β -ENDORPHIN INDUCES GENERAL ANAESTHESIA BY AN INTERACTION WITH OPIATE RECEPTORS

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ABSTRACT

β -endorphin, administered into the cerebral ventricles of rats, provokes a sequence of behavioural and electroencephalographic (EEG) responses similar to those observed with general anaesthetics used clinically. Initial behavioural and EEG excitation, motor inco-ordination and exaggerated responsiveness to sensory stimuli are followed by a stage of rigid immobility with maintenance of local reflexes (withdrawal, corneal) and EEG arousal in response to stimulation. Finally, there is immobility associated with both EEG and behavioural unresponsiveness to severely noxious stimuli. Such a state of unconsciousness with complete analgesia defines general anaesthesia. This state was completely and rapidly reversed by the specific opiate antagonist, naloxone. The induction of general anaesthesia by a water-soluble neurohormonal peptide acting at specific receptor sites has important implications for traditional theories of anaesthesia.

THE IDENTIFICATION in the brain of opiate receptors¹⁻³ and their endogenous ligands⁴⁻⁹ has initiated a new era in research on central mechanisms of pain and analgesia.¹⁰⁻¹³ Beta-endorphin is apparently localized intraneuronally and has been implicated as a neuromodulator or neurohormone.¹⁴⁻²⁰ It is assumed that centrally administered β -endorphin produces its effects by an interaction with opiate receptors. Opioids given intracerebroventricularly (ICV) in low doses cause restlessness, excessive grooming, "wet-dog shaking" behaviour and epileptiform electroencephalographic (EEG) activity.^{11,14,21-26} Higher doses, in contrast, induce motor immobility and muscle rigidity. This akinetic state has been equated by some with schizophrenic catatonia¹⁴ and by others²⁶ to the cataleptic state.

We have observed that β -endorphin, 50 μ g ICV, induces a sequence of behavioural and EEG responses with three distinct phases. There is an initial stage of motor and EEG excitation, followed by a state of immobility during which there

is EEG arousal in response to external stimuli. This stage progresses to one where EEG arousal is abolished, even upon extensive surgical skin incisions. Because the behavioural and EEG profiles in the last stage indicate unconsciousness accompanied by complete analgesia, this latter state may be referred to as general anaesthesia.

METHODS

Male Sprague-Dawley rats weighing 250–300 g were anaesthetized and stainless steel cannulae²⁷ were implanted into the right lateral ventricle while bipolar platinum electrodes of 3 mm separation were placed bilaterally over the sensorimotor cortex. One week or more after surgery animals were placed individually in a soundproof and electrically shielded chamber, 20 $\times$ 35 cm, having a grid floor with 10 mm separation between bars. The grid floor enabled us to monitor continuously motor co-ordination and maintenance of balance. Camel β -endorphin (Peninsula Labs. Inc.) 50 μ g, was dissolved in 10 μ l sterile saline and infused ICV over 5.5 minutes. Techniques for polygraphic recording in rats and Fast Fourier Transform (FFT) analysis of the EEG have been described elsewhere.^{28,29}

RESULTS

Sleeping animals awakened during infusion of peptide and wakeful animals became restless. Exploring movements became frantic and jerky and were often interrupted by "frozen stares" and wet-dog shakes. As a rule, initial episodes of wet-dog shaking were preceded by EEG spiking

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FIGURE 1 EEG response to ICV infusion of 50 μ g β -endorphin (top). (A) Stage of excitation during the first 15 minutes after β -endorphin administration. There is EEG activation with bursts or single discharges. Epileptic-like EEG is accompanied by wet-dog shaking behaviour. Animals showed excessive grooming and exploring activity, "frozen stares", and eventually became unco-ordinated. (B) a "catatonic" state of about 15 minutes duration in which animals became rigidly immobile. When rats remained in a quiet environment, the EEG became deactivated (left part of the record). External stimulation, however, resulted in pronounced EEG activation (a-auditory and visual stimulation; b-tail pin-prick; c-corneal stimulation) which gradually diminished. (C) Surgical anaesthesia (about 60 minutes' duration). The EEG became deactivated and exhibited constant hypersynchrony. Auditory and visual (a), painful (b) and corneal (c) stimulation failed to evoke EEG or motor response. Incision of the skin ((d) and dotted line) resulted in a slight diminution of EEG hypersynchrony. (D) Recovery period (about 45–60 minutes duration) EEG hypersynchrony gradually disappeared. Animals began to show spontaneous and co-ordinated motor activity. EEG and motor responsiveness to stimuli recovered.

