Canadian Journal of Physiology and Pharmacology

Published by
THE NATIONAL RESEARCH COUNCIL OF CANADA

Journal canadien de physiologie et pharmacologie

Publié par
LE CONSEIL NATIONAL DE RECHERCHES DU CANADA

Volume 57 Number 11 November 1979 Volume 57 numéro 11 novembre 1979

The role of the cholinergic systems in the central control of thermoregulation in rats

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Received April 2, 1979

LIN, M. T., CHEN, F. F., CHERN, Y. F., and FUNG, T. C. 1979. The role of the cholinergic systems in the central control of thermoregulation in rats. Can. J. Physiol. Pharmacol. **57**, 1205–1212.

Systemic and central administration of methacholine (a synthetic choline derivative) both produced dose-dependent decreases in rectal temperature in rats at all the ambient temperatures studied. Both at room temperature (22°C) and in the cold (8°C), the hypothermia in response to methacholine application was brought about by both a decrease in metabolic heat production and an increase in cutaneous circulation. In the heat (29°C), the hypothermia was due solely to an increase in respiratory evaporative heat loss. Furthermore, the methacholine-induced hypothermia was antagonized by central pretreatment of atropine (a selective blocker of cholinergic receptors), but not by the central administration of either 6-hydroxydopamine (a relative depletor of catecholaminergic nerve fibers) or 5,6-dihydroxytryptamine (predominately a serotonin depletor). The data indicate that activation of the cholinergic receptors within brain with methacholine decreases heat production and (or) increases heat loss which leads to hypothermia in rats.

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L'administration systémique et centrale de méthacholine (un dérivé synthétique de la choline) produit dans les deux cas des diminutions de la température rectale qui dépendent de la dose et ce à toutes les températures ambiantes auxquelles nous avons fait cette étude. L'hypothermie résultant de l'application de méthacholine est amenée à la fois par une diminution de la production de chaleur métabolique et une augmentation de la circulation cutanée, tant à la température de la pièce (22°C) qu'au froid (8°C). A la chaleur (29°C), l'hypothermie est uniquement due à une augmentation de la perte de chaleur par évaporation respiratoire. De plus, l'hypothermie induite par la méthacholine est antagonisée par un prétraitement central à l'atropine (un bloqueur sélectif des récepteurs parasympathomimétiques), mais ne l'est pas par l'administration centrale soit de 6-hydroxydopamine (un amoindrisseur relatif des fibres de nerfs catécholaminergiques) soit de 5,6-dihydroxytryptamine (qui est de façon prédominante un amoindrisseur de la sérotonine). Ces données indiquent que l'activation par la méthacholine des récepteurs cholinergiques à l'intérieur du cerveau, diminue la production de chaleur et (ou) augmente la perte de chaleur qui chez le rat, amènent l'hypothermie.

[Traduit par le journal]

ABBREVIATIONS: T_a, ambient temperature(s); 6-OHDA, 6-hydroxydopamine; 5,6-DHT, 5,6-dihydroxytryptamine; M, metabolic rate; E_{res}, respiratory evaporative heat loss; T_r, rectal temperature; T_{back}, back skin temperature; T_t, tail skin temperature; T_f, foot skin temperature; l.c.v., lateral cerebroventricle; sc, subcutaneous.

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0008-4212/79/111205-08$01.00/0

Introduction

There is total disagreement over the role of cholinergic systems within brain in the regulation of body temperature in rats. For example, intracerebral administration of acetylcholine or cholinomimetics produced hyperthermia at T_a of 5–24°C (Avery 1970, 1972; Myers and Yaksh 1968). In contrast, administration of the same drugs via the same routes was shown to produce hypothermia in the same species of animals by many other investigators (Beckman and Carlisle 1969; Hulst and De Wied 1967; Kirkpatrick and Lomax 1970; Lomax et al. 1969; Meeter 1971).

The present study was an attempt to quantify any changes in the thermoregulatory outputs (such as respiratory, metabolic, and vasomotor activity) induced by a synthetic choline derivative (methacholine) in rats, in order to define the possible role for central cholinergic systems in thermoregulation.

