channel currents recorded at high hydrostatic pressure. Proc. Natn. Acad. Sci., 84(10), 3229-3233.

Henderson, J.V. Jr. & Gilbert, D.L. (1975). Slowing of ionic currents in the voltage-clamped squid axon by helium pressure. Nature, 258, 351-352.

Hirota, K., Momosa, Y., Takeda, R., Nakanishi, S. & Ito, Y. (1986). Prolongation of the action potential and reduction of the delayed outward K^+ current by halothane in single frog atrial cells. European. J. Pharamacol., 126(3), 293-295.

Hirota, K., Ito, Y., Masuda, A. & Momose, Y. (1989). Effects of halothane on membrane currents in guinea pig atrial and ventricular myocytes. Acta. Anaesthesiol. Scand. 33(3), 239-244.

Holmes, J.C. & Schneider, F.H. (1973). Pentobarbitone inhibition of catecholamine secretion. Br. J. Pharmac., 49, 205-213.

Holtman, J.R. Jr. & Richter, J.A. (1983). Effects of convulsant barbiturates on endogenous acetylcholine release and sodium-dependent high affinity choline uptake. J. Pharmac. exp. Ther., 224(2), 398-403.

Hunter, W.L. & Bennett, P.B. (1974). The causes, mechanisms and prevention of the high pressure nervous syndrome. Undersea Biomed. Res., 1(1), 1-28.

Hotson, J.R. & Prince, D.A. (1980). A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. J. Neurophysiol., 43, 409-419.

Ikemoto, Y., Ono, K., Yoshida, A. & Akaike, N. (1989). Delayed activation of large-conductance Ca^{2+}-activated K^+ channels in hippocampal neurones of the rat. Biophys. J., 56, 207-212.

Iwasaki, M. (1989). Effects of pentobarbitone, ketamine and lignocaine on synaptic transmission in the rat olfactory cortex in vitro. Br. J. Anaesth., 63, 306-314.

Jacobson, I., Pocock, G. & Richards, C.D. (1989). Effects of pentobarbitone on current noise induced by nicotinic agonists in bovine adrenal medullary cells maintained in tissue culture. J. Physiol., 417, 84P.

Jessell, T.M. & Richards, C.D. (1977). Barbiturate potentiation of hippocampal IPSPs is not mediated by blockade of GABA uptake. J. Physiol., 269, 42P-44P.

Joas, T.A. & Eger, E.I. (1977). Electroencephalographic seizure activity in dogs diving anaestheia. Br. J. Anaesth., 43, 739-743.

Johnson, F.H., Brown, D.E.S. & Marsland, D.A. (1942). Pressure reversal of the actions of certain narcotics. J. Cell Comp. Physiol., 20, 269-276.

Johnson, F.H. & Flager, E.A. (1950). Hydrostatic pressure reversal of narcosis in tadpoles. Science, 112, 91-92.

Johansen, J., Yang, J., Zormumski, C.F. & Kleinhaus, A.C. (1989). Different actions of a short acting barbiturate on sodium and potassium conductances in invertebrate and vertebrate nerons. Neuropharmacology, 28(2), 153-160.

Jones, R.S.G. & Heinemann, U. (1988). Verapamil blocks the after-hyperpolarization but not the spike frequency adaptation of pyramidal cells in vitro. Brain Res., 462, 367-371.

Katz, B. (1949). Les constantes electriques de la membrane du muscle. Archs. Sci. Physiol., 3, 285-299.

Kendig, J.J. (1984). Ionic currents in vertebrate myelinated nerve at hyperbaric pressure. Am. J. Physiol., 246, (Cell Physiol. 15), C84-C90.

Kendig, J.J. & Cohen, E.N. (1976). Neuromuscular function at hyperbaric pressures: pressure-anaesthetic interactions. Am. J. Physiol., 230(5), 1244-1249.

Kendig, J.J., Grossman, Y. & MacIver, M.B. (1988). Pressure reversal of anaesthesia: a synaptic mechanism. Br. J. Anaesth., 60, 806-816.

Kendig, J.J., Schneider, T.M. & Cohen, E.N. (1978). Pressure, temperature, and repetitive impulse generation in crustacean axons. J. Appl. Physiol., 45, 742-746.

Kendig, J.J., Trudell, J.R. & Cohen, E.N. (1975). Effects of pressure and anaesthetics on conduction and synaptic transmission. J. Pharmac. Exp. Ther., 195, 216-224.

Knöpfel, T., Brown, D.A., Vranesic, I. & Gähwiler, B.H. (1990). Muscarinic and ß-adrenergic depression of the slow calcium-activated potassium conductance in hippocampal CA3 pyramidal cells is not mediated by a reduction of depolarisation-induced cytosolic calcium transients. Proc. Natnl. Acad. Sci., 87, 4083-4087.

Krnjević, K. (1972). Excitable membranes and anaesthetics. In: Cellular Biology and Toxicity of Anaesthetics. (B.R. Fink. Ed.) pp3-9. Williams and Wilkins, Baltimore.

Krnjević, K, Dalkara, T & Yin, C. (1988). Synchronization of pyramidal cell firing by ephaptic currents in hippocampus. In: Experimental Medicine and Biology Vol.203; Excitatory Amino Acids and Epilepsy. (R. Schwarz & Y. Ben-Ari. Eds.) pp413-420. Plenum Press, New York & London.

Lancaster, B. & Adams, P.R. (1986). Calcium-dependent current generating the after hyperpolarization of hippocampal neurons. J. Neurophysiol., 55, 1268-1282.

Lancaster, B. & Nicoll, R.A. (1987a). Properties of two calcium-activated hyperpolarizations in rat hippocampal neurones. J. Physiol., 389, 187-203.

Lancaster, B. & Nicoll, R.A. (1987b). Small conductance Ca^{2+} activated K^{+} channels in hippocampal neurones. Neurosci. Abstr., 13, 176.

Lancaster, B., Nicoll, R.A. & Perkell, D.J. (1991). Calcium activates two types of potassium channels in rat hippocampal cells in culture. J. Neurosci. 11(1), 23-30.

Larrabee, M.G. & Posternack, J.M. (1952) Selective actions of anaesthetics on synapses and axons in mammalian sympathetic ganglia. Journal of Neurophysiology, 15,92-114.

Lee, A.G. (1976). Model for the action of local anaesthetics. Nature, 262, 545-547.

Lever, M.J., Paton, W.D.M., Smith, R.A. & Smith, E.B. (1971). Pressure reversal of anaesthesia. Nature, 231, 368-371.

Lipton, P. & Whittingham, T.S. (1971). The effect of hypoxia on evoked potentials in the in vitro hippocampus. J. Physiol., 287, 427-438.

Little, H.J. (1982). The effects of ketamine and of high pressure on the responses to GABA of the rat superior cervical ganglion in vitro. Br. J. Pharmac., 77, 209-216.

Little, H.J. & Atkinson, H.D. (1984). Ketamine potentiates the responses of the rat superior cervical ganglion to GABA. Eur. J. Pharmac., 98, 53-59.

Lodge, D. & Anis, N.A. (1982). Effects of phencyclidine on excitatory amino acid activation of spinal interneurones in the cat. Euro. J Pharmac., 77, 203-204.

Lodge, D. & Anis, N.A. (1984). Effects of ketamine and three other anaesthetics on spinal reflexes and inhibitions in the cat. Br. J. Anaes., 56, 1143-1147.

Lodge, D., Anis. N.A. & Burton, N.R. (1982). Effects of optical isomers of ketamine on excitation of cat and rat spinal neurones by amino acids and acetylcholine. Neurosci. Let., 29, 281-286.

Løyning, Y., Oshima, T. & Yokota, T. (1964). Site of action of thiamylal sodium on the monosynaptic spinal reflex pathway in cats. J. Neurophsyiol., 27, 408-428.

MacIver, M.B. & Kendig, J.J. (1989). Enflurane-induced burst discharge on hippocampal CA1 neurones is blocked by the NMDA antagonist APV. Br. J. Anaes., 63, 296-305.

MacIver, M.B. & Roth, S.H. (1987). Enflurane-induced burst firing of hippocampal CA1 neurones. Br. J. Anaesth., 59, 369-378.

MacIver, M.B. & Roth, S.H. (1988). Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. Br. J. Anaesth., 60, 680-691.

Madison, D.V., Lancaster, B. & Nicoll, R.A. (1987). Voltage-clamp analysis of cholinergic action in the hippocampus. J. Neurosci., 7, 733-741.

Madison, D.V. & Nicoll, R.A. (1982). Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. Nature, 299, 636-638.

Madison, D.V. & Nicoll, R.A. (1984). Control of repetitive discharge of rat pyramidal neurones in vitro. J. Physiol., 354, 319-331.

Madison, D.V. & Nicoll, R.A. (1986). Actions of noradrenaline recorded intracellularly in rat hippocampal CA1 pyramidal neurones, in vitro. J. Physiol., 372, 221-244.

Malenka, R.C. & Nicoll, R.A. (1986). Dopamine decreases the calcium-activated after hyperpolarization in hippocampal CA1 pyramidal cells. Brain Res., 379, 210-215.

Marietta, M.P. Way, W.L. Castagnoli, N. & Trevor, A.J. (1977) On the pharmacology of ketamine enantiomorphs in the rat. J. Pharmac. Exp. Therap. 202, 157-165.

Matthews, E.K. & Quilliam, J.P. (1964). Effects of central depressant drugs upon acetylcholine release. Brit. J. Pharmac., 22, 415-440.

Metcalfe, J.C. & Richards, C.D. (1978). The effects of temperature on the local anaesthetic action of primary alcohols. J. Physiol., 281, 35-36P.

Meyer, K.H. (1937). Contributions to the theory of narcosis. Trans. Faraday Soc., 33, 1062-1068.

Miller, K.W., Firestone, L.L. & Forman, S.A. (1987). General anesthetic and specific effects of ethanol on acetylcholine receptors. Annals of the New York Academy of Science, 492, 71-87.

Miller, K.W., Paton, W.D.M., Smith, R.A. & Smith, E.B. (1972). Physio-chemical approaches to the mode of action of general anaesthetics. Anesthesiology, 36, 339-351.

Miller, K.W., Paton, W.D.M., Smith, R.A. & Smith, E.B. (1973). The pressure reversal of general anesthesia and the critical volume hypothesis. Mol. Pharmacol., 9, 131-143.

Miu, P. & Puil, E. (1989). Isoflurane-induced impairment of synaptic transmission. Exp. Brain Res., 75, 354-360.

Moore, J.W., Ulhricht, W. & Tateata, M. (1964). Effect of ethanol on the sodium and potassium conductanes of the squid axon membrane. J. Gen. Physiol., 48, 280-295.

Moore, S.D., Madamba, S.G., Joels, M. & Siggins, G.R. (1988). Somatostatin augments the M-current in hippocampal neurons. Science, 239, 278-280.

Mori, K., Kawamata, M., Mitani, H., Yamazaki, Y. & Fujita, M. (1972). A neurophysiologic study of ketamine anesthesia in the cat. Anesthesiology, 35, 373-383.

Morris, M.E. (1978). Facilitation of synaptic transmission by general anaesthetics. J. Physiol., 284, 307-325.

Mullins, L.J. (1954). Some physical mechanisms in narcosis. Chem. Rev., 54, 289-323.

Nakajima, Y., Nakajima, S., Leonard, R.J. & Yamaguchi, K. (1986). Acetylcholine raises excitability by inhibiting the fast transient potassium current in cultured hippocampal neurones. Proc. Natl. Acad. Sci. USA., 83, 3022-3026.

Nestoros, R.N. (1980). Ethanol selectively potentiates GABA-mediated inhibition of single feline cortical neurons. Life Sciences, 26, 519-523.

Newberry, N.R. & Nicoll, R.A. (1985). Comparison of the action of baclofen with gamma-amino butyric acid on rat hippocampal pyramidal cells. J. Physiol., 360, 161-185.

Nicoll, R.A. (1972). The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J. Physiol., 223, 803-814.

Nicoll, R.A. (1975). Presynaptic action of barbiturates in the frog spinal cord. Proc. Natn. Acad. Sci. USA., 72(4), 1460-1465.

Nicoll, R.A. & Alger, B.E. (1981). Synaptic excitation may activate a calcium dependent K^+ conductance in hippocampal pyramidal cells. Science, 212, 957-958.

Nicoll, R.A., Eccles, J.C., Oshima, T. & Rubia, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature, 258, 625-627.

Nicoll, R.A. & Madison, D.V. (1982). General anaesthetics hyperpolarise neurones in the vertebrate central nervous system. Science, 217, 1055-1057.

Niggli, A., Rudisuli, P., Maurar, P. & Weingart, R. (1989). Effects of general anaesthetics on current flow across membranes in guinea pig myocytes. Am. J. Physiol., 256,(Cell Physiol. 25), C273-C281.

Nowycky, M.C., Fox, A.P. & Tsien, R.W. (1985). Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature, 316, 440-443.

Numann, R.E., Wadman, W.J. & Wong, R.K.S. (1987). Outward currents of single hippocampal cells obtained from the adult guinea-pig. J. Physiol., 393, 331-353.

Oshima, E. & Richards, C.D. (1988). An in vitro investigation of the action of ketamine on excitatory synaptic transmission in the hippocampus of the guinea-pig. European J. Pharmac., 148, 25-33.

Owen, D.G. (1987). Three types of inward rectifier currents in cultured hippocampal neurones. Neurosci. Lett., (suppl.), 29, 518.

Parmentier, J.L., Shrivastav, B.B. & Bennett, P.B. (1981). Hydrostatic pressure reduces synaptic efficiency by inhibiting transmitter release, Undersea Biomed. Res., 8(3), 175-183.

Pearce, P.C., Halsey, M.J., Ross, J.A.S., Luff, N.P., Bevilaqua, K.A. & MacLean, C.J. (1989). A method of remote physiolgical monitoring of a fully mobile primate in a single animal cage. Lab. Animals., 23, 180-187.

Pearce, R.A., Stringer, J.L. & Lothman, E.W. (1989). Effect of volatile anesthetics on synaptic transmission in the rat hippocampus. Anesthesiology, 71, 591-598.

Peters, J.A., Kirkness, E.F., Callachan, H., Lambert, J.J. & Turner, A.J. (1988). Modulation of the $GABA_A$ receptor by depressant barbiturates and pregnane steroids. Br. J. Pharmac., 94, 1257-1269.

Pincus, J.H. & Hsiao, K. (1981). Calcium uptake mechanisms are affected by some convulsant and anticonvulsant drugs. Brain Res., 217, 119-127.

Po, B.T., Watson, R.L. & Hansen, H.R. (1968). Arousal time following intravenous anesthetic agents, methohexital and thiopental: effect of doxapram hydrochloride. Anesthesia and Analgesia, 47, 446-451.

Pocock, G., & Richards, C.D. (1987). The action of pentobarbitone on stimulus-secretion coupling in adrenal chromaffin cells. Br. J. Pharmac., 90, 71-80.

Pocock, G. & Richards, C.D. (1988). The action of volatile anaesthetics on stimulus-secretion coupling in bovine adrenal chromaffin cells. Br. J. Pharmac., 95, 209-217.

