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Bohr effect
congenital heart disease
2,3-diphosphoglycerate
newborns
P_{50}
shunt hypoxemia

Oxygen Transport in Congenital Heart Disease: Influence of Fetal Hemoglobin, Red Cell pH, and 2,3-Diphosphoglycerate

H. T. VERSMOLD,[37] C. LINDERKAMP, C. DÖHLEMANN, AND K. P. RIEGEL

Division of Neonatology, Kinderklinik der Universität, Munich, Federal Republic of Germany

Extract

In 48 individuals (age 1 day to 13 years) with congenital heart disease, blood oxygen transport function was studied in order to evaluate adaptive changes in shunt hypoxemia and to investigate the *in vivo* regulation of erythrocyte 2,3-diphosphoglycerate concentration (RBC 2,3-DPG) in the presence of fetal hemoglobin (HbF). Arterial pO_2 and oxygen content, oxygen capacity, acid base status, oxygen affinity, HbF fraction, plasma pH, red cell pH, and RBC 2,3-DPG were determined. During the first 50 days of life values of standard P_{50} ($stdP_{50}$) (37°, pH 7.4), actual *in vivo* P_{50} ($actP_{50}$), RBC 2,3-DPG, O_2 capacity, arterial plasma pH, and red cell pH were scattered around the normal range, although tending to low values for $stdP_{50}$ and arterial plasma pH and to high values for O_2 capacity. After the third month, $stdP_{50}$ $actP_{50}$, RBC 2,3-DPG, O_2 capacity, and red cell pH were found to be elevated. Plasma pH and $actP_{50}$ were scattered around the normal range (Figs. 1 and 2).

Intraerythrocytic pH in hypoxemic infants was increased compared with normal children when related to plasma pH (Fig. 3). A close to normal intraerythrocytic pH was therefore found in the hypoxemic infants with low plasma pH, and an increased intraerythrocytic pH in the hypoxemic children with normal plasma pH (Fig. 1). A significant negative correlation exists between erythrocyte H^+ ion and 2,3-DPG concentration (Fig. 5); regression constants derived from data at high (mean 47%) and low (mean 9%) fractions of HbF are not significantly different (Regression Equations 8 and 11 in Table 1). Thus, the known difference in 2,3-DPG binding to fetal or adult deoxyhemoglobin does not measurably influence the erythrocyte 2,3-DPG concentration, indicating that *in vivo* the 2,3-DPG synthesis in hypoxia is virtually regulated by the erythrocyte pH, which in turn is determined by plasma pH and the oxygenation state of hemoglobin.

Speculation

In young infants and older children with cyanotic heart disease an identical negative correlation between the concentrations of 2,3-DPG and hydrogen ions within the erythrocyte was found. The *in vivo* regulation of 2,3-DPG synthesis thus appears to be controlled by the erythrocyte pH which, in turn, is determined by plasma pH and oxygen saturation of hemoglobin. Apparently the well established difference of 2,3-DPG binding to fetal or adult deoxyhemoglobin does not measurably influence the erythrocyte 2,3-DPG concentration, indicating that *in vivo* a relief of product inhibition of the diphosphoglycerate mutase does not contribute significantly to the regulation of 2,3-DPG synthesis in hypoxemia.

Chronic hypoxemia in congenital heart disease is associated with low affinity of blood for oxygen (high P_{50} value), both in adults (19, 24) and in children older than 4 months (19, 24), because of an increased erythrocyte 2,3-DPG concentration (20, 24, 31). It has been suggested that low hemoglobin O_2 affinity improves oxygen delivery to tissues, as has been experimentally confirmed for anemic hypoxia on isolated perfused livers (27).

In the human newborn blood oxygen affinity depends mainly on the relation of fetal to adult hemoglobin (HbA) and on red cell pH. HbF reacts to a lesser extent with RBC 2,3-DPG. In this age group lowered blood oxygen affinity and elevated 2,3-DPG have been described (12) in cyanotic infants with congenital cardiac malformations. Detailed information on parameters of blood oxygen transport in infants during the first months of life is lacking in regard to congenital heart disease.

