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Effects of Cholinomimetic Drugs and Their Antagonists Injected into Vertebral Artery of Unanaesthetized Dogs

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(Received 18 May 1976)

HARANATH, P. S. R. K., G. INDIRA AND A. KRISHNAMURTHY *Effects of cholinomimetic drugs and their antagonists injected into vertebral artery of unanaesthetized dogs.* PHARMAC. BIOCHEM. BEHAV. 6(3) 259–263, 1977. – In unanaesthetized dogs, cholinomimetic drugs and their antagonists, catecholamines and 5HT were injected into vertebral artery placed in a skin loop. Acetylcholine (1 mg), pilocarpine (1–2 mg), nicotine (250–500 μg). eserine (100 μg) and neostigmine (250 μg) produced sleep apart from a few other peripheral effects. Sleep for longer periods followed injections of tubocurarine (1 mg), atropine (50 μg) and hexamethonium (500 μg). Adrenaline (50 μg) and noradrenaline (50–250 μg) did not produce significant effects on behaviour and sleep. 5HT (250–500 μg) also caused sleep.

Injections Vertebral Artery Autonomic Drugs Conscious Dogs

HARANATH, Sunanda-Bai and Venkatakrishna-Bhatt [8] described the effects of intracarotid injections or infusions of cholinomimetic drugs and their antagonists in conscious dogs. They observed that sleep was a prominent effect following the intracarotid injections of both groups of drugs. In the present study the above groups of drugs were injected into the vertebral artery of conscious dogs and the effects are described.

METHOD

Vertebral arterial loops were prepared in adult mongrel dogs (12–14 kg) under aseptic conditions according to the method described by Himwich, Costa, Canham and Goldstein [12]. Briefly the procedure was as follows. The common carotid artery was severed close to its bifurcation and its cardiac cut end was anastomosed with the vertebral artery severed at its origin from the subclavian artery, using 6/0 arterial silk sutures. The patent anastomosed arterial loop was placed in a skin pedicle. After 1 month when the wound healed and the oedema subsided, drugs were injected into the arterial loop and they will reach the brain via the vertebral artery. Six such dogs were used in the present study. The patency of the loop was confirmed finally by injecting a dye and postmortem examination of the brain.

During the observations the dogs were generally unrestrained. Gentle restraint was used when recording electroencephalogram (EEG). To record the EEG, silver wires were stitched to the skin over the fronto-parietal and occipital regions on each side under aseptic conditions and the leads so formed were connected to a 16 channel Schwarzer electroencephalograph. A minute to minute record of the behaviour of the animal was maintained. After control observation for 1 hr with or without saline injection into the vertebral artery, drug solutions made in sodium chloride 0.9% were injected in a volume of 0.5 ml into the vertebral artery with due aseptic precautions.

The observations were done at different parts of the day and in different places, at different noise levels to exclude conditioning of the responses. The state was recorded as a sleep period, if the animal was in a sleeping posture, closed its eyes for more than 3 min with slow and deep respiration and a slow wave EEG pattern when recorded. After establishing an effective dose, EEG was recorded in subsequent experiments. During sleep when twitchings occurred of the vibrissae, muscles of the eye, muzzle and paws accompanied by desynchronised EEG it is deemed REM sleep.

Generally there was an interval of more than 2 days between drug injections. The drug injections were carried out over several months in the same dog. Each drug at effective doses was studied twice in the same dog after interval of some weeks and in three different dogs.

Drugs

Acetylcholine chloride (E. Merck), physostigmine sulphate (T&H Smith), neostigmine methylsulphate (Hoffmann La Roche), pilocarpine nitrate (Boehringer, Ingelheim), nicotine (BDH), atropine sulphate (E. Merck), (+) tubocurarine chloride (Koch-Light, England), hexamethonium tartarate (May & Baker), adrenaline (E. Merck), noradrenaline (Fluka-Buchs) and 5-hydroxytryptamine creatinine sulphate (E. Merck) were used in these studies. The doses refer to the salts.

RESULTS

Sleep was a prominent effect observed following injections of cholinomimetic drugs and their antagonists into vertebral artery. The sleep observed was natural and the

animals could be readily awakened. The sleep lasted for continuous periods of 5–15 min. Sometimes the animals slept for nearly 20–40 min opening their eyes for a few seconds at 5–10 min intervals. Towards the end of the sleep periods, the animals showed movements of the vibrissae, eyelids, eye balls and muscles of the muzzle and the paws which correspond to Rapid Eye Movement (REM) sleep. The results in detail are as follows.

