Urinary Excretion of Adrenaline and Noradrenaline in Holstein Calves during Heat and Cold Exposure

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Abstract The effects of environmental temperature upon the sympathetic nervous system and adrenal medulla of Holstein calves were examined to assess characteristics of the self auto-regulation system. To these aims this study describes the urinary excretion of adrenaline and noradrenaline of Holstein calves housed in a climate controlled room at 10°C, 20°C and 30°C was measured by high-performance liquid chromatography with the trihydroxyindole fluorimetric procedure. The experimental animals were housed in a climate room at 20°C for five days, then exposed for four or five days to 10°C, 20°C and 30°C. The animals were fed two liters of milk twice daily, with no other supplemental food nor water. Urine was collected continuously by a self-retaining urethral catheter. The mean value of daily urine volume was maximum at 10°C, and decreased with increasing environmental temperature. The mean value of daily urine volume at 30°C was significantly ($P<0.05$) smaller than the values obtained during other environmental temperatures. When the calves were exposed to 10°C, the mean value of noradrenaline excretion increased significantly ($P<0.05$) from 20.9 µg/day at 20°C to 38.1 µg/day at 10°C. The mean value of adrenaline excretion increased from 3.2 µg/day at 20°C to 4.1 µg/day at 10°C. During exposure at 30°C, daily excretion of noradrenaline and adrenaline was not significantly affected.

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Urinary excretion of adrenaline and noradrenaline has been used by several investigators for evaluation of the sympathetic nervous system and adrenal medullary activity during heat and cold exposure. During heat and cold exposure, an increase in urinary noradrenaline has been observed in the rat^{9,13,15} and in man^{4,7,8}. The increase in urinary excretion of adrenaline (A) and noradrenaline (NA) during exposure to heat and cold implies an important role of the adrenergic nervous system and the adrenal glands³. Despite the prevalence of studies on excretion of A and NA, observations on the ruminant are scarce^{1,2,12}. Our experi-

ment was designed to study the effects of cold (10°C) and heat exposure (30°C) on the sympathetic nervous system and adrenal medulla of Holstein calves by measuring urinary excretion of A and NA.

Materials and Methods

Four young (1-2 weeks old) female Holstein calves (body weight : 34.0, 48.5, 49.5, 50.0 kg) were housed in a climatic room. The animals were secured by a neck chain, and were fed two liters of milk twice daily at 9 a.m. and 4 p.m., with no other supplemental food nor water. Light was provided from 6 a.m. to 6 p.m. The

experimental schedule of treatment was divided into four periods. The animals were exposed for four or five days to each climatic condition. In the first and third period, the environment was kept at 20°C and 60% relative humidity. In the second period, the environment was kept at 10°C and 80% relative humidity. In the fourth period, the environment was kept at 30°C and 60% relative humidity. Changes in the climatic condition in the room at the end of the each period were begun at 10 a.m. and completed within one hour.

Urine was collected continuously by a self-retaining urethral catheter (Bardex foley catheter No. 8, Bard Inc., U.S.A.). The bladder was drained by continuous siphonage into a polyethylene bottle containing 20 ml of 6 N HCl to keep urine below pH 3¹⁰. Urine was collected each 12 hours of daytime (6 a.m.–6 p.m.) and nighttime (6 p.m.–6 a.m.) using an electromagnetic valve and time switch. Urine volume was measured by a graduated cylinder after 12 hours of collection, and the samples were stored at –20°C until later analysis.

Vaginal temperature was measured using a thermistor probe. The R-R interval was measured from one peak to the next peak in the ECG with sampling frequency of 1 ms. Then heart rate was converted from each R-R interval employing a microcomputer. Respiration rate was measured using a thermistor sensor attached to a nose ring of calf. These data were stored every 24 hours on a hard disk in a microcomputer via I/O interface. The physiological responses in calves during each environmental condition were obtained from the stored data between 1 p.m. to 2 p.m. in every-day in this study.

