Possible Role of Vasopressin in the Thermoregulatory Response to Chlorpyrifos in the Rat

Yong Lu Yang and Christopher J. Gordon

Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, U.S.A.

(Received August 9, 2001; Accepted January 17, 2002)

Abstract: Arginine vasopressin is a naturally occurring antipyretic which is released into the CNS to prevent excessive elevations in body temperature during fever. Circulating levels of arginine vasopressin may also have a role in the tonic control of body temperature. We have found that the organophosphate insecticide chlorpyrifos will raise blood pressure and lower body temperature in the rat. Because arginine vasopressin is a potent hypertensive agent and is capable of lowering core temperature, we suspected that arginine vasopressin may be involved in the thermoregulatory response to chlorpyrifos. To this end, core temperature and motor activity of male and female Sprague-Dawley rats were monitored before and after treatment with the corn oil vehicle or chlorpyrifos (15 mg/kg in females; 30 mg/kg in males; oral) concomitant with injection of a saline vehicle or a type 1 arginine vasopressin antagonist (20 μg/kg in females; 30 μg/kg in males; intraperitoneally). Rats dosed with chlorpyrifos and saline underwent a 2–3°C reduction in core temperature >50% decrease in motor activity. The V1 antagonist attenuated the hypothermic effect of chlorpyrifos in both sexes. Chlorpyrifos-induced inhibition in motor activity was unaffected by the V1 antagonist. In another experiment, the V1 antagonist (30 μg/kg) was co-administered with saline or 0.2 mg/kg oxotremorine, a muscarinic agonist that stimulates a heat loss response and partially mimics the effects of chlorpyrifos. The V1 antagonist attenuated the hypothermic effect of oxotremorine in both sexes. Plasma arginine vasopressin levels were determined in male rats 3 hr after corn oil or 30 mg/kg chlorpyrifos. There was no significant effect of chlorpyrifos on plasma levels of arginine vasopressin. That the V1 antagonist blocked the hypothermic effect of chlorpyrifos suggests that the thermoregulatory response to chlorpyrifos is mediated by central and/or systemic vasopressin release. The lack of a significant increase in plasma vasopressin after chlorpyrifos suggests that localized release of vasopressin may be involved in the thermoregulatory response to chlorpyrifos.

Vasopressin is one of many peptides that has been shown to be involved in the control of body temperature. It has also been shown to be critical in antipyresis, or the recovery of body temperature following a fever (Wilkinson and Kasting 1987; Kasting 1989; Pittman & Wilkinson 1992). Central infusion of vasopressin to the febrile rat elicits a marked antipyretic response (i.e., decrease in set-point) associated with a decrease in heat production, increase in heat loss, and subsequent lowering of core temperature. On the other hand, the same antipyretic dose of vasopressin administered to the afebrile rat has little effect on baseline core temperature (Kasting, 1989). During fever, vasopressin is released into the ventral septal area and binds to V1 type vasopressin receptors that appear to drive heat loss responses (Pittman & Wilkinson 1992). Vasopressin may also be involved in other types of thermoregulatory responses. For example, intravenous infusion of vasopressin will cause a reduction in body temperature whereas infusion of the V1 receptor antagonist leads to an elevation in temperature (Steiner et al. 1998). Central infusion of arginine vasopressin also reduces the magnitude of psychological stress-induced hyperthermia, a response akin to that of a fever (Terlouw et al. 1996). Central nitric oxide release has been shown to be operative in the mediation of vasopressin-induced hypothermia in the rat (Steiner et al. 1998).

We have found that the organophosphate insecticide chlorpyrifos affects body temperature and blood pressure regulation. Oral dosing with chlorpyrifos leads to a transient period of hypothermia followed by a delayed fever that persists for several days after exposure (Gordon et al. 1997). In addition, our laboratory has also shown that chlorpyrifos leads to a prolonged elevation in blood pressure in the rat (Gordon & Padnos 2000). Blood pressure remains elevated for 24 hr or more following oral exposure to 10 and 25 mg/kg chlorpyrifos. The elevated pressure persists in spite of normal heart rate and minimal changes in body temperature.

