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Effects of Environmental Hyperthermia on Cardiovascular Function in the Rat Embryo

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ABSTRACT. Effects of hyperthermia on the cardiovascular function of the mammalian embryo have not been well defined. The effect of hyperthermia on the blood flow and umbilical artery blood pressure was studied in rat embryos at gestational d 12 by using a method developed in our laboratory. When the temperature was changed from 37 to 42°C, the heart rate increased by 15% (n = 33). Mean umbilical artery blood pressure, measured by a servo-null micropressure system, decreased from 0.64 ± 0.05 to 0.53 ± 0.04 mm Hg (n = 11), whereas blood flow velocity at the conotruncus, a measure of cardiac output, obtained by a 20-MHz pulsed Doppler ultrasound flowmeter, increased by 36 ± 11% (n = 11). Mean umbilical artery blood flow increased by 66 ± 13% (n = 11) and its vascular resistance, calculated by ratio analysis, decreased from 3.7 (median) to 1.8 units. These changes returned to baseline values when the temperature was returned to 37°C. The change in blood pressure was different from that seen in the chick embryo, indicating that there is species difference in the hemodynamic effect. (*Pediatr Res* 30: 505–508, 1991)

Hyperthermia is an environmental hazard to the mammalian embryo and is reported to be a teratogen, although this is not unanimously accepted (1–3). We previously found that hyperthermia increased blood pressure and cardiac output in chick embryos (4), which was considered to be causally related to the cardiovascular pathogenesis, at least in part. The effects of high temperature on cardiovascular function of the mammalian embryo, however, have not been studied. We have found that various interventions have different hemodynamic effects in chick and rat embryos (5). Therefore, the purpose of the present study was to investigate the hemodynamic effect of high temperature in the rat embryo and to compare the results with those in the chick embryo.

MATERIALS AND METHODS

The methods used in the present study were modified from one already reported elsewhere (6). Wistar-Imamichi rats (Imamichi Institute for Animal Reproduction, Saitama, Japan) were bred overnight, and fertilization was confirmed by vaginal smear on the next morning, when the gestational day was counted as d 0. At d 12, pregnant rats were anesthetized with an intraperitoneal injection of α-chloralose (80 mg/kg) or pentobarbital-Na (50 mg/kg). The uterus was exposed through an abdominal incision, and a part of the uterus containing one conceptus was isolated and put into a bath of Hanks' solution. Then, a part of the uterus wall was pinned down on the rubber sheet at the bottom of the bath and the yolk sac was opened to expose the embryo and umbilical vessels (5, 6). In the modified system, the perfusate was bubbled with 100% oxygen gas in a glass flask, which was warmed to 37°C on an electric heating pan, and a constant flow through the bath was maintained by a roller pump (5) (Fig. 1). The Po_2 of perfusate in the bath ranged from 60 to 67 kPa. The hemodynamic condition was stable for at least 30 min in this system.

The umbilical artery blood pressure was measured with a servo-null micro-pressure system (model 900; WP Instruments, Inc., Sarasota, FL) by inserting a micro-glass pipette into the umbilical artery. The blood flow velocity at the conotruncal region was obtained with a 20-MHz pulsed Doppler velocity meter (University of Iowa) by placing a piezoelectric crystal as the Doppler beam passed through the axis of the outflow tract of the embryo heart. Although cardiac output could not be obtained because simultaneous measurement of the diameter of that portion was not feasible, any change in velocity would be parallel to that of cardiac output, assuming that the diameter of the outflow tract was not affected by the intervention (6). Thus, a value obtained by dividing the velocity by heart rate would represent stroke volume (this value will be designated as stroke volume index in the following discussion). The flow velocity was also taken at the umbilical artery and the diameter of this vessel was directly measured under a microscope. We placed the Doppler probe in such a way that the Doppler beam passed through the center of the vessel. From mean velocity and the diameter, blood flow was calculated. Each of these measurements was done in separate embryos. Baseline data were obtained at 37°C, then the perfusate was warmed by a heating lamp while the temperature was monitored with a needle thermoprobe, which was kept as close to the embryo as possible in the bath. Data were again taken at 42°C. Because Doppler velocity measurement is known to be dependent on angle between flow and Doppler beam, we stuck pins on the rubber sheet around embryos to make their positions stable or when embryos moved we adjusted the angle of Doppler beam to obtain the maximum velocity at the new position. In the velocity study, temperature was then lowered by turning off the lamp while recording the velocity, and data were again taken at 37°C. Thus, velocity data were obtained at three points (at 37, 42, and 37°C) in each embryo. In the blood pressure study, however, lowering of the temperature was done in different groups of embryos; *i.e.* the pressure was first measured at 42°C, then after lowering to 37°C the data were obtained. This was done because the zero-balance of the system can change with time, so zero should be confirmed each time to ensure the accuracy of the data obtained. Because the flow and pressure were obtained in separate embryos, vascular resistance of the umbilical artery was calculated using the pressure and flow data by ratio analysis (7) and expressed as median ± 95% confidence interval.

