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Sleep induced by small doses of tubocurarine injected into cerebral ventricles of dog

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Summary

In unanaesthetized dogs, sleep with the typical sleep pattern in the E.E.G. was produced with (+)-tubocurarine when injected in a small dose (500 ng) into, or perfused in low concentration (100 ng/ml) through the cerebral ventricles.

Introduction

In previous experiments it was shown that (+)-tubocurarine injected into the cerebral ventricles of cats produced increased reflex excitability followed by convulsions and seizure activity on the electrocorticogram (Salama & Wright, 1950; Feldberg & Sherwood, 1954). The seizure activity was found to originate in the hippocampus (Feldberg & Fleischhauer, 1962, 1963). In the present experiments on unanaesthetized dogs, it was found that tubocurarine either injected into or perfused through the cerebral ventricles in amounts much smaller than those required to elicit these responses, produced sleep with the typical sleep pattern on the electro-encephalogram (E.E.G.). This observation is of particular interest in view of the recent finding that small amounts of tubocurarine pass into the cerebrospinal fluid (c.s.f.) after its intravenous administration (Devasankaraiah, Haranath & Krishnamurty, 1973).

Methods

Twenty dogs of either sex weighing between 7·5 and 11·5 kg were used. For the intraventricular injections of tubocurarine a Collison cannula was implanted in an aseptic operation under pentobarbitone sodium anaesthesia (30 mg/kg i.v.) into the left lateral cerebral ventricle according to the method described for cats by Feldberg & Sherwood (1953). For perfusion of tubocurarine through the cerebral ventricles a polyethylene tube (i.d. 0·75 mm; o.d. 1·3 mm) was inserted in the same operation into the cervical subarachnoid space after implantation of the Collison cannula. For this purpose the arches of two upper lumbar vertebrae were removed and the spinal cord with its meningeal coverings was exposed. A small opening was made into the subarachnoid space sufficient to admit the polyethylene tube which was pushed up into the cervical subarachnoid space as far as it would go. When it was withdrawn a few mm, c.s.f. flowed out from the outer end of the tube which was stoppered with a stilette. The tube was then fixed to the muscles and the skin by stitches and the wound was closed in layers.

To record the E.E.G. silver wires were stitched with aseptic precautions to the skin over the frontal, parietal and occipital regions of either side, and the leads so formed were connected to a 16 channel Schwarzer electro-encephalograph.

For the intraventricular injections, the tubocurarine was injected in a volume of 0·5 ml 0·9% w/v NaCl solution. Observations were made from the day after the operation. Several observations were made on the same dog on different days with different doses of tubocurarine injected each time. The injections were usually made up to 10 days after the operation and in a few instances even up to the 38th day. A minute to minute record of the behaviour of the animal was made during a control period of 1 h after injection of 0·5 ml 0·9% w/v NaCl solution before the tubocurarine was injected. For perfusion of the cerebral ventricles sterile artificial c.s.f. was perfused at a rate of 0·1 ml/minute. The composition of the artificial c.s.f. was (g/litre): NaCl 8·1 ; KCl 0·25 ; $CaCl_2$ 0·14 ; $MgCl_2$ 0·11 ; $NaHCO_3$ 1·76 ; NaH_2PO_4 0·07 ; urea 0·13 and glucose 0·61. The perfusions were made either on the second or third day or on both days after the operation.

(+)-Tubocurarine chloride (Koch-Light, England) was used. The values refer to the salt.