The mechanism of the *in vivo* regulation of 2,3-DPG synthesis during hypoxia is still under discussion (2, 13, 14, 21, 23). Prevailing influences of pH or of 2,3-DPG binding to deoxyhemoglobin (13) are alleged. Since 2,3-DPG is less bound to deoxy-HbF than to deoxy-HbA (11), a comparison of findings in young infants and older children with cyanotic heart disease should give useful information regarding those factors which control the erythrocyte 2,3-DPG concentration and, thus, the red cell adaptation to hypoxemia.

METHODS

PATIENTS

Fifty-three children with congenital heart disease were examined, 39 of whom were cyanotic (transposition of the great vessels, $n = 23$). Seven infants were studied on two, and one infant on five occasions. Sixteen examinations were performed at an age < 30 days, 35 < 4 months, 47 < 1 year. The acyanotic children did not suffer from severe cardiac failure. Data are compared with those for normal infants (29) who were studied simultaneously by the same methods. At the time of study all individuals had normal body temperature.

PROCEDURES

In all individuals blood was withdrawn at routine measures for diagnosis (e.g., during cardiac catheterization) or for clinical control with informed consent of parents. At each observation a total of approximately 3 ml arterial and venous blood were collected in heparinized glass syringes. Specimens were processed within 2 hr and kept at 4° until analysis. The methods used have been described in detail elsewhere (29). Oxygen content was measured by means of gas chromatography (Beckman GCM), oxygen tension with a Clark-type oxygen electrode (Instrumentation Laboratories pH/gas analyzer, model 113), pH with a microglass-electrode (Instrumentation Laboratories). Red cell pH was measured directly (16) using a freeze-thaw technique. Fetal hemoglobin was measured by alkali denaturation (7), and RBC 2,3-DPG enzymatically by a kinetic test (8). Whole blood $stdP_{50}$ was determined graphically using a Bohr factor ($\Delta \log pO_2/\Delta pH$) $= -0.50$ (29); $actP_{50}$ was derived from the actual *in vivo* data.

Linear regression lines were compared with regard to the probability of nonidentity of the slope (b) and the intercept (a) (15).

RESULTS

INFANTS WITH ACYANOTIC HEART DISEASE

The parameters studied (Figs. 1 and 2) did not differ from the values for healthy children in their age-dependent course. These findings are in agreement with those reported for adults with acyanotic heart disease and minor cardiac failure (18, 32).

INFANTS WITH CYANOTIC HEART DISEASE

Arterial oxygen tension (paO_2) of this group ranged from 14.4 to 61.4 Torr. Arterial pCO_2 was found to be low or normal; two infants had moderate hypercapnia.

