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Cardiovascular responses to injections of cholinomimetic drugs into the cerebral ventricles of unanaesthetized dogs

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Summary

1. The cardiovascular and behavioural effects of cholinomimetic drugs injected through a cannula chronically implanted into a lateral cerebral ventricle were examined in unanaesthetized dogs.

2. Acetylcholine (ACh) (10–20 μg) produced an increase in arterial pressure and heart rate, the dogs became more alert, moved their heads, licked and swallowed and then became drowsy.

3. The responses to ACh were potentiated by intraventricular physostigmine (5 μg), were abolished by intraventricular atropine (100 μg) but were unaffected by intraventricular mecamylamine (250 μg). The responses to ACh were reproducible on any one day if injections were given again after a 30 min interval but tolerance developed when ACh was injected repeatedly over periods of several days.

4. Methacholine (40 μg) produced similar behavioural and cardiovascular effects to ACh but of a longer duration. The responses to methacholine were abolished by intraventricular atropine (100 μg).

5. Nicotine (20–60 μg) produced a biphasic cardiovascular response of an initial brief pressor response and tachycardia followed by a secondary increase in arterial pressure and heart rate which was greater in magnitude and duration. The secondary cardiovascular effects were associated with restlessness and vomiting.

6. The responses to nicotine were abolished by prior injection of mecamylamine (250 μg) but were unaffected by atropine (100 μg). The responses to nicotine were not reproducible if injections were repeated on the same day but could be again produced if a few days were allowed to elapse between injections.

7. An increased heart rate occurred during the pressor response to the cholinomimetic drugs but when a comparable pressor response was produced by intravenous infusion of noradrenaline in the same unanaesthetized dogs pronounced reflex bradycardia resulted.

8. The results indicate that the activation of both muscarinic and nicotinic cholinergic mechanisms leads to cardiovascular and behavioural effects in the conscious dog although the site of action and peripheral mechanisms have not been determined.

Introduction

Evidence for a central transmitter role of acetylcholine (ACh) is indirect and is based upon the presence and distribution of ACh, choline acetylase and choline

esterase (Curtis, Ryall & Watkins, 1965). The ACh is contained within synaptic endings in the central nervous system (Ryall, 1963).

Feldberg & Sherwood (1954) found that 0·1–0·5 μg of ACh injected into the lateral ventricle of unanaesthetized cats produced a state resembling akinetic seizure whereas larger doses (1 mg) caused convulsions followed by sleep and stupor, sometimes reaching a catatonia-like condition. Bhattacharya & Feldberg (1958) perfused the cerebral ventricles in anaesthetized cats and found that ACh was released from the brain into the effluent. This finding indicated to them central cholinergic neuronal activity.

Central cardiovascular effects of choline and its esters are well documented. In an early study on cats Suh, Wang & Lim (1935) obtained a rise in blood pressure when ACh was introduced intracisternally. Later Bhawe (1958) obtained both pressor and depressor responses with large doses of ACh injected into the lateral ventricles of anaesthetized cats and dogs. Pressor responses after intraventricular injection of carbachol and ACh in the anaesthetized dog were also reported by Dhawan, Gupta, Dixit & Chandra (1965) and Sinha, Dhawan, Chandra & Gupta (1967). According to Srimal, Jaju, Sinha, Dixit & Bhargava (1969) intraventricular choline in anaesthetized dogs produces first a rise and then a prolonged fall in arterial blood pressure, and Sinha *et al.* (1967) suggested that cholinomimetic substances stimulate the vasomotor centre.

Nicotine injected into the cerebral ventricles of cats produces variable effects on the arterial blood pressure; the most consistent response obtained was a fall (Armitage & Hall, 1967). Pradhan, Bhattacharya & Atkinson (1967) observed a more consistent pressor component. According to Domino (1969) an intact functional central nervous system is necessary in order to obtain optimal cardiovascular effects with nicotine. He demonstrated that both its depressor and pressor responses were reduced in anaesthesia.

The following study was undertaken to determine the effects of cholinomimetic substances injected into the cerebral ventricles of unanaesthetized dogs. As no evidence is available concerning the nature of the cholinoceptive receptors involved in centrally mediated cardiovascular responses to cholinomimetic substances this problem was investigated as well.

