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INTRACAROTID INJECTIONS AND INFUSIONS OF CHOLINOMIMETIC DRUGS AND THEIR ANTAGONISTS IN CONSCIOUS DOGS

BY

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Dikshit (1935) injected acetylcholine in doses of 0.1–1.0 μg into the lateral ventricles of conscious cats and observed a condition resembling sleep that lasted for 2–3 hr. Feldberg & Sherwood (1954a) observed drowsiness or stupor in cats after similar injections of acetylcholine in doses of 1 μg or less. However, with doses of 10–20 μg they observed that the cats emitted a high-pitched cry and retched, and later went into a condition resembling akinetic seizure. When large doses of 1 mg acetylcholine were injected into the cerebral ventricles of cats general convulsions were produced, followed later by stupor (Feldberg & Sherwood, 1954b). No studies are available, however, on the effects of intracarotid injections of acetylcholine in conscious animals, which would expose the brain to a higher concentration of the drug than the rest of the body. Bradley (1960) injected small amounts of acetylcholine (5 ng) into the carotid artery of the cat (*encéphale isolé* preparation) and observed activation of the electroencephalogram.

In the present experiments with conscious dogs, the effects of intracarotid injections, as well as infusions of acetylcholine, were studied. In addition to acetylcholine, its antagonists, atropine, *d*-tubocurarine and hexamethonium, and the anticholinesterase drugs neostigmine and physostigmine were administered into the carotid artery, and the effects were observed.

METHODS

The experiments were performed on 31 dogs of either sex weighing 6.8–14.9 kg.

Operative procedures

Carotid loops

The carotid arteries were placed in skin loops under aseptic conditions in five dogs under pentobarbitone anaesthesia. A procedure similar to that described by Himwich, Costa, Canham & Goldstein (1960) was followed with some modifications. The common carotid artery was dissected distally to about 2 cm above the origin of the thyroid artery (which was ligated and severed) and proximally to about 3 cm above the root of the neck. The freed artery was included in a skin pedicle. Care was taken not to close too tightly the sites of entry and exit of the artery in the skin pedicle, so that the artery was not constricted by fibrous tissue at the time of healing. In one dog the external carotid artery was ligated before it divided into branches.

Indwelling carotid cannulae

Under aseptic conditions, in 21 dogs anaesthetized with pentobarbitone, a skin incision was made in the midline starting about 5 cm above the larynx and ending about 7 cm below it. The common carotid artery was exposed and cleaned, care being taken not to injure its thyroid branch. A polythene tube (Clay Adam PE 60 or PE 90) filled with heparin was placed in the common carotid artery through its thyroid branch. To the outer end of the polythene tube was attached a metal cannula with rubber diaphragm through which drugs could be injected or infused without causing pain. In two dogs the external carotid artery was ligated before it branched out.

Heparin (0.2–0.5 ml. of 1% solution) was injected into the cannula at the time of operation and also each day at the end of the experiment. The cannula remained patent for 2–7 days. When block occurred, it was generally due to a small clot on the arterial wall in contact with the tip of the cannula. The clot was adherent to the arterial wall and did not usually fill the entire lumen of the artery. It was sometimes possible to pass a finer polythene tube (PE 10) through the original cannula to render it patent. In a few dogs an anticoagulant (ethyl biscoumacetate (Tromexan, Geigy), 150–300 mg) was given daily or on alternate days to help keep the cannula patent for longer periods. The results were not influenced by anticoagulant therapy.

Tracheostomy

Simultaneously with insertion of the carotid cannula, a tracheostomy was performed and a Fuller's tracheal cannula consisting of a winged outer tube and an inner tube was left in position.

Experimental procedures

Conscious dogs

Dogs with carotid loops were preferred for single injections of drugs, since they were more active than animals with indwelling cannulae. They were used 15–20 days after operation, when the skin had healed completely and no oedema was present. During the observations, the dogs were not restrained. The same drugs were injected into each animal on at least two different days.

Dogs with indwelling cannulae were used for infusions since the solutions could be infused for any length of time without causing pain. The dogs were used from the day after operation, as long as the cannula was patent. Respiration was recorded on a kymograph in these animals by connecting the inner tube of the tracheal cannula to a tambour. The dogs were lightly restrained by the attendant so that the connexions for the infusion and the respiration record were not disturbed. In one experiment atropine was infused intravenously in a conscious dog through a fine polythene tube passed into the saphenous vein through a wide bore intravenous needle and kept in position.

