ENFLURANE-INDUCED BURST FIRING OF HIPPOCAMPAL CA 1 NEURONES

In Vitro Studies using a Brain Slice Preparation

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EEG activation and seizure activity can be produced by cyclopropane (Mori, 1973), ether (Joas and Eger, 1971), ketamine (Bowyer and Winters, 1981) and enflurane (Black, 1979; Steen and Michenfelder, 1979). Of these, enflurane is the only agent in clinical use which produces seizure activity during anaesthesia (Persson, Peterson and Wahlin, 1978); it exhibits a profile of excitatory and depressant CNS effects similar to those of phencyclidine, gamma-hydroxybutyrate, and alpha-chloralose (Winters, 1982). The convulsive actions of enflurane may result from excitation or depression, or both, in the CNS, since both have been observed during enflurane anaesthesia (Darimont and Jenkins, 1977; Steen and Michenfelder, 1979). Although the neurophysiological basis of anaesthetic-induced excitatory and convulsive actions is unknown, it is unlikely that a simple depression of neuronal excitability or synaptic transmission could account for all of the pharmacological actions of these agents (Roth, 1980; Richards, 1983).

A study using a chronic recording technique in cats demonstrated that enflurane-induced seizure activity was associated with concentration-dependent excitatory and depressant actions on reticular neurone firing, cortical evoked responses, and EEG activity (Stevens et al., 1984). Increases in brain stem firing activity and cortical responses were observed at sub-anaesthetic concentrations, while depression of reticular activity occurred at concentrations which produced surgical anaesthesia. In the cortical EEG, seizure activity was observed when reticular activity was depressed, although cortical evoked responses were enhanced (Stevens et al., 1984). These observations suggest that anaesthetic-induced seizure activity could be associated with either a release of brain stem inhibition or a direct excitation of cortical neurones.

SUMMARY

Enflurane can produce seizure activity in the cortical EEG, in vivo, at concentrations associated with surgical anaesthesia. The present study was designed to determine whether this seizure-like burst activity could occur in isolated cortical neurones. Enflurane altered synaptic transmission in the in vitro rat hippocampal slice preparation and produced seizure-like burst discharges of CA 1 neurones, at vapour concentrations equivalent to those obtained during anaesthesia (2–6 vol %; 0.5–1.5 mmol litre⁻¹). Burst discharges occurred both spontaneously and in response to stimulation of stratum radiatum fibres in the CA 1 pyramidal region, but not in the dentate area. Low concentrations of enflurane (approx. 0.75 mmol litre⁻¹), decreased the field potential responses of CA 1 neurones; however, dentate granule neurone responses were increased. Input/output analyses of field excitatory post-synaptic potential (EPSP) and population spike amplitudes revealed that the enflurane-induced depression of field potential responses was associated with decreases in synaptic input, whereas burst activity resulted from a decrease in the threshold of CA 1 neurones.
In the present study, the effects of enflurane were examined on the hippocampal brain slice preparation to determine whether this anaesthetic agent could produce activation and seizure-like activity in isolated cortical neurones. Effects on CA 1 pyramidal and dentate granule neurones were compared to determine whether the two populations of cortical neurones demonstrate different susceptibilities to anaesthetic-induced seizure activity. Such differential actions between CA 1 and dentate granule neurones would also provide support for selective, pathway-specific sites of action, as previously reported for other general anaesthetic agents (Roth, Bland and MacIver, 1983; Roth, Tan and MacIver, 1986).

Preliminary results have been published in an abstract (MacIver, Harris and Roth, 1984).

MATERIALS AND METHODS

Preparation

Experiments were conducted on 31 hippocampal slices; 18 for CA 1 responses, and 13 for dentate granule (DG) neurones. Rats were anaesthetized with ether, the heart stopped by a blow to the back of the thorax and the brain removed rapidly and placed in pre-cooled (10 °C) oxygenated artificial cerebrospinal fluid (CSF). Transverse slices (400 μm) of hippocampus were cut using a vibratome (Campden Instruments, U.K.), following dissection of the dentate-hippocampal formation. Slices were placed on a nylon mesh screen at the gas-liquid interface in a tissue chamber (Richards and Tegg, 1977). Oxygenated (95% oxygen-5% carbon dioxide), prewarmed (35 °C) CSF was continuously perfused through the bath at a rate of 1–1.5 ml min⁻¹.

