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Human Temperature Regulation During Exercise After Oral Pyridostigmine Administration

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Four healthy males exercised in two experiments at ambient temperatures of 22, 29, and 36°C with the relative humidity at 30% in all environments ($T_{dp} = 3.9, 9.9, \text{ and } 15.8^\circ\text{C}$). One experiment in each environment was done 150 min after 30 mg oral pyridostigmine bromide (PYR) administration, and the second experiment was done on a separate day with no medication (CON). Red blood cell cholinesterase was $39 \pm 7\%$ lower after PYR (11.8 vs. $7.2 \mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$). Esophageal (T_{es}) and mean skin temperature (T_{sk}), forearm blood flow (FBF), forearm sweating, and skin blood flow (SkBF) were measured twice each minute during a 15-min rest period and during 30-min of seated cycle exercise at $\sim 58\% \text{ Vo}_2$ peak. Whole body sweating was determined from weight changes before and after exercise. PYR decreased heart rate at rest and during exercise at 29°C and 36°C (8bpm, $p < 0.05$). Resting SkBF was 40% lower at 29°C and 30% lower at 36°C after PYR. During exercise, SkBF was 40% lower at 29°C and 50% lower at 36°C after PYR compared to CON ($p < 0.05$). There was no effect of PYR on heat production at rest or during exercise. T_{sk} was different in the three conditions by design, but was unchanged by PYR. T_{es} was not different at rest in any condition, but was elevated during exercise at 36°C (0.1°C , $p < 0.05$) in PYR compared to CON. These data suggest that pyridostigmine ingestion decreased skin blood flow, which may limit exercise thermoregulation in more severe environments.

PYRIDOSTIGMINE, a carbamate, is a reversible anticholinesterase with a half-life of approximately 2.5 h (3,18). Pyridostigmine and other anticholinesterases exert their actions at cholinergic synapses, as acetylcholine, which is released upon neutral stimulation, is not hydrolyzed and continues to bind with available receptors in the synaptic area (17-19). Individuals taking pyridostigmine or similar medications may experience

increased salivation, sweating, or bradycardia, which may affect an individual's ability to withstand hot environments. Pyridostigmine is used routinely in the clinical management of myasthenia gravis (18), and is used by the armed forces as a pretreatment drug for soldiers who may subsequently be exposed to nerve agents.

Exposure to hot environmental temperatures and/or increasing deep body temperature during exercise is associated with increased sweat secretion and vasodilation of surface vessels to transfer heat from the body core to the surface and, subsequently, to the environment (13,14). The sweat glands are innervated primarily by cholinergic fibers, although sweating can be induced through adrenergic stimulation (15). Skin and muscle blood flow are believed to have cholinergic components as well (6,13,14). Thus, any change in effector stimulation (sweat glands or vasomotor elements) which may result from increased cholinergic activity after pyridostigmine treatment may affect heat dissipation or perhaps body fluid status.

The present study characterized changes in thermoregulatory function at rest and during moderate, short-term exercise following a single 30-mg oral dose of pyridostigmine bromide. The 30-mg dose was selected as it is the dose contained in one tablet given to military personnel facing nerve agent exposure. We characterized temperature regulation in three environments chosen to provide three distinct mean skin temperatures at rest: the first was associated with significant vasoconstrictor activity (13,14); the second was in the thermal neutral or comfort zone; and the third was associated with active vasodilation. Since skin temperature influences sweat secretion and vasomotor activity, studying the effects of cholinesterase inhibition on sweating and vasomotor function at different skin temperatures provided a thorough representation of drug effects.

METHOD

Four healthy adult males participated in this study following approval by the human subjects review board.