Pocock, G. & Richards, C.D. (1991). Cellular mechanisms in general anaesthesia. Br. J. Anaesth., 66, 116-128.

Regan, M.G. & Eger E.I. II (1967) Effect of hyperthermia in dogs on anaesthetizing and apneic doses of inhalation agents. Anesthesiology. 28, 689-693.

Richards, C.D. (1972). Potentiation and depression of synaptic transmission in the olfactory cortex of the guinea pig. J. Physiol, 222, 209-233.

Richards, C.D. (1972). On the mechanism of barbiturate anaesthesia. J. Physiol., 227, 749-767.

Richards, C.D. (1973). On the mechanism of halothane anaesthesia. J. Physiol., 233, 439-456.

Richards, C.D. (1973). Does trichloroethylene have a different mode of action from other general anaesthetics? J. Physiol., 233, 25-27P.

Richards, C.D. (1980). The mechanisms of general anaesthesia. In: Topical reviews in anaesthesia. Vol. 1. Eds: Norman, J. & Whitman, J. John Wright, Bristol. pp1-84.

Richards, C.D. (1982). The actions of pentobarbitone, procaine and tetrodotoxin on synaptic transmission in the olfactory cortex of the guinea-pig. Br. J. Pharmac., 75, 639-646.

Richards, C.D. (1983). Actions of general anaesthetics on synaptic transmission in the CNS. Br. J. Anaesth., 55, 201-207.

Richards, C.D., Martin, K., Gregory, S., Keighley, C.A., Hesketh, T.R., Smith, G.A., Warren, G.B. & Metcalfe, J.C. (1978). Degenerate perturbations of protein structure as the mechanism of anaesthetic action. Nature, 276, 775-779.

Richards, C.D., Russell, W.J. & Smaje, J.C. (1975). The action of ether and methoxyflurane on synaptic transmission in isolated preparations of the mammalian cortex. J. Physiol., 248, 121-142.

Richards, C.D. & Smaje, J.C. (1976). Anaesthestics depress the sensitivity of cortical neurones to L-glutamate. Br. J. Pharmac., 58, 347-357.

Richards, C.D. & Strupinski, K. (1986). An analysis of the action of pentobarbitone on the excitatory postsynaptic potentials and membrane properties of neurones in the guinea-pig olfactory cortex. Br. J. Pharmac., 89, 321-325.

Richards, C.D. & White, A.E. (1975). The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. J. Physiol., 252, 241-257.

Richards, C.D. & White, A.E. (1981). Additive and non additive effects of mixtures of short acting intravenous anaesthetic agents and their significance for theories of anaesthesia. Br. J. Pharmac., 74, 161-170.

Richter, J.A. & Waller, M.B. (1977). Effects of pentobarbitone on the regulation of acetylcholine content and release in different regions of the rat brain. Biochem. Pharmac., 26, 609-615.

Rosenberg, P.H., Eihl, H. & Steir, A. (1975). Biphasic effects of halothane on phospholipid and synaptic plasma membranes: a spin label study. Mol. Pharmacol., 11, 879-882.

Rosenberg, P.H., Jansson, S.E. & Gripenberg, J. (1979). Effects of halothane, thiopental and lidocaine on fluidity of synaptic plasma membranes and artificial phospholipid membranes. Anesthesiology. 46(5), 322-326.

Rostain. J.C. (1980). Le syndrome nerveux des hautes pressions chez l'homme et le singe Papio papio. These de doctorat es sciences naturelles (part 1: 195 pages, part 2: 130 pages). Universite d'Aix-Marseille.

Roth, S.H., Smith, R.A. & Paton, W.D.M. (1976). Pressure antagonism of anaesthetic-induced conduction failure in frog peripheral nerve. Br. J. Anaesth., 48, 621-628.

Rowland-James, P., Wilson, M.W. & Miller, K.W. (1981). Pharmacological evidence for multiple sites of action of pressure in mice. Undersea Biomed. Res., 8(1), 1-11.

Rudy, B. (1988). Diversity and ubiquity of K^+ channels. Neuroscience, 25(3), 729-749.

Sah, P., French, C.R. & Gage, P.W. (1985). Effects of noradrenaline on some potassium currents in CA1 neurones in rat hippocampal slices. Neurosci. Lett., 60, 295-300.

Sandorfy, C. (1978). Intermolecular interactions and anesthesia. Anesthesiology, 48, 357-359.

Sauter, J.F., Braswell, L., Wankowicz, P. & Miller, K.W. (1981). The effects of high pressure of inert gases and cholinergic receptor binding and function. In: Underwater Physiology VII Proceedings of the Seventh Symposium on Underwater Physiology, pp. 629-638. Eds. A.J. Bacharach & M.M Matzen. Undersea Biomedical Society: Bethesda, Maryland.

Sato, M., Austin, G.M. & Yai, H. (1967). Increase in permeability of the postsynaptic membrane to potassium produced by "Nembutal". Nature, 215, 1506-1508.

Sawada, A. & Yamamoto, C. (1985). Blocking action of pentobarbital on receptors for excitatory amino acids in the guinea-pig hippocampus. Exp. Brain Res., 59, 226-231.

Scharff, O. & Foder, B. (1989). Halothane inhibits hyperpolarization and potassium channels in human red blood cells. Euro. J. Pharmacol., 159, 165-173.

Scholfield, C.N. (1980). Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex in vitro. Pflugers Arch., 383, 249-255.

Schwarzkroin, P.A. & Stafstrom, C.E. (1980). Effects of EGTA on the calcium activated after hyperpolarization in hippocampal CA3 pyramidal cells. Science, 210, 1125-1126.

Schwindt, P.C., Spain, W.J., Foehring, R.C., Stafstrom, C.E., Chubb, M.C. & Crill, W.E. (1988). Multiple potassium conductances and their functions in neurons from cat sensorimotor cortex in vitro. J. Neurophysiol., 59, 424-449.

Segal, M. & Barker, J.L. (1984). Rat hippocampal neurons in culture: potassium conductances. J. Neurophysiol., 51, 1409-1433.

Segal, M., Rogawski, M.A. & Barker, J.L. (1984). A transient potassium conductance regulates the excitability of cultured hippocampal and spinal neurones. J. Neurosci., 4, 404-609.

Shearer, A.J., Ross, J.A. & Manson, H.J. (1981). Althesin, ketamine and methohexitone at pressure:- duration of action of a single i.v. dose. Br. J. Anaesth., 53, 455-461.

Shimoji, K., Fujikoa, H. & Ebata, T. (1984). Anesthetics block excitation with various effects on inhibition in MRF neurons. Brain Res., 295, 190-193.

Somjen, G.G. & Gill, M. (1963). The mechanism of blockade of synaptic transmission in the mammalian spinal cord by diethyl ether and thiopental. J. Pharmac. & Exp. Ther., 140, 19-30.

Somjen, G.G. (1963). Effects of thiopental on spinal presynaptic terminals. J. Pharmac. & Exp. Ther., 140, 396-402.

Sowton, S.C.M. & Sherrington, C.S. (1905). On the relative effects of chloroform upon the heart and other muscular organs. Br. Medical J., 2, 181-182.

Spyropoulos, C.S. (1957a). The effects of hydrostatic pressure upon the normal and narcotized nerve fibre. J. Gen. Physiol., 40(6), 840-857.

Spyropoulos, C.S. (1957b). Response of single nerve fibres at different hydrostatic pressures. Am. J. Physiol., 189(1), 214-218.

Stanfield, P.R. (1988). Intracellular Mg^{2+} may act as a co-factor in ion channel function. Trends Neurosci., 11, 475-477

Stevens, J.E., Fuginage, M., Oshima, E. & Mori, K. (1984). The biphasic pattern of the convulsive property of enflurane in cats. Br. J. Anaesth., 56, 395-403.

Storm, J.F. (1987a). Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. J. Physiol., 385, 733-759.

Storm, J.F. (1987b). Intracellular injection of a Ca^{2+} chelator inhibits spike repolarization in hippocampal neurons. Brain Res., 435, 387-392.

Storm, J.F. (1988). Temporal integration by a slowly inactivating K^+ current in hippocampal neurons. Nature, 336, 379-381.

Storm, J.F. (1989). An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells. J. Physiol., 409, 171-199.

Study, R.E. & Barker, J.L. (1981). Diazepam and (-)- pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of Gamma-aminobutyric acid responses in cultured central neurones. Proc. Natn. Acad. Sci. USA., 78, 7180-7184.

Takara, H., Wada, M., Arita, K., Sumikawa, K. & Izumi, F. (1986). Ketamine inhibits $^{45}Ca^{2+}$ influx and catecholamine secretion by inhibiting $^{22}Na^{+}$ influx in cultured bovine adrenal medullary cells. European J. Pharmac., 125, 217-224.

Thompson, S.H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. J. Physiol., 265, 465-488.

Trudeau, G., Dumas, J.M., Dupuis, P., Guérin, M. & Sandorfy, C. (1980). Intermolecular interactions and anesthesia: infrared spectroscopic studies. Topics in Current Chemistry, 93. Springer, Berlin, 91-124.

Trudell, J.R. (1977) A unitary theory of anaesthesia based upon lateral phase separation in membranes. Anesthesiology, 46, 5-10.

Trudell, J.R, & Cohen, E.N. (1975). Anesthetic induced nerve membrane fluidity as a mechanism of anesthesia. In: Molecular Mechanisms of Anesthesia. Ed. B.R. Fink. Progress in Anesthesiology, Vol 1, Raven Press, New York. 315-321.

Trudell, J.R., Payan, D.G. & Chin, J.H. (1975) The antagonistic effect of an inhalation anaesthetic and high pressure on the phase diagram of mixed dipalmitoyl-dimyristoylphosphatidylcholine bilayers. Proc. Natnl. Acad. Sci. USA. 72, 210-213.

Uehara, Y. (1960). Narcotic and NaCl-deficiency as blocking agents. Japanese Journal of Physiology, 10, 267-274.

Ungartedt, U. (1986). Microdialysis: a new bioanalytical sampling technique. Current Separation, 7, 43-46.

Wachtel, R.E. (1988). Ketamine decreases the open time of single channel currents activated by acetylcholine. Anesthesiology, 68, 563-570.

Wann, K.T., Harper, A.A., Wilcock, S.E. & MacDonald, A.G. (1977). Excitatory and inhibitory effects of pressure on neuronal membranes. Med. Subaqua. Hyperbare, 16, No. 63, 284-286.

Wann, K.T. & MacDonald, A.G. (1988). Actions and interactions of high pressure and general anaesthetics. Progress in Neurobiology, 30, 271-307.

Wann, K.T., MacDonald, A.G., Harper, A.A. & Wilcock, S.E. (1979). Electrophysiological measurements at high hydrostatic pressure: methods for intracellular recording from isolated ganglia and for extracellular recording in vivo. Comp. Biochem. Physiol., 64A, 141-147.

Wann, K.T., MacDonald, A.G. & Harper, A.A. (1979). The effects of high hydrostatic pressure on the electrical characteristics of Helix neurones. Comp. Biochem. Physiol., 64A, 149-159.

Watkins, J.C. (1984). Excitatory amino aids and central synaptic transmission. Trends Pharmacol., 5, 373-376.

Weakly, J.N. (1969). Effect of barbiturates on quantal synaptic transmission in spinal motoneurones. J. Physiol., 204, 63-77.

Werz, M.A. & Macdonald, R.L. (1985). Barbiturates decrease voltage-dependent calcium conductance of mouse neurones in dissociated cell culture. Mol. Pharmacol., 28(3), 269-277.

Yamamoto, H.A. & Harris, R.A. (1983). Effects of ethanol and barbiturates on Ca^{2+}-ATPase activity of erythrocyte and brain membranes. Biochem. Pharmac., 32(18), 2787-2791.

Yoshimura, M., Higashi, H., Fujita, S. & Shimoji, K. (1985). Selective depression of hippocampal inhibitory postsynaptic potentials and spontaneous firing by volatile anaesthetics. Brain Res., 340, 363-368.

Zbicz, K.L. & Weight, F.F. (1985). Transient voltage and calcium-dependent outward currents in hippocampal CA3 pyramidal neurons. J. Neurophysiol., 53, 1038-1058.

Zinebi, F., Fagni, L. & Hugon, M. (1988a). The influence of helium pressure on the reduction induced in field potentials by various amino acids and on the GABA mediated inhibition in the CA1 region of hippocampal slices in the rat. Neuropharmac., 27(1), 57-65.

Zinebi, F., Fagni, L. & Hugon, M. (1988b). Decrease of recurrent and feed forward inhibition under high pressures of helium in rat hippocampal slices. European J. Physiol., 153(2-3), 191-199.

APPENDIX

WORK PUBLISHED/IN PRESS (1987-1991)

Southan, A.P. & Wann, K.T. (1989). In vitro actions of ketamine and methohexitone in the rat hippocampus. British Journal of Anaesthesia, **63**, 574-580.

Southan, A.P. & Wann, K.T. (1989). Inhalational anaesthetics block accommodation of pyramidal cell discharge in the rat hippocampus. British Journal of Anaesthesia, **63**, 581-586.

Southan, A.P. & Wann, K.T. (1989). High helium pressure modifies the repetitive discharge of CA1 pyramidal neurones in the rat hippocampus in vitro. Journal of Physiology, **418**, 17P.

Southan, A.P. & Wann, K.T. (1991). Methods for intracellular recording from hippocampal brain slices under high helium pressure. Journal of Applied Physiology, **71(1)**, 365-371.

Wann, K.T. & Southan, A.P. (1991). The effects of high pressure on rat hippocampal CA1 pyramidal neurones - an intracellular study. In: J. Drouet, J.J. Rizzo & J.C. Rostain (Eds.), Proceedings of the Second International Meeting on High Pressure Biology, Toulon, 1990 (in press).

Wann, K.T. & Southan, A.P. (1991). The action of anaesthetics and high pressure on neuronal discharge patterns. Abstract, 1991 SEB annual meeting. University of Birmingham.

Wann, K.T. & Southan, A.P. (1991). The actions of anaesthetics and high pressure on neuronal discharge patterns. In: W. Winlow (Ed.), Neural Mechanisms of General Anaesthesia. Manchester University Press (in press).

High helium pressure modifies the repetitive discharge of CA1 pyramidal neurones in the rat hippocampus *in vitro*

BY A. P. SOUTHAN and K. T. WANN. *H.P.N.S. Group, Division of Anaesthesia, Clinical Research Centre, Watford Road, Harrow HA1 3UJ*

CA1 pyramidal neurones respond to long depolarizing current pulses with a burst of action potentials which slow or accommodate and then stop. A hyperpolarization mediated by at least two classes of K^+ channels follows this response (Madison & Nicoll, 1984; Storm, 1989). We have examined, in rat hippocampal brain slices, the effect of high helium pressure on both the action potential discharge and the after-hyperpolarization at resting potentials between −68 and −72 mV. Experiments were carried out in a 58 l high-pressure chamber.

