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# OBSERVATIONS ON CHLORALOSE-INDUCED MYOCLONUS IN GUINEA-PIGS

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1 The physiological, biochemical and pharmacological features of α-chloralose-induced myoclonus in the guinea-pig have been studied.

2 EMG bursts in muscles jerking in chloralose-induced myoclonus are long, and are not time-locked to any cortical event recorded in the EEG, although they are evoked by auditory or peripheral nerve stimuli.

3 The efferent conduction velocity down the spinal cord of the signals generating the EMG bursts is fast but the afferent conduction velocity up the cord for stimulus-evoked jerks is slow, in distinction to the reverse characteristics of the spino-bulbo-spinal reflex arc.

4 α-Choralose did not cause any consistent change in 5-hydroxytryptamine (5-HT) or 5-hydroxyindoleacetic acid levels in any brain area, nor did it alter 5-HT turnover as judged by the depletion of 5-HT after *p*-chlorophenylalanine pretreatment.

5 Pretreatment of animals with drugs that increase brain 5-HT action (L-tryptophan with a monoamine oxidase inhibitor, or 5-hydroxytryptophan), or antagonize the action of 5-HT (cyproheptadine) did not abolish or obviously increase chloralose-induced myoclonus.

6 Chloralose-induced myoclonus is not similar to 5-HT-sensitive reticular reflex myoclonus in man.

## Introduction

Early clinical descriptions recognised that some forms of myoclonus were exacerbated by voluntary movement or sensory stimuli. Such action-induced, reflex myoclonus is characteristic of the syndrome described by Lance & Adams (1963) of intention or action myoclonus following cerebral anoxia. Two forms of reflex myoclonus have been identified in patients after cerebral anoxia. The first, reticular reflex myoclonus (Hallett, Chadwick, Adams & Marsden, 1977) arises as a result of a brain stem discharge which activates both cerebral cortex and muscle. The second, cortical reflex myoclonus (Hallett, Chadwick & Marsden, 1979) arises from a discharge in the cerebral cortex which activates sequentially both bulbar and limb muscles. Both forms of myoclonus are triggered by peripheral stimuli. That arising in the brainstem, reticular reflex myoclonus, appears to be associated with a functional deficiency of 5-hydroxytryptamine (5-HT) and may be treated successfully by 5-HT replacement therapy with its precursor 5-hydroxytryptophan or with tryptophan plus a monoamine oxidase inhibitor (Lhermitte, Marteau & Degos, 1972; Van Woert & Sethy, 1975; Chadwick, Hallett, Harris, Jenner, Reynolds & Marsden, 1977).

There are a number of animal models of reflex myoclonus (see Halliday, 1975, for review) but their relevance to human myoclonus is uncertain. The aim of such studies has been to establish an animal model suitable for refinement of drug therapy for the human condition. In this paper we describe certain physiological, biochemical and pharmacological features of the myoclonus provoked by α-chloralose with particular reference to whether or not there are suitable mimics of human action-induced, reflex-sensitive, 5-HT-dependent human post-anoxic myoclonus.

First described by Adrian & Moruzzi (1939), chloralose-induced myoclonus exhibits the characteristics of stimulus-sensitivity. Any abrupt sensory stimulus is sufficient to evoke muscle jerking in such animals. Each jerk is accompanied by a recognisable electrical discharge in the motor cortical area, and a subsequent corresponsing discharge of impulses down the pyramidal tract. While it was believed initially that chloralose-induced myoclonus was dependent on the motor cortex, subsequent investigation established that it could still be recorded after de-cortication

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(Alvord & Whitlock, 1954; Ascher, Jassik-Gerschenfeld & Buser, 1963) suggesting that it arose within the brainstem. Indeed, Shimamura & Yamauchi (1967) suggested that chloralose-induced myoclonus may be mediated by a spino-bulbo-spinal reflex arc. Certainly, such myoclonus survives bilateral section of the pyramidal tract at the medullary level (Ascher *et al.*, 1963) but is abolished by acute high spinal cord transection (Alvord & Fuortes, 1954).

Chloralose-induced myoclonus resembles reticular reflex post-anoxic myoclonus in man in that it appears to be dependent upon brainstem structures and is stimulus-sensitive. Chloralose-induced myoclonus therefore might be a suitable model to evaluate therapeutic agents for possible use in human post-anoxic action myoclonus. However, the present physiological, biochemical and pharmacological findings indicate that chloralose-induced myoclonus is not dependent upon 5-HT mechanisms.

## Methods

Guinea-pigs (Duncan-Hartley, 200 to 300 g) of either sex were used in all studies.

