Activation of 5-HT\textsubscript{1A} receptors in the paragigantocellularis lateralis decreases shivering during cooling in the conscious piglet


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Submitted 20 November 2006; accepted in final form 3 April 2007


STUDIES IN ANESTHETIZED ANIMALS have demonstrated that many neurons located in the medullary raphé, including serotoninergic (5-HT) neurons, project to the intermediolateral cell column (IML) of the spinal cord. Some of these modulate sympathetic outflow to thermoregulatory effector mechanisms, including brown adipose tissue (BAT) thermogenesis and peripheral vasoconstriction (9, 41, 45, 64, 66). Studies in anesthetized and conscious animals have shown that activation of 5-HT\textsubscript{1A} receptors in the medullary raphé with 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, or DPAT hereafter) attenuates sympathetic outflow to BAT and peripheral vessels when sympathetic activity is previously elevated by LPS, leptin, or cooling (5, 40, 46, 49, 50). In the medullary raphé 5-HT\textsubscript{1A} receptors are located on both 5-HT and non-5-HT neurons (21).

Thus, exogenous activation of 5-HT\textsubscript{1A} receptors in the medullary raphé with DPAT would inhibit both 5-HT and any non-5-HT neurons expressing 5-HT\textsubscript{1A} receptors. Whether the effects of exogenous 5-HT\textsubscript{1A} receptor activation with DPAT on BAT thermogenesis and peripheral vasoconstriction are due to a decrease in the activity (via inhibitory autoreceptors) of IML projecting 5-HT neurons or to postsynaptic inhibition of other IML projecting neurons, such as glutamatergic neurons, or to some combination, remain unclear. The idea that medullary raphé 5-HT neurons modulate sympathetic outflow to thermoregulatory effector mechanisms is supported by evidence indicating that 5-HT neurons increase their firing rates in response to cooling or PGE\textsubscript{2} administration and that the increase is positively correlated with BAT temperature (36, 47). Moreover, local application of 5-HT into the IML increases sympathetic outflow to BAT and positively modulates the excitatory effect of locally applied NMDA (34).

Whereas there is mounting evidence that medullary 5-HT neurons modulate sympathetically mediated thermoregulatory mechanisms, little is known about the role of medullary 5-HT neurons in shivering thermogenesis. Shivering is an involuntary tremor which, as described by Schäfer and Schäfer (58–60), is caused by an oscillatory instability resulting via fusimotor innervation of skeletal muscle. A role for medullary raphé 5-HT neurons in modulating shivering is suggested by recent evidence that fusiform muscle fiber activity during skin cooling can be attenuated by the microinjection of glycine into the medullary raphé (65). Moreover, microinjection of DPAT or lidocaine into the ventral medial medulla attenuates shivering activity in conscious rats (4).

Interestingly, both nonshivering and shivering thermogenesis and peripheral vasoconstriction are greatly attenuated, or even eliminated, during rapid eye movement (REM) sleep in most animal species (19, 53), a time when 5-HT neurons are thought to be at their lowest level of activity (23, 26). These observations support a state-related modulatory role for medullary 5-HT neurons in controlling thermoregulatory sympathetic activity. Thus both a decrease in 5-HT neuronal activity and exogenous activation of 5-HT\textsubscript{1A} receptors are associated with an attenuation of thermoregulatory effector mechanisms.

With respect to thermoregulation, most interest has been focused on the midline raphé. However, extensive counting of piglet medullary tryptophan hydroxylase immunoreactive neu-
rons and examining their three-dimensional distribution have demonstrated substantial numbers of 5-HT neurons in parallel columns lateral to the midline extending from the pontomedullary junction to just caudal to the caudal border of the facial nucleus (12, 48). We previously demonstrated that these lateral columns containing 5-HT neurons may be important for sleep homeostasis. Moreover, activation of 5-HT₁₅A receptors with DPAT in these lateral columns of neurons, which include the paragigantocellularis lateralis (PGCL), decreases muscle activity and body temperature during non-rapid eye movement (NREM) sleep (12).

