Intraventricular administration of isoproterenol inhibits both heat production and heat loss mechanisms in rats[1]

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Summary. At an ambient temperature (T_a) of 8 °C, intraventricular administration of isoproterenol inhibited metabolic heat production and led to hypothermia in rats. In contrast, at a T_a of 22 °C and of 30 °C, isoproterenol decreased cutaneous circulation and led to hyperthermia. The data indicate that isoproterenol inhibits both heat production and heat loss mechanisms in rats.

Isoproterenol is the most active of the sympathomimetic amines that act almost exclusively on beta receptors. The drug is employed clinically as a bronchodilator in respiratory disorders and as a cardiac stimulant in heart block, cardiogenic shock after myocardial infarction, and septicemic shock[2-5]. However, little is known about the effect of central administration of isoproterenol on thermoregulation. Therefore, the present study was an attempt to determine the effects of central administration of isoproterenol on the thermoregulatory functions (including metabolic, respiratory and vasomotor activities, as well as body temperatures) of conscious rats to different ambient temperatures.

Materials and methods. Adult male Sprague-Dawley rats weighing between 250 and 300 g were used in all experiments. Measurements were obtained from conscious animals which were trained to sit quietly under minimal restraint in rat stocks. Between experiments the animals were housed individually in wire-mesh cages in a room at 25± 1.0 °C with a 12 h light/12 h dark cycle. The animals were given free access to tap water and granular chicken feed. For intraventricular injection, the ventricular cannulae were chronically implanted in the animals under general anesthesia (sodium pentobarbital, 6 mg/100 g, i.p.). Implantation of ventricular cannulae were carried out according to the DeGroot coordinates: AP, 7.0; Lat., 1.0; and Hor., 0.1 mm[6]. A 27-gauge Hamilton syringe 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 or drug solutions into the lateral cerebral ventricle under gravity. At least 2 weeks were allowed for the animals to recover from the operation. All drug solutions were prepared in pyrogen-free glassware which was baked at 180 °C for 5 h before use. A 5-µl aliquot containing 50–400 µg of isoproterenol (Sigma) was administered into the lateral cerebral ventricle through a guide tube. Metabolic rate (M), respiratory evaporative heat loss (E_{res}) and vasomotor activities were measured in a small calorimeter[7,8]. M was calculated from the animal's oxygen consumption and expressed as W/kg b.wt. E_{res} was calculated by measuring the increase in water vapor content in the expired air. Evaporative heat loss expressed as W was calculated from evaporative water loss[9,10]. 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 PE 200 tubing, sealed at one end, inserted 60 mm into the rectum. All measurements were taken once per min throughout the experiments, each variable being measured as a direct current potential on a Hewlett-Packard digital voltmeter (DVM 3465) interfaced to an on-line CPU 9825 computer. Every min all temperatures, M and E_{res} were calculated instantaneously by the computer and relayed immediately back to the laboratory where they were displayed by an on-line printer HP 9871. Animals were permitted 120 min to attain thermal balance before each drug injection. The maximal changes in T_r, T_f, T_t, M and E_{res} produced within a 60-min period after isoproterenol injection were expressed as ΔT_r,

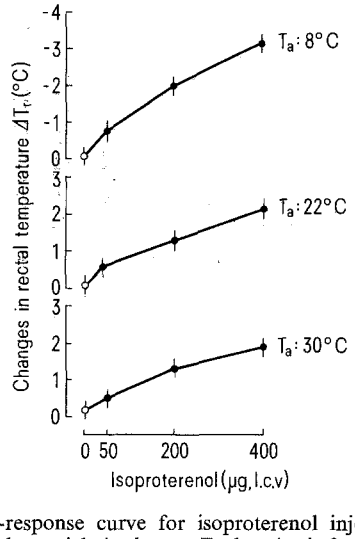

Fig. 1. Dose-response curve for isoproterenol injected into the lateral cerebral ventricle in the rat. Each point is from 8 animals at various ambient temperatures (T_a). The points (solid circle, various doses of isoproterenol; opening circle, saline vehicle) represent the mean change in rectal temperature (T_r in °C) and the vertical bars denote± SEM.

