Heart rate responses to cooling in emu hatchlings


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Abstract

Among fluctuations of instantaneous heart rate (IHR) in newly hatched chicks, heart rate (HR) oscillation with a mean frequency of 0.7 Hz has been designated as Type II HR variability characterized by low frequency (LF) oscillation [Comp. Biochem. Physiol. Part A 124 (1999) 461]. In response to exposure to lowered ambient temperature (Ta), chick hatchlings raised their HR baseline accompanied with the production or augmentation of Type II HR oscillation, indicating that LF oscillation is a phenomenon relating to thermoregulation [J. Therm. Biol. 26 (2001) 281]. In emu hatchlings that are precocial like chickens, Type II HR oscillation also occurred, but less frequently in comparison with chick hatchlings [Comp. Biochem. Physiol. Part A 131 (2002) 787]. This present experiment was conducted to elucidate how IHR of emu hatchlings responds to changes in Ta. Six hatchlings were measured for IHR and skin temperature (Ts) during a 3-h period when they were exposed to controlled Ta (ca. 35°C), lowered Ta (ca. 15–30°C) and again the controlled Ta for individual 1-h periods. In response to all the cooling and re-warming procedures, HR baseline changed depending upon the intensity of the Ta differences; i.e. large differences of Ta produced large changes in HR. HR fluctuations tended to augment during cooling with a few exceptions, but LF oscillation was not produced. Thus, LF oscillation, which was scarce even at the controlled Ta, could not be used as a thermoregulatory indicator in emus.

Keywords: Emu hatchling; Chick hatchling; Cooling; Instantaneous heart rate; Heart rate fluctuations; Low frequency oscillation; Thermoregulation; Skin temperature

1. Introduction

In the precocial domestic fowl (Gallus gallus domesticus) and emu (Dromaius novaehollandiae), patterns of instantaneous heart rate (IHR) fluctuations have been elucidated for both developing embryos and hatchlings (Höchel et al., 1998; Kato et al., 2002; Moriya et al., 1999, 2000, 2002; Tazawa et al., 1999, 2002a). IHR fluctuations are comprised of heart rate variability (HRV) that tends to be cyclic and oscillating and heart rate irregularities (HRI) that are irregular with transient decelerations and/or accelerations. Particularly, in the domestic fowl hatchlings that were measured for the first time for the distinctive patterns of IHR fluctuations, the patterns were categorized into three types; Types I, II and III (Moriya et al., 1999). Type I HRV is characterized by a widespread baseline HR that is due to respiratory sinus arrhythmia (RSA). Type II HRV comprises HR oscillation and Type III HR fluctuations are non-cyclic irregularities dominated by HR accelerations. Due to Type I HRV and Type II HRV having a mean oscillatory frequency of approximately 0.7 Hz and 0.07 Hz, respectively, they were designated...
as high frequency (HF) oscillation and low frequency (LF) oscillation of HR, respectively (Tazawa et al., 2002b). In emu hatchlings, IHR fluctuations were also categorized as Types I, II and III (Moriya et al., 2002). Type I HR oscillation had a mean frequency of 0.37 Hz which was approximately half that in chickens. Type II HRV had a mean frequency of 0.06 Hz, which was similar to that of chickens, although the appearance was less frequent when compared with chick hatchlings.

In the meantime, an experiment was previously made to investigate an origin of Type II HRV in chick hatchlings (Tazawa et al., 2002b). Type II HRV was produced or augmented by exposure of hatchlings to lowered ambient temperatures (Ta) and it was eliminated by exposure to elevated Ta. The hatchlings that were exposed to large temperature decreases tended to increase HR more than those exposed to small temperature decreases. As a result, it was concluded that LF oscillation which was accompanied by an elevation of HR baseline was in response to cooling and might be a phenomenon relating to thermoregulation of chick hatchlings (Tazawa et al., 2002b).

In emu hatchlings, LF oscillation of IHR also occurred, but less frequently in comparison with chick hatchlings (Moriya et al., 2002). Emus are as precocial as chickens and their hatchlings are already covered with dense feathers at hatching. It seems that emu hatchlings are also provided with a thermoregulatory capacity right after hatching. It is hypothesized that if emu hatchlings have some capacity of thermoregulation, they will also respond to the cooling exposure with an increase in HR and production of Type II HR oscillation. The present experiment is designed to examine this hypothesis.

