SHIVERING THERMOGENESIS IN THE NEONATAL PIG

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(Received 28 April 1994; accepted in revised form 23 August 1994)

Abstract—(1) Shivering intensity and metabolic rate were determined in Large White pigs aged 2, 24, 48 h and 5 d, at temperatures ranging from thermoneutrality (36°C) to cold (20°C). (2) Shivering is the main heat producing mechanism, the absence of nonshivering thermogenesis being implied by both the absence of delay between the onset of shivering (Stt) and the increase in metabolic rate (Lm) and by the linearity of the relationship between metabolic rate and shivering intensity in the cold. (3) For a comparable thermal demand, shivering intensity decreased with age whereas cold induced heat production remained constant, which suggests that the thermogenic efficiency of shivering is improved during the first 5 days of life.

Key Word Index: Shivering; piglet; metabolic rate; newborn.

INTRODUCTION

At birth, the newborn piglet usually experiences a sudden and dramatic 15 to 20°C decrease in its thermal environment. Because the newborn pig is poorly insulated, maintenance of homeothermia depends almost exclusively on its capacity to produce heat. Unfortunately, unlike most of the other newborn mammals, the pig does not possess brown adipose tissue, as suggested by both the absence of response to the injection of noradrenaline during the first week of life (LeBlanc and Mount, 1969) and by the immunoblotting studies of Trayhurn et al. (1989) on the uncoupling protein. Consequently, neonatal pigs are assumed to rely essentially on muscular thermogenesis for thermoregulatory purpose (Brück et al., 1969). In fact, newborn pigs shiver vigorously from birth (Mount, 1968), but measurement of shivering has only been performed on pigs aged 7 or 8 weeks (Heath and Ingram, 1983). In addition, non-shivering thermogenesis of muscular origin cannot be completely excluded in the newborn pig since loosely coupled mitochondria have been previously isolated from the rhomboideus muscle of 8-week old cold-acclimated pigs (Herpin and Barré, 1989; Herpin and Lefaucheur, 1992).

The purpose of our study was, therefore, to determine the occurrence and the magnitude of shivering during the neonatal period. To evaluate the relation between the metabolic response to cold and the intensity of shivering and therefore, to determine the thermogenic capacity of shivering, both responses were measured simultaneously at temperatures ranging from thermoneutrality (36°C) to cold (20°C). This allowed to compare the ambient temperature eliciting the first increase in metabolic rate in the cold (Lm) with the threshold of ambient temperature for directly recorded shivering (Stt) and, hence, to question the possible existence of nonshivering thermogenesis.

MATERIALS AND METHODS

Animals

Forty newborn Large White pigs from 17 litters were used. Parturition was induced with an i.m. injection of a prostaglandin analogue (ICI 80996) on day 113 of gestation, to ensure farrowing on day 114. Sows were kept in the usual farrowing house conditions, at an ambient temperature of 20°C. Piglets were provided with straw bedding and 2 infrared lamps of 250 W on each side of the sow in order to minimize heat loss. Within each litter, piglets with birth weight ranging from 1.0 to 1.4 kg were selected for the measurements of metabolic rate and shivering intensity at 2, 24, 48 h and 5 days of age.

Measurements

Metabolic rate was measured by indirect calorimetry as previously described (Berthon et al., 1993) using 3 open-circuit temperature-controlled respiration chambers. Briefly, piglet O2 consumption and CO2 production were determined during successive 200 s period from the difference in O2 and CO2...
concentrations between the air entering and leaving each chamber, and from the flow-rate of the extracted air, a correction being applied for any change in the O₂ and CO₂ content of the chamber during this period. O₂ and CO₂ were measured using a paramagnetic O₂ analyzer (Oxygor, Maihak, Colombes, F) and an infrared CO₂ analyzer (Finor, Maihak, Colombes, F). The flow-rate of the extracted air was measured continuously using a flowmeter (HFM, Hastings, Nozay, F).