Methods

Mongrel dogs of either sex, weighing 10–20 kg were used. The method of recording arterial blood pressure was similar to that described by Lang, Gershon & Holan (1963). Dogs were surgically prepared beforehand with exteriorized carotid arteries and were trained to stand in harness in a Pavlov-type stand. Arterial pressure was measured in mmHg (1 mmHg$\equiv$1·333 mbar) with a Statham transducer by inserting an Intramedic (R) PE50 polythene cannula through a thin-walled 18 gauge needle into the carotid artery. The ECG was obtained from standard limb leads, and heart rate integrated with a cardiotachometer coupler to give a continuous record. Both heart rate and blood pressure were recorded on an Offner dynograph. Behaviour of the dogs was observed from outside the experimental room through a one-way mirror.

To facilitate injections of drugs into the cerebral ventricles, a modified Collison cannula which served as a permanent guide tube (9 mm long, 19 gauge needle) was

implanted into the skull above the left lateral ventricle, at a point approximately 15 mm anterior to the inter-aural plane and 5 mm lateral to the sagittal suture.

The guide tube was fixed onto the skull with dental acrylic cement and screws. Before each experiment, a 25 gauge needle was lowered through the guide tube until a fall in pressure and a flow of c.s.f. occurred. Drugs were injected through this needle, in 0·1–0·2 ml of a 0·9% NaCl solution and washed in with 0·1 ml of this solution. The positioning of the guide tube and needle was determined at the end of the experiments by injection of 0·3 ml of a 0·5% bromophenol blue solution and examination of the ventricular stain at post-mortem. The brain was fixed by perfusion with formalin through the carotid and vertebral arteries.

Drugs used were acetylcholine chloride (Acacholine, Anglo-French Drug Co.), atropine sulphate, mecamylamine hydrochloride (Merck, Sharp and Dohme (Aust.) Pty. Ltd.), methacholine chloride (Sigma), physostigmine sulphate (Macfarlan Smith) and nicotine hydrogen tartrate (B.D.H.). All doses quoted are in terms of the salt.

Results

Acetylcholine and methacholine

Intraventricular injections of 10 or 20 μg ACh produced behavioural and cardiovascular effects, but with 10 μg significant responses were obtained in 8 out of 11 dogs only.

Following the ACh injections, the dogs moved their heads from side to side, licked and swallowed, became more alert and held their bodies rigid for a few minutes. Then they became drowsy for at least 30 minutes.

The cardiovascular effects consisted of a quickening of the heart rate and a rise in arterial blood pressure. Following the injection of 10 μg ACh, the mean increase in heart rate was 11 ± 4 beats/min and the mean rise in pressure 25 ± 8 mmHg (mean $\pm$ S.E.) in 11 dogs. In the 3 dogs in which 10 μg ACh failed to produce a significant response, after 20 μg the mean increase in heart rate was 23 ± 3 beats/min and the mean rise in pressure 27 ± 7 mmHg.

When the injections of 10 or 20 μg ACh were repeated at 30 min intervals, reproducible behavioural and cardiovascular responses were obtained, but when the injections were repeated over a series of days, some tolerance developed and the dose of ACh had to be increased to become effective. The onset of tolerance varied in different dogs but generally developed after two weeks of giving injections twice daily on two occasions each week. In the top records of Fig. 1 are shown the cardiovascular responses to two injections of 10 μg ACh given with a 30 min interval between them. In the bottom records, obtained from the same dog the reduced sensitivity to ACh is shown after 8 injections of 10 μg ACh given in the course of two weeks. The injection of 10 μg ACh was no longer effective, but 30 μg now produced a cardiovascular response similar to that originally obtained with 10 μg.

The tachycardia produced by an intraventricular injection of ACh is indicative of profound stimulation of cardiac controlling centres which over-rides the influence of reflex bradycardia which in the unanaesthetized dog would result from a rise in arterial blood pressure when produced by peripheral vasoconstriction. For instance, in the same three dogs in which intravenous infusions of noradrenaline lead to a mean rise in arterial blood pressure of 43 ± 7 mmHg with a mean decrease in heart