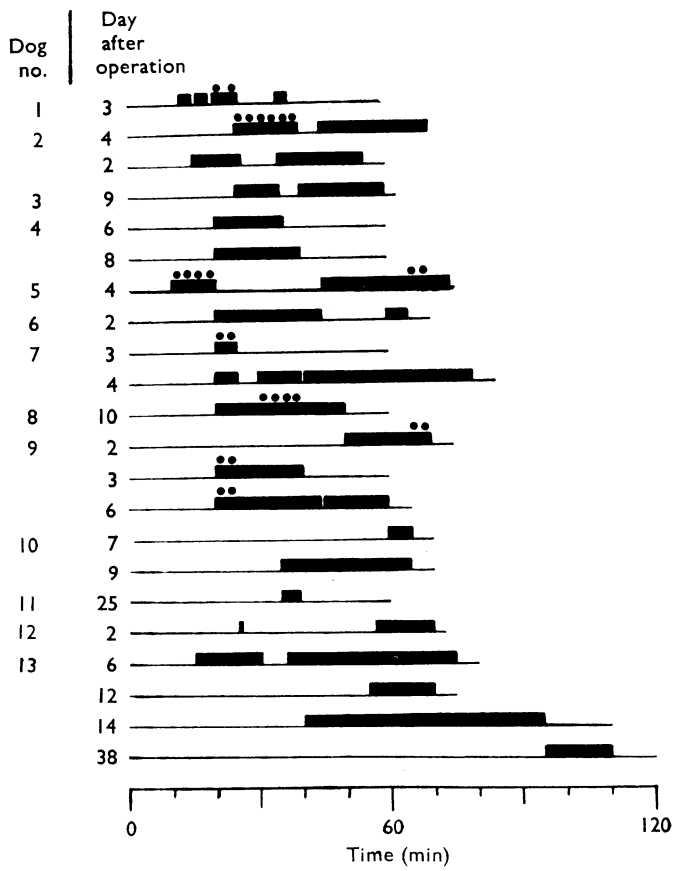

FIG. 1. Sleep periods indicated by horizontal black bars in 22 experiments on 13 dogs. At zero time intraventricular injection of 500 ng (+)-tubocurarine. The black dots over the bars indicate periods of movements of eyes, muzzle and paws during sleep. The numbers in the first column indicate the dog nos. and in the second column the day after operation when the injection was made.

Results

Intraventricular injections

The responses varied according to the dose injected. With a dose of tubocurarine above 25 μg, convulsions occurred, and with a dose above 2 μg there were signs of excitation in the form of occasional or continuous muscle twitches but no signs of sleep.

Following the injection of 500 ng the dog first moved about the room exploring and there were some licking and scratching movements, but after a few minutes the dog settled down in a corner and appeared to doze. Later, sometimes as early as 10 min, sometimes as late as 60 min, but usually about 20 min after the injection, the dog went into spells of sleep which varied in duration. The results of 22 experiments performed on 13 dogs on different days after operation are illustrated in Figure 1. The sleep periods are indicated by the horizontal bars. In some experiments, movements of the eye-balls, eye-lids, muzzle and paws occurred during the sleep periods. They are indicated in Fig. 1 by dots above the bars and correspond to rapid eye movement (REM) sleep. During the sleep periods the dogs were easily roused. In the intervals between sleep periods and when roused, the behaviour of the dogs was normal.

Figure 2 gives the E.E.G. in dog 1 of Figure 1. It shows at A the low voltage fast waves of the wakeful state before the tubocurarine injection, at B slow waves of high voltage characteristic of sleep 14 min after the injection, and again, at C, 10 min later (during a subsequent sleep period) when the dog was

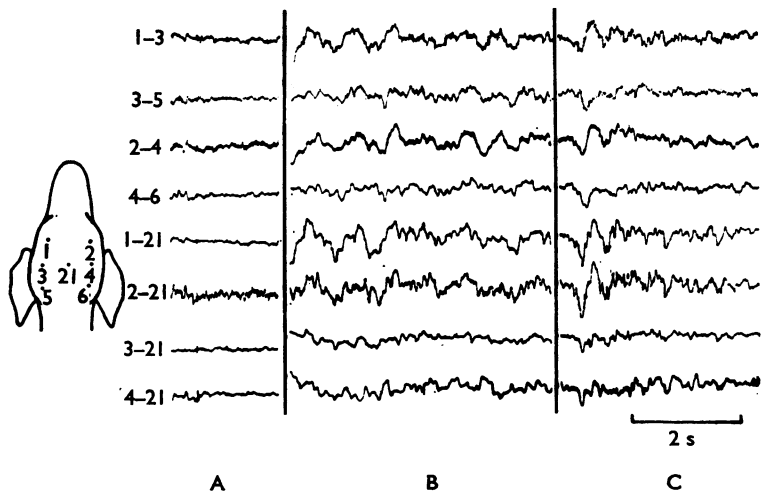

FIG. 2. E.E.G. from an unanaesthetized dog before (A), and 14 and 25 min after (B and C) an intraventricular injection of 500 ng (+)-tubocurarine. Dog was awake at A, asleep without eye movements at B, and with eye movements and movements of muzzle and paws at C. (Same experiment as with dog no. 1 of Figure 1).

showing movements of the eyes, muzzle and paws. In contrast to the general observation that REM sleep is associated with fast low voltage waves in the E.E.G., the eye and other movements seen after the tubocurarine injection during a sleep period sometimes occurred while the E.E.G. showed a slow wave pattern.