Electrode placement

A bipolar nichrome stimulating electrode was placed on perforant path fibres or in the stratum radiatum to activate excitatory synaptic inputs to DG or CA 1 pyramidal neurones, respectively (fig. 1). Similarly, antidromic responses were produced by stimulation of the alveus or mossy-fibre pathways. Extracellular recording electrodes (sodium chloride 2 mol litre⁻¹, 2–10 MΩ) were placed in the cell body regions of DG and CA 1 areas to record synaptically evoked field potentials (FP) and unit discharge activity. Paired stimulus pulses of 0.01–0.05 ms duration (25–70 μA) were

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**Fig. 1.** Diagram of a hippocampal slice showing placement of stimulating (S) and recording electrodes (R). Sample recordings from CA 1 and DG demonstrate paired pulse potentiation in both pathways. Arrows indicate portions of recordings used to determine EPSP slope (dV/dt) and population spike (PS) amplitude. Calibration: 2.0 mV and 10 ms.
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delivered at 0.1 Hz. Stimulus intensity was varied to determine input/output relationships at a fixed interstimulus interval of 25 ms (Andersen et al., 1980; Abraham, Bliss and Goddard, 1985). In addition, interpulse interval delays were varied between 5 and 120 ms, in 5-ms increments, to examine the time course of short-term potentiation as a measure of synaptic facilitation and local inhibition. Recorded signals were amplified (×1000), filtered (1 Hz to 10 kHz, bandpass), and stored digitally for later analysis. Population spike (PS) amplitudes were measured from threshold to peak negativity and excitatory post-synaptic potential (EPSP) rise times (dV/dt) were measured on the initial EPSP slope (fig. 1). Rise times were determined by linear regression analysis of data between 20 and 80 % of the EPSP peak positivity.

Administration and analysis of enflurane

Enflurane was applied as a vapour to the tissue chamber via the prewarmed and humidified oxygen–carbon dioxide gas stream above the brain slices, using a calibrated commercial vaporizer (Ohio Medical Products, Madison, Wis.). Concentrations, expressed as volume percent (vol %), refer to settings on the vaporizer dial. Anaesthetic concentrations equivalent to minimum alveolar concentrations required to maintain anaesthesia (MAC = 2–4 vol %) (Stevens et al., 1984) were used. To reach equilibrium, vapour concentrations were applied for a minimum period of 30 min. Accurate measurements of enflurane concentrations in the perfusate were obtained using a Hewlett-Packard 5880A gas chromatograph fitted with a glass column, packed with Poropak Q, and a flame ionization detector. The carrier gas was nitrogen delivered at a flow rate of approximately 40 ml min⁻¹. Temperatures of the injector, oven and detector were 155, 140 and 170 °C, respectively. Test samples (20 µl) were taken from the recording chamber with a gas-tight Hamilton syringe and injected directly to the column. Peak:area ratios from the test samples were plotted against those from known standards to determine bath concentrations (fig. 2). Dextrose was used as an internal standard in the artificial CSF. Column retention times were approximately 3.5 and 7.5 min for enflurane and dextrose, respectively.

Materials

Adult male Sprague–Dawley rats (200–300 g) were obtained from the University of Calgary Biosciences Vivarium. Enflurane was obtained from Ohio Medical Products (Pointe Claire, Que.). The artificial CSF physiological solution had the following composition (mmol litre⁻¹): NaCl 134; KCl 4.5; CaCl₂ 1.6; KH₂PO₄ 1.25; MgSO₄ 2; NaHCO₃ 16; dextrose 10 (modified from Richards, Russell and Smaje (1975)). Calcium and potassium concentrations were adjusted to the approximate values measured in vivo (Krnjevic, Morris and Reiffenstein, 1982).

