Adrenocorticotropic hormone and corticosterone responses to acute hypoxia in the neonatal rat: effects of body temperature maintenance

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PERIODS OF ACUTE HYPOXIA are among the more common neonatal stresses, particularly with prematurity (11, 21–23). Neonatal hypoxia can become a devastating condition requiring mechanical ventilation, O2 therapy, corticosteroids, and other supplemental therapies (29, 42, 43). Coordinated physiological and metabolic responses to hypoxia and a complete understanding of the development of these responses are crucial for positive clinical outcomes (13, 14, 25, 27).

Neonatal hypoxia leads to spontaneous hypothermia in a variety of mammalian species and may have the salutary effect of decreasing total body O2 consumption and metabolism (6, 12, 26, 45). To our knowledge, there are no specific guidelines for the control of body temperature in infants during periods of acute hypoxia. The consensus appears to be that prevention of hypothermia during hypoxia is warranted, even though this may place an additional neural and metabolic stress on the infant. Conversely, the use of therapeutic hypothermia following periods of neonatal hypoxia to minimize hypoxic-ischemic encephalopathy has received much attention, as it has been shown to decrease brain injury during reoxygenation (16, 28, 39).

We previously demonstrated that the neonatal corticosterone response to acute hypoxia shifts from relative ACTH independence to ACTH dependence between postnatal day 2 (PD2) and postnatal day 8 (PD8) (4). We also documented a profound spontaneous decrease in body temperature during acute hypoxia. This phenomenon has been termed the “hypoxic thermal response” and is thought to protect an organism from severe metabolic stress and brain damage by allowing a decrease in whole body O2 consumption (33, 40).

The current study evaluated the ACTH and corticosterone responses to acute hypoxia in PD2 and PD8 rats. We assessed responses in pups allowed to become spontaneously hypothermic compared with those in which hypothermia was prevented by maintenance of body temperature. Assuming that the hypothermic response to hypoxia is a beneficial adaptation, as has been suggested previously (26), we hypothesized that preventing the decrease in body temperature would constitute an additional stress to the animal. Therefore, we hypothesized that aggressive maintenance of isothermia would augment pituitary-adrenocortical responses to acute hypoxia in the neonatal rat.

METHODS

Animal treatment and experimental protocol. The Aurora Health Care Institutional Animal Care and Use Committee approved the animal protocol. Timed-pregnant Sprague-Dawley rats at gestational day 15 or 18 (n = 117) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and maintained on a standard diet and water ad libitum in a controlled environment (lights-on from 0600 to 1800, 23°C room temperature). The size of litters born in-house was normalized (12–14 rats/litter, mixed sexes). On the morning of experimentation, litters without dams were placed in an environmental chamber. Pups were placed on a standard heating pad (Moore Medical, Farmington, CT) overlaid with an adequate amount of bedding. Litters were kept separate during the experiment, with each litter occupying a ~6 × 12 in. space in the chamber. For the first 30 min (prehypoxia period), 21% O2 was supplied to the chamber at a rate of ~8 l/min, and rectal temperature was recorded in one sentinel pup per litter using RET-3-Iso probes and a BAT-12 digital thermometer connected to a SBT-5 switchbox (Physitemp Instruments, Clifton, NJ). The base of the temperature probe was taped to the pup’s tail to keep the probe in place. Pups were allowed to huddle together at the beginning of the experiment, with care taken to keep the sentinel pup within the huddle. All pups were allowed to move freely once experimentation was commenced.
Results

Initial body temperature (obtained immediately upon instrumentation) at the start of the 30-min prehypoxia period was 32.6 ± 0.3°C in PD2 rats and 34.4 ± 0.3°C in PD8 rats (P < 0.001). Values at both ages were similar to those previously reported for pups allowed to huddle in the absence of the dam (1, 10). Changes in body temperature during the 30-min prehypoxia (control) period are shown in Fig. 1A. Body temperature in group I pups (no external heat) significantly decreased at both ages during the prehypoxia period (F1,51 = 82.4, P < 0.001). Use of servo-controlled heat during the prehypoxia period (group II) maintained body temperature near isothermia at both ages. Application of external heat to maintain body temperature (group II) during the prehypoxia period elicited a significant increase in plasma ACTH at both ages (Fig. 1B).

