0031-3998/03/5406-0875
PEDIATRIC RESEARCH
Copyright © 2003 International Pediatric Research Foundation, Inc.
Vol. 54, No. 6, 2003
Printed in U.S.A.

Na^+-Channel Modulation, a New Principle of Inotropic Intervention: Effects on Hemodynamic and Myocardial Energetics in the Immature Rabbit Heart

HOLGER SCHIFFMANN, VICKY RIZOULI, FRANK LÜERS, FRANK HACKMANN, DYRKEN HOEBEL, ANNETTE PFAHLBERG, AND GERHARD HELLIGE

Departments of Pediatric Cardiology and Intensive Care [H.S., V.R., F.L., F.H., D.H.] and Anaesthesiological Research [G.H.], University of Goettingen, Goettingen D-37075, Germany; and Department of Statistics [A.P.], University of Erlangen, D-91058 Erlangen, Germany

ABSTRACT

Na^+-channel modulators exert their positive inotropic action without affecting the adenylate-cyclase pathway by an increase in the open probability of the sarcolemmal Na^+ channels. Although inotropic effects in neonatal hearts are less pronounced compared with adult hearts, the Na^+-channel modulator BDF 9148 increases contractility and relaxation velocity in immature myocardium. Effects on hemodynamics and myocardial energetics are not known. Therefore, we studied the Na^+-channel modulator BDF 9148 in isolated antegrade perfused rabbit hearts of different ages (2-28 d) and compared the effects with isoproterenol, enoximone, and ouabain. ANOVA showed significant effects in the concentration response curves for heart rate, stroke volume, cardiac output, and oxygen consumption but not for myocardial efficiency ($p = 0.06$). Age-dependent differences were observed for heart rate and stroke volume. Administration of BDF 9148 resulted in a maximal increase in stroke volume and cardiac output up to 25% in neonatal and 40% to 60% in adult preparations. Heart rate decreased by 15% in adult hearts only. Myocardial oxygen consumption was increased in a concentration-dependent manner between 25% in neonatal and 50% in adult hearts. Myocardial efficiency was increased by 35% in adult and by 10% in neonatal preparations. Although positive hemodynamic and energetic effects were less pronounced in immature compared with adult hearts, neonatal hearts also profited from the administration of the Na^+-channel modulator BDF 9148. Further studies are necessary to clarify the risk of arrhythmia during application of Na^+-channel modulators such as BDF 9148. **(*Pediatr Res* 54: 875–884, 2003)**

Abbreviations

CO, cardiac output
Eff, myocardial efficiency
HR, heart rate
pLV, left ventricular pressure
SV, stroke volume
V_{cor}, coronary flow

Immediately after birth, the performance of the neonatal heart is challenged by the transition from fetal to adult hemodynamics. To meet all requirements of ontogeny, an increase in contractility, diastolic relaxation, and a remarkable improvement in stroke volume (SV) and cardiac output (CO) can be observed during maturation (1, 2). However, the performance of the fetal and neonatal heart generally seems to be restricted and the response to inotropic interventions reduced compared with the adult heart. Thus, maladaptation during transition from neonatal to adult hemodynamics may easily lead to heart failure. Despite the wide use of various inotropic medications in neonates, small infants, or even preterm infants, there is a lack of information about the ontogeny and the effects of inotropic intervention on hemodynamics and myocardial energetics in these patients. This is mainly due to ethical and technical considerations that restrict studies in neonates or preterm infants. For instance, there is some controversy about the response of the neonatal heart to β-adrenergic stimulation. *In vitro* experiments showed either an increased sensitivity to sympathomimetic interventions from a reduced baseline level onward (3, 4) or a decreased response in neonatal compared with adult myocardium (5–8). *In vivo* studies in the fetal and neonatal lamb revealed a deficiency of the myocardial contractile reserve on sympathomimetic stimulation (9, 10). We recently demonstrated a marked deficiency in diastolic relaxation and a slight reduction of systolic function as one major factor behind the reduced performance of the neonatal rabbit heart

Received July 26, 2002; accepted May 6, 2003.
Correspondence: Holger Schiffmann, Ph.D., Department of Pediatric Cardiology and Intensive Care, University of Goettingen, Robert-Koch-Strasse 40, D-37075 Goettingen, Germany; email: schiffi@med.uni-goettingen.de

DOI: 10.1203/01.PDR.0000091286.21994.E5

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

(11). Generally, in this study, the effect of different inotropic interventions on contractility and relaxation was decreased compared with the adult heart. Although the effect of the Na^+-channel modulator BDF 9148 was reduced compared with mature myocardium, the immature heart profited from this new principle of inotropic support. However, the effects on hemodynamics and myocardial energetics are not known. We therefore studied the ontogeny of myocardial performance in an isolated heart model using antegrade-perfused rabbit hearts ("working hearts") in neonatal (2 d), immature (8 d), juvenile (14 d), and adult (28 d) animals. The rabbit heart is known to be immature at birth, characterized by a high Na^+-Ca^{2+} exchanger expression and a sparse sarcoplasmic reticulum (SR) (11–13). The aim of our study was to analyze the effects of Na^+-channel modulation on hemodynamic and myocardial energetics, particularly immediately after birth, and to compare the effects with other cAMP-dependent and cAMP-independent inotropic interventions.

METHODS

Timed pregnant New Zealand white rabbits were housed in our animal care facility so that the age of the newborn animals was known exactly. All animals were cared for in compliance with the recommendations of the Declaration of Helsinki and the National Institutes of Health Guiding Principles in the Care and Use of Animals. The study was approved by the local bioethical board and was announced to the supervision authority "Bezirksregierung Braunschweig."

Hearts from neonatal (2 d), immature (8 d), juvenile (14 d), and adult (28 d) animals of either sex were used. Hearts were removed quickly during general anesthesia (xylazoline, ketamine i.v. or i.p.). After abdominal incision and transection of the diaphragm, the hearts were cooled *in situ* with Ringer lactate solution of 4°C, then excised and perfused retrograde *via* the aorta according to Langendorff with a modified Krebs-Henseleit solution (all mmol/L: 120 NaCl, 4 KCl, 2 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 5 glucose). The preparation was finished according to Neely *et al.* (14) as a modified antegrade-perfused "working heart" model. The perfusion medium was equilibrated to a pH of 7.4 (at 38°C) with CO_2. Oxygenation was performed using a membrane oxygenator (Quadrax, Hohlfaser Membran Oxygenator, HMO 100, Jostra Medizintechnik, Hirrlingen, Germany). Circulation of the perfusate was maintained using a roller pump (Typ 10-20-00; Stöckert Instrumente, Munich, Germany), and temperature was controlled using a heat exchanger. Preload and afterload were adjusted at 10 cm H_2O and 35 cm H_2O in neonatal hearts and 15 cm H_2O and 50 cm H_2O in the other hearts.