Neurons in the PGCL receive inputs from many areas, including the nucleus of the solitary tract, A1 region, parabigeminal nucleus, Kölliker-Fuse nucleus, periaqueductal gray, and the hypothalamus. The more rostral (juxtafacial) PGCL also receives polymodal inputs from the inferior colliculus, the dorsal column nuclei, and the medial geniculate nucleus (69, 70). The PGCL projects to areas important for alertness and arousal, including the locus coeruleus (1, 2) and to the dorsal and ventral horns of the spinal cord (25) important for motor control, via extensive collateralization supplying multiple spinal cord segments (7, 28).

Abnormalities in thermoregulatory responses have been implicated in many disorders, including the sudden infant death syndrome (SIDS). There are significant postnatal changes in thermoregulatory control occurring in the first few months after birth, the period of greatest risk for SIDS (17), and several risk factors for SIDS suggest defective thermoregulation, e.g., prone positioning (56), elevations in environmental temperature (39), and overbundling (16). In addition, some SIDS infants have persistence of BAT at autopsy (44, 68) and are found with elevated body temperatures at the time of death (61). Abnormalities in 5-HT₁₅A binding and numbers of 5-HT neurons have been reported in SIDS cases in three independent data sets in the medullary raphe and extra-raphe regions, including the PGCL (29–31, 51, 55), groups of neurons homologous to those have been found to be important in thermoregulatory control in animals (6, 41).

In this study, we tested the idea that shivering, a major thermoregulatory effector mechanism, would be modulated by the level of 5-HT₁₅A receptor activation in the PGCL. The goal of the current study was to determine whether activation of 5-HT₁₅A receptors in the PGCL of conscious piglets during mild cooling would attenuate both shivering and peripheral vasoconstriction. In addition, we wanted to compare the effects of 5-HT₁₅A receptor activation with those of naturally occurring constriction. Moreover, we wanted to compare the effects of cooling would attenuate both shivering and peripheral vasoconstriction. In addition, we wanted to compare the effects of cooling would attenuate both shivering and peripheral vasoconstriction.

Surgical instrumentation. Our surgical procedures have been described in detail previously (10–12). Briefly, under sterile conditions and using isoflurane anesthesia, a dual-lumen catheter was placed via the femoral artery into the abdominal aorta, and a telemetric thermistor was placed into the peritoneal cavity. A microdialysis guide tube was stereotaxically placed with its tip between the midline and the medial border of the right facial nucleus near the ventral surface of the medulla. EEG electrodes were screwed into the left frontal and right occipital regions of the skull and referenced to a right parietal electrode. Electrooculogram (EOG) electrodes were sewn into the muscleateral surface of each eye, and bipolar electromyogram (EMG) electrodes were sewn into the muscleateral surface of each eye, and bipolar electromyogram (EMG) electrodes were sewn into the neck muscles on the right side, near the midline. All wires were tunneled and connected to two plastic pedestalsthat were then cemented to the skull along with the microdialysis guide tube. The femoral catheter was tunneled through the skin and exited the subcapsular region on the back. After surgery, the animals were provided with analgesia and antibiotics, allowed to recover, and returned to the sow and siblings in the animal care facility.

Protocols. Animals were first studied 24–48 h after surgery. The piglet was suspended in a sling inside a double-walled barometric plethysmograph (3, 14) modified to allow continuous gas flow (52). Air flowing through the plethysmograph was heated (~38°C at the heater/humidifier) and fully humidified. Heart rate (HR) and mean arterial pressure (MAP) were calculated from continuous measurements of arterial pressure (World Precision Instruments, Sarasota, FL). Respiratory measurements were derived from plethysmograph pressure fluctuations (Validyne, Northridge, CA). EEG, EOG, and EMG signals were amplified and band-pass filtered (0.1–300 Hz for EEG and EOG and 10–300 Hz for EMG). The percent carbon dioxide (CO₂) in the outlet air of the plethysmograph (Capstar-100; CWI; Ardmore, PA) was continuously measured to estimate CO₂ production. Plethysmograph air, piglet right ear skin, and core temperatures were continuously measured (YSI, Yellow Springs, OH and DSI, St. Paul, MN). In addition to ear surface temperature measured on the right side, an index of left ear capillary blood flow was derived from Doppler flowmetry signals (Periflux PF3; Perimed, Stockholm, Sweden). All electronic signals were digitized at 1,000 Hz and recorded using a computerized data acquisition system (PowerLab; ADInstruments, Sydney, Australia). Throughout the experiment, piglet behavior was also video recorded and digitized for later scoring. Shivering was assessed by measuring the percent time shivering, the number of shivering bouts/min, and changes in integrated neck EMG activity.

