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CHOLINERGIC MECHANISMS IN CENTRAL THERMOREGULATION IN PIGEONS

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1 In unanaesthetized pigeons the effect on cloacal temperature was studied of acetylcholine (ACh), carbachol, atropine and (+)-tubocurarine injected into a cannulated lateral cerebral ventricle. The experiments were carried out at an ambient temperature of 19-25° C.

2 ACh or carbachol injected intraventricularly produced hyperthermia, and in larger doses hyperthermia followed by hypothermia. These were central effects because they were not obtained when these drugs were injected in the same doses intravenously.

3 Atropine injected intraventricularly produced hypothermia which was greater and longer lasting than the hypothermia produced with the same dose of atropine injected intravenously. After the intraventricular injection of atropine the hyperthermic effects of ACh and of carbachol were abolished.

4 (+)-Tubocurarine injected intraventricularly produced a long-lasting hyperthermia in doses which had no effect on temperature when injected intravenously. After the intraventricular injection of tubocurarine the hypothermic effects of ACh and of carbachol were abolished.

5 It is concluded that the effects of ACh and carbachol imitate the effects of ACh released from cholinergic neurones in the central pathway involved in temperature regulation. The hypothermic effect of atropine is attributed to unmasking the activity of continuously released ACh acting on nicotinic receptors, and the hyperthermic effect of tubocurarine to unmasking the activity of continuously released ACh acting on muscarinic receptors.

Introduction

Burn & Dutta (1948) were the first to suggest involvement of cholinergic mechanisms in temperature regulation because atropine lowered body temperature in mice. Since then abundant evidence has been obtained in support of this suggestion in a number of mammals by studying the effects of cholinomimetic substances on body temperature following their injection into the cerebral ventricles or into different regions of the hypothalamus. Experiments of this kind were done in mice (Friedman & Jaffe, 1969), rats (Hulst & de Wied, 1967; Meeter & Wolthuis, 1968; Myers & Yaksh, 1968; Lomax, Foster & Kirkpatrick, 1969; Meeter, 1969, 1971; Avery, 1972; Baird & Lang, 1973), sheep, goats and rabbits (Bligh, Cottle & Maskrey, 1971), cats (Baird & Lang, 1973) and monkeys (Myers & Yaksh, 1969). The nature of the thermoregulatory response to cholinomimetic substances was found to differ in different species and to be dependent on the dose of the substance injected, the ambient temperature, and when injected into the hypothalamus on the actual site of injection.

Evidence that in birds cholinergic neurones are involved in central control of body temperature is provided by the results obtained in the present experiments. In these the temperature effects of cholinomimetic substances were examined when injected into a cannulated lateral cerebral ventricle of pigeon before and after atropine or (+)-tubocurarine similarly injected.

Methods

Healthy pigeons of either sex and weighing 200-300 g were used. Food and water were withheld during the period of the experiment. Room temperature varied between 19-25° C.

The methods of implanting a cannula into the right lateral ventricle under intravenous pentobarbitone sodium (30 mg/kg) anaesthesia, of injecting drugs through this cannula, and of verifying its correct placement were the same as described elsewhere (Chawla, Johri, Saxena & Singhal, 1974). Experiments were started not earlier than 2-3 days after the operation when the birds had recovered; injections were made on the same bird not more frequently than once every second day.

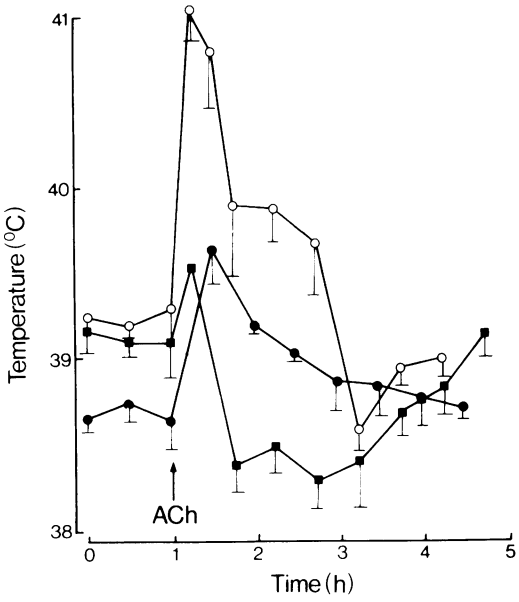

Figure 1 Records of mean cloacal temperature obtained from unanaesthetized pigeons. At the arrow injection into a lateral ventricle of 100 μg acetylcholine (ACh) (•), 200 μg ACh (○) and 200 μg ACh plus 100 μg physostigmine (▪). The vertical bars (shown in one direction only) indicate s.e. mean of six experiments

