Peripheral cholinergic pathway modulates hyperthermia induced by stress in rats exposed to open-field stress

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ROWSEY, Pamela Johnson, Yong-Lu Yang, and Christopher J. Gordon. Peripheral cholinergic pathway modulates hyperthermia induced by stress in rats exposed to open-field stress. J Appl Physiol 92: 789–794, 2002. First published October 19, 2001; 10.1152/japplphysiol.00240.2001.—Exposure to an open field is psychologically stressful and leads to an elevation in core temperature (Tc). Methyl scopolamine (MS), a muscarinic antagonist, and pyridostigmine (PYR), a carbamate that inhibits acetylcholinesterase, do not cross the blood-brain barrier and have little effect on Tc in resting, nonstressed animals. However, we have found that MS has an antipyretic effect on Tc that is caused by handling and cage-switch stress. PYR should act pharmacologically to reverse the effects of MS. To this end, we assessed the effects of MS and PYR on stress-induced hyperthermia. Male Sprague-Dawley rats at 90 days of age were housed individually at an ambient temperature of 22°C. Tc and motor activity were monitored by radiotelemetry in an open-field chamber. Rats were dosed intraperitoneally at 1200 with 1.0 mg/kg MS, 0.1 mg/kg PYR, a combination of MS and PYR, or saline and placed immediately inside the open-field chamber for 60 min. Stress-induced hyperthermia was suppressed immediately by MS and enhanced by PYR. Tc only increased by 0.3°C in the MS-treated animals. The hyperthermic response in the PYR group was nearly 0.6°C above that of rats dosed with saline. Co-administration of PYR and MS led to a stress-induced hyperthermia response nearly identical to that of rats injected with saline. Overall, open-field stress exacerbated the effects of MS and PYR on body Tc, and provides support for a peripheral cholinergic mechanism that mediates stress-induced hyperthermia.

body temperature; fever; chlorpyrifos; circadian rhythm; handling

ONE RESPONSE TO PSYCHOLOGICAL stress is an elevation in core temperature termed stress-induced hyperthermia. A portion of this elevation in core temperature can be blocked with cyclooxygenase inhibitors, such as sodium salicylate (3, 17) and indomethacin (5). The rise in core temperature encountered by a rat placed in a novel environment (open-field paradigm) provides a measure of the stress it encounters. Because prostaglandin inhibitors block part of this stress-induced rise in core temperature, it has been hypothesized that this increase in core temperature is a type of fever mediated by central nervous system (CNS) thermoregulatory centers (17).

Our laboratory found that a peripheral cholinergic pathway may be operative in the modulation of stress-induced hyperthermia. Methyl scopolamine, a peripheral muscarinic antagonist, does not cross the blood-brain barrier but exhibited antipyretic properties under certain conditions when body temperature was elevated (16). Administration of methyl scopolamine (1 mg/kg ip) resulted in a rapid recovery of core temperature in rats exposed to chlorpyrifos, an organophosphate pesticide, which causes a delayed increase in core temperature 1 day after exposure (7). We also reported that methyl scopolamine blocked the stress-induced increase in core temperature associated with cage switch and handling (16). These data indicate that a peripheral cholinergic pathway may be operative in the febrile response to chlorpyrifos and stress.

As has been shown in other types of stress, methyl scopolamine should block the hyperthermic response to open-field stress (16). If this marked reversal in core temperature during open-field stress is occurring through cholinergic-receptor activation, then stimulating these receptors should enhance this response. Because of its low toxicity, pyridostigmine is an ideal drug to test the reversible effects of methyl scopolamine. Thus the purpose of this study is to determine the effectiveness of methyl scopolamine in blocking open-field-induced hyperthermia and whether or not the effects of methyl scopolamine can be reversed by pyridostigmine.

MATERIALS AND METHODS

Animals

Animals used in this study were male rats of the Sprague-Dawley strain, obtained from Charles River Laboratories (Raleigh, NC) at 90 days of age. The animals were housed individually in acrylic cages lined with wood shavings at an ambient temperature of 22°C. Tc and motor activity were housed individually in acrylic cages lined with wood shavings at an ambient temperature of 22°C. Tc and motor activity were housed individually at an ambient temperature of 22°C. Tc and motor activity were housed individually at an ambient temperature of 22°C.
ambient temperature of 23°C and a 12:12-h light-dark photoperiod. They had access to food and water ad libitum.

The animals were surgically implanted with radiotelemetry transmitters (model TA10TA-F40, Data Sciences) to measure core temperature and motor activity. They were anesthetized with pentobarbital sodium (50 mg/kg), and a small incision was made in the abdominal cavity to allow insertion of the transmitter. The abdominal muscle was sutured, and the skin was closed with surgical wound clips. After surgery, rats were administered 30,000 units of penicillin (intramuscularly) and an analgesic (buprenorphine, 0.03 mg/kg sc). All animals were returned to their home cages after implantation and allowed at least 1 wk recovery time before any experimental manipulations occurred.