Control observations. The animals were observed for a period of 1 hr before the drug injections and a minute to minute record of the behaviour of the dog was noted. No experiments were done if the animal was drowsy. The observations were made at different times of the day and at different places to exclude the possibility of conditioned reflexes. Injections of 0.5 ml sodium chloride 0.9% did not produce any behavioural changes. The dogs were active and explored the surroundings as usual.

Cholinomimetic drugs

Acetylcholine. When acetylcholine 1000 μg was injected into the vertebral artery in three dogs, the dog immediately gave a loud cry and in the next few minutes had salivation, defecation or urination. The dog went to sleep sometimes immediately and at others about 30 min later. Figure 1 shows the sleep periods following injection of acetylcholine 1000 μg in three such experiments. Eye movements were present in the sleep periods at places noted with a black dot on top of each block. Even doses of 400 μg acetylcholine produced similar effects but less prominent. Figure 2 shows the EEG from one such experiment after injection of acetylcholine 400 μg into the vertebral artery. In Fig. 2A is given the EEG 15 min after injection of acetylcholine showing fast waves of low voltage, in B 35 min after the injection with the animal asleep showing high voltage slow waves. Figure 2C is taken 40 min after the injection when eye movements were present during sleep and shows fast waves. Acetylcholine 250 μg produced only salivation and defecation but no sleep.

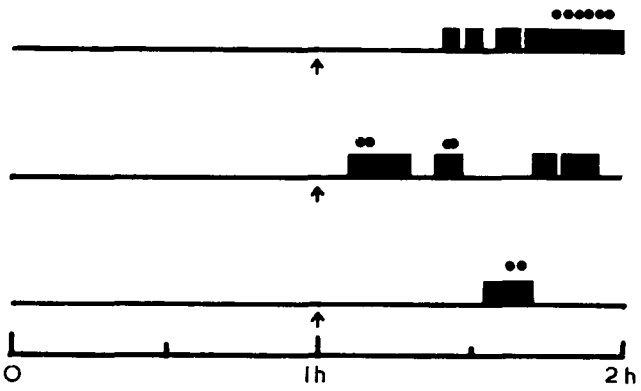

FIG. 1. Sleep periods indicated by horizontal black bars in 3 experiments. At the arrow acetylcholine 1 mg was injected into the vertebral artery. Black dots over the bars indicate periods of movement of eye, paws and muzzle during sleep.

Anticholinesterases. With eserine 100 μg injected into the vertebral artery the dog showed immediate circling movements with its head turned towards the side of the injection, which lasted for about 5 min. Later the dog appeared to be having intense itching as it started scratching all over the body and rolled over the ground for relief which lasted for 10–15 min. Panting and salivation were observed. The gait was unsteady for about 25 min. Sleep which occurred about 1/2 hr after the injection lasted for 10–15 min and was observed more with doses of 100 μg eserine than with 500 μg.

Neostigmine 250 μg caused twitching of muscles, urina-

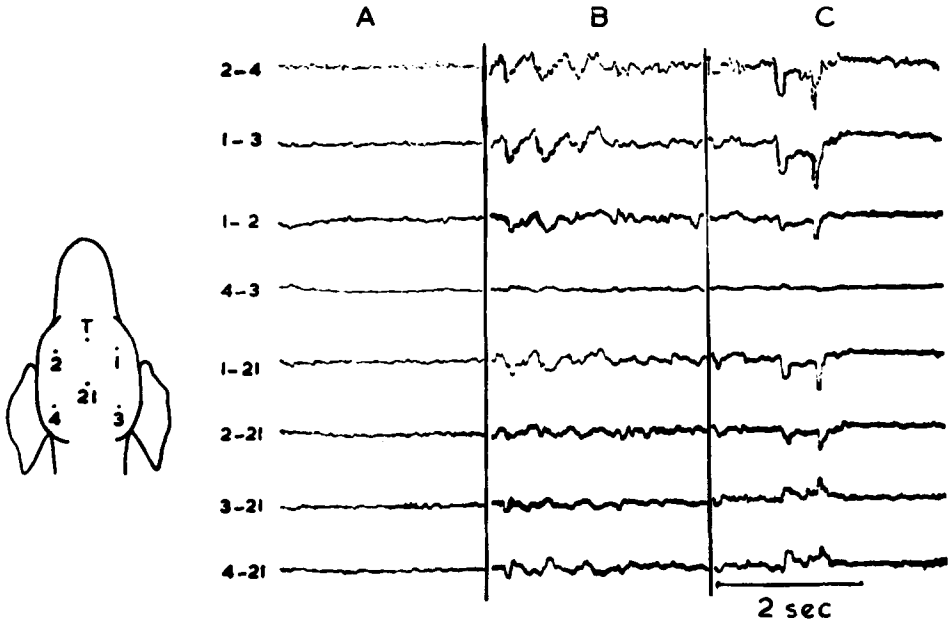

FIG. 2. EEG from an unanaesthetized dog. A was taken with dog awake 15 min after injection of acetylcholine 400 μg into vertebral artery. B and C were taken with the dog asleep 35 and 40 min after injection. In C the dog was showing movements of eye, paws and muzzle.

tion and emesis. Sleep occurred only 45–50 min after the injection and lasted for about 10–15 min which included eye movements.