Group I: prehypoxia body temperature not controlled. Body temperature was allowed to decrease during the prehypoxia period (i.e., thermoneutrality was not maintained). After the prehypoxia period, the O2 concentration of the chamber was decreased to 8% for 3 h. Body temperature was allowed to spontaneously decrease (hypoxia-hypothermia) or was maintained using servo-controlled external heat (hypoxia-isothermia). Servo-controlled heat entailed small adjustments to the heating pad setting in response to changes in body temperature. In the hypoxia-isothermia group, the target body temperature was set at the level at the end of the prehypoxia period. A separate set of litters was exposed to 21% O2 for 3 h, and their body temperature was not controlled (normoxia; time-control).

Group II: prehypoxia body temperature controlled. Thermoneutrality was maintained during the prehypoxia period using servo-controlled heat, as described above. Again, a separate set of litters was exposed to 21% O2 for 3 h, but body temperature was maintained using servo-controlled external heat (normoxia-isothermia). During the hypoxic exposure, body temperature was manipulated as follows: 1) spontaneous decrease (hypoxia-hypothermia; no external heat), 2) spontaneous decrease, but with constant external heat set at the level required to maintain isothermia in normoxic pups (hypoxia-hypothermia; low external heat), or 3) isothermia maintained using servo-controlled external heat (hypoxia-isothermia). For all rats in group II, the target body temperature was defined as the value obtained at the onset of the prehypoxia period.

Sample collection. Baseline measurements were obtained following the 30-min prehypoxia period, at which time half of each litter was removed from the chamber and killed. Trunk blood was pooled and collected in EDTA, and adrenal glands were pooled and quickly frozen in liquid nitrogen (2–3 rats per plasma or adrenal sample). After 3 h at 8% or 21% O2, the remaining pups were removed from the chamber and killed, and samples were collected as they were at baseline.

Hormone assays. Plasma ACTH and corticosterone were measured by radioimmunoassay, as described previously (MP Biomedicals, Solon, OH) (31).

RNA isolation and real-time PCR analysis. Real-time PCR was performed on total RNA isolated from adrenal glands using the RNeasy Mini protocol (Qiagen, Valencia, CA). The concentration of RNA was quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA). All RNA samples were diluted to a final concentration of 10 –20 ng/μl in the PCR assay. The Taqman One-Step RT-PCR protocol and premade primers and probes (Applied Biosystems, Foster City, CA) were used for all real-time assays. The final reaction volume of 25 μl consisted of 1× AmpliTaq Gold DNA polymerase mix, 1× RT enzyme mix containing MultiScribe reverse transcriptase and RNase inhibitor, 1× primer/probe mix, and 50–100 ng of total RNA. The following thermal cycler conditions were used during amplification and detection performed with the ABI Prism 7900HT Sequence Detection System: 48°C for 30 min reverse transcription, 95°C for 10 min, and 40 cycles at 95°C for 0.25 min and 60°C for 1 min. Each sample was assayed in triplicate. The number of cycles required to reach a predetermined threshold value in the intensity of the PCR signal [cycle threshold (Ct) value] was used to quantify gene expression.

Statistical analyses. Hormone and real-time PCR data were analyzed by two-way ANOVA. Body temperature data were analyzed by two-way ANOVA for repeated measures; P < 0.05 was considered significant. All post hoc analyses were performed by Student-Newman-Keuls method for multiple comparisons (SigmaStat 2.03).
compared with ACTH in group I pups at the same age ($F_{1,82} = 26.2$, $P < 0.001$). In addition, the ACTH response was more pronounced in group II PD8 than group II PD2 pups ($F_{1,82} = 9.9$, $P = 0.002$). There was no significant effect of body temperature maintenance during the prehypoxia period on plasma corticosterone (Fig. 1C), while plasma corticosterone was significantly lower in PD8 pups, regardless of the strategy used to maintain body temperature ($F_{1,83} = 22.8$, $P < 0.001$). Baseline ACTH and corticosterone values were similar to those previously reported by us in pups maintained with their lactating dams (31).

Figure 2 shows changes in body temperature during 3 h of normoxia (time control) in group I and II pups at both ages. In PD2 pups exposed to normoxia without application of external heat (heat off), body temperature decreased significantly and progressively between each time point ($P < 0.002$), except between 90 and 120 min ($P = 0.345$). PD8 pups exposed to normoxia for 3 h (heat off) did not exhibit significant changes in body temperature between time points; however, body temperature decreased significantly by the end of the 3-h period compared with baseline (0.8°C; $P = 0.019$). While there were fluctuations in body temperature in PD2 pups exposed to normoxia for 3 h (servo-controlled heat), changes were not significant. PD8 pups from the servo-controlled group exhibited a small decrease in body temperature between 0 and 30 min ($P < 0.001$), but body temperature was subsequently unchanged throughout the remainder of the 3-h period.