### *Physiological*

For electroencephalographic (EEG) observations circuit board pins, insulated along their length with lacquer, except at the tips, were implanted bilaterally into frontal, central and occipital regions under ether anaesthesia. Animals were allowed to recover for at least 24 h before recordings were made. Electromyograms (EMG) were recorded using teflon-coated platinum wire with hook ends inserted into appropriate muscle-bellies via a needle. EEG and EMG data were processed and amplified using Devices 3160 preamplifiers and Devices 3120 amplifiers. Recordings were processed, stored and averaged by a DEC PDP-12 computer. The computer could be triggered by the same square wave pulse that triggered either an electrical stimulus to a limb or an auditory stimulus. Electrical stimuli were delivered by a Devices 3073 stimulator via ring electrodes placed 1 cm apart distally on the appropriate limb. Such stimuli were of 500 ms duration and 100 V in strength. Auditory stimuli of a duration of 20 ms and frequency of 8,000 Hz were delivered by a loudspeaker placed by the animal's head.

### *Biochemical*

Guinea-pigs received α-chloralose (80 mg/kg i.p. in 0.9% w/v NaCl solution (saline)) or an equivalent volume of saline 30 min before death. Animals were killed by cervical dislocation and decapitation and the brain rapidly removed and cooled to 4°C on ice. The brain was quickly dissected into pons, mid-brain, corpus striatum, hippocampus, cerebellum, hypothalamus, mesolimbic area (nucleus accumbens, plus tuberculum olfactorium) and cortex. Brain parts were cooled to −20°C until dissection was complete and then weighed and homogenized in acidified *n*-butanol. Following homogenization, in an Ultraturrax homogenizer, and centrifugation at 3000 *g* for 10 min, the supernatant was removed and stored at −70°C overnight. The 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) content of the samples was assayed the next day by the fluorimetric technique of Curzon & Green (1970).

In some experiments animals were pretreated with *p*-chlorophenylalanine methyl ester hydrochloride (300 mg/kg i.p.) 48 h, 24 h and 1.5 h before the administration of α-chloralose (80 mg/kg) or saline.

### *Pharmacological*

Guinea-pigs were treated with α-chloralose (80 mg/kg i.p. in saline) and the following agents were administered: L-5-hydroxytryptophan (Cambrian Chemicals) dissolved in warm acidified saline administered subcutaneously in a dose of 100 mg/kg; L-tryptophan (Sigma Chemical Co.) dissolved in warm acidified saline, administered subcutaneously in a dose of 200 mg/kg, 45 min after pargyline hydrochloride (Abbott Laboratories) 75 mg/kg i.p.; clonazepam (Roche) 1 mg/kg in an appropriate volume of diluent; cyproheptadine (Merck, Sharpe & Dohme Ltd.) 10 mg/kg, i.p. Unless otherwise stated, drugs were dissolved in saline.

These agents were administered both 30 min before intraperitoneal chloralose, and following the induction of anaesthesia. The effect on the frequency of sensory evoked myoclonus was studied.

## Results

### *The EMG in chloralose myoclonus*

EMG records obtained from biceps brachialis and tibialis anterior following an electrical stimulus to the contralateral hind limb showed a polyphasic EMG response, corresponding to a myoclonic jerk, with a duration of approximately 30 ms, after a latency of some 28 to 30 ms to onset. Whilst the latency to onset of EMG activity was relatively constant, and varied only with the distance of the site of stimulation from the central neuraxis (see below), the duration of the EMG response varied from between 20 to 50 ms in both fore-limb and hind-limb muscles. The onset of the EMG potential in biceps brachialis always preceded that in tibialis anterior, usually by 1 to 2 ms,

irrespective of the site of sensory stimulation. On occasions, however, the difference in latency to onset between fore-limb and hind-limb muscles could be as little as 0.5 ms or as much as 4.0 ms.

Table 1 presents the latency to onset of the myoclonic response provoked either by an auditory stimulus or by electrical stimuli to the fore-limb and hind-limb. The response to auditory stimuli occurred earlier than that to fore-limb stimuli by approximately 10 ms. The latency of the response to fore-limb stimuli was some 10 ms earlier than that to hind-limb stimuli.

If it is assumed that the difference in time taken for afferent and efferent conduction in peripheral nerve in hind- and fore-limb is negligible, then the time taken for the efferent volley to descend from cervical to lumbar cord is constant at 1 to 2 ms, no matter whether an auditory stimulus or an electrical stimulus to fore- or hind-limb is used to evoke the myoclonus. (This value is derived by deducting the latency to onset of the response in biceps brachialis from the latency to onset of the response in tibialis anterior: Table 1).