Pilocarpine. When pilocarpine 1000 μg was injected the animal showed some licking movements. Salivation occurred 5–15 min later and scratching after 30 min. Sleep for short period was observed 30 min after the injection. But when 2 mg pilocarpine was injected, salivation occurred within 5 min and sleep followed after 10 min and lasted for nearly 40–60 min. Eye movements were present in two out of three observations.

Nicotine. When nicotine 500 μg was injected into the vertebral artery the dog immediately let out a loud cry similar to that following injection of acetylcholine. During the next 10 min the dog lay down and showed salivation, lacrimation, dilation of pupils, scratching and panting, and was later able to move. Thirty minutes later it slept for nearly half hour with gaps of few seconds once or twice. No eye movements were observed with this dose. Nicotine 250 μg produced similar effects but less in intensity. But 1000 μg doses of nicotine produced effects in a more intense form and for longer duration but no sleep. Narrowing of palpebral fissure occurred on the side of injection for nearly 20 min.

Cholingergic Antagonists

Atropine. Atropine 50 μg when injected did not produce any obvious effects up to about 15–20 min. Later the animal relaxed and slept for nearly 15–20 min. Towards the end of the sleep periods eye movements were observed. Similar effects were observed with atropine 100 μg.

Tubocurarine. When 1 mg tubocurarine was injected into vertebral artery no paresis of limbs was observed and the dog continued to be active for about 10–15 min. Thereafter it went to sleep from which it was not easily disturbed. During sleep there were frequent spells of eye movement. Figure 3 shows EEG from one such experiment. It shows in A low voltage fast waves with the animal awake taken 11 min after injection of tubocurarine 1 mg. The animal was asleep in B and C taken 30 and 40 min after the injection showing high voltage slow waves. In B there is a spike. During sleep when eye movements were present the EEG generally showed low voltage fast waves (paradoxical sleep). But occasionnally (for example see Fig. 4), the EEG retained slow wave nature even while the eye movements were present. Similar observation was made by Haranath and Shyamalakumari [7] during sleep following injection into or perfusion of cerebral ventricles with tubocurarine. Spikes were frequently seen in the EEG after tubocurarine injection into vertebral artery.

Hexamethonium. After 500 μg hexamethonium injected into vertebral artery there was no immediate change in behaviour. But the dog became drowsy after about 20–30 min and slept for periods varying from 10–20 min. During sleep eye movements were observed.

Other Drugs

Adrenaline. When 50 μg adrenaline was given into the vertebral artery it did not produce any behavioural effects during 1 hr period of observation. When similar injections were made into the carotid artery, Haranath (unpublished) observed that there was no change in behaviour, but intense spasm of the lingual blood vessels on the side of injection occurred as noticed by the blanching of ipsilateral half of the tongue.

Noradrenaline. Noradrenaline given in 50–250 μg doses into vertebral artery produced slight mydriasis on both sides immediately after injection. Later the animal was restless for some period roaming about the room and later lay down but did not sleep.

