THE BIPHASIC PATTERN OF THE CONVULSIVE PROPERTY OF ENFLURANE IN CATS

J. E. STEVENS, M. FUJINAGA, E. OSHIMA AND K. MORI

SUMMARY

The nature of the epileptoid state produced by enflurane was examined in the concentration range 2–5% in cats. A biphasic pattern in the convulsant property was revealed. The ease of induction of seizure by repetitive peripheral stimulation, the duration of seizures, and the activation of reticular neuronal firing during seizure activity showed peak values between 3 and 4%, whereas the values were significantly lower both above and below this range. The amplitudes of the somato-sensory evoked potential also showed a biphasic pattern which correlated well with the severity of the epileptoid state as judged by the aforementioned indices. These findings, when compared with evidence on the actions of other convulsant drugs, and with the known depressant actions of enflurane, suggest a combination of depressant and convulsant properties, the balance of which varies depending on the depth of anaesthesia.

Drugs in current use as experimental convulsant agents, such as pentylenetetrazol, strychnine and picrotoxin, produce generalized excitation of the central nervous system (CNS) before the development of seizure activity and, the larger the dose, the greater the excitation, which leads to seizures and only then, to depression in the post-ictal period (Franz, 1980). Enflurane, on the other hand, in common with the other anaesthetic agents known to produce seizure activity, such as ether (Joas and Eger, 1971; Mori, Mitani and Fujita, 1971), cyclopropane (Mori, 1973), and ketamine (Mori et al., 1971), produce varying degrees of depression, both in CNS electrical activity and in behaviour, before the stage of seizure is reached. Furthermore, enflurane is the only agent which produces seizure activity at doses normally used clinically. Most investigators have considered EEG seizure activity to be a sign of overdosage, and have advised decreasing the concentration of this agent if signs of CNS stimulation are encountered (Persson, Peterson and Wahlin, 1978; Black, 1979). As a result, all the work published on the epileptogenic property of enflurane has been undertaken using low concentrations (1–3%). This balance of depression and excitation also raises the possibility that, at higher doses, the depressant element may again become dominant, producing a biphasic picture. As high doses of enflurane produce profound cardiovascular and respiratory depression which may alter CNS activity, the present study was undertaken in artificially ventilated cats and the arterial pressure was supported pharmacologically, in an attempt to demonstrate this biphasic response.

MATERIALS AND METHODS

Eleven cats (weights 2.2–3.7 kg) were anaesthetized in a 50-litre Perspex box, with 5% enflurane in oxygen. Tracheal intubation was performed with the aid of alcuronium (initial dose 5 mg, supplemented as required). Mechanical ventilation was instituted and anaesthesia maintained with 3% enflurane in oxygen using a 3-litre flow through an Enfluratec vaporizer (Cyprane). Cannulae were inserted to the femoral artery to permit the monitoring of arterial pressure and the withdrawal of blood for blood-gas analysis, and to the cephalic vein for the administration of fluids and drugs. Blood-gas analysis was performed at 1–2-h intervals, and PCO2 and pH values of 29–31 mm Hg and 7.40–7.44 respectively (Herbert and Mitchell, 1971) maintained by adjustment of the tidal volume and the administration of NaHCO3. A rectal thermometer was sited, and temperature maintained at 38–39°C with a warm water blanket.

Stainless steel screws (2.0 mm diameter) were inserted in the frontal bone, as reference electrodes, and over the primary sensory area of the cortex to record the sensory evoked potential (SEP) and the EEG. (Details of the location of the primary sensory receiving area in the cat, were described in full by Stevens, Oshima and Mori (1983).) Parallel stainless steel wire electrodes (0.2 mm o.d.) were inserted bilaterally in the mesencephalic reticular formation.

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obtained by inserting either a 15-kΩ resistor or a mated by the difference between the d.c. levels firing of a population of units. This signal was measured using the multi-unit activity technique was as follows: the wide-band signal ob-
repeated. The resulting traces were plotted on an X—Y plotter (Watanabe WX442). Generalized EEG seizure was produced by the same peripheral stimulus as the SEP, delivered at 2 Hz. (Stevens, Oshima and Mori (1983) described in detail the recording methods and the induction of seizures.)