Pulmonary venous and coronary O_2 and CO_2 concentrations were determined at each point of measurement (ABL 500; Radiometer, Copenhagen, Denmark). CO and coronary flow (V_{cor}) were measured with an ultrasonic flowmeter (T206X, Transonic Systems, Ithaca, NY, U.S.A.). CO was determined at the left atrial cannulation, and V_{cor} was determined in the pulmonary trunk cannulation. Left ventricular pressure (pLV) was measured using a fine canula inserted through the apex in the left ventricle (Sterikan L17L, 24 Gauge, Braun Melsungen, Germany) directly placed on a pressure transducer (Braun Melsungen) and monitored continuously on an oscilloscope (Hellige Servomed, Freiburg, Germany). Stability of function was guaranteed for >3 h for all age groups, which included the whole time course of the experimental protocol described below.

Data acquisition and calculations. For data acquisition on a PC, an AD exchanger (PCL-818L; Advantech Corp., Taipei, Taiwan) and commercially available software modified for our experimental protocol was used (DASY Lab Data Acquisition System Laboratory Version 3, Datalog, Mönchengladbach, Germany). pLV, CO, and V_{cor} were monitored online. The maximal developed pressure velocity and the peak negative pressure velocity were calculated and monitored continuously. The SV was derived from CO and heart rate (HR). Double registration was performed for each point of measurement. Data were stored on a PC, and representative data were analyzed off-line (Excel 7.0; Microsoft Corp., Redmond, WA, U.S.A.). The system was calibrated with a blood pressure simulator (Bio-Tek Model 601A, Blood pressure system calibrator; Bio-Tek Instruments, Winooski, VT, U.S.A.).

The myocardial efficiency (Eff) was calculated according to Neely *et al.* (14) as follows:

$$Eff = \frac{E_{out}}{E_{in}} * 10^2 \text{ [\%]}$$

where E_{in} = energy input per minute, E_{out} = energy output per minute

$$E_{out} = W_p + W_{kin} \text{ [mW]}$$

where W_p = pressure minute work and W_{kin} = kinetic work. The kinetic work comprises only 3% of the total amount of the external work and therefore is negligible in this working heart model (15).

Pressure minute work, energy input, and oxygen consumption per minute (MVO_2) were calculated as follows:

$$W_p = \left(\frac{pLV}{760}\right) * atm * CO * \left(\frac{1}{60}\right) * 10^2 * HW^{-1} \text{ [mW * g}^{-1}\text{]}$$

$$E_{in} = MVO_2 * atm * \left(\frac{1}{60}\right) * E_{aqu} * 10^3 \text{ [mW * g}^{-1}\text{]}$$

$$MVO_2 = Vcor * (pO_{2art} - pO_{2cor}) * \left(\frac{\omega}{760}\right) * 10^2 * HW^{-1} \text{ [ml * min}^{-1} \text{ * 100g}^{-1}\text{]}$$

where HW = heart weight, E_{equ} = energy equivalent of glucose (21 J * ml^{-1}), ω = Bunsen solubility coefficient (0.0227), atm = 1.013 bar, and 1/60 is a factor for the conversion from minute to second.

Experimental protocol. The total sample site of the investigation consisted of 128 hearts (32 per drug comprising eight of each age). Hearts were allowed to equilibrate for 30 min, and baseline values were recorded. Hearts that did not fulfill a baseline standard of maximal developed pressure velocity

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

>1000 mm Hg/s and CO appropriate for age (2 d, >8 mL/min; 8 d, >10 mL/min; 14 d, >15 mL/min; 28 d, >20 mL/min) were excluded. Drugs were administered into the left atrium inflow canula using a microinfusion pump (Perfusor Secura; Braun, Melsungen, Germany). In each step of the concentration range, the velocity of the infusion pump was adjusted according to the current CO to achieve correct drug concentration. Isoproterenol was administered from 0.5 nmol/L to 1 μmol/L, and enoximone was administered from 10 nmol/L to 10 μmol/L. Perfusion with BDF 9148 (provided by Beiersdorf-Lilly, Hamburg, Germany) was performed in a concentration range from 0.1 to 100 μmol/L. Each step was continued until a new steady-state level was reached (usually within 3–5 min) and subsequently higher concentrations were added. The compound BDF 9148, a carbonitrile-derivate (4-[3'-1″-benzhydryl-azetidine-3″-oxy)-2'hydroxypropoxyl]-1H-indole-2-carbonitrile), acts as an Na^+-channel modulator. This substance exerts its positive inotropic action without affecting the adenylylcyclase pathway by an increase in open probability of the Na^+ channels. Hence, the exact mechanism of action is not completely known. One explanation may be an increase of intracellular Na^+ concentration, which would decrease the Ca^{2+} efflux *via* the Na^+-Ca^{2+} exchanger and increase the Ca^{2+} transient by diastolic Ca^{2+} accumulation (16). However, the dynamics and the extent of the transsarcolemmal sodium flux might explain some of the observed differences between both substances. Inhibition of the Na^+-K^+-ATPase may lead to a slow increase of subsarcolemmal sodium concentration, whereas the fast increase of intracellular Na^+ as a result of Na^+-channel influx may reverse the mode of the Na^+-Ca^{2+} exchanger. Consequently, Ca^{2+} influx *via* Na^+-Ca^{2+} exchanger acts as a trigger for SR Ca^{2+} release, predominantly in adult myocardium. In immature myocytes, sarcolemmal Ca^{2+} influx may predominantly interact in a direct way with the myofilaments. The different mechanisms of action may explain the slow onset of action of ouabain compared with the fast onset of BDF 9148 (11). The cardiac glycoside ouabain (g-Strophanthin; Jenapharm, Jena, Germany) was given in a concentration of 0.1 μmol/L for 20 min, followed by a washout period of 60 min. Data were registered every 2 min during drug administration and at t = 25, 30, 35, 45, and 60 min during washout. Wet weight of the heart was determined immediately after the end of perfusion.