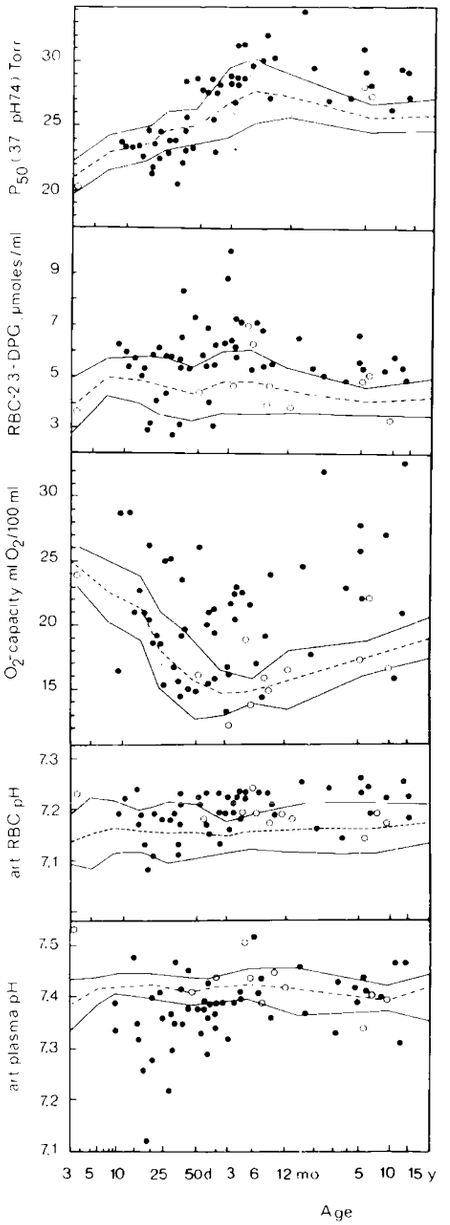

Fig. 1. Postnatal course of standard P_{50} value (pH 7.4; 37°), red cell 2,3-diphosphoglycerate (*RBC-2,3-DPG*) concentration, oxygen capacity, red cell pH, and arterial plasma pH in infants suffering from cyanotic heart disease (●) and acyanotic heart disease (O) as compared with the mean ( ) ± SD (——) of normal individuals (data from Reference 29).

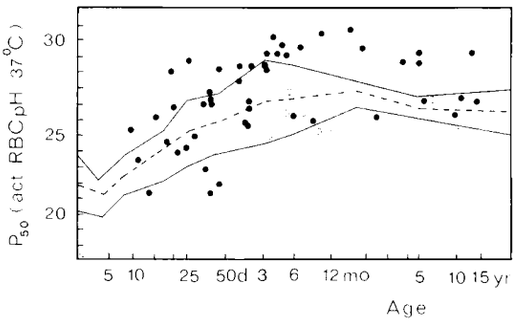

Fig. 2. Postnatal course of *in vivo* P_{50} at actual red cell (*act. RBC*) pH, 37°. ●, cyanotic heart disease; O, acyanotic heart disease as compared with the mean (---) ± SD (——) of normal indivduals.

During the first 50 days of life the RBC 2,3-DPG levels were scattered around the normal range; after about the third month they were found to be generally elevated (Fig. 1). No arterial *vs* venous differences of RBC 2,3-DPG were found, in samples collected from arterial and venous sites in the same individual during cardiac catheterization ($n = 15$; paired t-test).

$StdP_{50}$ values (pH 7.4; 37°) were low or normal during the first 50 days, and elevated after the third month (Fig. 1). The $actP_{50}$ values were scattered around the normal range during the first 50 days and were moderately elevated later on (Fig. 2).

Blood O_2 capacity was normal or elevated during the first 50 days of life. As in healthy individuals, there was a reduction in O_2 capacity in the first trimester, although on a higher level. With increasing age the O_2 capacity of cyanotic children exceeded that of normal individuals (Fig. 1). The substitution of HbA for HbF in the cyanotic infants was not different from that observed in healthy individuals. At comparable HbA/HbF ratios the correlations of $stdP_{50}$ *vs* RBC 2,3-DPG (Regression Equations 12 and 13 in Table 1) are in accordance with the literature (10, 29).

Arterial plasma pH was found to be decreased most frequently during the first 3 months of age, and to be normal later on. In contrast, the RBC pH was not significantly reduced, and was elevated or normal after the third month (Fig. 1). The distribution ratio of hydrogen ions between plasma and red cells (H_e^+/H_i^+) in the hypoxemic infants is increased at any given plasma pH as compared with normoxemic control subjects (Fig. 3), independently of age. This means that at comparable plasma pH the RBC pH is elevated in hypoxemia. The data coincide with regressions found *in vitro* under similar conditions (5).