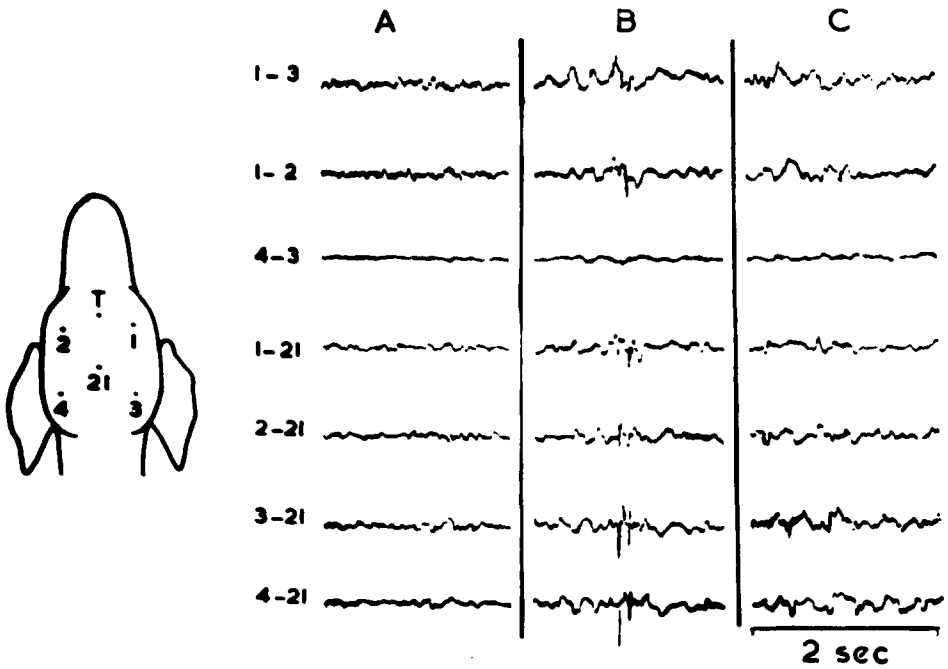

FIG. 3. EEG from an unanaesthetized dog. A was taken with dog awake 11 min after the injection of tubocurarine 1 mg into vertebral artery. B and C were taken with dog asleep 30 and 40 min after the injection. B shows a spike.

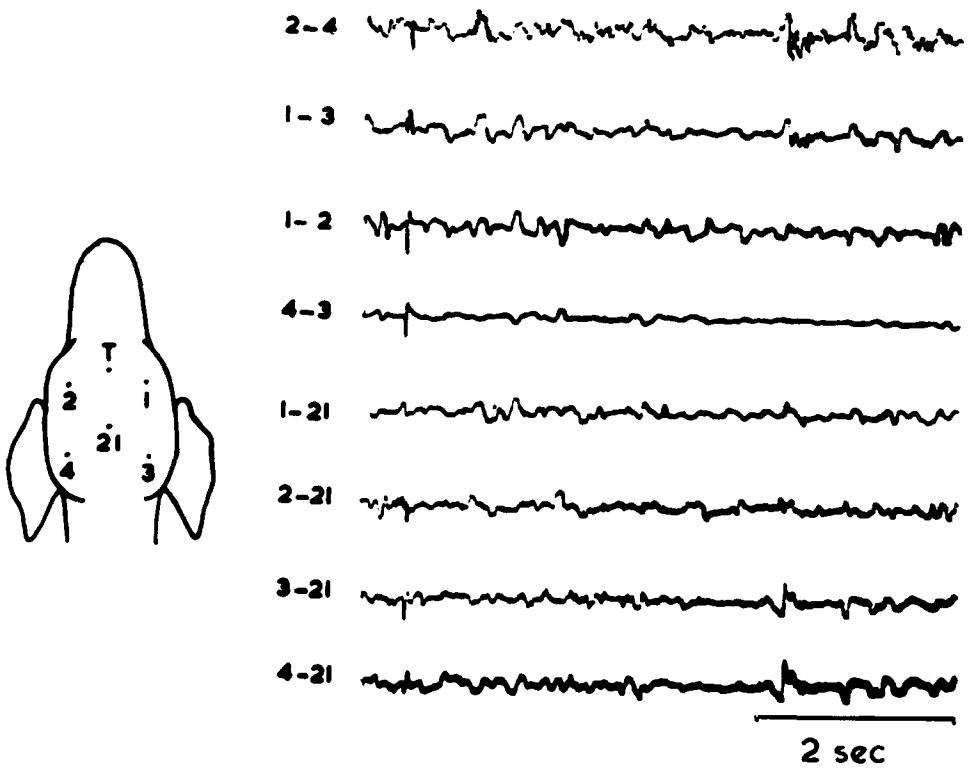

FIG. 4. EEG from an unanaesthetized dog taken with the dog asleep showing movements of eye, paw and muzzle, 25 min after the injection of tubocurarine 1 mg into vertebral artery. Note absence of activation (desynchronisation) and the presence of a spike.

5-Hydroxytryptamine. When 5-Hydroxytrptamine (5HT) was injected in doses ranging from 50–1000 μg, the dog cried soon after injections of 5HT 250 μg or more. With doses of 100–500 μg, the dogs slept after about 20 min and showed eye movements during sleep. With 5HT 1000 μg doses there was defecation and urination but no sleep. No other behavioural effects were observed.