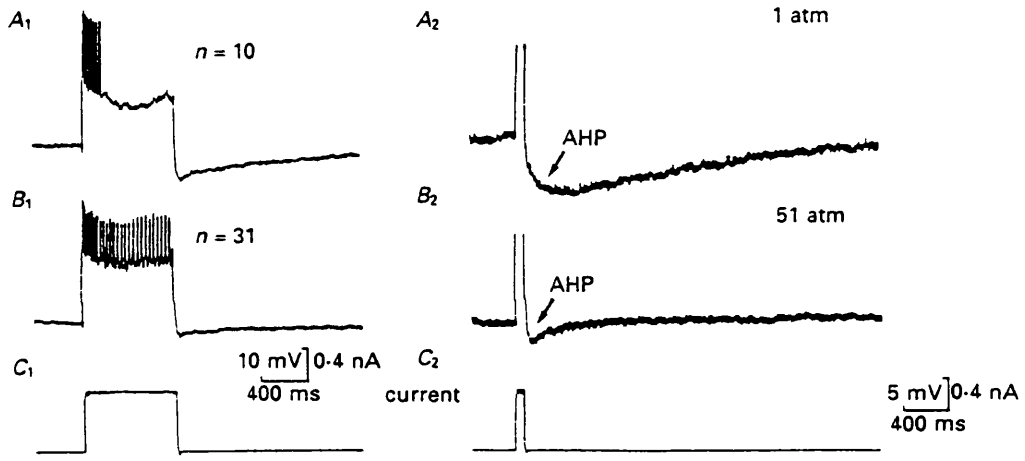


Fig. 1. Chart recordings showing the number of action potentials (n) and the after-hyperpolarization (AHP) in response to an 800 ms and following an 80 ms current pulse. Temperature. 31 °C (*A*. cell 1) and 36 °C (*B*. cell 2).

Recordings were made outside the chamber (30–33 °C), within the chamber (1·3 atm, 0·5 atm O_2, 36–37 °C), or at 51 atm (0·5 atm O_2, 36–37 °C). The number of action potentials in response to an 800 ms/0·7 nA current pulse increased from $8·7 \pm 0·76$ S.E.M. ($n = 7$) at 1 atm to $19·0 \pm 2·94$ S.E.M. ($n = 6$) at 51 atm. The AHP was also decreased (see Fig. 1). From these data we conclude that the K^+ channels of hippocampal neurones may be more sensitive to high pressure than other ion channels (e.g. Harper *et al.* 1981).

REFERENCES

HARPER, A. A., MACDONALD, A. G. & WANN, K. T. (1981). *J. Physiol.* **311**, 325–339.
MADISON, D. V. & NICOLL, R. A. (1984). *J. Physiol.* **354**, 319–331.
STORM, J. F. (1989). *J. Physiol.* **409**, 171–190.

Br. J. Anaesth. (1989), **63**, 581–586

INHALATION ANAESTHETICS BLOCK ACCOMMODATION OF PYRAMIDAL CELL DISCHARGE IN THE RAT HIPPOCAMPUS

A. P. SOUTHAN AND K. T. WANN

SUMMARY

The effects of the three inhalation anaesthetics enflurane, isoflurane and halothane were tested in vitro *on accommodation of rat CA1 neurones. At near clinical concentrations (approximately 2.5%) the anaesthetics slightly depressed antidromic field potential responses. At the same concentrations the anaesthetics also blocked accommodation reversibly and reduced the after hyperpolarization of CA1 neurones. No significant changes in the threshold potential were observed, although the resting membrane potential was often increased in the presence of the anaesthetics. The action of enflurane was not blocked by propranolol 20 μmol litre^{-1} and enflurane had no obvious effect on the duration of Ca^{2+} spikes of CA1 neurones. It is concluded that the anaesthetics may have a direct effect on membrane K^+ channels such as the Ca^{2+}-activated K^+ conductance and that the block of accommodation is unlikely to account for the proconvulsant action of enflurane.*

It is well known that certain anaesthetics have proconvulsant actions at clinical concentrations. Such effects occur with the inhalation agents enflurane [1, 2] and ether [3] and the i.v. anaesthetic ketamine [4]. *In vitro* work with both mammalian hippocampal neurones and invertebrate molluscan neurones has shown that the inhalation agents produce interesting changes in the firing activity of such cells. However, there has been little indication that enflurance acts differently. For example, in hippocampal CA1 neurones, halothane, isoflurane and enflurane initially increase, then decrease spontaneous firing [5, 6]. In squid axon, both methoxyflurane and isoflurane induce spontaneous firing [7], although these agents are not generally considered proconvulsant.

More recently, MacIver and Roth [8] reported that in field potential studies there was an increase in excitability of hippocampal CA1 neurones in the presence of isoflurane and enflurane and, most importantly, that this occurred despite depressed excitatory synaptic transmission and enhanced synaptic inhibition. This suggests that isoflurane and enflurane have a direct action on postsynaptic excitability and this could account for proconvulsant effects. However, previous intracellular work with hippocampal CA1 neurones showed that the threshold potential for spike generation was shifted in the depolarizing direction [5] in the presence of such anaesthetics. Such changes in the threshold potential can probably be attributed to the shifts in the activation–inactivation curves for the Na^+ current which are observed most readily in squid axon [9]. These shifts are not consistent with an "excitant action". The precise postsynaptic action of these anaesthetics is thus unclear and, given their possible relevance to the proconvulsant actions of anaesthetics, we have investigated such effects further.

Factors controlling postsynaptic excitability include the resting membrane potential and the threshold potential. The firing pattern of hippocampal cells is under the control of K^+ channels such as the Ca^{2+}-activated or muscarinic receptor coupled (M) K^+ channels [10–12]. Consequently, we have paid particular attention to the action of the anaesthetics enflurane, isoflurane and halothane on a form of repetitive activity of hippocampal CA1 neurones under the control of

A. P. SOUTHAN, B.SC.,; K. T. WANN, B.SC., PH.D.; HPNS Group, Clinical Research Centre, Watford Road, Harrow, Middx HA1 3UJ. Accepted for Publication: March 17, 1989.
Correspondence to K.T.W.

K^- channels. Our study examined two questions: Is such repetitive discharge sensitive to the action of anaesthetics? Are there any effects which might explain the proconvulsant profile of enflurane?

MATERIALS AND METHODS

Slice preparation and recording

All experiments were carried out on CA1 pyramidal neurones of rat hippocampal slices using methods described previously [8]. Orthodromic responses were produced by placing a unipolar metal electrode of tungsten wire 50 μm in diameter (WT-2T, Clark Electromedical) in the stratum radiatum. Antidromic responses were evoked by stimulation (0.1 Hz, 1–40 volts, 40–80 μs) of the alveus using a similar electrode. Intracellular measurements were made on neurones with resting membrane potential $\geqq -55$ mV, input resistance $\geqq 30$ MΩ and action potential amplitude $\geqq 60$ mV. Experiments were conducted at 28–32 °C.

Drugs

Enflurane (Ethrane) and isoflurane (Forane) were obtained from Abbott, and halothane (Fluothane) from I.C.I. All anaesthetics were delivered in 5% carbon dioxide in oxygen (flow rate $0.6-1$ litre min^{-1}) using commercial vaporizers (e.g. Enfluratec) to a 50-ml reservoir of artificial cerebrospinal fluid (aCSF) at 42 °C. The flow rate from the reservoir to the tissue chamber (volume 5 ml) was approximately 3 ml min^{-1}. The halothane vaporizer was calibrated by infra-red analysis at the carrier gas flow rate (600 ml min^{-1}) and corrections were applied for water vapour pressure at the temperature of the equilibration chamber. The partial pressure of the anaesthetic in the tissue chamber was calculated from the partial pressure in the equilibration chamber by multiplying by the ratio of the gas/aCSF partition coefficients at the two different temperatures. The concentration of halothane in the tissue chamber (measured by high pressure liquid chromatography) was constant within 15 min of the start of bubbling and reached 70% of the concentration in the equilibration chamber, the difference being the result of unavoidable loss from the tissue bath. Taking all these factors into account, the effective partial pressure of halothane (as percentage of a standard atmosphere) in the aCSF of the tissue bath was approximately 50% of that of the concentration indicated on the dial of the vaporizer,—that is, 2.5% instead of 5%. This would be the driving partial pressure for entry of the anaesthetic into the brain slices. The same considerations apply to enflurane and isoflurane. However, in all the experiments described here the concentrations given are those indicated on the dial of the vaporizer.

DL-Propranolol hydrochloride, tetraethylammonium hydrochloride (TEA) and tetrodotoxin (TTX) were obtained from Sigma.

RESULTS

Antidromic field potential studies

Any changes in postsynaptic excitability of the CA1 neurones can be inferred from the amplitude of the population spike activated by antidromic stimulation. The amplitude of the population spike was therefore measured in the absence and presence of anaesthetics. In each case, the anaesthetic was applied for 30 min. In five experiments 5% enflurane (nominal; for effective partial pressure, see Methods) decreased the amplitude of the population spike over the whole range of voltages tested (fig. 1). The decrease (to 0.74 of the control value) was significant ($P < 0.05$) only at the 5 × threshold voltage (fig. 1c). In three experiments 3% enflurane (nominal) produced a smaller decrease. In five experiments with 5% isoflurane (nominal) a similar decrease was observed (to 0.83 of control value at 5 × threshold), and in three experiments with 5% halothane (nominal), a more marked depression was recorded (to 0.70 of control value at 5 × threshold). There was thus no evidence of an enhancement of excitability in any case. The action of the anaesthetics on the properties of CA1 neurones was then examined in intracellular studies in the results presented below.

Effects on passive membrane properties

At the nominal concentrations tested (1–5%), none of the anaesthetics had any marked effect on resting membrane potential, although there was a tendency for neurones to hyperpolarize. Thus in 12 neurones exposed to 5% enflurane the mean resting membrane potential changed from -71.0 (SEM 1.9) mV to -74.5 (2.4) mV. Nine neurones hyperpolarized by 2–7 mV and three neurones showed no change in the resting membrane potential with 5% enflurane. There was no detectable change in the input resistance from the

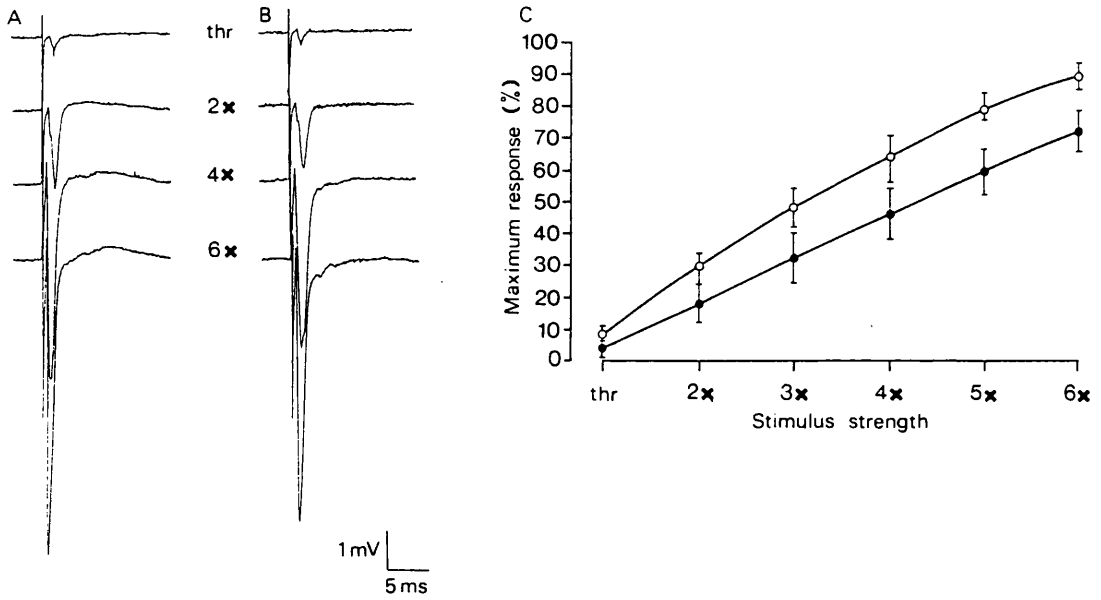


FIG. 1. A = Control. B = 5 % enflurane. C: Antidromic potentials in response to stimulation at threshold (thr) and at 2 ×, 4 × and 6 × threshold. Effects of a 30-min exposure to 5 % enflurane (nominal) on the hippocampal CA1 neurone antidromic population spike amplitude *v.* stimulus strength curve (mean, SEM; $n = 5$). ○ = Control; ● = 5 % enflurane. Temperature = 32 °C.

control value of 38.6 (1.2) MΩ ($n = 6$). Similar results were obtained with 5 % isoflurane and 5 % halothane. Thus the mean changes in resting membrane potential were 3.2 (0.5) mV and 3.2 (0.6) mV, respectively ($n = 4$). In all cases these effects of the anaesthetics on resting membrane potential were highly significant ($P < 0.001$).

Effects on accommodation

When CA1 neurones are subjected to long (> 400 ms) depolarizing pulses, they respond with a burst of 7–8 spikes and then adapt or accommodate [11] (fig. 2A). Enflurane 3–5 % reduces such accommodation rapidly and reversibly (fig. 2). The anaesthetic often hyperpolarized CA1 neurones and in all of the experiments reported here the cells were held at the control resting membrane potential whilst accommodation or the after hyperpolarization was studied. In five neurones the number of spikes increased from an average of 5.4 (SEM 0.5) in the control to 14.4 (1.2) ($P = 0.0001$) after 15 min in 5 % enflurane. To investigate the basis of this action of enflurane, we examined its action on the hyperpolarization of several seconds duration which follows the response to depolarization. The reduction of accommodation is accompanied by a decrease of the

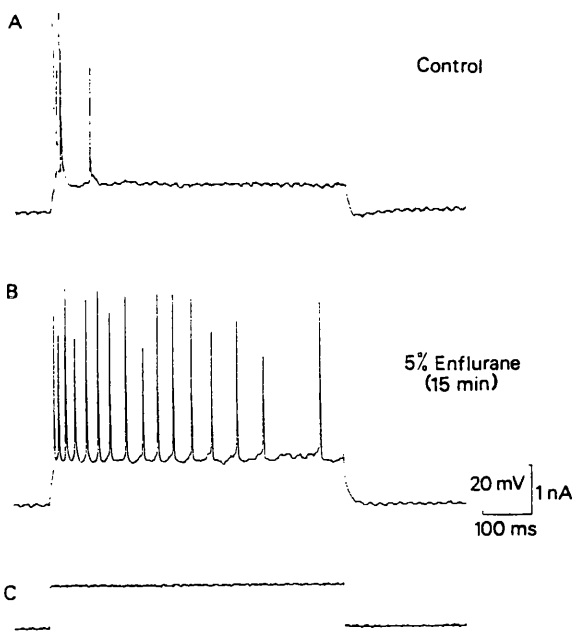


FIG. 2. Effect of 5 % enflurane on accommodation in the CA1 pyramidal neurone. A: Control; B: 15-min exposure to 5 % enflurane (nominal); C: current pulse (approximately 600 ms/0.9 nA). Resting membrane potential was −71 mV in A and −76 mV in B. Temperature = 32 °C.