Table 1 lists plasma ACTH and corticosterone concentrations in group I and II pups following 3 h of normoxia. In group I PD2 pups (heat off), plasma ACTH and corticosterone were significantly increased following 3 h of normoxia ($P = 0.017$ and $P < 0.001$, respectively). Preventing the decrease in body temperature (Fig. 2) in normoxic PD2 pups with servo-controlled heat (group II) also prevented the increase in ACTH and corticosterone. In fact, plasma corticosterone in group II PD2 pups had decreased to below baseline levels following 3 h of normoxia ($P = 0.017$). The 3-h normoxic period had no significant effect on plasma ACTH or corticosterone in PD8 pups without (group I) or with (group II) maintenance of body temperature with external heat. Thus any effect of 3 h of maternal separation on ACTH and corticosterone was absent in PD8 pups.

Figure 3 displays changes in body temperature during 3 h of hypoxia in group I pups. Hypoxia (heat off) resulted in a significant decrease in body temperature after 30 min of exposure in PD2 ($F_{3,18} = 18.0$, $P < 0.001$) and PD8 ($F_{3,15} = 11.0$, $P < 0.001$) pups, and body temperature continued to decrease during most of the 3-h time period. The magnitude of the decrease in body temperature was greater in PD8 than PD2 pups after 2 h of hypoxia ($F_{1,1} = 5.8$, $P = 0.034$). Servo-controlled heat successfully maintained isothermia during acute hypoxia (albeit at the lower baseline levels shown in Fig. 1), as there were no significant changes in body temperature in PD2 or PD8 pups.

Plasma ACTH and corticosterone concentrations in group I PD2 and PD8 pups are shown in Fig. 4. In PD2 pups, hypoxia (heat off) did not affect plasma ACTH compared with normoxic values, and plasma ACTH was also not affected by hypoxia with servo-controlled heat. Hypoxia (heat off) in PD8

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**Table 1. ACTH and corticosterone concentrations following 3 h of normoxia (21% O2): time control**

<table>
<thead>
<tr>
<th></th>
<th>ACTH, pg/ml</th>
<th>Corticosterone, ng/ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Normoxia</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Normoxia</td>
</tr>
<tr>
<td>Group I (no heat)</td>
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<tr>
<td>PD2</td>
<td>59 ± 2</td>
<td>104 ± 6*</td>
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<tr>
<td></td>
<td>45 ± 8</td>
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</tr>
<tr>
<td></td>
<td>20 ± 4</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Group II (servo-controlled heat)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD2</td>
<td>86 ± 8</td>
<td>85 ± 7</td>
</tr>
<tr>
<td></td>
<td>43 ± 7</td>
<td>22 ± 4*</td>
</tr>
<tr>
<td>PD8</td>
<td>117 ± 4</td>
<td>98 ± 3</td>
</tr>
<tr>
<td></td>
<td>22 ± 3</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 21–35$ (baseline) and 8–9 (normoxia). Thermoneutrality was not maintained during prehypoxia period in group I pups, nor was external heat applied during 3-h experimental period; the opposite was true in group II pups. Baseline represents values obtained after 30-min period preceding 3-h normoxic exposure. PD2 and PD8, postnatal days 2 and 8. *Significantly different from baseline within the specified age group ($P < 0.02$).

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Fig. 2. Changes in body temperature during 3 h of normoxia (21% O2; time control) in group I and II pups. Values are from group I and II pups at PD2 and PD8; $n = 3–5$ measurements per group (PD2 and PD8). *Significant decrease in body temperature from the previous time point within the specified age group ($P < 0.05$). +Significant difference from baseline (0 min) value within the same age group ($P < 0.05$).