Afferent cord conduction time can similarly be derived from the data in Table 1. This is given by deducting the latency of the myoclonic response in biceps brachialis or tibialis anterior following a fore-limb stimulus from the latency of the response in the same muscle following a hind-limb stimulus. This gives a slow afferent conduction time of 10 to 11 ms from lumbar to cervical cord. Similarly, afferent conduction time from cervical cord to brainstem can be derived from the latency of the myoclonic response following an auditory stimulus from that following an electric stimulus to the fore-limb. A value of 9 to 10 ms is thus obtained although some of this may be accounted for by longer peripheral conduction following a fore-limb stimulus.

*The EEG in chloralose myoclonus*

Bipolar cortical EEG records from all regions in animals anaesthetized with chloralose were flat with occasional generalised spikes or spike-slow wave complexes. However, such spikes bore no fixed relationship either to the application of any sensory stimuli or the occurrence of a myoclonic jerk. EEG spikes could occur synchronously with an evoked myoclonic jerk (Figure 1a), or in isolation (Figure 1b), or myoclonus frequently occurred without cortical spikes (Figure 1c).

We were unable to record enhanced cortical evoked potentials to peripheral electrical stimuli in our animals but following auditory click stimuli there was a consistent generalized enhanced evoked response recorded from cortical electrodes. This occurred 7.0 to 10.0 ms after an auditory stimulus (mean latency 8.4 ± 0.25 (s.e. mean) for 16 observations) and preceded the onset of the EMG response in the fore-limb by 2.0 to 4.0 ms.

*Regional brain concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid*

Regional concentrations of 5-HT and 5-HIAA 30 min following α-chloralose are compared to control groups in Table 2. Only mid-brain 5-HT was increased above control values but no change in mid-brain 5-HIAA occurred.

When synthesis of 5-HT was blocked by pretreatment with *p*-chlorophenylalanine, the administration of α-chloralose only increased 5-HT concentration in the corpus striatum and cerebellum compared to values in control animals, suggesting that α-chloralose may diminish 5-HT turnover in these regions (Table 3). However, such an interpretation is not supported by the concentrations of 5-HIAA which did not alter in these brain areas after chloralose (Table 2).

*Effect of drugs altering central 5-hydroxytryptamine metabolism on chloralose-induced myoclonus*

Because of the extreme variation from minute to minute in the susceptibility of animals under chloralose anaesthesia to sensory evoked myoclonus, observations on the influence of a pharmacological agent on the frequency of myoclonus were difficult. However, the administration of the 5-HT precursors, L-tryptophan (in combination with a monoamine oxidase in-

**Table 1** Latency to onset of myoclonus following administration of α-chloralose (80 mg/kg i.p. in saline) to guinea-pigs

| | *Latency to onset of myoclonus* (mean (ms) ± s.e. mean) | |
|---|---|---|
| | *Biceps brachialis* | *Tibialis anterior* |
| Auditory stimulus | 10.1 ± 0.9 (14) | 11.7 ± 0.9 (17) |
| Fore-limb stimulus | 19.2 ± 1.3 (13) | 21.5 ± 0.9 (13) |
| Hind-limb stimulus | 30.5 ± 3.2 (11) | 31.8 ± 3.3 (11) |