RBC 2,3-DPG increased with advancing hypoxemia in older children (HbF $\leq$ 25%; mean 9%) if related to paO_2 (Equation 5, Table 1) or to O_2 content of arterial blood (CaO_2) (Equation 3, Table 1). In young infants (HbF > 35%; mean 47%) a significant correlation of RBC 2,3-DPG *vs* paO_2 or CaO_2 is found only if data of infants with a plasma pH < 7.3 are excluded (Equations 4 and 2, Table 1). The correlations appear to be closer in older children; however, the slopes of the regressions are not significantly different among young and older infants, indicating that the 2,3-DPG response to hypoxemia is quantitatively the same in these two age groups at comparable plasma pH.

The dependence of RBC 2,3-DPG on both the plasma H^+ concentration (H_e^+) and on O_2 saturation of hemoglobin is represented in Figure 4*a* for older children (mean HbF 9%) and in Figure 4*b* for young infants (mean HbF 47%). In both age groups there is a negative correlation between RBC 2,3-DPG and plasma H^+; at a given plasma H^+ concentration the RBC 2,3-DPG levels in hypoxemia exceed the values found in normoxemia by approximately 1.5 mM. Since high plasma H^+ concentrations (low plasma pH) prevail in the young hypoxemic group, their average RBC 2,3-DPG does not exceed that of the young normoxemic group (Fig. 4*b*).

The relation between RBC 2,3-DPG and the actual intraerythrocytic H^+ concentration (H_i^+) is represented in Figure 5*a* for

Table 1. *Constants of regression equation:* $y = bx + a$[1]

Equation no.	n	y	=	b (slope)	x	+	a (intercept)	s_{yx}	r	P	Conditions	Comment
1	22	2,3-DPG	=	0.014	CaO_2	+	4.898	1.433	0.046	n.s.	HbF > 35%	
2	18	2,3-DPG	=	0.167	CaO_2	+	7.394	1.102	0.564	<0.05	HbF > 35%; pH ≤ 7.30	
3	38	2,3-DPG	=	0.155	CaO_2	+	7.800	0.934	0.613	<0.001	HbF ≤ 25%; pH ≤ 7.30	
4	18	2,3-DPG	=	0.056	paO_2	+	7.165	0.119	0.502	<0.05	HbF > 35%; pH ≤ 7.30	
5	38	2,3-DPG	=	0.043	paO_2	+	7.630	0.925	0.638	<0.001	HbF ≤ 25%; pH ≤ 7.30	
6	22	2,3-DPG	=	0.193	H_i^+	+	17.810	0.520	0.826	<0.001	HbF > 35%; SaO_2 > 90%	2
7	16	2,3-DPG	=	0.148	H_i^+	+	15.162	0.966	0.753	<0.001	HbF > 35%; SaO_2 25–75%	2
8	38	2,3-DPG	=	0.177	H_i^+	+	16.854	0.981	0.731	<0.001	Nos. 6 and 7 combined	2
9	34	2,3-DPG	=	0.182	H_i^+	+	16.850	0.470	0.738	<0.001	HbF ≤ 25%; SaO_2 > 90%	2
10	22	2,3-DPG	=	0.150	H_i^+	+	15.496	0.938	0.617	<0.01	HbF ≤ 25%; SaO_2 25–75%	2
11	56	2,3-DPG	=	0.126	H_i^+	+	19.383	0.743	0.818	<0.001	Nos. 9 and 10 combined	2
12	21	$stdP_{50}$	=	1.192	2,3-DPG	+	17.512	1.239	0.810	<0.001	HbF > 35%	
13	35	$stdP_{50}$	=	1.705	2,3-DPG	+	18.988	1.248	0.827	<0.001	HbF ≤ 25%	

[1] 2,3-DPG: red cell 2,3-diphosphoglycerate (in millimolar concentration); CaO_2: arterial oxygen (in milliliters of O_2 per 100 ml); paO_2: arterial oxygen tension (Torr); H_i^+: intraerythrocytic hydrogen ion (in nanomolar concentration); $stdP_{50}$: P_{50} (pH 7.4, 37°) (Torr); SaO_2: arterial oxygen saturation (percentage).

