Hemodynamic Effects of Environmental Hyperthermia in Stage 18, 21, and 24 Chick Embryos

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ABSTRACT. Environmental hyperthermia is a hazard to the poikilothermic chick embryo. We studied effects of hyperthermia on mean vitelline arterial blood pressure and mean dorsal aortic blood flow in stage 18, 21, and 24 chick embryos. The pressure was measured with a servo-null micropressure system, and the blood flow was measured with a 20 MHz pulsed Doppler flowmeter. Temperature was monitored with a needle thermoprobe positioned adjacent to the embryo. Data were obtained at 37° C, after warming to 40° C, and then after cooling to 37° C. At stage 21, the pressure increased from 0.96 ± 0.05 ($\pm$ SE) to 1.04 ± 0.06 mm Hg on warming and returned from 1.05 ± 0.04 to 0.87 ± 0.04 mm Hg on cooling. Pressure measurements during warming and cooling were performed in two separate groups of embryos because of technical problems. The blood flow, studied using different groups of the embryo from the pressure study, also increased from 0.65 ± 0.06 to 0.75 ± 0.06 mm³/s on warming and returned to 0.56 ± 0.05 mm³/s. The heart rate increased from 173 ± 2 to 211 ± 3 at 40° C and returned to 170 ± 3 at 37° C. Stroke volume (flow/heart rate) did not change during the temperature variation. Vascular resistance, the quotient of pressure to blood flow obtained by a ratio analysis, changed from 1.53 ± 0.33 (median $\pm$ 95% confidence interval) to 1.42 ± 0.29 mm Hg/mm³/s on warming and changed to 1.60 ± 0.32 mm Hg/mm³/s on cooling. Similar results were obtained at stages 18 and 24. The data indicate that the primary effect of hyperthermia is increasing heart rate, bringing about the increase of cardiac output and arterial pressure. (*Pediatr Res* 20: 1213-1215, 1986)

MATERIALS AND METHODS

One hundred twenty-eight embryos of the white Leghorn chick were used at the Hamburger-Hamilton stages 18, 21, and 24 (5). We used the same experimental procedures as previously reported (1, 2). After exposing the embryo and the vitelline arteries, the dorsal aortic blood flow was measured with a 20 MHz pulsed Doppler flowmeter with a probe of 1 mm diameter. The vitelline arterial blood pressure was measured by a servo-null micropressure system (WP Instruments model 900) with a glass pipette of a tip diameter of 5 μ m. Data for pressure and flow were obtained in separate groups of embryos because of technical difficulty.

Temperature was monitored by a needle thermoprobe, a tip of which was inserted just underneath or very close to the embryo (Fig. 1). A heat lamp was used to control the temperature. To avoid drying the embryo and vitelline circulation, we limited an opening of the shell membrane as small as possible and pipetted 0.3-0.5 ml chick Ringer solution. The latter procedure had been reported to have no hemodynamic effect in this preparation (1).

Data were obtained at 37° C, and the embryo was warmed to 40° C when data were again recorded. Then temperature was lowered to 37° C where pressure and flow were measured. The flow data were recorded continuously during the temperature change in each embryo. But the pressure study was performed in two separate groups of the embryo at each stage. One group was during warming and the other during cooling. This was because the ionic balance between chick embryo blood and 1 N KCl solution within the glass pipette could change with time,

Evidence is now being accumulated on the functional development of the cardiovascular system, which should help understand normal or abnormal organogenesis. We have reported the effects of environmental hypothermia on hemodynamics of the chick embryo (1, 2). High temperature during early stages of development causes embryo death or malformations of various organs including the cardiovascular system (3, 4). Therefore, we studied the hemodynamic effect of environmental hyperthermia in the chick embryo. We found that hyperthermia brought about the increase of cardiac output through the increase of heart rate, which resulted in the increase of vitelline arterial blood pressure.

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Fig. 1. Positioning of a needle thermoprobe. Upper right, cross-sectional view details relation of needle probe to embryo.

thus causing a shift of zero balance of the system. The number of embryos used in each protocol was presented in Table 1. The arterial vascular resistance was calculated from the flow and pressure data by a ratio analysis (6). Statistical analysis was performed by analysis of variance or repeated-measures analysis of variance and Newman-Keuls test; $p < 0.05$ was accepted as significant.

RESULTS

Heart rate increased by 17–22% at the high temperature and returned to base line on cooling to 37° C (Table 2).

On warming to 40° C, the mean arterial blood pressure increased from 0.80 ± 0.02 ($\pm$ SE) to 0.92 ± 0.03 mm Hg in stage 18 ($n = 16$), from 0.96 ± 0.05 to 1.04 ± 0.06 mm Hg in stage 21 ($n = 14$), and from 1.15 ± 0.05 to 1.34 ± 0.06 mm Hg in stage 24 ($n = 13$). This parameter decreased to base line on cooling to 37° C (Fig. 2).

