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Clonidine-induced hypothermia: Possible involvement of cholinergic and serotonergic mechanisms

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Summary. 1. The thermoregulatory effects (including metabolic, vasomotor and respiratory activities) produced by an injection of clonidine (1–3 μg in 0.5 μl) into the preoptic anterior hypothalamus were assessed in conscious rats at ambient temperatures (T_a) of 8, 22 and 30° C.

2. Intrahypothalamic administration of clonidine caused a dose-dependent fall in rectal temperature at T_a 8° C and 22° C. The hypothermia in response to clonidine was due to decreased metabolic heat production and/or cutaneous vasodilation. There was no change in respiratory evaporative heat loss.

3. The clonidine-induced hypothermic response was attenuated by pretreatment of the rats with either 5,7-dihydroxytryptamine (10 μg, administered intrahypothalamicly, 14 days before clonidine injection), yohimbine (0.2 μg, administered intrahypothalamicly, 10 min before clonidine injection), cyproheptadine (1 μg, administered intrahypothalamicly, 10 min before clonidine injection), or atropine (0.1 μg, administered intrahypothalamicly, 10 min before clonidine injection).

4. The data indicate that clonidine may act on α-adrenoceptors located on a serotonin-acetylcholine pathway within the preoptic anterior hypothalamus to induce hypothermia by promoting a reduction in metabolic heat production and/or an enhancement in dry heat loss in rats.

Key words: Clonidine – Hypothalamus – 5-Hydroxytryptamine – Acetylcholine – Thermoregulation

Introduction

It has been demonstrated that central administration of clonidine caused hypothermia in rats (Tsoucaris-Kupfer and Schmitt 1972; Lin et al. 1981a), goats and sheep (Maskrey et al. 1970), and rabbits (Lin et al. 1981b). The clonidine-induced hypothermia was brought about by both decreased metabolic heat production and cutaneous vasodilation (Lin et al. 1981a, b). However, the mode and the site of the hypothermic effects of clonidine are uncertain.

In addition, it was found that central administration of either acetylcholine (Hulst and Wied 1967; Lin et al. 1979, 1980; Lin 1983a) or 5-hydroxytryptamine (5-HT; serotonin) (Cox et al. 1980; Lin et al. 1983) also produced a dose-dependent fall in rectal temperature in the rat. Again, the hypothermia induced by acetylcholine or 5-HT was due to decreased metabolism and cutaneous vasodilation.

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The present investigation was an attempt to delineate the possible involvement of hypothalamic acetylcholine and 5-HT in the clonidine-induced hypothermia in rats. The thermal responses of rats, which were pretreated with 5,7-dihydroxytryptamine to deplete hypothalamic 5-HT, or with cholinoceptor, adrenoceptor or 5-HT receptor antagonists, to intrahypothalamic administration of clonidine were compared with those of control rats.

Methods

1. Preparation of experimental animals. Male Sprague-Dawley rats weighing between 250 and 300 g were used. They had been maintained at 22 ± 2.0° C and were previously accustomed to being restrained for several hours at a time. The animals had freedom to move their limbs and neck and were conscious. Between experiments they were individually housed in wire-mesh cages with a 12-h light-dark cycle and were fed with dry powder chow, with tap water available ad libitum.

2. Cannula implantation. For the administration of drugs into the preoptic anterior hypothalamus, stainless-steel cannulae consisting of a guide tube (0.81 mm o.d.) with a snugly fitting trocar, and a cannula insert which was introduced into the tube just before injection, were used. The cannula guide tubes with trocars were implanted using the stereotaxic atlas and coordinates of König and Klippel (1963). The following co-ordinates were used: A, 6.3–7.2 mm, L, 0.2–1.2 mm and H, −0.8– −2.4 mm (Lin et al. 1983). After holes had been drilled into the parietal bones, two self-tapping screws were attached and the cannula guide tubes were inserted to the desired depth through the holes. They were anchored with fast drying dental cement to the cranial surface which had been scraped clean of periosteum. The reflected skin was replaced, and the wound dressed with antibiotic ointment (Furacin; Eaton Laboratories, Norwich, NY, USA). At the time of drug injection, a cannula filled to the tip with drug solution was introduced into the guide tube. The cannula was connected to a 10-μl Hamilton microsyringe by P.E. 10 polyethylene tubing, and the syringe and tubing were filled with silicone fluid to act as a piston. The volume of injection down each cannula was 0.5 μl. The cannula inserts were sterilized by boiling in sodium carbonate solution and were subsequently flushed with pyrogen-free sterile water and then autoclaved.