Methods

Adult male Sprague–Dawley rats weighing between 250 and 300 g were used in all experiments. The experiments were performed on the unanesthetized animals minimally restrained in special rat stocks (Lin, Chern, Liu et al. 1979). Between experiments the animals were housed individually in wire-mesh cages in a room of 25 ± 1.0°C with natural light–dark cycles. The animals were given free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation.

Surgical Techniques

For the intracerebroventricular injection, the cerebroventricular cannulae were implanted in the animals under general anesthesia (sodium pentobarbital, 6 mg/100 g, ip). Implantation of cerebroventricular cannulae were carried out according to the DeGroot (1959) coordinates : AP 7.0–7.1; Lat. 0.9–1.0; Hor. 0.01–0.1. A 27-gauge injecting needle was connected via PE 10 tubing to a 50-μL Hamilton syringe. During the surgery the correct positioning of each guide tube was verified by the rapid flow of saline into the l.c.v. under gravity. A period of 2 weeks was allowed to permit the animals to recover before they were used.

Drug Solutions

All drug solutions were prepared in pyrogen-free glassware which was baked at 180°C for 5 h before use. Methacholine (Sigma, 1–3 mg/kg for sc and 50 μg for l.c.v. injection) was freshly prepared in 0.9% saline. The 6-OHDA (Sigma, 100 μg/50 μL for l.c.v.injection) hydromate was freshly prepared in 0.9% saline containing 0.1% ascorbic acid. The 5,6-DHT (Sigma, 100 μg/50 μL for l.c.v. injection) was freshly prepared in 0.9% saline containing 0.1% ascorbic acid. Atropine sulfate (Retired Service Pharmaceutical Co., 0.5 mg/kg for intraperitoneal injection and 20 μg/50 μL for l.c.v. injection) was prepared in 0.9% saline. For sc injection, drugs with doses expressed as milligrams of free base per kilogram of body weight were administered in a volume of 1 mL/kg body weight.

Measurement of Thermoregulatory Parameters

The effects of methacholine on metabolic, vasomotor, and respiratory function as well as body temperatures were assessed in a small partitional calorimeter.

The chamber was 0.76 m × 0.80 m in internal dimension and the chamber's temperature was regulated to ±0.3°C over the range of T_a studied by opposing a refrigeration unit of fixed outputs against a series of heaters controlled through a thermostatic proportional controller. The air in the chamber was mixed by a series of fans, but the air movement over the animal was less than 8 m/min.

The M was calculated from the animal's oxygen consumption. Oxygen consumption was measured using a modified open flow draw technique described previously (Lin, Pang et al. 1978; Lin, Chern, Liu et al. 1979; Lin, Chow et al. 1978). Air was drawn at a constant rate (350 mL/min) through a Plexiglas helmet enclosing the animal's head so that all the animal's expired gas was drawn into the chamber effluent tube. The deficit in oxygen content of the effluent air was measured downstream by passing a dry sample of this air through a S-3A oxygen analyzer (Applied Electrochemistry Inc., California). Total air flow was measured by a wet gas meter at the end of the system. The M was then calculated in watts assuming an RQ = 0.83 so that 1 L of oxygen consumed per hour was equivalent to a heat production of 5.6 W (Lin, Chern, Liu et al. 1979; Lin, Chow et al. 1978; Lin 1978*a*).

The E_{res} was calculated by measuring the increase in water vapor content in the helmet effluent air over that of the ambient air. Two pairs of wet and dry bulb thermocouples were used. The first pair, placed in the environmental chamber, gave a measure of the water content of the inspired air, while the second pair, located downstream from the Plexiglas helmet, continuously monitored the water content of the air containing the expired air of the animals. The difference between these measurements, corrected for air flow, provided respiratory water loss by the equation: water loss = (water content of circuit air − water content of ambient air) × air flow. The E_{res} expressed as watts was calculated from evaporative water loss assuming the latent heat of the evaporization of water to be 0.7 W·h/g (Lin 1978*b*).

The T_r, T_{back}, T_t, and T_f were measured using copper–constantan themocouples. The T_r was measured with a copper–constantan thermocouple enclosed in PE 200 tubing, sealed at one end, inserted 6 cm into the rectum.

Measurements were obtained every minute as a DC potential with a Hewlett-Packard digital voltmeter (DVM 3455) interfaced on-line to a CPU 9825 computer which calculated temperatures, M, and E_{res}, and relayed them on an on-line HP printer 9871.