Organophosphate insecticides irreversibly inhibit acetylcholinesterase activity, leading to cholinergic stimulation of the CNS and peripheral tissues (Ballantyne & M arrs 1992). Since cholinergic stimulation of the hypothalamic area has been shown to elicit release of vasopressin (Gregg 1985; Raber et al. 1994), one might expect that exposure to a cholinesterase inhibitor such as chlorpyrifos could lead to vasopressin release. Considering the evidence that vasopressin can mediate a hypothermic response in the febrile and afebrile rat, we hypothesize that it could be involved in the mediation of the thermoregulatory and other physio-
logical responses to chlorpyrifos and possibly other organophosphate insecticides. The purpose of this study was to assess if pre-administration of an vasopressin antagonist blocks the thermoregulatory effects of chlorpyrifos in the male and female rat.

Materials and Methods

Animals used in this study were male and female rats of the Sprague-Dawley strain obtained from Charles River Laboratories (Raleigh, NC, USA). The rats were obtained at 60 days of age and housed individually at a $T_a$ of 22°C, a relative humidity of 50%, and a 12:12 light:dark photoperiod.

Core temperature and motor activity were monitored in undisturbed rats using radiotelemetry (Data Sciences International, St. Paul, M N, U.S.A). Details of the telemetry system have been published (Gordon et al. 1997). Briefly, rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.) and an abdominal incision was made for the implantation of the transmitter (TA10TA-F40) into the abdominal cavity. The abdominal muscle was sutured and the skin was closed with wound clips. Following surgery, rats were administered a penicillin antibiotic (30,000 units; intramuscularly) and analgesic (buprenorphine; 0.03 mg/kg; subcutaneously). The rats were allowed at least 10 days of recovery before testing.

Protocol. Chlorpyrifos (>99% purity) was obtained from Chem Services (West Chester, PA, U.S.A). The vasopressin blocker [beta-mercapto-beta-cyclopentamethyleneglycine]1.0-Et-Tyr2,Val4, Arg8vasopressin] was obtained in 100 mg aliquots from Sigma (St. Louis, MO, U.S.A). It was dissolved in sterile saline into a stock solution of 100 μg/ml and stored at −22°C until the day of an experiment. Core temperature and motor activity of male and female rats were monitored by radio telemetry for at least 24 hr prior to dosing. Several experiments were performed in each sex to assess the effects of the V1 antagonist on the thermoregulatory response to chlorpyrifos.

Experiment 1. Male rats were administered the corn oil vehicle or 30 mg/kg chlorpyrifos by oral gavage (0.1 ml/100 g body weight) at 3 p.m. Immediately after the corn oil/chlorpyrifos treatment the rats were dosed intraperitoneally with the saline vehicle or 30 μg/kg V1 antagonist (0.1 ml/100 g body weight). Note that Steiner et al. (1998) reported that intraperitoneal doses of 10 and 40 μg/kg of the V1 antagonist are effective for altering rat body temperature. The temperature data were monitored for at least 48 hr after dosing. Twelve rats were tested at one time in each experiment; each treatment was assigned randomly to 3 of 12 rats. The rats were allowed to recover for at least 10 days from this treatment and then they were subjected to a cross-over design where rats that initially had been dosed with corn oil were given chlorpyrifos and rats that had been given chlorpyrifos were dosed with corn oil.

Experiment 2. Female rats were administered the corn oil vehicle or 15 mg/kg chlorpyrifos by oral gavage (0.1 ml/100 g body weight) at 3 p.m. Immediately after the corn oil/chlorpyrifos treatment the rats were dosed intraperitoneally with the saline vehicle or 20 μg/kg V1 antagonist (0.1 ml/100 g body weight). The lower dose of chlorpyrifos was used in female rats because of their increased sensitivity to this insecticide (Gordon et al. 1997). A hypothermic effect of 30 mg/kg body weight was observed in male rats but not detected in females.

Experiment 3. The effect of the V1 antagonist on the hypothermic response to the muscarinic agonist oxotremorine was assessed in male and female rats. Rats were implanted with radiotransmitters as described earlier and allowed to recover for at least 10 days. A core temperature and motor activity were recorded for at least 24 hr. The rats were injected at 10 a.m. subcutaneously with saline or 0.2 mg/kg oxotremorine dissolved in a solution of 1.0 mg/ml methyl scopolamine (0.1 ml/100 g body weight). The methyl scopolamine (nitrate salt; Sigma) was given to block the peripheral cholinergic effects of oxotremorine. Immediately after oxotremorine the rats were injected intraperitoneally with saline or 30 μg/kg V1 antagonist. The rats were returned to their cages and monitored for at least 24 hr. The rats were subjected to a cross-over design as described above.