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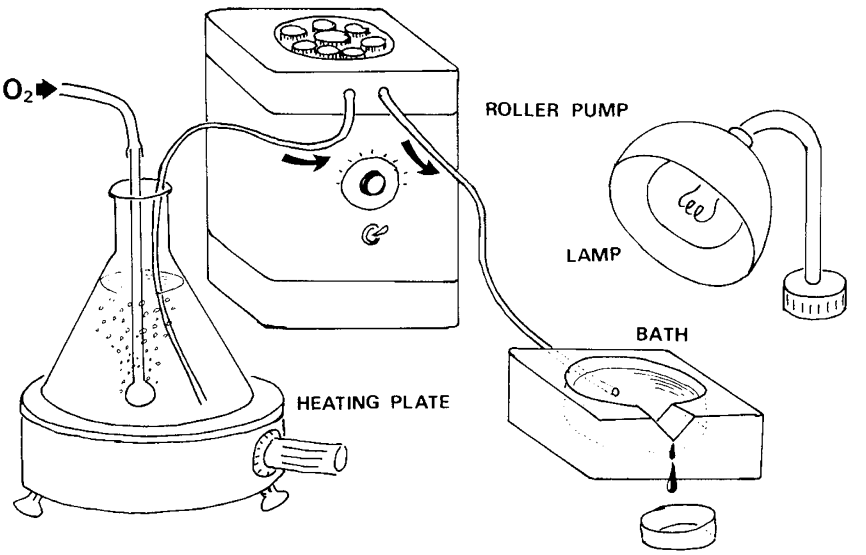

Fig. 1. A bath system: Hanks' solution, warmed to 37°C on a heating plate, was bubbled with 100% oxygen. The solution was perfused by a roller pump through the bath with a constant flow. The temperature was finally controlled by a heating lamp.

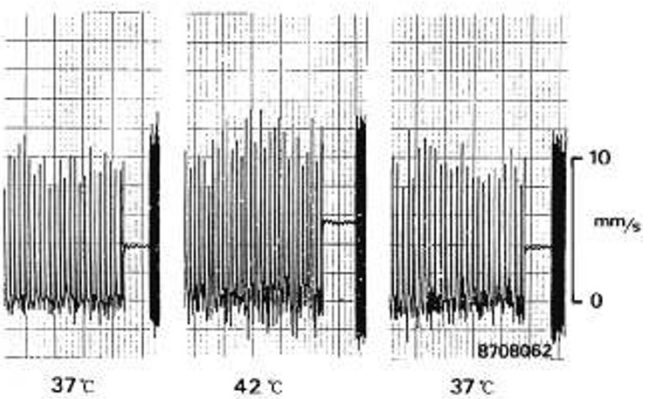

Fig. 2. A tracing of blood flow velocity at the conotruncal region. The velocity increased during hyperthermia.