Two ml of thawed urine was hydrolyzed at 100°C for 20 min in a water bath incubator. To a 2 ml volume of hydrolyzed urine, 20 mg of alumina (02120, Woelm Inc., Germany) and 0.8 ml of Tris buffer, pH 8.6, and 100 μ l of 0.15 M EDTA-2 Na solution, pH 7, were added⁶. The pH of the mixture was adjusted between 8

to 9 with 1 N NaOH. The tube was then placed in a shaker and was shaken for 20 min. The alumina was then washed two times by mixing for a few seconds with 5 ml of 3 mM EDTA-2 Na solution, pH 7⁶. The amines were eluted from the alumina by voltexing for 3 min with 200 μ l of 1 N acetic acid. After centrifugation, 50 μ l of the supernatant layer was analyzed by high-performance liquid chromatography.

High-performance liquid chromatography with the trihydroxyindole fluorimetric procedure^{10,16} was used to measure levels of A and NA. These catecholamines were detected by a spectrofluorophotometer (FP-540D, Japan Spectroscopic Co. Ltd., Tokyo) at an excitation wavelength of 395 nm and an emission wavelength of 500 nm and were integrated as peak areas. Furthermore, these peaks were calculated quantitatively from the values of the catecholamine standard which was passed through the test procedure simultaneously.

Students' t-test was used to determine differences between means.

Results

Chromatograms obtained for 2 ml of urine and the standard of 250 pg A and NA are shown in Fig. 1. Fluorimetric detection limits were 10 pg for A and 30 pg for NA (signal/peak-to-peak noise ratio of 2). The within-day coefficient of variation for identical standard solutions was 6.8% for A (n = 4) and 2.0% for NA (n = 4). Absolute recoveries (means $\pm$ S.D.) of A and NA were 61.6 $\pm$ 5.9% (n = 6) and 57.7 $\pm$ 4.1% (n = 6), respectively, when the amount of standard added was varied over a wide range (100 pg – 10 ng/ml).

Changes in daily vaginal temperature, heart rate and respiration rate are shown in Fig. 2. The vaginal temperatures and respiration rate increased at 30°C. The mean value of vaginal temperatures at 30°C was significantly (P < 0.05) higher than the values obtained at 10°C. Heart rates showed a slight tendency to in-

Fig. 1. Chromatograms of catecholamine standard (250 pg each of noradrenaline (NA) and adrenaline (A)) and calf urine sample.

Conditions: sample size $50\ \mu\text{l}$; column, Catechopak ($150\ \text{mm} \times 4.5\ \text{mm I.D.}$); mobile phase, $0.1\ \text{M KH}_2\text{PO}_4$, pH 3.2; flow-rate, $0.8\ \text{ml/min}$; column temperature, 40°C ; spectrofluorophotometer excitation wavelength, $395\ \text{nm}$; emission wavelength, $500\ \text{nm}$.

crease at 10°C . The increase in the heart rates, however, was not statistically significant (Table 1). Respiration rate at 30°C was significantly ($P < 0.05$) higher than the values obtained during other environmental temperatures (Table 1). Changes in daily urine volume are shown in Fig. 3. The daily urine volume was maximum at 10°C , and decreased with increasing environmental temperature. The

mean value of daily urine volume at 30°C was significantly ($P < 0.05$) smaller than the values obtained during other environmental temperatures (Table 1).

Changes in the daily excretion of urinary NA are shown in Fig. 3. Mean value of NA excretion was $20.9\ \mu\text{g/day}$ at 20°C (Table 1.). When the environmental temperature was decreased to 10°C , NA excretion increased significantly

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Fig. 2. Physiological responses in calves before, during cold (10°C) and heat exposure (30°C). Each point represents the average value of four Holstein calves. Vertical bars, S.D. of each average value.