Statistics. Descriptive information on the measurements in different groups and their precision is given as mean ± SEM. ANOVA for repeated measures was used for statistical analysis of concentration-response curves. This model differentiates the effects of the three factors "concentration of medication," "age," and "parallelism of the concentration-response curves" for different groups. This last factor is incorporated in the ANOVA model as the interaction between the first two factors. For validity, however, the data must satisfy the typical sphericity assumption in the repeated measurement ANOVA model. For circumventing the biasing effects of nonsphericity, evaluation of significance was performed with the ϵ-correction according to Greenhouse and Geisser (17). $p < 0.05$ was considered significant. All statistical analyses were performed using the statistical software package SAS (Version 6.12; SAS Institute, Cary, NC, U.S.A.).

RESULTS

The mean wet weight of the hearts were 0.46 ± 0.12 g (2 d), 0.83 ± 0.20 g (8 d), 1.24 ± 0.27 g (14 d), and 3.44 ± 0.78 g (28 d). Baseline data for hemodynamic and myocardial energetic parameter before inotropic intervention are given in Table 1.

Absolute values of pLV, CO, and SV rose continuously during development. Relative CO and SV decreased during maturation. Mean HR remained constant during the first 2 wk of life and was significantly lower at day 28. No significant differences were observed for V_{cor} and O_2 consumption between neonatal, immature, and adult hearts. Juvenile preparation (14 d) showed a significantly higher V_{cor} and myocardial O_2 consumption. Eff decreased significantly from day 2 to day 28.

Results of the statistical significance tests within the repeated measurement ANOVA model for the relative changes of the concentration-response curves compared with baseline data are given in Table 2 for selected hemodynamic parameters and in Table 3 for the myocardial oxygen consumption and efficiency.

Concentration-dependent changes in response to inotropic intervention were observed for HR, SV, and CO, with exception of SV during enoximone and HR during ouabain administration. Significant age-dependent differences in the response to increasing concentrations could be demonstrated for isoproterenol and ouabain (all hemodynamic parameters) and BDF 9148 (HR, SV) but not enoximone.

Myocardial O_2 consumption significantly increased in a concentration-dependent manner in response to isoproterenol, enoximone, and BDF 9148 but decreased in response to ouabain. Eff increased significantly in response to ouabain and enoximone. No significant age-dependent differences in the

Table 1. *Baseline data for hemodynamics and myocardial energetic*

	2 d	8 d	14 d	28 d
Peak pLV [mmHg]	49 ± 0.3	67 ± 0.5	72 ± 0.7	77 ± 0.9
Relative CO [ml · min^{-1} · g^{-1}]	28.0 ± 0.2	18.2 ± 0.2	18.1 ± 0.3	9.4 ± 0.1
HR [1 · min^{-1}]	256 ± 3	260 ± 4	252 ± 4	226 ± 5
Relative SV [μl · g^{-1}]	111 ± 3	71 ± 3	73 ± 3	44 ± 2
Coronary minute volume [ml · min^{-1} · g^{-1}]	4.3 ± 0.1	4.5 ± 0.2	5.8 ± 0.2	4.0 ± 0.1
O_2 consumption [ml · min^{-1} · 100g^{-1}]	6.3 ± 0.2	6.7 ± 0.2	8.9 ± 0.3	6.5 ± 0.2
Eff [%]	15.5 ± 0.4	14.3 ± 0.4	11.2 ± 0.3	8.3 ± 0.2

N = 128, each group of age n = 32, all data mean ± SEM. For all parameters, significant differences between the age groups were observed (ANOVA).

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

Table 2. *ANOVA with repeated measures for relative changes of the hemodynamic parameters compared with baseline data*

	Isoproterenol			Enoximone			Ouabain			BDF 9148		
	HR	SV	CO	HR	SV	CO	HR	SV	CO	HR	SV	CO
Concentration	0.001	0.045	0.001	0.001	0.122	0.001	0.189	0.001	<0.001	0.028	<0.001	<0.001
Age	0.03	0.007	0.029	0.166	0.8	0.1	0.02	0.001	0.012	0.038	0.015	0.504
Interaction concentration + age	0.098	0.042	0.015	0.415	0.95	0.709	0.489	0.457	0.737	0.935	0.005	0.042

Corrected p value (Greenhouse-Geisser ε-correction) for relative changes of concentration, age, and interaction of the first two factors for each variable compared with baseline data are shown. Each condition $n = 32$, each age group $n = 8$.

Table 3. *ANOVA with repeated measures for relative changes of myocardial oxygen consumption and efficiency compared with baseline data*

	Isoproterenol		Enoximone		Ouabain		BDF 9148	
	O_2 consumption	Eff	O_2 consumption	Eff	O_2 consumption	Eff	O_2 consumption	Eff
Concentration	0.001	0.135	0.05	0.009	0.026	<0.001	<0.001	0.062
Age	0.201	0.341	0.993	0.714	0.298	0.705	0.628	0.179
Interaction concentration + age	0.214	0.561	0.118	0.904	0.28	0.547	0.348	0.342

Corrected p value (Greenhouse-Geisser ε-correction) for relative changes of concentration, age, and interaction of the first two factors for each variable compared with baseline data are shown. Each condition $n = 32$, each age group $n = 8$.

response to increasing concentrations could be demonstrated for myocardial oxygen consumption and efficiency.

The corresponding concentration-response curves are shown in Figures 1–5. A fast response within 2–3 min to a new concentration step was recorded for isoproterenol, enoximone, and BDF 9148, whereas a slow onset of action was observed during ouabain administration.

No arrhythmias were observed during administration of BDF 9148, ouabain or enoximone, but sporadic ectopic beats during high concentration of isoproterenol were recorded.

During maturation, the positive chronotropic effect of isoproterenol significantly increased in a concentration-dependent manner (Fig. 1). In neonatal preparation at a maximal concentration of 1 μmol/L, HR increased by 40%, in adult preparations by 75%. SV (Fig. 2) significantly decreased in neonatal (−20%) and adult (−25%) hearts, whereas immature and juvenile preparations showed an increase up to 20%, followed by a decrease just above baseline values at maximal concentration of 1 μmol/L. Thus, CO (Fig. 3) increased in 8-d and 14-d preparations up to 50–75%, whereas in adult and neonatal hearts, an increase of 20–25% was observed. Enoximone administration resulted in a significant increase of HR up to 5–15% at a maximal concentration of 10 μmol/L, without significant changes in SV and a significant increase in CO (5–15%). No significant age-dependent differences could be observed.

