

SLEEP INDUCED BY DRUGS INJECTED INTO THE INFERIOR HORN OF THE LATERAL CEREBRAL VENTRICLE IN DOGS

P.S.R.K. HARANATH & H. VENKATAKRISHNA-BHATT

Department of Pharmacology, Kurnool Medical College, Kurnool A.P. 518 002, India

1 In unanaesthetized dogs, cholinomimetic drugs and their antagonists were injected into the inferior horn of the left lateral cerebral ventricle. Injection volumes of 5 μ l were used to limit spread of the drugs beyond the inferior horn. The effects on EEG and behaviour were recorded and compared with the effects of the same doses given into the body of the right lateral ventricle a little behind the foramen of Monro.

2 Injections of cholinomimetic drugs into the inferior horn (acetylcholine 1–2 μ g, physostigmine 1.0 μ g, pilocarpine 100 μ g and nicotine 10 μ g) induced sleep during the following hour. The same doses injected into the body of the lateral ventricle did not produce sleep.

3 Cholinolytic drugs (atropine 10–20 μ g, hyoscine 0.4–1.6 μ g, (\pm)-tubocurarine 10–20 ng and hexamethonium 40 μ g) injected into the inferior horn also produced sleep, but the same doses injected into the body of the lateral ventricle were without effect. The EEG recorded after tubocurarine showed high voltage slow waves during sleep and desynchronized activation during rapid eye movement sleep.

4 Noradrenaline (10 μ g) injected into the inferior horn produced sleep whereas the same dose given into the body of lateral ventricle did not produce sleep. The results with 5-hydroxytryptamine were equivocal.

5 It is suggested that the site for induction of sleep lies in structures lining the inferior horn of the lateral cerebral ventricle and that the cholinomimetic drugs probably act by a depolarizing block and the acetylcholine antagonists by a competitive block.

Introduction

Haranath & Shyamalakumari (1973) described sleep following tubocurarine injected into the lateral ventricle in small doses (500 ng) or perfused in low concentration (10 ng/min) through the cerebral ventricles. They postulated that the site for induction of sleep could be in the structures lining the inferior horn of the lateral cerebral ventricles. Earlier Haranath, Sunanda-bai & Venkatakrisna-Bhatt (1967) had injected cholinomimetic drugs like acetylcholine, physostigmine and neostigmine and their antagonists atropine, tubocurarine and hexamethonium into the carotid arteries of unanaesthetized dogs and observed sleep following both groups of drugs. The present study was designed to establish whether the site of action for sleep induced by the above groups of drugs could indeed be in the structures lining the inferior horn. The effects of noradrenaline and 5-hydroxytryptamine (5-HT) were also studied since they too are involved in sleep mechanisms (Jouvet, 1969).

Methods

The studies were made on dogs of either sex (6.5–14 kg).

Cannulation of the inferior horn and body of the lateral cerebral ventricle

In dogs under pentobarbitone anaesthesia, a Collison cannula described by Feldberg & Sherwood (1953) and modified by McCarthy & Borison (1966) was screwed into the skull under aseptic conditions 12 mm in front of the zero plane and 17 mm lateral to midline. The shaft length of the cannula was 25 mm so that its tip penetrated the inferior horn of the left lateral ventricle.

Another ventricular cannula, with shaft length 17 mm, was implanted on the opposite side into the body of the right lateral ventricle, a little behind the foramen of Monro (8 mm behind the coronal suture and 5 mm lateral to midline). This position will be referred to as the body of the lateral cerebral ventricle.

EEG

To record the EEG, silver wires were stitched to the skin bilaterally on the frontal, parietal and occipital regions of the head and were connected to an Encardio-Rite polygraph at the time of recording.