![Figure 2](image-url)

**Fig. 2.** Linear relationships between standard concentrations (Std), vaporizer dial settings (Dial) (vol %) and integrated peak area ratios (Area) are shown. Each point is the mean ± SD of at least four determinations. Examples of gas chromatograph recordings are shown in the insets for 1.5 mmol litre⁻¹ STD and 4.0 vol % samples (peak a = enflurane; peak b = dextrose).
RESULTS

Enflurane-induced burst activity

Figure 3 shows typical recordings from CA 1 and DG regions during enflurane-induced field bursts. CA 1 burst discharges were concentration-dependent, with low concentrations (0.5–2.0 vol %; 0.5–0.9 mmol litre\(^{-1}\)) producing only small amplitude discharges, and higher concentrations (2.5–5.0 vol %; 1.0–1.3 mmol litre\(^{-1}\)) producing large amplitude field bursts which increased in frequency. Both spontaneous and stimulus-evoked burst activities were observed in the CA 1 area. Burst discharges were periodic and did not occur in response to each stimulus. Instead, burst activity occurred intermittently throughout the period of exposure to enflurane. Spontaneous burst discharges were not observed in DG neurones, and perforant path evoked responses appeared relatively normal at concentrations of enflurane which produced marked seizure-like activity in the CA 1 area. Enflurane-induced burst firing of CA 1 neurones could, occasionally, be recorded in the dentate area (volume conduction), and resulted in a late "ringing" of the perforant path to DG neurone evoked responses which occurred 15–20 ms after the DG population spike (figs 3 and 4). These late responses appeared to be CA 1 neurone bursts which were synchronized with the perforant path stimulus (fig. 3), perhaps triggered via DG neurone activation of CA 3 neurones which subsequently synapse onto CA 1 cells (Andersen, 1975), or via direct perforant path activation of CA 1 neurones. CA 3 neurone firing patterns were not altered in the presence of enflurane (data not shown; three experiments), and multiple fibre-volleys were not recorded in stratum radiatum at concentrations which produced spontaneous or evoked seizure discharges in CA 1 neurones. Multiple firing of DG neurones was never observed, even in the presence of vapour concentrations which produced increased field potential responses (for example: 0.5–2.5 vol %; 0.5–1.0 mmol litre\(^{-1}\)). Thus, enflurane-induced seizure activity appeared to be localized to the CA 1 region; however, a more detailed study of CA 1 and CA 3 responses is required to determine how perforant path activation may result in CA 1 neurone burst firing.

Analysis of field potentials

A characteristic field potential was recorded from the cell body layers when input fibres to either DG or CA 1 cells were electrically stimulated. Weak stimuli (10–15 μA) produced a positive waveform (field EPSP) which originated from the synchronous depolarization of postsynaptic neurones in response to the release of transmitter from input fibres (fig. 1) (Andersen, Blackstad and Lomo, 1966; Langmoen and Andersen, 1983). When the intensity of the stimulation was increased or potentiation occurred, or both, the positive waveform increased in amplitude and a negative spike appeared superimposed on the EPSP (compare responses to 1st and 2nd stimulus pulses in figure 1). This negative waveform reflects the synchronous discharge of postsynaptic neurones (Andersen, Bliss
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Fig. 4. DG: Multiple sweeps of field potentials produced by perforant path inputs to DG neurones at various stimulus intensities. These data were used for the input/output curve in figure 5. In the presence of enflurane 0.9 mmol litre\(^{-1}\) both EPSP and PS amplitudes were increased. At greater concentrations (1.4 mmol litre\(^{-1}\)) PS responses were depressed, although EPSP amplitudes remained above control. On washout (W), responses returned to control (C) values. CA 1: Enflurane depressed both the EPSP and PS of stratum radiatum to CA 1 evoked field potentials. Calibration: 5 mV and 20 ms (DG), 10 ms (CA 1).

and Skrede, 1971; Bliss and Richards, 1971). The relationship between the positive synaptic potential (EPSP) and the amplitudes of the negative population spike (PS) describes an input/output function (E–S curve) for the pathway (Richards and White, 1975; Andersen et al., 1980; Abraham, Bliss and Goddard, 1985). The intercept on the EPSP axis provides a measure of postsynaptic discharge threshold, and the slope of the curve describes depolarization–excitation coupling (fig. 5) (Lomo, 1971; Richards, Russell and Smaje, 1975; Abraham, Bliss and Goddard, 1985).