During maturation, the negative chronotropic effect of the Na^+ channel agonist BDF 9148 and ouabain was continuously increased (Fig. 1). Administration of BDF 9148 resulted in a significant age-dependent increase of SV (Fig. 2) and CO (Fig. 3) up to 25% in neonatal hearts and 40–60% in adult preparation. In contrast, the application of ouabain increased SV (30%) and CO (5–15%) in the neonatal and adult but not in the immature and juvenile hearts.

Myocardial oxygen consumption was significantly increased by isoproterenol and BDF 9148 in a concentration-dependent manner without differences in age, whereas application of ouabain resulted in a significant decrease of O_2 consumption (Fig. 4). Eff (Fig. 5) was significantly increased by ouabain (20–30%) and enoximone (5–10%) without differences in age. BDF 9148 increased Eff in adult preparation by 35% and by 5–15% in the other groups without reaching statistical significance in the repeated measurement ANOVA model ($p = 0.062$).

DISCUSSION

Adequate CO meets the basic requirements, particularly of oxygen, of the organism. Sufficient supply of the organs necessitates specific and rapid adjustment of CO to real demands. If these demands are not met despite sufficient left ventricular filling volume, then cardiac insufficiency is present (18). Preterm infants as well as neonates can be affected by the same myocardial insufficiency as is seen in adults.

Effects of the Na^+-channel modulator BDF 9148. In the present study, a significant influence of ontogenetic development on the effects of the Na^+-channel agonist BDF 9148 was demonstrated. The positive hemodynamic effects reflect the increase in contractility and diastolic relaxation as described by us and others. Our findings in adult isolated hearts correspond to *in situ* data. Baumgart *et al.* (19) and Raap *et al.* (20) reported in adult dogs an increase in SV and CO of 50%. Positive inotropic effects of BDF 9148 were also demonstrated in isolated myocardial preparations in various mammals (16, 21). Flesch *et al.* (22) showed in failing human myocardium with its increased Na^+-Ca^{2+} exchanger activity that the increase in contractility was higher than in healthy myocardium. The age dependence of the positive inotropic effect was dem-

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

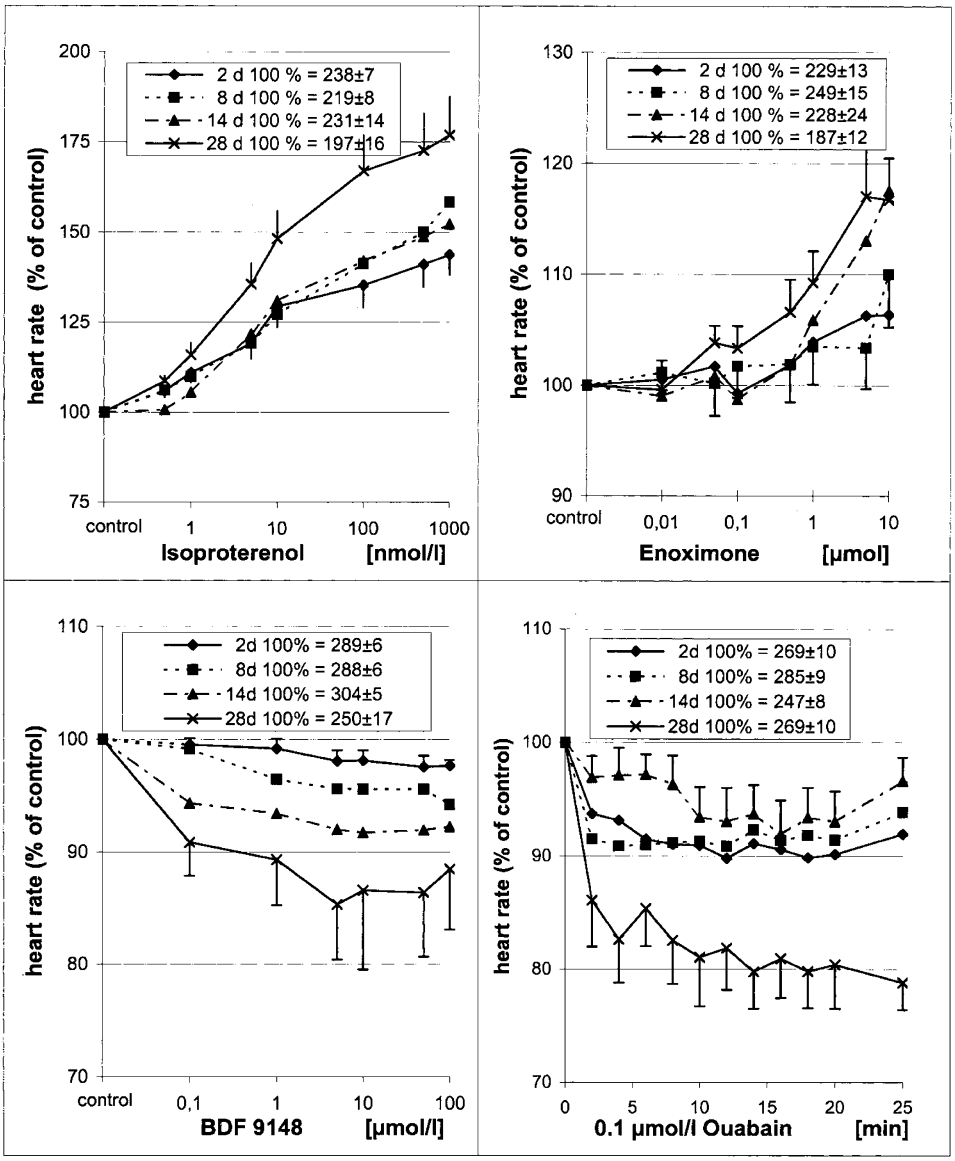

Figure 1. Effect of isoproterenol, enoximone, BDF 9148, and ouabain on HR. Relative changes in percentage of control before inotropic intervention, baseline data are given in the legend. Significances of the analysis of variance as corrected p value (Greenhouse-Geisser ϵ-correction) for the effect of concentration and age are given in Table 2.

onstrated by us for the first time (11). In contrast to the results of Flesch *et al.* (22), however, the effects on immature hearts—which are characterized by increased Na^+-Ca^{2+} exchanger expression as in failing myocardium—are less marked than in adult myocardium.