Experiment 4. The effects of chlorpyrifos on plasma levels of vasopressin were assessed in 90-day-old male rats. The rats were housed individually and left undisturbed in the laboratory overnight with food and water provided ad libitum. At 10 a.m. the rats were gavaged with either the corn oil vehicle or 30 mg/kg chlorpyrifos. Three hr after dosing the rats were terminated with a commercial cervical dislocating device (Cervical Dislocators, Inc., Schofield, WI, U.S.A). Care was taken to terminate rats quickly with little stress or disturbance to the remaining animals to be tested. Blood was drawn by cardiac puncture into heparinized syringes and stored on ice. The plasma was separated in a refrigerated centrifuge (5°C; 4000×g for 15 min.). Vasopressin levels were measured by radioimmunoassay according to the methods of Matsuguchi et al. (1981).

Analysis. Core temperature and motor activity data were averaged into 60 min. bins for the chlorpyrifos study and 30 min. bins for the oxotremorine study. The core temperature data were analyzed for statistical significance using a two-way repeated measures ANOVA. For the chlorpyrifos experiments, the temperatures were analyzed over a 12 hr period beginning immediately after dosing. This time period represents the approximate time for animals to remain hypothermic following chlorpyrifos treatment (Gordon et al. 1997). For the oxotremorine experiments, the temperature data were analyzed over a 90 min. period which represents the time of maximum change in core temperature for this drug. Motor activity is generally suppressed during the night following dosing with chlorpyrifos (Gordon et al. 1997; Gordon, 1997). Thus, the motor activity data were averaged over the entire dark phase following injection of chlorpyrifos. These data were then analyzed for effects of chlorpyrifos and V1 antagonist using a two-way ANOVA. Blood levels of vasopressin were analyzed using a two-tailed Student’s t-test.

Results

Aministration of the corn oil vehicle and chlorpyrifos led to a transient elevation in core temperature that was attributed to the handling and injection procedure (fig. 1). While motor activity returned to near baseline levels, core temperature of the control groups (corn oil-saline and corn oil-V1 antagonist) remained elevated as the nocturnal phase approached. There was a marked elevation in core temperature of the corn oil-V1 antagonist group at −6 p.m. Otherwise, the saline and V1 antagonist given to the corn oil controls had similar effects on core temperature. Following the transient increase in core temperature, rats dosed with chlorpyrifos and saline became markedly hypothermic for approximately 12 hr. On the other hand, rats dosed with chlorpyrifos and given the V1 antagonist did not become hypothermic (fig. 1A). Their core temperature was 1.5°C higher than that of the rats given chlorpyrifos and saline; however, the V1 antagonist did not completely block the hypothermic effects of chlorpyrifos when compared to that of rats dosed with corn oil. Motor activity during the first night was significantly suppressed in rats dosed with chlorpyrifos (fig. 1B). The V1 antagonist had no effect on the chlorpyrifos-induced suppression in activity.
Fig. 1A. Time-course of core temperature of male rats administered the corn oil vehicle or 30 mg/kg chlorpyrifos by oral gavage followed immediately by a subcutaneous administration of saline or 30 μg/kg V1 antagonist. Repeated measures ANOVA results assessing effect of saline and V1 antagonist treatment for 12 hr after injection: corn oil, treatment, not significant (NS); treatment×time, NS; chlorpyrifos, treatment, F(1,10)=6.2, P=0.03; treatment×time, NS. Horizontal bars indicate periods of darkness. 1B. Mean levels of motor activity during the first night following administration of corn oil/chlorpyrifos and the saline/V1 antagonist treatments in male rats. ANOVA: chlorpyrifos treatment, F(1,1)=11.2, P=0.003; V1 treatment, NS; interaction, NS. * P<0.05; refers to significant difference when compared to corn oil group.