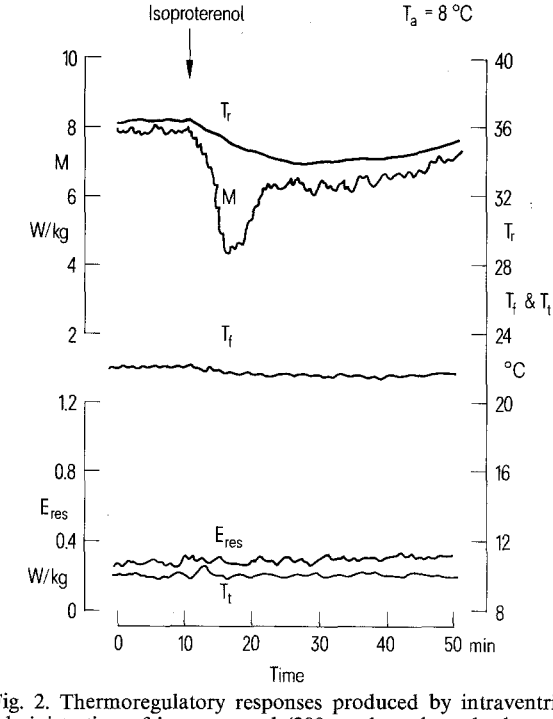

Fig. 2. Thermoregulatory responses produced by intraventricular administration of isoproterenol (200 µg, lateral cerebral ventricle) in an unanesthetized rat at an ambient temperature (T_a) of 8 °C.

The maximal changes in thermoregulatory responses produced by an injection of isoproterenol into the lateral cerebral ventricle (l.c.v.) of conscious rats at various ambient temperatures (T_a)

Dosage and route of administration	T_a (°C)	ΔT_r (°C)	ΔT_f (°C)	ΔT_t (°C)	ΔE_{res} (W/kg)	ΔM (W/kg)
0.9% saline, l.c.v. (n=8)	8	0.1±0.07	0.5±0.22	−0.5±0.25	0.05±0.02	−0.5±0.09
Isoproterenol 200 µg, l.c.v. (n=8)	8	−2.0±0.21*	−0.6±0.19	0.4±0.20	0.04±0.02	−3.5±0.34*
0.9% saline, l.c.v., (n=8)	22	−0.1±0.06	0.4±0.18	0.5±0.21	−0.05±0.03	−0.6±0.15
Isoproterenol 200 µg, l.c.v. (n=8)	22	1.3±0.18*	−4.1±0.49*	−2.2±0.17*	0.03±0.01	0.5±0.14
0.9% saline, l.c.v. (n=8)	30	0.2±0.09	0.4±0.21	0.6±0.25	0.06±0.03	0.5±0.13
Isoproterenol 200 µg, l.c.v. (n=8)	30	1.2±0.10*	−5.6±0.62*	−2.3±0.24*	0.07±0.03	0.4±0.15

* Significantly different from corresponding control value before the drug injection, $p<0.05$ (1-way analysis of variance). The values are expressed as the mean ± SEM. n, number of rats tested.

ΔT_f, ΔT_t, ΔM and ΔE_{res}, respectively. The data were collected at 3 different ambient temperatures (T_a) of 8, 22 and 30 °C.

Results and discussion. Figure 1 shows that the administration of isoproterenol into the lateral cerebral ventricle produced a dose-dependent hypothermia in rats at 8 °C T_a. The hypothermia was brought about solely by a decrease in M (figure 2 and table). The maximal reduction in M within a 60-min period after the injection of isoproterenol was about 3.5 W/kg (ΔM). There was no change in either the cutaneous circulation or the E_{res}. On the other hand, intraventricular administration of isoproterenol produced a dose-dependent hyperthermia in rats both at 22 and at 30 °C T_a (figure 1). The hyperthermia was due to cutaneous vasoconstriction (figures 3 and 4). The maximal reduction in T_f and T_t within a 60-min period after the injection of isoproterenol were about 5.3 °C (ΔT_f) and 2.2 °C (ΔT_t), respectively (table). There was no change in either M or E_{res}. The data indicate that the activation of central adrenergic receptors with the synthetic agonist isoproterenol inhibits both heat production and heat loss mechanisms which lead to an alteration in body temperature.

The present results support the model dealing with the monoaminergic mechanisms of temperature regulation deduced by Bligh, Cottle and Maskrey[11] from the effects of intraventricular injections of norepinephrine in sheep, goat and rabbit; the effects of injected norepinephrine are interpreted as indicating that endogenous hypothalamic norepinephrine functions as an inhibitory transmitter substance on both the heat-production and heat-loss pathways. In addition, in rats measurements of the turnover of norepinephrine in the hypothalamus showed an increase in high ambient temperature, but a similar increase was found during cold exposure[12]. Thus, the increased norepinephrine turnover in response to heat and cold, which was confined to the hypothalamus, would support the role proposed for norepinephrine as a crossed inhibitory transmitter between heat production and heat loss pathways.