Figures in parentheses represent number of trials

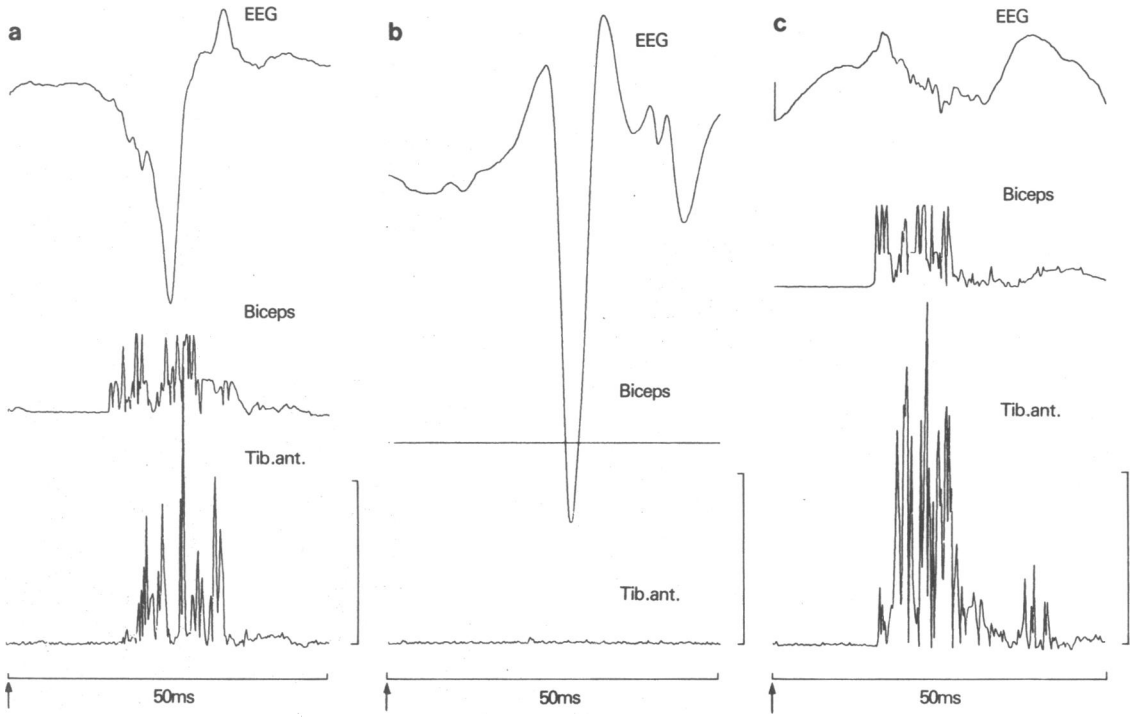


**Figure 1** EEG recordings (from electrode, inserted into the central region contralateral to the limb from which EMG records were taken, referred to an electrode in the opposite central region) (upper trace), and simultaneous rectified EMG records from biceps brachialis (middle trace) and tibialis anterior (lower trace) in a chloralose-anaesthetized guinea-pig to demonstrate: (a) EEG spikes occurring synchronously with an evoked myoclonic jerk; (b) EEG spikes occurring in isolation and (c) myoclonus occurring without cortical spikes. An electric stimulus to the hind-limb contralateral to the limbs from which EMG recordings were made has been applied at the start of each recording, and EEG and EMG activity was recorded for the subsequent 50 ms. Calibration marker indicates 0.2 mV for EEG trace and 5 mV for EMG traces. It can be seen that EMG activity and cortical spikes can occur independently of each other.

**Table 2** Effect of α-chloralose (80 mg/kg i.p. in saline 30 min previously) on regional concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in guinea-pig brain

| | | *Pons* | *Mid-brain* | *Corpus striatum* | *Hippocampus* | *Cerebellum* | *Hypothalamus* | *Mesolimbic* | *Cerebral cortex* |
|---|---|---|---|---|---|---|---|---|---|
| 5-HT (ng/g) | Saline controls | 836 ± 59 | 563 ± 35 | 586 ± 50 | 454 ± 35 | 131 ± 13 | 2307 ± 284 | 1191 ± 145 | 390 ± 38 |
| | α-Chloralose | 867 ± 50 | 746 ± 38* | 583 ± 30 | 462 ± 29 | 127 ± 8 | 2105 ± 151 | 1232 ± 79 | 363 ± 19 |
| 5-HIAA (ng/g) | Saline controls | 204 ± 24 | 209 ± 16 | 254 ± 39 | 154 ± 14 | 43 ± 9 | 484 ± 66 | 297 ± 42 | 110 ± 13 |
| | α-Chloralose | 254 ± 28 | 242 ± 28 | 170 ± 31 | 169 ± 21 | 44 ± 8 | 350 ± 65 | 189 ± 44 | 101 ± 9 |

* $P < 0.05$ (Student's $t$ test)
Each value is the mean ± 1 s.e. mean; $n$ = at least 6 for each value.

hibitor) and L-5-hydroxytryptophan, and of a 5-HT receptor antagonist, cyproheptadine, did not have any obvious influence on the frequency or intensity of myoclonus following α-chloralose. Certainly none of these drug pretreatments greatly increased or abolished chloralose-induced myoclonus.

## Discussion

The purpose of this investigation was to compare the characteristics of chloralose-induced myoclonus with those of human reticular reflex myoclonus (Hallett *et al.*, 1977). Both forms of myoclonus involve proximal limb muscles, and although both flexor and extensor muscles are activated simultaneously, the myoclonus usually results in a rapid flexion movement (Alvord & Fuortes, 1954; Hallett *et al.*, 1977).