Fig. 3. Excretion of noradrenaline, adrenaline and urine volume in calves before, during cold (10°C) and heat exposure (30°C). Each column represents the average value of four Holstein calves ($\square$; daytime, hatched ; nighttime). Vertical bars, S.D. of each average value.

($P < 0.05$) and reached $41.7 \mu\text{g/day}$ after four days. When the environmental temperature was returned to 20°C , NA excretion in the first

day was about equal to the end of 10°C , but then decreased markedly after two days, reaching $19.6 \mu\text{g/day}$ after three days. When the

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Table 1. Mean values with standard deviations for urine volume, urinary excretion of catecholamines and physiological responses in Holstein calves at different environmental temperatures

Parameter	Environmental temperature (°C)			
	20	10	20	30
Urine volume (ml/day)	2829±444 ^a	3148±193 ^a	3050±193 ^a	2123±274 ^b
Noradrenaline (μg/day)	20.9±7.3 ^a	38.1±7.2 ^b	25.1±3.2 ^a	16.9±3.2 ^a
Adrenaline (μg/day)	3.2±1.4 ^a	4.1±1.2 ^a	2.9±0.5 ^a	2.4±0.5 ^a
Vaginal temperature (°C)	38.8±0.0 ^{ab}	38.7±0.3 ^a	38.9±0.1 ^{ab}	39.4±0.6 ^b
Heart rate (beats/min)	74±11 ^a	77±19 ^a	73±15 ^a	75±17 ^a
Respiration rate (breaths/min)	26±5 ^a	14±3 ^a	24±10 ^a	111±62 ^b

^{a, b}: Values having same letters in the same row are not significantly different ($P < 0.05$).

environmental temperature was increased to 30°C, NA excretion was about equal to the end of the previous period.

Changes in the daily excretion of urinary A are shown in Fig. 3. Mean value of A excretion was 3.2 μg/day at 20°C. When the environmental temperature was decreased to 10°C, A excretion increased after two days, and reached 4.3 μg/day after four days. The increase in A excretion, however, was not statistically significant (Table 1). When the environmental temperature was returned to 20°C, A excretion decreased to 2.7 μg/day after two days. When the environmental temperature was increased to 30°C, A excretion was about equal to the end of the previous period.

Discussion

The finding that daily urine volume decreased with increasing environmental temperature suggests a high output of water vapor through the respiratory organs due to an increase in respiration rate (Table 1). According to AXELROD and REISINE³, urinary excretion of NA and A reflects sympathetic nervous system and adrenal medullary activity, respectively. In the calf, the adrenal medulla secretes both A and NA^{5,14}. Thus, urinary output of these catecholamines in calves is correlated to both sympathetic and adrenal medullary activity.

In the present study, urinary NA excretion in calves increased by 180 percent from a ther-

moneutral (20°C) to a cold environment (10°C), suggesting a high output of NA in cold environment, and providing indirect evidence of higher sympathetic activity during this exposure. The sympathetic response to cold in the calves observed in this study appears to be similar to the findings for NA reported ANDERSSON *et al.*²⁾ for the goat and SASAKI *et al.*¹²⁾ for the sheep. ANDERSSON *et al.*²⁾ reported that cold exposure at -3°C gradually caused a 300 percent increase in NA excretion, whereas A excretion was only slightly elevated. SASAKI *et al.*¹²⁾ found that percentage increases from thermoneutral to cold exposure at 0°C of NA and A excretion into sheep urine was 270 and 390, respectively. The percentage increase of A excretion from thermoneutral to cold in our study was 130, which was considerably lower than the results of SASAKI *et al.*¹²⁾. They also reported the increase in heart rate during cold exposure at 0°C. However, no significant increases in heart rate were found in this study. Lower critical temperature in newborn calves was estimated nearly 10°C¹¹⁾. It appears that cold exposure at 10°C in this study was not sufficiently low temperature to activate thoroughly adrenal medulla in young (1-2 weeks old) Holstein calf. A slight but significant increase (21%) in NA excretion at 2°C was reported in the cow¹⁾. The relatively small increase in catecholamine excretion in the cow exposed to cold could be explained by the fact

that the duration of exposure to the cow was not sufficiently long. As demonstrated in the present study, catecholamine excretion did not increase to maximum within one or two days after cold exposure. Our results agree with those reported by YOUNG and LANDSBERG¹⁵⁾ for the rat. They found NA excretion reached a maximum several days after cold exposure.