It should be noted that the increase in CO developed without any significant change in HR, a fact that must be considered favorable for cardiac economy. However, independent of age, myocardial O_2 consumption was found to be higher compared with resting state (Fig. 4). In contrast, Baumgart *et al.* (19) did not observe any significant effect on coronary perfusion and O_2 consumption when applying BDF 9148 after artificial coronary stenosis in a dog model.

The influence of Na^+-channel modulators on the degree of myocardial efficiency has up to now never been investigated. In our study, the Eff increased after application of the substance in all age groups; however, the increase remained just below the 5% level of significance in the variance analysis (Table 3). At maximal stimulation, adult hearts improved their degree of Eff an average of 40% and the other preparations by between 10% and 20% (Fig. 5).

Na^+-channel agonists have been suggested to provoke arrhythmia as a result of action potential prolongation. In some electrophysiologic studies, an extension of the QT interval was detected after application of Na^+-channel modulators such as BDF 9148 (16, 21). Further investigation is necessary to clarify the risk of arrhythmia, particularly torsade des pointes.

Comparison of the effects of ouabain with BDF 9148. Despite that ouabain and BDF 9148 work *via* an increase of intracellular Na^+ concentration, the observed different effects might be explained by the dynamics and extent of transsarcolemmal sodium flux. Inhibition of the Na^+-K^+-ATPase may lead to a slow increase of subsarcolemmal sodium concentration, whereas the fast increase of intracellular Na^+ as a result

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

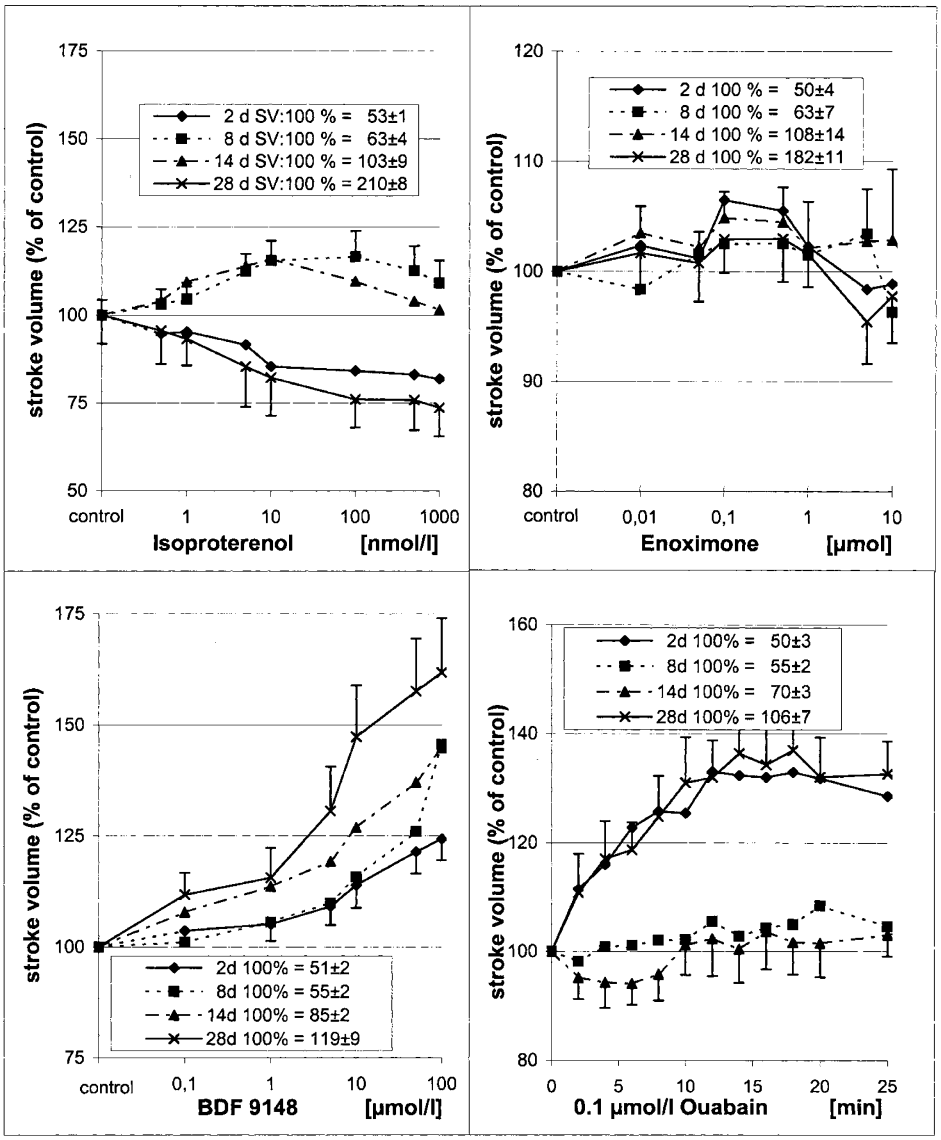

Figure 2. Effect of isoproterenol, enoximone, BDF 9148, and ouabain on SV. Relative changes in percentage of control before inotropic intervention, baseline data are given in the legend. Significances of the analysis of variance as corrected p value (Greenhouse-Geisser ϵ-correction) for the effect of concentration and age are given in Table 2.

of Na^+-channel influx may reverse the mode of the Na^+-Ca^{2+} exchanger. Consequently, Ca^{2+} influx *via* Na^+-Ca^{2+} exchanger acts as a trigger for SR Ca^{2+} release, predominantly in adult myocardium. In immature myocytes, sarcolemmal Ca^{2+} influx may predominantly interact in a direct way with the myofilaments (13). The different mechanisms may explain the slow onset of action of ouabain compared with the fast onset of BDF 9148. In contrast to BDF 9148, ouabain did not show any strict parallels between age and effectiveness. In all age groups, the degree of effectiveness was significantly improved by ouabain, independent of ontogenetic maturity (30%; Fig. 5). The influence of digitalis on the energetics of immature hearts has hardly been investigated to date. In a study on ischemic tolerance in isolated neonate rabbit hearts, Konishi and Apstein (23) reported unchanged coronary perfusion at low concentrations (7×10^{-8} M) with ouabain. The higher concentration of glycoside of 4×10^{-7} used by the authors had toxic effects and led to a significant increase in coronary perfusion. Lorell *et al.* (24) were not able to show any change in myocardial oxygen consumption in adult isolated rabbit hearts after application of $0.5–6 \times 10^{-7}$ ouabain. Hasenfuss *et al.* (25) used microcalorimetry in failing human myocardium to demonstrate that ouabain, in contrast to catecholamine, causes a neutral energetic balance at the cellular level.