To obtain electroencephalographic records, silver wires were stitched to the skin with aseptic precautions on the fronto-parietal and the occipital regions on each side, and the leads so formed were connected to a 16 channel Schwarzer electroencephalograph. A complete minute to minute record was maintained of the behaviour of the animal and of the times it closed its eyes and slept. Solutions used for injections into the carotid arteries were prepared in sterile (0.9%) sodium chloride solution and were injected with due aseptic precautions. Single injections of drugs did not exceed 0.5 ml. in volume. If necessary the injections were repeated at the end of half an hour to enhance the effects observed. In experiments where drug solutions were infused with a continuous slow injector, the rate of infusion was 0.3 ml./min.

After all the observations were completed the dog was anaesthetized with pentobarbitone, and methylene blue 1% was either infused for 5 min, or injected in the same way as drugs into the carotid artery. The animal was immediately killed with an overdose of pentobarbitone and a post mortem examination was carried out. The staining of the brain was examined to confirm the patency of the carotid artery.

Anaesthetized dogs

In a few experiments in dogs under pentobarbitone anaesthesia, a polythene carotid cannula was placed as described above and drugs were infused at the same rate as in conscious dogs. The femoral blood pressure (mercury manometer) and respiration were recorded simultaneously.

Drugs

Acetylcholine chloride (E. Merck), neostigmine methylsulphate (Hoffmann-la Roche), physostigmine salicylate (T. & H. Smith), atropine sulphate (E. Merck), *d*-tubocurarine chloride (L. Light & Co.) and hexamethonium tartrate (May & Baker) were used in these experiments. The doses mentioned in the text refer to their salts.

RESULTS

Acetylcholine, *d*-tubocurarine, atropine and hexamethonium were administered, in conscious dogs, as single injections into carotid loops, and also as continuous slow infusions into the carotid arteries through the indwelling cannulae. Neostigmine and physostigmine were given only as single injections. The drugs were administered on different days in each animal. When the same drug was given on a second or third occasion the intensity of effects obtained was sometimes diminished.

Intracarotid injections

Control observations

Dogs with carotid loops which were used for single injections were observed for 1 hr before each drug injection. They were found exploring the room, active and attentive to signals given or voices heard. Sometimes they lay down for short periods and closed their eyes for a few seconds. The injection of 0.5 ml. saline did not produce any significant effects on their behaviour.

The dogs did not sleep during control observations. One one occasion, when the dog was observed after it had eaten, it slept for short periods of 2–4 min. During sleep both eye-lids twitched. We also saw, outside the laboratory, sleeping dogs who showed twitchings of the eye-lids and snout muscles, and movements of the feet. Similar twitchings of limbs, vibrissae and ears were observed in cats during normal sleep by Dement (1958). He further described that the electroencephalogram showed an activated pattern during these phases of sleep.

Acetylcholine

Injections of acetylcholine 1,000 μg into the carotid artery elicited a peculiar high-pitched cry, and later the dog stood still, apparently dazed. In the ipsilateral side the eye-lids went into a spasm, and nasal and tear secretions appeared. Salivary secretion was also present. The animal slowly lay down with the eyes closed but head raised. For a brief period the ipsilateral pupil was dilated. The spasm of the eye-lids was present for 5–10 min. Salivary secretion diminished in about 5 min. The eye changes and secretions from the same side were absent in the dog with the external carotid artery ligated.

At the end of 10 min the animal was drowsy, though the eyes were open and head raised. After about 20 min the animal rested its head on the front limbs and closed its eyes for periods of 1–2 min, opening them briefly for a few seconds. This state continued for about an hour, and during it two out of the five dogs studied slept continuously for periods of 5–15 min. During sleep the animals showed twitchings of the eye-lids and snout muscles and movements of the feet which were similar to those observed during normal sleep. The dogs, though sleepy, responded to calls and were attentive to noise. At

the end of 1–2 hr they were once more active. Respiration was generally rapid. The animal occasionally passed urine and faeces.

Injections of 500 μg acetylcholine also elicited a cry. Salivation occurred and respiration was rapid for about 10 min, after which the dog was drowsy for a short period. In about 30 min the animal was alert and was actively moving about the room.

d-*Tubocurarine*

d-Tubocurarine was injected in doses of 500 μg into the carotid artery. Sometimes the injections were repeated after half an hour. The animals were observed for 1–2 hr. Immediately on injection there was ptosis of the ipsilateral eye-lid which lasted for 10–15 min. Paresis of the limbs was noticed in only one experiment in which the second dose was administered less than 10 min after the first one. Otherwise, in the other experiments no systemic paretic effects were observed. Respiration was generally rapid after the injection but became slow when the animal slept later.