Observations

The dogs had recovered completely on the day after implantation of the cannulae and drugs were injected into the cerebral ventricles on the following days. A minute to minute record of the behaviour of the animal was kept. Closure of the eyes for more than 3 min in a sleeping posture with slow and deep respiration and a high voltage slow wave EEG (when recorded) was considered to be sleep. The time until the animal awoke was recorded as a continuous sleep period. Eye movements were seen and were often associated with movements of the muzzle and paws and were confirmed as rapid eye movement (REM) sleep by the EEG changes. The times when these movements occurred were noted in the record. At the effective (sleep-inducing) doses the drug was tried in three or four different dogs and sometimes in the same dog more than once. Only one drug was tried on each day. The animals were generally unrestrained during observations. The dose of a drug necessary to induce sleep was first established without the EEG leads attached to the polygraph. In subsequent experiments with this dose EEG was recorded and mild restraint was necessary at the beginning of the experiment. Soon after the injection the dogs became quiet and later went to sleep when no further restraint was necessary.

Sterilized solutions of drugs were injected into the inferior horn in a volume of 5 μ l to limit the spread of the drug only to a small area around the tip of the cannula in the inferior horn. The injections were made with an Agla brand micrometer syringe (Burroughs Wellcome) or Hamilton syringe. For injections into the body of the lateral ventricle the drugs were given in a volume of 100 μ l. After completion of all observations methylene blue (0.1%) was injected in the same volume as the drugs into both cannulae and the distribution of the dye was studied *post mortem*. In all the experiments the dye injected into the inferior horn 15 min before the dog was killed was restricted to a 5–10 mm area around the tip of the cannulae and only in a few experiments did it spread up to the genu of the lateral ventricle.

Drugs

Acetylcholine chloride (E. Merck), physostigmine salicylate (T. & H. Smith) pilocarpine nitrate (Boehringer, Ingelheim), nicotine (BDH), atropine sulphate (Merck), (+)-tubocurarine hydrochloride

(Koch-Light), hexamethonium tartrate (May and Baker), noradrenaline (Fluka-Buchs) and 5-hydroxy-tryptamine creatinine sulphate (Merck) were used in these studies. The doses refer to their salts.

Results

Control observations

Control observations were made for 1 h after injection of 5 μ l of 0.85% sodium chloride (saline) into the inferior horn of the lateral cerebral ventricle or for 1 h without any injection. There were no changes of behaviour and no sleep occurred during the first or second hour after saline injection. The observations were made at different times of the day and in different places to exclude conditioning of the responses. If the animal was drowsy during the control observation no drug was injected on that day.

Cholinomimetic drugs

Acetylcholine injected into the inferior horn of the lateral cerebral ventricle in doses of 1–2 μ g immediately produced ipsilateral ptosis of varying degrees. The dog attempted to wipe its eyes and face with its paws. There was slight salivation. Drowsiness followed in 10–15 min and the animal slept for 3–10 min periods at a time with brief intervals of wakefulness during the following hour. Figure 1 shows the sleep periods from typical experiments after injection of cholinomimetic drugs. Sleep was for longer periods with 2 μ g acetylcholine. But with 10 μ g acetylcholine the animal showed restlessness, panting and increased depth of respirations but no sleep. When injected into the body of the lateral ventricle, acetylcholine 2 μ g did not produce any sleep. It required 20 μ g at this site to produce sleep.

Physostigmine in doses of 0.01–0.5 μ g injected into the inferior horn did not produce any effect. But doses of 1 μ g and above produced sleep (Figure 1). Injections of 10 μ g physostigmine produced sleep for a long period with frequent twitchings of the muscles of the eyelids, eye balls, muzzle and paws. These are identical with the REM phase of sleep. Feldberg & Sherwood (1954) found cats did not sleep after injection of 10 μ g physostigmine into the body of the lateral ventricle. They observed catatonia with large doses.