Biochemical Assays

The monoamine assays on the animals treated with 6-OHDA or 5,6-DHT were made 7 days after the injection. Animals were decapitated and their brains were rapidly removed. The methods used for the determinations of dopamine, norepinephrine, and 5-hydroxytryptamine, respectively, were based on those of Walters and Roth (1972), von Euler and Lishajko (1961) and Boadle-Biber et al. (1970), and Atack and Lindqvist (1973). Each sample of brain was used for assay of dopamine, norepinephrine, and serotonin.

Histological Verification

After the completion of the experiments, the animals

TABLE 1. The thermal responses produced by an injection of methacholine into the sc tissue or l.c.v. of conscious rats at three different T_a (8, 22, and 29°C)

Dosage and route of administration	No. of animals	T_a, °C	ΔT_r, °C	ΔT_{back}, °C	ΔT_t, °C	ΔT_f, °C	ΔE_{res}, W/kg	ΔM, W/kg
2 mg/kg, sc	8	8	−2.1±0.23*	−2.0±0.25*	2.0±0.32*	5.0±0.72*	0.03±0.02	−3.8±0.31*
50 μg, l.c.v.	8	8	−2.5±0.33*	−2.3±0.35*	2.5±0.42*	5.5±0.86*	0.05±0.02	−4.2±0.37*
2 mg/kg, sc	8	22	−1.7±0.26*	−1.9±0.31*	5.0±0.75*	5.4±0.84*	0.04±0.03	−2.0±0.18*
50 μg, l.c.v.	8	22	−2.0±0.28*	−1.9±0.35*	5.2±0.81*	6.0±0.76*	0.05±0.03	−2.2±0.21*
2 mg/kg, sc	8	29	−0.6±0.09*	−0.7±0.08*	−3.5±0.38*	−3.1±0.41*	0.25±0.04*	0.2±0.09
50 μg, l.c.v.	8	29	−0.8±0.08*	−0.6±0.06*	−3.4±0.39*	−3.3±0.36*	0.31±0.03*	0.1±0.08

*Significantly different from corresponding control value before the drug injection, $p < 0.05$ (one-way analysis of variance).
NOTE: The values are expressed as the mean ± SEM.

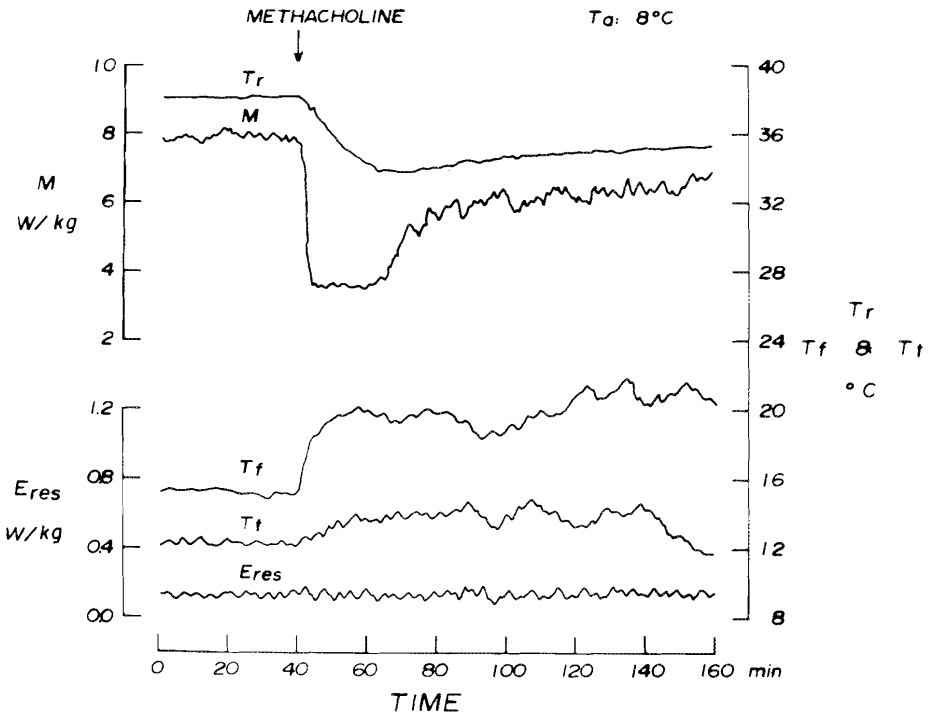

FIG. 1. Changes in T_r, M, T_f, T_t, and E_{res} produced by a sc injection of 2 mg of methacholine/kg at an T_a of 8°C.

were killed with an overdose of sodium pentobarbital. Later, sections of the fixed brain were cut and stained with hematoxylin–eosin so that the stereotaxic coordinates of cerebroventricular cannulae were verified.