3. Drug solutions. All drug solutions were prepared in pyrogen-free glassware which was heated for 5 h at 180° C

Table 1. Maximal changes in rectal temperature (T_r), foot skin temperature (T_f), tail skin temperature (T_t), metabolic rate (M) and respiratory evaporative heat loss (E_{res}) produced by an unilateral injection of clonidine into the preoptic anterior hypothalamus in rats at various ambient temperatures (T_a)

Treatment of animals	T_a (°C)	ΔT_r (°C)	ΔT_f (°C)	ΔT_t (°C)	ΔM (W/kg)	ΔE_{res} (W/kg)
0.9% Saline ($n = 8$)	8	0.12 ± 0.04	-0.44 ± 0.17	-0.53 ± 0.18	-0.32 ± 0.08	0.02 ± 0.01
Clonidine 1 µg ($n = 8$)	8	-0.63 ± 0.06*	-0.54 ± 0.21	-0.42 ± 0.19	-1.73 ± 0.22*	0.05 ± 0.02
Clonidine 2 µg ($n = 8$)	8	-1.14 ± 0.08*	0.43 ± 0.18	0.51 ± 0.20	-2.84 ± 0.12*	0.03 ± 0.01
Clonidine 3 µg ($n = 8$)	8	-1.62 ± 0.09*	0.52 ± 0.19	0.43 ± 0.21	-3.51 ± 0.15*	0.03 ± 0.01
0.9% Saline ($n = 8$)	22	-0.23 ± 0.08	0.50 ± 0.19	-0.42 ± 0.20	0.32 ± 0.10	0.03 ± 0.01
Clonidine 1 µg ($n = 8$)	22	-0.71 ± 0.06*	2.23 ± 0.38*	1.84 ± 0.35*	-1.04 ± 0.11*	0.03 ± 0.01
Clonidine 2 µg ($n = 8$)	22	-1.23 ± 0.09*	4.01 ± 0.51*	3.52 ± 0.44*	-1.63 ± 0.15*	0.03 ± 0.01
Clonidine 3 µg ($n = 8$)	22	-1.64 ± 0.08*	5.52 ± 0.57*	5.33 ± 0.61*	-2.02 ± 0.14*	0.04 ± 0.02
0.9% Saline ($n = 8$)	30	-0.12 ± 0.09	-0.52 ± 0.21	0.42 ± 0.19	0.41 ± 0.11	0.04 ± 0.02
Clonidine 1 µg ($n = 8$)	30	0.23 ± 0.08	0.62 ± 0.27	0.54 ± 0.22	-0.34 ± 0.11	0.04 ± 0.02

* Significantly different from corresponding control values before the drug injection, P values less than 0.05 (Student's t-test). The values are expressed as the mean ± SEM; n = numbers of rats studied

before use. A 0.5-µl aliquot containing 0.9% saline plus 0.1% ascorbic acid, 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT, Sigma Chemical, St. Louis, MO, USA; 10 µg), 0.9% saline alone, clonidine hydrochloride (Boehringer, Ingelheim, FRG; 1–3 µg), yohimbine hydrochloride (Sigma; 0.2 µg), cyproheptadine hydrochloride (Sandoz, Basel, Switzerland; 1 µg), or atropine sulfate (Sigma; 0.1 µg) was administered into the hypothalamus via the previously implanted guide tubes. Desmethylimipramine hydrochloride (20 mg/kg) was administered subcutaneously. Doses refer to the salts.

4. Measurements of thermoregulatory parameters. The effects of intrahypothalamic administration of either vehicle or drugs on metabolic, respiratory and vasomotor activities as well as body temperature in conscious rats were assessed in a small animal's partitional calorimeter (Lin 1978, 1980; Lin et al. 1983).

Metabolic rate (M) was calculated from the animal's oxygen consumption. Metabolic rate was 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.

Respiratory evaporative heat loss (E_{res}) was calculated by measuring the increase in water vapor content in the helmet effluent air over that of the ambient air. The amount of water evaporated by the animal was calculated according to the equation: water loss = (water content of circuit air − water content of ambient air) × airflow. Evaporative heat loss, expressed as watts, was calculated from evaporative water loss assuming the latent heat of the vaporization of water to be $0.7\ W \cdot h^{-1} \cdot g^{-1}$.