Experiments were performed on piglets 6–15 days old, of either sex, weighing 1.8–3.9 kg at the time of study. All surgery and experimental protocols were approved by the Institutional Animal Care and Use Committee of Dartmouth College. The piglets were housed with the sow and siblings in a farrowing crate located in the Dartmouth College Animal Resource Center and were maintained at a constant ambient temperature of 21°C and 12:12-h light-dark cycles. Piglets were brought to the laboratory on one or more days before surgery to acclimatize them to the experimental environment.
thermoregulatory environment, the wall temperature was initially adjusted to 5°C below thermoneutral in the same manner as in the control experiment. Experiments were also performed in a second group of animals (n = 6), where the sequence of events was identical to that described above, except that the dialysate containing DPAT was replaced with aCSF.

For the second protocol (effect of DPAT in a continuous cool environment), the wall temperature was initially adjusted to ~5°C below the lower critical threshold of the thermoneutral zone, and this environment was maintained for the duration of the experiment. Following a 60–90 min baseline period with aCSF dialysis, the dialysate was switched to 30 mM DPAT for 30 min and then switched back to aCSF for an additional 60–90 min. Time-control experiments were performed in a second group of animals (n = 3), in which the identical protocol was used, except that aCSF was substituted for DPAT in the dialysate.

Table 1. Baseline values for cardiorespiratory, thermoregulatory, and environmental variables during NREM sleep in baseline thermoneutral and cool environments

<table>
<thead>
<tr>
<th>Variables Number</th>
<th>Thermoneutral (n = 6)</th>
<th>Cool (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>181.3±9.9</td>
<td>181.2±10.1</td>
<td>0.993</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>91.2±1.7</td>
<td>96.2±3.7</td>
<td>0.327</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>43.3±6.3</td>
<td>55.6±4.0</td>
<td>0.112</td>
</tr>
<tr>
<td>Tidal volume, ml/kg</td>
<td>7.5±1.1</td>
<td>8.8±1.0</td>
<td>0.410</td>
</tr>
<tr>
<td>Minute ventilation, ml/kg/min</td>
<td>295.9±39.1</td>
<td>470.1±62.5</td>
<td>0.050</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>39.4±0.6</td>
<td>39.7±0.3</td>
<td>0.625</td>
</tr>
<tr>
<td>Ear Surface temperature, °C</td>
<td>37.1±0.5</td>
<td>29.8±0.4*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plethysmograph air temperature, °C</td>
<td>27.7±0.6</td>
<td>24.7±0.1*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plethysmograph CO2 concentration, %</td>
<td>0.40±0.1</td>
<td>0.52±0.1*</td>
<td>0.028</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. Baseline values for animals studied in both protocols during non-rapid eye movement (NREM) sleep. In protocol 1, the animals were in a thermoregulatory environment before rapid cooling, and in protocol 2, the animals were maintained in a continuous cool environment for the entire experiment. On average, in the cool environment, there was more vasoconstriction (lower ear surface temperature) and an increase in metabolic rate (higher plethysmograph CO2 concentration) in the cool environment. Some variables are not shown because they were not measured in both groups. Asterisks and P values indicate a significant difference between thermoneutral and cool baseline values.

Drugs. Thirty millimolar DPAT were used in the dialysate in the current study. This is a relatively high concentration compared with those used in dialysis experiments in the dorsal raphe (57). They are otherwise consistent with those used in our previous experiments (12, 37) and those done by other investigators in the caudal brain stem (4). Higher concentrations in the dialysate may be necessary for several reasons. First, the estimated tissue concentration is approximately one-tenth of the dialysate concentration (13). Second, compared with dorsal raphe 5-HT neurons, caudal medullary 5-HT neurons have faster firing rates (23), may have fewer 5-HT1A autoreceptors (67), and appear to be less sensitive to 5-HT1A agonists (24). Our prior data using fluorescein and 5,7-dihydroxytryptamine indicated that we were affecting 5-HT1A receptors in an area restricted to the juxtafacial PGCL and a portion of the retrotrapezoid nucleus (10, 12).