**Effects of enflurane on evoked field potentials**

Enflurane produced concentration-dependent biphasic effects on perforant path to DG evoked field potentials, but only a monophasic depression of CA 1 responses. Low concentrations (0.5–2.0 vol %; 0.5–0.9 mmol litre\(^{-1}\)) produced a facilitation of transmission in DG, while higher concentrations (2.5–4.0 vol %; 0.5–1.2 mmol litre\(^{-1}\)) produced depression. Half-maximal (ED\(_{50}\)) depression of perforant path inputs occurred at approximately 3.0 vol % (1.0 mmol litre\(^{-1}\)) Stratum radiatum to CA 1 evoked responses appeared to be more sensitive to the depressant actions of enflurane, since half-maximal effects occurred at 2.0 vol % (0.9 mmol litre\(^{-1}\)) (fig. 4).

Depression of stratum radiatum inputs to CA 1 neurones appeared to involve synaptic actions, because reductions in PS amplitudes were always accompanied by depression of synaptic potentials (fig. 4). In the dentate area, in contrast, enflurane produced a depression of PS responses which was not accompanied by decreases in EPSP amplitudes, suggesting a predominantly postsynaptic site of action (see input/output analysis below). Conversely, in both the CA 1 and DG areas, response latencies were increased in the presence of all effective concentrations of enflurane, including the lowest which increased the amplitude of the DG neurone field potential. Effects on field potential responses appeared to be independent of
stimulus intensity for both pathways studied (data shown for DG responses in figure 4). Thus, enflurane-induced enhancement or depression of responses occurred to the same extent at both low and high degrees of stimulation, indicating that recruitment of afferent fibres was not affected.

The effects of enflurane on evoked potentials in both CA 1 and DG areas were often associated with increased background unit firing activities (not shown). Recording electrodes were located below the cell body layers where basket cell inhibitory interneurone somas are most prevalent (Schwartzkroin and Knowles, 1983). This, together with the observation that firing activities tended to be grouped into short bursts which are characteristic of basket cell firing (Schwartzkroin and Knowles, 1983), suggests that increases in background discharge may be partially attributed to enflurane-induced activation of inhibitory interneurones (see also Latency Analysis below).

**Enflurane effects on input/output relationships**

To determine whether enflurane altered postsynaptic responses, input/output analyses of the two synaptic pathways were performed. This method of analysis involves a comparison of the strength of synaptic input with the amplitude of postsynaptic response (output), termed E–S curve analysis (Andersen et al., 1980). Rate of rise (dV/dt) of the field EPSP does not correlate directly with the apparent EPSP amplitude (compare figures 4 and 5); however, dV/dt provides a better measure of synaptic input for two reasons. First, responses are measured before possible alteration by postsynaptic currents contributing to discharge and in response to polysynaptic drive (Turner, Richardson and Miller, 1984). Second, the rate of rise of the EPSP is known to correlate better with discharge probability than EPSP amplitude and is commonly used for this type of analysis (Abraham, Bliss and Goddard, 1985). As shown in figure 5, a steep relationship between EPSP and PS occurred at low stimulus intensities, whereas PS responses approached a maximum at higher intensities (see also: Andersen et al., 1980; Ault and Nadler, 1983).

In the presence of enflurane 0.75 mmol litre⁻¹, PS amplitudes in DG were increased, although perforant path EPSP dV/dt responses were slightly reduced; thus, for a given amount of synaptic input, postsynaptic discharge was increased (fig. 5). This suggests that, in the DG area, postsynaptic facilitation underlies the enhancement of transmission observed at these low concentrations. In the presence of enflurane 1.2 mmol litre⁻¹, however, PS failure was almost complete, although EPSP dV/dt responses were
not markedly depressed. Higher concentrations (1.4 mmol litre\(^{-1}\)) produced complete depression of the PS, and decreases in EPSP \(dV/dt\) and amplitude (data not shown). Thus, the excitatory actions on perforant path inputs appear to involve primarily postsynaptic sites. Synaptic responses (EPSP) were only depressed in the presence of concentrations greater than those required to block postsynaptic discharge.