(5–6 discharges per second) of increasing amplitude which became maximum during shaking (Figure 1). Subsequently, wet dog shakes continued on the background of a normal desynchronized EEG. There were additional symptoms of central excitation, such as stereotyped circular movements, stiff or erect tail, excessive grooming, penile erection, defaecation and urination. These activities lasted for 10–15

minutes after infusion. Responsiveness to external stimuli during this excitatory phase of β -endorphin action was exaggerated and associated with aggressive behaviour. Animals exhibited motor incoordination progressing to immobility. Concomitantly with increasing akinesia the desynchronized low-amplitude EEG of the excitatory phase became progressively transformed to high amplitude, slow-wave activity (Figure 1). During the first 15 minutes of this inhibitory phase of β -endorphin action ("CATATONIA", Figure 1), acoustic, visual or tactile stimulation evoked EEG arousal, but with minimal behavioural components. Noxious stimulation (pin-prick to the paw) still provoked both an EEG response and limb-withdrawal reflex, while the corneal reflex was positive and associated with EEG arousal (Figure 1).

Behavioural and EEG responses to noxious and non-noxious stimulation became less and less pronounced with time after infusion of the β -endorphin. Responses to pin-prick stimulation disappeared in the sequence: tail and hind legs, trunk, front legs, head and ears. The corneal reflex and its EEG component were extinguished concurrently with inhibition of the responses to noxious stimulation of ears ("SURGICAL ANAESTHESIA"; a, b, c, Figure 1). Approximately 30 minutes after infusion of β -endorphin, deep and extensive skin and fascial incision failed to elicit either behavioural or EEG responses. Thus, there was unconsciousness associated with complete unresponsiveness to stimuli, a condition which defines general anaesthesia. The test animal's respiration was never markedly depressed even during the deepest state of surgical anaesthesia and only minimal darkening of mucous membranes and superficial capillary beds (snout, maw, ears) was observed. Thus, hypoxia could not be implicated as a cause of the unconscious state we observed. This state of general anaesthesia lasted about 60 minutes and was characterized by a constant pattern of high voltage, slow-wave EEG activity with occasional single spikes (Figure 1).

There were changes from the awake state in the frequency components of the EEG recorded during the state of general anaesthesia induced by β -endorphin (Figure 2). Spectral analysis²⁹ of the EEG recorded during the anaesthetic state showed significant increases in power for all frequency bands examined over the range of 1.5 to 25 Hz (Figure 2). During this stage the averaged power spectrum (SUM in Figure 2) was eighteen times greater than that of the awake state and

FIGURE 2 EEG power spectra. (A) During the awake state after ICV infusion of vehicle. (B) During deep, slow-wave sleep. (C) During surgical anaesthesia induced by 50 μ g of β -endorphin. Frequency bands: D2 = 1.56–3.51 Hz; T1 = 3.61–5.57 Hz; T2 = 5.66–7.52 Hz; A1 = 7.62–9.47 Hz; A2 = 9.57–12.5 Hz; B1 = 12.6–17.48 Hz; B2 = 17.58–25 Hz. Presented are percentage changes or values averaged from 6 to 8 animals. Vertical lines indicate S.E. ** $p \leq 0.01$.

three times greater than that of deep slow-wave sleep. We have defined this type of EEG as "hypersynchrony".²⁵

The behavioural and EEG components of the endorphin-induced anaesthetic state could be immediately and completely reversed by the intraperitoneal injection of naloxone HCl (1.0–3.0 $\text{mg} \cdot \text{kg}^{-1}$), as could all other phases of the total response to β -endorphin. Within one to two minutes after injection of the opioid antagonist the EEG pattern became essentially that of the normal awake state and the rats displayed normal locomotor and grooming activity.

DISCUSSION

The β -endorphin-induced state, in which animals are unresponsive to noxious stimulation but where palpebral and corneal reflexes are absent and the animals are rigidly immobile, has been described as "catatonia".¹⁴ We believe, however, that this syndrome, which can be induced by a variety of central depressants, corresponds to a state which differs from catatonia but resem-

bles, in all essential aspects, the condition of general anaesthesia. In clinical catatonia the schizophrenic patient is rigid and immobile but remains conscious, can recall events which occur during the catatonic period and retains sensory perception and pain sensation.³⁰ The EEG recorded from such a patient displays the typical awake pattern with desynchronization and shows arousal in response to stimulation.^{31,32} These criteria are met during the phase designated "CATATONIA" in Figure 1. This contrasts with the subsequent state in which sensory stimuli, including intensely noxious interventions, cause neither behavioural or EEG arousal.