Data reduction and calculations. Data reduction, including sleep scoring, was done using custom programs written in MATLAB (MathWorks, Natick, MA) and have been described in detail previously (12). Briefly, sleep-state scoring was accomplished using a wavelet-based analysis that derived frequency information from the EEG and periods of NREM sleep, REM sleep, and wakefulness (WAKE) were identified using the combination of EEG, EOG, and
nuchal EMG data. State identification was confirmed by observing the piglet’s behavior using synchronized video recordings.

Body, ear, and plethysmograph temperature data and relative estimates of ear capillary blood flow (Flux) were resampled to 1 Hz. In addition to measurements of Flux, an estimate of resistance was calculated as FLUX/Mean Arterial Blood Pressure (BPm). Since shivering was suspended during REM sleep, it was quantified only during NREM sleep by determining for each epoch, the percent of time shivering, the number of shivering bursts per minute, and integrated neck EMG activity. This was accomplished by first confirming the presence of shivering during a given epoch of NREM sleep in the video recording and then determining the percent of time spent in shivering activity for that epoch by manually measuring the duration of each rhythmic burst (bout) of neck EMG activity. The number of shivering bouts and the sum of their durations were used to calculate the number of shivering bouts per minute and percent time spent in shivering, respectively. To determine changes in neck EMG activity, the raw signal was resampled at 100 Hz, rectified, and integrated over 5-s bins and was then averaged for each NREM epoch.

For cardiorespiratory variables, the original digitized data were resampled at rates appropriate for each variable. For respiratory calculations, the maximum and minimum of each breath-related pressure fluctuation were determined using an automated peak detector followed by manual correction, if necessary. Tidal volume (VT) was calculated from the pressure fluctuations using methods used previously (3). Minute ventilation was calculated as the product VT and instantaneous respiratory rate calculated from the inter-breath interval. The peak of each blood pressure pulse was determined similarly and was used to calculate beat-to-beat HR. BPm was calculated from the arterial pressure waveform. For these studies, CO2 production was estimated by measuring [CO2] in the outflow of the plethysmograph.

For each variable, artifact-free segments of the recording were averaged and archived as to the time before or after DPAT dialysis, and state (NREM, REM, or WAKE). For the comparison of NREM and REM, all NREM-REM pairs during the baseline period of protocol 2 were examined. Differences for each pair were averaged for each animal (n = 8). To determine the effects of DPAT dialysis, two methods were used depending on the protocol. For protocol 1 (rapid cooling before and after DPAT), data were averaged during two phases of cooling. The first phase, COLD 1, consisted of the first half of the decrease in temperature, and COLD 2, the remaining cooling until the desired temperature (5°C below thermoneutral) was reached. The time in which the temperature was increased to reestablish thermoneutrality was not analyzed, as it represents a period of “warming”. The mean values for cardiorespiratory and thermoregulatory variables, including the percent time shivering and change in integrated neck EMG activity during COLD 1 and COLD 2 NREM epochs, were compared with the last epoch of NREM sleep before cooling. For protocol 2, values of cardiorespiratory and thermoregu-

![Fig. 2. A typical example of changes in sleep, cardiovascular, and thermoregulatory related variables on transition from non-rapid eye movement (NREM) to rapid eye movement (REM) sleep in a continuous cool environment in a single animal. NREM sleep is characterized by a high-voltage, low-frequency EEG, minimal rapid eye movements in the electrooculogram (EOG), and relatively low neck EMG (nEMG) activity. REM sleep is accompanied by low-voltage, higher-frequency EEG, evidence of rapid movement in the EOG, and low neck EMG activity. Wakefulness (WAKE) is indicated by an increasing EEG amplitude, and an abrupt increase in neck EMG activity. Shivering is indicated by phasic activity in the neck EMG. During REM, there is a decrease in blood pressure (BP) and heart rate (HR), plethysmograph CO2 concentration (CO2), and body temperature (Tbody), and increases in ear capillary blood flow (Flux) and ear surface temperature (Tear). Thus, during REM, there is both an attenuation of shivering and peripheral vasoconstriction indicated by the lack of phasic neck EMG activity, and increases in ear capillary blood flow and ear skin temperature. The decrease in plethysmograph CO2 concentration and HR suggest a decrease in metabolic rate. In a cool environment the loss of both heat-conserving mechanisms and heat production is reflected in a progressive decrease in body temperature.](http://ajpregu.physiology.org/Downloadedfrom)
Table 2. Changes in cardiovascular and thermoregulatory variables on transition from NREM to REM sleep in a cool environment