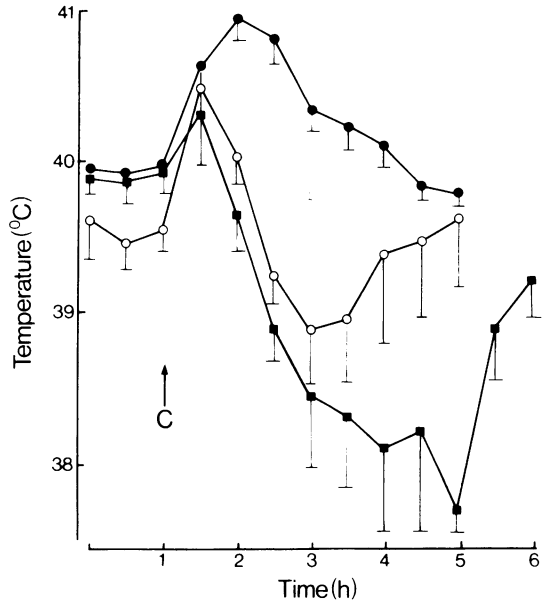

Figure 2 Records of mean cloacal temperature obtained from unanaesthetized pigeons. At the arrow injection into a lateral cerebral ventricle of 2.5 μg (•), 5 μg (○) and 7.5 μg (▪) of carbachol (C). The vertical bars (shown in one direction only) indicate s.e. mean of six experiments

Cloacal temperature was taken with a thermistor probe inserted about 2 cm deep into the cloaca, held in position with a piece of tape and connected to a multichannel telethermometer. Readings were taken every 6 min at the beginning and every 15 min later on in the experiment. The temperature records of Figures 1-4 were plotted from these readings.

The drugs used were acetylcholine chloride (ACh), physostigmine sulphate, carbamylcholine chloride (carbachol), atropine sulphate and (+)-tubocurarine chloride. All doses given in the text refer to the salts. The drugs were dissolved in pyrogen-free distilled water. For injections into the lateral ventricle the volume was always 0.02 ml whatever dose was tested. Injections of 0.02 ml distilled water served as controls. For intravenous injections into the pectoral vein the drugs were injected in a volume of 0.1 ml. Injections were made after the cloacal temperature had been relatively stable for at least one hour.

Results

Apart from a transient rise which occurred in some pigeons as a result of excitement due to handling of the birds, the cloacal temperature was not affected by control injections of distilled water into the cerebral ventricle or into the pectoral vein.

Cholinomimetic substances

Intravenous injections of ACh and carbachol produced dose-dependent hypothermias. The mean fall produced in six experiments with each drug was $0.4 \pm 0.12^{\circ}$C for 200 μg of ACh and $0.6 \pm 0.15^{\circ}$C for 7.5 μg of carbachol.

Intraventricular injections of ACh alone or of ACh with physostigmine produced changes in cloacal temperature, restlessness and mild convulsions which lasted for a few minutes. With the smaller doses of ACh the sole effect on temperature was a rise, with the larger doses the rise was followed by a fall and a biphasic response was obtained. When the ACh was injected together with physostigmine the initial rise in temperature was cut short by a steep fall which dominated the picture. These temperature effects are illustrated in Figure 1 which gives the mean changes obtained with six injections of either 100 or 200 μg of ACh or of 200 μg ACh together with 100 μg of physostigmine. With 100 μg ACh, the temperature