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The mean (\pm S.D.) age was 22 ± 4 years, height 175.4 ± 10.2 cm, weight 75.5 ± 5.4 kg and peak aerobic power 3.37 ± 0.45 L \cdot min $^{-1}$. Each subject was tested twice in each of three different environments for a total of six tests. The three environments were 22°C ($T_{dp} = 3.9^\circ\text{C}$), 29°C ($T_{dp} = 8.9^\circ\text{C}$); and 36°C ($T_{dp} = 15.8^\circ\text{C}$); the relative humidity was 30% for all three environments. Air movement was less than 0.1 m \cdot s $^{-1}$, considered as still air. There was no radiant heat load. All tests were conducted at the same time of day to minimize the circadian variability in heat loss (16). The order of experiments was balanced for both environment and drug. On three test days, the subject dressed in running shorts, shoes, and socks came into the laboratory at 0700 hours having not eaten or consumed caffeine-containing beverages in the previous 12 h, and ingested 30 mg pyridostigmine bromide (PYR, Roche UK, Lot BK94626) with 200 ml spring water. Immediately preceding and at 150 min after receiving the medication, a blood sample was taken to determine red cell cholinesterase activity (8). Blood samples were taken to determine red cell cholinesterase activity the three control days (CON) at the same time of day as in PYR. Immediately after the second blood sample for red cell cholinesterase determination (150 min post-drug) or at the same clock time on control days, the subject swallowed a catheter containing a thermocouple into his esophagus for the measurement of core temperature. The thermocouple was inserted 25% of the subject's height and adjusted to a point at which the highest temperature was recorded. The use of esophageal temperature in these studies was critical, as this site is the only one routinely used which responds very quickly and closely mimics changes in blood temperature. We routinely observe a rapid increase in esophageal temperature at the beginning of exercise and use this increase to evaluate changes in sudomotor and vasomotor responses (10). Eight surface thermocouples were taped to the skin to calculate a mean weighted skin temperature as $\bar{T}_{sk} = 0.07 T_{forehead} + 0.175 T_{chest} + 0.175 T_{back} + 0.07 T_{upperarm} + 0.07 T_{forearm} + 0.005 T_{hand} + 0.19 T_{thigh} + 0.20 T_{calf}$ (12).

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (9,20). Briefly, the forearm was suspended at the wrist with a sling anchored at two points, thereby minimizing movement artifact as the arm and strain gauge moved in translation with the torso. Blood flow from the hand was excluded from the measurement as the wrist cuff was inflated to exceed systolic pressure. The measurement of FBF included flow through the skin, muscle, adipose tissue, and bone. Cutaneous vascular perfusion was measured as an index of skin blood flow (SkBF) by laser Doppler velocimetry (MED PACIFIC). This system used a 2 mW HeNe laser and fiber optic system to measure blood flow through the skin of the forearm. The flow measurements reported are mV values proportional to the quantity of moving red blood cells multiplied by the average velocity of the red blood cells within the sample volume of the capillary tissue measured. Laser probe and strain gauge (for FBF) placement were identical for each experiment for each subject. Forearm sweating (\dot{m}_s) was measured on the contralateral forearm using a ventilated dew-point sensor attached firmly to the skin (7).

This sensor was ventilated with ambient air from the chamber at a flow rate of 600 ml \cdot min $^{-1}$, which did not artificially dry the skin under the capsule but allowed sufficient evaporation. Heart rate was measured from the EKG. Oxygen consumption was measured at rest and frequently during exercise (SENSORMEDICS). The percent change in plasma volume was calculated from hematocrit and hemoglobin measurements of blood samples taken at rest and during steady-state exercise (20 min) from an indwelling venous catheter.

The subject sat in a contour chair placed behind the pedals of a cycle ergometer so that, during exercise, his legs were parallel to the floor. After instrumentation and establishment of thermal equilibrium, 15 min of resting data were collected. There was a 30-min period of exercise at $\sim 58\%$ peak aerobic power* immediately following the rest period. All thermoregulatory variables were measured twice each minute.

Data were analyzed by a three-way analysis of variance (drug by activity by environment) and are presented as the mean and the standard deviation. Reported differences are at $p < 0.05$ unless otherwise indicated.