The firing of a population of reticular neurones was measured using the multi-unit activity technique described previously (Winters et al., 1967; Mori et al., 1971; Mori and Winters, 1975). Briefly, the technique was as follows: the wide-band signal obtained from the preamplifier of the polygraph was introduced to a high frequency band-pass filter, the peak frequency response of which was centred at 1300 Hz, with 3-dB decrease at 600 and 2500 Hz. Since the conventional ink-writing oscillograph could not follow such high frequency activities, the output was rectified and smoothed with an electronic circuit, with a smoothing time constant of 50 ms and was expressed as an oscillation of d.c. voltage: the higher the d.c. level, the greater the firing of a population of units. This signal was recorded on a slow-moving oscillograph (Sanei Rectigraph 8s) with a paper speed of 10 mm min⁻¹.

The noise level of the recording system was estimated by the difference between the d.c. levels obtained by inserting either a 15-kΩ resistor or a short across the input in place of the animal. The signal was measured as the distance from the lower limit of MUA tracing to the 15-kΩ resistor line (the signal to noise ratio exceeded 10 in all cases). By this recording technique neuronal discharges were picked up from an area of 1 mm radius around the tip of the electrode (Halas and Beardsley, 1968). Since the level of multi-unit activity recorded is dependent not only on the amount of bioelectrical activity but also on the impedance of the electrode, calibration of the level of MUA was not practical and changes in the lower limit of spontaneous activity of the MUA trace with increasing enflurane concentrations were measured and expressed as a percentage of the level during the inhalation of 2% enflurane. The degree of activation during seizure was also measured from the lower limit of the trace and expressed as a percentage of the highest level achieved during seizure in each animal. These results were averaged for all 10 cats.

Drug administration

After completion of the surgical preparation, the enflurane concentration was decreased to 2%, the level at which sensory evoked seizures could not be produced in any animal in previous experiments (Stevens, Oshima and Mori, 1983). After 30 min at this level, the SEP was elicited and the induction of seizures attempted. The anaesthetic concentration was then increased by 0.5% every 30 min and seizure induction and SEP elicited immediately before each increment. When the systolic arterial pressure decreased towards 100 mm Hg, methoxamine 0.1 mg ml⁻¹ in saline was infused i.v. so as to maintain the systolic arterial pressure greater than 100 mm Hg. In six cats blood enflurane concentra-

RESULTS

Results were collected from 10 of the 11 cats, the other being excluded because of heavy blood loss from a disconnection of the arterial line. Body temperature, PCO₂, pH and PO₂ were well maintained by the methods described. The arterial pressure decreased rapidly with increasing enflurane concentra-
TABLE I. Changes in arterial pressure (mean ± SEM) with increasing enflurane concentration (n = 10)

<table>
<thead>
<tr>
<th>Enflurane concentration (%)</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic (mm Hg)</td>
<td>130±5</td>
<td>111±5</td>
<td>107±3</td>
<td>109±2</td>
<td>107±3</td>
<td>105±2</td>
<td>105±2</td>
</tr>
<tr>
<td>Diastolic (mm Hg)</td>
<td>76±6</td>
<td>70±4</td>
<td>63±4</td>
<td>61±3</td>
<td>63±6</td>
<td>61±3</td>
<td>53±5</td>
</tr>
<tr>
<td>No. of animals with methoxamine infusion</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Sensation (table I), but it was possible to maintain a value greater than 100 mm Hg systolic with the infusion of methoxamine in all cats, the concentration of enflurane at which support became necessary varying considerably from 2.5 to 4.0% (table I).

**Electroencephalograph**

The basic EEG pattern at the various levels agreed with that seen in previous reports (Mori, 1973 (fig. 7); Steen and Michenfelder, 1979), and consisted of high-amplitude slow waves with very occasional spikes of more than 500 μV with 2% enflurane. With increasing concentration, the slow waves disappeared and the high voltage spikes initially increased rapidly in both frequency and amplitude (fig. 1), and then decreased — although more gradually (Mori, 1973 (fig. 9)). Peak frequency, 26.5 ± 3.3 s⁻¹, occurred at 2.5%, whereas peak amplitude, 1430 ± 150 μV, was at 3.0%. The decrease after the peak became statistically significant at 3.5–5.0% (P < 0.01) for frequency, whereas it only reached significance at 4.5% (P < 0.05) and 5.0% (P < 0.01) in the amplitude measurement.

