The effect of systemic atropine (ATR, 2 mg, im) or pyridostigmine (PYR, 30 mg, oral) at rest and during moderate seated cycle exercise was evaluated in two separate protocols at an ambient temperature of 30°C. Esophageal (Tes) and mean weighted skin temperatures were measured continuously, as was forearm sweating rate, forearm blood flow (FFB, venous occlusion plethysmography) and cutaneous perfusion (SkBF, laser doppler velocimetry). Whole body sweating decreased 55% (P<0.05) in ATR, while heart rate increased 30 bpm (P<0.05). ATR increased the slope (central thermosensitivity) of FBF:Tes by 90% (P<0.05) compared to control. The Tew for sweating onset was increased 0.3°C (P<0.05) after ATR. 
TEMPERATURE REGULATION FOLLOWING SYSTEMIC ANTICHOLINERGIC OR ANTICHOLINESTERASE THERAPY.

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INTRODUCTION

The systemic administration of either cholinolytic or anticholinesterase drugs affects temperature regulation in humans. Cholinergic receptors are blocked by an anticholinergic such as atropine, thus at effector organs a given concentration of neurotransmitter produces an attenuated response. Two consequences are 1) the inhibition of cardiac vagal stimulation and therefore increased heart rate at rest and during exercise; and 2) attenuated secretion from eccrine sweat glands. Conversely, pyridostigmine, an anticholinesterase, increases cholinergic stimulation at receptors because acetylcholine is not rapidly hydrolyzed and binds with available receptors in the synaptic area. By this action, 1) eccrine sweat glands secrete more fluid which becomes available for evaporative heat loss and 2) bradycardia predominates by an enhanced vagal stimulation.

This paper concerns an investigation of alterations in skin blood flow which occur after systemic anticholinergic or anticholinesterase drug application. The anticholinergic drug used in these studies was atropine sulfate (ATR), administered i.m. in a dose which decreased whole body sweating by 55% and increased resting heart rate by 30 b\textsuperscript{-}min\textsuperscript{-1}. The second set of experiments examined the effect of oral administration of the anticholinesterase, pyridostigmine bromide (PYR) in a dose which decreased red blood cell cholinesterase activity by 39% and caused bradycardia (10 b\textsuperscript{-}min\textsuperscript{-1}).

METHODS

Two independent groups of subjects were tested in the two studies (Table 1).

TABLE 1. Mean (±SD) characteristics of the four subjects in the atropine study and of the five subjects in the pyridostigmine study.

<table>
<thead>
<tr>
<th></th>
<th>Age (yr)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>(\dot{V}O_2)peak (L\textsuperscript{-}min\textsuperscript{-1})</th>
<th>(A_o) (m\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATR</td>
<td>21.3 (2.2)</td>
<td>1.82 (0.9)</td>
<td>81.3 (9.8)</td>
<td>3.71 (0.4)</td>
<td>2.03 (.16)</td>
</tr>
<tr>
<td>PYR</td>
<td>25.8 (6.9)</td>
<td>1.74 (0.9)</td>
<td>72.2 (7.0)</td>
<td>3.38 (0.5)</td>
<td>1.86 (.12)</td>
</tr>
</tbody>
</table>
The methodology of the two sets of experiments was essentially identical except for two events. Exercise started 30 minutes after the injection of 2 mg atropine into the vastus lateralis. Exercise was initiated 150 minutes after pyridostigmine ingestion (30 mg). The ambient temperature was 30°C with an ambient water vapor pressure of 1.0 kPa. In all experiments subjects were dressed in running shorts, shoes and socks. A cannula containing a copper-constantan thermocouple was threaded through the nose and swallowed into the esophagus (T_e), advanced to a position at approximately heart level. Skin thermocouples were attached to eight sites for calculation of a mean weighted skin temperature (T_w). Forearm sweating (\( \dot{m}_s \)) was measured with a ventilated (600 ml min\(^{-1} \)) dew point sensor. Forearm blood flow (FBF) was measured by venous occlusion plethysmography in all experiments, and forearm skin blood flow or cutaneous perfusion (SKBF) was measured by laser doppler velocimetry in the pyridostigmine study. Each subject exercised (55% VO\(_{2}\)peak) for 30 minutes during control experiments and on a separate day after the appropriate drug therapy. Slopes and esophageal temperature intercepts were calculated for each subject during the exercise transient for sweating, forearm blood flow or skin blood flow.

Data were analyzed by analysis of variance procedures with repeated measures. Data in the results are given as the mean ± standard deviation. Linear regressions the transient responses of sweating, forearm blood flow and skin blood flow were calculated.

RESULTS

The results of the atropine study are summarized in Figure 1. Whole body sweating was reduced 55% by atropine treatment and heart rate at rest and during exercise was increased 30 b•min\(^{-1}\).

\( T_e \) was significantly higher after 25 minutes of exercise in atropine experiments. \( T_e \) and FBF were higher in atropine experiments by ten minutes of exercise. Table 2 gives the mean slopes and \( T_e \) thresholds for FBF and \( \dot{m}_s \). The slope of FBF to \( T_e \) was 90% higher after atropine. The \( T_e \) threshold for forearm sweating was 0.3°C higher in atropine experiments.