Open-Field Stress

The open-field chamber consisted of a 61 × 61 × 61-cm Plexiglas box illuminated by two fluorescent lights suspended over the chamber. The sides of the chamber were white, and the bottom was brown. The top was open to the air. The chamber was placed inside a temperature-controlled chamber. A receiver board placed under the open-field box to monitor temperature and motor activity signals of the rat as it moved about the chamber detected the signal from the radiotransmitter. The receiver board was connected to a multiplexer (BMX-10) that automatically detected the strongest signal and processed it to a computer for data analysis. Data were recorded every 2 min to assess the dynamics of the open-field stress. The temperature within the open-field box and temperature chamber was the same (23°C) as that of the animal’s home cage.

Protocol

Open-field stress paradigm. Rats were tested individually in the open-field chamber. The day before an experiment, animals were transported from the animal room, weighed, and returned to their home cage, which was then placed in an environmental chamber maintained at 23°C. The environmental chamber also housed the open-field chamber (located above the rat’s cage, supported by a metal rack). The rat was allowed to acclimate to its environment overnight and remained in the same 12:12-h photoperiod as in the animal facility.

The next day, at 1200, each rat was quickly removed from its cage and dosed intraperitoneally (0.1 ml/100 g body wt) with physiological saline, methyl scopolamine (1.0 mg/kg), pyridostigmine (0.1 mg/kg), or a coadministration of methyl scopolamine (1.0 mg/kg) and pyridostigmine (0.1 mg/kg). Syringes with the drugs were prepared beforehand to facilitate a rapid injection and placement of the rat in the open field. After the injection, each animal was immediately placed inside the open-field box for 60 min. Then the rat was removed from the open-field box and monitored for a recovery period of 3 h in its home cage.

Baseline effects. To evaluate the effects of these drugs on core temperature, in the absence of open-field stress, we used the same protocol as the open field with the exception that rats were injected with saline, methyl scopolamine, pyridostigmine, or both drugs and returned to their home cages. Syringes with the drug were prepared beforehand to facilitate a rapid injection and placement of the rat in its home cage. Each rat was taken out of the home cage, quickly injected, and returned to its home cage for 4 h. Four rats were tested at once in the environmental chamber, and data were collected at 5-min intervals.

Cholinesterase measurements. The amount of cholinesterase (ChE) inhibition produced by pyridostigmine was determined in both nonstressful and stressful environments. In the first experiment, rats (n = 10) were injected with either physiological saline or pyridostigmine (0.1 mg/kg ip) and returned to their home cages where they were left undisturbed for 30 min. Each rat was euthanized by CO₂ asphyxiation at 30 min postinjection (i.e., comparable to the midpoint of the open-field experiment), and a blood sample was taken via cardiac puncture into a heparinized syringe and placed on ice. Plasma was separated (4,000 g × 15 min at 4°C), aliquoted into 1-ml vials, and stored at −22°C.

Plasma ChE activity was analyzed by spectrophotometry by measuring the breakdown of acetylthiocholine to 2-nitro-5-mercapto benzoate under controlled conditions (Boehringer Mannheim, Indianapolis, IN). ChE activity was measured in dimensions of 1 U/l, which converts a 1 μmol/l of substrate per minute under standard conditions at a temperature of 30°C.

In the second experiment, a new group of animals (n = 12) was tested to determine the amount of ChE inhibition produced by pyridostigmine under stressful conditions. Again, rats were injected with either physiological saline or pyridostigmine (0.1 mg/kg ip) and were immediately placed in the open-field box and left undisturbed for 30 min. Each rat was immediately euthanized by CO₂ asphyxiation after 30 min in the open-field box. Blood sampling and ChE analysis were performed in the same manner as described above.

Data analysis. The change in core temperature over the 1-h period in the open-field chamber was integrated with time to calculate the area under the curve. Motor activity over the 1-h open-field period was averaged for analysis. One-way ANOVA, followed by Dunnett’s t-test, was applied to each set of measurements comparing drug treatments to that of saline to assess the effects of each drug treatment on open-field hyperthermia.

RESULTS

Open-Field Hyperthermia

Rats displayed a minimal level of motor activity and a core temperature of ~37°C before injection of all drugs (Fig. 1). From the time of injection of saline until the core temperature was measured for the first time in the open field (2 min), core temperature decreased ~0.1°C, whereas motor activity increased abruptly. Core temperature of control animals then increased by 0.7°C during open-field exposure. Removing the rat from the open field to its home cage led to another transient elevation in core temperature followed by a gradual decrease over the next 90 min.