DISCUSSION

The present study shows that sleep is a prominent effect following injection of cholinomimetic drugs or their antagonists into vertebral artery of unanaesthetized dogs. Sleep following intracarotid injections of the above groups of drugs in unanaesthetized dogs was described by Haranath *et al.* [8]. Himwich and Inman [13] studied the distribution of dye injected into vertebral arterial loops similar to those in the present study and showed that the dye is distributed to both sides of the hind brain, of the midbrain, both hippocampus and caudate nucleus and pyriform lobe of the cerebral hemispheres. The dye did not extend to the anterior portion of the cerebral hemispheres. Hence the site of action for the effects produced by drugs injected into the vertebral arterial loop in the present study could be at any of the above areas.

Haranath and Venkatakrishna-Bhatt [10] observed that sleep followed injection of as little as 20 ng tubocurarine into the inferior horn of lateral ventricle. Also sleep was induced by injections into the inferior horn of acetylcholine 1–2 μg, eserine 1 μg, pilocarpine 100 μg, nicotine 10 μg, Hyoscine 0.4–1.6 μg, and atropine 10–20 μg. It is possible that the sleep-inducing effects of drugs injected into vertebral artery could also be in the structures lining the inferior horn like hippocampus. But since the substances injected into the vertebral artery have a wider distribution, the possibility of drugs injected into the vertebral artery exerting actions in other areas cannot be excluded.

Hernandez-Peon, Chavez-Ibarra, Morgane and Timo-Iaria [11] described cholinergic pathways in the limbic system in induction of sleep. They observed sleep following application of crystals of acetylcholine to a very circumscribed pathway extending from upper medial preoptic region into the medial pontine tegmentum along the medial forebrain bundle, interpeduncular nucleus, Bechterew's and Gudden's nuclei. Yamaguchi, Ling and Marczynski [19] reported sleep following application of acetylcholine 30 μg to preoptic region of both sides. Myers [16] reported various effects of enhanced emotional activity or alertness but no sleep on application of acetylcholine to hypothalamus, thalamus preoptic region and Cajal's intestitial nucleus.

Though both cholinomimetic drugs and their antagonists produced sleep on intracarotid or intravertebral arterial injections, the doses of cholinomimetic drugs which do so are much larger than the doses of cholinergic antagonists. Is it possible that both act by producing a block at the cholinergic pathways cholinomimetics by a depolarizing block and their antagonists by a competitive block? The doses of nicotine required to produce sleep after intravertebral arterial injection is less than that for pilocarpine (a muscarinic agent). But neither drug appears as effective as their blocking agents in producing sleep. Therefore it is difficult to conclude whether muscarinic or nicotinic receptors are involved. It is also shown that more acetyl-

choline is released during wakeful state and REM sleep from perfused cerebral ventricles [9], from perfused cortex [14] and from perfused caudate nucleus [5]. This fits into the concept of increased activity of cholinergic mechanisms during wakefulness and REM sleep.

The central effects of some of the neurohormones injected into the cerebral ventricles appear different to their effects on systemic administration. Administration of adrenaline and noradrenaline into the vertebral artery did not produce any sleep in our experiments. Their systemic administration was shown to produce heightened alertness [2,18]. But intracerebroventricular administration of catecholamines produced sleep [4]. When drugs are given either systemically into circualtion or into the cerebral ventricles into the c.s.f., the drugs are distributed over a large area. In studies with topical application of neurohumors applied to discrete areas of brain Yamaguchi *et al.* [19] observed alerting features after application of noradrenaline to preoptic region, but sleep (both slow wave and REM phases) on application to nucleus centralis medialis. The difference in effects with the different routes of administration i.e. systemically or directly into the cerebral ventricles may be ascribed to factors like ability of the neurohormones to cross blood-brain or blood-c.s.f. barriers, the distribution in brain after systemic administration, concentrations achieved at the target areas which may produce either stimulation or block depending on the concentration and finally the actions on more than one area which may be mutually antagonistic or synergistic.

The anticholinesterases and the cholinergic blocking agents used in this study have been shown to pass from blood to c.s.f. though in small amounts. Eserine and neostigmine [1], atropine [6], hexamethonium [17] and tubocurarine [3] were shown to pass from blood into c.s.f. in small amounts. Since small doses of tubocurarine 500 ng injected into lateral cerebral ventricles [7] or 20 ng into the inferior horn [10] are known to produce sleep it may be possible that some of these drugs may produce sleep after passing first into the c.s.f.

Jouvet [15] suggested a role both to catecholamines and 5HT in the production of sleep. But on injection into the vertebral artery only in doses of 100–500 μg 5HT produced sleep but not adrenaline or noradrenaline.

ACKNOWLEDGEMENT

The work was carried out with a grant-in-aid from Indian Council of Medical Research, which is gratefully acknowledged. We thank Dr. M. V. Raghava Reddy, Neurologist, Osmania General Hospital, Hyderabad for the facilities of recording the EEG.

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