<table>
<thead>
<tr>
<th>Variables</th>
<th>REM vs. NREM (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>-17.1±4.6*</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>-4.2±0.9*</td>
<td>0.003</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>0.06±0.20*</td>
<td>0.457</td>
</tr>
<tr>
<td>Ear skin temperature, °C</td>
<td>0.036±0.046</td>
<td>0.459</td>
</tr>
<tr>
<td>Ear capillary blood flow, au</td>
<td>0.54±0.17*</td>
<td>0.001</td>
</tr>
<tr>
<td>%Change</td>
<td>(17.3±3.3)</td>
<td></td>
</tr>
<tr>
<td>Ear capillary resistance, au</td>
<td>-15.1±6.9*</td>
<td>0.001</td>
</tr>
<tr>
<td>%Change</td>
<td>(-15.8±2.7)</td>
<td></td>
</tr>
<tr>
<td>Plethysmograph outlet [CO₂], %</td>
<td>-0.025±0.005*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

While in a continuous cool environment, transition from NREM to rapid eye movement (REM) sleep is associated with significant decreases in mean heart rate, mean blood pressure, body temperature, plethysmograph CO₂ concentration, and ear capillary resistance, as well as significant increases in ear capillary blood flow. These changes are consistent with a loss of thermoregulatory control during REM sleep. The data are derived from all of the NREM-REM pairs during the control period (prior to DPAT dialysis) in protocol 2. All values are expressed as means ± SE and represent the absolute differences between NREM and REM sleep. *Significant differences between REM and NREM.

RESULTS

Suspension of thermoregulation mechanisms during REM sleep. When maintained in a continuous cool environment, the piglets consistently demonstrated decreases in body temperature, HR, and BPm on transition to REM sleep. The plethysmograph outlet [CO₂] also decreased, consistent with a decrease in VCO₂. Ear skin temperature consistently increased, and when measured, skin capillary blood flow also increased. A typical transition from NREM sleep to REM sleep in a single animal illustrating the decrease in body temperature, blood pressure and outlet [CO₂], and the progressive increase in ear capillary blood flow (Flux) and ear skin temperature is shown in Fig. 2. Summary data derived from all NREM to REM transitions in eight piglets during the control (pre-DPAT dialysis) period in protocol 2 are shown in Table 2.

Activation of 5-HT₁A receptors in the PGCL attenuates shivering during rapid cooling. In this protocol, the animals were cooled both before and after DPAT dialysis. The plethysmograph temperature profile during the cooling episodes in the experimental group is shown in Fig. 3 and was similar for the control group experiments. The timing and degree of cooling were similar before and after DPAT dialysis. A typical example of acute cooling before and after DPAT dialysis in a single

![Fig. 3. Mean plethysmograph temperature profiles, expressed as the absolute change in temperature, during rapid cooling before (CONTROL) and after 8-hydroxy-2-(di-n-propylamino)tetrabutylammonium (8-OH-DPAT, DPAT in figure) dialysis into the of paragigantocellularis lateralis (PGCL) obtained from six animals. C1 indicates the first half of cooling, and C2 indicates the second half. Solid lines are the means and the dashed lines indicate ± SE.]
animal is shown in Fig. 4 and illustrates the lack of shivering in the EMG channel both during REM before DPAT dialysis, and during NREM after DPAT dialysis. We were unable to analyze shivering during REM after DPAT dialysis, as REM sleep is almost completely absent under these conditions (12). Moreover, in piglets, like most mammals, shivering is completely suspended during REM sleep. The percent time shivering during cooling in NREM sleep was significantly attenuated after DPAT dialysis. In contrast, in control experiments, in which aCSF was substituted for DPAT in the dialysate, there was no difference in the percent time shivering during the first and second cooling period. In these control experiments, shivering occurred 79.7 ± 7.5 percent of the time in the first cooling exposure (COLD 1 and COLD 2 combined) and 71.8 ± 5.3 percent of the time in the second exposure ($P = 0.45$). The changes in the percent of time shivering during NREM sleep before and after DPAT or aCSF dialysis for the experimental and control groups are shown in Fig. 5. An increase in shivering during cooling was reflected in an increase in integrated neck EMG activity as shown in Fig. 6, and the increase was attenuated after DPAT dialysis. Rapid cooling also resulted in an increase in blood pressure and heart rate and a small decrease in body temperature. Cooling after DPAT dialysis resulted in similar changes in blood pressure but an attenuated increase in heart rate. Moreover, body temperature decreased more after DPAT dialysis (Fig. 7). In the control experiments where aCSF was substituted for DPAT, the increases in blood pressure (2.4 ± 0.6 vs. 2.3 ± 0.3 mmHg-COLD2) and heart rate (11.2 ± 2.2 vs. 8.4 ± 3.3 bpm-COLD2), and the decreases in core temperature (−0.21 ± 0.09 vs. −0.15 ± 0.06°C-COLD2) were not statistically different between the two cooling periods.