Fig. 3. Changes in body temperature during 3 h of hypoxia in group I pups. External heat was not used during the prehypoxia period; therefore, pups were not thermoneutral at baseline. Values represent 6–8 measurements per experimental treatment at each age. *Significant decrease from the previous time point within the specified age group ($P < 0.05$). +Significant difference in the magnitude of reduction compared with PD2 at the specified time point (hypothermia only; $P < 0.05$).
pups resulted in a large increase in plasma ACTH compared with normoxic values (P < 0.001), and this response was further augmented with the maintenance of isothermia via servo-controlled heat (P < 0.001). Hypoxia per se also had a significant effect on plasma corticosterone at both ages (F3,63 = 237.2, P < 0.001). At PD2, hypoxia (heat off) only tended to increase plasma corticosterone concentrations compared with normoxic values (P = 0.057). Maintenance of isothermia greatly augmented the corticosterone response to hypoxia in PD2 rats (P < 0.001). Hypoxia (heat off) also increased plasma corticosterone in PD8 rats (P < 0.001), and maintenance of isothermia elicited a further increase (P < 0.001).

Table 2 lists adrenal real-time PCR data for group I pups. In PD2 adrenals, hypoxia (heat off) did not affect mRNA expression of any of the selected genes. When isothermia was maintained in PD2 pups via servo-controlled heat, melanocortin 2/ACTH receptor (Mc2r) mRNA expression was decreased compared with baseline values (P = 0.003). In PD8 adrenals, hypoxia (heat off) elicited a significant increase in LDL receptor (Ldlr) mRNA expression compared with baseline (P = 0.005). Hypoxia with isothermia in PD8 pups increased steroidogenic acute regulatory protein (Star) and Ldlr mRNA expression compared with baseline (P = 0.001 and P = 0.002, respectively).

Figure 5 depicts changes in body temperature during 3 h of hypoxia in group II pups. Recall that body temperature was maintained during the prehypoxia period via servo-controlled heat in these pups. Isothermia was successfully maintained during hypoxia via servo-controlled heat, as body temperature was not different from baseline throughout the 3-h hypoxic period (both ages). When only modest hypothermia was allowed (low heat) in PD2 pups, body temperature significantly decreased between 30 and 60 min of hypoxia (P = 0.021) but remained constant throughout the remainder of the hypoxic period. In PD8 pups exposed to hypoxia with low heat, body temperature decreased after 30 min (P = 0.003) but did not change significantly for the remainder of the hypoxic period. By contrast, when no external heat was applied (heat off), body temperature decreased during the first 90 min of the hypoxic period in PD2 and PD8 pups (P < 0.001). By the end of 3 h of hypoxia, body temperature had decreased by ~10°C in PD2 and PD8 pups.

Plasma ACTH and corticosterone concentrations in group II pups are shown in Fig. 6. Plasma ACTH levels in group II normoxic pups (both ages) were not different from the corresponding baseline values listed in Table 1 (F1,72 = 2.1, P = 0.156). In PD2 pups, plasma ACTH was not different from normoxic values, regardless of the strategy used to maintain body temperature during the hypoxic period. By contrast, plasma ACTH in PD8 pups was significantly increased by hypoxia without external heat (heat off; P < 0.001), was increased further when low heat was applied (P < 0.001), and was again augmented when body temperature was aggressively maintained (P < 0.001). Interestingly, plasma corticosterone in group II normoxic (PD2) pups was lower than the corresponding baseline value listed in Table 1 (P = 0.017). There was no

Table 2. Adrenal real-time PCR in group I pups

<table>
<thead>
<tr>
<th>Gene</th>
<th>Baseline</th>
<th>Hypoxia (no external heat)</th>
<th>Hypoxia (servo-controlled heat)</th>
<th>Baseline</th>
<th>Hypoxia (no external heat)</th>
<th>Hypoxia (servo-controlled heat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Star</td>
<td>18.50 ± 0.11</td>
<td>18.71 ± 0.09</td>
<td>18.96 ± 0.32</td>
<td>22.62 ± 0.17</td>
<td>22.23 ± 0.25</td>
<td>21.16 ± 0.11 *</td>
</tr>
<tr>
<td>Ldlr</td>
<td>28.50 ± 0.32</td>
<td>28.14 ± 0.57</td>
<td>26.58 ± 0.20</td>
<td>32.47 ± 0.39</td>
<td>31.09 ± 0.17 *</td>
<td>30.62 ± 0.12 *</td>
</tr>
<tr>
<td>Mc2r</td>
<td>24.29 ± 0.19</td>
<td>24.57 ± 0.07</td>
<td>25.26 ± 0.15 *</td>
<td>30.02 ± 0.22</td>
<td>30.70 ± 0.10</td>
<td>29.84 ± 0.31</td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed as number of cycles required to reach a predetermined threshold value (Ct); n = 4 pooled adrenal samples per experimental group at each age. A decrease in Ct indicates increased mRNA expression. Thermoneutrality was not maintained during hypoxia period in group I pups. Baseline represents values obtained after 30-min prehypoxia period and immediately preceding hypoxic exposure. Litters were stratified into treatments during 3 h of hypoxia (8% O2). Star, steroidogenic acute regulatory protein; Ldlr, LDL receptor; Mc2r, melanocortin 2/ACTH receptor. *Significantly different from baseline within the specified age group (P < 0.05).
effect of 3 h of normoxia on corticosterone in PD8 pups compared with baseline ($P = 0.679$). Plasma corticosterone was significantly increased in PD2 pups exposed to hypoxia without external heat ($P < 0.001$) and was further increased by application of low external heat ($P < 0.001$) but was not augmented further when body temperature was aggressively maintained ($P = 0.392$). In PD8 pups, plasma corticosterone responses were also increased by hypoxia without external heat ($P = 0.011$) and were further increased by application of low external heat ($P = 0.04$). The magnitude of the corticosterone responses was much greater in PD2 than PD8 pups ($F_{1,90} = 83.6, P < 0.001$).