The results observed here have shown that the behavioural and EEG responses to the ICV infusion of β -endorphin bear a remarkable resemblance in character and sequence to those observed with various classical and clinically used inhalational anaesthetics.³³ Interestingly, a clinically useful counterpart to these effects has previously been evident in procedures where relatively high (0.5 to 3.0 $\text{mg} \cdot \text{kg}^{-1}$) doses of the alkaloid opiate, morphine, have been given intravenously to achieve morphine anaesthesia.^{34,35} Complete absence of operative pain in open-heart surgery was universally reported, with morphine as the sole anaesthetic agent. Amnesia, a distinctive feature of the surgical anaesthetic state and an important goal of anaesthetic administration,^{33,36} was reported as varying from complete³⁴ to sporadic.³⁵ The latter was reported largely for patients who were not seriously ill. Production of an amnesic state, difficult to assess in animal experiments, is a strong indication that the observed morphine anaesthesia was genuinely equivalent to the anaesthetic state induced by classical inhalation anaesthetics. This corresponds with our own concept that the opioid β -endorphin, given ICV as in the present study, produces the equivalent of general anaesthesia rather than that of catatonia where amnesia is absent.³⁰

Classical theories of general anaesthesia originate from the correlation noted between anaesthetic potency and the oil-water partition coefficient of the anaesthetic substance. Such theories state that anaesthesia results from dissolution of the drug into the lipid layers of neuronal membranes.³⁷ In recent years, several reports have concluded that polar as well as non-polar chemical moieties are involved in the action of general anaesthetics and that the target site may include protein.^{38–42} For example, Franks and Lieb have shown that the partition

coefficients for general anaesthetics in octanol-water is much more closely correlated with anaesthetic potency than is that for vegetable oil/water or hexadecane/water. Those investigators concluded that the anaesthetic target site has solubility characteristics similar to those of the polar octanol. Others⁴³ point out that anaesthesia produced by the steroid alphaxalone is antagonized by a non-anaesthetic analog, delta-16 alphaxalone. This phenomenon cannot be accommodated by any of the traditional hypotheses of anaesthetic action; classical theories predict that these two closely related lipid substances would be additive in their effects, since solubility of drug in membranes should be the major determinant of anaesthetic action.

The endorphins are a family among a large group of neuropeptides stored within nerve terminals and believed to act as neuromodulators by combining with specific receptor sites on target neurons¹⁵⁻¹⁸. Our results show that general anaesthesia can be induced by β -endorphin, and immediately and completely reversed by a specific narcotic antagonist. This is clearly incompatible with traditional theories of anaesthesia based solely on lipid-lipid (hydrophobic) interactions or on occupation of a critical volume of the cell membrane. Instead, general anaesthesia can apparently result from the administration of a water-soluble peptide acting at very low tissue concentrations and upon specific receptors which occupy only a minute proportion of the cell surface of a few groups of target neurons. This observation has important implications for traditional theories of anaesthesia as well as for the possible development of or new class of general anaesthetic agents.

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RÉSUMÉ

L'injection de β -endorphine dans les ventricules cérébraux provoque chez le rat une séquence d'altérations du comportement et du tracé électroencéphalographique qui correspondent bien à l'observation clinique lors de l'administration d'anesthésiques généraux. L'agitation comportementale et l'excitation de l'EEG initiales, l'incoordination motrice et la réponse exagérée aux stimuli sensoriels sont suivies d'une période d'immobilité rigide avec maintien des réflexes locaux (retrait et cornée) ainsi qu'un tracé d'éveil à l'EEF après stimulation. Finalement, s'ensuit une immobilité associée à une absence de réponse électroencéphalographique et comportementale aux stimuli les plus nocifs. Un tel état d'inconscience avec analgésie complète définit bien l'anesthésie générale. Cet état fut complètement renversé par l'antagoniste spécifique aux opiacés, la naloxone. L'induction de l'anesthésie générale par une neurohormone peptidique hydrosoluble agissant sur des récepteurs spécifiques a d'importantes implications sur les théories traditionnelles de l'anesthésie.