Investigations on ontogenetic aspects of the effects of glycosides mainly deal with the activity and sarcolemmal density of Na^+-K^+-ATPase, receptor affinity of the substance, and the pharmacokinetics of newborns and children; the results reported are in part contradictory (23, 26–29). Systematic studies on the effects of glycosides on the hemodynamics of juvenile organism are not available. The increase in SV and CO observed in this study can be explained by the different age-dependent effects of ouabain on myocardial contractility and relaxation, as we had shown recently (11).

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

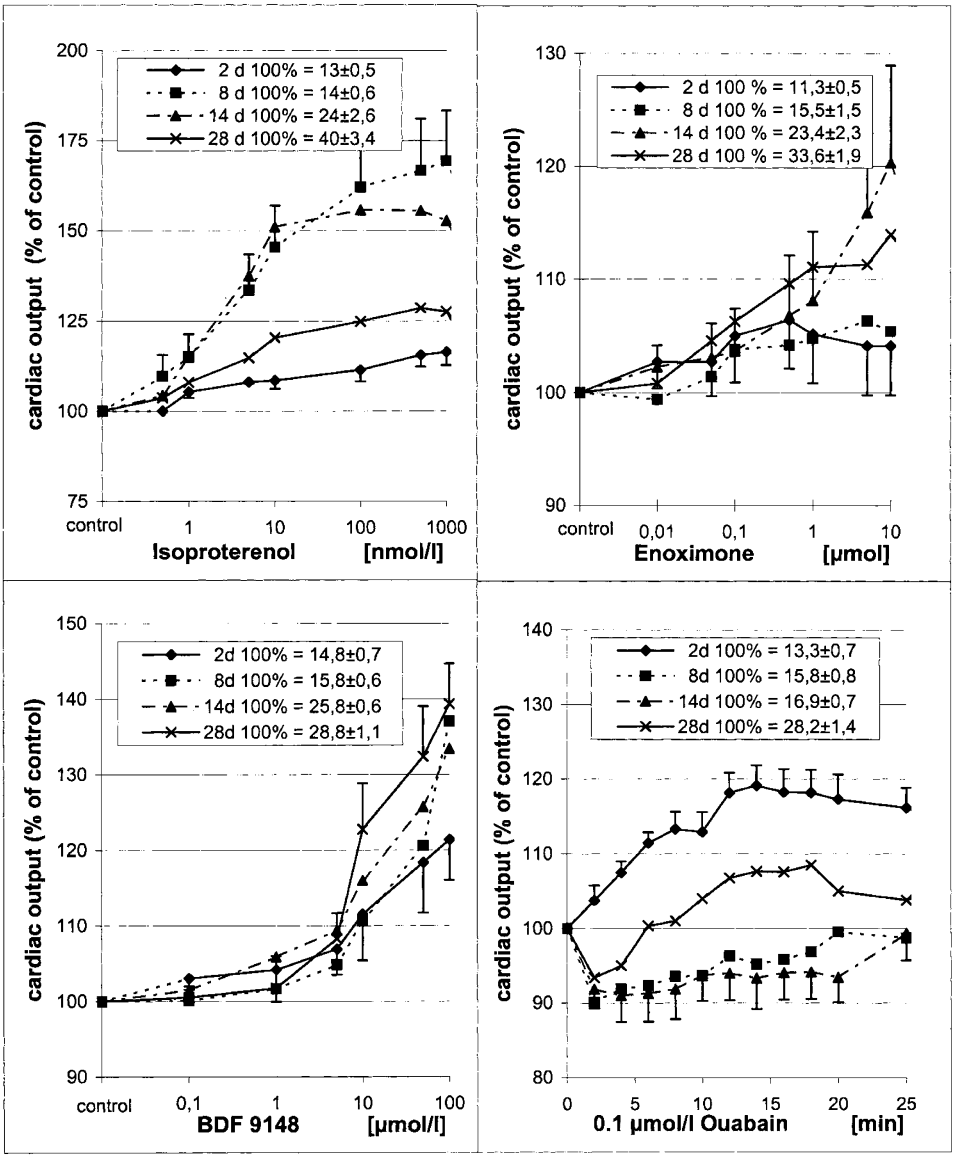

Figure 3. Effect of isoproterenol, enoximone, BDF 9148, and ouabain on CO. Relative changes in percentage of control before inotropic intervention, baseline data are given in the legend. Significances of the analysis of variance as corrected p value (Greenhouse-Geisser ϵ-correction) for the effect of concentration and age are given in Table 2.

Comparison of the effects of isoprenaline with BDF 9148. Isoprenaline has shown the strongest chronotropic effects in all age groups of all substances investigated (Fig. 1). The relative increase in neonatal hearts was significantly lower than in the other age groups, which reflects an increased sympathetic tonus and a reduced myocardial reserve (9, 10). SV of neonate and adult hearts was reduced by isoprenaline in a concentration-dependent manner (Fig. 2). Apparently, the end-diastolic left ventricular volume was rendered insufficient by the distinctly positive chronotropy of the substance. In adult hearts, an insufficient preload during tachycardia was most likely responsible for the reduction in ventricular volume. In neonatal hearts, this is probably due to poor diastolic relaxation ("stiff ventricle") (11). The increase in CO was respectively less in both age groups than in the immature and juvenile hearts. The inotropic potency of the substance, which showed a clear increase in contractility and maximal development of strength, therefore could not be optimally transformed into a positive circulatory effect. Driscoll *et al.* (30) reported reduced inotropy of isoprenaline in newborn puppies in comparison with adult dogs but did not mention any findings for SV and end-diastolic volume. Pridjian *et al.* (31) observed a positive effect of dobutamine infusion on the recovery of CO after ischemia in isolated rabbit hearts aged 7–10 d.