[2] Regression Equations 6–11 not significantly different with regard to slope (b) and intercept (a).

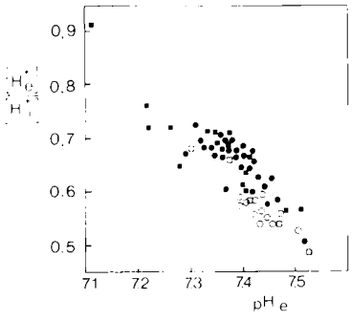

Fig. 3. Distribution ratio of extraerythrocytic over intraerythrocytic H^+ concentration (H_e^+/H_i^+) plotted *vs.* arterial plasma pH (pH_e), at different arterial oxygen saturations (SaO_2) in congenital heart disease. ●, ■: SaO_2 <80%; ○, □: SaO_2 <85%. Relative red cell alkalinity in the hypoxemic subjects at any given plasma pH is evident. Values for young infants (□, ■: mean fetal hemoglobin 47%) and older children (○, ●: mean adult hemoglobin 91%) do not differ significantly.

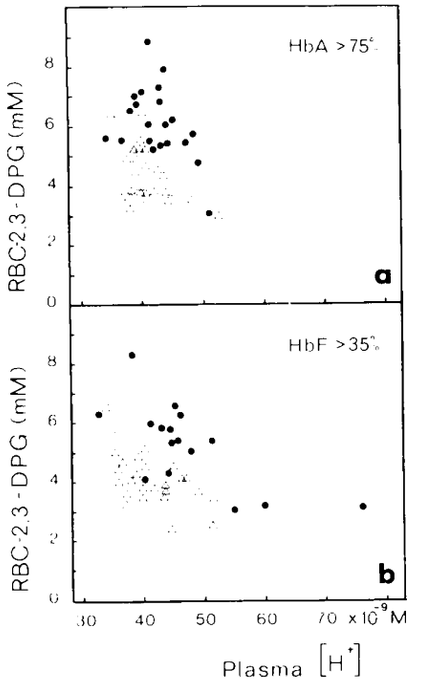

Fig. 4. Dependency of red cell 2,3-diphosphoglycerate (*RBC-2,3-DPG*) concentration on plasma H^+ concentration. *a*, older children (adult hemoglobin (*HbA*) >75%; mean 91%); *b*, young infants (fetal hemoglobin (*HbF*) >35%, mean 47%). Normoxemic individuals (Δ), SaO_2 >90% (data from Reference 29); hypoxemic patients (●), SaO_2 25–75%.

older children, and in Figure 5*b* for young infants, grouped according to normoxemia and hypoxemia. Both in the young infants and the older children RBC 2,3-DPG correlates closely with H^+ (Equations 8 and 11, Table 1). In these correlations the values of the hypoxemic and normoxemic groups coincide (Equations 6 and 7, Table 1, for young infants; Equations 9 and 10, Table 1, for older children). There is no significant difference between these correlations of RBC 2,3-DPG *vs* H_i^+ calculated for young infants and older children.

DISCUSSION

IN VIVO REGULATION OF 2,3-DPG IN CHRONIC HYPOXEMIA

The 2,3-DPG increase in hypoxemia appears to be controlled by the oxygenation state of hemoglobin (2), although contradictory results have been described (23). *In vitro* two control mechanisms for RBC 2,3-DPG by the O_2 saturation of hemoglobin are established. Their relative importance *in vivo* is still under debate.

Mechanism 1. 2,3-DPG is preferentially bound to adult deoxyhemoglobin (6, 9, 14); a drop of free 2,3-DPG in deoxygenated erythrocytes has been discussed to enhance 2,3-DPG synthesis in hypoxemia via a relief of product inhibition of DPG mutase (6, 14, 21). Because of the low binding of 2,3-DPG to HbF (11), a lower response to hypoxemia should be expected in fetal erythrocytes. This has indeed been observed *in vitro* (21).