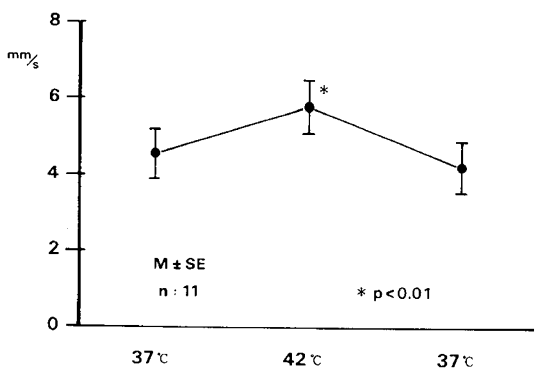

Fig. 3. Blood flow velocity at the conotruncal region increased by 36 ± 11% when temperature of the perfusate was elevated from 37 to 42°C. The velocity returned to the control level when the temperature was lowered to 37°C. M ± SE, mean ± standard error.

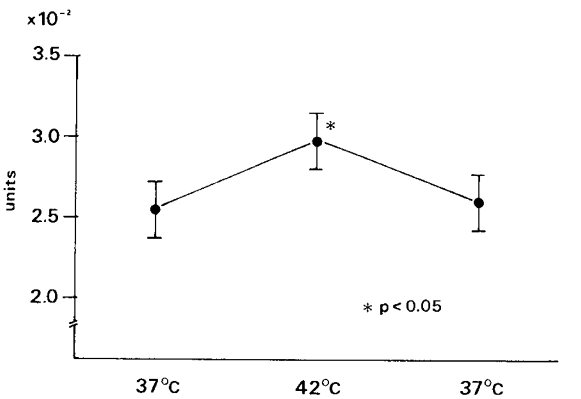

Fig. 4. The stroke volume index, a measure of stoke volume of the embryonic heart, increased by 22 ± 11% during hyperthermia.

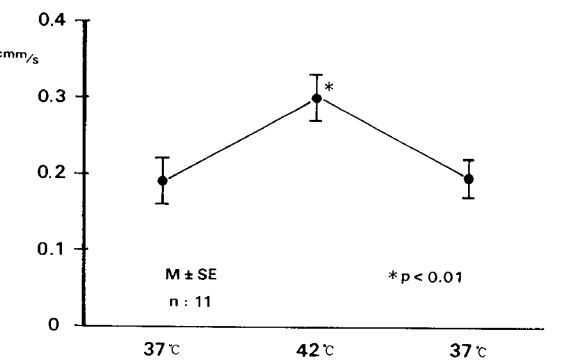

Fig. 5. Umbilical artery blood flow increased by 66 ± 13% during hyperthermia and was returned to baseline after cooling to 37°C. M ± SE, mean ± standard error.

The data were expressed as mean ± SEM and were analyzed by repeated measurement analysis of variance with a post hoc analysis of Newman-Keuls method. Changes in blood pressure were analyzed by paired t test. A p value less than 0.05 was considered significant.

RESULTS

The blood flow velocity at the conotruncus was 4.50 ± 0.69 mm/s at 37°C, which increased to 5.80 ± 0.69 mm/s at 42°C and returned to 4.18 ± 0.67 mm/s at 37°C ($p < 0.01$, $n = 11$; Figs. 2 and 3). The stroke volume index was 2.51 ± 0.34 × 10^{-2} at control, increased to 2.95 ± 0.33 × 10^{-2}, and returned to 2.52 ± 0.36 × 10^{-2} ($p < 0.05$; Fig. 4). The umbilical artery blood flow was 0.194 ± 0.029 mm^3/s at 37°C, increased to 0.301 ± 0.030 mm^3/s at 42°C, and returned to 0.195 ± 0.025 mm^3/s at 37°C ($p < 0.01$, $n = 11$; Fig. 5). Stroke volume at this artery was 1.26 ± 0.60 × $10^{-3}mm^3/s$ at control, increased to 1.65 ± 0.58 × 10^{-3} mm^3/s, and returned to 1.34 ± 0.57 × 10^{-3} mm^3/s ($p < 0.01$; Fig. 6). The umbilical artery blood pressure decreased from 0.64

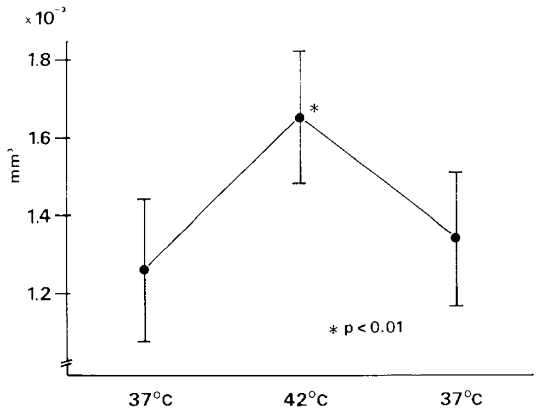

Fig. 6. The stroke volume at the umbilical artery increased by 34 ± 9% during hyperthermia.