**Sensory evoked potentials**

Typical traces are shown in figure 2. An increase

![Fig. 1. Changes in mean frequency per minute (—) and amplitude (—) (μV x 10²) of spontaneous EEG spikes of 500 μV or greater, during enflurane anaesthesia (mean ± SEM). The frequency of spontaneous spikes reached the maximum during administration of 2.5% enflurane and that of amplitude during 2.5–3.0% enflurane.](http://bja.oxfordjournals.org/)

![Fig. 2. Typical SEP traces (from an actual recording) seen during enflurane anaesthesia. The SEP was recorded at the primary sensory cortex and the stimulation was applied at the contralateral forepaw. See text for further description.](http://bja.oxfordjournals.org/)
in amplitude of components P₁ and N₂ (figs 3, 4) was seen on increasing the inhaled concentration of enflurane. This increase peaked at 3.5% (P<0.02 for P₁ and P<0.001 for N₂ cf. 2%) and then returned towards the amplitude seen at 2%. Although this partial recovery was not statistically significant with this sample number, the trend was identical (that is, the amplitude at 5% was less than that at 3.5%) in all animals. The latent period of the peak of both these elements measured from the administration of stimulus was prolonged in a linear dose-related manner (table II). At the higher concentrations the N₂ element first became wider and then separated into an early peak, with a latency consistent with N₂ and either a number of later negative peaks or a period of sustained negativity without individual peaks. This was in distinct contrast to the SEP at 2% where the trace returned to the isoelectric point immediately after N₂ (fig. 2).

Reticular multi-unit activity

The changes in R-MUA confirmed our previous report (Mori, 1973) and a typical trace is shown in figure 5. The disappearance of slow waves and appearance of an isoelectric pattern with spikes in the EEG was associated with a sharp decline in the lower limit of background activity coupled with phasic enhancement. The lower limit of the trace reflects the level of activity during the isoelectric periods of the EEG, and the degree of phasic enhancement the spikes and bursts of the typical enflurane EEG. The decrease of the lower limit of R-MUA reached a maximum at 57.1±6.9% (P<0.001) (fig. 6) after which slight recovery occurred, which was not significant. The increase of the R-MUA during seizure peaked at 3% and declined rapidly, the decline being significant at 4% and greater (P<0.02 at 4% and P<0.01 at 4.5 and 5.0%) (fig. 6).

Seizures

The occurrence of seizures (table III) increased rapidly to 100% at 3.5% enflurane, and remained constant at 4.0 and 4.5%. In one animal seizures did not occur at 5%. The ease of induction of seizure activity (fig. 7), calculated as the reciprocal of the number of shocks required to initiate generalized seizure × 100 (see Stevens, Oshima and Mori,
FIG. 5. Typical trace of R-MUA during enflurane anaesthesia. An upward deflection indicates an increase in firing rate of a population of units and downward deflection a decrease. Attempted seizure induction by repetitive peripheral stimulation at 2 and 3% caused only temporary increases in the firing rate, representing an activation of CNS activities by peripheral stimulation during light anaesthesia, but not seizure. From 3.0 to 5.0%, identical stimulations induced seizures which were associated with a rapid increase of the neuronal firing. The degree of increase in neuronal firing was maximum at 3% and then decreased with each increment of enflurane. The width of the spike-like increase of the trace during seizure is proportional to the duration of the seizure. After the seizure a short period of post-ictal depression of spontaneous activity, represented by a decrease in the width of the trace, is seen.

FIG. 6. Changes in the degree of activation of R-MUA during seizure, and the background value (---). The maximum activation during seizure was seen at 3.0 and 3.5% enflurane and then the activation decreased, showing the biphasic nature. The differences of background value from 3.5 to 5.0% are not statistically significant.

FIG. 7. Changes in seizure induction index by incremental concentration of enflurane. The maximum ease of seizure induction by peripheral stimulation was seen at 3.5–4.0% enflurane, showing the biphasic nature of epileptoid state. Otherwise see text.
TABLE II. Changes (mean ± SEM) in peak latent periods of P₁ and N₂ components of SEP with increasing enflurane concentration (n = 10)

<table>
<thead>
<tr>
<th>Enflurane concentration (%)</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁ (ms)</td>
<td>11.6±0.4</td>
<td>12.0±0.4</td>
<td>12.4±0.3</td>
<td>12.6±0.3</td>
<td>12.8±0.5</td>
<td>13.4±0.5</td>
<td>14.4±0.7</td>
</tr>
<tr>
<td>P₁ (P)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>N₂ (ms)</td>
<td>27.0±1.0</td>
<td>28.8±0.8</td>
<td>29.8±0.7</td>
<td>30.2±0.6</td>
<td>31.6±0.6</td>
<td>33.2±0.8</td>
<td>34.0±1.0</td>
</tr>
<tr>
<td>N₂ (P)</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.05</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