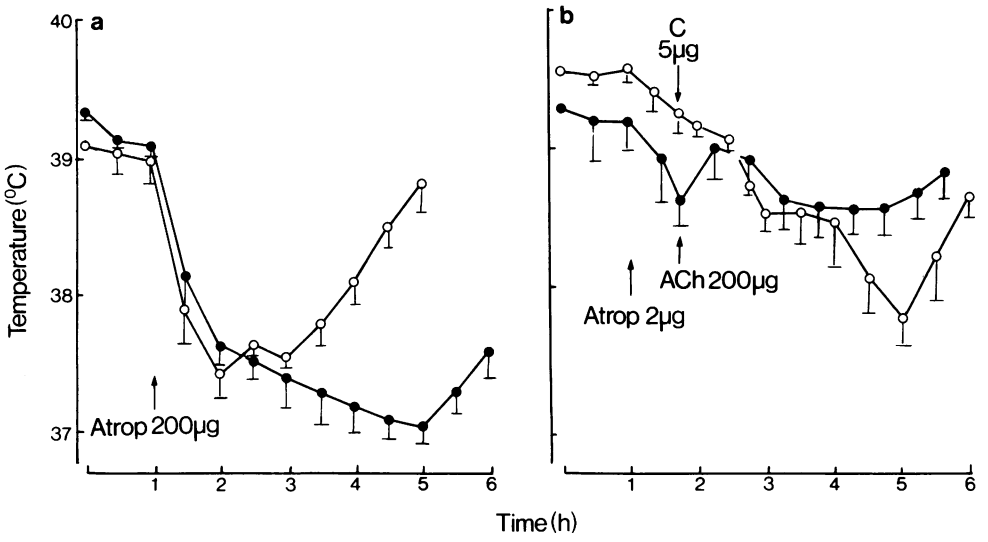

Figure 3 Records of mean cloacal temperature obtained from unanaesthetized pigeons. (a) At the arrow injection of 200 μg atropine (Atrop) into a lateral cerebral ventricle (•) or intravenously (○). (b) At the first arrow injection of 2 μg atropine (Atrop) into a lateral cerebral ventricle. At the second arrow, 45 min later, injection of 200 μg acetylcholine (ACh) or 5 μg carbachol (C) into a lateral cerebral ventricle. The vertical bars (shown in one direction only) indicate s.e. mean of six experiments in (a) and of eight ACh or 10 carbachol experiments in (b).

rose by 1° C, with 200 μg ACh it rose by 1.75° C, and the rise was followed after about 2 h by a fall to 0.7° C below the pre-injection level. When 200 μg ACh was injected together with 100 μg physostigmine, the initial rise of temperature amounted to only 0.35° C; it was followed within about 30 min by a steep fall to 0.9° C below the pre-injection level and temperature then remained at this level for about 1.5 hours.

The changes in cloacal temperature following intraventricular injections of carbachol were similar to those produced by ACh or ACh together with physostigmine. In addition, there occurred excitement, miosis, salivation lasting 20-30 min and in some birds defaecation. Figure 2 illustrates the mean changes in cloacal temperature produced by six injections of either 2.5, 5, or 7.5 μg carbachol. With 2.5 μg the response consisted of a rise of 1° C; with 5 μg, the temperature rose initially more rapidly than with 2.5 μg; but in less than 30 min when the temperature had risen 0.9° C, it began to fall and fell during the following 80 min to 1.1° C below the pre-injection level. With 7.5 μg the initial rise in temperature during the first 30 min after the injection was only 0.35° C; the temperature then began to fall and continued to do so during the following 4 h when it had fallen to 2.2° C below the pre-injection level.

Atropine

Injected intravenously or intraventricularly in doses between 2 and 200 μg atropine produced a dose-dependent fall in cloacal temperature. The only difference in the effects produced with the two routes of administration was that on intraventricular injection the fall was greater and more prolonged. This is seen for 200 μg atropine in the two records of Figure 3a.

It was further found that on intraventricular injection, a dose of 2 μg atropine was sufficient to prevent the hyperthermic effect of a subsequent intraventricular injection of either ACh or carbachol. This is illustrated by Figure 3b. The two records show that 2 μg of atropine which itself produced a small fall in temperature, prevented the rise usually produced by an intraventricular injection of 200 μg ACh or of 5 μg of carbachol, which were injected 45 min after the atropine. It is difficult to be certain whether the atropine also prevented the late hypothermic effect produced by intraventricular injections of ACh and carbachol since the atropine injection itself produced a fall in temperature. From the prolonged large fall seen in Figure 3b when the intraventricular injection of 2 μg of atropine was followed by one of carbachol it would appear that