Data Collection and Analysis

Animals were permitted a period of 120 min at each level of T_a to attain thermal balance before the drug injections were made. The maximal changes in T_r, T_{back}, T_t, T_f, M, and E_{res} produced within a 120-min period after the injection of drugs were expressed as ΔT_r, ΔT_{back}, ΔT_t, ΔT_f, ΔM, and ΔE_{res}, respectively. These data were collected at three different T_a (8, 22, and 30°C) and are displayed in both tabular and figure forms.

Results

Effects of Methacholine Treatment on Thermoregulatory Responses of Rats to Different T_a

Either subcutaneous or intraventricular administration of methacholine produced a fall in T_r at all the T_a studied. The results are summarized in Table 1 and a more detailed description is given below.

In the Cold

At a T_a of 8°C, the hypothermia in response to methacholine administration was brought about by

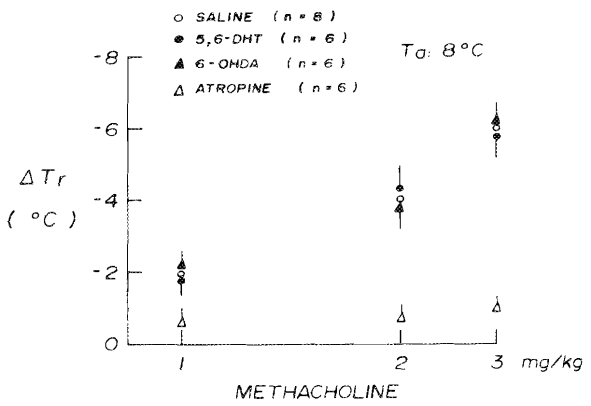

FIG. 2. Effects of 5,6-DHT (100 μg, 3rd ventricle), 6-OHDA (100 μg, 3rd ventricle), and atropine (20 μg, 3rd ventricle) on the hypothermia induced by methacholine (sc) at an T_a of 8°C.

both a decrease in metabolic heat production and an increase in cutaneous circulation (Table 1 and Fig. 1). Cutaneous vasodilatation was shown by an increase in both T_t and T_f. There was no change in E_{res}. The dose–response relation for the hypothermia under external cold conditions is shown in Fig. 2.

At Room Temperature

At a T_a of 22°C, the hypothermia was due to an increase in T_t, an increase in T_f, and a decrease in M (Fig. 3). Again, there was no change in E_{res}. The dose–response relation for the hypothermia at room temperature is shown in Fig. 4.

In the Heat

At a T_a of 29°C, methacholine administration produced a slight hypothermia. The hypothermia was due solely to an increase in E_{res} (probably an increase in salivary secretion) (Fig. 5). There was no change in M. However, methacholine administration did produce a decrease in both T_t and T_f (Fig. 5). The dose–response relation for hypothermia in the heat is shown in Fig. 6.

Control injections of 0.9% saline into the peritoneal cavity or l.c.v. produced insignificant changes in T_r and other thermoregulatory functions at all the T_a studied.

Effects of 6-OHDA, 5,6-DHT, and Atropine Treatment on the Methacholine-induced Hypothermia

The effects of these drugs on the methacholine-induced hypothermia at the three different T_a studied are illustrated in Figs. 2, 4, and 6. The hypothermia induced by the systemic administration of methacholine was greatly reduced by the central administration of atropine, but not by pretreatment with either 6-OHDA or 5,6-DHT. The reduction in methacho-