Mechanism 2. The O_2 saturation of hemoglobin controls 2,3-DPG synthesis and breakdown by influencing the intraerythrocytic pH (10). *In vitro* (1, 5, 13) and also *in vivo* (Fig. 3) the intraerythrocytic pH increases when blood is deoxygenated, at any given plasma pH, because of proton binding to deoxyhemoglobin (33) and a redistribution of diffusable charges, according to the Donnan equilibrium (26).

From the present data (Fig. 5), it appears that *in vivo* the RBC 2,3-DPG concentration is virtually regulated by the RBC pH.

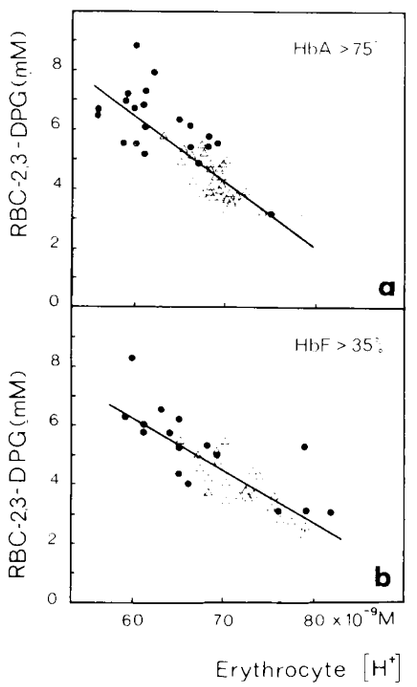

Fig. 5. Dependency of red cell 2,3-diphosphoglycerate (*RBC-2,3-DPG*) concentration on intraerythrocytic H^+ concentration (H^+_i). *a*, older children; *b*, young infants. For conditions and symbols see Figure 4. The correlation of 2,3-DPG *vs* H^+ is not significantly different in young infants and older children (see Regression Equations 8 and 11, Table 1). *HbA*, adult hemoglobin; *HbF*, fetal hemoglobin.

RBC pH, in turn, depends on the oxygenation state of hemoglobin and on plasma pH.

From the comparison of the presented correlations of RBC 2,3-DPG *vs* H^+, which are not significantly different in hypoxemic young infants (Fig. 5*b*) and older children (Fig. 5*a*), it may be concluded that *in vivo* the difference in 2,3-DPG binding to fetal or adult deoxyhemoglobin does not measurably influence the RBC 2,3-DPG concentration.

In hypoxemia, RBC pH and, thus, 2,3-DPG were found to be elevated, provided plasma pH was normal. Low plasma pH was found to counteract the effect of hypoxemia on RBC pH and 2,3-DPG, as in most of the young infants studied. Similar counteracting effects of low plasma pH and hypoxemia on RBC 2,3-DPG have been observed in the respiratory distress syndrome of the newborn (10, 12, 22).

Additional factors, *e.g.*, plasma inorganic phosphate, may contribute to the 2,3-DPG increase in hypoxemia. However, the correlation of 2,3-DPG *vs* H^+_i which we observed in hypoxemia is strictly the same as that found in patients with metabolic acid base disorders (3). This underlining the fact that RBC pH is indeed the predominant regulator of 2,3-DPG metabolism.

Keitt and coworkers (17) excluded inorganic phosphate as a factor determining RBC 2,3-DPG in patients with chronic obstructive lung disease. Furthermore, although these authors did not consider red cell pH, a rough estimation of red cell pH from their data on the basis of our regression equations suggests that 2,3-DPG may correlate with red cell pH in their patients, both during sustained hypoxemia and after acute changes of the oxygenation state.