Rectal (T_r), foot skin (T_f) and tail skin (T_t) temperatures were measured using copper-constantan thermocouples. Rectal temperature was measured with a copper-constantan thermocouple enclosed in P. E. polyethylene tubing, sealed at one end, inserted 60 mm into the rectum. All measurements were taken once per minute throughout the experiments, each variable being measured as a DC potential on a Hewlett-Packard digital voltmeter (DVM 3455) interfaced to an on-line computer (Hewlett-Packard 9825). Each min all temperatures, M and E_{res} were calculated instantaneously by the computer and relayed back to the laboratory where they were displayed by an on-line printer Hewlett-Packard 9871.

5. Histological verification. After the experiments were completed, the animals were killed with an overdose of sodium pentobarbital and the internal carotid arteries were perfused with 0.9% saline, followed by a 10% formalin solution. Later, sections of the fixed brain were cut at 40 µm and stained with thionin to verify the proper placement of the cannula.

6. Assay of 5-HT. In some experiments, the 5-HT content of the hypothalamus was quantified by spectrophotofluorometry as described previously (Lin et al. 1981a).

7. Data collection and analysis. Temperatures are indicated in °C. Animals were permitted at each level of ambient temperature (T_a) for at least 90 min to attain thermal balance before drug injections were made. The maximal changes in T_r, T_f, T_t, M and E_{res} occurring during the 60-min period after drug injections were made, as compared to pre-injection values, were expressed as ΔT_r, ΔT_f, ΔT_t, ΔM and ΔE_{res}, respectively. Each animal received several injections at an interval of 5–7 days.

The significance of the differences between means was determined by Student's t-test. A value of $P < 0.05$ was taken to be significant.

Results

1. Effects of administration of clonidine into the hypothalamus on thermoregulation

Rats with implanted cannulae were equilibrated in a partitional calorimeter for a period of 90 min at the selected T_a. Clonidine (1–3 µg in a volume of 0.5 µl) injected unilaterally into the preoptic anterior hypothalamus produced a dose-dependent fall in rectal temperature in conscious rats at T_a's of both 8°C and 22°C (Table 1). The hypothermia in response to clonidine was brought about solely by a decrease in metabolic heat production at T_a = 8°C, while at T_a = 22°C the hypothermia was brought about by both a decrease in metabolism and an increase in cutaneous temperature (Table 1 and Fig. 1). However, at T_a = 30°C, intrahypothalamic administration of clonidine (3 µg) produced no significant change in thermoregulatory parameters. There was no change in respiratory evaporative

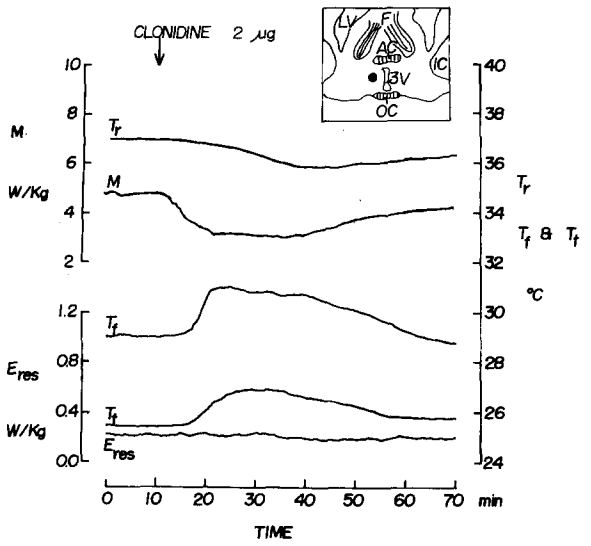

Fig. 1. Changes in rectal temperature (T_r), metabolic rate (M), foot skin temperature (T_f), tail skin temperature (T_t) and respiratory evaporative heat loss (E_{res}) produced by an intrahypothalamic injection of clonidine in a conscious rat at an ambient temperature of 22° C. The site of the microinjection in the anterior hypothalamus is denoted in the histological inset by the solid circle (●), *F*, fornix; *LV*, lateral ventricle; *AC*, anterior commissure; *3V*, third ventricle; *OC*, optic chiasma; *IC*, internal capsule

heat loss in response to clonidine injection at all T_a's studied (Table 1).

2. Effects of intrahypothalamic pretreatment with 5,7-DHT, yohimbine, cyproheptadine or atropine on the clonidine-induced hypothermia

Table 2 contains a summary of thermal responses of the rats which received an ipsilateral intrahypothalamic dose of either 5,7-DHT, yohimbine, cyproheptadine or atropine to intrahypothalamic administration of clonidine (1 – 3 µg) at T_a = 22° C, and a comparison of these responses to those of vehicle-treated animals. Intrahypothalamic administration of cyproheptadine (1 µg), yohimbine (0.2 µg) or atropine (0.1 µg) alone caused no change in thermoregulatory parameters. However, the hypothermia induced by intrahypothalamic injection of clonidine was significantly attenuated ($P < 0.05$) after pretreatment of the animals with either 5,7-DHT, yohimbine, cyproheptadine or atropine.