Pilocarpine (100 μ g) injected into the inferior horn produced sleep within 10–15 min and each sleep period lasted for 5–10 min at a time with brief intervals of wakefulness. Rapid eye movements were sometimes observed during sleep (Figure 1). In 50 μ g doses pilocarpine produced drowsiness, and sleep

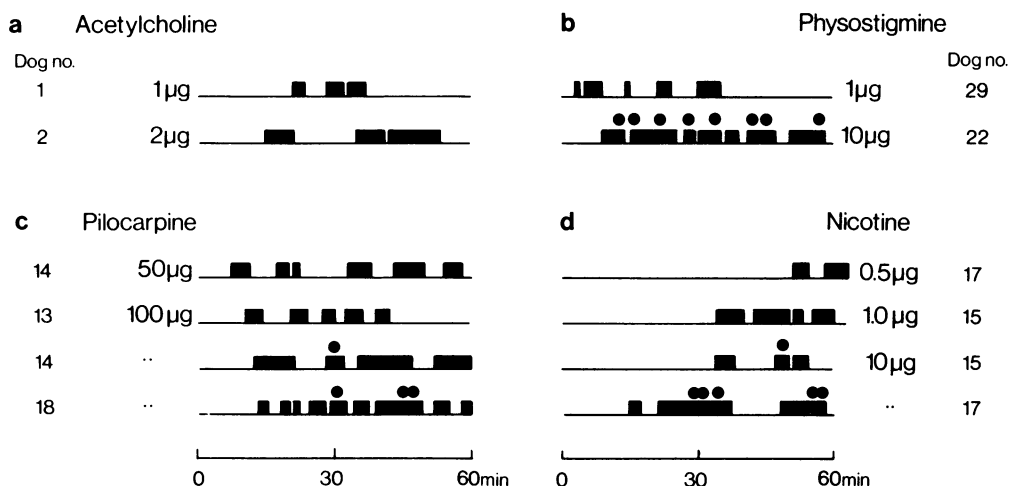


Figure 1 Sleep periods indicated by horizontal black bars in different experiments. At zero time acetylcholine (a), physostigmine (b), pilocarpine (c) or nicotine (d) was injected into the inferior horn of the lateral cerebral ventricle in conscious dogs, in a 5 μ l volume in the doses shown. The black dots over the bars indicate periods of movements of eyes, muzzle and paws during sleep. The numbers given in the margins are the code numbers of the individual dogs.

occurred only in a few instances. In 10 μ g doses it produced some salivation and lacrimation but no sleep; 1–2 μ g doses had no effect. Pilocarpine 100 μ g injected into the body of the lateral ventricle a little behind the foramen of Monro did not produce drowsiness or sleep but only wiping of the face and salivation.

Nicotine in doses of 0.05–0.5 μ g injected into the inferior horn caused restlessness, retching and increased activity. Doses of 1 μ g produced drowsiness and, in some experiments, sleep. The animal wiped its eyes and face on the ipsilateral side for some time. With 10 μ g nicotine the animal slept for varying periods after 15 min as can be seen from Figure 1. Rapid eye movements were also observed during sleep. Nicotine 10 μ g injected into the body of the lateral ventricle failed to produce sleep or any other effect.

Cholinolytic drugs

Atropine 10–20 μ g injected into the inferior horn produced immediately increased activity for about 10 min followed by intermittent sleep for nearly 1 h (Figure 2). There was less sleep after 50 μ g atropine. Atropine 1 μ g was ineffective, while 5 μ g produced some drowsiness. In doses of 20–50 μ g injected into the body of the lateral ventricle, atropine did not produce sleep.

Hyoscine 0.4–1.6 μ g injected into the inferior horn produced sleep for varying periods as can be seen

from Figure 2. Higher doses of 3.2 μ g and lower doses of 0.01–0.2 μ g hyoscine did not produce sleep. When injected into the body of the lateral ventricle hyoscine 1 μ g produced intense salivation but no sleep. Sleep occurred only with 5–100 μ g hyoscine injected into the body of the lateral ventricle. With 1 mg hyoscine injected into the body of the lateral ventricle, the dog defaecated, micturated, became restless and slept after 50 minutes.