The animal was quiet within 5 min and lay down on the ground and closed its eyes occasionally. About 20 min later the dog had spells of sleep lasting up to 15 min at a time. Usually it slept for 5–10 min periods. Towards the end of each such period twitchings of eye-lids and snout muscles, movements of the feet and masticatory movements occurred. Sleep occurred usually in all the injected dogs and lasted much longer than with acetylcholine. Twitchings occurred frequently. Sometimes the animals slept only after the second injection. Figure 1 shows three electroencephalographic records from a dog after curare injection. They were taken while it was awake, asleep, and while its muscles were twitching during sleep. The electroencephalogram shows a high voltage slow wave pattern during sleep and an activated pattern when twitchings occurred during sleep.

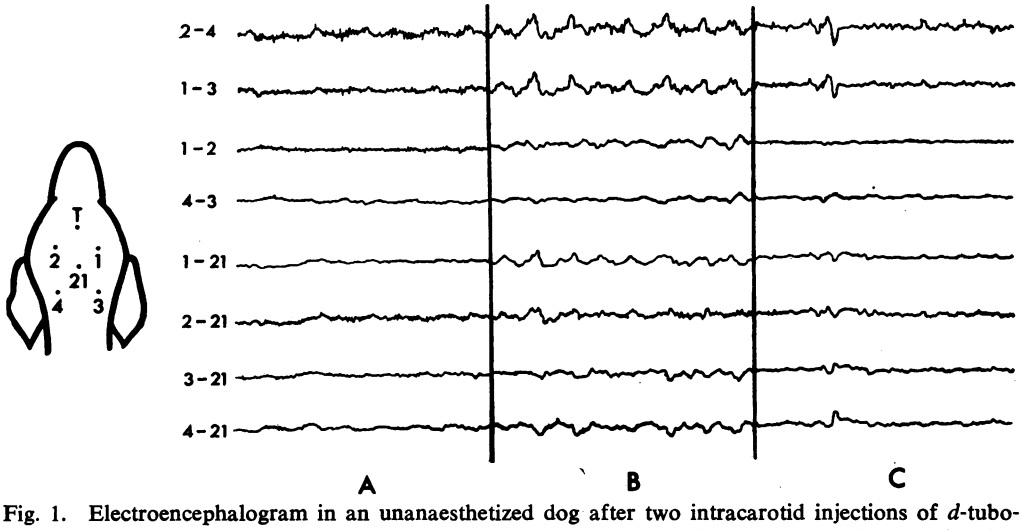

Fig. 1. Electroencephalogram in an unanaesthetized dog after two intracarotid injections of *d*-tubocurarine 500 μg each, given at 40 min interval. A was taken with the dog awake, 16 min after the first injection; B with the dog asleep, 30 min after the second injection; C with the dog asleep and showing twitchings of eye-lids and snout muscles, 50 min after the second injection.

Atropine

When atropine was injected in doses of 50 μg into the carotid arteries there was immediate dilatation of the ipsilateral pupil. There was no apparent change in the animal's behaviour for about 10 min. Later it became drowsy, and lay down in a dark corner closing its eyes now and then. It slept for about 10 min at a time once or twice during the 90 min period of observation. While asleep, the animal showed a characteristic slow wave pattern in the electroencephalograms. There were twitchings of the eye-lids and snout muscles, masticatory movements and movements of feet during sleep and the electroencephalogram showed an activated pattern. Respiration was rapid soon after injection but later gradually slowed. The pupil on the ipsilateral side returned to normal size by the next day.

Hexamethonium

Hexamethonium was administered in doses of 500 μg into the carotid artery. Within the first few minutes no obvious effects were noticed. In 10–15 min the dog was drowsy, closing its eyes occasionally and later had spells of sleep. The periods of sleep usually lasted for 10–15 min each and at most for 28 min. During sleep, twitching of the muscles of the face and movements of the toes occurred. In the intervals, when the animal was awake, it was alert. The duration and depth of sleep observed with hexamethonium was similar to that produced by curare.

Neostigmine

Neostigmine was injected into carotid loops and also into the cannulated carotid arteries in doses of 250 μg. This dose was again repeated at the end of 20–40 min. Immediately after injection there were nasal and tear secretions from the same side and salivation. In 1–2 min there was intense fasciculation of the temporal muscle of the same side. Later this extended to the neck muscles and muscles of the rest of the body. These muscle fasciculations lasted for about 15 min and later subsided. The pupil on the same side contracted. In animals with the external carotid artery ligated, the ipsilateral temporal muscles were not involved in the fasciculations and no miosis or salivary secretion was observed. The animals were sleepy after 20 min and slept for about 10 min periods. Sometimes sleep was observed only after the second injection. The electroencephalogram taken while the animal was asleep showed a slow wave pattern. Twitchings of the eye-lids, etc., observed in sleep with other drugs were also observed in these animals. Sometimes they passed urine and faeces.