Fig. 2A. Time-course of core temperature of female rats administered the corn oil vehicle or 15 mg/kg chlorpyrifos by oral gavage followed immediately by a subcutaneous administration of saline or 20 μg/kg V1 antagonist. Repeated measures ANOVA results assessing effect of saline and V1 antagonist treatment for 12 hr after injection: corn oil, treatment, NS; treatment×time, NS; chlorpyrifos, treatment, F(1,24)=5.4, P=0.028; treatment×time, F(23, 552)=2.4, P=0.0002. 2B. Mean levels of motor activity during the first night following administration of corn oil/chlorpyrifos and the saline/V1 antagonist treatments in female rats. ANOVA: chlorpyrifos treatment, F(1,1)=42.3, P<0.0001; V1 treatment, NS; interaction, F(1,30)=8.4, P=0.006). * P<0.05, ** P<0.01 refers to significant difference when compared to corn oil group. *** P<0.01 when compared to corn oil/saline group.
The pattern of core temperature of female rats dosed with corn oil and then given saline or the V1 antagonist was similar to that of male rats (fig. 2A). Core temperature and motor activity increased transiently following injection. Core temperature started to recover then increased abruptly with the dark phase. As observed in male rats, the higher core temperature of rats given the V1 antagonist at the start of the dark phase was also seen in the females. The V1 antagonist blocked the hypothermic effects of chlorpyrifos in female rats in a similar pattern as was seen in males (fig. 2A). Rats dosed with chlorpyrifos and saline underwent a rapid drop in core temperature, reaching a nadir of 36.2°C at 6 hr after dosing with recovery over then next 12 hr. On the other hand, core temperature decreased to only 37.2°C by 6 hr after treatment with chlorpyrifos and the V1 antagonist. As with the male rats, the female rats treated with chlorpyrifos and the V1 antagonist failed to exhibit the normal nocturnal rise in core temperature during the first night after dosing. That is, the V1 antagonist did not completely block the hypothermic effects of chlorpyrifos. The V1 antagonist had no effect on chlorpyrifos-induced reductions in motor activity during the first night after dosing (fig. 2B). However, in rats dosed with corn oil, the V1 antagonist led to a significant increase in activity during the night after dosing.

Oxotremorine administered with methyl scopolamine led to a rapid reduction in core temperature of male and female rats (fig. 3A, B). The duration of the hypothermic response was only 2 hr. The V1 antagonist affected the hypothermic effects of oxotremorine in both male and female rats. In both sexes the V1 antagonist reduced the maximum hypothermic effect of oxotremorine by 0.5°C. The handling and injection of the control vehicle led to a transient elevation in core temperature that persisted for approximately 2 hr. Interestingly, the V1 antagonist resulted in a higher core temperature of the control rats approximately 2 hr after injection. At a time when the temperature of the rats receiving two injections of saline was returning to baseline, the core temperature of the saline-V1 antagonist group remained elevated. The V1 antagonist had no significant effects on motor activity in either the saline- or oxotremorine-treated rats (data not shown).

Administering 30 mg/kg chlorpyrifos to male rats had
Oil and chlorpyrifos had vasopressin levels of 2.4 ng/ml measured 3 hr after treatment (Fig. 4). Rats dosed with corn oil had vasopressin levels of 2.4±0.56 and 3.15±0.56 ng/ml, respectively.

Discussion

The data suggest that vasopressin is involved in mediating the hypothermic effects of chlorpyrifos in the male and female rats. Administering the V1 antagonist at the same time as dosing rats with chlorpyrifos led to a marked attenuation in the hypothermic effects of chlorpyrifos. However, the V1 antagonist had no effect on chlorpyrifos-induced reductions in motor activity, corroborating another study showing that organophosphate-induced reductions in motor activity are independent of changes in body temperature (Gordon et al. 1997). The V1 antagonist also attenuated the hypothermic effect of the muscarinic antagonist, oxotremorine. However, the effect of the antagonist on oxotremorine was not nearly as profound as the effect on the chlorpyrifos-induced hypothermia. The experiments with the V1 antagonist suggest that muscarinic stimulation of the CNS and the subsequent activation of heat loss processes involves the vasopressin system. On the other hand, plasma levels of vasopressin measured 3 hr after administration of chlorpyrifos were slightly but not statistically higher than that of rats given corn oil. This would suggest that the role of vasopressin in the mediation of the thermoregulatory effects of chlorpyrifos could involve subtle elevations in vasopressin in the peripheral tissues and possibly, the CNS.

The V1 antagonist administered peripherally would be expected to have little if any penetration into the CNS. Peptide hormones such as vasopressin do not cross the blood brain barrier and the polypeptide V1 antagonist would likewise have poor penetration into the brain when administered peripherally (Deyo et al. 1986). Systemically administered vasopressin can bind in the circumventricular organs (Ermisch et al. 1993; Jurzak & Schmid 1998). Hence, it is possible that the effect of the V1 antagonist on thermoregulation would involve certain parts of the CNS with “leaky” blood brain barriers that are not completely insulated from the circulation. On the other hand, vasopressin and its antagonism by the V1 antagonist may affect CNS function indirectly through alterations in blood pressure and cardiovascular tone. Systemic administration of relatively high doses of vasopressin that elevate blood pressure results in EEG arousal in the rat (Ebenezer 1994). This effect is blocked by peripheral administration of the V1 antagonist, suggesting that the vasopressin effects on the EEG are mediated by peripheral V1 receptors. A similar scenario could also occur following chlorpyrifos administration where subtle changes in vasopressin outside of the CNS are affected by systemic administration of the V1 antagonist.