2. Materials and methods

Fertile eggs were collected in breeding yards of the Cross Timbers Emu Ranch, Flower Mound, Texas and incubated at a temperature of 36.5 °C with relative humidity of approximately 30% in a laboratory of the University of North Texas. The eggs were incubated for approximately six weeks in Texas and then transported to the Muroran Institute of Technology by flight and car. The eggs were put into a corrugated cardboard box and exposed to low ambient temperatures during transportation of approximately 20 h. After arrival at the laboratory in Muroran, incubation was resumed at a temperature of 36.5 °C and relative humidity of approximately 40% in a forced draught incubator, which was also used for experiments with chicken eggs. Egg turning was manually carried out three times a day (Kato et al., 2002). Fresh eggs were also brought to Japan and incubated in Muroran. When all eggs hatched, the hatchlings were kept in a box and warmed at a temperature of approximately 35 °C (referred to as a control temperature-box). Six hatchlings were examined for cooling response of IHR. Two of the hatchlings, which hatched from the fresh eggs in Muroran, were examined once and then they were used for other experiments. The remaining four were subjected to multiple exposures (i.e. 13, 12, 8 and 8 times, respectively) to cooling during two weeks of postnatal life in order to assure whether or not IHR responses always occurred.

IHR was determined from an electrocardiogram (ECG) that was measured with three flexible Ag/AgCl disk electrodes as described previously (Moriya et al., 1999). On the day of experiment, the ECG electrodes were attached to the thoracic wall and the abdomen by adhesive gel after a thermistor disk probe 3 mm in diameter was placed between the skin and an ECG electrode to measure skin temperature (Ts in °C). The hatchling carrying wires from the ECG-electrodes and the thermistor probe was accommodated in a small metal mesh cage (referred to as a measuring cage). All the wires were fixed to the measuring cage, and another thermister probe was attached to the cage to measure Ta ( °C). The Ta to which hatchlings were exposed ranged from approximately 30 °C to 15 °C and was produced by placing the animal in the cage into a thermo-stated box (referred to as low temperature box) or laboratory space. The measuring cage containing the hatchling was first placed in the control temperature-box. After approximately 1 h had elapsed, ECG measurements were made for the next 3-h period that was equally divided into three 1-h measuring periods. The first and the last 1-h periods were control periods when the hatchling was measured, for control IHR at 35 °C (referred to as pre-exposure and post-exposure control periods, respectively), and the middle 1-h period was the cooling period, when responses of IHR to exposure to Ta ranging from 30 °C to 15 °C, were determined.

Power spectrum analysis of IHR patterns was made by fast Fourier transform to assure the HR
Fig. 1. Three-hour recordings of instantaneous heart rate (IHR, shown by points), skin temperature (Ts, solid line) and ambient temperature (Ta, broken line) of a hatchling determined on day 6 of postnatal life. The average value of Ta was 34.8, 23.7 and 34.8 °C during the first, middle and last 1-h periods, respectively. The averaged value of Ts during individual 1-h periods was 38.7, 35.5 and 37.7 °C, respectively. The spontaneous, irregular accelerations of IHR occurred sporadically during the pre- and post-exposure control periods. The spontaneous accelerations that occurred just prior to cooling exposure were particularly large. Upon exposure to cooling, HR baseline was elevated and fluctuations were augmented.

oscillation and evaluate oscillatory frequency (Moriya et al., 1999). Normalized power of frequency spectrum was presented.

3. Results

Fig. 1 shows an example of IHR recorded for a 3-h period when a six-day-old hatchling was exposed to a room temperature of approximately 24 °C for 1 h during the cooling phase. Ts and Ta were also recorded by solid and broken lines, respectively. The transfer of the measuring cage containing the hatchling between the control-temperature box and laboratory space was made at a time indicated by thick dotted vertical lines. Mean Ts during exposure to the control temperature (34.8 °C) was 38.7 °C, which was approximately 4 °C higher than Ta, and it decreased to 35.5 °C on average during the 1-h exposure to a room temperature of 23.7 °C. The drop of Ts caused by the decrease in Ta of approximately 11 °C was approximately 3 °C, with Ts remaining approximately 12 °C higher than Ta, indicating that the thermoregulatory function was elicited in the hatchling. In response to exposure to cooling, HR baseline was raised and widened compared with the pre- and post-exposure controls. The mean value of IHR (MHR) during the pre-exposure control was 106 ± 17 (S.D.) bpm (N=6265) and MHR increased markedly to 158 ± 31 bpm (N=9066) during the cooling exposure. After the cooling exposure, the HR returned to the pre-exposure control level; i.e. MHR during post-exposure control was 104 ± 17 bpm (N=6152).