With single injections of 500 μg neostigmine the animal immediately rushed to a corner and defaecated. There was some spasticity of gait and the muscle fasciculations lasted for a longer time. The animals slept longer.

Physostigmine

When physostigmine was administered in doses of 100 μg into the carotid loops, salivation and nasal secretions were produced immediately. Later the animals appeared to be itching all over its body. As it rolled on the ground it licked itself and started scratching and biting all accessible areas. It also wiped its face with its paws repeatedly.

With 500 μg doses, however, the dogs immediately began circling in the opposite direction with the neck appearing to look over the opposite shoulder. This lasted for 5–10 min. No effects were observed on the pupils. When the injection was repeated at the end of 20 min it caused drowsiness alternating with phases of scratching. Injections of 500 μg physostigmine into the carotid artery with the external carotid branch ligated did not result in circling movements, suggesting that their origin was peripheral. With the external carotid artery ligated the internal carotid and occipital arteries receive a greater share of the drug injected into the common carotid artery. In such animals muscle fasciculation (similar to those produced with neostigmine) appeared on the same side in the neck muscles extending up to the ear. When the same dose was repeated at the end of 20 min there was intense coughing, frothing at the mouth and spastic gait. After an hour the dog slept for about 10 min. During sleep it showed the characteristic twitching of the eye-lids and snout muscles.

Rothballer, Jarvik & Jacobs (1961) observed contraversive circling with injections of 50–60 μg physostigmine into the carotid artery in conscious cats, and they considered it a central effect. Feldberg & Sherwood (1954b) injected 10–100 μg physostigmine into the lateral ventricles of conscious cats and observed behaviour suggestive of intense itching and irritation, stiffness of muscles, and clumsiness of movements which was later followed by catatonic stupor.

Intracarotid infusions

Since drowsiness and sleep were produced about 10–20 min after intracarotid injections these could be residual effects of the drugs that reached the brain with each single injection. It was therefore decided to study the effects of continuous slow infusion of small quantities of these drugs into the carotid artery, so that the brain received a higher concentration of the drug over a long period while the rest of the body received insignificant amounts. The drugs were infused at the rate of 0.3 ml./min for an hour or more into the carotid arteries of conscious dogs with indwelling cannulae.

Control observations

Dogs with indwelling cannulae appeared less active than those with carotid loops and only 10 out of 21 dogs did not sleep during the control observations. The other animals were sluggish, either on days immediately after the operation or a few days later, and slept for short periods during control observations. During the periods of sleep their eye-lids and snout muscles twitched, and their toes moved. Effects of drug infusions were taken into account only when the dogs did not sleep for more than 3 min at a time during control observations.

Respiration recorded from the trachea was rapid, irregular and of small amplitude when the animal was awake; and the excursions of the tambour were large when the dog cleared its throat or whined. Respiration during sleep was slow and regular.

The infusion of saline at the same rate as drug solutions (0.3 ml./min) for 1 hr periods did not produce any marked effects.

Acetylcholine

Acetylcholine infused at 30 μg/min into the carotid artery produced salivation, and nasal and tear secretions on the ipsilateral side which continued throughout the period of infusion. Within 10–15 min the dog lay down, curled itself up, closed its eyes and went to sleep. The muscles relaxed and the head sagged. Figure 2 shows the electroencephalogram of a dog recorded during acetylcholine infusion. It was taken with the animal awake as well as asleep, and shows the slow wave pattern denoting sleep. During sleep induced with acetylcholine the animals regularly showed twitchings of the eye-lids and snout muscles, and movements of the feet, the front feet generally preceding the hind ones. The animal was fully awake within 10 min of stopping the infusions. Only two out of 17 animals failed to sleep with acetylcholine infusion.