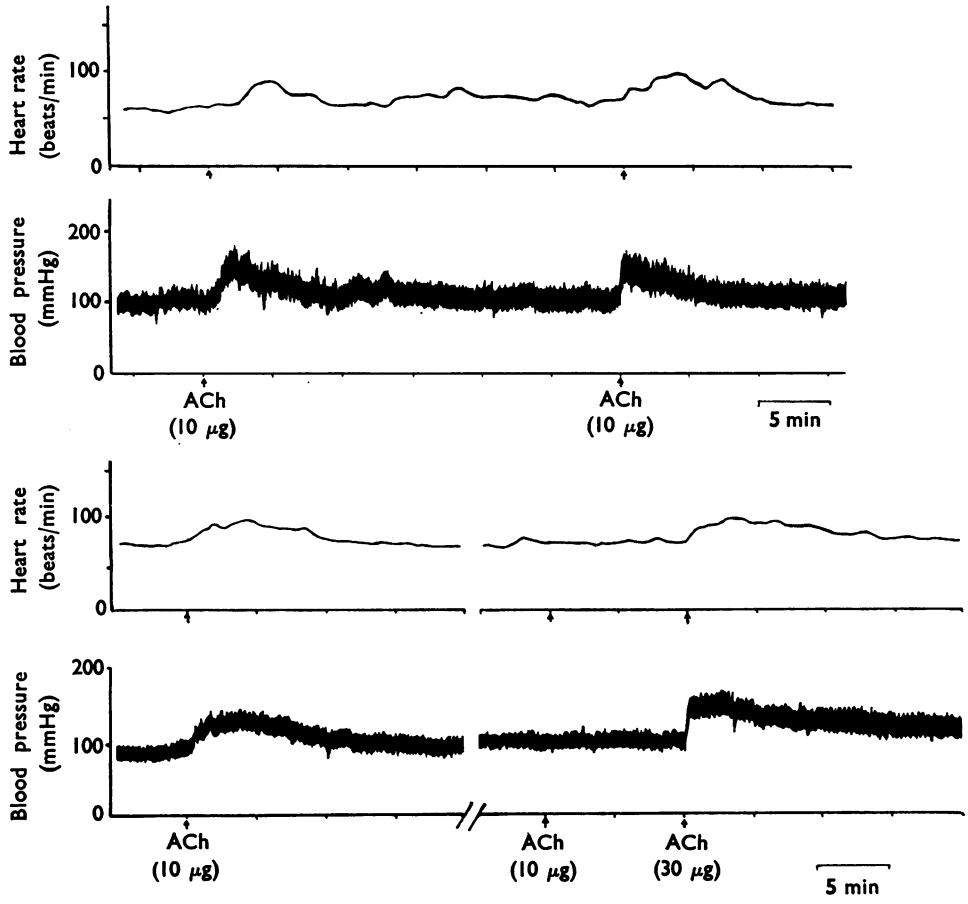

FIG. 1. Heart rate and arterial blood pressure from an unanaesthetized dog. At the arrows, injection into the cerebral ventricles of 10 or 30 μg acetylcholine (ACh) as indicated. The lower two records were obtained four days after the upper records. The break in the lower records represents an interval of 14 days during which the dog received six injections of 10 μg ACh on three separate days.

rate of 35 ± 3 beats/min, intraventricular injections of 10 μg ACh produced a mean rise in arterial blood pressure of 48 ± 3 mmHg with a mean increase in heart rate of 25 ± 3 beats/min. The results obtained in one of the three dogs are illustrated in Figure 2.

The effects of injections of methacholine into the cerebral ventricles were examined in five dogs. The behavioural and cardiovascular effects produced by 40 μg were similar to those produced by 10 or 20 μg ACh, except that drowsiness developed earlier, within 5 min, and that the effects lasted longer. The cardiovascular effects obtained in one of the five dogs are illustrated in Figure 3. They reached a maximum about 5 min after the injection and lasted about 35 minutes. The mean pressor response was 52 ± 5 mmHg and the mean increase in heart rate 36 ± 6 beats/minute.