NA and A excretion maintained relatively high levels within 24 hours after re-exposure to 20°C, which indicates that the augmented sympathetic nervous system and adrenal medulla activity remained at high levels within 24 hours after cessation of cold exposure. It is suggested that acclimation of the adrenergic nervous system and adrenal medulla of the calves was achieved, at least partially, by five days exposure to 10°C, and that the enhanced nervous system and adrenal medulla activity deacclimated after more than two days exposure to 20°C. The rate of cold deacclimation of sympathetic nervous system in the calves found here differs from that observed in the sheep¹²⁾. SASAKI *et al.* reported that the augmented adrenergic nervous system of the sheep did not deacclimate within one week of exposure to 20°C. Our results agree with that reported by LEDUC⁹⁾ for the rat. He reported that rats housed in a cold room for one week, one month or two months recovered normal NA excretion values within two days at room temperature.

In regard to catecholamine excretion during heat exposure, no significant increases in NA nor A were found in this study. SHUM *et al.*¹³⁾ reported an increase of 210% in the urinary excretion of NA in the rat during heat exposure at 36°C. ALVAREZ and JOHNSON¹⁾ reported an increase of 21% in the urinary excretion of NA in the cow during heat exposure at 37°C. It should be noted, however, that the environmental temperatures used by these investigators during heat exposure differed from that employed here in calves. The data presented are indicative that urinary excretion of NA and

A in calves were not affected during heat exposure at 30°C. In this study, catecholamine excretion during heat exposure was not significantly affected. However, exposure to more severe heat condition may influence catecholamine excretion.

In summary, the percentage increases in urinary NA and A excretion in Holstein calves from thermoneutral (20°C) to cold exposure (10°C) were 180 and 130, respectively. Heat exposure at 30°C, however did not significantly affect NA nor A excretion.

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暑熱および寒冷時におけるホルスタイン子牛の尿中 アドレナリンおよびノルアドレナリン排泄量

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新生子牛の交感神経系および副腎髄質活動に対する環境温度の影響を調べるため，10℃，20℃ および 30℃ に制御された環境調節室内におけるホルスタイン子牛の尿中アドレナリンおよびノルアドレナリン排泄量を，高速液体クロマトグラフィートリハイドロキシインドール法により蛍光定量した。生後 1～2 週齢のホルスタイン雌子牛 4 頭を供試し，尿道カテーテルを膀胱に留置して全尿採取した。子牛は 20℃ で 5 日間飼育後，10℃，20℃ および 30℃ にそれぞれ 4 ないし 5 日間感作した。子牛には 1 日に 2 回，各 2 l の牛乳を給与し，他の飼料や水は与えなかった。1 日当りの平均尿量は 10℃ のときが最大で，環境温度が上昇するに従って減少した。30℃ のときの 1 日当りの平均尿量は，他の環境温度に比べて有意 ($P < 0.05$) に少なかった。子牛を 10℃ に感作すると，1 日当りの平均ノルアドレナリン排泄量は，20℃ の $20.9 \mu\text{g}$ から， $38.1 \mu\text{g}$ へと有意 ($P < 0.05$) に増加した。1 日当りの平均アドレナリン排泄量は，20℃ の $3.2 \mu\text{g}$ から，10℃ の $4.1 \mu\text{g}$ へと増加した。30℃ の感作では，尿中アドレナリンおよびノルアドレナリン排泄量は 20℃ に比べて有意な影響は認められなかった。

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