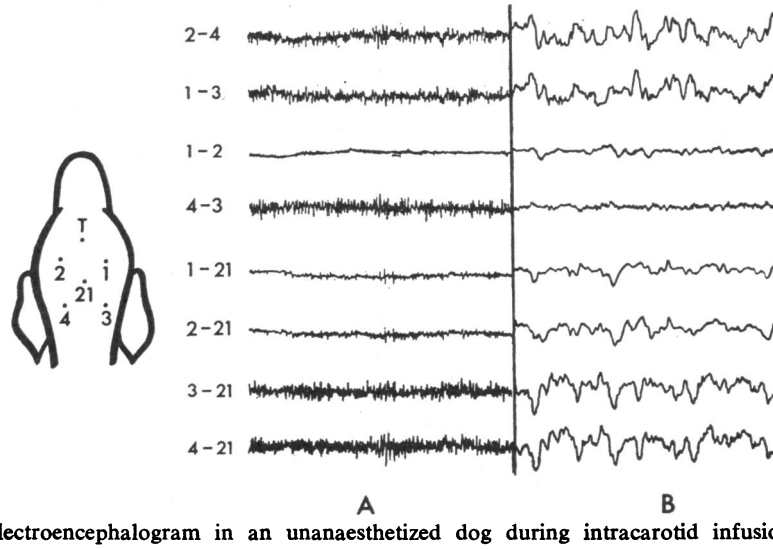

Fig. 2. Electroencephalogram in an unanaesthetized dog during intracarotid infusion of acetylcholine 30 μg/min. A was taken with the dog awake, 15 min after infusion started; B with the dog asleep, 64 min after the infusion started.

Figure 3 shows the respiration during acetylcholine infusion at 30 μg/min in one experiment. Initially respiration was of low amplitude. But after the infusion was started there were excursions of the tambour record when the animal tried to clear its throat. The respiration became regular, slow and increased in amplitude during sleep.

In doses of 3 or 10 μg/min acetylcholine produced salivation and tear secretion a little late, and no significant effects of sleep were observed. But when 300 μg/min acetylcholine was infused the secretions were profuse and the sleep induced was frequently interrupted because of the secretions.

The administration of 0.5 to 1 mg atropine into the femoral vein ½–1 hr before infusion did not reduce the secretions nor did it influence the sleep pattern.

Atropine

In eight animals atropine was infused at 3 μg/min into the carotid artery. The ipsilateral pupil dilated within 5 min. Sleep ensued within 10–20 min. and lasted for periods

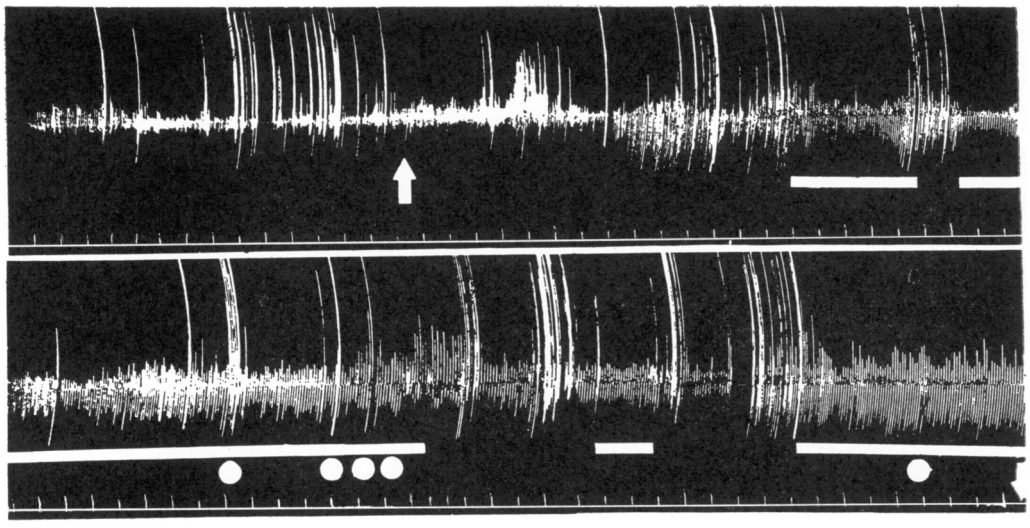

Fig. 3. Respiration in an unanaesthetized dog during intracarotid infusion of acetylcholine at 30 μg/min. Bottom record is a continuation of top record. The white arrows indicate the beginning and end of infusion, the white horizontal bands the periods when the dog closed its eyes and slept, and the white dots where the dog showed twitchings of eye-lids and snout muscles during sleep. Time 1 min.

of 10–15 min. The dogs were still sleepy in the intervening wakeful periods, as well as for nearly an hour beyond the period of infusion. The mydriatic effect was restricted to the ipsilateral pupil and disappeared within 24 hr, thus showing that little atropine reached the systemic circulation. Figure 4 shows the respiratory record and the sleep periods in one such experiment. The respiration was only slightly slowed during sleep and was regular. The amplitude of the respiratory movements progressively increased. When muscle twitchings occurred in sleep, respiration became irregular. The electro-encephalogram taken when the animal was asleep showed a slow wave pattern which changed to an activated pattern when twitchings of the muscles occurred.