Physostigmine

An injection of 5 to 20 μg into the cerebral ventricles produced restlessness. Respiration increased in depth and rate, heart rate increased slightly and the arterial

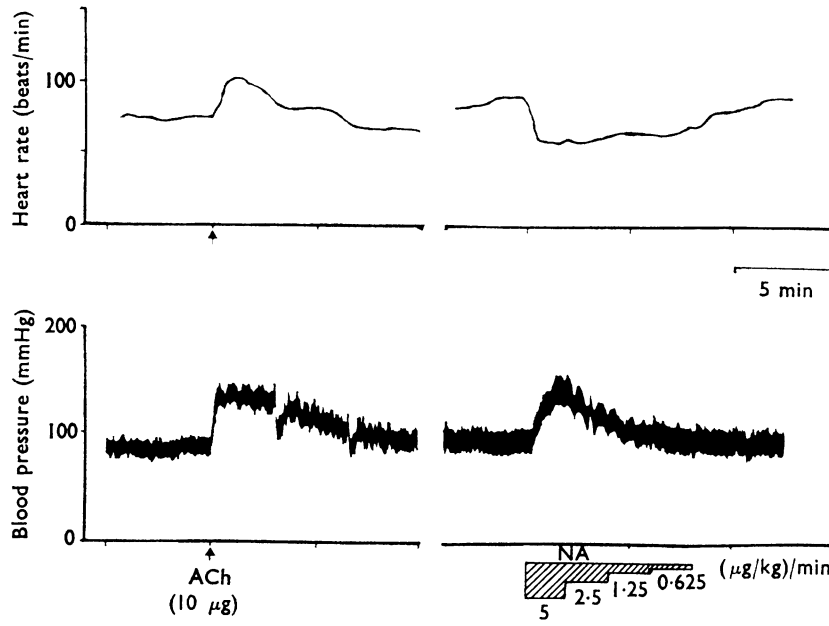

FIG. 2. Heart rate and arterial blood pressure from a 10 kg unanaesthetized dog. At the arrow injection of 10 μg acetylcholine (ACh) into the cerebral ventricle. The horizontal striped area beneath the lower record indicates 8 min intravenous infusion of noradrenaline (NA) beginning at a rate of 5 (μg/kg) per min, and halving the rate every 2 minutes.

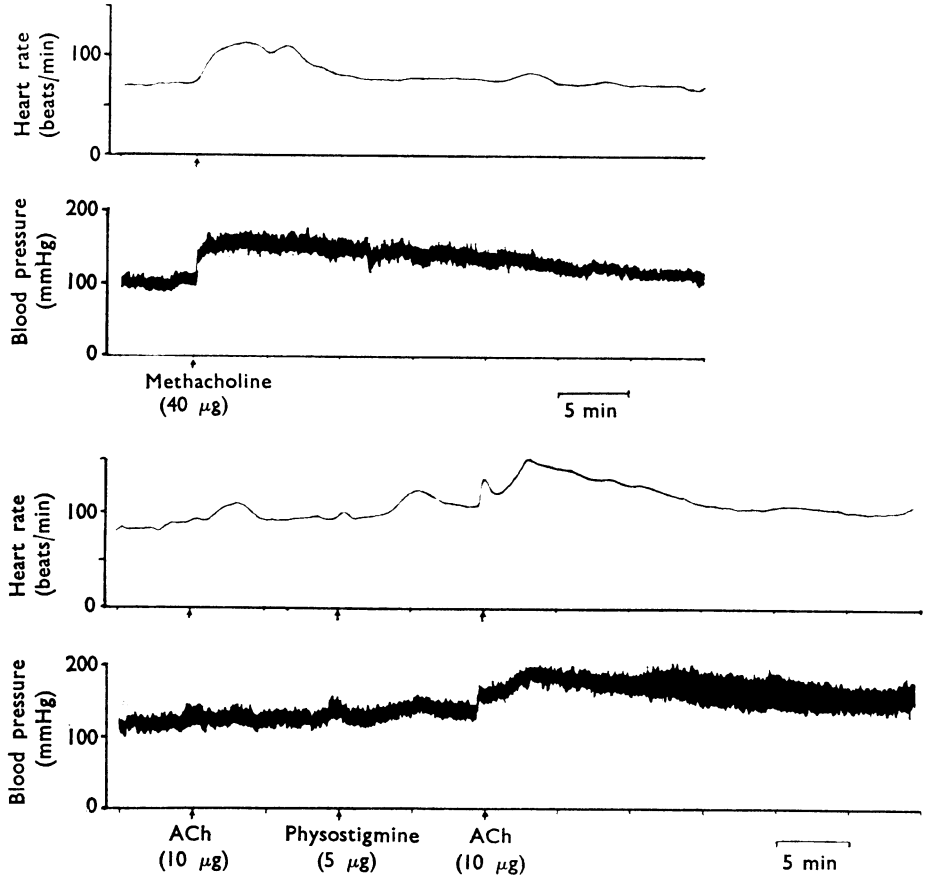

FIG. 3. Heart rate and arterial blood pressure from an unanaesthetized dog. In the upper two records at the arrow injection into the cerebral ventricles of 40 μg methacholine. The lower two records were obtained 21 days after the upper records. At the arrows, injections into the cerebral ventricles of 10 μg of acetylcholine (ACh) and 5 μg of physostigmine.