In human reticular reflex myoclonus, the EMG correlate of the myoclonus is a brief (10 to 30 ms) hypersynchronous muscle action potential (Hallett *et al.*, 1977). This differs from the EMG correlate of the myoclonic jerk in the chloralose-anaesthetized animals, which consists of a prolonged (20 to 50 ms) polyphasic burst (see below) similar to the pattern of activity observed by others in recordings from ventral roots and peripheral nerves (Alvord & Fuortes, 1954; Shimamura & Yamauchi, 1967). (Early spinal responses to sensory stimuli are not seen in our recordings, because we have recorded from the limb contralateral to the stimulus).

The most prominent feature of the EEG in human reticular reflex myoclonus is the presence of generalized spike/slow wave activity. Animals lightly anaesthetized with α-chloralose show high amplitude irregular slow waves. In deeper anaesthesia the EEG becomes flat, with generalized spike/slow wave activity in response to sensory stimuli (Moruzzi & Magoun, 1949). In both forms of myoclonus there is no fixed temporal relationship between the EEG spike and the myoclonic jerk. Myoclonus may occur in the absence of an EEG spike, and *vice versa*. When both occur together they do not appear 'time-locked' to each other (see above, and Hallett *et al.*, 1977).

In human reticular reflex myoclonus, the cortical somatosensory evoked potential (s.e.p.) is usually of normal amplitude (Hallett *et al.*, 1977; Chadwick *et al.*, 1977). Whilst we have been unable to record enlarged s.e.ps from our experimental animals, King (1956) has recorded enlarged s.e.ps using lower doses of chloralose. He found that the amplitude of these responses diminished with higher doses, but still remained enhanced. The reasons for our failure to record enhanced s.e.ps are not clear, but we have been able to demonstrate enlarged auditory evoked responses.

In human reticular reflex myoclonus there is good presumptive evidence of disorder of 5-HT metabolism, which may be causally related to the occurrence of myoclonus. Thus, patients have low lumbar CSF 5-HIAA concentrations and they respond dramatically to the 5-HT precursor, 5-hydroxytryptophan, and to clonazepam, a benzodiazepine drug that may also affect central 5-HT metabolism (Chadwick *et al.*, 1977). However, we have been unable to demonstrate an influence of 5-HT on chloralose-induced myoclonus in the guinea-pig. Chloralose does not appear to alter regional 5-HT metabolism, and 5-HTP and L-tryptophan do not appear to suppress chloralose myoclonus.

Both human reticular reflex myoclonus and chloralose-induced myoclonus are mediated by reflexes involving the lower brainstem, but their precise mechanisms differ. The detailed neurophysiological evidence derived from the experimental study of chloralose myoclonus cannot, therefore, be directly applicable to this form of human myoclonus.

Shimamura & Livingston (1963) have shown that afferent nerve stimuli elicit motor responses in many segments of the spinal cord. These appear to be dependent on a spino-bulbo-spinal reflex arc. Shimamura & Yamauchi (1967) suggested that chloralose myoclonus may be mediated by such a pathway. However, the experiments of Shimamura & Livingston (1963) imply that the spino-bulbo-spinal arc used ascending pathways with a conduction velocity approximately twice that of the descending pathways. The data published here demonstrate that in

**Table 3** Effect of α-chloralose (80 mg/kg i.p. in saline 30 min previously) on regional, brain 5-hydroxytryptamine (5-HT) concentrations in guinea-pigs following *p*-chlorophenylalanine pretreatment (300 mg/kg 48 h, 24 h and 1.5 h before α-chloralose administration)

| | *Pons* | *Mid-brain* | *Corpus striatum* | *Hippocampus* | *Cerebellum* | *Hypothalamus* | *Mesolimbic* | *Cortex* |
|---|---|---|---|---|---|---|---|---|
| Saline controls | 349 ± 60 | 220 ± 17 | 299 ± 50 | 216 ± 42 | 78 ± 12 | 1134 ± 303 | 705 ± 156 | 258 ± 50 |
| α-Chloralose | 373 ± 37 | 270 ± 23 | 452 ± 31* | 212 ± 15 | 153 ± 19* | 1607 ± 283 | 801 ± 85 | 277 ± 34 |

Each value = mean ± s.e. mean; $n$ = at least 6 for each value.
* $P < 0.05$ (Student's $t$ test)

chloralose-induced myoclonus, ascending conduction velocity is much slower than descending conduction velocity, a finding also noted by Alvord & Fuortes (1964). It seems probable that chloralose myoclonus uses pathways other than the spino-bulbo-spinal reflex arc as described by Shimamura & Livingston (1963), and that it arises both in cortex and reticular formations, neither source obviously being related to 5-HT activity.

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