Such effects were not observed with intravenous infusion. In one experiment in which atropine 10 μg/kg/min was infused into the saphenous vein in a conscious dog, the animal did not sleep, but panted and was restless. Both the pupils were fully dilated at the end of 20 min infusion.

d-*Tubocurarine*

d-Tubocurarine was infused at 30 μg/min into the carotid artery in eight dogs. No peripheral neuromuscular paralytic action or weakness was noticed in the animals at the end of 60 min infusion, nor was there even ipsilateral ptosis. In all the experiments sleep occurred within 15 min of starting the infusion. Sleep periods sometimes lasted for as long as 20 min at a time. The animals were not roused by quiet conversation. Twitchings of the muscles of the eye-lids and face, and movements of the feet occurred more frequently and continuously. In some experiments, jerky movements of limbs and

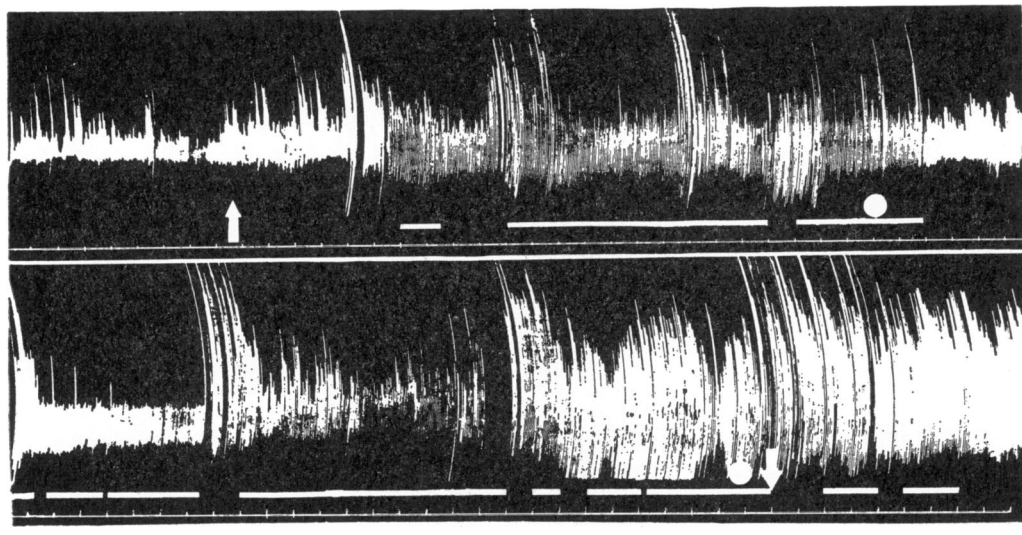

Fig. 4. Respiration in an unanaesthetized dog during intracarotid infusion of atropine at 3 μg/min. Bottom record is continuation of top record. The white arrows indicate beginning and end of infusion, the white horizontal bands the periods when the dog closed its eyes and slept, and the white dots where the dog showed twitchings of eye-lids and snout muscles during sleep. Time 1 min.

masticatory movements were observed. Sleep lasted beyond the 1 hr period of infusion. Sometimes the onset of sleep was delayed for over 30 min and when the infusion was continued beyond 1 hr, sleep ensued. Figure 5 illustrates the respiration and sleep periods in one experiment. Respiration was slow, regular and deep when the animal was asleep.