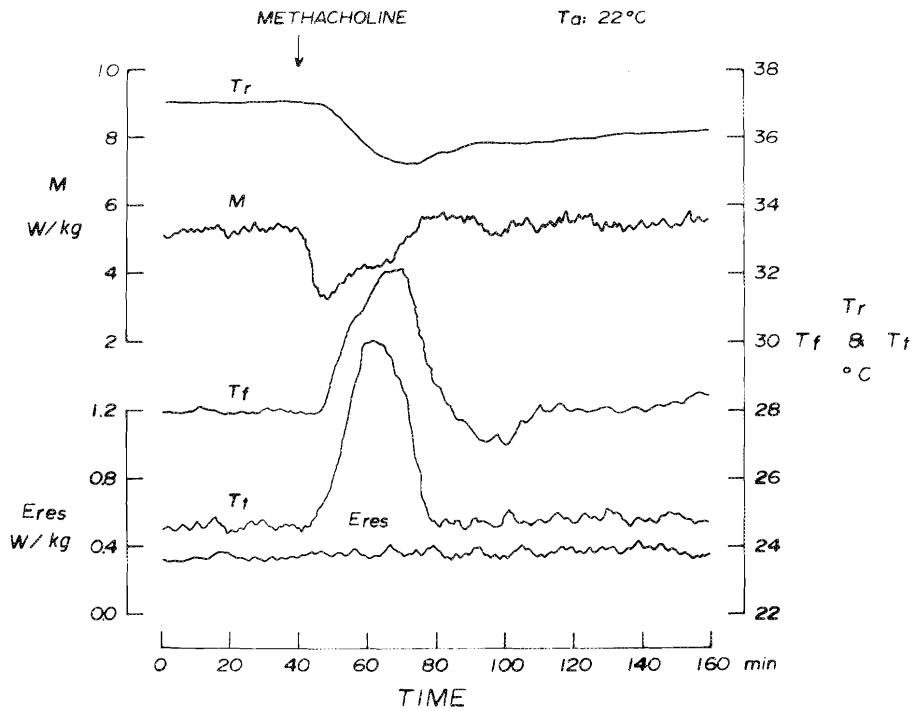

FIG. 3. Changes in T_r, M, T_f, T_t, and E_{res} produced by a sc injection of 2 mg of methacholine/kg at an T_a of 22°C.

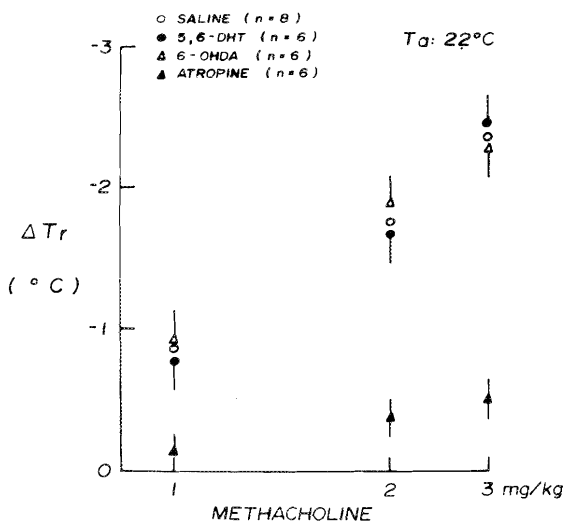

FIG. 4. Effects of 5,6-DHT (100 μg, 3rd ventricle), 6-OHDA (100 μg, 3rd ventricle), and atropine (20 μg, 3rd ventricle) on the hypothermia induced by methacholine (sc) at an T_a of 22°C.

line hypothermia was due to the reduced metabolic and vasomotor responses at T_a of 8 and 22°C (Fig. 7) or the reduced respiratory responses at T_a of 29°C after the methacholine injection as compared with those of the controls (Fig. 8).

Effects of 6-OHDA and 5,6-DHT Treatment on Monoamine Contents of the Rat Brain

The effects of 6-OHDA and 5,6-DHT on monoamine contents of the rat brain during the time at which the thermal experiments were being conducted are summarized in Table 2, and the values are compared with those of control animals. Intracerebroventricular treatment of 6-OHDA produced a significant reduction in brain catecholamine levels with a negligible change in brain serotonin level. On the other hand, intracerebroventricular administration of 5,6-DHT produced a significant reduction in brain serotonin level with a negligible change in brain catecholamine levels.

