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# Changes in body temperature produced by cholinomimetic substances injected into the cerebral ventricles of unanaesthetized cats

G. H. HALL

*Department of Neuropharmacology, Tobacco Research Council Laboratories, Harrogate, Yorkshire, England*

**Summary**

1. The effects on body temperature of a number of substances injected into a lateral cerebral ventricle were examined in the unanaesthetized cat.

2. Nicotine (50 and 100 $\mu$g) caused a fall in body temperature ranging from 0·95–2·1° C associated with skin vasodilatation, tachypnoea and panting. These responses were prevented by the intraventricular injection of hexamethonium or mecamylamine, but not of atropine or phentolamine.

3. Carbachol (5 $\mu$g) caused a rise in temperature associated with skin vasoconstriction, piloerection and severe shivering. These effects were prevented by the intraventricular injection of atropine, but not of hexamethonium or mecamylamine.

4. An acetylcholine/eserine mixture (10 $\mu$g of each) had no reproducible effect on body temperature. Because of the variability in the response, interaction studies with antagonist drugs were not performed.

5. Hexamethonium (100 $\mu$g) or mecamylamine (100 $\mu$g) caused a prolonged rise in temperature, together with skin vasoconstriction, piloerection and intense shivering. Atropine (200 $\mu$g) was without effect on body temperature.

6. Noradrenaline (100 $\mu$g) caused a fall in temperature with skin vasodilatation. The fall was converted to a rise with skin vasoconstriction, piloerection and vigorous shivering following an intraventricular injection of phentolamine (100 $\mu$g).

7. These observations suggest the existence of cholinergic heat loss and heat gain mechanisms in the hypothalamic thermoregulatory pathways of the cat and the interaction studies with hexamethonium, mecamylamine and atropine, support the involvement of 'nicotinic' and 'muscarinic' receptors within the cholinergic thermoregulatory system.

## Introduction

A possible role for cholinergic mechanisms in the central control of body temperature has been considered for the rat by Lomax, Foster & Kirkpatrick (1969), for the monkey by Myers & Yaksh (1969), and for the rabbit, goat and sheep by Bligh, Cottle & Maskrey (1971). The cat has been used extensively for studies of the thermoregulatory response to the catecholamines (Feldberg & Myers, 1964; 1965), but it has received little attention with regard to thermoregulatory effects of cholinomimetic substances.

Nicotine perfused through the cerebral ventricles of the unanaesthetized monkey was found to produce a profound fall in body temperature (Hall & Myers, 1971). Similar responses were obtained with acetylcholine and noradrenaline, suggesting an interrelationship in the thermoregulatory response to these substances. In further experiments, it was found that nicotine could evoke hyper- and hypothermic responses when injected into the hypothalamus of the monkey (Hall & Myers, 1972). Nicotine caused a rise in temperature when injected in the posterior hypothalamus but a fall when injected into the anterior hypothalamus, suggesting that the hypothermia evoked by nicotine on perfusion through the cerebral ventricles, was due to an action on cells of the anterior pre-optic area.

The catecholamines exert similar actions on body temperature in the cat and monkey (for references see Myers, 1970). To find out if this would apply to cholinomimetic substances as well, the effects on body temperature of nicotine, carbachol and acetylcholine, injected into the cerebral ventricles of unanaesthetized cats, were examined. Further, interaction studies with antagonists of cholinomimetic substances suggest that 'nicotinic' and 'muscarinic' receptors exist at thermoregulatory sites in the hypothalamus of the cat.

## Methods

Experiments were performed on twelve male cats weighing 2·9–3·1 kg. For intraventricular injections a Collison cannula was implanted in the left lateral ventricle, 6 mm caudal to the coronal suture and 4 mm lateral to the saggital suture, as described by Feldberg & Sherwood (1953). The cannula, with a side hole 1 mm from the closed cannula tip, was positioned with its opening directed medially towards the foramen of Monro. Dye studies indicated that the greater proportion of any injected material passed directly into the third ventricle. Implantation of the cannula was carried out aseptically under pentobarbitone anaesthesia, at least a week before the effects of intraventricular injections were examined without anaesthesia. At least 40 h elapsed between each experiment, and for each experiment not more than three injections were given within a 6 h period. The volume for each injection was 0·2 ml except when the period between injections was less than 2 h, then the volume was reduced to 0·1 ml.