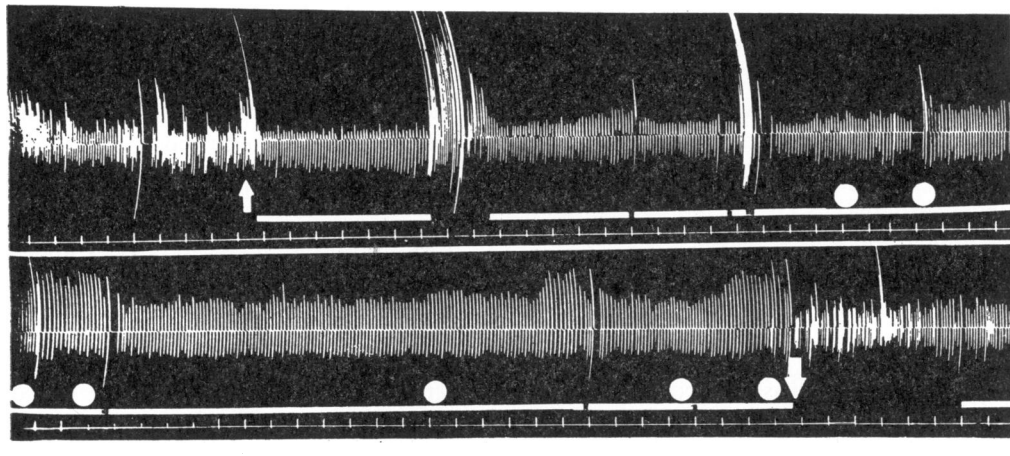

Fig. 5. Respiration in an unanaesthetized dog during intracarotid infusion of *d*-tubocurarine at 30 μg/min. Bottom record is continuation of top record. The white arrows indicate beginning and end of infusion, the white horizontal bands the periods when the dog closed its eyes and slept, and the white dots where the dog showed twitchings of eye-lids and snout muscles during sleep. Time 1 min.

Hexamethonium

Hexamethonium was infused into the cannulated carotid artery in seven dogs at 30 μg/min. The animals went to sleep generally in about 10 min and slept for periods of up to 20 min. The intervening wakeful periods were sometimes as short as a minute or as long as 15–20 min. Respiration was slow and regular during sleep periods. Twitchings of the eye-lids occurred during sleep. No other significant effect was observed.

Intracarotid infusion in anaesthetized dogs

In dogs under pentobarbitone anaesthesia, the drugs were infused into the carotid arteries cannulated in the same way as in conscious dogs, and their effect on blood pressure and respiration was studied.

When acetylcholine was infused at a rate of 30 μg/min the blood pressure showed only mild fluctuations of about 10 mm Hg and there was no overall change at the end of the 1 hr period of infusion. With 300 μg/min, however, blood pressure gradually fell about 30 mm Hg by the end of 1 hr infusion. Atropine, *d*-tubocurarine and hexamethonium, infused in the same doses as in conscious dogs for 1 hr periods, did not produce any significant changes in blood pressure and respiration.

DISCUSSION

The doses of acetylcholine used in our experiments may appear large but were based on the following considerations. Only about a third of the drug injected into the common carotid artery reaches the internal carotid, since the external carotid is a much larger branch in dogs. The injected drug is diluted in the fast-flowing blood stream and may partly be destroyed by the cholinesterase in blood. The doses injected directly into the cerebral ventricles of conscious cats by Feldberg & Sherwood (1954a) are 10–20 μg, and, therefore, to obtain corresponding effects a much larger dose has to be injected into the blood stream. We tried in our experiments to produce a sustained effect of drugs reaching the brain from the blood stream without exposing the rest of the body to similar concentrations. This we did either by infusing small amounts of the drug into the carotid artery over a period of an hour or by injecting a single large dose which would leave a residual effect on the brain for some time. Significant effects could be obtained only with the doses used.

The immediate ipsilateral effects occurring within the first 5–10 min after the intracarotid administration of drugs were the result of the drugs reaching the target glands or muscles directly through the branches of the external carotid and occipital arteries. These effects were absent or modified when the external carotid artery was ligated. The immediate effects observed with acetylcholine were increased nasal, salivary and tear secretions, and, with large doses, spasm of the eye-lids. *d*-Tubocurarine produced ipsilateral ptosis, and atropine mydriasis. There appear to be differences in the peripheral effects of the anticholinesterase drugs, neostigmine and physostigmine. While neostigmine (250 μg) produced intense muscle fasciculation and miosis, physostigmine caused circling movements and only with high doses (500 μg) produced feeble muscle fasciculations and even then no miosis. The origin for the circling movements observed with large doses

of physostigmine has to be looked for outside the central nervous system, since it is absent in animals with the external carotid artery ligated. These peripheral effects produced by the drugs lasted only for a short time after the injections.

A common delayed effect observed after about 20 min in our experiments with all the drugs was either sleepiness or regular sleep for varying periods. This effect is not likely to be due to their action on the baroreceptors of the carotid sinus or the chemoreceptors in the carotid body for the following reasons. The effects were delayed and were better observed with continuous slow infusion of about 0.3 ml./min of drug solution, a procedure unlikely to increase the pressure in the carotid sinus region. Since both cholinomimetic drugs as well as acetylcholine antagonists cause sleep, it is not likely that this effect is due to an action on chemoreceptors. The sleep-inducing effects are probably due to the action of drugs reaching the brain *via* the internal carotid artery. The drugs may reach the brain substance from the blood stream either by crossing the blood-brain barrier or by first entering the cerebrospinal fluid, and later penetrating the brain. In cats atropine was shown to reach the cerebrospinal fluid from the blood stream by Haranath, Premalatha & Sunanda-Bai (1966). The passage of *d*-tubocurarine into the cerebrospinal fluid in man after its systemic administration was described by Mahfouz (1949), but was doubted by Cohen (1963). However, when administered directly into the cerebral ventricles, atropine causes liveliness and *d*-tubocurarine convulsions (Feldberg & Sherwood, 1954a). Hence the sleep-inducing action of these drugs is probably the result of their reaching brain substance across the blood-brain barrier.