<table>
<thead>
<tr>
<th>( T_e ) Threshold (°C)</th>
<th>Slope</th>
<th>CON</th>
<th>ATR</th>
<th>CON</th>
<th>ATR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBF</td>
<td>36.75</td>
<td>36.73</td>
<td>15.02</td>
<td>27.72*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.42)</td>
<td>(0.16)</td>
<td>(6.23)</td>
<td>(2.99)</td>
<td></td>
</tr>
<tr>
<td>( \dot{m}_s )</td>
<td>36.49</td>
<td>36.81*</td>
<td>1.09</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.32)</td>
<td>(0.23)</td>
<td>(0.30)</td>
<td>(0.30)</td>
<td></td>
</tr>
</tbody>
</table>

* ps0.05
Pyridostigmine ingestion inhibited red blood cell cholinesterase activity by 39% which was associated with lower resting and exercise heart rates (10 b·min⁻¹) and increased sweating rate (13%). The results of the pyridostigmine study are summarized in Figure 2.

Skin blood flow (measured by laser doppler velocimetry) was decreased both at rest and during exercise by approximately 50%. FBF was unchanged by PYR indicating potential increased muscle blood flow. Table 3 gives the mean slopes and $T_{ss}$ thresholds for SkBF. The $T_{ss}$ threshold for the onset of cutaneous perfusion was elevated 0.2°C after PYR. The slope of SkBF:$T_{ss}$ was 40% (p=0.16) lower after PYR.
Figure 2. Time course for $T_{es}$, $T_{sk}$ and cutaneous perfusion for control and pyridostigmine experiments. The data are the mean ±SD. * p≤0.05.

**TABLE 3.** Mean (±SD) esophageal temperature threshold and skin blood flow sensitivity during the pyridostigmine study.

<table>
<thead>
<tr>
<th>$T_{es}$ Threshold (°C)</th>
<th>Slope (mV°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CON</strong></td>
<td><strong>PYR</strong></td>
</tr>
<tr>
<td>SkBF</td>
<td>36.84</td>
</tr>
<tr>
<td></td>
<td>(0.29)</td>
</tr>
</tbody>
</table>

* p≤0.05

**CONCLUSIONS**

In general, sensible heat flux by radiative and convective heat exchange at the skin at rest and during exercise is affected by systemic treatment of either an anticholinergic (atropine sulfate) or an anticholinesterase (pyridostigmine bromide). These experiments show increased blood flow to the skin surface after atropine which increases heat loss, and decreased skin blood flow after pyridostigmine which decreases heat loss.
The presence of cholinergic vasodilator fibers to the vessels near the skin surface has never been proven. However, evidence has accumulated showing indirectly that vasodilation is a cholinergic event. Briefly, the intra-arterial injection of acetylcholine or methacholine results in flushing of the skin\textsuperscript{3,5}; and atropine, when injected intra-arterially, delays the skin dilation which occurs during body heating.\textsuperscript{7} These events would suggest a cholinergic component to dilation of the skin surface vessels. Furthermore, isolated vascular strips with intact endothelial surfaces relax when incubated with a solution containing acetylcholine.\textsuperscript{8} This evidence is directly opposite to what was observed in the current studies.

Cholinergic inhibition was apparent after atropine, evidenced by increased heart rate and decreased sweat secretion. The physiologic responses involving skin blood flow (Table 2) are consistent with a peripheral modification of thermoregulatory control, as the slope of skin blood flow to increasing core temperature was increased.\textsuperscript{9} Possible mechanisms by which atropine increased skin blood flow may be 1) a direct local action at the blood vessel, 2) a release of vasoconstriction mediated by CNS receptor activity, or 3) the presence of a direct vasodilatory substance associated with the sweat gland stimulation or function.\textsuperscript{10} This study suggests that the vasodilation after atropine is distinct from acetylcholine or acetylcholine-like mediated vasodilation, which requires an endothelium derived releasing factor. It is possible that the vasodilation may be mediated by a release of a vasoactive substance associated with sweat gland stimulation, not necessarily secretion.

Sweating and heart rate responses after acute PYR treatment are consistent with an increased accumulation of ACh at cholinergic receptors of the sweat gland and heart. Therefore, it could be hypothesized that increased ACh accumulation occurred at cholinergic neuronal sites affecting skin blood flow. The increased accumulation of ACh at the paravertebral sympathetic ganglion might be a site where increased cholinergic receptor stimulation could promote increased vasoconstrictor tone. This possibility is consistent with the observation of decreased skin blood flow at rest and during exercise. It is also consistent with the observation that the T\textsubscript{a} threshold for onset of cutaneous vasodilation was increased with PYR treatment (Table 3). A shift in the latter threshold has been classically interpreted as a central modulation of the thermoregulatory system.\textsuperscript{9} Some other possible mechanisms for the reduced skin blood flow observed with PYR treatment may be: 1) increased vasomotor tone in the skin resulting from ACh accumulation at the autonomic ganglion; 2) the accumulation of ACh to a great enough extent to diffuse into the brain to cause a central effect; 3) excessive accumulation of ACh at cholinergic vasodilatory fibers to cause autoinhibition\textsuperscript{11}; or 4) bradycardia resulting from PYR treatment which promotes relative vasoconstriction through a baroreflex response.

In summary, the systemic administration of an anticholinergic or an anticholinesterase results in skin blood flow changes which are not totally consistent with cholinergic dilation at the skin surface.
ACKNOWLEDGEMENTS

The views, 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 voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

REFERENCES

4. Carmichael EA, Fraser FR (1933) Heart 16: 263-274