Table 3 lists adrenal real-time PCR data for group II pups. At PD2, 3 h of normoxia decreased adrenal Star and Ldlr mRNA expression compared with baseline ($P = 0.006$ and $P = 0.025$, respectively). None of the three treatment protocols employed during hypoxia affected mRNA expression in PD2 adrenals (compared with baseline); however, Star mRNA expression was increased to a similar extent by all three treatments compared with normoxia values ($P < 0.007$). At PD8, Star mRNA expression increased during normoxia, hypothermia (low external heat), and isothermia compared with baseline. In addition, Ldlr mRNA expression in PD8 adrenals was increased during hypoxia compared with baseline, regardless of treatment protocol.

**DISCUSSION**

The present study evaluated body temperature, ACTH, and corticosterone responses to acute hypoxia in PD2 and PD8 rats. We assessed differences in these responses between pups allowed to become spontaneously hypothermic and those in which hypothermia was prevented by control of external heat. We also examined the effects of these treatments on expression of adrenal Star, Ldlr, and Mc2r mRNA, genes that encode upstream proteins involved in the steroidogenic pathway. Hypoxia alone (no external heat) decreased body temperatures by $\sim 10^\circ$C in pups at both ages. Hypoxia with spontaneous hypothermia increased plasma corticosterone at both ages, although plasma ACTH was increased in PD8 pups only. Maintenance of isothermia during hypoxia via servo-controlled external heat more than tripled the corticosterone response in PD2 rats and nearly doubled the corticosterone response in PD8 rats. Star and Ldlr mRNA expression was increased in PD8 adrenals when these pups were maintained at isothermia during the hypoxic exposure.

Baseline plasma ACTH and corticosterone values in the present studies were similar to those of pups maintained with their dams (31), indicating that the pups were not stressed by 30 min of maternal separation (prehypoxia period). We previously showed that a 4-h period of maternal separation under normoxic conditions does not effect body temperature, ACTH, or corticosterone in PD8 pups (4). Data from the present study confirm these findings, as 3 h of normoxia had no effect on plasma ACTH or corticosterone at PD8. In contrast, 3 h of normoxia in PD2 pups elicited a decrease in body temperature and increases in plasma ACTH and corticosterone. Maintenance of body temperature with servo-controlled heat prevented these increases, indicating that decreased body temperature elicited the ACTH and corticosterone responses, and not maternal separation per se. Interestingly, maintaining thermoneutrality with servo-controlled external heat during the 30-min prehypoxia period resulted in a small increase in plasma ACTH at both ages.

![Fig. 5. Changes in body temperature during 3 h of hypoxia in group II pups. External heat was used during the prehypoxia period; therefore, pups were thermoneutral at baseline. Values represent 4–6 measurements per experimental treatment at each age. For all time points at $\geq 60$ min, there was a significant difference between each of the three treatment groups, regardless of age (statistical symbols not shown). *Significant decrease in body temperature from the previous time point within the specified age group ($P < 0.05$).](http://ajpregu.physiology.org/)

**Fig. 5.** Changes in body temperature during 3 h of hypoxia in group II pups. External heat was used during the prehypoxia period; therefore, pups were thermoneutral at baseline. Values represent 4–6 measurements per experimental treatment at each age. For all time points at $\geq 60$ min, there was a significant difference between each of the three treatment groups, regardless of age (statistical symbols not shown). *Significant decrease in body temperature from the previous time point within the specified age group ($P < 0.05$).