While sleep was observed with both acetylcholine-like drugs and their antagonists it was more marked with the antagonists. Can there be a common explanation for the sleep observed with both these groups ? It could be due to a blocking action by acetylcholine antagonists as well as excess amounts of acetylcholine on some neurones of the brain. It could also be that both groups cause an accumulation of acetylcholine in the brain, and the sleep observed could be due to such an accumulation of acetylcholine. All the drugs used by us can be expected to increase acetylcholine concentration in the brain. The intracarotid administration of acetylcholine can do so. Bhattacharya & Feldberg (1958) gave physostigmine and neostigmine systemically into anaesthetized cats and demonstrated an increase in acetylcholine release into perfused cerebral ventricles. Similar increase in acetylcholine release has also been described with systemic administration of hyoscine and atropine by Polak (1965). A sleep-like condition (Dikshit, 1935) and stupor and catatonia (Feldberg & Sherwood, 1954a) were described with intracerebroventricular injections of acetylcholine in cats. Feldberg (1963) suggests that large amounts of undestroyed acetylcholine present after intraventricular injection of anticholinesterases or acetylcholine could by " impairment of function of nerve cells " produce catatonia. It is not possible at this stage to decide whether the sleep-inducing effects observed by us are the results of stimulation or depression of nerve cells by these drugs either directly or through acetylcholine release.

In our experiments, at some stage during sleep, the animals showed twitchings of the eye-lids and snout muscles and movements of the feet. The electroencephalogram (EEG) during these periods showed an activated pattern while the animal was still asleep. Such a phenomenon was described by Dement (1958) in cats. This phase of sleep is generally referred to as paradoxical sleep (Jouvet, 1961). It is considered to be " deeper " when

compared with sleep showing high voltage slow waves in the EEG, since it is associated with reduced or no electrical activity (EMG) of the neck muscles. In our experiments we did not record EMG of neck muscles and are therefore unable to say if the sleep observed during these phases with twitchings was deep sleep. But to all outward appearances during these periods, the dogs appeared deeply asleep and needed a greater than usual noise level to be awakened.

Two of the drugs used in our experiments—atropine and physostigmine—are known to produce dissociation between behaviour and EEG pattern. Atropine administered systemically in dogs (Wikler, 1952), in cats (Bradley & Elkes, 1957) and in rats (Bradley, 1964) produces an EEG pattern of high voltage slow waves similar to those seen in sleep, while the animal is active. However, in our experiments with intracarotid injection, it produced sleep, as well as slow waves in EEG denoting sleep. Bradley & Elkes (1957) observed that, after intraperitoneal injection of physostigmine, the cat was quiet and drowsy but its EEG was activated. We did not take EEG records during experiments with physostigmine. However, with neostigmine we observed sleep as well as slow wave pattern of EEG during sleep.

SUMMARY

1. In conscious dogs cholinomimetic drugs and their antagonists were injected into the carotid arteries placed in skin loops or were infused for 1 hr periods into the indwelling cannulae placed in the carotid arteries.

2. Acetylcholine (1,000 μg) injection produced immediately a high-pitched cry and a dazed state, followed by drowsiness and sometimes sleep. Its infusion at 30 μg/min increased nasal, salivary and tear secretions, and also induced sleep.

3. Neostigmine (250 μg) injections produced muscle fasciculations in the ipsilateral temporal muscles spreading to the rest of the body, and later drowsiness and sleep.

4. Physostigmine (500 μg) injections caused contraversive circling movements. This effect was absent if the external carotid artery was ligated. It also induced sleep.

5. Tubocurarine (500 μg) injections produced ipsilateral ptosis of short duration followed by drowsiness and sleep. During its infusion at 30 μg/min sleep occurred for long periods.

6. Atropine injected in doses of 50 μg or infused at 3 μg/min produced ipsilateral mydriasis followed 20 min later by sleep.

7. Hexamethonium injected in doses of 500 μg or infused at 30 μg/min induced sleep in about 20 min.

8. During sleep induced with various drugs, the dogs showed in some phases of sleep twitchings of the eye-lids and snout muscles and movements of the feet. With all drugs, high voltage slow waves appeared in the EEG records when the dogs were asleep and these were replaced by an activated pattern when there were muscle twitchings during sleep.

9. Respiration recorded during sleep was slow, regular and deep.

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