In response to the cooling exposure, IHR fluctuations augmented as indicated by the widened baseline during the second segment of 1-h recording in Fig. 1. Fig. 2 shows time-expanded 10-min recordings of IHR during pre-exposure control (top panel), cooling exposure (middle panel) and post-exposure control (bottom panel) periods, respectively, and the normalized power of the frequency spectrum. As small peaks in the top and bottom panels of the power spectrum indicate, the baseline HR oscillated with high frequency of 0.20 Hz and 0.22 Hz, respectively, during the pre- and post-exposure controls (Fig. 2). These are Type I HRV that are thought to be associated with respiratory
Fig. 2. Ten-minute recordings of instantaneous heart rate (IHR) corresponding to recordings beginning from 20, 80 and 140 min of Fig. 1 (left panels) and the normalized power spectrum (right panels). Mean Ta is shown in each of the left three panels. A small peak of the power pointed by an arrow in the right top and bottom panels is located at 0.20 Hz and 0.22 Hz, respectively.

In response to cooling, the HR baseline was elevated and fluctuation became predominant (middle left panel). However, as no peaks are indicated at any frequencies by power spectrum analysis (middle right panel), IHR did not oscillate with any detectable pattern during cooling.

This hatchling was also examined with the cooling experiment on different days in order to assure whether a similar HR response always occurred. The results are summarized in Fig. 3. The top panel shows Ts (triangles) and Ta (rhombuses) before, during and after cooling exposure; that is, mean values of Ts and Ta during pre-exposure control, cooling and post-exposure control periods are connected with solid lines. In the bottom panel, MHR (circles) during pre-exposure control, cooling and post-exposure control periods are also connected with solid lines. The plots of Ts, Ta and MHR on day six correspond to respective mean values of Fig. 1. The intensity of the increase in MHR produced by cooling exposure seemed to be related to the intensity of the decrease in Ta on all days examined.

Fig. 4 shows the results obtained from a hatchling that was exposed to cooling more than once a day in order to examine whether or not the intensity of the increase in MHR produced by sinus arrhythmia (RSA).
Fig. 4. Average values of skin temperature (Ts), ambient temperature (Ta) and heart rate during individual 1-h periods of pre-exposure control, cooling exposure and post-exposure control in another hatchling that was subjected to cooling test more than once in a day. Symbols are the same as in Fig. 3.

Fig. 5. The 3-h recordings of skin temperature (Ts, solid line), ambient temperature (Ta, broken line) and instantaneous heart rate (IHR, points) of a hatchling on day 12 when it was placed in the low-temperature box set at approximately 16 °C for 1-h period in the middle of experiment. Although the temperature of the box changed in a sinusoidal fashion, the change befell accidentally due to inadequate adjustment of temperature control. The mean values of Ta during individual 1-h periods of pre-exposure control, cooling exposure and post-exposure control were 34.4, 15.6 and 34.7 °C, respectively, while those of Ts were 39.9, 36.5 and 38.6 °C, respectively. The mean value of IHR during these three 1-h periods were 136±15 bpm (N=8109), 199±20 bpm (N=11 735) and 130±19 bpm (N=7650), respectively.
cooling was related to the intensity of the decrease in Ta. The hatchling was exposed to cooling twice on days six and eight and thrice on days 12 and 13. It responded to multiple cooling exposures on the same day with HR increases corresponding to differences of Ta. On day 12, for instance, the largest drop in Ta induced the largest increase in HR. Fig. 5 shows the large increase in IHR produced by exposure to a low Ta of approximately 16 °C. The box-temperature (Ta) changed in a sinusoidal fashion, which seemed to influence the changing pattern of the HR baseline. However, the HR baseline, which accelerated irregularly during pre-exposure control period (i.e. Type III HRI), was elevated predominantly during cooling, and returned to the original level during post-exposure control period. The average increase in HR due to the average drop of approximately 19 °C in Ta was not less than 60 bpm in this experiment. The elevated baseline became wide early in the cooling-exposure due to augmented HR fluctuations and then narrow towards the end of cooling. Fig. 6 presents time-expanded 10-min recordings of IHR taken from three individual 1-h segments in Fig. 5 and the normalized power spectrum. The spectrum analysis showed a small peak at a high frequency of 0.51 Hz (arrow) during pre-exposure control period. During the later period of cooling, HR baseline seemed to be oscillating (middle left panel), but no particular peak was shown at any frequency ranges (middle right panel).