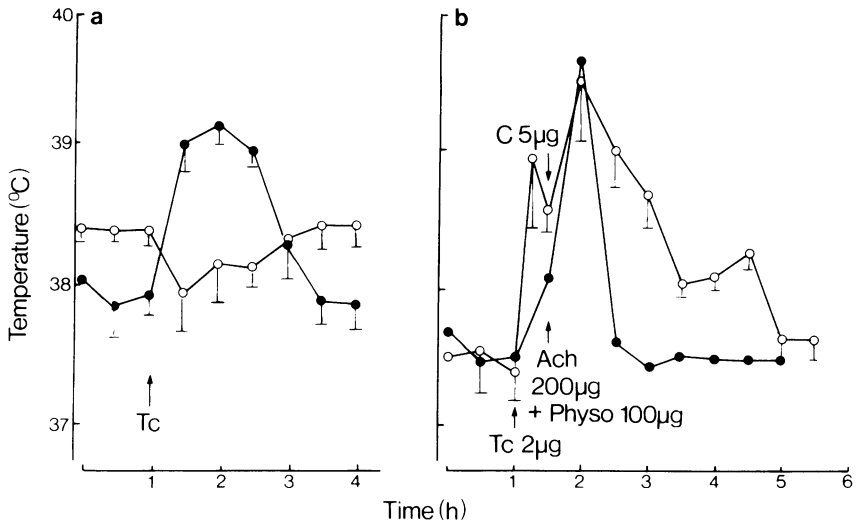

Figure 4 Records of mean cloacal temperature obtained from unanaesthetized pigeons. (a) At the arrow injection of 2 μg (+)-tubocurarine (Tc) into a lateral cerebral ventricle (•) and of 8 μg (+)-tubocurarine intravenously (○). (b) At the first arrow injection of 2 μg (+)-tubocurarine into a lateral ventricle. At the second arrow, 30 min later, injection of 200 μg acetylcholine (ACh) plus 100 μg physostigmine (Physo) or of 5 μg carbachol (C) into a lateral cerebral ventricle. The vertical bars (shown in one direction only) indicate the s.e. mean of six experiments in (a), and of six ACh or eight carbachol experiments in (b).

the hypothermic effect of atropine had summed with that of carbachol.

(+)-Tubocurarine

Injected intravenously, 2 μg of (+)-tubocurarine had no effect on cloacal temperature and 8 μg produced a slight fall as illustrated in Figure 4a. Injected intraventricularly, tubocurarine produced convulsions and a rise in temperature. With 2 μg, short-lasting bouts of tonic-clonic convulsions occurred in many but not in all birds during the first 5-10 min after the injection; temperature rose regularly, whether convulsions occurred or not, and remained high long after the convulsions had come to an end. Figure 4a shows for six birds the mean rise of 1.2°C lasting about 2.5 hours. Intraventricular injections of 8 μg of (+)-tubocurarine were regularly followed by strong tonic-clonic convulsions which resulted in death within a few minutes.

The effect of an intraventricular injection of 2 μg of (+)-tubocurarine on an intraventricular injection, given 30 min later, of ACh with physostigmine, or of carbachol is illustrated in Figure 4b. The rise in temperature produced by the two injections following each other was greater than that produced by either injection alone. This suggests that (+)-tubocurarine, unlike atropine, does not prevent the hyperthermic effect produced either by ACh injected together with physostigmine or by carbachol. On the other hand, the late hypothermic response which these substances produced and which appeared not to be affected by atropine was abolished by (+)-tubocurarine.

Discussion

The cholinomimetic substances, ACh and carbachol, were found to produce hyperthermia when injected into the cerebral ventricles of pigeons, and in larger doses the hyperthermia was followed by hypothermia. These are central effects because they were not produced when the substances were injected intravenously. The action of ACh on the thermoregulatory system of pigeons appears to be similar to that in cats and monkeys, but different from that in rats.

From the results obtained with atropine and tubocurarine it is evident that different receptors are involved. The hyperthermia can be attributed to activation of muscarinic receptors because it was prevented by atropine. Since atropine did not seem to affect the hypothermic response it is

probably not due to activation of muscarinic receptors. The fact that it was prevented by (+)-tubocurarine would suggest that it is due to activation of nicotinic receptors. However, this conclusion depends on the assumption that (+)-tubocurarine inhibits nicotinic receptors in the central nervous system in the same way as in the peripheral nervous system, i.e. at the motor endplates or the synapses of autonomic ganglia.