3. Effects of 5,7-DHT on the concentration of 5-HT in the hypothalamus

The concentrations of 5-HT in the hypothalamus of both the vehicle-treated and 5,7-DHT-pretreated rats are summarized in Table 3. The 5-HT assays were made 14 days after the injection, the point of the period during which the thermoregulatory studies were conducted on these animals. One intrahypothalamic dose of 10 µg 5,7-DHT caused a significant depletion of 5-HT to 23.5% of control values.

Discussion

After intrahypothalamic injection in the rat, clonidine, like 5-HT or acetylcholine (see Introduction), caused a dose-dependent fall in rectal temperature. The clonidine-induced hypothermia was due to decreased metabolism and/or cutaneous vasodilation. In addition, the hypothermia induced by clonidine was attenuated after intrahypothalamic pretreatment of rats with either cyproheptadine (a 5-HT receptor antagonist), atropine (a cholinoceptor antagonist), or yohimbine (an α-adrenoceptor antagonist). Furthermore, depletion of hypothalamic 5-HT to 23.5% of control values with 5,7-DHT (a depletor of 5-HT nerve fibers) reduced the clonidine-induced hypothermia. Thus, it appears that clonidine may act on the α-adrenoceptors located on a 5-HT-acetylcholine pathway (Lin 1983b) within the preoptic anterior hypothalamus to exert its hypothermic action by pro-

Table 2. Effects of intrahypothalamic pretreatment with 5,7-dihydroxytryptamine (5,7-DHT), yohimbine, cyproheptadine or atropine on the hypothermia induced by unilateral intrahypothalamic injection of clonidine in rats

Treatment of animals	Maximal changes of rectal temperature, Δ° C
1. Vehicle-treated rats (n = 8)[a]	
0.9% Saline	−0.12 ± 0.05
Clonidine 1 µg	−0.63 ± 0.07
Clonidine 2 µg	−1.24 ± 0.08
Clonidine 3 µg	−1.51 ± 0.09
2. 5,7-DHT-treated rats (n = 8)[a]	
0.9% Saline	0.11 ± 0.06
Clonidine 1 µg	−0.22 ± 0.07*
Clonidine 2 µg	−0.34 ± 0.06*
Clonidine 3 µg	−0.43 ± 0.05*
3. Saline-treated rats (n = 8)[b]	
0.9% Saline	0.23 ± 0.08
Clonidine 1 µg	−0.72 ± 0.08
Clonidine 2 µg	−1.14 ± 0.09
Clonidine 3 µg	−1.42 ± 0.11
4. Yohimbine-treated rats (n = 8)[b]	
0.9% Saline	−0.21 ± 0.06
Clonidine 1 µg	0.14 ± 0.05 **
Clonidine 2 µg	−0.24 ± 0.07**
Clonidine 3 µg	−0.33 ± 0.08**
5. Cyproheptadine-treated rats (n = 8)[b]	
0.9% Saline	0.13 ± 0.05
Clonidine 1 µg	−0.21 ± 0.06**
Clonidine 2 µg	−0.43 ± 0.08**
Clonidine 3 µg	−0.43 ± 0.08**
6. Atropine-treated rats (n = 8)[b]	
0.9% Saline	0.12 ± 0.06
Clonidine 1 µg	−0.32 ± 0.05**
Clonidine 2 µg	−0.33 ± 0.08**
Clonidine 3 µg	−0.41 ± 0.08**

[a] Vehicle (0.9% saline plus 0.1% ascorbic acid) or 5,7-DHT (10 µg in 0.5 µl) injections were preceded 45 min by SC injection of desmethylimipramine, 20 mg/kg in 0.05 ml saline; clonidine injections were made 14 days after vehicle or 5,7-DHT injection

[b] Normal saline, yohimbine (0.2 µg in 0.5 µl), cyproheptadine (1 µg in 0.5 µl) or atropine (0.1 µg in 0.5 µl) injections were made 10 min before clonidine injection

* Significantly different from corresponding control values (vehicle groups). At $P < 0.01$ (Student's t-test)

** Significantly different from corresponding control values (saline groups), at $P < 0.01$ (Student's t-test). The values are expressed as the mean ± SEM; n = numbers of rats tested