The influence of β-sympathomimetics on Eff has long been a matter of controversy (32); the literature on myocardial energetics deals exclusively with adult myocardium. Despite the seemingly neutral or even slightly positive energetic balance at the organ level, the positive inotropic effects of the β-sympathomimetic substances must be considered unfavorable (15, 25, 32). In the present study, most of the hearts presented a trend toward higher effectivity after application of the β-sympathomimetic substances. In neonatal hearts, the degree of effectivity using isoprenaline deteriorated and

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

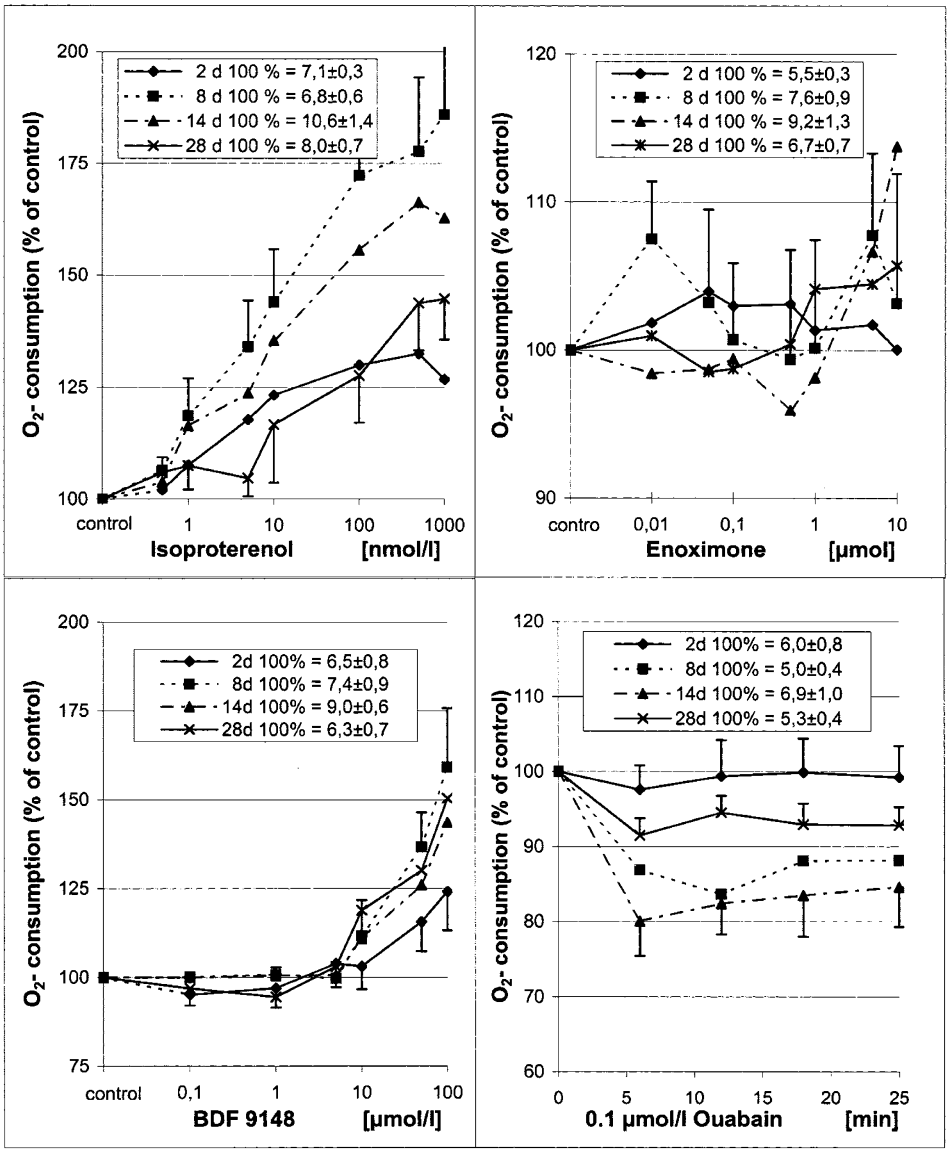

Figure 4. Effect of isoproterenol, enoximone, BDF 9148, and ouabain on myocardial oxygen consumption. Relative changes in percentage of control before inotropic intervention, baseline data are given in the legend. Significances of the analysis of variance as corrected p value (Greenhouse-Geisser ϵ-correction) for the effect of concentration and age are given in Table 3.

the energetic balance in this age group was particularly negative.

Comparison of the effects of enoximone with BDF 9148. In contrast to the negative chronotropy of BDF 9148, administration of enoximone leads to an increase in HR of approximately 120%, independent of age. SV remained unchanged; thus, the increase in CO must be interpreted as solely being due to an effect of HR. Pridjian *et al.* (31) reported positive effects of the phosphodiesterase inhibitor milrinone on the recovery of CO after ischemia in isolated rabbit hearts of 7–10 d. Schranz *et al.* (33) showed that an increase in CO without change in blood pressure and HR in newborn pigs was an expression of the positive inotropy of the medication. Enoximone, as isoprenaline, acts on cAMP as a second messenger, which is why effects on energetics similar to application of β-adrenergic stimulation could be expected. In clinical studies in patients with myocardial insufficiency (NYHA II-IV), some authors reported only a slight reduction or no change in oxygen consumption (32, 34, 35), whereas other groups recorded a significant increase in myocardial oxygen consumption (36–38). Age-specific differences in myocardial energetics has to date not been recorded for phosphodiesterase inhibitors. Our study and data collected thus far indicate that there is an overall negative energetic balance at the cellular level for phosphodiesterase inhibitors in immature as well as in mature myocardium (39).

CONCLUSIONS

Glycosides and Na^+-channel modulators, in contrast to catecholamines and phosphodiesterase inhibitors, improve myocardial function without influencing cellular cAMP concentration. Our investigation shows that this has a positive effect on hemodynamics, myocardial O_2 consumption, and cardiac economy. Further studies are necessary to clarify the incidence of

