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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 vaso-pressin receptor antagonist [beta-Mercapto-beta, beta-cyclopenta-methylenepropionyl(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 simulta-neously 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.

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_{sk} 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_c, 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_c along with an increase in motor activity. Injections of equal volumes of saline produced no significant changes in T_c 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_1 receptor antagonist on PHY-induced hypothermia

Control animals subcutaneously injected with saline or AVP V_1 receptor antagonist combined with intraperitoneal injection of saline underwent a transient increase in T_c and motor activity that was attributed to the stress from handling and injection. The rats dosed with PHY and the AVP V_1 receptor antagonist did not show a hypothermic response (Fig. 2). Their T_c was 1.0 °C higher than that of the rats given PHY and saline. However, the AVP V_1 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_1 group that reached a nadir at approximately the same time as animals dosed only with PHY. The AVP V_1 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.
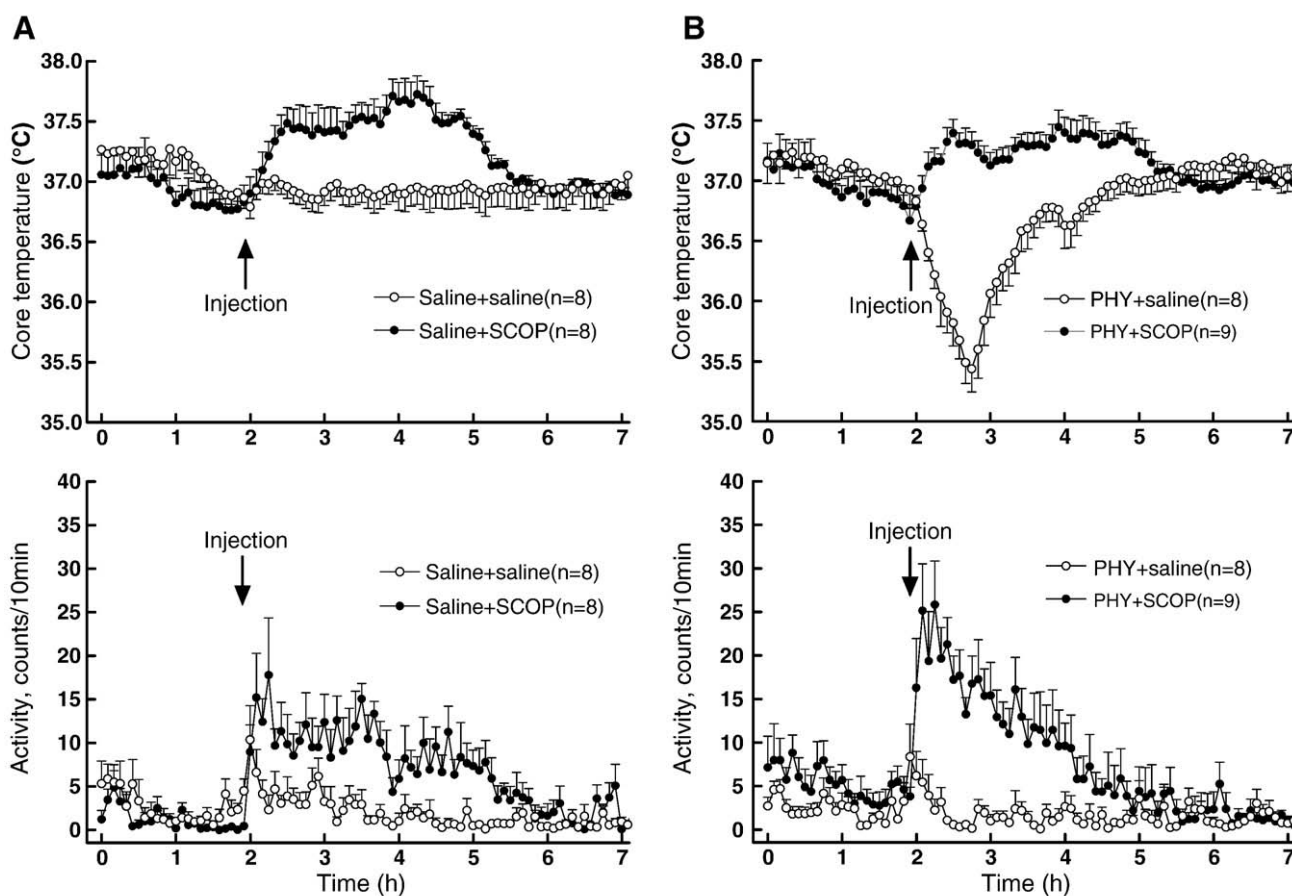

Fig. 1. Time-course of core temperature and motor activity of rats administered saline (A) or 200 μg/kg PHY (B) by a subcutaneous (sc) injection followed immediately by an intraperitoneal (ip) administration of saline or 1 mg/kg scopolamine (SCOP). Data plotted as mean ± S.E. *n* number of animals used 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, $p < 0.001$).