RESULTS

The oral administration of pyridostigmine bromide decreased the activity of red blood cell cholinesterase by $39 \pm 7\%$ from an average 11.8 ± 0.7 $\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ before PYR treatment to an average of 7.2 ± 1.0 $\mu\text{mol} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ 150 min after treatment. Resting heart rate was unchanged at 22°C, but was reduced by PYR 8 and 9 b \cdot min $^{-1}$ at 29°C and 36°C, respectively. Resting SkBF was not different at 22°C, but was lower in PYR by 40% at 29°C (25 vs. 15 mV) and by 30% at 36°C (57 vs. 39 mV). FBF was unchanged at rest in all environments, which may indirectly show increased muscle blood flow, as skin blood flow was lower. The mean (\pm S.D.) thermoregulatory variables measured at rest for the three environments are given in Table I for both CON and PYR. Resting esophageal temperature (T_{es}) was not different in any of the six experiments. Mean skin temperature by design, was different in each environment, but was not affected by PYR at rest.

There was significant bradycardia during exercise at both 29°C and 36°C in PYR (Fig. 1). During exercise at 29°C and 36°C, SkBF was decreased by 40 and 50% by PYR, respectively (Fig. 2). Data for a single subject during all six experiments are shown in Fig. 3, and show the change in SkBF between CON and PYR experiments. Furthermore, the differences in SkBF at the three distinct skin temperatures are also evident. T_{es} was not different between PYR and CON during exercise at 22°C or 29°C (Table II). However, T_{es} was higher during exercise at 36°C in PYR compared to CON. T_{es} was higher at 36°C during exercise in both PYR and CON than in the other two environments. The change in

* The peak aerobic power was measured in the week prior to the first day of testing during incremental exercise in this seated position. The oxygen uptake peak was determined as that point where no further change in oxygen uptake occurred following an increase in the ergometer resistance.

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TABLE 1. MEAN (\pm S.D.) THERMOREGULATORY VARIABLES MEASURED AT REST FOR FOUR SUBJECTS IN CON AND PYR EXPERIMENTS IN THREE ENVIRONMENTS.

| | T_{es} ($^{\circ}$ C) | T_{sk} ($^{\circ}$ C) | FBF ($ml \cdot 100 ml^{-1} \cdot min^{-1}$) | \dot{m}_s ($mg \cdot cm^{-2} \cdot min^{-1}$) |
|---------------------|-----------------------------|-----------------------------|--|--|
| CON 22 $^{\circ}$ C | 36.51 (0.10) | 30.21 (0.55) | 0.5 (0.2) | 0.09 (0.03) |
| PYR 22 $^{\circ}$ C | 36.51 (0.15) | 30.02 (0.75) | 0.8 (0.4) | 0.12 (0.09) |
| CON 29 $^{\circ}$ C | 36.38 (0.09) | 33.43 (0.07) | 2.3 (1.3) | 0.13 (0.03) |
| PYR 29 $^{\circ}$ C | 36.41 (0.13) | 33.40 (0.17) | 2.3 (1.4) | 0.19 (0.08) |
| CON 36 $^{\circ}$ C | 36.49 (0.16) | 35.63 (0.31) | 3.6 (1.7) | 0.24 (0.11) |
| PYR 36 $^{\circ}$ C | 36.44 (0.12) | 35.45 (0.43) | 4.5 (2.5) | 0.28 (0.15) |

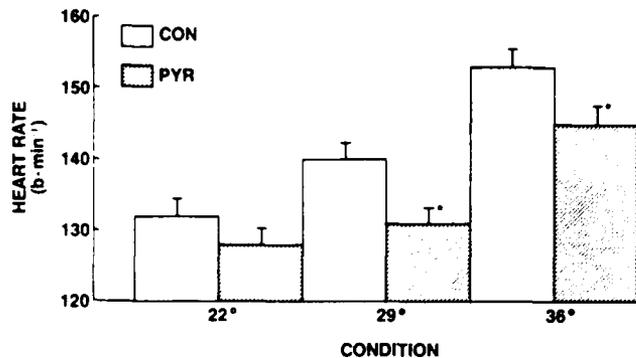


Fig. 1. Mean (\pm S.D.) heart rate during exercise for the four subjects in the three environmental conditions of the study.