(+)-*Tubocurarine* injected into the inferior horn in doses as little as 10–20 ng produced sleep (Figure 2). There were movements of eyes, muzzle and paws during the sleep so produced. Figure 3 shows the EEG taken before and after injection of (+)-tubocurarine 10 ng into the inferior horn. In the record taken 30 min after injection of (+)-tubocurarine the dog was awake and showed increased neuronal activity and desynchronization. After 47 min, the dog was asleep and showed high voltage slow waves. In the 50th min the dog was still asleep but showed rapid eye movements and EEG desynchronization. The same doses of 10–20 ng tubocurarine did not produce any effect when given into the body of the lateral ventricle. The injection of 500 ng (+)-tubocurarine into the body of the lateral cerebral ventricle was required to produce sleep (Haranath & Shyamalakumari, 1973).

Hexamethonium 40 μ g injected into the inferior horn produced sleep after 10 min and rapid eye movements were frequently observed (Figure 2). No sleep occurred when the dose injected into the inferior horn

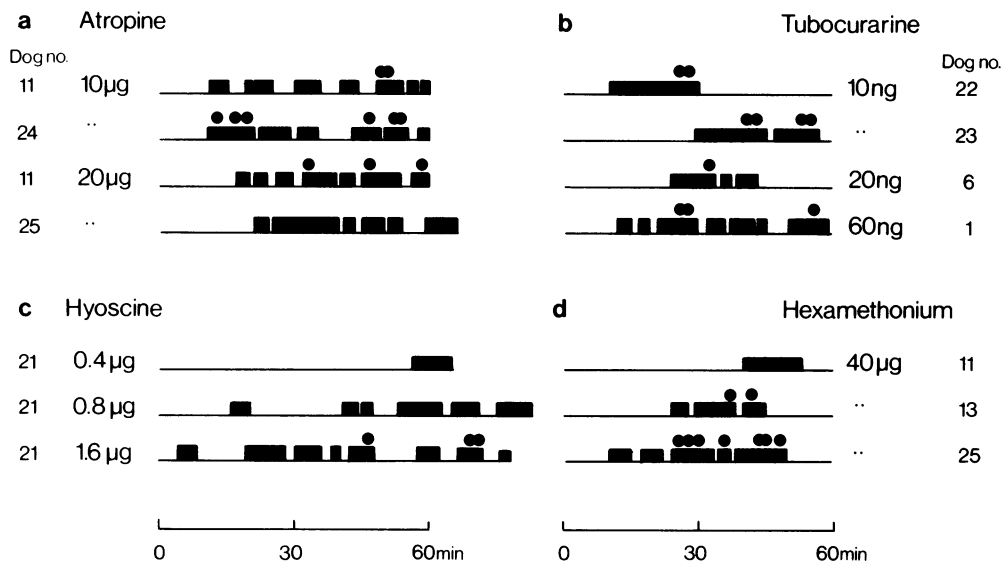


Figure 2 Sleep periods indicated by horizontal black bars in different experiments. At zero time atropine (a), (+)-tubocurarine (b), hyoscine (c), or hexamethonium (d) was injected into the inferior horn of the lateral cerebral ventricle in conscious dogs in a 5 µl volume in the doses shown. The black dots over the bars indicate periods of movements of eyes, muzzle and paws during sleep. The numbers given in the margins are the code numbers of the individual dogs.

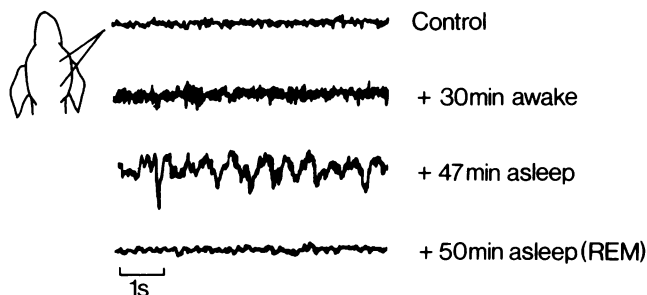


Figure 3 EEG from an unaesthetized dog. Top record taken before and the rest after injection of (+)-tubocurarine 10 ng into the inferior horn of the left lateral cerebral ventricle. Time of taking the record after the injection and the behavioural state of the animal is given opposite each record.

was reduced to 10 µg, nor when 40 µg hexamethonium was given into the body of the lateral cerebral ventricle.