Pyrogen-free glassware was used throughout the experiments. Syringes and injection needles were stored in 70% alcohol. All drugs were dissolved in Krebs-Ringer solution and were sterilized by passage through a Millipore Swinnex Filter Unit (SX002500). Rectal temperatures were recorded with flexible YSI thermistor probes inserted into the colon to a depth of 10 cm and held in place by adhesive tape wrapped gently around the base of the tail. Temperatures were monitored on YSI telethermometers and plotted continuously on a Servoscribe potentiometric recorder. Experiments were carried out in an air-conditioned laboratory at temperatures which varied between 21 and 23° C.

The compounds used were as follows: acetylcholine perchlorate, atropine sulphate, carbachol chloride, hexamethonium bromide, mecamylamine hydrochloride, nicotine hydrogen tartrate, (±)-noradrenaline hydrochloride, phentolamine methane sulphonate and physostigmine sulphate (eserine). When acetylcholine and eserine were used in combination, the mixture contained 10 $\mu$g of each substance in terms of the base. Doses of the other drugs also refer to the base.

**Results**

Ear twitching, salivation, tachypnoea and panting, have previously been described following injections of nicotine into the cerebral ventricles of the cat (Hall & Reit, 1966). Since panting is an evaporating heat-loss mechanism, body temperature should fall. It has now been found that hypothermia occurs after an intraventricular injection of 50 or 100 $\mu$g of nicotine, but this response is only partly the result of panting. Within 60 to 90 s after the injection, respiratory rate increased and with the larger dose, panting occurred. The constricted ear vessels became dilated, the ears pink and warm. Tachypnoea and panting ceased after 10–12 min, but skin vasodilatation persisted and temperature continued to fall. The maximum fall was reached between 15 and 45 min after the injection and temperature then began to rise, returning to its pre-injection level. The rise was associated with skin vasoconstriction, piloerection and intense shivering. Temperature responses to 50 and 100 $\mu$g of nicotine injected intraventricularly are illustrated in Fig. 1. The injection of 50 $\mu$g caused a fall of 1° C, which was associated with skin vasodilatation and tachypnoea, but not panting. When body temperature had attained its pre-injection level, a second injection, this time of 100 $\mu$g, caused a fall in temperature of 2·1° C, which was accompanied by skin vasodilatation, tachypnoea, and also pronounced panting. When a cat had received two or more intraventricular injections of nicotine, even at intervals of several days, tachypnoea and panting usually no longer occurred and the hypothermic response was reduced by approximately 50%. Thereafter, the magnitude of the response was usually maintained following subsequent injections of nicotine.

When nicotine was given twice within a 6 h period, the second injection was sometimes less effective than the first, but the response was never abolished (Fig. 2A). It was, however, abolished by the intraventricular injection of the ganglion blocking drugs, hexamethonium (100 $\mu$g) or mecamylamine (100 $\mu$g), both of which caused a prolonged rise in temperature which was associated with skin vasoconstriction, piloerection and intense shivering. Figure 2B illustrates not only the hyperthermic response to hexamethonium which, in this experiment, was preceded by a slight hypothermia, but also that this ganglion blocking agent abolished the hypothermic response to nicotine. Fig. 3 illustrates a hyperthermic response to intraventricular mecamylamine. The response occurred shortly after the injection

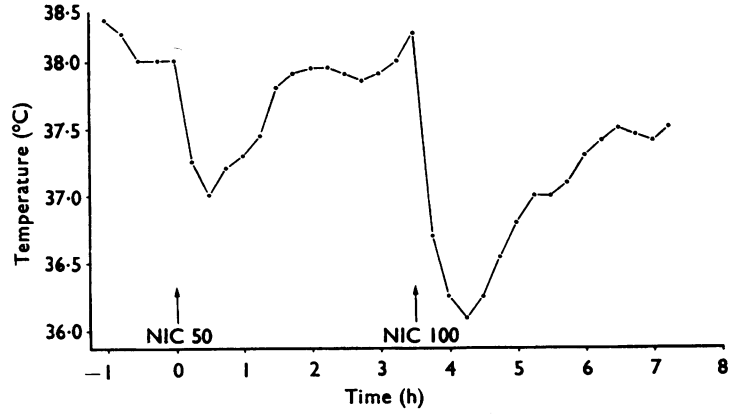


FIG. 1. Effect on body temperature of nicotine (NIC) 50 and 100 $\mu$g injected into a lateral cerebral ventricle in the unanaesthetized cat.

and was accompanied by skin vasoconstriction, piloerection and vigorous shivering. Temperature continued to rise for 3 h, attaining a peak of 1·4° C above the pre-injection level. The Figure also illustrates the effect of a control injection of Krebs-Ringer solution.