PHYSIOLOGIC RELEVANCE OF OXYGEN AFFINITY IN SHUNT HYPOXEMIA

A decrease in oxygen affinity (high P_{50} value) of blood enhances the release of oxygen from hemoglobin to the tissues (27) and thus compensates for a decreased cardiac output and/or reduced O_2 content of blood (4). The generally accepted assumption, however, that a high P_{50} value implies an increased O_2 unloading capacity of blood, is not applicable at low arterial oxygen tensions (22, 25, 31), *i.e.*, in the steep part of the oxygen equilibrium curve of hemoglobin. With increasing shunt hypoxemia the effect of P_{50} on O_2 unloading diminishes (22, 25, 30, 31). In shunt hypoxemia the observed changes of the actual *in vivo* P_{50} (Fig. 2) should be of minor relevance, whereas adequate tissue oxygenation depends primarily on oxygen content of blood and thus mainly on red cell mass (28).

SUMMARY

In older children suffering from cyanotic heart disease an adaptive increase in O_2 capacity, 2,3-DPG, and P_{50} occurs. *In vivo* the 2,3-DPG increase in hypoxemia appears to be controlled by the increased intraerythrocytic pH which, in turn, depends on the low O_2 saturation of hemoglobin. In the first weeks of life 2,3-DPG is not elevated in infants with cyanotic heart disease because the low plasma pH frequently found in these babies is balancing the effect of hypoxemia on red cell pH.

During hypoxemia, oxygen delivery to tissues depends on the O_2 capacity rather than on the O_2 affinity. Therefore, an insufficient increase of RBC 2,3-DPG and of blood P_{50} in response to hypoxemia may not represent a serious disadvantage. Low hemoglobin concentration, however, must be considered a serious cause of hypoxia.

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37. Requests for reprints should be addressed to: H. Versmold, M.D., Universitäts-kinderklinik, D-8 München 2, Lindwurmstr. 4, Federal Republic of Germany.
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Response of the Neonatal Heart to a New Inotropic Agent, RO2-2985 (X537A)

PAUL C. GILLETTE,[22] RONALD G. MUNSON, ROBERT M. LEWIS, AND ARNOLD SCHWARTZ

Department of Cell Biophysics, Division of Myocardial Biology, and Department of Pediatrics, Section of Cardiology, Baylor College of Medicine and the Fondren-Brown Cardiovascular Research and Training Center, The Methodist Hospital, Houston, Texas, U.S.A.

Extract

The ionophore RO2-2985 did not produce a positive inotropic response in 1-day-old canine isolated ventricle. A gradual increase in inotropic response was seen with age. Isolated atria, however, exhibited a positive inotropic response at birth (50% increased dF/dt), which became progressively greater with age (100% increase in dF/dt at 15 days of age). In the neonatal heart *in situ* there was a positive inotropic response in 1-day-old puppies (40% increase in left ventricular dF/dt) with progressively greater responses with age (135% increase in left ventricular dF/dt at 11 days of age). There was a positive chronotropic (75–125% increase) response to RO2-2985 at all ages studied. The drug elevated systemic arterial pressure (150% increase in mean arterial pressure) to a similar degree in all ages studied. RO2-2985 depressed total calcium binding by both neonatal and adult isolated cardiac sarcoplasmic reticulum approximately 50%.

Speculation

Although the exact mechanism of action of RO2-2985 on the heart remains unproven, the temporal association of the increase in ventricular and atrial sympathetic nerve endings with development reported by previous authors and the increase in response to RO2-2985 and tyramine suggest that the drug might act in part by releasing a humoral substance. The discrepancy between *in situ* and isolated studies tends to support this view. A more complete delineation of all subcellular fractions of the developing heart will be necessary to clarify the differential actions of this and other drugs on the developing heart. RO2-2985 may be of value in the treatment of low cardiac output states in neonates as well as adults, but specific testing in neonates will be necessary.

The antibiotic ionophore RO2-2985 (Hofmann-LaRoche), in exerting a significant effect on the cardiovascular system,