Shivering but not peripheral vasoconstriction produced by a cold environment is inhibited after activation of 5-HT1A receptors. In this protocol, the animal was in a cool environment throughout the experiment. A typical example of an experiment in a single piglet is shown in Fig. 8. During baseline REM sleep periods, there are decreases in outlet CO₂, heart rate, mean blood pressure, and body temperature but increases in ear capillary blood flow (Flux) and ear skin temperature consistent with a suspension of thermoregulatory mechanisms. After the onset of DPAT dialysis, sleep becomes fragmented with no REM and short periods of NREM alternating with periods of wakefulness (WAKE). In this example, there are small decreases in body temperature and heart rate after DPAT dialysis. However, there are no consistent changes in mean blood pressure, ear skin temperature, ear capillary blood flow, or CO₂. Soon after the onset of DPAT dialysis into the PGCL, increased motor activity is frequently observed during periods of WAKE, whereas during periods of NREM, there is general hypotonia (12). This might explain the apparent early increase in CO₂ since the large volume of the plethysmograph and the short alternating epochs of NREM and wakefulness may mask brief decreases in CO₂ production during NREM. On average,

![Diagram of EEG, EOG, and EMG channels showing shivering and control conditions.](image-url)

Fig. 4. An example of rapid cooling before and after unilateral DPAT dialysis into the PGCL of one animal, showing changes in EEG, EOG, and neck EMG activity. NREM sleep is characterized by a high-voltage, low-frequency EEG, minimal REMs, and relatively low neck EMG activity. REM sleep is accompanied by low-voltage, higher-frequency EEG, evidence of rapid movement in the EOG, and low neck EMG activity. Thermoneutral periods are indicated as WARM and rapid cooling as COLD. Shivering is indicated by an increase in activity of the neck EMG that occurs during cooling. Only NREM sleep is shown after DPAT dialysis, because under these conditions, REM sleep is almost completely abolished (12). Note that before DPAT dialysis (control period), shivering occurs during cooling only during NREM sleep and is completely absent after transitioning to REM sleep. Shivering is absent during NREM sleep after DPAT dialysis.

Fig. 5. Changes in the percentage of time spent shivering during NREM sleep resulting from rapid cooling from a thermoneutral environment before and after unilateral dialysis of DPAT (A), or artificial CSF (B) into the PGCL. COLD1 is the first half of cooling, and COLD 2 is the second half. Values are expressed as means ± SE. *Significant change from values obtained during thermoneutrality (at least $P < 0.05$). #Response after DPAT dialysis was significantly different from that before DPAT dialysis (at least $P < 0.05$).
there were significant decreases in HR and body temperature after DPAT dialysis but no significant changes in ear surface temperature, ear capillary blood flow or resistance, or outlet CO₂ concentration (Table 3). In three control experiments, in which aCSF was substituted for DPAT, there were also small decreases in heart rate and body temperature that were statistically not different from the decreases noted in the experimental group. In contrast, shivering was dramatically attenuated in the experimental group compared with the control group after DPAT and aCSF dialysis, respectively ($P \leq 0.003$). The time course for the percent time spent shivering for the two groups is shown in Fig. 9. Similarly, the number of shivering bouts per minute was significantly reduced in the DPAT group compared with the control group ($72.8 \pm 13.1$ vs. $9.1 \pm 7.7$ percent decrease, $P < 0.01$). The reduction in shivering was reflected in a significant decrease in average integrated neck EMG activity after DPAT dialysis ($33.1 \pm 9.7$ percent decrease, $P < 0.05$).