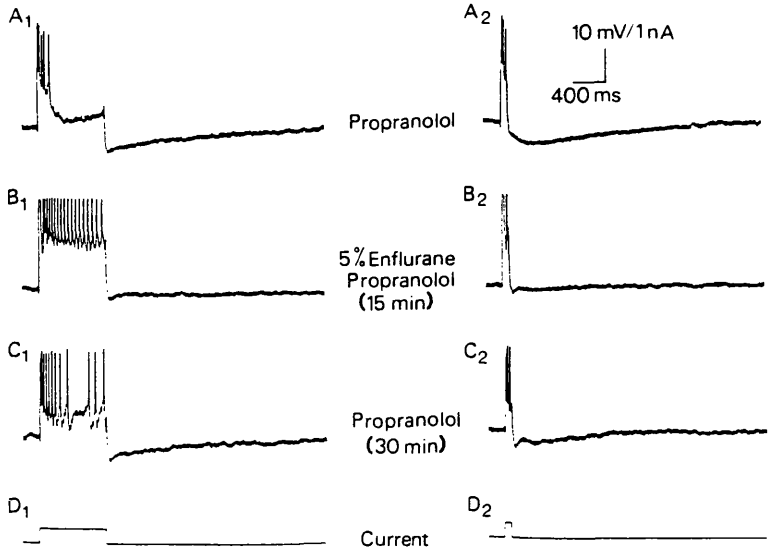


FIG. 3. Action of a 15-min exposure to 5% enflurane (nominal) in the presence of propranolol 20 μmol litre^{-1} (throughout A, B and C) on the accommodation (A_1, B_1 and C_1) of a CA1 neurone to depolarizations of long duration (800 ms) and the after hyperpolarization (A_2, B_2 and C_2) following 80-ms depolarizing pulses. The current pulse was 0.5 nA in each case. Temperature = 32 °C.

amplitude of the after hyperpolarization (AHP) which can be seen following either long (800 ms) or short (80 ms) depolarizing pulses (fig. 3A). In three neurones the AHP amplitude (80-ms pulse) was decreased by 5% enflurane from a control value of 6.1 (0.8) mV to 3.8 (0.6) mV ($P = 0.04$). A typical recording is shown in figure 3.

Madison and Nicoll [11] reported that noradrenaline blocks accommodation and reduces the AHP of CA1 neurones. To test if enflurane exerts its effect by releasing noradrenaline, we studied the action propranolol 20 μmol litre^{-1} on the effect of enflurane. Figure 3 shows the result of a typical experiment. The slices were pretreated with propranolol for 20 min. The antagonist had no action on accommodation during this period. Enflurane 5% (15-min exposure) in the presence of propranolol blocked accommodation and reversibly decreased the AHP. In six other neurones, enflurane was similarly effective in the presence of propranolol. The mean number of spikes during an 800-ms pulse increased from 5.7 (SEM 0.8) to 12.7 (2.4) ($n = 7$) ($P = 0.008$) and the AHP amplitude following an 80-ms pulse decreased, from 5.5 (1.1) mV to 2.2 (0.6) mV ($n = 7$) ($P = 0.008$). Both of these effects were reversed on returning to anaesthetic-free aCSF.

Isoflurane and halothane also blocked the accommodation of CA1 neurones. The effect occurred at the same concentration with the same time course, and it would appear that the three anaesthetics were almost equieffective (figs 3, 4). Thus, for four neurones exposed to 5% isoflurane, the average number of spikes increased from 5.8 (SEM 0.5) to 13.5 (0.6) ($P = 0.0001$) and the AHP amplitude (80 ms) decreased from 5.8 (0.3) mV to 3.9 (0.5) mV ($P = 0.009$). In the case of 5% halothane (fig. 4), for four neurones the average number of spikes increased from 6 (1.2) to 15.0 (1.5) ($P = 0.02$) and the AHP amplitude (80 ms) decreased from 4.0 (0.9) mV to 2.6 (0.3) mV ($P = 0.09$).

Effects on threshold behaviour

The effects of the inhalation anaesthetics on accommodation occurred in the absence of changes in either resting membrane potential or threshold current required to elicit a single spike or, in many instances, the neurones hyperpolarized and the threshold current (80-ms depolarizing pulse) actually increased, despite accommodation being blocked (fig. 2). No reduction in threshold current was observed for any of the anaesthetics.

Action on Ca^{2+} channels

Accommodation and the AHP of CA1 neurones is thought to be controlled by a Ca^{2+}-activated K^+

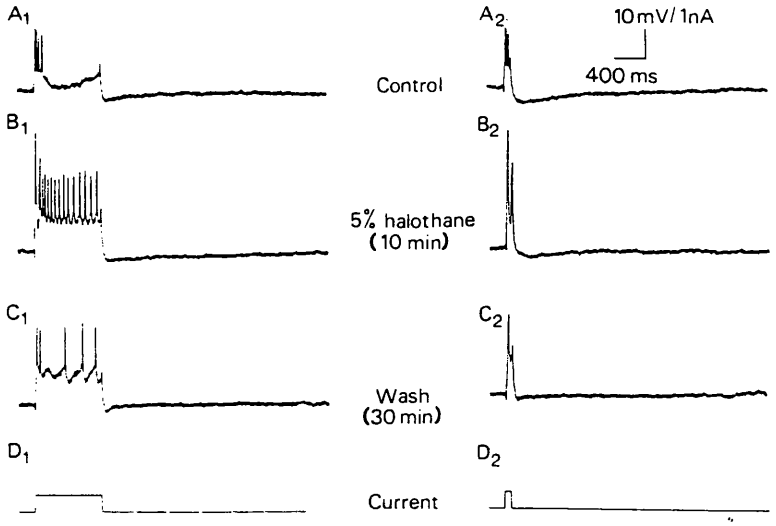


FIG. 4. Effect of a 10-min exposure to 5 % halothane (nominal) on the accommodation of a CA1 neurone to depolarizations of long duration (800 ms) (A_1, B_1 and C_1) and the afterhyperpolarization (A_2, B_2 and C_2) following 80-ms depolarizing pulses. A_1, A_2: Control; B_1, B_2: 5 % halothane; C_1, C_2: wash. Current pulse 0.5 nA. Temperature = 30 °C.

conductance. Thus Ca^{2+} entry into the neurone is necessary. We therefore tested the possibility that the anaesthetics reduced Ca^{2+} entry, thereby preventing the activation of the K^+ conductance. CA1 neurones were bathed in tetrodotoxin (TTX) 10^{-6} mol litre^{-1} and tetraethylammonium (TEA) 5×10^{-3} mol litre. Under these conditions the action potentials are Ca^{2+}-based and duration increased from < 2 ms to approximately 40 ms. The AHP was increased also (up to 8 mV). In four neurones 5 % enflurane had no effect on the Ca^{2+} spike duration (measured at 50 % amplitude or the base), although the subsequent hyperpolarization was reduced from 6.9 (0.6) mV to 2.8 (0.3) mV ($P = 0.001$). This effect was fully reversible on returning to anaesthetic-free aCSF.

DISCUSSION

We have found that high concentrations of the inhalation anaesthetics enflurane, isoflurane and halothane both decreased postsynaptic excitability and enhanced the repetitive discharge of CA1 neurones. In particular, the anaesthetics blocked neuronal accommodation, probably by a direct action on the neuronal K^+ channels. Previous work has shown that general anaesthetics decrease the activity of axonal voltage-gated K^+ channels [13, 14] and enhance the activity of other less well defined K^+ conductances [15, 16]. More recently, two lines of investigation have drawn attention to the fact that a component of K^+ current in molluscan neurones is extremely sensitive to general anaesthetics [17, 18]. In the case of neurones in the ganglion of *Lymnea stagnalis*, general anaesthetics activate an outward current producing hyperpolarization [17], and in the case of squid axon, general anaesthetics reduce an outward component of K^+ current producing depolarization [18].

Our results show that the general anaesthetics enflurane, isoflurane and halothane, at relatively high concentrations (approximately 2.5 %) modified K^+ channel function in CA1 neurones. It is possible that the anaesthetics have a dual action on the K^+ conductances of hippocampal neurones. Thus the anaesthetics both block the accommodation of these neurones by reducing the Ca^{2+}-activated K^+ current and possibly the M current, and hyperpolarize by enhancing a K^+ conductance which contributes to the resting membrane potential. It seems likely that the small hyperpolarization observed intracellularly was responsible for the reduction in excitability observed in the field potential studies. As the action of the anaesthetics is not modified by propranolol and the anaesthetics do not modify the shape of Ca^{2+} spikes recorded in the presence of TTX and TEA, we propose that the effects of the anaesthetics on the Ca^{2+}-activated K^+ current are

direct rather than mediated via changes in noradrenaline release or the Ca^{2+} flux into the neurone. However, voltage-clamp experiments are required to establish fully the ionic basis of such effects.

In our experiments it was not possible to distinguish between the effects of enflurane, an anaesthetic with proconvulsant properties, and isoflurane or halothane which have no proconvulsant actions. It is consequently difficult to ascribe the proconvulsant effects of enflurane to an action on membrane K^+ channels. The dual action of these anaesthetics is reminiscent of the action of noradrenaline [11]. As Madison and Nicoll have suggested [11], the hyperpolarization and decreased response to threshold current pulses indicate that anaesthetics effectively suppress weak depolarizing inputs. The block of accommodation by the anaesthetics effectively enhances strong depolarizing inputs. Future studies are required to determine if these effects are shared by other anaesthetics, including i.v. agents.

ACKNOWLEDGEMENT

We are grateful to Dr M. Landon for measuring anaesthetic concentrations.

REFERENCES

1. Black GW. Enflurane. *British Journal of Anaesthesia* 1979; **51**: 627–640.
2. Stevens JE, Fujinaga M, Oshima E, Mori K. The biphasic pattern of the convulsive property of enflurane in cats. *British Journal of Anaesthesia* 1984; **56**: 395–403.
3. Nunn JF. An anaesthetist looks at Malaya. *Lancet* 1954; **1**: 361–367.
4. Bowyer JF, Winters WD. The effects of various anaesthetics on amygdaloid kindling seizures. *Neuropharmacology* 1981; **20**: 199–209.
5. Yoshimura M, Higashi H, Fujita, S, Shimoji K. Selective depression of hippocampal inhibitory postsynaptic potentials and spontaneous firing by volatile anaesthetics. *Brain Research* 1985; **340**: 363–368.
6. Fujiwara N, Higashi H, Nishi S, Shimoji K., Sugita S, Yoshimura M. Changes in spontaneous firing patterns of rat hippocampal neurones induced by volatile anaesthetics. *Journal of Physiology (London)* 1988; **402**: 155–175.
7. Urban BW, Haydon DA. The actions of halogenated ethers on the ionic currents of the squid giant axon. *Proceedings of the Royal Society B* 1987; **231**: 13–26.
8. MacIver MB, Roth SH. Enflurane-induced burst firing of hippocampal CA1 neurones. *British Journal of Anaesthesia* 1987; **59**: 369–378.
9. Haydon DA, Urban BW. The effects of some inhalation anaesthetics on the sodium current of the squid giant axon. *Journal of Physiology (London)* 1983; **341**: 429–440.
10. Halliwell JV, Adams PR. Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* 1982; **250**: 71–92.
11. Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature (London)* 1982; **299**: 636–638.
12. Madison DV, Nicoll RA. Control of the repetitive discharge of rat CA1 pyramidal neurones *in vitro*. *Journal of Physiology (London)* 1984; **354**: 319–331.
13. Bean BP, Shrager P, Goldstein DA. Modification of sodium and potassium channel gating kinetics by ether and halothane. *Journal of General Physiology* 1981; **77**: 233–253.
14. Haydon DA, Elliott JR, Hendry BM. Effects of anesthetics on the squid giant axon. In: *The Squid Axon. Current Topics in Membranes and Transport* 1984; **22**: 445–482.
15. Chalazonitis N. Effects of anesthetics on neural mechanisms: selective actions of volatile anesthetics on synaptic transmission and autorhythmicity in single identifiable neurons. *Anesthesiology* 1967; **28**: 111–123.
16. Nicoll RA, Madison DV. General anaesthetics hyperpolarise neurons in the vertebrate central nervous system. *Science* 1982; **217**: 1055–1057.
17. Franks NP, Lieb WR. Volatile general anaesthetics activate a novel neuronal K^- current. *Nature (London)* 1988; **333**: 662–664.
18. Haydon DA, Requena J, Simon AJB. The potassium conductance of the resting squid axon and its blockage by clinical concentrations of general anaesthetics. *Journal of Physiology (London)* 1988; **402**: 363–374.

Br. J. Anaesth. (1989), **63**, 574–580

IN VITRO ACTIONS OF KETAMINE AND METHOHEXITONE IN THE RAT HIPPOCAMPUS

A. P. SOUTHAN AND K. T. WANN

SUMMARY

The effects of ketamine and methohexitone have been tested in vitro *on rat CA1 pyramidal neurones using conventional extracellular and intracellular recording techniques. Ketamine 20–200 μmol litre^{-1} predominantly increased excitability by a postsynaptic action: it enhanced the amplitude of the antidromic (field) potential response in extracellular recordings; in intracellular studies depolarized or did not change the resting membrane potential; increased intrinsic excitability (assessed by direct stimulation); and reduced accommodation properties of CA1 neurones. Methohexitone 10–100 μmol litre^{-1} did not affect the amplitude of the antidromic field potential responses, tended to hyperpolarize and reduce the intrinsic excitability, but did not alter accommodation properties. At these concentrations these agents either did not affect or, in the case of ketamine, enhanced excitatory synaptic transmission on to the CA1 pyramidal neurones. Methohexitone 50 and 100 μmol litre^{-1} also induced a large, slow (several seconds) after depolarization which followed the conventional orthodromic response and may lead to action potential discharge. It is clear that these agents have multiple actions on CA1 pyramidal neurones* in vitro *and that ketamine and methohexitone* in vitro *influence excitability by different mechanisms.*

Several general anaesthetics have allegedly excitant or "convulsant" actions *in vivo*. This is so for inhalation agents such as enflurane [1] or diethyl-ether [2] and for i.v. agents including ketamine [3, 4] and methohexitone [5]. The cellular basis of such excitant effects is poorly defined. Electrophysiological analysis of the action of anaesthetics may provide a partial explanation [6, 7]. Morris [6] argued that ketamine and methohexitone enhance transmitter release in the cuneate nucleus. Further, in the case of enflurane it is clear that the postsynaptic excitability of the CA1 neurones in the hippocampus is increased [7]. We have also reported that inhalation anaesthetics such as enflurane have an excitant postsynaptic effect, blocking the accommodation of hippocampal CA1 neurones to long-lasting depolarizing currents [8].

In the experiments reported here we have analysed antidromic field potential responses and intracellular responses to test if ketamine and methohexitone possess postsynaptic actions in rat hippocampal CA1 pyramidal neurones. Our results show that these agents act differently from each other and from the inhalation agents which we have tested previously.