TABLE III. Frequency of seizures

<table>
<thead>
<tr>
<th>No. of animal* with EEG seizure</th>
<th>0</th>
<th>5</th>
<th>9</th>
<th>10</th>
<th>10</th>
<th>10</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enflurane concentration (%)</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>3.5</td>
<td>4.0</td>
<td>4.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

1983), peaked less rapidly than other indices, at 4.0%, but also declined after the peak, although this was not statistically significant. The mean duration of seizure (fig. 8) reached a peak at 3.0% of 29.0 ± 4.1 s, and then decreased rapidly to 9.7 ± 0.4 s at 5.0% (P < 0.001).

Enflurane concentrations

The results from six cats are shown in figure 9. As the arterial pressure and ventilation were controlled, values varied considerably from one animal to another, mainly because of variation in hematocrit as a result of different degrees of blood loss during the surgical preparation, each animal also showed good linearity (correlation of arterial whole blood enflurane concentration and vaporizer setting: Y = 13.82X - 0.39; r = 0.92; P < 0.01).

![Fig. 8](image-url)  
**Fig. 8.** Changes in duration of seizures with incremental concentration of enflurane. The seizure of maximum duration was seen at 2.5–3.5% enflurane, and the duration decreased above this concentration range, showing the biphasic nature of epileptoid state.

![Fig. 9](image-url)  
**Fig. 9.** Whole blood enflurane concentrations with increasing inspired concentration (mean ± SEM). A line of best-fit was calculated by the least-square method.
DISCUSSION

The main difficulty in defining the SEP from human scalp recordings resides in the fact that the signal is small, and buried in the background of EEG oscillations, and that the components of each response fluctuate from time to time in accordance with fluctuation in excitability of the receptive neuronal elements (Jones, 1982). This difficulty is overcome by summating a certain number of responses, which serves to increase the signal-to-noise ratio and averages out the fluctuation in individual responses. For example, Perot and Vera (1982) summated 250 responses and Cracco and colleagues (1982) more than 1000. However, in the present study it was defined by summating only 10 responses, for a number of reasons. First as the recording electrode was placed directly in the skull touching the dura mater over the primary receiving area, extremely large amplitude responses were achieved with very little background noise. Second, the application of stimuli of supramaximal intensity and stability in the excitability of the receptive neuronal elements combined to give very little fluctuation in the evoked response during surgical depth of anaesthesia, when arousability is completely blocked (French, Verzeano and Magoun, 1953). Further, summating a large number of responses was practically not an easy task in the present study, since the background EEG consisted of spikes and electrical silence during deep anaesthesia, and any SEP responses which coincided with the spontaneous spikes had to be repeated.

The present study has shown a definite biphasic nature in the convulsive property of enflurane in cats: the ease of induction of seizure by repetitive peripheral stimulation increased as the depth of anaesthesia was increased from 2.0% enflurane in oxygen, reaching maximum with 3.5–4.5% enflurane and then, finally, decreased at the deepest level (5.0%). A similar biphasic pattern was demonstrated in the maximum level of activation of reticular neuronal firing and the duration of seizures. This biphasic nature of the convulsive property also correlated well with the changes in amplitude of the somatosensory evoked potentials, but did not correlate with the frequency and amplitude of spontaneous spike activity, which showed maximum values in the concentration ranges of 2.5–3.5% enflurane.

A review of previous reports on the actions of enflurane on neuro–electrical activities shows that most reports cover a relatively narrow concentration range, 0–3.5%, and report a dose-related monophasic stimulation of the CNS (Steen and Michenfelder, 1979). Neigh, Garra and Harp (1971) and Darimont and Jenkins (1977) reported a biphasic pattern in the change in spontaneous EEG spike frequency, which was confirmed by the present study, but these reports did not examine the convulsive property.

Hypotension (Beecher, McDonough and Forbes, 1938) and hypercapnoea (Clark and Rosner, 1971) have been shown to depress the SEP, and hypercapnoea (Joas and Eger, 1971) also depresses enflurane-induced seizure activity and, therefore, such a biphasic pattern would be anticipated in spontaneously breathing untreated animals as a result of progressive depression of cardiovascular and respiratory function with increasing concentrations of enflurane. In the present study, when arterial pressure was supported by methoxamine, and P\textsubscript{CO}\textsubscript{2} and pH were controlled, there was still a definite decrease in the amplitudes of SEP at higher concentrations of enflurane. Therefore, it seems likely that this late depression is a true drug-induced effect, resulting from a direct action on the neuro–electric processes in the CNS.