Carbachol had the opposite effect to nicotine and caused a steep rise in temperature accompanied by piloerection and vigorous shivering. The effects on the skin vessels varied, mostly the ear vessels constricted, but in some experiments they dilated during the rise. For instance, skin vasodilatation occurred in the experiment of Fig. 4A in which the injection of 5 $\mu$g carbachol caused a rise of 1·2° C. With 2 $\mu$g, temperature rose less than half a degree and with 10 $\mu$g.

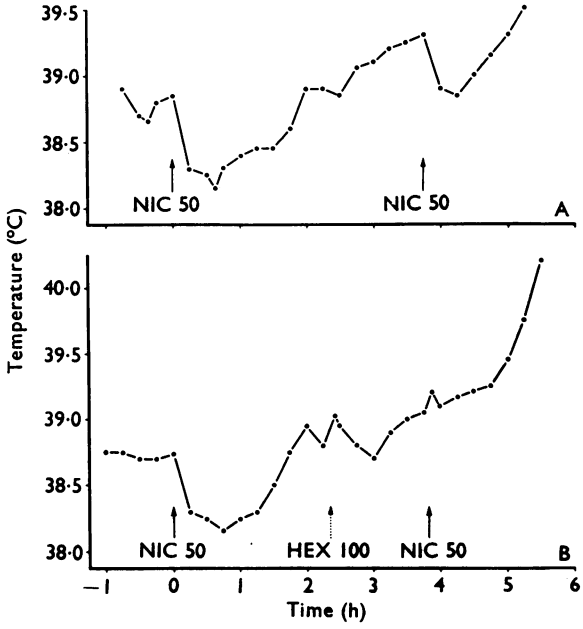


FIG. 2. Effect of hexamethonium (HEX) 100 $\mu$g on the hypothermia produced by nicotine (NIC) 50 $\mu$g, both substances injected intraventricularly in the unanaesthetized cat.

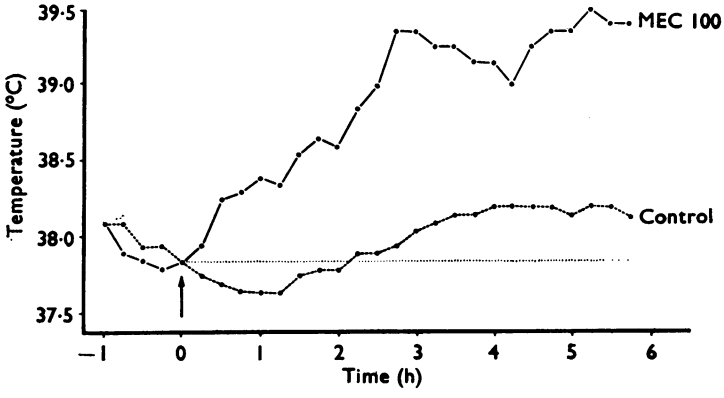


FIG. 3. Effect on body temperature of mecamylamine (MEC) 100 $\mu$g and Krebs-Ringer solution (CONTROL) 0·2 ml injected into a lateral cerebral ventricle in the unanaesthetized cat.

behavioural disturbances such as excitement and bouts of vigorous scratching augmented the strong hyperthermic response.

An intraventricular injection of atropine (200 $\mu$g) prevented the hyperthermia as well as the behavioural changes produced by carbachol. When atropine was injected 10 to 15 min after the carbachol, during the steep rise of temperature, temperature ceased to rise within a few minutes and fell abruptly to below the level existing before the carbachol injection. This is shown in Fig. 4B. The fall was associated with the disappearance of piloerection and of shivering, and when the ear vessels had been constricted by the carbachol they became dilated.

Acetylcholine or eserine injected intraventricularly had no effect on temperature. This is shown for acetylcholine in Fig. 5A. The effect of an acetylcholine/eserine mixture, 10 $\mu$g of each, varied in different experiments even in the same cat. For instance, in the experiment of Fig. 5A the acetylcholine/eserine mixture caused a prolonged rise in temperature, thus acting like carbachol. However, two days later a fall was produced as shown in Fig. 5B.