On the central nervous system (+)-tubocurarine exerts an excitatory strychnine-like action due to depolarization of the central synapse. This action does not manifest itself on intravenous administration because (+)-tubocurarine does not readily pass the blood-brain barrier. The depolarizing effect was first described by Chang (1953) who applied (+)-tubocurarine topically to the cerebral cortex of cats and rabbits. The effect has been confirmed by many authors who introduced tubocurarine by micro-injection into the grey matter of the cerebral cortex or into the hippocampus (for references see Banerjee, Feldberg & Georgiev, 1970). The depolarization is thought to be due, at least in part, to 'disinhibition', i.e., to depression of inhibitory potentials. The tonic-clonic convulsions observed in pigeons after intraventricular injections of tubocurarine can be attributed to an excitatory action on the hippocampus because they also occur in cats when tubocurarine is injected intraventricularly, and in this species were shown to result from an excitatory action on the hippocampus reached from the posterior half of the lateral ventricle (Feldberg & Fleischhauer, 1963). In cats tubocurarine applied intraventricularly produces in addition shivering due to an excitatory action on the hypothalamus which is reached from the third ventricle (Carmichael, Feldberg & Fleischhauer, 1962). Both convulsions and shivering would raise body temperature, so the rise in temperature produced in pigeons could be due to an excitatory action on these two structures. However, temperature remained elevated for over 2 h whereas the convulsions came to an end after a few minutes and shivering was not observed. It is difficult therefore, to attribute the hyperthermia produced by (+)-tubocurarine as well as the prevention of the late hypothermia produced by acetylcholine and carbachol to a remnant of the excitatory action of (+)-tubocurarine on the hippocampus and hypothalamus. Instead they could well be due to blocking of nicotinic receptors on the assumption that tubocurarine exerts such an action on the central synapse in addition to its depolarizing effect.

Both muscarinic and nicotinic receptors have been postulated to explain the effects on body temperature obtained with acetylcholine injected into the cerebral ventricles or introduced by microinfusion into different parts of the hypothalamus. Its hyperthermic effect is thought to be due to an action on muscarinic, its hypothermic effect to an action on nicotinic receptors. In monkeys, Myers & Yaksh (1969) found that microinfusions of ACh as well as of carbachol into various regions throughout the hypothalamus produced hyperthermia, but microinfusion into one circumscribed region at the caudal border of the posterior hypothalamus produced hypothermia. This would localize the muscarinic receptors throughout the hypothalamus and the nicotinic receptors to its posterior portion. Nicotine itself produces hypothermia when perfused in monkeys through the cerebral ventricles (Hall & Myers, 1971a) but on microinfusion into the hypothalamus, hyperthermia from the posterior, and hypothermia from the anterior hypothalamus (Hall & Myers, 1971b). To explain these two effects as being due to actions on nicotinic receptors one would have to postulate inhibitory and excitatory nicotinic receptors. Baird & Lang (1973) obtained in cats hyper- and hypothermic responses to ACh and metacholine and hypothermic responses to nicotine when these drugs were injected intraventricularly. The hyperthermic responses were prevented by atropine and the hypothermic responses by mecamylamine. They therefore postulated muscarinic receptors to account for the hyper-, and nicotinic receptors to account for the hypothermic responses.

The fact that in pigeons the hyperthermic effect was obtained with smaller doses of ACh or carbachol than the hypothermia which, when it occurred, followed the hyperthermia, is explained on the assumption that the sites of the muscarinic receptors are more readily accessible on penetration from the ventricular lumen than the site of the nicotinic receptors. The possibility of a difference in accessibility of the two receptor sites from the ventricular lumen was first envisaged by Hall & Myers (1971a).

The finding that atropine injected intraventricularly into pigeons produced a fall in cloacal temperature can be explained by its blockade of a muscarinic action of continuously released acetylcholine, whereas the rise produced by (+)-tubocurarine, if not due to its depolarizing action, could be explained by blockade of a nicotinic action of released acetylcholine. A tonic activity in the cholinergic neurones subserving the thermoregulatory system has also been observed in cats and rats. In cats, Baird & Lang (1973) obtained hypothermia on intraventricular injection of atropine and in rats, in which the main thermoregulatory effect of acetylcholine is hypothermia, atropine raises temperature (Kirkpatrick & Lomax, 1967).

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