The dorsal aortic blood flow increased on warming from 0.40 ± 0.03 to 0.46 ± 0.03 mm³/s and returned to 0.36 ± 0.03 mm³/s on cooling in stage 18 ($n = 13$); from 0.65 ± 0.06 to 0.75 ± 0.06 mm³/s, then to 0.56 ± 0.05 mm³/s in stage 21 ($n = 18$),

Table 1. Number of embryos in each protocol

	Flow study	Pressure study	
		Warm-up	Cool-down
Stage 18	13	16	12
Stage 21	18	14	16
Stage 24	12	13	14

Table 2. Changes in heart rate*

Temperature (°C)	Stage 18	Stage 21	Stage 24
Pressure study			
37	162 $\pm$ 4	174 $\pm$ 3	192 $\pm$ 7
40	189 $\pm$ 3	204 $\pm$ 4	227 $\pm$ 7
40	192 $\pm$ 4	203 $\pm$ 5	225 $\pm$ 5
37	159 $\pm$ 4	167 $\pm$ 4	187 $\pm$ 5
Flow study			
37	163 $\pm$ 3	173 $\pm$ 2	186 $\pm$ 2
40	196 $\pm$ 4	211 $\pm$ 3	224 $\pm$ 5
37	159 $\pm$ 4	170 $\pm$ 3	188 $\pm$ 3

* Mean $\pm$ SE.

Fig. 2. Effect of hyperthermia on mean vitelline arterial blood pressure. Pressure increased in 14 of 16 embryos in stage 18, in 10 of 14 in stage 21, and in 11 of 13 in stage 24, whereas it decreased in only one embryo in each stage. These data resulted in a statistically significant change in each stage, although the amount change was very small.

Fig. 3. Effect of hyperthermia on mean dorsal aortic blood flow. Flow increased significantly ($p < 0.05$) in each stage.

Fig. 4. Stroke volume (mean dorsal aortic flow/heart rate) did not significantly change during temperature change.

Fig. 5. Vitelline arterial vascular resistance showed no statistically significant changes.

and from 1.27 ± 0.15 to 1.53 ± 0.16 mm³/s, then to 1.17 ± 0.10 mm³/s in stage 24 ($n = 12$), respectively (Fig. 3). The stroke volume (dorsal aortic blood flow $\times$ 60/heart rate) was not significantly altered by the temperature change (Fig. 4).

The change of vitelline arterial vascular resistance was not statistically significant (Fig. 5).

DISCUSSION

The present study demonstrated that the increase in environmental temperature brought about the increase of dorsal aortic blood flow. The flow obtained at the dorsal aorta did not include flows to the head and myocardium, thus underestimating cardiac output (7). We assumed that the distribution of the systemic circulation of the embryo was constant during the change of temperature. Then we could conclude that environmental hyperthermia resulted in increased cardiac output.

We also found that stroke volume was not altered by hyperthermia. These findings indicated that the increase of cardiac output was a result of the increase in heart rate. Heart rate of the chick embryo is sensitive to temperature variation (1, 2, 8, 9).

In the mature subject, environmental hyperthermia results in a reduction of blood pressure through peripheral vasodilation, and this is compensated by an increase in heart rate brought about by the autonomic reflex and by an increase in cardiac output partly through augmented emptying of the ventricle (10, 11).

In the present study on the embryo, the calculated peripheral vascular resistance showed no significant reduction during hyperthermia. The lack of statistical significance might be due to the wide range of 95% confidence intervals of the data because of the technical problem as described in "Materials and Methods." Yet the reduction of vascular resistance might be physiologically significant.

Conversely, the vasculature may not actually show any active response to hyperthermia as the statistics showed. In the stages of embryo we used, the cardiovascular innervation was not established (9); thus vascular smooth muscle might be already in fully relaxed state. If this were the case, any intervention could not actively dilate the vessels. In fact, a potent β -agonist, isoproterenol, caused an increase, but not a decrease, of peripheral vascular resistance (12). Hawkins *et al.* (13) reported that caffeine induced vasodilation (13). In their study, blood flow and pressure were increased by caffeine, so it could not be concluded that the vessels were actively dilated; but vasodilation was likely due to the increase in cardiac output.

The vascular bed of the embryo of these stages is responsive to vasoactive substances such as phenylephrine, norepinephrine, and isoproterenol (12, 14). It is also known that egg yolk contains epinephrine and norepinephrine (15); in addition, Wallace reported that the chick embryo started to produce serotonin as early as stage 10 (16). Thus the temperature change may have induced the release of these intrinsic substances that diffused into the embryonic circulation. The result that hyperthermia was not associated with a significant decrease of vascular resistance might be explained by speculation that passive vasodilatation due to

increased cardiac output was counteracted by vasoconstriction brought about by the released vasoconstrictive substances.

Nilsen (4) reported that incubation at 41 and 42° C was associated with various cardiovascular malformations in the chick embryo. They included dilation of various vessels, congested and dilated microvascular segments in the pellucid area, perivascular swelling, cardiac deformations, and irregular defects in the endocardial lining of the cardiac bulb with streaks of fluorescent material used in the study within the wall. Although the experimental conditions between our study and the study of Nilsen were quite different, the increased blood flow and pressure could dilate the vessels and damage the ventricular wall through an increase of wall stress. Thus, the present data could explain part of the mechanisms of the observations made by Nilsen (4).

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