Table 1
Plasma cholinesterase (ChE) activity measured in rats 50 min after administration of drug combinations of saline, PHY, and scopolamine.

Groups	Plasma ChE (U/L)	Decrease from control (%)
Saline/saline (8)	369 ± 36.2	
PHY/saline (8)	$183.5 \pm 21.6^*$	49.6
Saline/scopolamine (8)	391.3 ± 57.0	
PHY/scopolamine (7)	$206.2 \pm 38.5^*$	52.7

Data represent mean $\pm$ S.E. $^*p < 0.01$ compared with saline/saline group.

Effect of PHY, scopolamine and AVP V_1 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 V_1 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 V_1 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
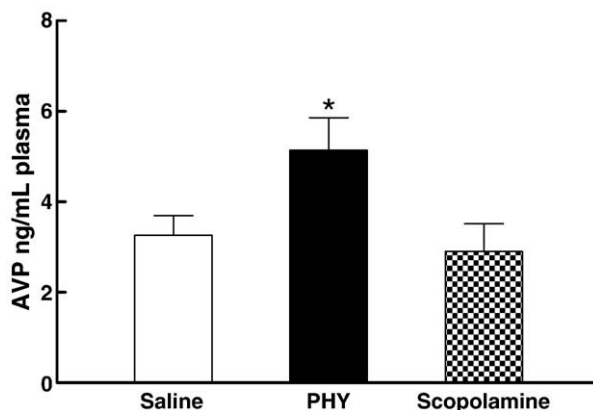

Fig. 3. Plasma levels of vasopressin (AVP) measured 50 min after rats were dosed with saline, or 30 mg/kg PHY or scopolamine. Data are mean $\pm$ S.E. of 8 rats. $^*p < 0.05$, significant difference compared to saline group.

two new key findings: (1) the hypothermic effect of PHY is essentially blocked by preadministration of a AVP V_1 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;
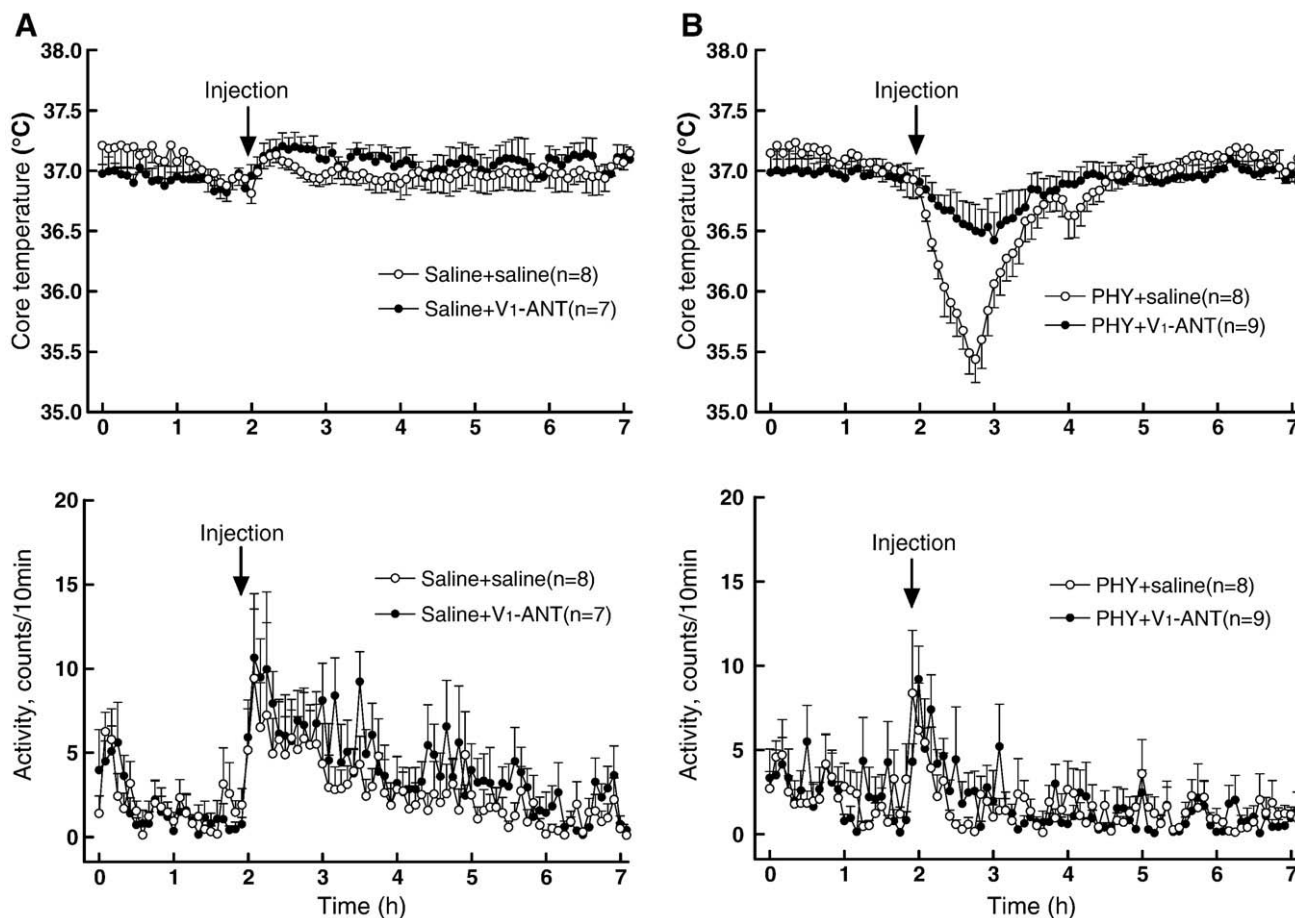