There is general agreement that bicuculline and picrotoxin should be classified as specific CNS stimulants, and their convulsant actions are attributed to interference with the inhibitory processes in the CNS that are mediated at receptors for glycine and GABA, putative inhibitory transmitters (Curtis et al., 1971; Johnston, 1978). The convulsant action of non-specific CNS stimulants, such as pentylentetrazol, are attributed to a variety of mechanisms including blockade of inhibition and direct neuronal excitation, which may involve an increase in the release of transmitter, labilization of the postsynaptic membrane or a decrease in synaptic recovery time (Franz, 1980).

The convulsive action of enflurane is generally taken as an expression of a "CNS excitatory action" (Marshall and Wollman, 1980). In addition to such "excitatory" action, various CNS depressive components have also been documented. The reticular neuronal firing (Mori, 1973), cerebral oxygen consumption (Wollman et al., 1969), and cerebral glucose utilization (Myers and Shapiro, 1979) are all suppressed in a concentration-related manner, including the concentration at which convulsions may occur, although during active convulsions, some recovery may be seen. Such non-specific depressive components, however, do not appear to have been reported in the studies of convulsants such as...
bicucculline, picrotoxin and pentylentetrazol. In addition to the general suppressive components of enflurane mentioned above, it has also been demonstrated that both non-convulsive and convulsive concentrations of enflurane show definite anticonvulsant action in several experimental models of epilepsy, such as amygdaloid kindling, bicucculline- and penicillin-induced convulsions (Urzebe et al., 1981).

Each of the animals in the present study underwent generalized seizures at least six times with inter-ictal periods of 30–40 min. The possibility arises that the induction of seizures per se might depress the ability of the animal to respond with a further seizure at a later stimulation. It has been shown that enflurane seizures are of short duration and are self-limiting (Marshall and Wollman, 1980), the cerebral metabolic oxygen requirement during active seizure does not exceed that during wakefulness (Wollman et al., 1969), and seizure induction during enflurane anaesthesia has been shown to produce no cerebral impairment on waking in man (Marshall and Wollman, 1980).

Furthermore, in a previous paper (Stevens, Oshima and Mori, 1983) the stability and reproducibility in the technique of seizure induction used here was shown.

These factors all indicate that enflurane-induced seizures produce only minimal CNS disturbance and, considering the frequency with which seizures of equal or increasing intensity may occur during status epilepticus in man when ventilation is controlled, it is likely that an inter-ictal period of 30 min allows adequate recovery.

The present study was not designed to elucidate the exact neuronal or neurohumoral mechanisms of the convulsive action of enflurane to a similar level as that achieved in the studies of bicucculline or picrotoxin. However, if we accept the prevailing view that the convulsive action of a given non-specific CNS stimulant is a result of either blockade of inhibition, or direct excitation, of neuronal tissue, as was postulated by Franz (1980), the biphasic nature of the convulsive action of enflurane may best be explained by postulation of a mixture of so-called “excitatory” or convulsive actions and non-specific depressive actions, and variation in the dominance of one effect over the other at the different doses.

acknowledgements

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References

CNS ACTIONS OF ENFLURANE IN CATS

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ASPECT BIPHASIC DES PROPRIETES CONVULSIVANTES DE L'ENFLURANE CHEZ LE CHAT

RESUME

La nature de l'état épileptique induit par l'enflurane a été étudiée chez le chat pour des concentrations de 2 à 5%. Cette action convulsive s'est révélée biphasique. La facilité d'induction des crises par des stimulations périphériques répétitives, la durée des crises et l'activation des décharges neuronales reticulaires au cours des crises passaient par des valeurs de pic entre 3 et 4%, alors que les valeurs étaient significativement plus basses à la fois au-dessus et au-dessous de cette valeur. Les amplitudes du potentiel évoqué somatosensorial ont également monté un aspect biphasique bien corrélé avec la sévérité de l'état épileptique estimé selon les paramètres déjà décrits. Ces données, confrontées à celles obtenues sur l'action d'autres agents convulsivants, et avec l'action dépressive connue de l'enflurane, laissent présager une association de propriétés convulsivantes et dépressives, dont la résultante varie selon la profondeur de l'anesthésie.