Shivering intensity was assessed as the integrated electromyographic activity (integrated EMG) of *longissimus dorsi* muscle. This muscle was chosen because it was easy to reach, wide and thick enough to insert the electrodes precisely. Attempts were also made to measure EMG on the interscapular muscles, particularly on the *rhomboides* because of its potentially unique role in thermoregulation (Herpin and Barré, 1989; Herpin and Lefaucheur, 1992; Harrison et al., 1994). However, it was too thin to ensure a correct and repetitive insertion of the electrodes and permanent insertion of the electrodes under light anaesthesia affected the thermoregulatory function for several hours. Besides, because piglets were returned to the sow between each measurement and because fighting frequency was high in the early neonatal period, maintenance of functional permanent electrodes was questionable. Direct EMG was recorded on an electrocardiograph (Alvar, Reega Minihuit TR, Montreuil, F) calibrated in voltage by means of an internally generated signal, using 3 monopolar electrodes insulated except for the tips (Stabilohm 110, 0.12 mm diameter, Johnson Matthey Metals, Roissy, F). The measurement of the electrical activity was determined between 2 electrodes while the third one was connected with the ground. The electrodes were inserted 5 mm apart in the *longissimus dorsi* at the level of the ninth pair of rib, 1 cm apart from the spinal column. The same position was used for all the animals and each animal was utilized twice, the electrodes being inserted only once in each muscle, on each side of the spinal column. The electrodes were removed at the end of each measurement before returning the piglets to the sow. The amplified signal was high-pass filtered, rectified and summed for 10 s periods with an integrator made as indicated by Latour and Ferre (1985) and recorded on a polygraph (Labograph E586). The simultaneous recording of direct and integrated EMG is particularly useful in identifying and eliminating the periods of movement of the animal.

Procedure

During the experiments, piglets were restrained in a comfort sling in order to minimize physical movement. Metabolic rate and integrated EMG were recorded over 15 min after an initial 40 min period which was necessary to reach thermal equilibrium and metabolic steady state after each change in ambient temperature (Berthon et al., 1993). During this 15 min period, EMG was recorded at least 3 times over a 1 min period while piglets rested quietly. Within the respiratory chamber, constant ambient temperatures ranging from thermoneutrality (36°C) to moderate cold (20°C) were successively maintained to determine the ambient temperature eliciting the first increase in metabolic rate corresponding to the lower critical temperature (Lc₁) and to compare it to the threshold of ambient temperature for shivering (S_th).) To take into account the decrease in Lc with age (Berthon et al., 1993), the measurements were performed at ambient temperatures ranging from 36°C to 25°C at 2 h and 24 h, from 34°C to 25°C at 48 h and from 34°C to 20°C at 5 days of age. Correct recording of integrated EMG was questionable at temperatures lower than 20°C because piglets were too restless and exhibited severe muscular shaking. To minimize the time spent in the chamber and the time of food deprivation, each animal was only used at 3 or 4 temperatures, which included at least a thermoneutral one. Before each measurement, rectal temperature and body weight of piglets were recorded to ensure homeothermia and positive growth rate. Between each measurement, piglets were returned to the sow and allowed to suckle ad libitum.

Calculations

Metabolic rate was calculated according to Brouwer (1965) and expressed in kJ h⁻¹ kg⁻¹ BW. Changes in metabolic rate with ambient temperature were subject to analysis by means of an unweighted least squares fit to a two phases model. In this analysis, 2 straight lines were fitted to the data, the line relating the metabolic rate to the higher ambient temperatures having a zero slope. This provided the following informations: (a) the rise in metabolic rate as ambient temperatures fell over the lower range of ambient temperatures, (b) the minimal metabolic rate, (c) the intersection of the lines determining in (a) and (b) which gave the Lc₁.

The minimal integrated EMG was defined as the basal EMG activity at thermoneutrality, *i.e.* at or just above Lc₁ and was expressed in mV min⁻¹. The S_th was the ambient temperature given by the linear regression relating integrated EMG to ambient temperature, when integrated EMG equals to the minimal integrated EMG.