blood pressure rose slowly but not more than 20 mmHg. This result was obtained in six dogs. In one dog, the injection of 5 μg physostigmine produced more pronounced changes including heaving respiration, tremor and agitation; arterial blood pressure rose 45 mmHg and heart rate 70 beats/minute.

In five of the dogs in which the injection of physostigmine had little effect on heart rate and blood pressure, it potentiated and prolonged the cardiovascular responses to an intraventricular injection of ACh given some minutes later but potentiation was not observed in the sixth dog. The results obtained in one of the five dogs are illustrated in the bottom records of Figure 3. The response lasted about as long as that produced by 40 μg methacholine in the same dog shown in the upper records of this figure.

If tolerance to the intraventricular injections of ACh had developed due to repeated ACh injections given during the previous fortnight, a subthreshold dose of ACh again became effective after physostigmine.

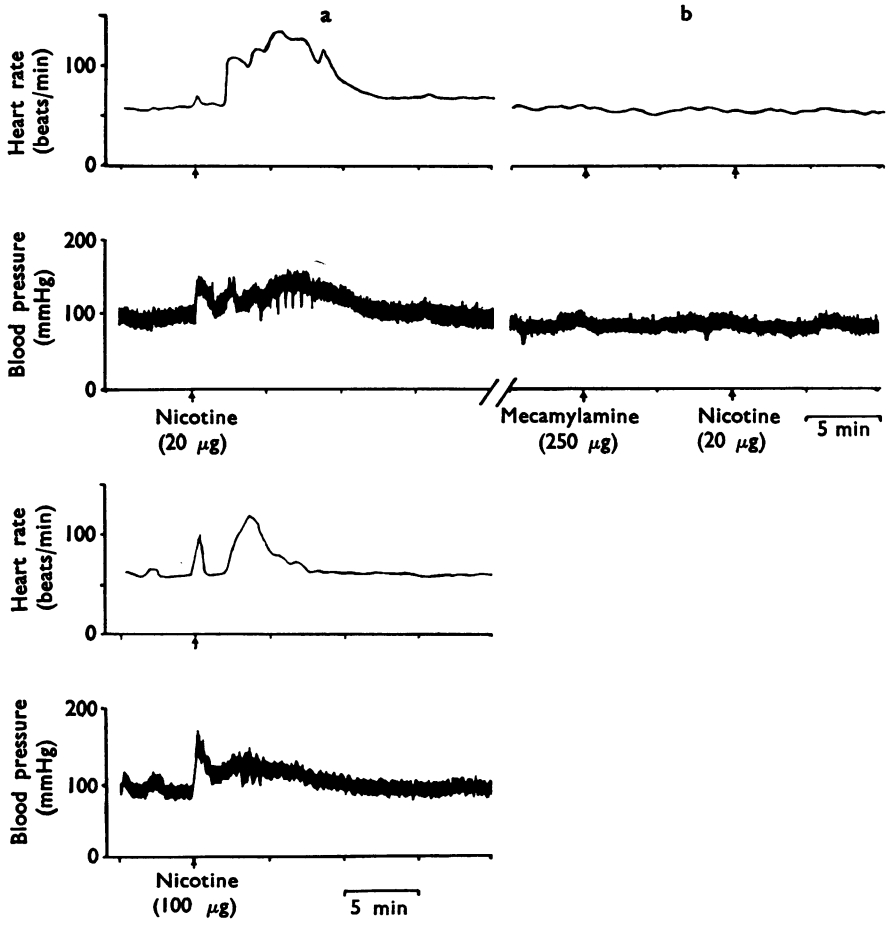

FIG. 4. Heart rate and arterial blood pressure from an unanaesthetized dog. In the upper two records, at the arrows, injections into the cerebral ventricles of 20 μg nicotine and 250 μg of mecamylamine as indicated. Record b was obtained 3 days after record a. In record b the interval between the injection of mecamylamine and nicotine was 10 min. In the lower two records, at the arrow injection into the cerebral ventricles of 100 μg of nicotine as indicated.