**DISCUSSION**

The major finding in this study is that in conscious piglets, activation of 5-HT₁A receptors located in the PGCL attenuates shivering during exposure to mild cooling. We also found that similar decreases in shivering occur during REM sleep when 5-HT neuronal activity is considered to be at a minimum. Shivering was assessed directly using video and neck EMG recordings, and decreases were indicated by a decrease in the percent time shivering, a decrease in the number of shivering bursts per minute, and a decrease in average integrated EMG activity. During continuous cooling (protocol 2), there were small decreases in heart rate and body temperature after DPAT dialysis, but these were not different from decreases observed...
Table 3. Unilateral dialysis of 8-OH-DPAT into the PGCL of animals maintained in a continuous cool environment: changes in cardiovascular and thermoregulatory variables during NREM sleep

<table>
<thead>
<tr>
<th>Variables</th>
<th>8-OH-DPAT (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>−15.5 ± 4.1*</td>
<td>0.007</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>−1.6 ± 1.7</td>
<td>0.387</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>−0.26 ± 0.08*</td>
<td>0.015</td>
</tr>
<tr>
<td>Ear skin temperature, °C</td>
<td>−0.07 ± 0.12</td>
<td>0.561</td>
</tr>
<tr>
<td>Ear capillary blood flow, au</td>
<td>0.02 ± 0.08</td>
<td>0.799</td>
</tr>
<tr>
<td>% Change</td>
<td>(2.2 ± 3.7)</td>
<td></td>
</tr>
<tr>
<td>Ear capillary resistance, au</td>
<td>−1.2 ± 4.9</td>
<td>0.819</td>
</tr>
<tr>
<td>% Change</td>
<td>(−1.9 ± 3.1)</td>
<td></td>
</tr>
<tr>
<td>Plethysmograph outlet [CO₂], %</td>
<td>−0.008 ± 0.007</td>
<td>0.263</td>
</tr>
</tbody>
</table>

For animals maintained in a continuous cool environment, unilateral dialysis of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) into the paragangiotocellularis lateralis (PGCL) resulted in a significant decrease in heart rate and body temperature, but no change in mean blood pressure, ear surface temperature, ear capillary blood flow, or resistance and plethysmograph CO₂ concentration. All values are the absolute difference between the last NREM epoch before DPAT dialysis and the averaged values of all subsequent NREM epochs and are expressed as means ± SE. The corresponding % changes are also shown for ear capillary blood flow and resistance. *Significant difference from baseline.

over time alone in a small number of control animals. During the rapid cooling (protocol 1), however, there were significant decreases in body temperature and heart rate that were not present in the control group. The differences in the results from the two protocols might be due to the small number of control animals in the continuous cooling protocol, or, possibly, they could be related to the varying effects of rapid cooling and a more constant cool environment. These differences will need to be confirmed in future experiments.

Previous evidence from our laboratory examining these cell groups with similar methodology showed that the effects of DPAT on sleep at thermoneutrality were largely abolished after destruction of 5-HT neurons (12). In these studies, however, effects on heart rate and body temperature were not attenuated, suggesting that these effects may have been due to activation of postsynaptic 5-HT₁A receptors located on non-5-HT neurons. We have not evaluated the effects of DPAT on shivering after destruction of 5-HT neurons. We hypothesize, however, that the effects of DPAT on shivering are due to activating 5-HT₁A autoreceptors located on the soma and dendrites of 5-HT neurons, thereby resulting in a decrease in 5-HT neuronal activity. The attenuation of shivering following activation of 5-HT₁A receptors in the PGCL suggests that serotonergic neurons in this region have an excitatory effect on shivering thermogenesis, most likely at the level of the spinal cord, and play a previously unreported role in the thermoregulatory response to cold exposure. Alternatively, the effects of DPAT on shivering that we observed could have resulted from activating postsynaptic receptors on other spinally projecting non-5-HT neurons involved in shivering thermogenesis.

In contrast, our data do not support a role for PGCL neurons in sympathetically mediated vasoconstriction. In contrast, neurons located in the midline medullary raphé appear to modulate many sympathetically driven thermoregulatory effector mechanisms, including brown adipose tissue (BAT) thermogenesis (42, 43), and peripheral vasoconstriction in rabbits (50) and rats (64). The current results in conscious piglets indicate that activation of 5-HT₁A receptors in the PGCL, a region containing a large number of serotonergic neurons located lateral to the raphé, decreases shivering thermogenesis but are not involved in peripheral vasoconstriction. This dichotomy may indicate a species-dependent difference, or the result of different experimental conditions. We believe, however, that the 5-HT₁A receptors located in the lateral medullary groups are less involved in control of peripheral vasomotor tone than in the control of shivering thermogenesis and therefore demonstrate an anatomical distribution of thermoregulatory function for serotonergic neurons in the medullary raphé and extra-raphé regions.