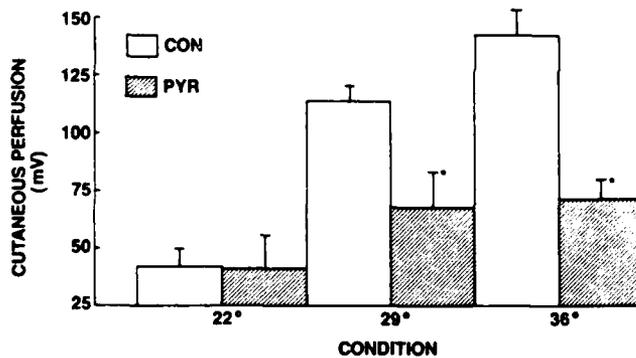


Fig. 2. Mean (\pm S.D.) cutaneous perfusion or skin blood flow during moderate exercise for the four subjects in the three environmental conditions of the study.

esophageal temperature from rest to steady-state exercise was not different between CON and PYR at 22 $^{\circ}$ C (0.69 $^{\circ}$ C vs. 0.68 $^{\circ}$ C) or 29 $^{\circ}$ C (0.84 $^{\circ}$ C vs. 0.92 $^{\circ}$ C). However, at 36 $^{\circ}$ C the increase in T_{es} averaged 0.93 $^{\circ}$ for CON and 1.06 $^{\circ}$ for PYR ($p < 0.05$). FBF was not different during exercise between CON and PYR in any environment, which may be indirect evidence of increased muscle blood flow. Whole body sweating during exercise was 15% higher at 29 $^{\circ}$ C. Forearm sweating during exercise was higher at 36 $^{\circ}$ C in PYR compared to CON (Table II).

The decrease in plasma volume calculated from hematocrit and hemoglobin values from rest and during cycle exercise averaged -9% in CON and -6% in PYR ($p = 0.22$).

DISCUSSION

Skin blood flow decreased at rest and during exercise after the oral ingestion of pyridostigmine bromide. This

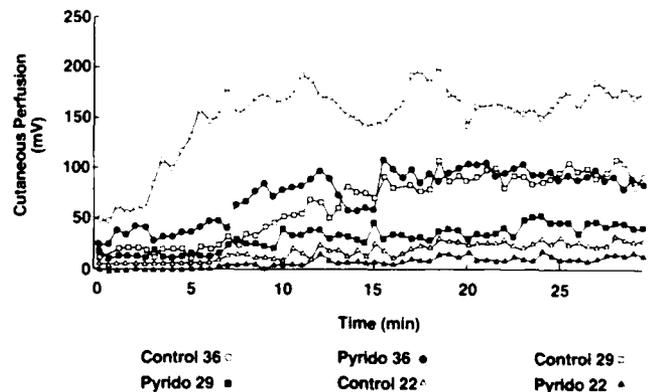


Fig. 3. Cutaneous perfusion or skin blood flow measured by laser doppler velocimetry (LDF) in a single subject during CON and PYR experiments in the three environments tested. The open symbols are for control experiments at 22 $^{\circ}$ C, 29 $^{\circ}$ C and 36 $^{\circ}$ C; the filled symbols are for pyridostigmine experiments at the same temperatures.

decrease in skin blood flow or cutaneous perfusion decreased skin temperature subtly in the hottest environment (0.2 $^{\circ}$ C), which precipitated the establishment of a less favorable temperature gradient for heat exchange between the skin and the environment, thus increasing heat storage. Decreased skin blood flow may have resulted from cholinergic stimulation centrally, at either pre- or postganglionic synapses, or directly at vasomotor elements. Pyridostigmine may have a direct effect as well. These sites and/or possible mechanisms of action cannot be addressed by the design or data of this study.