Fig. 2. Time-course of core temperature and motor activity of rats sc administered the saline (A) or 200 μg/kg PHY (B) followed immediately by an ip administration of saline or 30 μg/kg AVP V_1 receptor antagonist (V_1-ANT). Data plotted as mean $\pm$ S.E. n number of animals used for each experimental group. ANOVA analysis for saline comparing effects of saline/V_1-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).
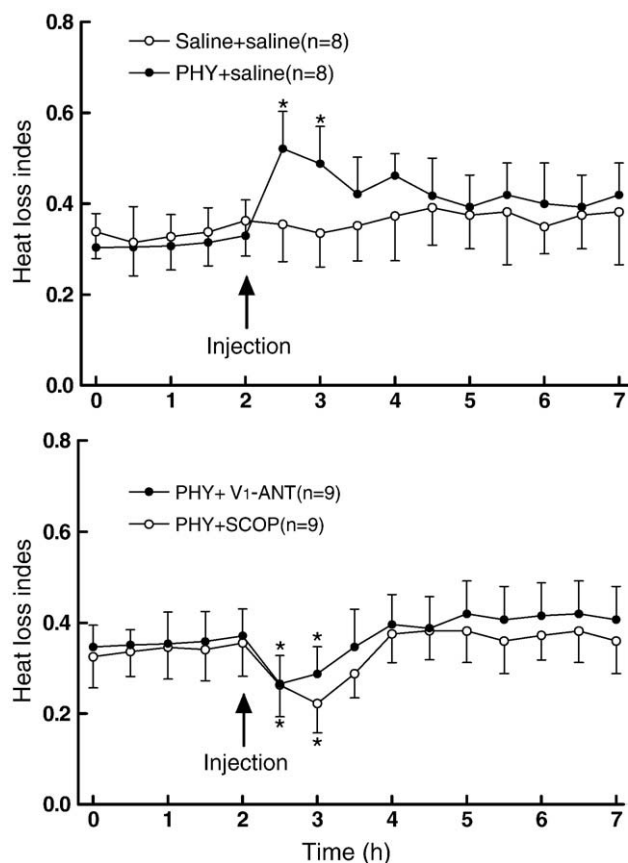
Fig. 4. Time-course of heat loss index (HLI) of rats administered the saline or PHY followed immediately by an administration of saline, scopolamine (SCOP) or AVP V$_1$ receptor antagonist (V$_1$-ANT). Data plotted as mean ± S.E. n number of animals used for each experimental group. *$p < 0.05$, **$p < 0.01$, significant difference compared to saline group.

Richmond 2003). In addition, preoptic administration of AVP induces grooming, a behavior that increases heat loss and may play a role in the thermoregulatory response to AVP (Lumley et al. 2001).