There was a transient decrease in core temperature immediately after methyl scopolamine injection that was similar to the saline response. However, as open-field exposure proceeded, core temperature rose during open-field stress by only 0.25°C and remained stable throughout the remainder of the test period. Removal of the rat to its home cage resulted in a transient increase in core temperature and then a gradual decrease over the next 2 h of recovery. Core temperature of the methyl scopolamine-treated rats remained significantly below that of the saline group throughout the open-field test and recovery phases (Fig. 1).
Pyridostigmine led to a significant enhancement of the hyperthermic response to open-field exposure compared with rats dosed with saline (Fig. 1). The hyperthermic response in this group was nearly 0.6°C above that of rats dosed with saline. When placed back in their home cage, the rats treated with pyridostigmine underwent a transient increase in core temperature and then a recovery that was similar to that of the saline group. Pyridostigmine coadministered with methyl scopolamine nearly abolished the effects of methyl scopolamine on core temperature during open-field stress. The response of rats given the mixture of pyridostigmine and methyl scopolamine was nearly identical to that of rats treated with saline during the open-field and recovery phases (Fig. 1).

Home-cage Hyperthermia

Core temperature before injection of drugs to rats in their home cages was ~37°C, and motor activity was near minimal levels (Fig. 2). All injections led to a transient decrease followed by an abrupt increase in core temperature for the first 15 min after injection. However, after this time, the core temperature of rats dosed with methyl scopolamine began a slow decrease for the next 1.75 h. Core temperature of rats dosed with saline and pyridostigmine was similar throughout the remainder of the 3-h monitoring period. Core temperature of rats dosed with the mixture of pyridostigmine and methyl scopolamine decreased below that of the saline group by 1 h after dosing. By 3.5 h after dosing, core temperature of rats treated with saline and pyridostigmine was notably higher than that of the rats receiving methyl scopolamine and rats receiving pyridostigmine coadministered with methyl scopolamine (Fig. 2).

Open-field vs. Home-cage Hyperthermia

The mean change in core temperature over the 1 h of exposure to open-field stress after injection of saline, pyridostigmine, and the pyridostigmine-methyl scopolamine mixture was significantly greater than the mean change in temperature for 1 h for rats housed in their home cages (Fig. 3). That is, open-field stress caused at least a doubling of the rise in core temperature after injection of these drugs compared with administration of the same drugs to rats housed continuously in their home cages. On the other hand, methyl scopolamine's
effect on core temperature was unaffected by placing rats in the open-field chamber.

Motor Activity

The motor activity averaged over a 1-h period after injection was generally unaffected by the drug and environment (Fig. 4). The level of activity during open-field stress was consistently higher compared with that of rats in their home cages. However, the absolute levels of activity between the two should not be directly compared because three receiver boards in the open-field generate a greater signal than the single board used in the home cage.

Plasma ChE Activity

Serum ChE activity of rats in their home cage, measured 30-min after intraperitoneal injection of saline and pyridostigmine, was $462 \pm 46.8$ and $204.2 \pm 36.8$ U/l, respectively. Thus the data suggest that pyridostigmine caused a 55% decrease ($F = 1.61; P = 0.0025$) in serum ChE activity at the halfway point of open-field exposure. A second experiment was conducted to verify pyridostigmine inhibition of ChE during the open-field condition. Serum ChE activity, measured 30-min into the open-field condition after intraperitoneal injection of saline and pyridostigmine, was $430.0 \pm 43.53$ and $230.3 \pm 29.25$ U/l, respectively. These data give further evidence that pyridostigmine caused a 54% decrease ($F = 2.22; P = 0.0034$) in serum ChE activity at the halfway point of open-field exposure. Overall, the inhibition in ChE activity was not affected by open-field stress.

DISCUSSION

Methyl scopolamine, a peripheral muscarinic antagonist, leads to a marked attenuation in the hyperthermic response to open-field stress. Methyl scopolamine also reduced core temperature in rats injected in their home cages. In this study, open-field exposure, an environment that leads to a marked thermogenic response, was nearly blocked by methyl scopolamine, and the findings suggest that muscarinic receptors outside of the CNS are involved in either the sensory or motor pathways leading to open-field hyperthermia. The block of cholinergic receptors with methyl scopolamine provides an incomplete picture of how cholinergic receptors may operate in open-field hyperthermia. That is, if blocking the receptors attenuates the hyperthermic response, then stimulating the receptors should enhance the response. Hence, the pyridostigmine data lend further support to a peripheral cholinergic mechanism that is involved in the control of open-field hyperthermia. Pyridostigmine, a peripheral anti-ChE agent that does not penetrate the blood-brain barrier, should lead to a transient stimulation of both muscarinic and nicotinic cholinergic receptors. Pyridostigmine did reverse the effects of methyl scopolamine and enhanced the open-field hyperthermic response compared with saline. These data support a peripheral cholinergic mechanism that controls open-field hyperthermia in the rat.