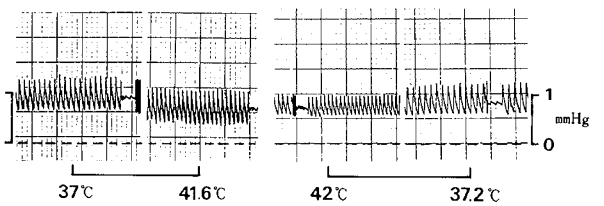

Fig. 7. Actual tracings of umbilical artery blood pressure are illustrated. The pressure decreased and heart rate increased when the temperature was raised. The responses were reversed when the temperature was lowered.

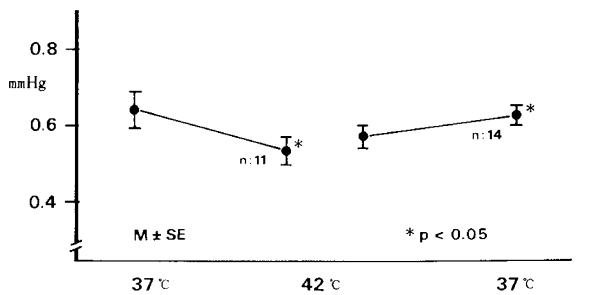

Fig. 8. An average of mean umbilical artery blood pressure decreased during hyperthermia by 16 ± 3%. It increased by 10 ± 5% when the temperature was lowered from 42 to 37°C.

± 0.05 mm Hg at 37°C to 0.53 ± 0.04 mm Hg at 42°C ($p < 0.01$, $n = 11$). In a separate group of embryos, this parameter increased from 0.57 ± 0.03 mm Hg at 42°C to 0.62 ± 0.02 mm Hg at 37°C ($p < 0.05$, $n = 14$; Figs. 7 and 8). Umbilical artery vascular resistance decreased from 3.7 ± 1.3 units (median ± 95% confidence interval) at 37°C to 1.8 ± 0.4 units at 42°C and returned to 3.4 ± 1.0 at 37°C (Fig. 9). Heart rate, averaged from all groups, increased from 158 ± 4 at 37°C ($n = 33$) to 181 ± 3 ($p < 0.01$) at 42°C ($n = 47$) and decreased to 149 ± 3 when the temperature was lowered to 37°C ($n = 36$).

DISCUSSION

In our previous studies using a nonoxygenated bath, we recognized that the physiologic condition of the embryo could be different from that of *in utero* status (6); thus, the system was not suitable for studies to be done during a certain length of time. To resolve this limitation, oxygen was bubbled into Hanks' solution in the present study as it is done in whole embryo culture (8). The heart rate of embryos in this new system was approximately 160 at 37°C, which was the same as that reported by Robkin *et al.* (9), who studied the effect of catecholamine on heart rate using a whole embryo culture technique. Thus, the

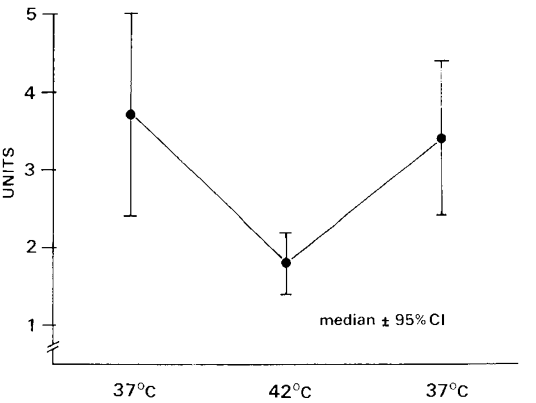

Fig. 9. Umbilical artery vascular resistance was decreased during hyperthermia and was returned to control value after returning temperature to normothermia. Data are presented as a median ± 95% confidence intervals.