The data indicate that PHY-induced hypothermia in the rat is accompanied by an increase in HLI. This corroborates the results of past studies showing that acute exposure to anti-ChE agents elicits an increase in heat loss from the tail of the rat (for review, see Gordon 2006). The vasodilatory effect of PHY was blocked by administration of scopolamine, a central muscarinic antagonist which penetrates through the blood-brain barrier. Previous studies have reported that, in conscious rats, injection of PHY into the lateral cerebral ventricle produces hypothermic effects brought by both decreased metabolic heat production and increased cutaneous vasodilation during rest and exercise (Fehlner and Gordon 1985; Maickel et al. 1991; Rodrigues et al. 2004; Pires et al. 2007). Overall, it appears that the PHY induces a regulated hypothermic response (i.e., reduction in set-point) as a result of its peripheral and central inhibition of ChE and subsequent cholinergic stimulation. Regulated hypothermia is indicated from evidence that thermoeffectors are activated to increase heat loss and suppress heat production, resulting in a reduction in body temperature. This provides further evidence that the hypothermic response to PHY is regulated by thermoregulatory centers involving muscarinic pathways (Rodrigues et al. 2004; Pires et al. 2007).

It is interesting to note that administration of scopolamine quickly reversed the hypothermic effect of PHY, and increased the motor activity. Scopolamine given to control animals also led to an elevation in T_c along with an increase in motor activity. Scopolamine has been shown to increase motor activity in many species, including rats (Sipos et al. 1999; Bertrand et al. 2001). The effect of scopolamine on

motor activity seemed to be more pronounced in rats dosed with PHY. 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 Rubin 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_c (Steiner et al. 1998). The effect of the AVP V$_1$ receptor antagonist on baseline T_c suggests that AVP V$_1$ receptors could be involved in tonic thermoregulatory processes. The AVP V$_1$ receptor antagonist is a peripheral antagonist (Kruzynski 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 (Ermisch 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 (Iitake 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 V1 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).

References

Bertrand F, Lehmann O, Galani R, Lazarus C, Jeltsch H, Casse JC. Effects of MDL 73005 on water maze performances and locomotor activity in scopolamine-treated rats. Pharmacology Biochemistry and Behavior 68, 647–660, 2001.

Chen X, Landgraf R, Pittman QJ. Differential ventral septal vasopressin release is associated with sexual dimorphism in PGE$_2$ fever. American Journal of Physiology 272, R1664–R1669, 1997.

Deyo SN, Shoemaker WJ, Ettenberg A, Bloom FE, Koob GF. Subcutaneous administration of behaviourally effective doses of arginine vasopressin results in brain uptake in only the median eminence. Neuroendocrinology 42, 260–266, 1986.

Dong J, Xie XH, Lu DX, Fu YM. Effects of electrical stimulation of ventral septal area on firing rates of pyrogen treated thermosensitive neurons in preoptic anterior hypothalamus from rabbits. Life Sciences 80, 408–413, 2007.

Ermisch A, Brust P, Kretzschmar R, Rühle H. Peptides and blood-brain barrier transport. Physiological Reviews 73, 489–527, 1993.

Fehlner KS, Gordon CJ. Effect of ambient temperature on thermoregulation in rats following preoptic–anterior hypothalamic injection of physostigmine. Neuropharmacology 24, 993–997, 1985.

Gordon CJ. Temperature regulation in experimental mammals and humans exposed to organophosphate and carbamate agents. In: Gupta, RC (Ed.), Toxicology of Organophosphate and Carbamate Compounds. Academic Press, San Diego, pp. 549–566, 2006.

Gordon CJ, Puckett E, Padnos B. Rat tail skin temperature monitored noninvasively by radiotelemetry: characterization by examination of vasomotor responses to thermomodulatory agents. Journal of Pharmacological and Toxicological Methods 47, 107–114, 2002.

Iitake K, Share L, Ouchi Y, Crofton JT, Brooks DP. Central cholinergic control of vasopressin release in conscious rats. The American Journal of Physiology 251, E146-El50, 1986.

Jurzak M, Schmid HA. Vasopressin and sensory circumventricular organs. Progress in Brain Research 119, 221-245, 1998.

Kruzynski M, Lammek B, Manning M, Seto J, Haldar J, Sawyer WH. [l-(β-mercapto-β, β-cyclopentamethylenepro-pionic acid), 2-(O-methyl)tyrosine] arginine vasopressin and [l-(-mercapto-β, β-cyclopentamethylenepropionic acid)-arginine vasopressin, two highly potent antagonists of the vasopressor respons to arginine vasopressin. Journal of Medicinal Chemistry 23, 364–368, 1980.