The effect of age on the various parameters studied and the linear regressions relating integrated EMG and metabolic rate to ambient temperature, or
Table 1. Effect of age on minimal metabolic rate (MMR, kJ h⁻¹ kg⁻¹ BW), lower critical temperature (L, °C) and shivering threshold temperature (Sₜ, °C) in pigs

<table>
<thead>
<tr>
<th>Age</th>
<th>2 h</th>
<th>24 h</th>
<th>48 h</th>
<th>5 days</th>
<th>Age effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMR</td>
<td>13.31 ± 1.15(9)</td>
<td>16.32 ± 0.89(12)</td>
<td>20.33 ± 0.76(13)</td>
<td>23.93 ± 0.57(13)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Lₜ</td>
<td>33.85 ± 0.34(9)</td>
<td>32.90 ± 0.82(12)</td>
<td>30.20 ± 0.75(13)</td>
<td>30.39 ± 0.45(13)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Sₜ</td>
<td>33.3 ± 0.7(13)</td>
<td>32.9 ± 0.5(14)</td>
<td>31.0 ± 1.0(13)</td>
<td>30.9 ± 0.5(13)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SEM (number of animals).

metabolic rate to integrated EMG, were analyzed using the GLM and REG procedures of SAS (1988). Results were expressed as means ± SEM.

RESULTS

Metabolic rate

At 2 h of age, piglets' body weight averaged 1.32 ± 0.02 kg (n = 40) and increased by 1.6-fold (P < 0.001) during the first 5 days of postnatal age. Over this period, the minimal metabolic rate increased by 80% (P < 0.001) and the Lₜ decreased by 3.5°C (P < 0.001) (Table 1). Below Lₜ, the metabolic rate (y, kJ h⁻¹ kg⁻¹) increased linearly (P < 0.01) with the decrease in ambient temperature (x, °C) (Fig. 1), the slopes of the regression lines being independent of age and averaging -1.99 ± 0.11 kJ h⁻¹ kg⁻¹. In other words, this indicated that for 1°C of coldness, the cold-induced increase in metabolic rate was similar over the first 5 days of life and that, for a given cold ambient temperature, metabolic rate did not change with age.

Shivering

A very low muscle electrical activity was recorded at ambient temperatures at or just above Lₜ. This activity, taken as the minimal muscle electrical activity, was constant over the studied period and averaged 4.14 ± 1.09 mV min⁻¹. Below Lₜ, integrated EMG increased linearly with the decrease in ambient temperature, as shown in Fig. 1.

![Graph showing changes in metabolic rate and integrated EMG with ambient temperature at 2, 24, 48 h and 5 days of age.](image-url)
temperature (Fig. 1) according to the following equations:

2 h of age,
\[ y = -20.3 (+1.5) \times +678.2 (+47.4) \]
\[ r = 0.909, \ n = 38, \]

24 h of age,
\[ y = -7.1 (+0.9) \times +244.0 (+26.9) \]
\[ r = 0.740, \ n = 52, \]

48 h of age,
\[ y = -5.6 (+1.2) \times +183.7 (+34.3) \]
\[ r = 0.589, \ n = 42, \]

5 days of age,
\[ y = -3.7 (+0.8) \times +116.2 (+20.8) \]
\[ r = 0.631, \ n = 34, \]

where \( y \) was the integrated EMG (mVmin \(^{-1}\)) and \( x \) the ambient temperature (°C). The slopes of the regression lines markedly decreased with age \((P < 0.001)\), and were significantly different from each other, except between 24 and 48 h and between 48 h and 5 days of life. This indicated that, for 1°C of coldness, the integrated EMG decreased by 82% between 2 h and 5 days of age. Similarly, at a given ambient temperature, shivering intensity decreased with age, averaging at 25°C, 176.9, 72.0, 39.4 and 26.6 mVmin \(^{-1}\) at 2, 24, 48 h and 5 days of age, respectively. The \( S_u \) decreased by 2.4°C \((P < 0.05)\) between 2 h and 5 days of age and there was no significant difference between \( L_{ac} \) and \( S_u \) (Table 1).