![Fig. 6. Plasma ACTH and corticosterone concentrations following 3 h of hypoxia in group II pups. External heat was used during the prehypoxia period; therefore, pups were thermoneutral at baseline. Values represent 9–20 measurements for each analyte. *Significantly different from normoxia within the same age group ($P < 0.05$). #Significantly different from hypoxia/hypothermia (heat off) within the same age group ($P < 0.05$). %Significantly different from hypoxia/hypothermia (low heat) within the same age group ($P < 0.05$).](http://ajpregu.physiology.org/)

**Fig. 6.** Plasma ACTH and corticosterone concentrations following 3 h of hypoxia in group II pups. External heat was used during the prehypoxia period; therefore, pups were thermoneutral at baseline. Values represent 9–20 measurements for each analyte. *Significantly different from normoxia within the same age group ($P < 0.05$). #Significantly different from hypoxia/hypothermia (heat off) within the same age group ($P < 0.05$). %Significantly different from hypoxia/hypothermia (low heat) within the same age group ($P < 0.05$).
Hydroxy with hypothermia was a significant physiological stressor and increased plasma corticosterone in PD2 and PD8 rats, as we showed previously (4). The present data clearly demonstrate that aggressive maintenance of body temperature amplifies the corticosterone response to acute hypoxia, regardless of age. The data also indicate that the major stimulus for the corticosterone response at PD2 was not increased plasma ACTH concentration, whereas this was the case in PD8 rats. The lower basal plasma corticosterone concentration in PD8 pups was likely due to decreased adrenal sensitivity to ACTH and decreased levels of corticosteroid-binding globulin (2, 7).

What mechanisms defined these age-dependent shifts in hypothalamus-pituitary-adrenal (HPA) activity during acute hypoxia? When body temperature in PD2 pups was not controlled during the prehypoxia period (group I), subsequent hypoxia with hypothermia increased plasma corticosterone without concomitant increases in ACTH. Maintenance of isothermia during hypoxia increased plasma corticosterone further, with only a relatively minor increase in ACTH. In contrast, in pups kept thermoneutral during the prehypoxia period (group II), this minor increase in ACTH did not occur. It is possible that increased corticosterone production during hypoxia in PD2 pups was partially driven by postganglionic sympathetic nerve input into the adrenal cortex (32). It is also likely that increased steroid production was at least partially driven by direct activation of chromaffin cells (41). Chromaffin cells from PD2 rats can synthesize and secrete catecholamines in direct response to stressful stimuli, such as hypoxia (37, 38). A local increase in catecholamine concentration might act in a paracrine fashion to stimulate steroid production, particularly since chromaffin cells are dispersed in the neonatal adrenal cortex (9, 36). This suggests that the corticosterone response to acute hypoxia at PD2 was not the result of increased plasma ACTH.

If the corticosterone response during acute hypoxia at PD2 was ACTH-independent, then the response at PD8 was likely to be ACTH-dependent. The dynamics of the ACTH and corticosterone responses at PD8 were similar to responses to acute stress in the adult rat. The ACTH responses observed in PD8 rats were, in turn, highly dependent on body temperature. As noted above, maintenance of isothermia during the prehypoxia period increased plasma ACTH at baseline. This effect had a significant influence on temperature-dependent changes in ACTH during hypoxia. Pups that were thermoneutral at baseline (group I) had more pronounced ACTH responses to hypoxia with low external heat and to hypoxia with isothermia than the same pups in group II (not thermoneutral at baseline). Hypoxia with hypothermia (no external heat) elicited similar ACTH responses in group I and II pups at PD8. Regardless of baseline body temperature, maintenance of isothermia during hypoxia augmented plasma ACTH compared with pups treated with minimal external heat. This finding suggests a positive correlation between ACTH responses and aggressive maintenance of body temperature during hypoxia in PD8 rats.