new system has greatly improved the experimental conditions, although it might not yet be completely satisfactory, inasmuch as heart rate declined in some embryos 30 min after the start of the experiment. Nevertheless, the stability of the pressure and flow data for 30 min has indicated that this system is suitable for studying hemodynamics of embryos at gestational d 12 for a period of at least 30 min. Criteria that exclude embryos from the study are a decline of heart rate by 10% or more in the control condition during the experiment and/or a progressive decline of any hemodynamic parameters. In the present experiment using these criteria, the study was completed at 21.4 ± 1.4 min after excision of embryos from dams when the flow velocity was measured at the outflow tract and 19.5 ± 1.6 min in the umbilical flow study. Thus, these studies were performed while the embryos were still viable.

Our previous study of the hemodynamic effects of hyperthermia in chick embryos showed that high environmental temperature resulted in an increase in heart rate and cardiac output, and an elevation of blood pressure (4). As seen in the present study, the response of heart rate and cardiac output was similar in rat and chick embryos, but the increase of stroke volume and the decrease of blood pressure were seen only in rat embryos. The lowering of blood pressure along with the increase in cardiac output indicates reduction of peripheral vascular resistance, which would have resulted from an active peripheral vasodilation and/or a reduction of viscosity of circulating blood secondary to the change in temperature. Because it was not technically possible to obtain the hemodynamic parameters at the aorta, we were not able to demonstrate that the blood pressure change at the umbilical artery was the same as at the aorta; thus, it was not clear that the resistant vessels of the embryo itself were dilated in response to hyperthermia. The response of embryonic vasculature to the intervention will be first clarified when we can measure the vascular resistance of the embryo alone in separation from placental circulation. It is unlikely that decrease of viscosity of the embryonic blood due to the elevation of temperature by only 5°C is large enough to correspond totally to the change of vascular resistance of the umbilical artery (10). We have wondered how the vascular bed of the placenta with its sinusoidal structure could have actively dilated in any circumstance. Because the elevation of temperature certainly increases embryonic metabolism, which is largely dependent upon the placental circulation, it is possible that this vasculature is able to actively reduce the resistance to blood flow in response to hyperthermia, although the precise mechanism is not clear at the present time.

The hemodynamics play an important role in normal and abnormal cardiovascular morphogenesis (11–13). Bruyere *et al.* (14) measured ventricular volume of chick embryos using a microscopic cine system and found that the increase in cardiac output evoked by epinephrine was associated with a cardiac

anomaly similar to tetralogy of Fallot, which was confirmed later in the same embryo. The intervention was not chronic in their study, yet the anomaly was produced, and it is also suggested that a transient epigenetic change is able to alter the cellular gene expression, which could affect morphogenesis (15). These indicate that chronic intervention is not necessarily mandatory to produce anomalies. There has been no detailed information whether hyperthermia during morphogenesis is causally related to cardiovascular anomalies. Spragget and Fraser (16) reviewed 1668 clinical records of mothers who had a child with a congenital anomaly and found that maternal fever during the first 5 mo of pregnancy was highly associated with congenital cardiac defect, hypospadia, and microphthalmia, but no further information was given in their abstract. Thus, we are not able to relate the hemodynamic changes directly to specific cardiovascular anomalies. Recent information obtained from fetal echocardiographic studies indicate that the abnormal intracardiac blood flow alters cardiac morphology as pregnancy goes on, even as late as during midtrimester of pregnancy (17). The developmental stage of the embryos that we used here corresponds to Horizon XIII, approximately d 28 of pregnancy, of human development, when septation of the heart is not yet finished but the looping has almost been completed and well developed endocardial cushions are functioning as inflow and outflow valves (18). Thus, the increase of cardiac output on hyperthermia seen in this study could modulate cardiovascular morphogenesis, although the intervention was not given during the teratologically vulnerable period.

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