**Relationship of shivering and thermogenesis**

Metabolic rate \((y, \text{kJ h}^{-1}\text{kg}^{-1})\) and integrated EMG \((x, \text{mV min}^{-1})\) were strictly linearly correlated \((P < 0.001)\) (Fig. 2). The slope of the relationship increased by 2.6-fold \((P < 0.0001)\) with age, averaging 0.09 ± 0.01 and 0.24 ± 0.04 at 2 h and 5 days, respectively. In other words, a 10 mVmin \(^{-1}\) increase in integrated EMG enhanced metabolic rate by 0.9 and 2.4 kJ h\(^{-1}\)kg\(^{-1}\)BW at 2 h and 5 days of age, respectively, as if the thermogenic capacity of shivering was really better with increasing age. Further, this is in agreement with the fact that shivering efficiency, assessed as the ratio of the increase in metabolic rate per 1°C coldness to the integrated EMG increased by 5.5-fold between 2 h and 5 days of age.

**DISCUSSION**

To our knowledge, this is the first measurement of shivering thermogenesis during the early postnatal period in pigs. It is shown that, in usual environmental conditions of the sty, (1) shivering is the major heat producing mechanism of the neonatal pig and, (2) the intensity of shivering decreases and its thermogenic efficiency increases over the first 5 days after birth.

First, the present data confirm the previous observations of Noblet and Etienne (1987) and Studinski et al. (1972), that the minimal metabolic rate rises continuously during the first 5 days of life. A similar result is also reported in other neonates including infants (Hill et al., 1965), lambs (Mercer et al., 1979) and rats (Spiers et al., 1986). This rise in MMR is probably involved in the postnatal increase of rectal temperature during the first day of life (Studzinski et al., 1972; Berthon et al., 1993). Simultaneously, the \( L_{ac} \) decrease by 3.5°C. This reduction is neither caused by an improvement of thermal insulation, as suggested by the fact that the rate of increase in metabolic rate by 1°C coldness remains constant over the first 5 days of age, nor by a higher level of feeding since milk intake, expressed in g kg\(^{-1}\)BW, declines during this period (Noblet and Etienne, 1987). Hence, it is very likely that this decrease in \( L_{ac} \) is largely due to the rise in minimal metabolic rate, as reported in lambs (Mercer et al., 1979).

Present results indicate that cold-induced non-shivering thermogenesis is not present in the newborn pig, and thus confirm the main role of shivering in neonatal thermogenesis. This is suggested by the lack of delay between the cold-induced increase in metabolic rate and the onset of shivering, since
Shivering thermogenesis in the neonatal pig

Table 2: Calculation of the thermogenic efficiency of shivering at 2, 24, 48 h and 5 days of age

<table>
<thead>
<tr>
<th>Age</th>
<th>2 h</th>
<th>24 h</th>
<th>48 h</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>22.7</td>
<td>25.1</td>
<td>31.2</td>
<td>34.1</td>
</tr>
<tr>
<td>MR-MMR</td>
<td>9.37</td>
<td>8.78</td>
<td>10.82</td>
<td>10.20</td>
</tr>
<tr>
<td>EMG-EMG</td>
<td>87.4</td>
<td>41.8</td>
<td>38.5</td>
<td>18.2</td>
</tr>
<tr>
<td>$S_{ET}$</td>
<td>1.8</td>
<td>3.5</td>
<td>4.7</td>
<td>9.3</td>
</tr>
</tbody>
</table>

To allow comparison between the different age groups, metabolic rate and integrated EMG were calculated for a comparable degree of coldness, 5°C below $L_{et}$, which correspond to 28.9, 27.9, 25.2 and 25.4°C at 2, 24, 48 h and 5 days of age, respectively. Shivering efficiency ($S_{ET}$) was assessed as the ratio of the difference between metabolic rate 5°C below $L_{et}$ and minimal metabolic rate (MR-MMR) to the difference between integrated EMG 5°C below $L_{et}$ and minimal integrated EMG, which averaged 4.14 mV min$^{-1}$ at all ages (EMG-EMG$_{et}$). The ratio was expressed in kJ kg$^{-1}$ BW$^{-1}$.

