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

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Abstract

Aim: It is well known that phystostigmine (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 V1 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 V1 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 V1 antagonist and associated with elevations in plasma AVP, suggesting a novel role for AVP in the mechanism of action of anti-ChE agents.

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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 (Tc). Intravenous infusion of AVP will cause a reduction in body temperature whereas infusion of the AVP V1 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 V1 receptors that appear to affect 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 V1 receptor antagonist, suggesting that the thermoregulatory response to chlorpyrifos is mediated by central and/or systemic AVP release (Yang and Gordon 2002).

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 V1 receptor antagonist, suggesting that the thermoregulatory response to chlorpyrifos is mediated by central and/or systemic AVP release (Yang and Gordon 2002).
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-cyclopenta-methylenerpropionyl][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 (30,000 units; intramuscularly). The rats were allowed to adapt to the chamber overnight. The following day the rats were dosed with drugs at 10 am. Tail 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<sub>1</sub> receptor antagonist on PHY-induced hypothermic responses

To evaluate the AVP V<sub>1</sub> receptor antagonist on PHY-induced hypothermic responses, rats received PHY (200 μg/kg; sc) or saline, followed immediately by an injection of AVP V<sub>1</sub> 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<sub>1</sub> receptor antagonist on heat loss index

Rats received a subcutaneous injection of PHY or saline followed by an intraperitoneal injection of AVP V<sub>1</sub> receptor antagonist or scopolamine, while tail skin temperature was measured simultaneously with T<sub>a</sub>. 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:

\[ HLI = \frac{(T_a - T_a)}{(T_c - T_a)} \]

HLI eliminates the passive effects of ambient temperature (T<sub>a</sub>) and T<sub>c</sub> on tail skin temperature (T<sub>c</sub>). 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 Jiangcheng 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.

Measurement of core temperature and motor activity

Core temperature (T<sub>c</sub>) 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. Tail and motor activity of the rat were monitored by radio telemetry for at least 12 h prior to dosing.
Statistical analysis

Data were analyzed statistically by two-way ANOVA followed by multi-range (or others) test to assess differences between groups. Core temperature and motor activity data were analyzed over the 2 h period starting from the time of injection of drugs. This time period represents the time of maximum change in core temperature and motor activity for drugs. T<sub>sk</sub> and HLI data were analyzed over the 5 h period starting with the administration of drugs. Blood levels of vasopressin were analyzed using a two-tailed Student’s t-test. Values of P < 0.05 were considered to be significantly different.

Results

Effect of scopolamine on PHY-induced hypothermia

Subcutaneous injection of PHY elicited a rapid drop in core temperature that was effectively blocked by preadministration of scopolamine (Fig. 1). The rats dosed with PHY underwent a rapid drop in T<sub>c</sub>, reaching a nadir of 1.5 °C below control levels at 50 min after treatment. Core temperature recovered toward baseline levels at 180 min after dosing. Motor activity was slightly decreased by PHY treatment. Administration of scopolamine quickly reversed the hypothermic responses of PHY and led to a 0.4 °C rise in core temperature along with a marked increase in motor activity; the period of hyperthermia persisted for 3.5 h. On the other hand, scopolamine given to control animals led also to a 0.6 °C elevation in T<sub>c</sub> along with an increase in motor activity. Injections of equal volumes of saline produced no significant changes in T<sub>c</sub> and motor activity.

Plasma ChE activity was significantly reduced by PHY but not by scopolamine (Table 1). Pooling all the samples show that plasma ChE activity was significantly decreased compared to controls when measured at 50 min after exposure to 200 μg/kg PHY (p < 0.01).

Effect of AVP V<sub>1</sub> receptor antagonist on PHY-induced hypothermia

Control animals subcutaneously injected with saline or AVP V<sub>1</sub> receptor antagonist combined with intraperitoneal injection of saline underwent a transient increase in T<sub>c</sub> and motor activity that was attributed to the stress from handling and injection. The rats dosed with PHY and the AVP V<sub>1</sub> receptor antagonist did not show a hypothermic response (Fig. 2). Their T<sub>c</sub> was 1.0 °C higher than that of the rats given PHY and saline. However, the AVP V<sub>1</sub> receptor antagonist did not completely block the hypothermic effects of PHY when compared to that of rats dosed with saline. There was a gradual 0.5 °C decline in core temperature of the PHY-AVP V<sub>1</sub> group that reached a nadir at approximately the same time as animals dosed only with PHY. The AVP V<sub>1</sub> receptor antagonist had no significant effects on motor activity in either the saline or PHY treated rats.

Plasma AVP concentration increased markedly at 50 min after PHY (P < 0.05 vs saline group) whereas scopolamine had no effect (Fig. 3). Overall, rats dosed with saline, PHY and scopolamine had AVP levels of 3.2 ± 0.43, 5.1 ± 0.61 and 2.9 ± 0.52 ng/ml, respectively.