Other drugs

Noradrenaline in doses of 10–20 µg injected into the inferior horn produced sleep lasting for varying periods starting 10–20 min after injection, in only two out of four experiments. But the same dose injected into the body of the lateral ventricle did not produce sleep.

5-Hydroxytryptamine injected into the inferior horn in doses of 0.01–0.1 µg produced ptosis and wiping of the face for about 10 min but no sleep. With 0.5 µg 5-HT, sleep occurred after 45 minutes. No sleep occurred when 1 µg 5-HT was given into the body of the lateral ventricle.

Discussion

The present study supports the suggestion of Haranath & Shyamalakumari (1973) that the site for

induction of sleep by small amounts of (+)-tubocurarine injected into the body of the lateral ventricle is in the structures lining the inferior horn. Tubocurarine, even in 10–20 ng doses injected in a 5 μ l volume into the inferior horn produced sleep, whereas 500 ng tubocurarine was required to induce sleep when injected into the body of the lateral ventricles. Some idea of the probable extent of the spread of the injected drug can be obtained from the experiments of McCarthy & Borison (1966). They reported that injections of 20 μ l volumes of radio-opaque material into the posterior lateral ventricle of a cat, close to the mouth of the inferior horn, outlined only the posterior and inferior portion of the ventricle. Only when 50 μ l was injected did the material spread anteriorly. On this basis, in the present experiments the 5 μ l injections into the most ventral portion of the inferior horn are unlikely to have spread very far. The distribution of injected methylene blue also supports this conclusion. Thus it appears that the site for induction of sleep by (+)-tubocurarine is in the structures lining the inferior horn. The sleep observed on injection of tubocurarine into the body of the lateral cerebral ventricle could be the result of diffusion into the inferior horn.

That the inferior horn is a site for induction of sleep was borne out not only with (+)-tubocurarine but by the cholinomimetic drugs like acetylcholine, physostigmine, pilocarpine and nicotine, and by the acetylcholine antagonists, atropine, hyoscine and hexamethonium. The above drugs induced sleep in small doses when injected into the inferior horn but the same doses were ineffective when injected into the body of the lateral ventricle.

Since both cholinomimetic drugs and their antagonists produce sleep, this common effect might be achieved through a depolarizing block by the cholinomimetics and a competitive block by the antagonists. The fact that the doses of cholinomimetic

drugs required to produce sleep are much higher than those of their antagonists supports this view. This also would fit into the concept of increased activity of cholinergic mechanisms in wakefulness and REM sleep. For example, Haranath & Venkatakrishna-Bhatt (1973) reported diminished release of acetylcholine into the perfused cerebral ventricles in unanaesthetized dogs just before and during sleep, and an increased release during REM sleep. Similarly Celesia & Jasper (1966) observed in cats diminished release of acetylcholine from the physostigmine-treated cerebral cortex during natural and barbiturate-induced sleep. Jasper & Tessier (1971) and Gadea-Ciria, Stadler, Lloyd & Bartholini (1973) have also reported diminished release of acetylcholine during slow wave sleep and an increase during REM sleep.

The inferior horn appears to be a site for induction of sleep by other mechanisms also, since noradrenaline induced sleep in much smaller doses when injected into the inferior horn than when given into the body of the lateral ventricle. The results with 5-HT were equivocal since it produced sleep only at a dose of 0.5 μ g but not higher or lower doses. Even at that dose, sleep occurred only after 45 minutes.

The important structures lining the inferior horn are the amygdala and the hippocampus. Although it is unlikely that a single neurohumoral mechanism can be solely responsible for induction of sleep at all levels (King, 1971), it is likely that cholinergic mechanisms are involved in some pathway situated in the limbic system (Hernandez-Peon, Chavez-Ibarra, Morgane & Timo-Iaria, 1963) and particularly in the hippocampal region (Kim, Choi, Kim, Kim, Kim, Park & Ahn, 1975).

This work was carried out with a grant-in-aid from the Indian Council of Medical Research, which is gratefully acknowledged.

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(Received January 26, 1976.
Revised September 28, 1976.)