Noradrenaline is known to produce a fall in temperature when injected into the cerebral ventricles and with repeated injections of the same dose, at intervals of a

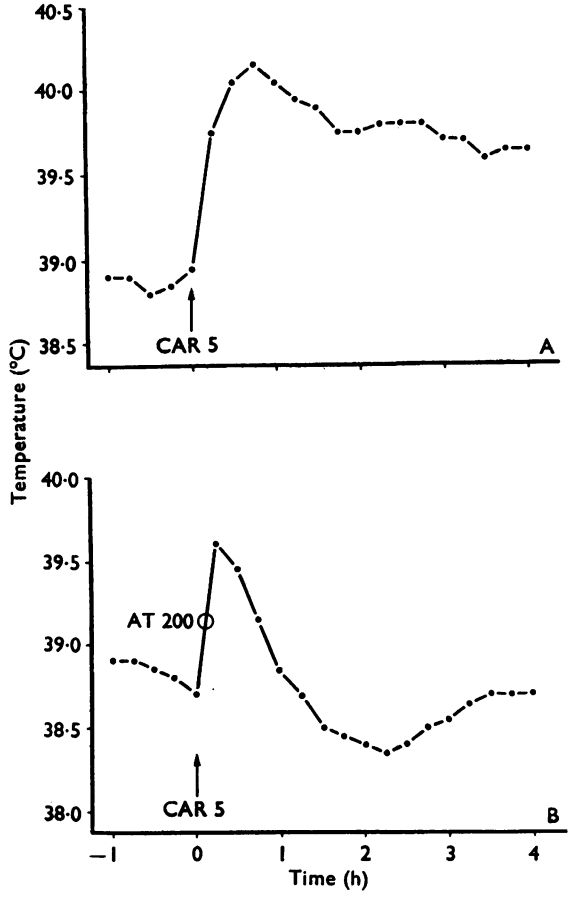


FIG. 4. Effect of atropine (AT) 200 $\mu$g on the hyperthermia produced by carbachol (CAR) 5 $\mu$g, both substances injected intraventricularly in the unanaesthetized cat.

few hours, reproducible responses are obtained. The noradrenaline hypothermia was found to be not only prevented, but reversed by phentolamine injected intraventricularly as shown in the experiment of Fig. 6. Before the injection of phentolamine (100 $\mu$g), noradrenaline (100 $\mu$g) produced a fall in temperature of 0·95° C with dilatation of the ear vessels. One hour after the injection, noradrenaline produced a sharp rise of 1·5° C which was associated with skin vasoconstriction, piloerection and vigorous shivering.

## Discussion

The present experiments show that the body temperature of cats responds to injections of nicotine into a lateral cerebral ventricle, with a fall in temperature in the same way as the monkey. In the cat, tachypnoea and panting is one mechanism by which the hypothermia is produced, but neither tachypnoea nor panting were observed in the monkey after intraventricular injection of nicotine. It is worth mentioning in this connexion that nicotine is so far the only substance which has been found to produce panting in cats on intraventricular injection.

In both species the effect on temperature of intraventricular nicotine resembles that of intraventricular noradrenaline, in that temperature is lowered. In monkeys, it has recently been shown (Hall & Myers, 1972) that microinjections of either

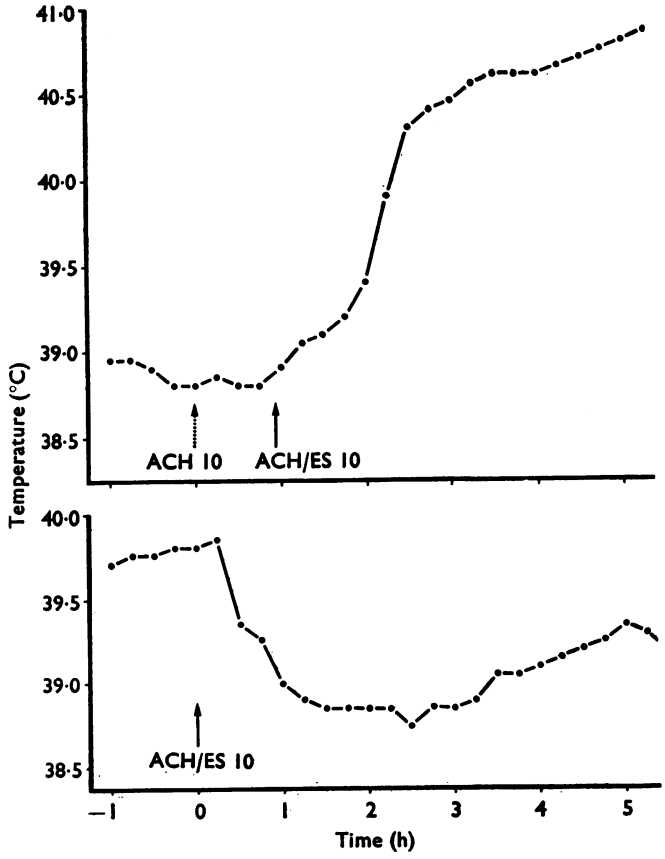


FIG. 5. Effect on body temperature of an acetylcholine/eserine mixture (ACH/ES, 10 $\mu$g of each) injected into a lateral cerebral ventricle in the unanaesthetized cat.