METHODS

Details of the preparation and the electrophysiological techniques have been described in detail elsewhere [7, 8; Granger, Southan and Wann, in preparation]. Antidromic field potential responses were elicited by stimulating the alveus whilst recording the population spike from the stratum pyramidale using a microelectrode filled with sodium chloride 4 mol litre^{-1} (resistances 2–10 MΩ). Intracellular recordings from 22 CA1 pyramidal neurones were made using microelectrodes (pulled with a Flaming-Brown puller, Sutter Instrument) filled with potassium acetate 4 mol litre^{-1} (resistances 50–130 MΩ). We selected only neurones exhibiting stable resting membrane potentials (≥ -55 mV) and action potentials of amplitude > 60 mV. The hippo-

A. P. SOUTHAN, B.SC.; K. T. WANN, B.SC., PH.D.; HPNS Group, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ. Accepted for Publication: April 24, 1989.

Correspondence to K. T. W.

campal slices were superfused with anaesthetic containing artificial cerebrospinal fluid for 30 min. All experiments were carried out at 28–32 °C.

Ketamine (Ketalar) was obtained from Parke–Davis, methohexitone (Brietal-sodium) from Eli–Lilly.

RESULTS

Antidromic field potential responses

An assessment of the effects of anaesthetics on CA1 neurone excitability can be obtained from an analysis of the antidromic field potential responses. The effects of both ketamine and methohexitone were studied therefore on such potentials if the responses were stable in the control medium over a 20-min period.

Ketamine enhanced excitability at low concentrations; in six experiments 20 μmol litre^{-1} produced significant ($P < 0.05$) shift to the left in the relationship between the antidromic field potential and the stimulus voltage (fig. 1). The mean enhancement after 30 min at 5 × threshold was 33% ($n = 6$). This was reversed only partially on prolonged washing (1 h). Such an enhancement in the antidromic field potential has not been observed previously with control slices over the duration of these experiments. In contrast, ketamine 200 μmol litre^{-1} produced no shift in the antidromic field potential responses over the full voltage range (four experiments). At greater concentrations (1000 μmol litre^{-1}), ketamine was irreversibly depressant.

Methohexitone 10 and 100 μmol litre^{-1} produced only small shifts (approximately 10%) to the left in the antidromic field potential *v.* stimulus voltage curve (four experiments at each concentration). These shifts were most noticeable at short exposure times (5–10 min), but were insignificant ($P > 0.05$) with 15–30 min exposures.

Intracellular responses

Only neurones with stable resting membrane

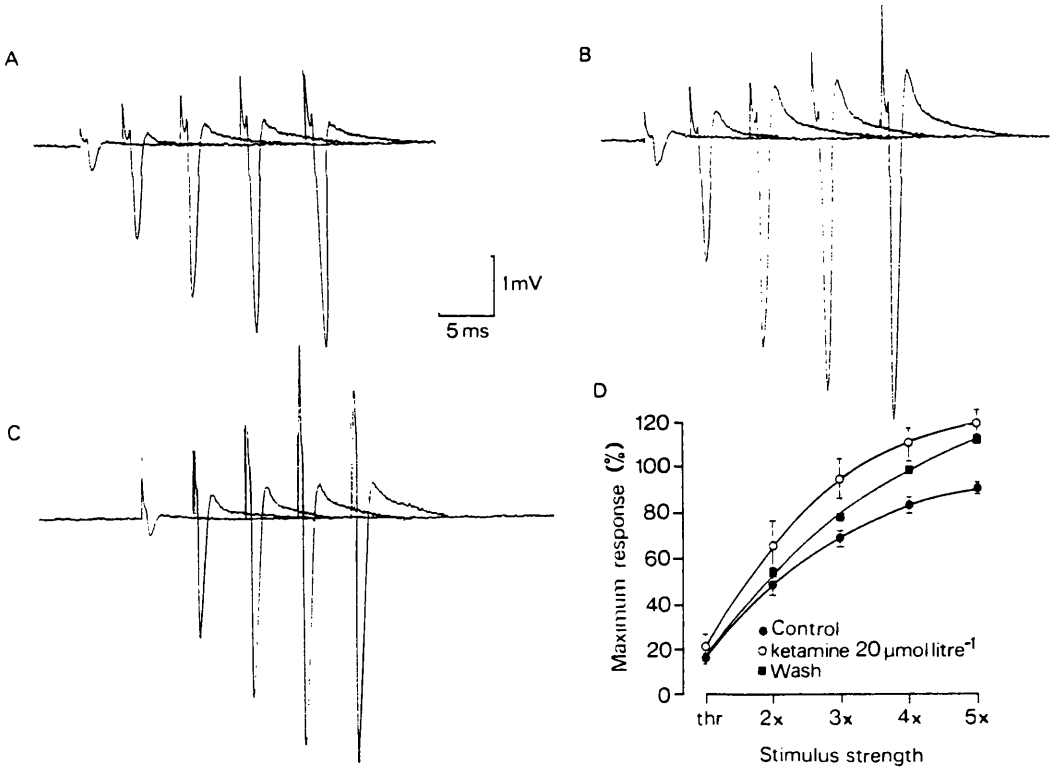


FIG. 1. The effect of ketamine 20 μmol litre^{-1} (30 min) on the amplitude of the antidromic field potential recorded from the CA1 pyramidal neurone field. A: Control; B: ketamine; C: wash. Responses were recorded at threshold (1 V) and 2 V, 3 V, 4 V and 5 V. D: Relationship between the amplitude of the antidromic field potential (percentage of the maximum control response) and the stimulus voltage (means, SEM; $n = 6$). ● = Control; ○ = ketamine 20 μmol litre^{-1}; ■ = wash (for clarity no SEM are shown). Temperature: A–C = 30 °C; D = 28–32 °C.

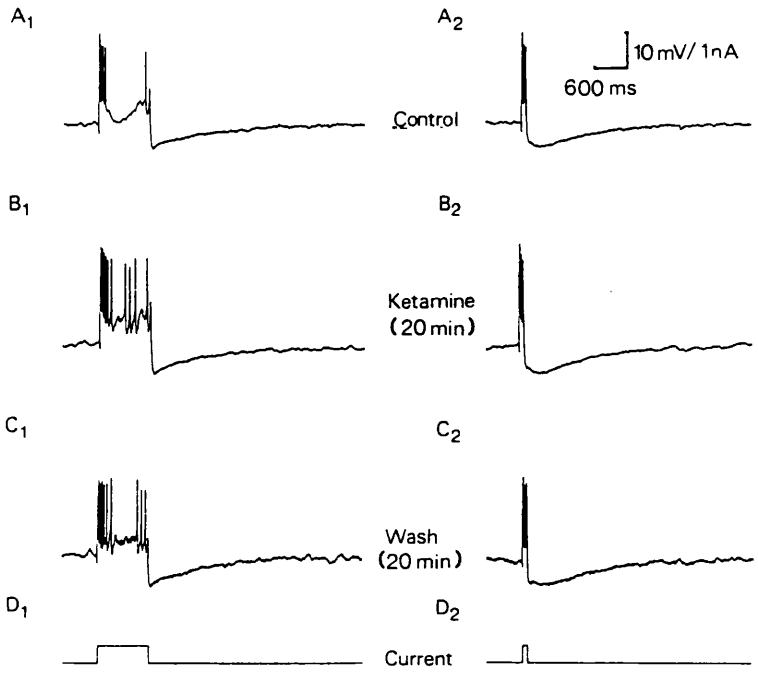


FIG. 2. Chart records of the effect of ketamine 200 μmol litre^{-1} (20 min) on the accommodation of a CA1 neurone to a depolarizing current of long duration (800 ms, 0.5 nA) (A_1, B_1 and C_1) and the after hyperpolarization (A_2, B_2 and C_2) following 80-ms depolarizing currents. A_1/A_2 = Control; B_1/B_2 = ketamine 200 μmol litre^{-1}; C_1/C_2 = wash (20 min). The number of action potentials elicited by the 800-ms pulse increased from 7 to 12 in the presence of ketamine. There was no change in the resting membrane potential (approximately 66 mV), but the stimulus variables required to stimulate orthodromically and directly changed from 6.5 V to 1 V and 0.05 nA to 0.02 nA, respectively. Temperature = 29 °C.

potentials ($\geqslant -55$ mV) which accommodate to depolarizing currents of long duration were used (approximately 50 % of neurones with stable resting membrane potentials).

Ketamine. We used concentrations of ketamine 20, 50, 200 or 400 μmol litre^{-1} as this is considered to span the anaesthetic range in the rat [9, 10]. Four neurones depolarized after 15–30 min in the presence of ketamine: one neurone of three tested depolarized by 7 mV in ketamine 20 μmol litre^{-1} and three neurones (eight tested) each depolarized by 4 mV in ketamine 200 μmol litre^{-1} after 30 min. The resting membrane potential changes were reversible, however, in only one neurone exposed to ketamine 200 μmol litre^{-1}. There were no resting membrane potential changes in the other nine neurones exposed to ketamine (two in 20 μmol litre^{-1}; one in 50 μmol litre^{-1}; five in 200 μmol litre^{-1}; one in 400 μmol litre^{-1}). There were no detectable changes (i.e. $\geqslant 20$ %) in input resistance at any concentration tested, which could imply that more than one ionic permeability change was involved in the changes in resting membrane potential. In eight of the 13 neurones (three exposed to ketamine 20 μmol litre^{-1} and five of eight exposed to 200 μmol litre^{-1}) there was a decrease in the current required to stimulate the CA1 neurone intracellularly (direct stimulation). In some cases, the current required to stimulate directly decreased to approximately 40 % of the control value. In no case was spontaneous firing observed. These changes in excitability were not reversed by washing for up to 1 h. The responses to orthodromic stimulation (via the Schaffer collaterals) were tested in two neurones exposed to ketamine 20 μmol litre^{-1} and five neurones exposed to 200 μmol litre^{-1}. Responses were not altered after 30 min in the case of the former group, but in four of the five neurones tested, there was usually a marked reduction in the current required to elicit an orthodromic action potential in ketamine 200 μmol litre^{-1}.

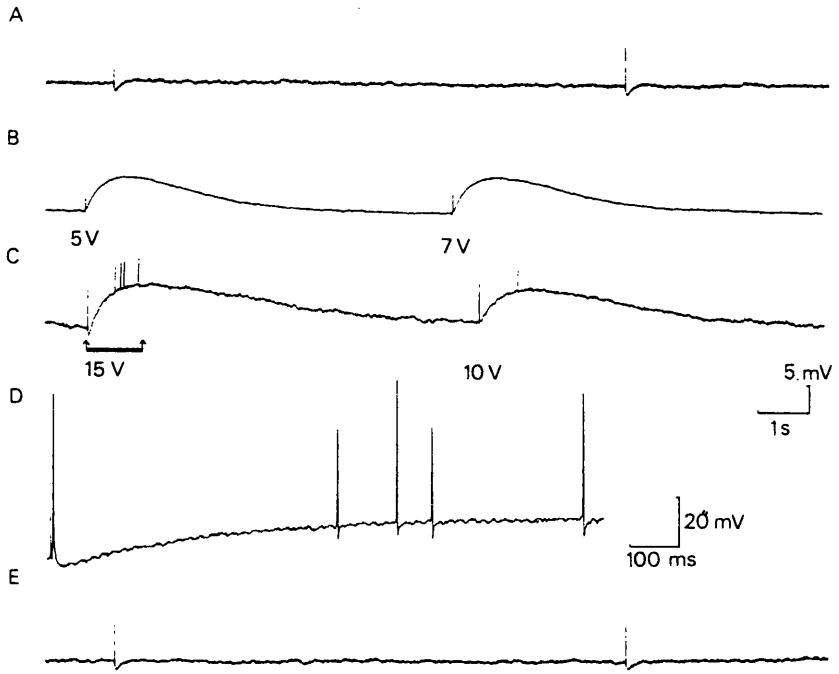


FIG. 3. Chart records of orthodromic responses of a CA1 pyramidal neurone elicited by stimulation of the Schaffer collaterals (5–15 V, 80 μs). A: Control responses to 50 V stimulus voltage. B–D: Methohexitone 50 μmol litre^{-1} throughout 15-min exposure. B: Response to 5 V then 7 V stimulus voltage. C: Response to 15 V followed by 10 V stimulus voltage. In B the response consists of an excitatory postsynaptic potential followed by a depolarization of long duration. In C the response consists of an action potential and an inhibitory postsynaptic potential followed by a depolarization of long duration. Note the action potentials on the depolarizing wave, truncated in C because of the limited frequency response of the pen recorder. D: "Hard copy" of an oscilloscope trace, showing more details of the response marked in C within the arrows. Note the variable action potential amplitude (resulting from limited resolution of the digital storage display). There was no change in the resting membrane potential, but the stimulus variables to activate orthodromically and directly decreased from approximately 50 V to 10 V and 0.74 nA to 0.63 nA, respectively. E: Recovery after 10-min wash, response to 40-V stimulus voltage. Temperature = 29 °C.

When subjected to depolarizing currents of long duration (> 400 ms), CA1 pyramidal neurones responded with an initial burst of action potentials and the neurones were then usually quiescent throughout the duration of the depolarization. This behaviour (accommodation) was observed in approximately 50 % of neurones with stable resting membrane potentials ($\geqslant -55$ mV) and was associated with a large slow after hyperpolarizing potential (AHP) (fig. 2). The AHP was observed readily with shorter depolarizing currents of approximately 80 ms duration (e.g. A_2 in figure 2). In four of eight neurones, ketamine 200 μmol litre^{-1} had a small effect on the accommodation, increasing (after 20–30 min) the number of action potentials elicited in response to an 800-ms pulse from 6.5 to 12.2. The voltage "sag" during the response was depressed also. A typical effect is shown in figure $2A_1$–C_1. In one of two neurones tested in the presence of ketamine 20 μmol litre^{-1}, accommodation was reduced also. The reduction in accommodation in all five neurones thus affected was accompanied by an increase in excitability as assessed by direct stimulation,—that is, there was a reduction in the threshold current (80-ms pulse). There was no change in the amplitude of the AHP following the response to an 80-ms current pulse (fig. 2 A_2–C_2). The effects of ketamine were not reversed on washing.

Methohexitone 50 μmol litre^{-1} or 100 μmol litre^{-1} often produced a small reversible hyperpolarization (2–9 mV within 10 min in four of

nine neurones studied). The resting membrane potential change was accompanied by a reversible reduction in the input resistance to 75 % ($n = 1$; methohexitone 50 μmol litre^{-1}) and 55 (SEM 10) % ($n = 3$; methohexitone 100 μmol litre^{-1}) of the control value. At these concentrations, methohexitone produced variable changes in the current required to stimulate directly or orthodromically, although there was a greater tendency to reduce the excitability to direct current stimulation. In these experiments the neurones were held at the control resting membrane potentials during testing. In all nine neurones tested, methohexitone consistently induced (within 10 min) a large-amplitude (up to 20 mV), long-lasting (approximately 5 s) depolarization which followed the action potential– (or excitatory postsynaptic potential–) inhibitory postsynaptic potential sequence elicited by orthodromic stimulation (fig. 3). The response was graded and in some instances gave rise to action potentials (fig. 3C, D).

Methohexitone had no effect on the accommodation of action potential discharge (measured at the control resting membrane potential), although in many instances the AHP was reduced or even abolished within 10–15 min (five of seven neurones).