Corticosterone responses to acute hypoxia in PD8 rats correlated with increased plasma ACTH levels, regardless of body temperature. A period of adrenocortical hyporesponsiveness to stimulation in the neonatal rat has been established, and PD8 occurs during the middle of this period (2, 44). The existence of such a period has been proposed as a protective mechanism to keep the rapidly developing brain from increased glucocorticoid exposure (35). Acute hypoxia with spontaneous hypothermia overrode this hyporesponsiveness, and maintenance of isothermia further activated the HPA axis. Increased plasma ACTH in PD8 pups likely had a stimulatory effect on the expression of adrenal Star and Ldlr mRNA expression, which in turn could contribute to increased corticosterone production (3). Increases in plasma corticosterone at PD8 may also have been facilitated by direct stimulation of the adrenal cortex via the splanchnic nerves, as we previously showed that postganglionic sympathetic blockade can inhibit corticosterone responses to chronic neonatal hypoxia (32). Furthermore, it is possible that neuronal stimulation of chromaffin cell catecholamine synthesis also played a role, and increased plasma epinephrine concentrations at PD8 would support this (15, 34).

Aggressive maintenance of isothermia without resolution of hypoxemia may expose the neonate to pathologically high glucocorticoid levels (18, 19). Blockade of corticosterone synthesis with metyrapone prevents hypoxia/ischemia-induced losses in hippocampal function that occur 1 day after hypoxic exposure (20). In addition, recent data suggest that the release of cortisol from corticosteroid-binding globulin is temperature-dependent.

### Table 3. Adrenal real-time PCR in group II pups

<table>
<thead>
<tr>
<th>Gene</th>
<th>Baseline</th>
<th>Normoxia (servo-controlled heat)</th>
<th>Hypothermia</th>
<th>Isothermia (servo-controlled heat)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PD2</td>
<td>Low external heat</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>No external heat</td>
<td>Low external heat</td>
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<tr>
<td>Star</td>
<td>18.34 ± 0.26</td>
<td>17.13 ± 0.37*</td>
<td>17.79 ± 0.11</td>
<td>17.21 ± 0.18*</td>
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<tr>
<td>Ldlr</td>
<td>23.40 ± 0.19</td>
<td>23.70 ± 0.71</td>
<td>22.71 ± 0.14*</td>
<td>23.44 ± 0.42*</td>
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<tr>
<td>Mc2r</td>
<td>26.15 ± 0.22</td>
<td>25.68 ± 0.20</td>
<td>26.53 ± 0.12*</td>
<td>26.33 ± 0.14</td>
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Values are means ± SE, expressed as number of cycles required to reach a predetermined threshold value (Ct); n = 4 pooled adrenal samples per experimental group at each age. Decrease in Ct indicates increased mRNA expression. Thermoneutrality was maintained during prehypoxia period in group II pups. Baseline represents values obtained after 30-min prehypoxia period and immediately preceding normoxic or hypoxic exposure. Litters were stratified into treatments, with minimal external heat. Significantly different from baseline within the specified age group (P < 0.05). †Significantly different from normoxia within the specified age group (P < 0.05).
sensitive (5). Maintenance of isothermia during hypoxia may significantly augment the concentration of free (unbound) glucocorticoid, thereby amplifying the effects of increased adrenocortical steroid production and, possibly, worsening the clinical outcome. Studies of the long-term consequences of glucocorticoid exposure (endogenous or exogenous) during the neonatal period have provided evidence of lasting defects in physiological function (8, 17, 30). On the other hand, generation of an augmented corticosterone response could be beneficial during neonatal hypoxia, if it resulted in improved pulmonary function (24).

What might be the central nervous system mechanism resulting in augmentation of the HPA response to hypoxia when isothermia was maintained? It is generally accepted that the hypoxic thermal response, also called hypoxia-induced anapnoea, is due to a direct hypothalamic effect on the set point for body temperature (40). Isothermic hypoxia may have resulted in a discrepancy between core temperature and this set point, resulting in increased hypothalamic drive to the adrenal cortex. This could have occurred through different sympathetic nervous system pathways in P2D pups or through corticotropin-releasing hormone neurons in the paraventricular nucleus, leading to large increases in plasma ACTH in P8 pups.

**Perspectives and Significance**

We have provided evidence that maintenance of isothermia during an episode of acute hypoxia in the neonate should be reconsidered. Therapeutic interventions aimed at maintaining body temperature during correction of the O2 deficit may prevent a beneficial hypometabolic survival response (45). The increased metabolic stress placed on a hypoxic neonate maintained at isothermia may have significant short- and long-term consequences. We suggest that studies be undertaken to develop a consensus on the proper approach to the control of body temperature and possible untoward effects on pituitary-adrenocortical stress responses in the hypoxic infant.

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**DISCLOSURES**

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