Our recent results showed that activation of central 5-HT receptors with either the 5-HT precursor[13] or the inhibitors of the uptake pump in 5-HT neurons[7] produced hypothermia in rats at room temperature (22 °C) and below it. Furthermore, activation of central dopaminergic receptors with apomorphine[9] or central cholinergic receptors with methacholine[14] was also shown to produce hypothermia in rats at the same T_a. The hypothermia incuced by either 5-HT, dopaminergic or cholinergic receptor activation was due to a decrease in M and (or) an increase in heat loss. These observations prompted us to realize that the activation of central serotoninergic, dopaminergic, cholinergic or

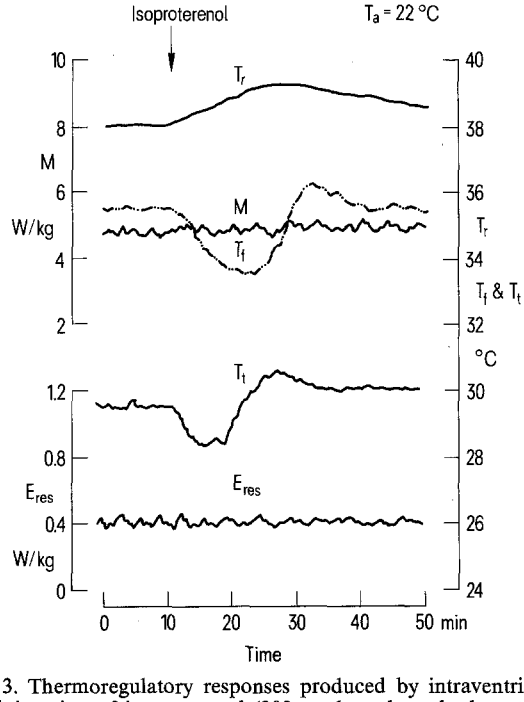

Fig. 3. Thermoregulatory responses produced by intraventricular administration of isoproterenol (200 µg, lateral cerebral ventricle) in an unanesthetized rat at an ambient temperature (T_a) of 22 °C.

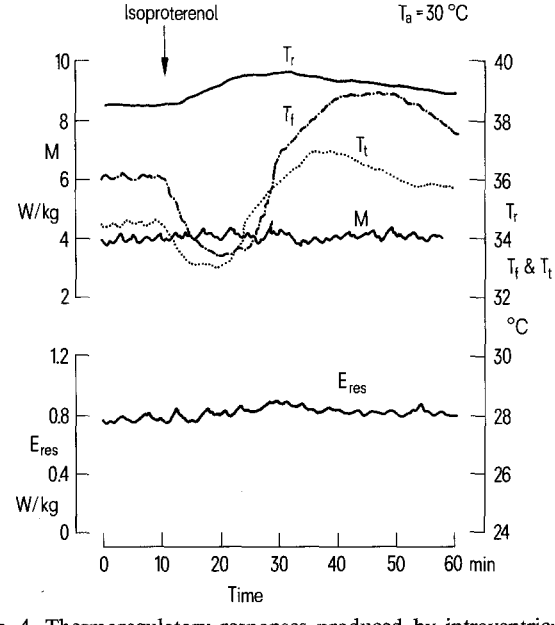

Fig. 4. Thermoregulatory responses produced by intraventricular administration of isoproterenol (200 µg, lateral cerebral ventricle) in an unanesthetized rat at an ambient temperature (T_a) of 30 °C.

adrenergic receptors inhibits the heat production mechanism. On the other hand, activation of central serotoninergic, dopaminergic or cholinergic receptors facilitates the heat loss mechanism, while activation of central adrenergic receptors inhibits the heat loss mechanism.

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Glutamate decarboxylase activity in brain regions of differentially-housed mice; a difference in the olfactory bulb

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Summary. Glutamate decarboxylase (GAD) activities were determined in homogenates of 8 brain regions of mice that had been differentially housed ('isolated' vs 'grouped') for 4–9 weeks. GAD activity was lower in whole forebrains and in olfactory bulbs of isolated mice, changes which might be associated with their increased aggressiveness.