Table 3. Effects of intrahypothalamic injection of 5,7-dihydroxytryptamine (5,7-DHT) on the concentration of 5-HT in rat hypothalamus

Treatment	Hypothalamic 5-HT concentration, ng/g
0.9% Saline+0.1% ascorbic acid[a] (n = 8)	965 ± 79.3
5,7-DHT 10 μg[a] (n = 8)	227 ± 30.8*

[a] The vehicle or 5,7-DHT injections were preceded 45 min by SC injection of desmethylimipramine, 20 mg/kg in 0.05 ml saline; rats were killed 14 days after injection

* Significantly different from control value (vehicle group), at $P < 0.05$ (Student's t-test). The values are expressed as the mean ± SEM; n = numbers of animals studied

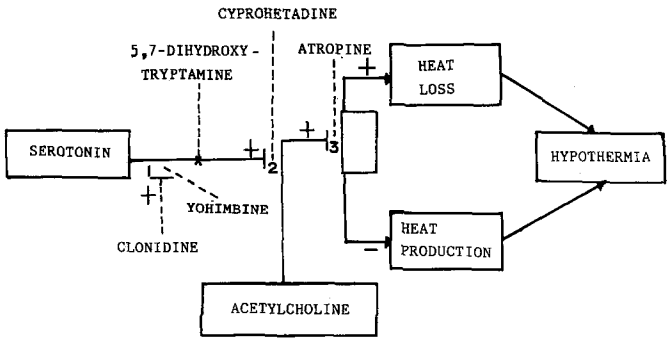

Fig. 2. A scheme proposed to explain the hypothermic action of clonidine. In the preoptic anterior hypothalamus, clonidine may act on α-adrenoceptors (*site 1*) located on a 5-hydroxytryptamine (5-HT or serotonin)-acetylcholine pathway to induce hypothermia by promoting a reduction in metabolic heat production and/or an enhancement in heat loss. The clonidine-induced hypothermia can be antagonized by either 5-HT depletion with 5,7-dihydroxytryptamine or blockade of 5-HT receptors (*site 2*, with cyproheptadine) or cholinoceptors (*site 3*, with atropine). See text for details

moting a decrease in heat production and/or an increase in heat loss in the rat.

In fact, evidence has accumulated to indicate that there exists a 5-HT-acetylcholine pathway in the hypothalamus which mediates hypothermic responses in the rat (as shown in Fig. 2). For example, the hypothalamus is richly innervated by both serotonergic and cholinergic nerve fibers (for review see Cooper et al. 1978). An increase in brain or hypothalamic 5-HT levels causes hypothermia, decreased metabolism and cutaneous vasodilation in rats (Lin 1978; Lin et al. 1978, 1983). In addition, the hypothermia induced by intrahypothalamic administration of 5-HT (Cox et al. 1980; Lin et al. 1983; Lin 1983b) is antagonized by intrahypothalamic preinjection of either atropine (Lin 1983b) or cyproheptadine (Cox et al. 1980; Lin 1983b). However, the hypothermia induced by intrahypothalamic administration of acetylcholine is antagonized by intrahypothalamic pretreatment with atropine, but not with cyproheptadine (Lin 1983b). This hypothesis is further supported by electrophysiological and biochemical data. For example, 5-HT applied by iontophoresis excites warm-sensitive neurons and depresses cold-sensitive neurons in the anterior hypothalamus of rats (Beckman and Eisenman 1970). A more recent report also demonstrated that almost all the warm-sensitive neurons and all the cold-sensitive neurons in the anterior hypothalamus are excited and depressed, respectively, during electrical stimulation of midbrain nuclei in rats (Matsumura et al. 1983). Furthermore, biochemical analysis revealed that the 5-HT turnover of the anterior hypothalamus increases as T_a is elevated (Corrodi et al. 1967; Simmonds 1970) and decreases as T_a is lowered (Simmonds 1970).

According to the scheme described in Fig. 2, intrahypothalamic administration of clonidine may have enhanced the release of 5-HT and acetylcholine and thus caused hypothermia. The clonidine-induced hypothermia can be antagonized by either depletion of hypothalamic 5-HT (with 5,7-DHT) or blockade of α-adrenoceptors, cholinoceptors or 5-HT receptors. In this hypothesis, clonidine has to activate hypothalamic 5-HT neurons. However, Starke and Montel (1973) as well as Göthert and Huth (1980) demonstrated that clonidine produced a decrease, rather than an increase, in the release of 5-HT from rat brain cortex slices. Thus while the results reported here are consistent with the hypothesis shown in Fig. 2, caution is necessary in this interpretation.

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