DISCUSSION

The principal findings of this study are that both ketamine and methohexitone at the concentrations tested had a variety of actions on the properties of CA1 pyramidal neurones. *In vivo* work with rats suggests that the effective plasma concentration of ketamine during anaesthesia is 15 μmol litre^{-1} [9]. Methohexitone is more potent than ketamine in the rat [11]. Thus the concentrations of ketamine and methohexitone used in our *in vitro* experiments ranged from near anaesthetic values to an order of magnitude greater. No particular concentration-dependent effect was observed, except in the field potential studies, where it was clear that the smaller concentration of ketamine (20 μmol litre^{-1}) enhanced the response, but the greater concentration (200 μmol litre^{-1}) had no effect.

Our main results may be summarized as follows. Ketamine depolarized CA1 neurones, increased excitability in response to depolarizing pulses, enhanced orthodromic responses and reduced accommodation. However, the AHP was not reduced. The enhancement of the field potential responses at low concentration may be attributed to these effects on excitability. In contrast, methohexitone often hyperpolarized CA1 neurones, reduced or increased excitability (in response to direct stimulation) and had a variable action on the threshold for orthodromic stimulation. There were no obvious changes in accommodation properties although, paradoxically, the AHP amplitude was depressed markedly or abolished. There was no significant change in the antidromic field potential responses at these concentrations of methohexitone. More importantly, methohexitone induced a large slow depolarization following the conventional orthodromic responses.

Previous work has shown that, at clinical concentrations, ketamine has a powerful postsynaptic depressant effect at the neuromuscular junction [12]. Depression of excitatory synaptic transmission in the central nervous system seems to require a higher concentration. For example, Oshima and Richards [10] reported that concentrations greater than 50 μmol litre^{-1} depressed transmission in the dentate gyrus of the hippocampus. Further, "excitant" effects of ketamine have been reported in the cuneate nucleus [6]. Ketamine is known to depress the postsynaptic responses to NMDA [13] and the effect of ketamine, on glutamate responses at least, may occur at lower concentrations than those required to block excitatory synaptic transmission [10]. Ketamine may also prolong the conductance change underlying the inhibitory postsynaptic potential [14, 15]. These effects of ketamine are usually reversible. In our experiments the dominant action of ketamine was to increase excitability. The lack of reversibility of the effects produced by ketamine in this study certainly deserves comment. First, the extracellular and intracellular variables remained stable in our preparations over the duration of an average ketamine experiment (approximately 2 h), so the changes observed are unlikely to result from long-term changes in the preparation itself. Second, we have shown that the actions of three inhalation anaesthetics [8] and methohexitone (this study) are reversed in hippocampal slice preparations. Finally, the effects of ketamine on excitability described here may not be relevant to anaesthesia, and for this reason might not be readily reversible. To what extent the changes we have observed *in vitro* can contribute to the convulsant effects of ketamine [3] remains to be established.

Previous work with methohexitone *in vivo* has shown that this anaesthetic depresses the acetyl-

choline-induced excitation of cortical neurones [16] and facilitates excitatory synaptic transmission within the cuneate nucleus [6]. More usually, barbiturates (including methohexitone) are known as depressants of excitatory synaptic transmission in the central nervous system [14, 17, 18], and often hyperpolarize neurones [19, 20]. They also enhance both pre- and postsynaptic inhibition [21] (see also [22] for review) by modifying GABA-ergic function [14, 23, 24]. Although the barbiturates often depress postsynaptic excitability [25], the block of excitatory synaptic transmission is ascribed usually to presynaptic factors [26]. In the hippocampus, MacIver and Roth [27] reported depression of CA1 pyramidal neurone field potential responses by barbiturates. In agreement with this, we often observed small reductions in intrinsic excitability with methohexitone.

The slow after depolarization induced by methohexitone in our studies is of considerable interest. A similar long after depolarization has been reported by Alger and Nicoll [28] who studied the action of both pentobarbitone and phenobarbitone on CA1 pyramidal neurones. The depolarization was more pronounced with methohexitone, even at the lower concentration tested (50 μmol litre^{-1}). This after depolarization has been attributed previously to enhanced feedforward inhibition [28]. Such inhibition is mediated allegedly via dendritic GABA receptors. We have reported a similar after depolarization in CA1 neurones following application of 4-aminopyridine [Granger, Southan and Wann, in preparation], and in both cases the after depolarization led to a discharge of action potentials (fig. 3). It seems paradoxical that enhancement of an inhibitory process should lead to an excitant or convulsant effect. The relevance of this after depolarization to the convulsive properties of methohexitone remains to be determined.

REFERENCES

1. Joas TA, Stevens WC, Eger EI II. Electroencephalographic seizure activity in dogs during anaesthesia. *British Journal of Anaesthesia* 1971; **43**: 739–745.
2. Nunn JF. An anaesthetist looks at Malaya. *Lancet* 1954; **1**: 361–367.
3. Mori K, Kawamata M, Mitani H, Yamazaki Y, Fujita M. A neurophysiologic study of ketamine anesthesia in the cat. *Anesthesiology* 1972; **35**: 373–383.
4. Bowyer JF, Winters WD. The effects of various anesthetics on amygdaloid kindled seizures. *Neuropharmacology* 1983; **20**: 199–209.
5. Po BT, Watson RL, Hansen HR. Arousal time following intravenous anesthetic agents, methohexital and thiopental: effect of doxapram hydrochloride. *Anesthesia and Analgesia* 1968; **47**: 446–451.
6. Morris ME. Facilitation of synaptic transmission by general anaesthetics. *Journal of Physiology (London)* 1978; **284**: 307–325.
7. MacIver MB, Roth SH. Enflurane-induced burst firing of hippocampal CA1 neurones. *British Journal of Anaesthesia* 1987; **59**: 369–378.
8. Southan AP, Wann KT. Inhalation anaesthetics block accommodation of pyramidal cell discharge in the rat hippocampus. *British Journal of Anaesthesia* 1989; **63**: 581–586.
9. Marietta MP, Way WL, Castagnoli N, Trevor AJ. On the pharmacology of ketamine enantiomorphs in the rat. *Journal of Pharmacology and Experimental Therapeutics* 1977; **202**: 157–165.
10. Oshima E, Richards CD. An *in vitro* investigation of the action of ketamine on excitatory synaptic transmission in the hippocampus of the guinea-pig. *European Journal of Pharmacology* 1988; **148**: 25–33.
11. Halsey MJ, Wardley-Smith B, Green CJ. Pressure reversal of general anaesthesia—a multi-site expansion hypothesis. *British Journal of Anaesthesia* 1978; **50**: 1091–1097.
12. Torda TA, Gage PW. Post synaptic effect of i.v. anaesthetic agents at the neuromuscular junction. *British Journal of Anaesthesia* 1977; **49**: 771–776.
13. Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *British Journal of Pharmacology* 1983; **79**: 565–575.
14. Scholfield CN. Potentiation of inhibition by general anaesthetics in neurones of the olfactory cortex *in vitro*. *Pflugers Archives* 1980; **383**: 249–255.
15. Gage PW, Robertson B. Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. *British Journal of Pharmacology* 1985; 85: 675–681.
16. Catchlove RFH, Krnjevic K, Maretic H. Similarity between effects of general anaesthetics and dinitrophenol on cortical neurones. *Canadian Journal of Physiology and Pharmacology* 1972; **50**: 1111–1113.
17. Loyning Y, Oshima T, Yokota T. Site of action of thiamylal sodium on the monosynaptic spinal reflex pathway in cats. *Journal of Neurophysiology* 1964; **27**: 408–428.
18. Richards CD. On the mechanism of barbiturate anaesthesia. *Journal of Physiology (London)* 1972; **227**: 749–767.
19. Sato M, Austin GM, Yai H. Increase in permeability of the postsynaptic membrane to potassium produced by 'Nembutal'. *Nature (London)* 1967; **215**: 1506–1508.
20. Nicoll RA, Madison DV. General anesthetics hyperpolarize neurons in the vertebrate central nervous system. *Science* 1982; **217**: 1055–1057.
21. Nicoll RA. The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. *Journal of Physiology (London)* 1972; **223**: 803–814.
22. Wann KT, Macdonald AG. Actions and interactions of high pressure and general anaesthetics. *Progress in Neurobiology* 1988; **30**: 271–307.

23. Barker JL, Ransom BR. Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. *Journal of Physiology (London)* 1978; **280**: 355–372.
24. Collingridge GL, Gage PW, Robertson B. Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. *Journal of Physiology (London)* 1984; **356**: 551–564.
25. Somjen G, Gill M. The mechanism of the blockade of synaptic transmission in the mammalian spinal cord by diethyl ether and by thiopental. *Journal of Pharmacology and Experimental Therapeutics* 1963; **140**: 19–30.
26. Weakly JN. Effect of barbiturates on "quantal" synaptic transmission in spinal motoneurones. *Journal of Physiology (London)* 1969; **204**: 63–77.
27. MacIver MB, Roth SH. Barbiturate effects on hippocampal excitatory synaptic responses are selective and pathway specific. *Canadian Journal of Physiology and Pharmacology* 1987; **65**: 385–394.
28. Alger BE, Nicoll RA. Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology (London)* 1982; **328**: 105–123.

Methods for intracellular recording from hippocampal brain slices under high helium pressure

A. P. SOUTHAN AND K. T. WANN
Division of Anaesthesia, Clinical Research Centre, Harrow, Middlesex HA1 3UJ, United Kingdom

SOUTHAN, A. P., AND K. T. WANN. *Methods for intracellular recording from hippocampal brain slices under high helium pressure.* J. Appl. Physiol. 71(1): 365-371, 1991.—A method for intracellular recording from rat hippocampal brain slices under helium pressure is described. The preparation is mounted on a horizontal mobile platform that is rolled into the pressure chamber and can be viewed at pressure. Remote manipulation of the glass microelectrodes is achieved by a high-resolution electrically driven commercially available system. The slice is superfused continuously from a closed system within the chamber. Temperature is maintained at 37°C and Po_2 at 0.5 atm within the pressure chamber. A pressure of 200 ATA can be obtained, although thus far recordings have been made up to only 130 ATA. The experiments demand that a number of sample recordings be made from the same slice at both ambient and high pressure, and tests have proved that, although difficult, this can be achieved. The resting membrane potential, the current-voltage relationship, and the action potential responses to short (8 ms), medium (80 ms), and long (800 ms) depolarizing current pulses have all been measured in CA1 pyramidal neurons.

rat hippocampus; hyperbaric electrophysiology; pressure chamber

HIGH PRESSURE produces profound motor disturbances in all unanesthetized animals so far studied, including humans, and also modifies anesthetic depth (4, 14). However, the presumed central neuronal hyperexcitability induced by high pressure is still an unexplained phenomenon without exact parallels in other convulsive states.

Experiments designed to analyze the cellular basis of the in vivo actions of high pressure have concentrated on the in vitro effects of high pressure (either gaseous or hydrostatic) on vertebrate peripheral axons and synapses, invertebrate neurons, and vertebrate and invertebrate muscle (for review see Ref. 14). Such preparations are technically relatively straightforward to manipulate and enable the investigator to carry out detailed electrophysiological analyses (e.g., voltage-clamp) of the effects of high pressure (1, 5, 9). Such in vitro experiments have yet to shed light on the basis of the in vivo effects of high pressure, namely, the ability to induce hyperexcitability and seizures, and to reverse anesthesia (cf. Ref. 10). These phenomena may or may not depend on the same cellular mechanisms.

Clearly an understanding of the in vivo central effects of high pressure demands that we learn more about the effects of high pressure on the function of central neurons. We have therefore chosen to investigate the action of high pressure on CA1 pyramidal neurons of the hippocampus in vitro. We elected to study this brain region because it is an area that develops seizurelike events and the hippocampal slice has been an important preparation in the study of epileptogenesis (6). Also *N*-methyl-D-aspartate receptors have been implicated in the motor disturbances at high pressure (13), and synaptic pathways involving both *N*-methyl-D-aspartate and γ-aminobutyric acid are amenable to investigation in the hippocampal slice. Furthermore CA1 pyramidal neurons possess a range of membrane K^+ channel subtypes that modulate neuronal excitability and may be relevant targets for high-pressure action.

We report our methods and first results from intracellular experiments with rat hippocampal CA1 pyramidal neurons under high helium pressure.

METHODS

The high-pressure chamber. The pressure chamber (Fig. 1) was manufactured by Magpie (Fraserburgh, Scotland) from a steel cylinder (ST 52-3) to which flanges were welded at either end. These flanges accept the two doors, the undercarriages of which are mounted on four wheels, which run on V-shaped rails (E in Fig. 1). The doors are secured by bolts (45 mm diam) and are pressure sealed with Neoprene (70 SH) O rings. The cylinder length is 50.5 cm, the bore diameter is 33 cm, and the internal volume of the chamber is ~58 liters. The cylinder wall thickness is 36 mm. The working and hydrostatic test pressures are 200 and 285 ATA, respectively. The chamber interior is kept at ~28°C by circulation of hot water through rubber tubing that is coiled around the chamber body. This minimizes the heat loss from the preparation due to the high thermal conductivity of helium. An external steel pipe connected in parallel with the main body of the chamber is designed to carry charcoal and two fans to permit removal of anesthetic gases. On the inner side of one door is mounted a direct-current (DC) fan (Papst multifan 8312) to aid gas mixing and a brass radiator through which water of various temperatures (10–60°C) can be circulated.

Gas lines. It is important that the flow of gas does not cause movement of the solution in the recording chamber or the microelectrode tip. For this reason the tissue chamber is positioned such that the gas inflow is not directed at the recording assembly. Gases are supplied from cylinders via a control panel on which are mounted

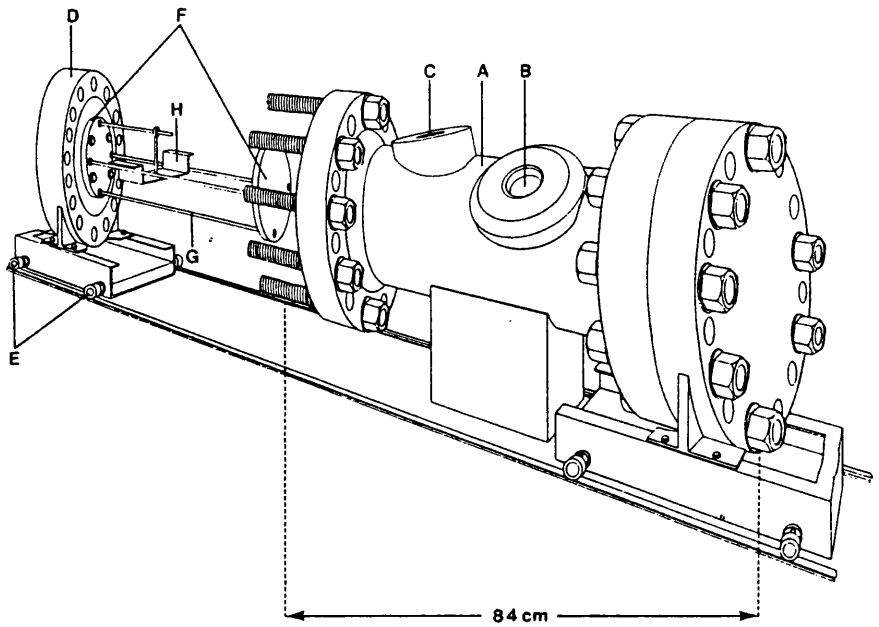


FIG. 1. Artist's impression of high-pressure chamber showing principal features of construction. A, chamber body with viewing (B) and lighting (C) ports. Ports are made from acrylic and are either conical or cylindrical in long section. D, mobile door, mounted on wheels (E), carrying the cradle, which consists of Perspex disks (F) and supporting rods (G). H, suspended tray, which holds 4-channel peristaltic pump and reservoir of artificial cerebrospinal fluid (volume 150 ml, not shown). A panel of electrical sockets and the "cradle," which supports all equipment normally required for electrophysiological work, are attached to disk bolted on door D. Four ¾ in. National Pipe Thread (NPT) holes (not shown) penetrate door D, and there are an additional two ½ in. NPT penetrators on the ventilation pipe (not shown). There are potentially 96 electrical connections to chamber interior, and at present we use routinely 2 × 16-way Conax electrical connectors (TG-20-16, Hellerman Electronic Components), which carry low DC voltages (at least ±15 V). Gas inflow or outflow occurs via ¼ in. NPT penetrators in chamber body (not shown), individual lines being used for helium (compression), oxygen, decompression, and anesthetic gases (e.g., N_2O). A fifth penetrator is connected to chamber pressure gauges, 0–20 and 0–133 atm (Negretti and Zambra), and associated relief valves (e.g., Nupio R3A, cracking pressure set to 133 atm).

high-pressure self-venting regulators (44-1100, Tescon; PR56, Go Products) and associated ball-and-needle valves (Whitey). All connections are made of copper tubing (4 mm ID, wall thickness 1 mm). Very slow compression rates can be achieved (e.g., 0.1 atm/min).