In contrast to DG neurone responses, enflurane-induced depression of CA 1 field potentials was associated with reduced EPSP responses. Figure 5 shows that depression of field potentials was accompanied by increased excitability (decreased threshold) in the CA 1 area; that is, a given EPSP produced larger PS responses in the presence of the anaesthetic. Field potential amplitudes, however, were not increased above control values because the EPSP responses which accompanied PS facilitation were smaller. It is difficult to attribute field potential depression to a postsynaptic failure given the observed increase in CA 1 pyramidal cell excitability. Furthermore, all effective concentrations produced a depression of the stratum radiatum EPSP \(dV/dt\) and this appeared to be the major mechanism underlying the depression of the field potential. Depolarization–discharge coupling did not appear to be altered until relatively high concentrations were applied (> 1.0 mmol litre\(^{-1}\)). Fibre-volley amplitudes were not affected by low concentrations; however, concentrations which produced greater than 90% depression of EPSP amplitudes were accompanied by small reductions in fibre-volley amplitudes in both the CA 1 and DG areas.

**Effects on antidromic field potentials**

Antidromic responses were used as a measure of enflurane effects on electrical excitability of DG and CA 1 neurones. Concentrations which produced enhanced perforant path to DG responses also increased dentate antidromic responses (fig. 3). Depression of antidromic spike amplitudes required concentrations greater than 5.0 vol % (1.3 mmol litre\(^{-1}\)), which reduced synaptic transmission by greater than 90%. Multiple antidromic discharges were produced in the CA 1 area when spontaneous and synaptically evoked seizure activity was present; however, antidromic bursts were not observed in the DG area.

**Latency analysis**

Latency analysis was used to examine effects on paired pulse potentiation and local synaptic inhibition. Potentiation was measured by applying paired pulses which were subthreshold for a PS response on the first pulse. The amplitude of the PS produced in response to the second pulse was measured, as shown in figure 6. Latency profiles were constructed by plotting the amplitude of PS responses, at various interstimulus intervals. Optimal intervals of 30 ms for CA 1 and 25 ms for DG control responses were observed. In the presence of enflurane, paired pulse potentiation still occurred and enhanced potentiation was associated with increased response amplitudes produced by low concentrations in the DG area;
reduced potentiation accompanied depression of responses in both pathways. Low concentrations also appeared to enhance inhibition in both pathways and higher concentrations resulted in further enhancement of late inhibition in DG neurones, with no additional effect in the CA 1 area. Increased inhibition was observed as a prolongation of the early component (optimal interstimulus intervals were shifted from 30 to > 40 ms) and steeper tailing in late responses. This interpretation of latency responses is supported by earlier observations which demonstrated similar effects produced by GABA and pentobarbitone (MacIver and Roth, unpublished observation; Gribkoff and Ashe, 1985; Ashton and Wauquier, 1985). Pentobarbitone has also been shown to enhance local inhibition in the hippocampal formation (Nicoll et al., 1975).

**DISCUSSION**

The results agree with recent (Stevens et al., 1984) and earlier (Darimont and Jenkins, 1977; Black, 1979) observations in mammalian in vivo preparations. These studies reported both excitatory and depressant actions of enflurane on CNS electrical activity. Our results extend these observations to include a cortical site of action for biphasic and convulsive effects of enflurane, since ascending afferent systems are not operative in the in vitro brain slice preparation. Drug effects on ascending inputs to cortical centres may exacerbate seizure-like actions in hippocampal and neocortical areas, in vivo. The present results demonstrate the intrinsic seizure susceptibility of the hippocampal formation and relate these anaesthetic-induced effects to actions at a neuronal level.