Following the injection of 50 to 250 ng tubocurarine the dogs became quiet

in approximately 15 min and in about half of the experiments they fell asleep after approximately 40 min and slept for 10–15 minutes.

Perfusions of the cerebral ventricles

On perfusion of tubocurarine 10 ng/min from lateral ventricle to upper cervical subarachnoid space the dogs became quiet and lay down within 5 minutes. Sleep occurred usually within 20 to 30 min after the beginning of the tubocurarine perfusion, but in one instance within 10 minutes. Sleep was continuous for 30 to 45 min or there were two or three periods of sleep lasting 5–20 min, interrupted by 5–20 min periods of wakefulness. During the sleep periods, movements of the eyes, muzzle and paws occurred frequently. They disappeared during the waking intervals. During the sleep periods the animals were easily woken up. The E.E.G. from such a perfusion experiment is shown in Figure 3.

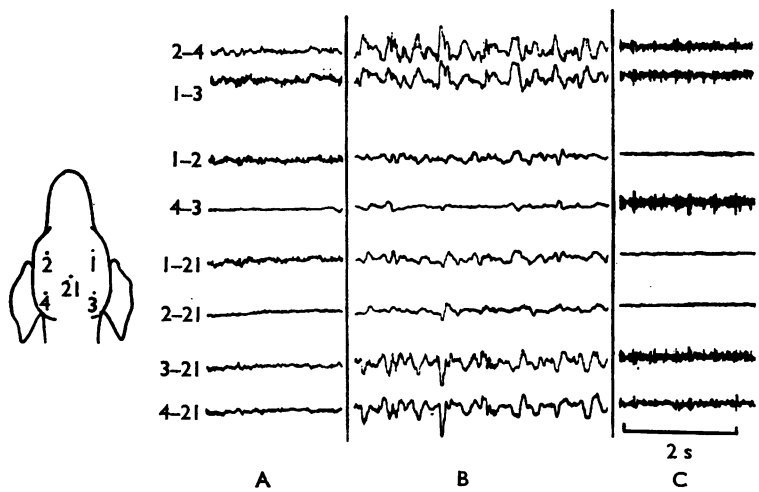

FIG. 3. E.E.G. from an unanaesthetized dog during perfusion of its cerebral ventricles at a rate of 0·1 ml/min with artificial c.s.f. without (A) and with 100 ng/ml (+)-tubocurarine (B and C). Dog was awake at A, asleep at B, and awake at C. B taken 11 and C 55 min after beginning of the tubocurarine perfusion.

In A, is seen the desynchronized pattern of the wakeful state during perfusion with artificial c.s.f. before the perfusion with tubocurarine began. In B, are seen the high voltage slow waves characteristic of sleep. A single spike is seen in the two top and bottom tracings. It suggests that this concentration of tubocurarine perfused through the cerebral ventricles was sufficient to produce signs of excitation. This is also suggested by the increased electrical activity in the leads attached to the occipital regions seen in record C obtained 55 min after the beginning of the tubocurarine perfusion at a time when the animal was awake and showed the activated E.E.G. pattern. In some experiments such increased electrical activity was prominent only in the records from the left occipital lead, i.e., on the ipsilateral side of perfusion of the lateral ventricle. The spikes and the increased electrical activity were not associated with any twitching of muscles.