Central and peripheral injections of vasopressin can elicit hyperthermia in the rat and the response that is blocked by the V1 antagonist; however, the effect appears to be most pronounced when the animal is stressed (Naylor et al. 1986; Steiner et al. 1998 & 1999). Because chlorpyrifos elevates blood pressure by cholinergic stimulation of the CNS, we reasoned that vasopressin release could be operative in the development of chlorpyrifos-induced hypertension. Moreover, in view of the hypothermic effects of vasopressin, it was thought that it could be involved in the hypothermic effects of chlorpyrifos. Chlorpyrifos had no significant effect on plasma vasopressin levels when measured 3 hr after exposure. On the other hand, the efficacy of the V1 antagonist to block the hypothermic effects of chlorpyrifos clearly suggests that V1 receptors are involved in mediating chlorpyrifos-induced hypothermia. It is possible that the sampling period of 3 hr after exposure was not the ideal time for assessing arginine vasopressin levels after chlorpyrifos exposure.

Steiner et al. (1998) found that the V1 antagonist injected intraperitoneally at a dose of 10 and 40 µg/kg led to a hyperthermic response when measured with a colonic probe one hour after injection. We also found that the control (corn oil) rats treated with V1 antagonist underwent a transient increase in core temperature persisting for about 90 min. at the start of the dark phase. We did not see a significant difference in core temperature one hour after injection because the core temperature of all control and treated groups was elevated by nearly 1.0° following the handling and injection procedures. The effects of the V1 antagonist on baseline core temperature suggests that V1 receptors could be involved in tonic thermoregulatory processes. It is also interesting to note that several studies have found that the V1 antagonist does not block hypoxic-induced hypothermia in the rat (Clark & Fewell 1994; Steiner et al., 1999). That cholinergic stimulation is critical in the chlorpyrifos-induced but not hypoxic-induced hypothermia suggests that cholinergic-induced vasopressin release is operative in the former but not the latter.

Although blood pressure was not measured in this study, we can assume that blood pressure was elevated by at least 25 mmHg at the time of the peak hypothermic effect of
chlorpyrifos treatment (Gordon & Padnos 2000). It follows that the V1 antagonist may have affected this blood pressure response which, in turn, could alter the thermoregulatory effects of chlorpyrifos. Shido et al. (1984) showed that the hypothermic effects of intravenous injection of vasopressin was attenuated following sino-aortic deafferentation. It was concluded that the hypertension resulting from intravenous vasopressin caused a suppression in non-shivering thermogenesis via activation of the sino-aortic baroreceptors. In our study, we can expect that injection of the V1 antagonist would have suppressed the hypertensive response of chlorpyrifos which would possibly have reduced the hypothermic effects. However, the decrease in core temperature following chlorpyrifos is marked and would not be expected to occur as a result of suppression of non-shivering thermogenesis. Indeed, we have found that chlorpyrifos mediates a hypothermic response through activation of heat dissipating thermoeffectors, including vasodilation of the tail and behavioral preference for cool ambient temperatures (Gordon 1997; Gordon & Yang, 2000). It remains to be shown if the hypertensive effects of chlorpyrifos are somehow related to these complex thermoregulatory processes.

Organophosphate insecticides cause cholinergic stimulation inside and outside of the CNS as a result of the irreversible inhibition of acetylcholinesterase activity (Ballantyne & Maris 1992). It is thought that the hypothermic response to organophosphate insecticides is due to the stimulation of muscarinic cholinergic receptors in CNS thermoregulatory centers (Gordon 1997). Vasopressin release from the hypothalamic area can also be driven by cholinergic stimulation (Gregg 1985; Raber et al. 1994). We do not know if the role of vasopressin on the thermoregulatory response to chlorpyrifos is due to a direct neuromodulator role of the peptide or to an indirect role such as through the modulation of blood pressure (see above). Nonetheless, the data of this study provide evidence for a vasopressin-mediated control of chlorpyrifos-induced hypothermia, a novel mechanism of action for an organophosphate insecticide.

Acknowledgements

We thank D. B. Farley of the College of Pharmacy, University of Iowa, for performing the vasopressin analysis. We also thank Dr. A. M. Rezvani and Robert Carroll for their review of the manuscript.

This paper has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

References