Enflurane did not appear to depress synaptic inhibition in either DG or CA 1 pathways; instead, enhanced inhibition was apparent from the paired pulse latency analysis. Increases in background firing activity of inhibitory interneurones also suggests an enhancement of inhibition. Stevens and co-workers (1984) noted that multiple unit activity in brainstem regions could be increased by enflurane, which may reflect a generalized stimulatory action on neuronal excitability in the CNS. Enflurane appeared to increase postsynaptic excitability in both CA 1 and DG regions, since increases in PS amplitudes were observed in response to equivalent EPSP input (fig. 5). Similar excitatory effects have been observed in the presence of other volatile anaesthetics in vivo (Winters, 1982).

**Enflurane-induced burst firing**

The seizure-like actions produced by enflurane were correlated with decreases in the threshold for discharge of CA 1 neurones. This was particularly evident from input/output analysis of recordings in the CA 1 region (fig. 5). All effective concentrations produced a shift to the left in the input/output relationship, indicating that less synaptic input was required to produce postsynaptic discharge. This shift in the intercept of the stratum radiatum EPSP axis is consistent with a decrease in threshold of the CA 1 neurones, and would be expected to accompany enflurane-induced spontaneous burst firing. A direct excitation of pyramidal neurones appears to be the most likely mechanism of enflurane-induced seizure activity in the present study, and has been previously postulated to underly the epileptogenic effects of kainate and folate (Olney, Fuller and deGubareff, 1981; Clifford and Ferrendelli, 1983). By comparison, DG neurone excitability did not appear to be altered by enflurane, since a shift in the intercept of the perforant path EPSP axis was not observed (fig. 5). This is consistent with a lack of enflurane-induced seizure activity in the dentate area. Enflurane-induced increases in DG field potential responses appear to be produced via a postsynaptic effect, since equivalent EPSP input resulted in larger PS amplitudes; however, this enhanced response was not associated with a lower threshold for discharge.

**Differential sensitivity of hippocampal regions**

Inputs to CA 1 neurones were altered by the lowest vapour concentrations of enflurane; significant depression occurred in this pathway, although DG responses remained virtually unchanged or enhanced. This differential sensitivity cannot be explained on the basis of drug distribution or kinetics, since the concentrations at these sites should be similar in thin (400-μm) slices of tissue. Furthermore, the onset times of the effects for the two pathways were identical and rapid (< 60 s). We propose that differences in drug efficacy are the result of a differential localization or composition, or both, of sites of action in the pathways studied. It is well known, for example, that DG neurones are less sensitive to the actions of other convulsants than the pyramidal regions of the hippocampus, and this may reflect dominant inhibitory mechanisms in the DG area (Schwartzkroin, 1983). Recent studies of penicillin and GABA antagonist actions...
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on DG neurones also demonstrated a relatively high resistance to convulsant-induced burst firing (Fricke and Prince, 1984), which was attributed to the apparent lack of inward calcium currents in these cells. CA 1 and CA 3 neurones are also more sensitive than DG cells to the non-specific effects produced by anoxia and mechanical damage (Kelly, 1982; Lipton and Whittingham, 1979). These latter two forms of perturbation produce seizure activity which is similar to that produced by enflurane; however, important differences are apparent. Anoxia and mechanical damage, for example, produced irreversible or only partially reversible alterations (Whittingham, Lust and Passonneau, 1984), whereas the effects of enflurane were readily reversible. Furthermore, enflurane-induced seizure activity tended to be intermittent, and burst responses did not correlate directly with the intensity of stimulation. Anoxic burst responses, in contrast, increased at higher intensities of stimulation and occurred in response to each stimulus (Maclver and Roth, unpublished observation; Schwartzkroin 1983).

In summary, we have demonstrated that enflurane produced excitatory and depressant effects at a synaptic level in vitro. The biphasic responses, previously observed in vivo, can include alterations of both neuronal excitability and synaptic transmission at cortical sites. Seizure-like activity appeared to be correlated with direct excitation of CA 1 neurones. Stratum radiatum to CA 1 evoked responses were depressed by enflurane via actions at a synaptic level. DG neurones, in contrast, did not produce burst firing and the biphasic (excitation–depression) responses appeared to involve postsynaptic sites of action. The differential effects produced on DG and CA 1 neurones also provide further evidence that anaesthetic sites of action are selective (Roth, 1980) and pathway specific (Roth, Bland and MacIver, 1983).

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REFERENCES


