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Physostigmine-induced hypothermic response in rats and its relationship with endogenous arginine vasopressin

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ABSTRACT

Aims: It is well known that physostigmine (PHY) and other anticholinesterase (anti-ChE) agents induce hypothermia in rodents but little is known about the mechanism of action. Because arginine vasopressin (AVP) has been found to be an endogenous antipyretic molecule in the CNS, we determined if PHY-induced hypothermia is linked to the endogenous release of AVP.
Main methods: Core temperature and motor activity were monitored by telemetry in rats maintained at an ambient temperature of 25 °C. Tail skin temperature was also measured at 30 min intervals to estimate nonevaporative heat loss. The central cholinergic antagonist, scopolamine (1 mg/kg; ip) and an AVP V_1 receptor antagonist (30 μg/kg; ip) were administered during the period of PHY (200 μg/kg; sc) induced hypothermia at 10 am. Plasma AVP concentration and plasma cholinesterase (ChE) activity were measured at 50 min after administration of PHY or scopolamine, respectively.
Key findings: PHY led to a rapid reduction in core temperature concomitant with a marked increase in heat loss from the tail. The hypothermic response of PHY was blocked by the AVP V_1 receptor antagonist. Administration of scopolamine also reversed the hypothermic responses and led to marked elevations in motor activity. Plasma AVP levels increased markedly at 50 min after PHY and plasma ChE activity was significantly reduced by PHY.
Significance: The results clearly demonstrate that PHY-induced hypothermia was blocked by the AVP V_1 antagonist and associated with elevations in plasma AVP, suggesting a novel role for AVP in the mechanism of action of anti-ChE agents.

Introduction

A large number of experimental and some clinical studies have demonstrated that arginine vasopressin (AVP) plays an important role in thermoregulation and fever (Pittman et al. 1998; Richmond 2003; Dong et al. 2007). Central infusion of AVP to the febrile rat elicits a marked antipyretic response associated with a decrease in heat production, an increase in heat loss, and subsequent lowering of core temperature (T_c). Intravenous infusion of AVP will cause a reduction in body temperature whereas infusion of the AVP V_1 receptor antagonist leads to an elevation in body temperature (Steiner et al. 1998). During fever, AVP is released into the ventral septal area and binds to AVP V_1 receptors that appear to drive heat loss responses (Pittman and Wilkinson 1992). Few studies have assessed the mechanism by which AVP evokes the drop in T_c. Evidence suggests that the fall in T_c after peripheral injection of AVP could be attributed, at least in part, to the sino-aortic baroreflex suppression of nonshivering thermogenesis (Shido et al. 1984). On the other hand, the ionotropic receptors of L-glutamate in the central nervous system (CNS) participate in peripheral AVP-induced hypothermia by affecting heat loss through the tail (Paro et al. 2003).

We have found that AVP is involved in mediating the hypothermic effects of chlorpyrifos (an organophosphate insecticide) in male and female rats (Yang and Gordon 2002). Chlorpyrifos is an anticholinesterase (anti-ChE) that irreversibly inhibits acetylcholinesterase activity leading to central and peripheral cholinergic stimulation. The cholinergic system is involved in temperature regulation (Ryan et al. 1996) and it is thought that the hypothermic response to chlorpyrifos and other anticholinesterase agents is due to the activation of muscarinic pathways in CNS thermoregulatory centers (Gordon 2006). Furthermore, cholinergic stimulation of the hypothalamic area has been shown to elicit the release of arginine vasopressin (Michels et al. 1991; Raber and Bloom 1996). Yang and Gordon (2002) found that chlorpyrifos-induced hypothermia was blocked by administration of an AVP V_1 receptor antagonist, suggesting that the thermoregulatory response to chlorpyrifos is mediated by central and/or systemic AVP release (Yang and Gordon 2002).

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Physostigmine (PHY) is a reversible cholinesterase inhibitor and has a short duration of action (i.e., compared to organophosphates). It is used clinically for the treatment of anticholinergic syndrome, myasthenia gravis, and Alzheimer's disease (Mach et al. 2004). In addition, PHY elicits hypothermia when injected peripherally or centrally into various species during rest and exercise (Fehlner and Gordon 1985; Maickel et al. 1991; Rodrigues et al. 2004; Pires et al. 2007). Central injection of cholinergic agonists produces hypothermia due to increased heat loss (Fehlner and Gordon 1985; Pires et al. 2007). However, little is known about the mechanism of PHY-induced hypothermia. Considering the evidence that PHY stimulates the AVP secretion from the hypothalamus (Rhodes et al. 2002; Rubin et al. 2006), we hypothesize that endogenous release of AVP could be involved in the mediation of the thermoregulatory and other physiological responses to PHY. A main goal of this study was to determine if pharmacological blockade of AVP affected PHY-induced hypothermia.