Visualization of the brain slice. Viewing and illumination of the tissue preparation within the pressure chamber is essential to make impalements of the CA1 pyramidal neurons either before compression or at high pressure. A conical viewport (136 × 50 mm, B in Fig. 1) provides a view of the length of the chamber interior and is used to view the preparation with the aid of a Nikon Stereozoom (SMZ-2B) microscope (working distance ~20 cm, magnification ×12). The flat viewport (C in Fig. 1) is set at a 20° angle to the conical viewport and is used to provide illumination from the end of the swan-neck fiber-optic pipes of a Schott KL 1500 light source.

Internal fittings. A principal consideration in the design of the internal fittings is that the recording chamber should be mechanically stable in order that long-duration (>1 h) intracellular recordings can be routinely carried out. To this end, a cradle was fixed to the inner face of one of the chamber doors (Fig. 1). An aluminum electrically driven heating block controls the temperature of the recording chamber artificial cerebrospinal fluid (ACSF) between 30 and 34°C at an ambient temperature of 20°C. Temperature measurement is made with a precision thermistor (model 44033, Yellow Springs Instrument) and a telethermometer (Yellow Springs Instrument). The tissue chamber and heating block (C and D, respectively, in Fig. 2, *top*) are mounted on a microscope stage that provides movement in two directions. This in turn is connected to a vertical adjustable rod attached to rod G (Fig. 1). Four coarse vertical brass manipulators (e.g., E in Fig. 2, *top*) are used to hold the inlet and outlet perfusion pipes and a restraining "bat" for the slice preparation. A coarse manipulator was also used to position stimulating electrodes on the slice (stratum radiatum or the alveus).

The slice preparation is mounted on a stainless steel wire mesh in the central well of the tissue chamber insert (5-ml volume) and is held by the strands of a small stainless steel bat (Fig. 2, *bottom*). To minimize solution movement around the slice, circulating solutions were delivered to the central well by gravity feed and withdrawn from a side compartment (Fig. 2, *bottom*). Solutions were delivered to the reservoir and withdrawn from the side compartment by a peristaltic pump, and to prevent overflow problems from the tissue chamber, solutions were circulated continuously even when the gas tensions of

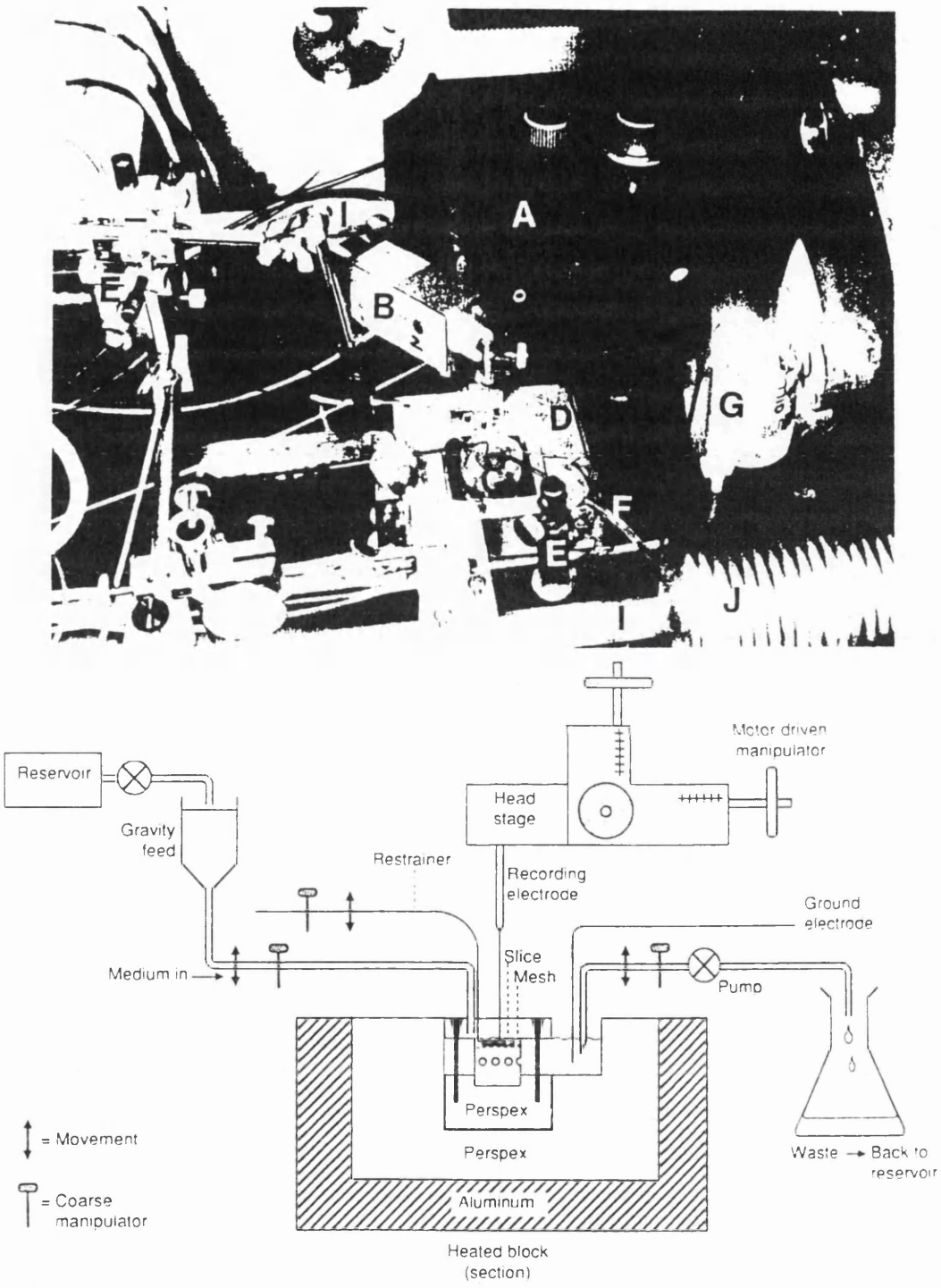


FIG. 2. *Top*: photograph of some of the apparatus mounted on cradle within chamber. A, electrically driven micromanipulator; B, axoclamp headstage; C, tissue chamber; D, heating block; E, course manipulators; F, inlet pipe to recording chamber; G, gravity-feed reservoir of artificial cerebrospinal fluid; H, Perspex disk; I, Duralumin rods; J, chamber bolt. *Bottom*: schematic diagram of tissue chamber (cross section), perfusion system, brain slice, and microelectrode.

the chamber atmosphere and the bathing solutions were at equilibrium (see below).

The voltage-recording microelectrode is connected to an Axoclamp 2A headstage amplifier (B in Fig. 2, *top*) via a AgCl wire or a AgCl half-cell (EH-IR/1.0, Clark Electromedical). The headstage amplifier is held by an electrically driven manipulator (DC-3 K Micro Instruments, A in Fig. 2, *top*) clamped to a supporting rod. A remote-control unit (MS-316) for the micromanipulator is mounted on a shelf below the viewport. The electrode can be advanced in steps as small as 1 μm.

Experimental protocol for intracellular measurements. Experiments were performed on rat hippocampal slices. Six-week-old male rats (180–200 g, Sprague-Dawley) were decapitated; the brain was quickly dissected out, chilled, and hemisected; and transverse slices of hippocampus (400 μm thick) were cut in chilled ACSF with a Vibroslice (Campden Instruments). After at least 1 h of storage at room temperature (18–25°C), one slice was transferred to an experimental chamber where it was continuously superfused with control ACSF, the flow rate being typically 2–3 ml/min. The standard ACSF contained (mM) 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, and 10 D-glucose and was maintained at pH 7.35–7.45 with 95% O_2-5% CO_2. Intracellular recordings are made using glass microelectrodes filled with 4 M K acetate (70- to 120-MΩ resistance). These are pulled from Kwik-fil capillaries (GC 100F-15, Clark Electromedical) on a Sutter Instruments Flaming-Brown puller (model P.80/PC).

Trial impalements are first made with the chamber door open to establish the stability and viability of the slice preparation. If satisfactory stable recordings (>10 min) are obtained, the microelectrode is withdrawn and the door is quickly and smoothly closed and secured while 0.3 atm of 95% O_2-5% CO_2 is added (measured using the low-pressure gauge 0–2 atm) until the chamber P_{O_2} was at least 0.5 ATA (see below). The chamber is preheated to ~28°C, and under these conditions the heating block maintains the temperature of the ACSF within the tissue chamber at 36–37°C. This temperature is reached within 10 min of closing the door.

In early experiments recordings were made at this stage (i.e., chamber pressure of 1.3 ATA), and these served as control data. In later experiments, recordings were only made having attained the desired pressure. The compression (with helium gas) was typically commenced within 10 min of closing the door and was usually 1–2 atm/min. During compression, the internal fan was run at a faster rate to ensure thorough gas mixing. The faster compression rate (e.g., 2 atm/min) often caused small temperature increases of 0.5–1.5°C, which could be minimized by temporarily switching off the heating block or the internal radiator. On reaching the desired pressure, the fan was turned down and recordings were attempted immediately after any temperature changes had dissipated (~1 min).

Headstage amplifier problems at high pressure. The operational amplifier in the Axoclamp headstage (Burr Brown OPA 103 CM) was initially pressure tolerant up to 65 ATA, at which point it ceased to work. Its function was restored to normal on decompression by 1 atm. Indeed compression and decompression at ~65 ATA showed that the amplifier was a reliable and sensitive indicator of the ambient pressure. The T099 can of the amplifier clearly dimpled at high pressure, and the problem was solved by drilling a small hole in the top surface. All the other electrical equipment, namely, the micromanipulator, peristaltic pump, heating block, thermistor, and fan, function normally during repeated compression and decompression.

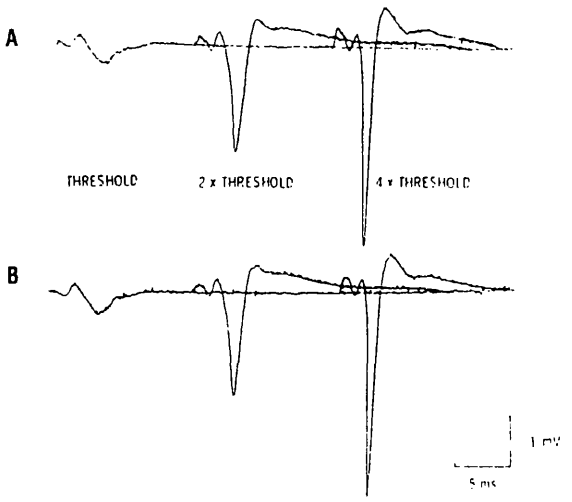


FIG. 3. Extracellular field potential responses to stimulation of Schaffer collaterals. Orthodromic responses were produced in hippocampal slice by a unipolar metal electrode of tungsten wire (50 μm diam; WT-2T, Clark Electromedical) in stratum radiatum. Stimulus parameters were 0.1 Hz, 1–10 V, 80 μs. Recording glass microelectrode was filled with 4 M NaCl. Temperature, 36°C. *A*: slice in chamber at 1.3 ATA after 5 min; *B*: slice in chamber at 1.3 ATA 45 min later.

RESULTS

Recording at 1–1.3 ATA. In our first experiments the viability of the hippocampal slice preparation within the chamber was assessed in field potential studies where the Schaffer collaterals were stimulated. Figure 3 shows typical orthodromic responses recorded from the CA1 field at 1.3 ATA. The population spike amplitude was stable, and in six experiments there was no evidence of multiple population spike responses after 1 h (maximum period tested). Subsequently experiments were confined to establishing the feasibility of intracellular recording from hippocampal CA1 pyramidal neurons by use of the pressure chamber door-cradle arrangement. The latter assembly clearly damps down undesirable vibration, ensuring reliable intracellular recording with the chamber door open. However, recordings cannot yet be maintained during chamber door closing. The selection criteria for satisfactory cells were resting membrane potential greater than −55 mV, input resistance >20 MΩ, and action potential height >60 mV (measured from threshold potential). Usually the firing of such neurons adapts or accommodates to long (e.g., 800 ms) depolarizing current pulses (12). Conditions were suitably stable to enable recordings to be made over periods of up to at least 2 h (maximum period tested). Mean data from 29 CA1 pyra-

TABLE 1. *CA1 pyramidal neuron parameters*

	Resting Membrane Potential, mV	Input Resistance, MΩ	Action Potential Amplitude, mV	Threshold Potential, mV
Out of chamber				
1 ATA	−70.17±0.30 (29)	33.60±1.23 (29)	71.9±1.54 (29)	−56.9±0.46 (29)
In chamber				
1.3 ATA	−69.00±2.70 (11)	33.10±1.89 (11)	74.5±2.49 (11)	56.5±0.74 (11)
51 ATA	−65.9±2.13 (10)	35.67±2.33 (8)	74.8±2.32 (10)	56.4±0.76 (10)
101 ATA	−65.2±1.98 (9)	34.2±1.54 (6)	73.4±3.09 (9)	55.4±0.97 (9)

Values are means ± SE of no. of rat hippocampal slices in parentheses.

midal neurons are shown in Table 1. The mean resting membrane potential, input resistance, and action potential amplitude were −70.17 mV (range −55 to −85 mV), 33.6 MΩ (range 24–45 MΩ), and 71.9 mV (range 60–90 mV), respectively.