Discussion

The results reported here show that both central and systemic administration of methacholine, a synthetic choline derivative, produced a fall in T_r at all the T_a studied in conscious rats. At room temperature (22°C) and below it, the hypothermia in response to methacholine application was brought about by both a decrease in metabolic heat production and an

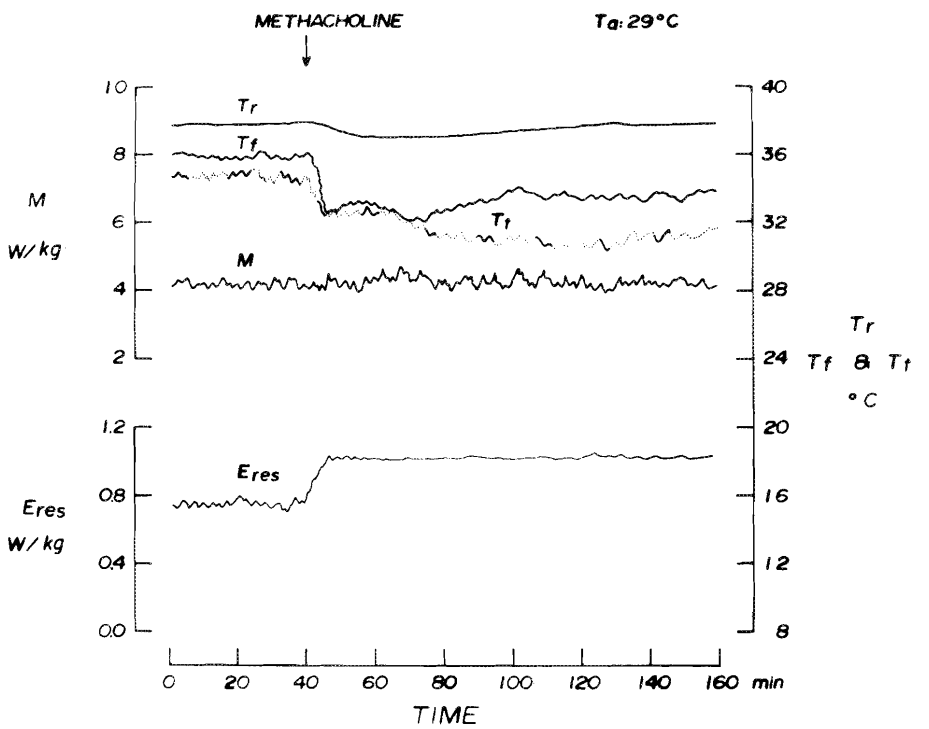

FIG. 5. Changes in T_r, T_f, T_t, M, and E_{res} produced by a sc injection of methacholine (2 mg/kg) at an T_a of 29°C.

TABLE 2. Effects of 6-OHDA and 5,6-DHT treatment on monoamine contents of the rat brain

Treatment	Brain concentration, ng/g		
	Norepinephrine	Dopamine	5-Hydroxytryptamine
0.9% saline, l.c.v.	603 ± 85.5 (5)	794 ± 87.4 (5)	552 ± 67.9 (5)
6-OHDA, 100 μg, l.c.v.	331 ± 49.6* (5)	368 ± 48.5* (5)	516 ± 54.7 (5)
5,6-DHT, 100 μg, l.c.v.	538 ± 41.6 (5)	824 ± 89.2 (5)	207 ± 44.8* (5)

*Significantly different from corresponding control value, $p < 0.05$ (one-way analysis of variance).
NOTE: The values are expressed as the mean ± SEM, followed by the numbers of animals in parentheses.

increase in cutaneous circulation (as indicated by an increase in the skin temperatures of both the tail and the feet). In the heat, the hypothermia was due solely to an increase in E_{res}. Probably, the increase in E_{res} was due to the increased saliva spreading caused by methacholine. In addition, in the present results, injections of small amounts (50 μg in total) of methacholine into the lateral cerebral ventricles produced almost the same temperature effect (−2.0°C at room temperature; see Table 1) as that (−1.7°C at room temperature; see Table 1) produced by a sc dose of 2 mg of methacholine per kilogram. The ratio between these two doses is 1:12 for a rat of 300 g, suggesting a central action of the drug. Furthermore, the present results show that the methacholine-induced hypothermia was antagonized by central pretreatment of atropine sulfate (a selective blocker of cholinergic receptors at this dose), but not by the central administration of either 6-OHDA (a relative depletor of catecholaminergic nerve fibers) or 5,6-DHT (predominately a serotonin depletor). Moreover, a more recent report demonstrated that intrahypothalamic administration of a cholinergic agonist (carbachol) produced a hypothermic response in rats (Netherton et al. 1977). These observations prompted us to realize that activation of the cholinergic receptors within brain (most probably the rostral hypothalamus) decreases heat production and (or) increases heat loss which leads to hypothermia in rats.