Dialysis of DPAT into the PGCL, the measurement of ear skin temperature, and neck EMG recordings were all performed on the right side. Measurements of ear surface capillary blood flow, however, were performed on the left ear. Changes in left ear surface capillary blood flow paralleled those of right ear surface temperature both before and after DPAT dialysis into the PGCL. Since sympathetic innervation of the blood vessels of the ears of rabbits and dogs is unilateral at the level of the spinal cord (15, 22), it is possible that the measurements of the left ear capillary blood flow would not be affected by right-sided PGCL DPAT dialysis. However, local administration of DPAT into the medullary raphé in rabbits does attenuate ear vessel vasoconstriction during cooling. If one assumes that unilateral inhibition of neurons in the PGCL will affect only ipsilateral vasoconstriction, our findings show, at least, that in our experiments, significant amounts of DPAT did not diffuse into the raphé. However, there is evidence that projections...
from some bulbospinal neurons in the PGCL and gigantocellular neurons are bilateral, with fibers crossing the midline to their regions of termination. Thus, it is also possible that if PGCL neurons were involved in vasocostriction, their unilateral inhibition would have bilateral sympathetic effects. In this case, the absence of changes in left-sided ear capillary blood flow after right-sided PGCL DPAT dialysis is consistent with our conclusions that neurons in the PGCL have little influence on peripheral vasocostriction during cooling.

The results of previous studies in our laboratory have shown decreases in skeletal muscle tone in REM sleep after DPAT dialysis into the PGCL (12), even when the animals were maintained in a thermoneutral environment. This may be similar to the muscle atonia commonly observed during REM sleep (27, 54). Magoun and Rhines (35) also observed an inhibitory effect of neurons in the caudal medulla on spinal motor activity, and several investigators have identified glycineric neurons in this region with projections to the spinal cord involved in muscle atonia occurring during REM sleep (20, 32, 33, 38), some of which appear to be serotonergic (18, 62). We believe that the hypotonia we previously observed after DPAT dialysis into the PGCL during thermoneutrality and the decrease in shivering after DPAT dialysis during cooling in the current study may be related to a dysfacilitation of serotonergic excitatory modulation of muscle tone at the level of the spinal cord.

Shivering is an involuntary tremor which, as described by Schäfer and Schäfer (58, 59), is caused by an oscillatory instability resulting via fusimotor innervation of skeletal muscle. It has been recently reported that cooling-induced fusimotor activation is inhibited after injecting glycine into the medullary raphe (65). This same study also reported injections in regions of the medulla located near the ventral surface, lateral to raphe pallidus as also having a significant, but more modulate, effect on fusimotor activation. These lateral groups may have included the PGCL, and when taken with the current findings, could be interpreted as resulting from a decrease in serotonergic activity in these regions.

In summary, we have reported the novel finding that serotonergic neurons located lateral to the midline medullary raphe are involved in the modulation of shivering in response to mild cooling. The activation of serotonergic 1A receptors was found to decrease the amount and intensity of shivering in the conscious animal. We speculate that excitatory inputs via serotonergic projections modulating fusimotor activity arise from the PGCL, as well as other areas of the medulla, including the raphe, and are essential for the generation of shivering tremor. Abnormalities in 5-HT1A receptors in these regions, as has been described in SIDS, could contribute to decreased thermoregulatory defense mechanisms.

ACKNOWLEDGMENTS

The authors would like to thank Eugene Nattie, Donald Bartlett, and Hannah Kinney for reviewing the manuscript and providing guidance; Laurie Hildebrandt for managing the laboratory; and Tracey Damon for identifying the location of our dialysis probe tips.

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GRANTS

Dr. Darnall is supported by the NIH (PO1HD36379; RO1HD045653) and a grant from the First Candle/SIDS Alliance.

REFERENCES


38. Kausz M.

Jacobs BL, Fornal CA.