Fig. 7 presents IHR changes produced by exposure to a room temperature of approximately 23 °C after a 2-h recovery from the exposure to 16 °C-environment (shown in Fig. 5). HR baseline fluctuated during cooling, but the increase in HR baseline was small compared with that shown in Fig. 5; i.e. the average increase in MHR produced by the average drop of approximately 11 °C in Ta was only 23 bpm. The time-expanded recordings of IHR during the 10-min period of cooling and spectrum analysis did not show LF oscillation of HR (Type II HRV).

Fig. 8 shows HR response to cooling in another hatchling that was examined on the day of hatching (day zero), indicating that the hatchling responded to cooling with an increase in HR even on day zero and also on days one–five when subsequent experiments were made.

Fig. 9 summarizes the increases in MHR produced upon cooling from the control temperature (ca. 35 °C) and the decreases in MHR upon returning from low Ta to the control Ta in six hatchlings. The changes in MHR during 1-h exposure to cooling and during 1-h re-warming were expressed by the following regression equation,

\[ \Delta MHR = -4.8 \Delta Ta - 0.04 \]  

\[(N=86, r=-0.917)\]

The correlation coefficient is statistically significant \((r=21.1, \ P<10^{-5})\). There was a consistent change in MHR that was dependent on the temperature change experienced by the hatchlings.

4. Discussion

Development of HR fluctuations was recently investigated in embryos and hatchlings of the chicken and the emu (Höchel et al., 1998; Kato et al., 2002; Moriya et al., 1999, 2000, 2002; Tazawa et al., 1999, 2002b). The various distinctive patterns of IHR fluctuations were found and their relations to autonomic nervous functions and physiological functions such as thermoregulation were suggested (Tazawa et al., 2002a,b). Comparative and ontogenetic studies on nascent thermoregula-
Fig. 7. The 3-h recordings of skin temperature (Ts, solid line), ambient temperature (Ta, broken line) and instantaneous heart rate (IHR, points) of the hatchling on day 12 when it was exposed again to low temperature of approximately 23 °C for a 1-h period in the middle of experiment. In this experiment, the measuring cage was transferred between the control temperature-box and the laboratory space. The average values of Ta during individual 1-h periods of pre-exposure control, cooling exposure and post-exposure control were 34.0, 23.4 and 33.9 °C, respectively, while those of Ts were 39.3, 36.3 and 38.6 °C, respectively. The average values of IHR during these three periods were 129 ± 21 bpm (N=7603), 152 ± 22 bpm (N=8941) and 120 ± 20 bpm (N=7100), respectively.

The metabolic responses of embryos and hatchlings to cooling in addition to temperature measurements (Freeman, 1964, 1967, 1970, 1971; Tazawa and Rahn, 1987 Matsunaga et al., 1989; Tazawa et al., 1988, 1989a,b Kuroda et al., 1990; Whittow and Tazawa, 1991; Nickelman et al., 2001). In this context, a previous experiment was attempted to study development of minute thermoregulatory competence in late chick embryos by measuring HR as an alternative of oxygen consumption (Tazawa et al., 2001). An embryo pipped the eggshell externally on day 20 of incubation, but failed to escape from the eggshell until day 22 when it hatched. Maturity of the embryo on day 22 might be the same as hatchling, and the embryo responded to egg cooling with marked raise of the HR baseline. In addition, the HR baseline began to oscillate in response to cooling, suggesting that Type II HR (i.e. LF) oscillation might be induced by exposure of the chick hatching to low Ta. In fact, in another experiment with newly hatched chickens (Tazawa et al., 2002b), Type II HR oscillation was induced or increased in oscillatory frequency by exposure of hatchlings to low Ta. Conversely, exposure to high Ta (38 °C) from low room temperature abolishes Type II HR oscillation. These changes were always accompanied with changes in the HR baseline.