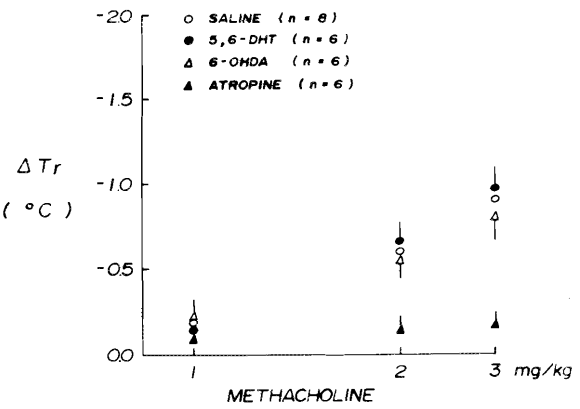

FIG. 6. Effects of 5,6-DHT (100 μg, 3rd ventricle), 6-OHDA (100 μg, 3rd ventricle), and atropine (20 μg, 3rd ventricle) on the hypothermia induced by methacholine (sc) in rats at an ambient T_a of 29°C.

In fact, methacholine is closely related chemically to acetylcholine and it is believed that it exerts its characteristic actions in a similar manner. However, methacholine is less readily hydrolyzed by cholinesterase and thus has a longer duration of action. In the present results, the methacholine-induced hypothermia was greatly arrested by atropine. This indicates that methacholine may elicit a central muscarinic receptor activation and lead to a fall in T_r.

Attempts have been made in rats to correlate the cholinergic sensitivity of single hypothalamic neurons with their sensitivity to hypothalamic temperature stimulation. Murakami (1973) tested warm- and cold-responsive neurons in the rostral hypothalamus with acetylcholine, but failed to find any regular association between acetylcholine and thermosensitivity. However, hypothalamic neurons have been described which were excited by warming the tail and also excited by local or systemic administration of acetylcholine (Knox et al. 1973).

Our recent findings revealed that activation of serotoninergic receptors with either the serotonin precursor 5-hydroxytryptophan (Lin, Chow, Chern et al. 1978) or the inhibitors of uptake pump in serotoninergic neurons such as Lilly 110140 and chlorimipramine (Lin 1978*a*) reduced body temperature in rats at room temperature and below it. Furthermore, activation of central dopaminergic receptors with apomorphine also produced dose-related hypothermia at the same T_a (Lin, Chern, Wang et al. 1979). The hypothermia induced by either serotoninergic or dopaminergic receptor activation was due to a decrease in heat production and (or) an increase in heat loss in rats. This is consistent with that of central cholinergic receptor activation demonstrated in the present study. Thus it can be stated that activation of either serotoninergic, dopami-