The disadvantage of intracellular work with CA1 pyramidal neurons is that no movement can be tolerated. The microelectrode has therefore to be withdrawn from the brain slice while the chamber door is wheeled forward, the bolts to secure the door tightened, and 95% O_2-5% CO_2 added to the chamber. Impalement of CA1 pyramidal neurons within the chamber by remote control with the reduced visibility is considerably more difficult but again, with practice, can be achieved with a tolerable success rate. (Successful recordings have been made at 1.3 ATA in ~40% of slices tested.) Stable recordings (criteria as above) have been made in the 0.5 ATA O_2 atmosphere within the chamber for periods of up to 2 h (Fig. 4*A*). The characteristics of CA1 pyramidal neurons at 1.3 ATA were indistinguishable from those at 1 ATA with the chamber door open (Table 1).

Measurement of ACSF Po_2, Pco_2, and pH. The gas tensions and pH of liquid samples from the tissue chamber (at 1.3 or 21 ATA) were measured by connecting fine tubing to a stainless steel pipe that penetrated the door. When a tap was opened on the outside of the door, ACSF could be drawn off. Typical values at 36 or 37°C after 1 h were Po_2 = 0.48 ATA, Pco_2 = 0.036 ATA, and pH = 7.54 (measured by blood gas analyzer, Instrumentation Laboratory System 1302). These values are within the range of values obtained when viable recordings are made from hippocampal slices outside the pressure chamber. The Po_2 of the gas in the chamber has also been sampled from the decompression line (chamber pressure 1.3 and 51 ATA) and analyzed using a Taylor Servomex O_2 analyzer (OA 272). The Po_2 was between 0.50 and 0.75 ATA. In independent experiments (conducted using the cradle assembly with the door open), the Po_2 of the ACSF was allowed to fall by switching off the delivery of gas (95% O_2-5% CO_2) to the reservoir of ACSF. When the Po_2 falls to low values (e.g., 0.2 ATA) and the pH rises (due to low Pco_2), the neurons depolarize markedly (15 mV) within minutes. This effect is usually reversible if the Po_2 is reduced for only a short period of time (e.g., 5 min). These experiments clearly indicate the consequences of reducing the O_2/CO_2 supply and assure us that adequate oxygenation is provided within the chamber.

Recording at high pressure. Maintaining intracellular recordings while compressing the chamber with helium has so far been generally unsatisfactory. Indeed recordings cannot be held reliably during either compression or decompression. In six experiments (when compression was commenced from 1.5 ATA), recordings have been lost at pressures ranging from 3 to ~30 ATA. In one experiment where an impalement was made at 20 ATA, recording was again lost at ~26 ATA during subsequent compression. In all cases the microelectrode is dislodged rapidly from the neuron. We believe that this is due to movement of the microelectrode itself and is not caused by movement of gases within the chamber. Compression and distortion of a component are probably responsible, and we are working to reduce this. Currently we usually compress to a specified pressure (usually 51 or 101 ATA) and then make intracellular recordings. In three experiments it has proved possible to maintain intracellular recording during compression above 51 ATA (see Fig. 4*C*). Stable recordings have been made from 32 neurons in 20 slices within the chamber at pressures between 1.3 and 120 ATA. Impalements have been made immediately on arriving at high pressure and up 1 h after reaching 51 or 101 ATA. Typical data are shown in Table 1 and Fig. 4. Measured resting membrane potential, input resistance, action potential amplitude, and threshold potential values of CA1 neurons at pressures of up to 101 ATA lay within the normal range (Table 1). The viability of the neurons at high pressure (in particular 51 and 101 ATA) indicated that there was no appreciable layering of gases within the chamber (O_2 is denser than He).

Variations in the DC potential of the input circuit were often observed during pressurization. These changes in asymmetry potential were small (≤3 mV over 1.3–101 ATA) and could be positive or negative and therefore were not readily attributable to an individual pressure-dependent junction potential (e.g., at the microelectrode tip). Because the resting membrane potential measurements were not derived from experiments where continuous recording during pressurization was made, no DC error from this source is introduced. In a few cases, unacceptable changes in microelectrode resistance occurred at high pressure and the experiment was abandoned.

If the microelectrode was broken at high pressure and then the chamber was decompressed, the door was un-

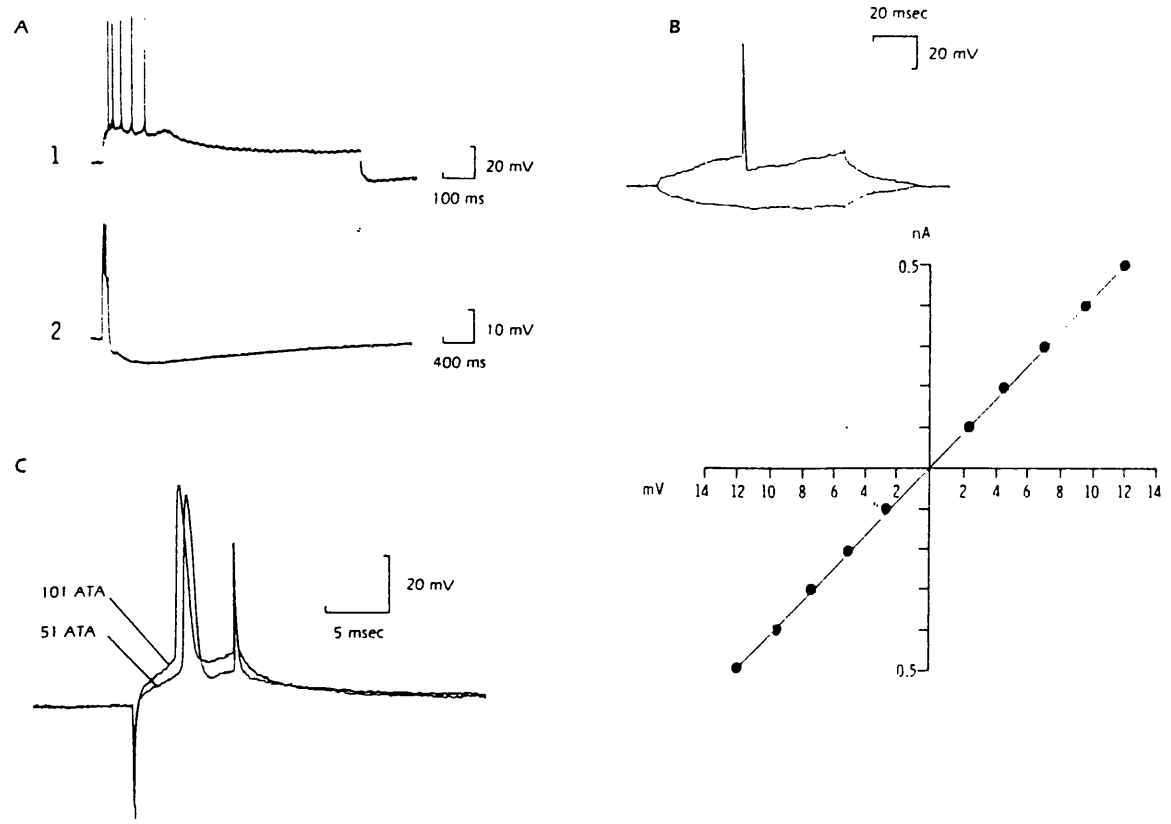


FIG. 4. Intracellular recordings from 3 CA1 pyramidal neurons impaled inside pressure chamber. *A*: 1.3 ATA; *trace 1* shows action potential response to an 800-ms/0.5 nA depolarizing current pulse; *trace 2* shows afterhyperpolarization following an 80-ms/0.5 nA depolarizing current. *Trace 2* was recorded within 10 s of *trace 1*. Temperature, 36.5°C; resting membrane potential, −60 mV; input resistance, 26 MΩ. *B*: current-voltage relationship of a neuron at 51 ATA. Current pulses were 80-ms duration; records show response to a 0.5-nA hyperpolarizing and 0.6-nA depolarizing current. Temperature, 36.5°C; resting membrane potential, −68 mV; input resistance, 24 MΩ. *C*: action potential responses to 8-ms depolarizing currents. Data for the same neuron at 51 and 101 ATA are shown. Resting membrane potential and input resistance, −68 mV and 32 MΩ, respectively; temperature, 37°C (51 ATA) and 38°C (101 ATA).

bolted and the slice was discarded. O_2 was not added to the chamber during decompression, so the Po_2 of the ACSF dropped markedly during this period (~20 min from 101 ATA). This factor, in addition to the bubble formation in the tissue chamber, made it impossible to obtain decompression data from successful recordings.

DISCUSSION

The pressure chamber and apparatus described here have enabled the first intracellular recordings to be made from a mammalian brain slice at high helium pressure. Previously, methods have been described for intracellular recordings from invertebrate neurons or vertebrate muscle at high pressure (7, 8, 11, 15); however, the technical demands of carrying out equivalent studies with mammalian central neurons are somewhat greater. The major problems here are those of vibration, visibility, and the need for quality remote manipulation. Such difficulties when added to the problem of working within a confined space, which at pressure is inaccessible to the investigator, make the experiment a challenging one.

Thus far it has not proved possible to record continuously from hippocampal neurons during pressurization. Previously, Wann et al. (15) have achieved twin-microelectrode measurements in invertebrate neurons during pressurization in a chamber where internal fittings were in principle of similar design. In the case of hippocampal neurons it has been necessary to adopt a different approach, namely, to make sample recordings from different neurons at 1.3 ATA (control) and 51 and 101 ATA (test). It is consequently necessary to make multiple stable impalements in each slice to obtain any high-pressure data.

The pressure chamber described here has a number of advantages over previous systems, which have been crucial to the success of our experiments with the hippocampal slice. First, the internal diameter and the arrangement of the internal fittings make it possible to put the headstage amplifier and a remotely driven micromanipulator inside the pressure chamber without compromising the viewing of the preparation. This would not be possible to achieve in a smaller chamber (8) and is obviously essential for intracellular work using high-resistance (80 MΩ) electrodes to optimize the frequency response and

electrical noise. Second, the position and relative orientation of the two viewports enable the CA1 field within the slice to be readily viewed within the chamber. By adjusting the orientation of the slice and the angle of the reflected light, visibility can be optimized, and we found it to be superior to that achieved by using transmitted light (2). Third, with the closed perfusion system adopted, it is possible to equilibrate the perfusing solutions with the chamber gas, which meant that we could dispense with the gassing (95% O_2-5% CO_2) of solutions within the chamber. This offered us the opportunity to equilibrate the bathing solutions with anesthetic gases at high pressure. The perfusion rate could be varied within limits by adjusting the pump delivery rate and, hence, the height of fluid in the gravity feed; however, the addition of drugs was not possible. (Use of a high-pressure liquid chromatography pump in subsequent studies enabled us to carry out such experiments, however, and also took us a step closer to a pure hydrostatic pressure study.) Fourth, the door carrying the cradle can be closed and secured during extracellular recording from the slice preparation. The first-hand experience of one of the authors (*KTW*) is that this can be achieved more quickly in the chamber described here than in the system of Fagni et al. (2). It seems unlikely that a door closure system that permits continuous intracellular recording from hippocampal neurons will be found, although previous work has shown that this can be achieved with less demanding preparations (7, 15).

Our chamber is fitted with only one remote-control manipulator rather than the four used by Fagni et al. (2). This in no way limited the intracellular measurements in our study but reduced the amount of information to be gained from corresponding field potential recordings (e.g., only one possible recording site).

Our present results show that the passive membrane properties (the resting membrane, input resistance) of CA1 pyramidal neurons are unaltered by pressure in the range 1–100 ATA. There is also no significant change in the action potential amplitude or the threshold potential at the pressures so far tested. The basis of the hyperexcitability induced by high pressure needs therefore to be investigated further in future experiments.

We acknowledge the invaluable assistance and guidance of the staff of Bioengineering and Bridget Wardley-Smith (Division of Anaesthesia).

Address for reprint requests: K. T. Wann, Dept. of Physiology, Royal Free Hospital School of Medicine, University of London, Rowland Hill St., London NW3 2PF, UK.

Received 11 July 1989; accepted in final form 5 March 1991.

REFERENCES

1. CONTI, F., R. FIORAVANTI, J. R. SEGAL, AND W. STÜHMER. Pressure dependence of the sodium currents of squid giant axon. *J. Membr. Biol.* 69: 23–34, 1982.
2. FAGNI, L., M. HUGON, A. FOLCO, AND G. IMBERT. A versatile chamber for microphysiologic studies with gas mixtures under high pressure. *Undersea Biomed. Res.* 14: 161–168, 1987.
3. FAGNI, L., F. ZINEBI, AND M. HUGON. Evoked potential changes in rat hippocampal slices under helium pressure. *Exp. Brain Res.* 65: 513–519, 1987.
4. HALSEY, M. J. Effects of high pressure on the central nervous system. *Physiol. Rev.* 62: 1341–1377, 1982.
5. HARPER, A. A., A. G. MACDONALD, AND K. T. WANN. The action of high hydrostatic pressure on the membrane currents of *Helix* neurones. *J. Physiol. Lond.* 338: 325–339, 1981.
6. HEINEMANN, U. Basic mechanisms of the epilepsies. In: *A Textbook of Clinical Neurophysiology*, edited by A. M. Halliday, S. R. Butler, and R. Paul. New York: Wiley, 1987, p. 497–534.
7. HENDERSON, J. V., JR., R. A. MORIN, AND E. H. LANPHIER. Apparatus for intracellular electrophysiological measurements at 200 ATA. *J. Appl. Physiol.* 38: 353–355, 1975.
8. HOGAN, P. M., H. C. ÖRNHAGEN, T. J. DOUBT, B. S. LARAWAY, R. A. MORIN, AND J. ZAHARKIN. Hyperbaric chamber for evaluating hydrostatic pressure effects on tissues and cells. *Undersea Biomed. Res.* 8: 51–58, 1981.
9. KENDIG, J. J. Ionic currents in vertebrate myelinated nerve at hyperbaric pressure. *Am. J. Physiol.* 246 (*Cell Physiol.* 15): C91–C95, 1984.
10. KENDIG, J. J., Y. GROSSMAN, AND M. B. MACIVER. Pressure reversal of anaesthesia: a synaptic mechanism. *Br. J. Anaesth.* 60: 806–816, 1988.
11. MACDONALD, A. G., AND K. T. WANN. Intracellular recording at high pressure (Abstract). *J. Physiol. Lond.* 322: 1P, 1982.
12. MADISON, D. V., AND R. A. NICOLL. Control of the repetitive discharge of rat CA1 pyramidal neurones in vitro. *J. Physiol. Lond.* 354: 319–331, 1984.
13. MELDRUM, B., B. WARDLEY-SMITH, M. HALSEY, AND J. C. ROSTAIN. 2-Amino-phosphonoheptanoic acid protects against the high pressure neurological syndrome. *Eur. J. Pharmacol.* 87: 501–502, 1983.
14. WANN, K. T., AND A. G. MACDONALD. Actions and interactions of high pressure and general anaesthetics. *Prog. Neurobiol.* 30: 271–307, 1988.
15. WANN, K. T., A. G. MACDONALD, A. A. HARPER, AND S. E. WILCOCK. Electrophysiological measurements at high hydrostatic pressure: methods for intracellular recording from isolated ganglia and for extracellular recording in vivo. *Comp. Biochem. Physiol. A Comp. Physiol.* 64: 141–147, 1979.