substance into the hypothalamus produce their hypothermic effect by an action on the same site, situated in the anterior hypothalamic area. In cats, it has long been known that this is the area where noradrenaline acts to produce hypothermia (Feldberg & Myers, 1965), and it is also most likely the site for the hypothermic effect of nicotine. Since nicotine causes an increased release of noradrenaline from the cat's hypothalamus (Hall & Turner, 1972) it was suggested that nicotine might produce its hypothermic effect by the release of noradrenaline. The present findings do not support this suggestion, since the nicotine hypothermia was found to be unaffected by phentolamine which, on the other hand, converted the hypothermic response to noradrenaline to a rise in temperature. In previous experiments Burks (1971) found that intraventricular phentolamine reduced the hypothermia caused by intraventricular noradrenaline in cats, but did not convert it into a hyperthermia. The difference between his results and the present observation is at present unexplained.

The finding that intraventricular carbachol had the opposite effect on temperature from that of nicotine, does not necessarily imply that the two substances act on different sites, particularly since carbachol produces a hyperthermic effect also in monkeys and the action is not only on the posterior, but also on the anterior hypothalamus (Myers & Yaksh, 1969). If the action of both substances is on the same site, within a cholinergic thermoregulatory system in the anterior hypothalamus, the nicotine hypothermia would have to be attributed to an action on 'nicotinic' receptors since it is abolished by the ganglion blocking agents hexamethonium and mecamylamine but not by atropine. On the other hand, the hyperthermic effect of carbachol would then have to be attributed to an action on 'muscarinic' receptors since it is abolished by atropine but not hexamethonium or mecamylamine. The prolonged hyperthermia which the two ganglionic blocking agents produced by themselves could also be explained by the existence of 'nicotinic' receptors in a cholinergic heat-loss pathway. The vasodepressor effect

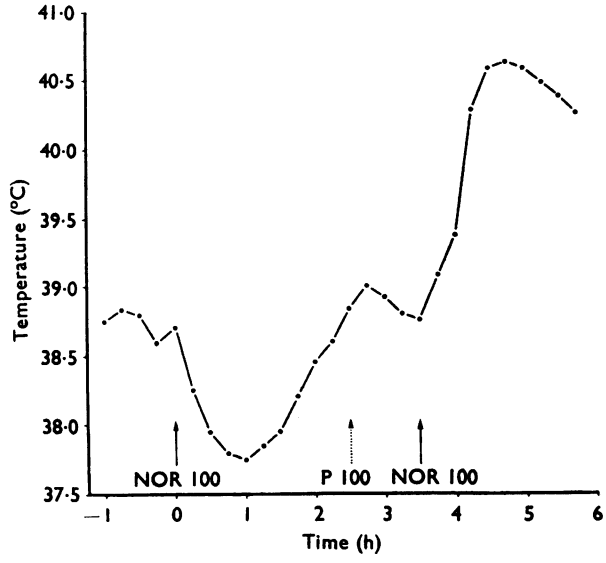


FIG. 6. Effect of phentolamine (P) 100 $\mu$g on the hypothermia produced by noradrenaline (NOR) 100 $\mu$g, both substances injected intraventricularly in the unanaesthetized cat.

which has been obtained with both nicotine and carbachol on intraventricular injection in cats, and found to be due to an action on structures near the ventral surface of the brain stem (Armitage & Hall, 1967a and b), is unlikely to have contributed to the temperature changes produced by the two substances since nicotine causes hypo- and carbachol hyperthermia.

The partial tolerance which developed to the hypothermic effect of nicotine on its repeated intraventricular injection is not the only central effect of nicotine to which tolerance develops. In the unanaesthetized rat, tolerance was found to develop to a hypothermic response following repeated microinjections of nicotine into the thermoregulatory centres (Lomax & Kirkpatrick, 1969) and to the behavioural effects of subcutaneous nicotine, but only to the depressant, not to the stimulant effects (Morrison & Stephenson, personal communication). Further, in anaesthetized cats, tolerance was shown to occur with regard to the circulatory effects (Armitage & Hall, 1967a) obtained on intraventricular and to the electrocortical effects (Domino, 1967) obtained on intravenous injections of nicotine.

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