Nonshivering thermogenesis is known to precede shivering in cold-exposed newborns having brown adipose tissue such as infants or rats (Hull and Hardman, 1970), and in cold-adapted birds which exhibit muscular nonshivering thermogenesis (Barré et al., 1985). Further, this is strengthened by the close relationship between metabolic rate and skeletal muscle electrical activity, which remains strictly linear, within the range of ambient temperatures studied. Similarly, in men who rely essentially on shivering thermogenesis for thermoregulatory purposes (Jacobs et al., 1994), Glickman et al. (1967) found a close and linear relationship between the progressive increase in muscle electrical activity and heat production during intense cold exposure. This is assumed to disprove the development of a concurrent NST in the cold. Further, the absence of NST is consistent with the absence of brown adipose tissue in the pig (Trayhurn et al., 1989). Therefore, the neonatal pig appears to rely exclusively on shivering for regulatory thermogenesis and, hence, represents a unique model to study the effects of shivering on the postnatal development of energy metabolism.

In the absence of regulatory nonshivering thermogenesis, the reduction of shivering intensity with age, for a given metabolic rate, must be regarded essentially as an adaptive mechanism reflecting an enhancement of the thermogenic efficiency of shivering. One can postulate that this mechanism is mainly related to the reduced cold sensitivity of the piglets with age, as shown by the reduction in $L_{et}$. However, the calculation presented in Table 2 assessed that, for a degree of coldness comparable between age groups, 5°C below $L_{et}$, shivering efficiency calculated as the ratio of the extra thermoregulatory metabolic rate to the integrated EMG, does increase with age. These results agree with the reduction of the energetic efficiency of muscular activity and in other words with the increase in heat produced by muscular activity, in cold-exposed rats (Brown et al., 1991). They support that the thermogenic efficiency of shivering is highly adaptable (Barré et al., 1985) and that variations in the efficiency of muscular work are implicated in the maintenance of homeothermy in mammals (Ivanov, 1989). Present results do not provide explanations for this specific metabolic adaptation. The thermogenic efficiency of shivering is known to be quite low (Hemingway, 1963; Kleinebeckel and Klussmann, 1990), because shivering occurs at the body periphery and, therefore, is associated with an enhancement in heat loss (Mount, 1968; Kleiber, 1975). However, in our study, the rise in the thermogenic capacity of shivering is not caused by a reduced heat loss during shivering thermogenesis, since, as mentioned above, piglet thermal insulation does not improve during the studied period. The mechanisms underlying an increase thermogenic capacity of muscular activity with age could include, (1) an increase in substrate availability through enzyme adaptation or change in muscle blood flow (Williams et al., 1979), (2) a change in the pattern of energy substrates utilized by the muscle, as there is an enhancement of oxidative capacities with age (Noblet and Le Dividich, 1981; Herpin et al., in press), (3) a shift in muscle fiber type, as type I fibers exhibit a higher oxidative metabolism and a greater contractile efficiency than type II fibers (Henriksson, 1990) and/or (4) an alteration of the capacities of ATP synthesis and utilization during muscular work (Ivanov, 1989).

Finally, the actual physiological significance of this improved thermogenic capacity of shivering with age is corroborated by the following statements. First, the longissimus dorsi muscle is assumed to be representative of the whole musculature in pigs (McMeekan, 1940). However, the uncertainty regarding the magnitude and the efficiency of shivering in a typically slow contracting muscle remains. Second, the intensity of the electrical activity depends on the size of the recorded area and, as the EMG was always recorded from the same portion of muscle, i.e. the electrodes were placed in the same region and at the same distance apart, it is possible to compare animals from different age groups. Third, it is reasonable to assume that the reduction of shivering intensity with age for a given metabolic rate is not associated with an increase in muscle weight in proportion of the body mass, since with an allometric coefficient close to 1.2 (Lefaucheur and Vigneron, 1986), relative weight of longissimus dorsi muscle increases only by 20% which is negligible when compared with the 5.5-fold increase in the thermogenic efficiency of shivering.
In conclusion, this physiological adaptation must be of significant importance for the establishment of postnatal homeothermia and justifies further comprehensive investigations.

Acknowledgements—The authors gratefully acknowledge the help of J. L. Rouanet and C. H. Malbert to set up the shivering measurement.

REFERENCES


Glickman N., Mitchell H. H., Keeton R. W. and Lambert 418 D. BERTHON


Differentiation postnatale des types de fibres musculaires chez le porc: caractérisation des récepteurs de l'insuline dans deux muscles aux propriétés contractiles et métaboliques différentes. Thèse de l'Ecole Nationale Supérieure Agronomique de Montpellier, p 55, France.