L-Glutamate-α-decarboxylase (L-glutamate-1-carboxylyase; E.C. 4.1.1.15; GAD), the rate-limiting enzyme in the synthesis of γ-aminobutyric acid (GABA), is present mainly in synaptosomes[1,2]. GABA-α-oxoglutarate transaminase (GABA aminotransferase; E.C. 2.6.1. c; GABA-T), the main enzyme involved in GABA catabolism, is associated, in part, with synaptosomal mitochondria[1,3,4]. Thus, changes in the activities of these enzymes could underlie certain behavioral modifications. In this regard, an inverse correlation has been found between cerebral GAD activity and exploratory activity in mice[5]. Changes in the central GABA-ergic system can also be produced by environmental modification; e.g., GABA binding to synaptosome-enriched fractions occurred to a lesser extent in 'isolated' (aggressive) male mice[6] and cerebral GABA content was lower in 'isolated' mice than in their 'grouped' counterparts[7,8]. Furthermore, olfactory deafferentation can induce mouse-killing in previously 'non-killer' rats, but isolation of the rats is necessary after the operation to elicit this behavior[9]. The GABA content of the olfactory bulbs is much lower in spontaneous 'killer' rats than in non-killers[10], and bilateral injections of GABA into the olfactory bulbs inhibits muricidal behavior[11]. Taken together, the above-mentioned results have revealed that a central GABA-ergic system, localized especially in the olfactory bulb, appears to be involved in the control of aggressive behavior, and that this system is subject to environmental regulation. Since this system provides a model for negative (inhibitory) control of behavior, we have compared the GAD activities of several brain regions of isolated vs grouped mice.

Materials and methods. Post-weanling, male mice (28 days of age) of the C3H/He/orl strain were used. 'Isolated' mice were kept singly in small (14 × 20 × 26 cm) cages and were prevented from seeing each other ; 'grouped' mice were kept in groups of 10–12 in large (28 × 31 × 50 cm) cages furnished with various objects which provided environmental enrichment. After 4–9 weeks of differential housing, the mice were decapitated and their whole forebrains (rostral to the inferior colliculi, excluding cerebellum) and regions of their brains were excised rapidly, weighed (regions from 2 brains/sample), and homogenized at 0 °C in 10 vols of K^+-phosphate buffer (5 mM, pH 7) containing 2-aminoethylisothiouronium bromide hydrobromide (1 mM), EDTA (5 mM), and pyridoxal phosphate (10 mg/l).

Aliquots (20 µl) of homogenates (representing about 0.2 mg protein) were incubated together with 100 µl of 0.1 M K^+-phosphate buffer (pH 7.0) which contained 0.5 mM pyridoxal phosphate, 1.0 mM 2-aminoethylisothiouronium bromide hydrobromide, 0.1 mM EDTA, and 15 mM L-glutamate (10.4 mCi/mmole) (final concentrations). Unlabelled L-glutamate (Sigma Chem. Corp.) and [U-^{14}C]L-glutamate (CEA, France; sp. act. 250 mCi/mmole) were used. Solutions were placed into 6-ml conical tubes, and the reaction was initiated by addition of homogenate. Tubes were sealed with rubber stoppers in which were fixed small cups containing 200 µl of hyamine hydroxide. After incubation at 37 °C for 35 min, 0.1 ml of 0.2 M sulfuric acid was injected through each stopper to terminate the reaction and samples were allowed to stand for an additional 60 min at 37 °C. Blanks were run under identical conditions using 20 µl of homogenizing buffer in place of homogenate. GAD activities were determined by 2 methods[12]. CO_2 evolution was measured by a modification of the method of Albers and Brady[13,14]. The contents of cups containing hyamine-plus-trapped $^{14}CO_2$ were washed into scintillation vials with 9 ml Omnifluor scintillation fluid (New England Nuclear Corp.; diluted 4 g/l of toluene). To determine [U-^{14}C]GABA (formed from labelled glutamate), incubated solutions were placed onto columns (0.5 × 4 cm) of Dowex AG-1 × 8 (200–400 mesh) anion exchange resin, previously equilibrated with 2 M Na^+-acetate[15,16]. After applying the samples to the columns, the tubes were washed with 2 ml of water, and the columns were washed with 3 × 1 ml of water; eluates were collected in scintillation vials containing 5 ml of Instagel (Packard Instr. Co.). Quench curves were constructed for both scintillation fluids to convert counts/min to disintegrations/min. An Intertechnique scintillation spectrometer was used to determine radioactivity.

Results and discussion. No differences in the protein concentrations of homogenates existed between whole forebrain or brain regions of isolated and grouped mice. GAD