Materials and methods

Animals

Experiments were performed on adult female Sprague–Dawley rats weighing 210–270 g (Institute of Laboratory Animal Sciences, Sichuan Academy of Medical Sciences, China), housed individually in acrylic cages lined with wood shavings and maintained at an ambient temperature of 25 °C, and exposed to a daily 12:12 light:dark photoperiod (lights on at 6 am). Animals were allowed free access to water and food. All animal studies complied with the WHO Guidelines of Humane Use and Care of Animals and approved by Institutional Animal Use and Care Committee.

Drugs

Physostigmine salicylate, scopolamine hydrobromide and vasopressin receptor antagonist [beta-Mercapto-beta, beta-cyclopentamethylenepropionyl(1),O-Me-Tyr(2), Arg(8)]-VP were purchased from Sigma Chemical Co. (St Louis, MO). The drugs were dissolved in pyrogen-free sterile saline.

Surgery

Animals were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally) and a small incision was made in the ventromedial section of the abdomen to allow for insertion of the transmitter (Data Sciences International, Model TA10TA-F40) into the peritoneal cavity. Following surgery, rats were administered a penicillin antibiotic (20,000 units; intramuscularly). The rats were allowed at least 7 days of recovery before testing.

Measurement of core temperature and motor activity

Core temperature (T_c) and motor activity were monitored in undisturbed rats using radiotelemetry (Data Sciences International, St. Paul, MN, USA). The output of the transmitter was monitored at 5 min intervals by a receiver board placed under each rat's cage. The rat's motor activity was measured from the change in position of the transmitter in relation to the antennae located in the receiver board. Data were monitored on line as well as stored on computer for later analysis.

Experimental protocol

In all experimental protocols, the environmental chamber was set at 25 °C. Each rat was weighed and returned to its home cage that was then placed in an environmental chamber at 5 pm. The rats were allowed to adapt to the chamber overnight. The following day the rats were dosed with drugs at 10 am. T_c and motor activity of the rat were monitored by radio telemetry for at least 12 h prior to dosing.

Effect of scopolamine on PHY-induced hypothermic responses

To evaluate the effect of scopolamine on PHY-induced hypothermia, rats were dosed subcutaneously with PHY (200 μg/kg body weight) or pyrogen-free saline (1 ml/kg) at 10 am. Immediately after PHY or saline the rats were dosed with scopolamine (1 mg/kg; ip) or saline (1 ml/kg). After injections, the rats were returned to their cages and monitored for changes in body temperature and motor activity.

Effect of AVP V_1 receptor antagonist on PHY-induced hypothermic responses

To evaluate the AVP V_1 receptor antagonist on PHY-induced hypothermic responses, rats received PHY (200 μg/kg; sc) or saline, followed immediately by an injection of AVP V_1 receptor antagonist (30 μg/kg; ip) or saline at 10 am. Saline (1 ml/kg) was used for control rat injections, at the same volume. The rats were returned to their cages and monitored for at least 8 h.

Effect of PHY, scopolamine and AVP V_1 receptor antagonist on heat loss index

Rats received a subcutaneous injection of PHY or saline followed by an intraperitoneal injection of AVP V_1 receptor antagonist or scopolamine, while tail skin temperature was measured simultaneously with T_c. A thermistor probe was taped to the dorsal side of the rat's tail and positioned approximately 2.0 cm from the base of the tail. The temperature from the thermistor probe was measured with a digital meter (SN2202, Beijing Sinan Instrument, China). Tail and core temperature were used to calculate the heat loss index (HLI).

HLI was calculated according to the formula:

$$HLI = (T_{sk} - T_a) / (T_c - T_a)$$

HLI eliminates the passive effects of ambient temperature (T_a) and T_c on tail skin temperature (T_{sk}). The HLI is essentially a measure of the active, nonevaporative heat exchange attributed to peripheral vasomotor mechanisms. The value of HLI will vary from 0 to 1.0, representing states of fully vasoconstricted to fully vasodilated, respectively (Gordon et al. 2002).

AVP measurement

The effects of PHY on plasma levels of vasopressin were assessed in adult female Sprague–Dawley rats. The rats were housed individually and left undisturbed in the laboratory overnight with food and water provided ad libitum. Plasma AVP concentration was measured by a radioimmunoassay at 50 min after PHY (200 μg/kg; sc), scopolamine (1 mg/kg; sc) or saline (1 ml/kg; sc) at 10 am. Blood was drawn by cardiac puncture into heparinized syringes and stored on ice. Plasma was separated in a refrigerated centrifuge (5 °C, 4000 × g for 15 min) and stored at −30 °C until analyzed for AVP. The AVP-RIA kit was obtained from DiaSorin (Saluggia, Italy).

Cholinesterase (ChE) measurement

Plasma ChE activity was measured by a spectrophotometry using a commercially ChE-kit (Nanjing Jiancheng Bioengineering Institute, China). The blood was taken by cardiac puncture into a heparinized syringe at 50 min after administration of PHY, scopolamine or saline. The blood samples were centrifuged at 4000 × g for 15 min at 5 °C and plasma was stored at −30 °C until analyzed for ChE.