We tested the effect of both core and surface temperature on sweating and skin blood flow at three distinct ambient temperatures. In the coolest environment (22 $^{\circ}$ C), the acute administration of the carbamate pyridostigmine did not affect heat production or dissipation. The effect or lack of an effect on temperature regulation at this ambient temperature was not surprising as the thermal gradient for dry heat loss was wide enough to dissipate heat produced during exercise. Little evaporating cooling occurred as sweat secretion was low. At 29 $^{\circ}$ C, decreased skin blood flow and bradycardia were observed at rest and during exercise. At this ambient temperature, heat transfer from the warmer skin to the cooler environment was sufficient to maintain core temperature. Skin and ambient temperature were closely matched at 36 $^{\circ}$ C. In fact, heat was transferred from the environment to the skin. This condition necessitated the evaporation of secreted sweat to maintain body temperature. Pyridostigmine decreased skin blood flow, thus

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TABLE II. MEAN (\pm S.D.) THERMOREGULATORY VARIABLES MEASURED DURING MODERATE EXERCISE FOR FOUR SUBJECTS IN CON AND PYR EXPERIMENTS IN THREE ENVIRONMENTS.

| | T_{es} (°C) | T_{sk} (°C) | FBF (ml · 100 ml ⁻¹ · min ⁻¹) | \dot{m}_s (mg · cm ⁻² · min ⁻¹) |
|----------|------------------|------------------|---|---|
| CON 22°C | 37.20 (0.24) | 31.11 (0.97) | 5.6 (5.2) | 0.58 (0.12) |
| PYR 22°C | 37.18 (0.16) | 30.54 (0.90) | 4.3 (2.4) | 0.47 (0.22) |
| CON 29°C | 37.22 (0.27) | 33.33 (0.77) | 14.2 (3.9) | 0.76 (0.29) |
| PYR 29°C | 37.33 (0.24) | 33.07 (0.57) | 14.2 (4.1) | 0.76 (0.14) |
| CON 36°C | 37.41 (0.37) | 35.47 (0.24) | 11.9 (6.2) | 1.03 (0.25) |
| PYR 36°C | 37.50 (0.45)* | 35.26 (0.12) | 18.3 (7.5) | 1.36 (0.27)* |

* $p \leq 0.05$.

creating an even larger thermal gradient between the ambient air and the skin, and the change in core temperature was greater compared to the control experiment.

As far as can be determined by a review of the available literature (1,4,5,11), there are no studies on human subjects which examine thermoregulatory consequences resulting from anticholinesterase therapy. Studies run on rodents (4,11) indicated that heat storage was increased and running time was compromised after acute pyridostigmine treatment at 26°C and 35°C. The effect of sustained oral pyridostigmine therapy (5 daily 0.4 mg/kg doses) in an exercising primate (*e. patas*) has recently been reported (1). Animals treated with pyridostigmine significantly increased running time compared to control experiments, which appeared to be the result of decreased heat storage due to a 60% increase in whole-body water loss.

Muscle blood flow, as measured indirectly by changes in limb blood flow, may have increased in an inactive arm at both rest and during leg exercise after the administration of an anticholinesterase in this study. This was not unexpected as blood vessels in the muscle have cholinergic innervation (2,6,13,14). If muscle blood flow increased, there was no adverse or beneficial effect during seated cycle exercise. However, in conditions where considerable venous pooling occurs, the possible increase in muscle blood flow may have adverse consequences if baroreflex activity is already affected by the accumulation of acetylcholine. This possibility should be investigated.

Decreased skin blood flow affected heat exchange at 36°C as evidenced by the slight, but statistically significant, increase in T_{es} during exercise and the change in T_{es} from rest to exercise compared to CON. At 36°C, heat production was approximately 350 W · m⁻²; approximately 360 W · m⁻² was eliminated through evaporation during steady-state exercise in control experiments. Thus, the small change in dry heat exchange (heat gain from the environment) after pyridostigmine ingestion, caused the higher core temperature. If the water vapor pressure of the environment were higher, such as occurs when chemical protective clothing is worn, heat exchange via the evaporation of secreted sweat would be limited by the low water vapor pressure gradient between the skin surface and the ambient air. In this instance, dry heat loss, the physical heat exchange by convection and radiation, becomes increas-

ingly important to maintain the deep body temperature. Blood flow from the core to the skin surface is critical to remove heat from the body. In addition, skin blood flow is the primary determinant of dry heat loss to the environment. Therefore, in any condition at rest or during exercise in which heat exchange from skin blood flow is critical, temperature regulation may be affected during pyridostigmine treatment.

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The opinions and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision unless so designated by other official documentation. Human subjects participated in these studies after giving their informed consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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