How can blockade of peripheral muscarinic receptors lead to an attenuation of stress-induced elevations in body temperature? Circulating levels of corticosteroids and key cytokines [e.g., interleukin (IL)-1, IL-6, tumor

Fig. 3. Mean change ($\Delta$) in core temperature of rats injected intraperitoneally with saline, methyl scopolamine, pyridostigmine, or a combination of methyl scopolamine and pyridostigmine when placed in open-field chamber and home cage. Values are means ± SE. Bonferroni corrected t-test: *$P = 0.05$, **$P = 0.01$.

Fig. 4. Motor activity of rats averaged over a 1-h period after intraperitoneal injection with saline, methyl scopolamine, pyridostigmine, or a combination of methyl scopolamine and pyridostigmine when placed in open-field chamber (A) and home cage (B). Values are means ± SE. Motor activity, NS.
necrosis factor (TNF) modulate stress-induced hyperthermia. High levels of corticosteroids attenuate stress-induced hyperthermia, as well as infection-induced rise in core temperature (fever), whereas adrenalectomy or administration of drugs to lower corticosteroid levels will augment fever (13). A corticosteroid mechanism of action is not expected to be operative to explain the methyl scopolamine and pyridostigmine effects in the present study, because the response to the cholinergic drugs was very rapid. The hypothalamic-adrenal axis has a relatively slow response that would not be seen in the time frame observed in the rats placed in the open field and given the cholinergic drugs.

There is growing evidence of peripheral pathways that modulate stress as well as infection-induced fevers (1, 4, 15). Subdiaphragmatic vagotomy has been found to attenuate the febrile response to lipopolysaccharide (10, 14). In addition, it has been shown that vagotomized rats displayed an attenuated response to stress-induced hyperthermia that is commonly observed after injection of control vehicles (8, 19). Vagotomy also blocks the fever response to IL-1β, a cytokine that is thought to be involved in the mediation of stress-induced hyperthermia (19). Antiserum to TNF also augments stress-induced hyperthermia (12). One or more cytokines, particularly IL-1, IL-6, and TNF, appear to be involved in the manifestation of stress-induced hyperthermia. A component of their efficacy involves stimulation of vagal afferents that leads to activation of thermogen centers in the CNS. It is conceivable that muscarinic and possibly other types of receptors may be involved at the peripheral level that activates stress-induced hyperthermia.

It is well known that there can be a marked release of some cytokines into the circulation during stress. In view of the role of cytokines and the mediation of fever (9), it is possible that modulation of peripheral cholinergic receptors during stress may affect the release of cytokines that mediate hyperthermia. Lipopolysaccharide-induced release of cytokines (IL-1α, IL-6, and IL-18) in vitro is dose-dependently inhibited by acetylcholine (2). Bilateral electrical stimulation of efferent vagus nerve activity significantly decreased serum levels of TNF (2). Although this evidence suggests that acetylcholine release could modulate the cytokine response to stress, the timing of the effects monitored in vivo does not support this mechanism of action. That is, a significant increase in proinflammatory cytokine IL-6 is not observed for at least 10–15 min after the initial exposure to stress (11). On the other hand, the onset of the open-field-induced hyperthermia observed in the present study occurred almost immediately after placement in the open field. Hence, there is no evidence that methyl scopolamine or pyridostigmine interacts with the neural-immune axis during stress; however, future work should address whether peripheral cholinergic stimulation alters blood cytokine levels during stress.

One may also question whether methyl scopolamine exerted an effect by penetrating the CNS. There are “leaky” parts of the blood-brain barrier that conceivably would allow for entrance of methyl scopolamine into CNS thermoregulatory centers. However, if that were the case, then one would expect a hyperthermic response, because other studies have shown that muscarinic receptors in CNS thermoregulatory centers are involved in driving heat-loss responses in the rat (6). Injection of scopolamine, a muscarinic antagonist that penetrates the blood-brain barrier, leads to an abrupt hyperthermic response in the rat (7). Hence, the effects of methyl scopolamine on core temperature are most likely attributed to pyrogenic signaling between the periphery and the brain.

It is of interest to note that, because of its protective effect against organophosphate poisoning, pyridostigmine has been used routinely by the military as a prophylactic measure to the threat of chemical warfare. In addition, pyridostigmine is used clinically in the treatment of myasthenia gravis (18). Our data, showing that a dose of pyridostigmine that has little effect on baseline core temperature but augments the stress-induced hyperthermia, may shed light on the possible deleterious effects of this drug when administered to humans under stressful conditions. A possible interaction between stress and the effects of pyridostigmine merits further study.

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

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