Nicotine

Injections of 20 to 100 μg nicotine into the cerebral ventricles produced licking, swallowing, increased alertness, reslessness, vomiting, tachycardia and a rise in arterial blood pressure. The cardiovascular response was biphasic and the vomiting occurred during its secondary phase.

Typical cardiovascular responses to 20 and 100 μg nicotine are illustrated in Figure 4. With both doses, the increase in heart rate was more pronounced during the secondary phase of tachycardia ; the difference was particularly great with 100 μg. With 20 μg behavioural and cardiovascular responses were not obtained in all dogs, but only in four out of six. On increasing the dose, the responses did not change qualitatively but became more intense.

When the injection of nicotine was repeated after an interval of 30 min it was ineffective, but when repeated after an interval of a few days it produced the same effect as that obtained with the first injection.

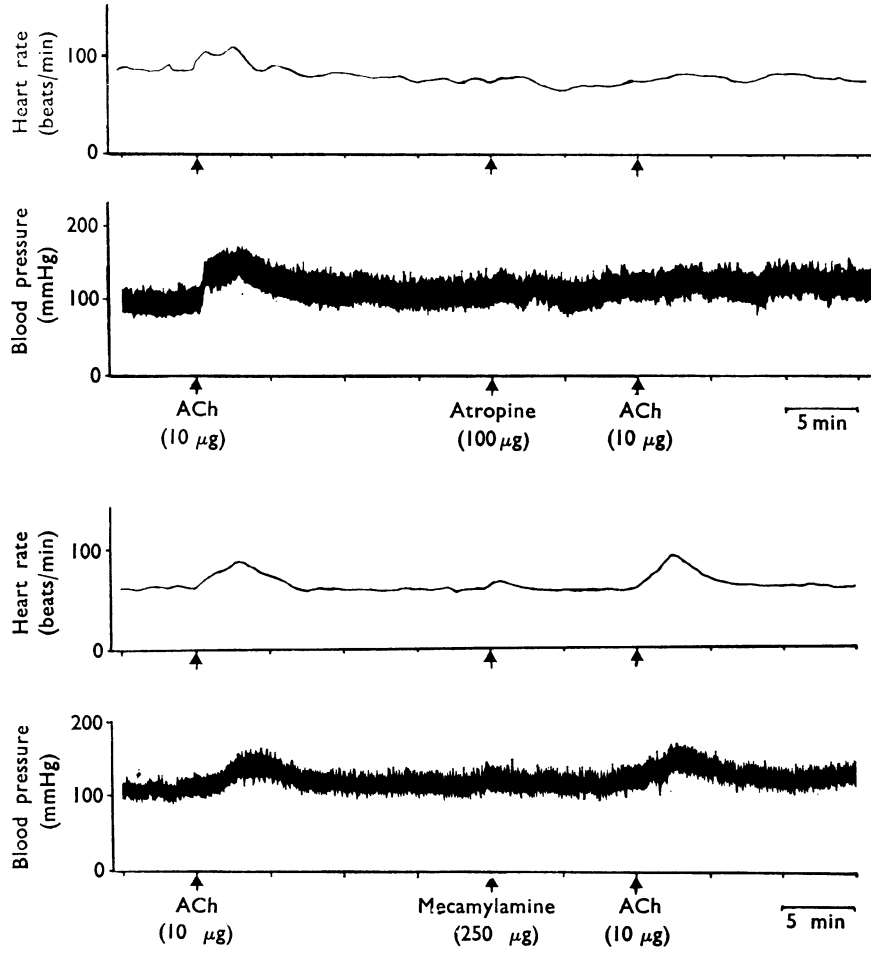

FIG. 5. Heart rate and arterial blood pressure from an unanaesthetized dog. In the upper two records, at the arrows, injections into the cerebral ventricles of 10 μg of acetylcholine (ACh) and 100 μg of atropine as indicated. In the lower two records, at the arrows, injections into the cerebral ventricles of 10 μg of ACh and 250 μg of mecamylamine as indicated.