Discussion

The present experiments show that tubocurarine produces sleep with the characteristic sleep pattern in the E.E.G. when acting from the cerebral ventricles in concentrations too low to produce excitatory effects or a seizure discharge in the E.E.G. Sleep was produced on intraventricular injection of 500 ng. As the injected tubocurarine will be diluted (in the cerebral ventricles) with about 5 ml c.s.f., its concentration should then be about 0·1 μg/ml. Devasankaraiah *et al.* (1973) found curare-like activity of the same or even higher order in the c.s.f. of men and dogs after intravenous administration of tubocurarine. In man the curare-like activity in lumbar c.s.f. corresponded to between 0·05 and 0·33 μg/ml after an intravenous injection of 0·43 to 0·63 mg/kg, and in dogs the activity in cisternal c.s.f. corresponded to between 0·1 and 0·75 μg/ml during an intravenous infusion of tubocurarine at a rate of 10 (μg/kg)/minute. These results were obtained in anaesthesia but it is unlikely that anaesthetics or the drugs used for preanaesthetic anaesthesia would have greatly influenced the passage of tubocurarine into the c.s.f. It is therefore possible that the amounts of tubocurarine reaching the c.s.f. after its systemic administration without anaesthesia exert pharmacological effects depending on the concentration of tubocurarine reached in the c.s.f., producing sleep in lower, and excitatory effects in higher concentrations. This may explain the controversial claims that tubocurarine has excitatory, inhibitory or no central effects on systemic administration. Blume (1934), Cohnberg (1946) and McCawley (1949) described excitatory effects in dogs and rats after systemic administration of tubocurarine. After intravenous injection of tubocurarine in dogs, McIntyre, Dunn & Tullar (1946) described an initial increase in the activity of E.E.G. followed by its depression. Sleep has been observed in unanaesthetized dogs following injections of small amounts of tubocurarine into the carotid or vertebral artery (Haranath, Sunanda-bai & Venkatakrishna-bhatt, 1967 ; Haranath & Indiranarayan, 1971). On the other hand, Smith, Brown, Toman & Goodman (1947) found no central effects of tubocurarine in an unanaesthetized subject (one of the authors weighing 80 kg) who was given an intravenous infusion of 30 mg in 15 min followed by three intravenous injections of 15 mg during the following 15 min and later by frequent intravenous injections of neostigmine. Any sleep-inducing effect of the tubocurarine may have been prevented by the continuous sensory stimuli arising from the intubated trachea, the accumulated secretions and from the constant questioning. Drowsiness occurred after 3 h when these stimuli were absent. In the present experiments on dogs too, sleep was produced by the intraventricular injections of tubocurarine, only when the dogs were left undisturbed.

Sleep has also been observed on injections of acetylcholine and of other cholinomimetic drugs into the cerebral ventricles or into the carotid artery. Dikshit (1935) found that injections of 0·1 to 0·5 μg of acetylcholine into the lateral ventricle of unanaesthetized cats produced a condition resembling sleep, lasting for two to three hours. In similar experiments, Silver & Morton (1936) observed only drowsiness in their cats. In unanaesthetized monkeys Light & Bysshe (1933) obtained no effect of this kind on intraventricular injections of a few milligrams of acetylcholine, and in man Henderson & Wilson (1936) found that such injections led to sleep in one, and to drowsiness in two out of eight patients. Haranath *et al.* (1967) who observed drowsiness and sleep with acetylcholine, neostigmine, physostigmine, hexamethonium and atropine injected into

the carotid artery of unanaesthetized dogs, discussed the role of cholinergic mechanism in sleep induced by cholinomimetic drugs and their antagonists. They suggested block of cholinergic receptors as a possible cause of sleep.

The seizure activity produced in the E.E.G. on intraventricular injection of tubocurarine in cats was found to originate in the hippocampus and to be most pronounced in the leads from the occipital regions (Feldberg & Fleischhauer, 1962, 1963; Feldberg & Lotti, 1970). Increased electrical activity recorded in the present experiments during the wakeful state on perfusion of tubocurarine through the cerebral ventricles also occurred solely in the occipital leads. Haranath *et al.* (1967) recorded in dogs similar increased electrical activity during intracarotid infusion of 30 μg/min acetylcholine during the wakeful state. Since Hernandez-Peon, Chavez-Ibarra, Morgane & Timo-Iaria (1963) obtained evidence that limbic cholinergic pathways are involved in sleep, the sleep induced by tubocurarine injected in small doses into the cerebral ventricles may be due to its action on the hippocampus or on associated areas.

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