Lumley LA, Robison CL, Chen WK, Mark B, Meyerhoff JL. Vasopressin into the preoptic area increases grooming behavior in mice. Physiology & Behavior 73, 451–455, 2001.

Mach M, Grubbs RD, Price WA, Paton SJ, Lucot JB. Behavioral changes after acetylcholinesterase inhibition with physostigmine in mice. Pharmacology Biochemistry and Behavior 79, 533–540, 2004.

Maickel RP, Kinney DK, Ryker DL, Nichols MB. Antagonism of physostigmine induced hypothermia and neuroendocrine changes following exposure to different environmental temperatures. Progress in Neuro-Psychopharmacology & Biological Psychiatry 15, 873–884, 1991.

Michels KM, Meeker RB, Hayward JN. Muscarinic cholinergic control of vasopressin secretion from the acute hypothalamoneurohypophysial explant. Neuroendocrinology 54, 219–226, 1991.

Paro FM, Almeida MC, Carnio EC, Branco LGS. Role of ʟ-glutamate in systemic AVP-induced hypothermia. Journal of Applied Physiology 94, 271–277, 2003.

Pires W, Wanner SP, La Guardia RP, Rodrigues LOC, Silveira SA, Coimbra CC, Marubayashi U, Lima NRV. Intracerebroventricular physostigmine enhances blood pressure and heat loss in running rats. Journal of Physiology and Pharmacology 58, 3–17, 2007.

Pittman QJ, Wilkinson MF. Central arginine vasopressin and endogenous antipyresis. Canadian Journal of Physiology and Pharmacology 70, 786–790, 1992.

Pittman QJ, Chen X, Mouihate A, Hirasawa M, Martin S. Arginine vasopressin, fever and temperature regulation. Progress in Brain Research 119, 383–392, 1998.

Raber J, Bloom FE. Arginine vasopressin release by acetylcholine or norepinephrine: region-specific and cytokine-specific regulation. Neuroscience 71, 747–759, 1996.

Rhodes ME, O'Toole SM, Wright SL, Czambel RK, Rubin RT. Sexual diergism in rat hypothalamic pituitary adrenal axis responses to cholinergic stimulation and antagonism. Brain Research Bulletin 54, 101–113, 2001.

Rhodes ME, Balestreire EM, Czambel RK, Rubin RT. Estrous cycle influences on sexual diergism of HPA axis responses to cholinergic stimulation in rats. Brain Research Bulletin 59, 217–225, 2002.

Richmond CA. The role of arginine vasopressin in thermoregulation during fever. The Journal of Neuroscience Nursing 35, 281–286, 2003.

Rodrigues AG, Lima NRV, Coimbra CC, Marubayashi U. Intracerebroventricular physostigmine facilitates heat loss mechanisms in running rats. Journal of Applied Physiology 97, 333–338, 2004.

Rubin RT, Rhodes ME, Miller TH, Jakab RL, Czambel RK. Sequence of pituitary-adrenal cortical hormone responses to low-dose physostigmine administration in young adult women and men. Life Sciences 79, 2260–2268, 2006.

Ryan PM, Kelly JP, Chambers PL, Leonard BE. The characterization of oxotremorine-induced hypothermic response in the rat. Pharmacology & Toxicology 79, 238–240, 1996.

Shellock FG, Rubin SA. Temperature regulation during treadmill exercise in the rat. Journal of Applied Physiology 57, 1872–1877, 1984.

Shido O, Kifune A, Nagasaka T. Baroreflexive suppression of heat production and fall in body temperature following peripheral administration of vasopressin in rats. The Japanese Journal of Physiology 34, 397–406, 1984.

Sipos ML, Burchnell V, Galbicka G. Dose–response curves and time-course effects of selected anticholinergics on locomotor activity in rats. Psychopharmacology (Berlin) 147, 250–256, 1999.

Steiner AA, Carnio EC, Antunes-Rodrigues J, Branco LGS. Role of nitric oxide in systemic vasopressin-induced hypothermia. The American Journal of Physiology 275, R937–R941, 1998.

Terlouw EM, Kent S, Cremona S, Dantzer R. Effect of intracerebroventricular administration of vasopressin on stress-induced hyperthermia in rats. Physiology & Behavior 60, 417–424, 1996.

Yang YL, Gordon CJ. Possible role of vasopressin in the thermoregulatory response to chlorpyrifos in the rat. Pharmacology & Toxicology 90, 311–316, 2002.

Young AA, Dawson NJ. Evidence for on-off control of heat dissipation from the tail of the rat. Canadian Journal of Physiology and Pharmacology 60, 392–398, 1982.