Fig. 1. Time-course of core temperature and motor activity of rats administered saline (A) or 200 μg/kg PHY (B) by a subcutaneous (sc) injection followed immediately by an intraperitoneal (ip) administration of saline or 1 mg/kg scopolamine (SCOP). Data plotted as mean ± S.E. n number of animals used for each experimental group. ANOVA analysis for saline comparing effects of saline/SCOP treatment for 180 min: core temperature (treatment, p < 0.001; treatment-time, p < 0.001); motor activity (treatment, p < 0.05; treatment-time, p < 0.01. ANOVA results for PHY: core temperature (treatment, p < 0.001; treatment-time, p < 0.001); motor activity (treatment, p < 0.001; treatment-time, p < 0.001).
Effect of PHY, scopolamine and AVP V₁ receptor antagonist on heat loss index

Conversion of the tail and core temperature data to heat loss index illustrates the marked increase in heat loss from the tail in rats treated with PHY and rapid reduction in heat loss following scopolamine or AVP V₁ receptor antagonist (Fig. 4). In animals that received PHY followed by saline, HLI increased from 0.32±0.03 to 0.51±0.04. In rats that received PHY combined followed with scopolamine or AVP V₁ receptor antagonist, HLI was reduced by over 0.19 and 0.11 at 30 min after treatment, respectively. HLI was not altered following subcutaneous or intraperitoneal injection of saline.

Discussion

The results of this study indicate a role of AVP release in the mediation of the thermoregulatory effects of PHY. This is supported by two new key findings: (1) the hypothermic effect of PHY is essentially blocked by preadministration of a AVP V₁ antagonist and (2) there was an association between a marked rise in plasma AVP and decrease in core temperature following injection of PHY. Several studies have shown that PHY stimulates AVP secretion in the rats (Rhodes et al. 2001, 2002; Rubin et al. 2006) and AVP is one of the main endogenous antipyretic molecules in the CNS (Chen et al. 1997).

Moreover, intracerebroventricular or intravenous administration of AVP leads to hypothermia (Terlouw et al. 1996; Pittman et al. 1998; Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma ChE (U/L)</th>
<th>Decrease from control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline/saline</td>
<td>369±36.2</td>
<td></td>
</tr>
<tr>
<td>PHY/saline</td>
<td>183.5±21.6*</td>
<td>49.6</td>
</tr>
<tr>
<td>Saline/scopolamine</td>
<td>391.3±57.0</td>
<td></td>
</tr>
<tr>
<td>PHY/scopolamine</td>
<td>206.2±38.5*</td>
<td>52.7</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E. *p<0.01 compared with saline/saline group.

Fig. 2. Time-course of core temperature and motor activity of rats sc administered the saline (A) or 200 μg/kg PHY (B) followed immediately by an ip administration of saline or 30 μg/kg AVP V₁ receptor antagonist (V₁-ANT). Data plotted as mean ± S.E. n number of animals used for each experimental group. ANOVA analysis for saline comparing effects of saline/V₁-ANT treatment for 120 min: core temperature (not significant); motor activity (not significant). ANOVA results for PHY: core temperature (treatment, p<0.001; treatment-time, p<0.001); motor activity (not significant).

Fig. 3. Plasma levels of vasopressin (AVP) measured 50 min after rats were dosed with saline, or 30 mg/kg PHY or scopolamine. Data are mean ± S.E. of 8 rats. *p<0.05, significant difference compared to saline group.
It is possible that PHY treatment sensitized the rats to the effects of scopolamine, because the subcutaneous administration of PHY can augment the stimulatory effects of scopolamine on motor activity.

The rat tail dissipates an equivalent of 25% of resting heat production (Young and Dawson 1982) and tail skin vasodilation is the primary mechanism of heat loss during exercise (Shellock and Richmond 1984). Our experiments showed that AVP $V_1$ receptor antagonist abolished PHY-induced hypothermia by reducing heat loss through the tail. The efficacy of the $V_1$ antagonist to block the heat loss processes of PHY clearly suggests that $V_1$ receptors are involved in mediating PHY-induced hypothermia. On the other hand, it has been reported that AVP $V_1$ receptor antagonist, when injected intraperitoneally, causes an increase in $T_r$ (Steiner et al. 1998). The effect of the AVP $V_1$ receptor antagonist on baseline $T_r$ suggests that AVP $V_1$ receptors could be involved in tonic thermoregulatory processes. The AVP $V_1$ receptor antagonist is a peripheral antagonist (Kruzyński et al. 1980), because peptide hormones such as AVP do not cross the blood-brain barrier and the polypeptide AVP $V_1$ receptor antagonist would likewise have poor penetration into the brain when administered peripherally (Deyo et al. 1986). Systemically administered AVP can bind in the circumventricular organs (Ernisch et al. 1993; Jurzak and Schmid 1998). Hence, it is possible that the effect of the $V_1$ antagonist on thermoregulation would involve certain parts of the CNS with “leaky” blood-brain barriers that are not completely insulated from the circulation.

**Conclusion**

It is thought that the hypothermic response to PHY is due to the stimulation of muscarinic cholinergic receptors in CNS thermoregulatory centers (Maickel et al. 1991; Rodrigues et al. 2004). Vasopressin release from the hypothalamic area can also be driven by cholinergic stimulation (Itake et al. 1986; Raber and Bloom 1996). The data of this study suggest that endogenous AVP could be involved in PHY-induced hypothermic processes, because PHY can increase plasma AVP and AVP $V_1$ receptor antagonist attenuated the hypothermia effect of PHY in the rat. This may be a novel mechanism of action for anti-ChE drugs such as PHY and related agents including organophosphates (Yang and Gordon 2002).

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