Atropine

An injection of 100 μg atropine caused increased alertness in all seven dogs in which the effect of atropine was examined, but no significant cardiovascular changes were produced. The injection, however, abolished the behavioural and cardiovascular responses to ACh injected intraventricularly, 10 min later. This is shown for the cardiovascular responses in the upper records of Figure 5. In each of the dogs the behavioural and cardiovascular response to intraventricular methacholine was abolished by the injection into the cerebral ventricles of atropine 100 μg, 10 min earlier. In contrast, in five dogs the behavioural and cardiovascular responses to intraventricular nicotine were unaffected by the injection of atropine 100 μg, into the cerebral ventricles, 10 min earlier.

Mecamylamine

An injection of 250 μg mecamylamine into the cerebral ventricles produced no behavioural or cardiovascular effects, nor did it prevent the changes produced by a subsequent intraventricular injection of ACh. If anything it slightly enhanced the cardiovascular response to ACh both in magnitude and duration. This is shown for 10 μg ACh in the lower records of Figure 5. In contrast, the injection of mecamylamine abolished the behavioural and cardiovascular response to nicotine. Since nicotine became inactive when given at 30 min intervals the effect of mecamylamine was studied three days after the cardiovascular effects of a first injection of 20 μg nicotine had been demonstrated. Such an experiment is illustrated in Figure 4. The two upper records show that the same dose of nicotine no longer produced tachycardia and a rise in arterial blood pressure when injected 10 min after the mecamylamine. The cardiovascular effects were again produced when the nicotine injection was repeated three days later.

Discussion

When the results obtained in the present experiments on unanaesthetized dogs are compared with those previously obtained in anaesthetized animals, it is evident that without anaesthesia a purely pressor response is obtained on the injection of cholinomimetic drugs into the cerebral ventricles, and that the effect is obtained with smaller doses than under anaesthesia when the response consists of both a pressor and a depressor component. Tsyrlin (1968) found that pentobarbitone strongly inhibited pressor responses to electrical stimulation of various regions of the brain. Such an inhibition appears to apply also to the pressor responses produced by cholinomimetic drugs acting from the cerebral ventricles and thus unmasking a depressor component when the substances are studied in anaesthesia.

As the pressor response, tachycardia and behavioural changes to ACh were abolished by intraventricular atropine but not by intraventricular mecamylamine they appear to be due to an action of ACh on central muscarinic receptors. This finding is in agreement with that of Sinha *et al.* (1967) who found that intraventricular atropine blocked the pressor response produced by the injection of large doses of ACh or carbachol into the cerebral ventricles of anaesthetized dogs. That the activation of muscarinic receptors by ACh causes cardiovascular changes is also supported by the results with intraventricular methacholine since this

compound has been shown to act predominantly on muscarinic receptors in a wide range of tissues (Koelle, 1970).

Armitage & Hall (1967) found both pressor and depressor responses after intraventricular injections of nicotine in unanaesthetized cats although they only quoted results from two animals. Anaesthesia with chloralose changed the pressor phase to a depressor one. Injection of nicotine into the vertebral artery produced a small pressor response followed by a prolonged fall in blood pressure (Schaeppi, 1967). This biphasic response was converted to an entirely depressor one after thiopentone. Not only ACh and methacholine but also nicotine therefore appears to be similarly affected by anaesthetics, which reduce the pressor component and thereby reveal a depressor effect.

In the unanaesthetized dog, an increased heart rate after an intraventricular injection of ACh, methacholine or nicotine occurs in association with a pressor effect. In contrast the pressor response resulting from peripheral vasoconstriction when produced by intravenous noradrenaline infusion was shown to be accompanied by pronounced reflex bradycardia. Therefore the tachycardia produced by the cholinomimetic substance injected intraventricularly indicates profound central stimulation of cardiac centres which over-rides the reflex bradycardia.

The cardiovascular effects produced by the cholinomimetic drugs on intraventricular injection suggest both muscarinic and nicotinic central receptors in the cholinergic pathways of blood pressure regulation. From the results obtained with atropine and mecamylamine it is evident that the central receptors for the pressor effect of ACh and of nicotine are different. Those for ACh are muscarinic as the responses are abolished by atropine and resistant to mecamylamine applied intraventricularly. On the other hand, the receptors for nicotine are nicotinic since these responses are abolished by mecamylamine but not by atropine applied intraventricularly.

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