Type II HR oscillation was recorded frequently in all the chick hatchlings that were measured for HR continuously during one-week of the post-hatching life (Moriya et al., 1999). The average frequency of 27 HR oscillations taken randomly was 0.07 Hz (range 0.04–0.10 Hz). The oscillation often continuously occurred for several hours, particularly in a low temperature environment. Meanwhile, LF oscillation in emu hatchlings occurred less frequently and lasted for a short period (e.g. 10–20 min) (Moriya et al., 2002). The frequency ranged 0.04–0.1 Hz with a mean of 0.06 Hz for 46 examples taken randomly. Since the range of the oscillatory frequency and mean value were similar in hatchlings of both species, we previously categorized LF oscillation of HR in emu into Type II HRV that was defined for chick hatchlings (Moriya et al., 1999, 2002), and that
suggested that the HR oscillations were related to the thermoregulation of the emu. However, in the present experiment, it was recorded that LF oscillation was not produced by the exposure of the emu hatchlings to cooling, or during control-temperature (ca. 35 °C) exposure (Figs. 1, 2, 5–8). While Type II HR oscillation in chick hatchlings formed sinusoidal patterns with magnitude of 20–50 bpm (Moriya et al., 1999), LF oscillation in emu hatchlings seemed to be repeated HR accelerations with magnitude exceeding 50 bpm (Moriya et al., 2002). Thus, although the oscillatory frequency was almost identical in both species, it is suggested that the origins may not be the same and LF oscillation in emu hatchling should not be designated as Type II HRV. From the patterns of LF oscillation in emu (Moriya et al., 2002), it is inferred that HR accelerations with magnitude exceeding 50 bpm occur repeatedly to form patterns that look like oscillation. This detail remains to be studied. In addition, whether or not Type II HRV in chick hatchlings is species-specific HR oscillation should be elucidated by measuring IHR in other precocial species such as duck hatchlings that may be provided with thermoregulatory capacity early in life.

On the other hand, HF oscillation of HR was also recorded frequently in the present measurement (Figs. 2 and 6) as was in the previous measurements for hatchlings of chickens and emus (Moriya et al., 1999, 2002). In chick hatchlings, HF oscillation was designated as Type I HRV with a mean oscillatory frequency of 0.74 Hz (range 0.4–1.2 Hz) and was respiratory sinus arrhythmia (RSA). HF oscillation indicated in Figs. 2 and 6 had an oscillatory frequency of approximately 0.20–0.22 Hz and 0.51 Hz, respectively, which corresponded to values within the range reported previously for emu hatchlings (0.2–0.7 Hz) (Moriya et al., 2002). HF oscillation (i.e. Type I HRV and RSA) was recorded only during control temperature exposure and was eliminated by cooling in the present experiment, which may indirectly indicate that sympathetic nervous function is relatively augmented during cooling.

Exposure of hatchlings to lowered Ta raised the HR baseline in all the measurements and the raised HR baseline returned to the original control level.
Fig. 9. The relationship between the temperature difference ($\Delta T$) and the mean heart rate ($\Delta MHR$) in six hatchlings is examined. The abscissa indicates the ambient temperature difference between the control and cooling. The difference made by decreasing from the control temperature to low temperature is minus (closed circles) and that made by increasing from low temperature to control temperature is plus (open circles).

Finally, it should be noted that HR responses to cooling occurred already on the day of hatching (day zero) and the HR baseline raised markedly during cooling exposure (Figs. 3 and 8). It seems that emus are provided with thermoregulatory competence on day zero and respond to cooling with increases of the HR baseline and HR fluctuations. Although HR fluctuations during cooling in emu hatchlings did not correspond to Type II HRV (i.e. LF oscillation) as in chickens, continuous measurements of HR before and during cooling indicate a relationship to the ability of thermoregulation in emus as well as chickens. In other words, although we did not see LF oscillation of HR in emus, the HR still responded to changes in temperature, indicating the ability to thermoregulate. In chickens, because the thermoregulatory capacity is minute before hatching, HR responses of externally pipped embryos were not large (Tazawa et al., 2001). On the other hand, in emus of which HR responses to cooling were detected from day zero, it is inferred that ability of thermoregulation may progress considerably before hatching compared with chickens. For comparison with chickens, the measurements of HR and $M_{O_2}$ responses to cooling should be made for late emu embryos, particularly during pipping periods. In ostriches that are larger than emus, hatchlings could maintain body temperature above 36 $^\circ$C at Ta of 20 $^\circ$C at 24 h of post-hatching life when measurements were made (Brown and Prior, 1999). Large size of embryos and hatchlings along with the well-developed control of HR in these ratite species may be favorable to thermoregulation.

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References