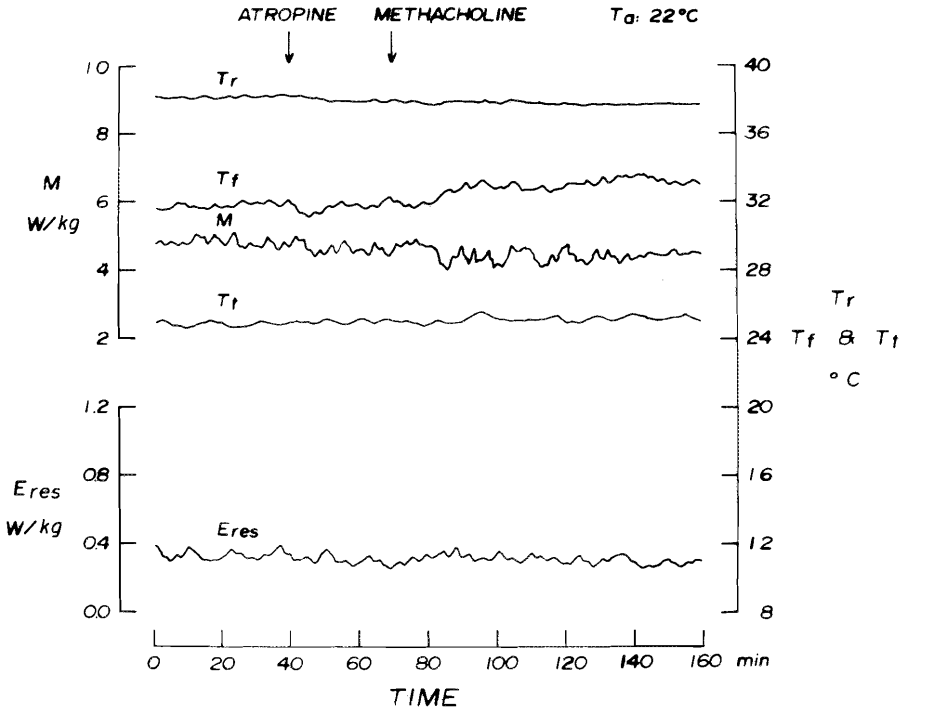

FIG. 7. Effects of atropine (20 μg, 3rd ventricle) treatment on the hypothermia induced by methacholine (2 mg/kg, sc) in a rat at an T_a of 22°C.

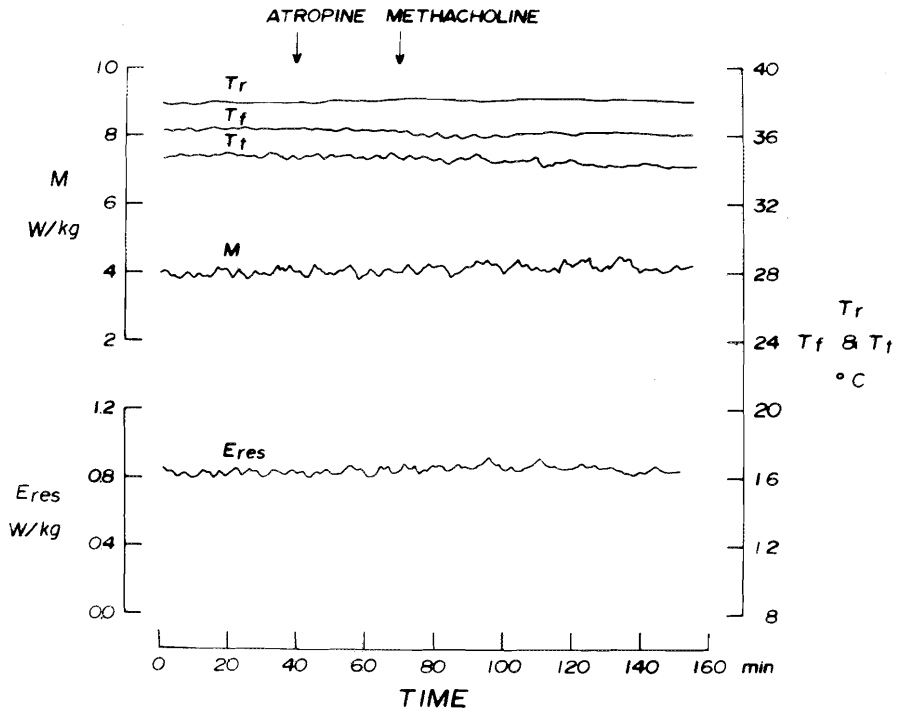

FIG. 8. Effects of atropine (20 μg, 3rd ventricle) treatment on the hypothermia induced by methacholine (2 mg/kg, sc) in a rat at an T_a of 29°C.

nergic, or cholinergic receptors within brain produces hypothermia in rats at room temperature (22°C) and below it. It is not known whether the temperature effects of these neurotransmitters are mediated by a common agent such as the second-messenger cyclic AMP or others.

Acknowledgments

The work was supported by the National Science Council (Republic of China) and the J. Aron Charitable Foundation (New York, U.S.A.). The authors are grateful to Dr. C. Y. Chai for his advice and support during experimentation, and Dr. C. J. Shih for his generous support.

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