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

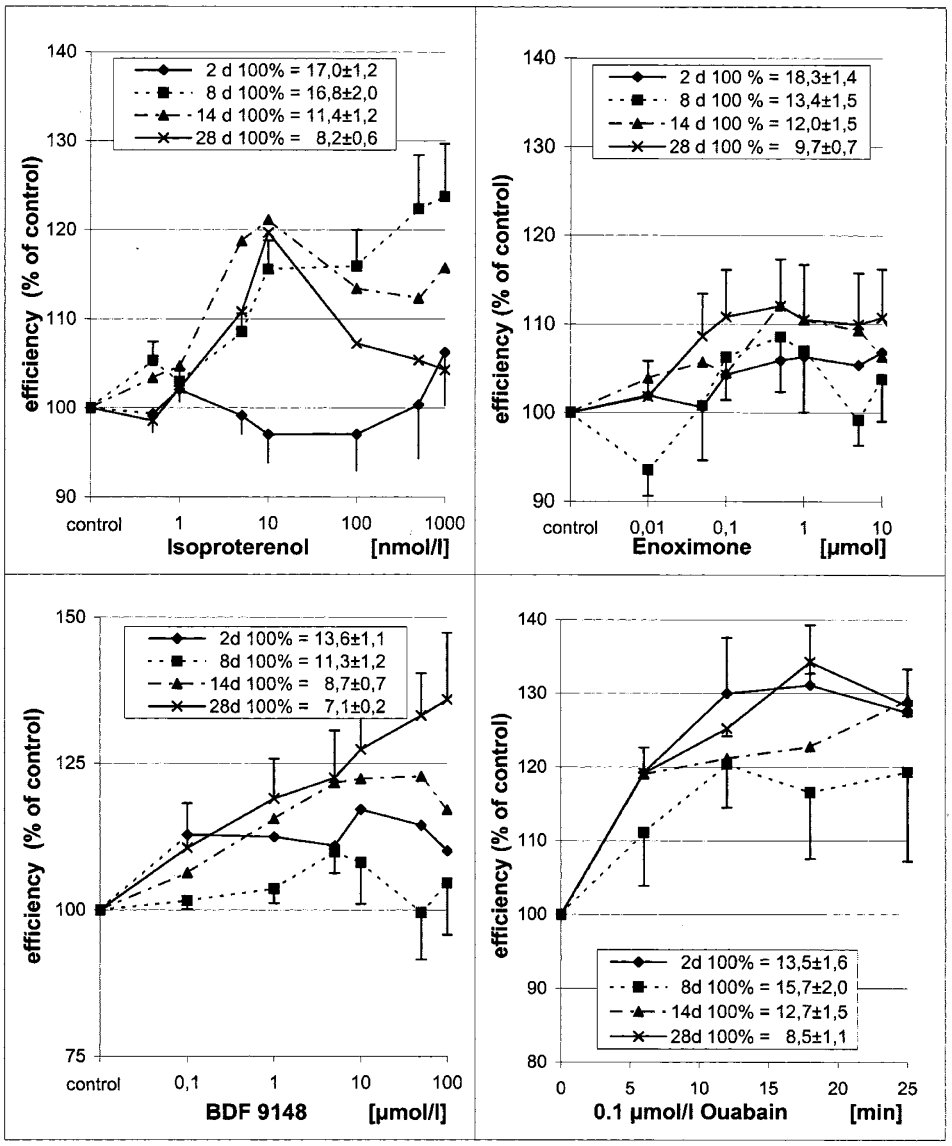

Figure 5. Effect of isoproterenol, enoximone, BDF 9148, and ouabain on Eff. Relative changes in percentage of control before inotropic intervention, baseline data are given in the legend. Significances of the analysis of variance as corrected p value (Greenhouse-Geisser ϵ-correction) for the effect of concentration and age are given in Table 3.

arrhythmia after application of Na^+-channel modulators such as BDF 9148.

REFERENCES

1. St. John Sutton MG, Gewitz MH, Shah B, Cohen A, Reichek N, Gabbe S, Huff DS 1984 Quantitative assessment of growth and function of the cardiac chambers in the normal human fetus: a prospective longitudinal echocardiographic study. Circulation 69:645–654
2. Teitel DF, Hoffman JI 1996 Coronary circulation and myocardial oxygen consumption. In: Gluckman PD, Heymann MA. Arnold (eds) Pediatrics and Perinatology. Arnold, London, pp 731–736
3. Artman M, Kithas PA, Wike JS, Strada SJ 1988 Inotropic responses change during postnatal maturation in rabbit. Am J Physiol 255:H335–H342
4. Nishioka K, Nakanishi T, George BL, Jarmakani JM 1981 The effect of calcium on the inotropy of catecholamine and paired electrical stimulation in the newborn and adult myocardium. J Mol Cell Cardiol 13:511–520
5. Feng ZP, Dryden WF, Gordon T 1989 Postnatal development of adrenergic responsiveness in the rabbit heart. Can J Physiol Pharmacol 67:883–889
6. Osaka T, Joyner RW 1992 Developmental changes in the beta-adrenergic modulation of calcium currents in rabbit ventricular cells. Circ Res 70:104–115
7. Park MK, Sheridan PH, Morgan WW, Beck N 1980 Comparative inotropic response of newborn and adult rabbit papillary muscles to isoproterenol and calcium. Dev Pharmacol Ther 1:70–82
8. Szymanska G, Grupp IL, Slack JP, Harrer JM, Kranias EG 1995 Alterations in sarcoplasmic reticulum calcium uptake, relaxation parameters and their responses to β-adrenergic agonists in the developing rabbit heart. J Mol Cell Cardiol 27:1819–1829
9. Teitel DF, Sidi D, Chin T, Brett C, Heymann MA, Rudolph AM 1985 Developmental changes in myocardial contractile reserve in the lamb. Pediatr Res 19:948–955
10. Teitel DF, Klautz R, Steendijk P, van der Velde ET, van Bel F, Baan J 1991 The end-systolic pressure-volume relationship in the newborn lamb: effects of loading and inotropic interventions. Pediatr Res 29:473–482
11. Schiffmann H, Flesch M, Häuseler C, Pfahlberg A, Böhm M, Hellige G 2002 Effects of different inotropic interventions on myocardial function in the developing rabbit heart. Basic Res Cardiol 97:76–87
12. Artman M 1992 Sarcolemmal Na+-Ca^2+ exchange activity and exchanger immunoreactivity in developing rabbit hearts. Am J Physiol 263:H1506–H1513
13. Mahony L 1996 Regulation of intracellular calcium concentration in the developing heart. Cardiovasc Res 31:E61–E67
14. Neely JR, Liebermeister H, Battersby EJ, Morgan HE 1967 Effect of pressure development on oxygen consumption by isolated rat heart. Am J Physiol 212:804–814
15. Bünger R, Sommer O, Walter G, Stiegler H, Gerlach E 1979 Functional and metabolic features of an isolated perfused guinea pig heart performing pressure-volume work. Pflugers Arch 380:259–266
16. Hoey A, Amos GJ, Ravens U 1994 Comparison of the action potential prolonging and positive inotropic activity of DPI 201-106 and BDF 9148 in human ventricular myocardium. J Mol Cell Cardiol 26:985–994

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.

17. Greenhouse SW, Geisser S 1958 On methods in the analysis of profile data. Psychometrika 32:95–112
18. Colucci W, Braunwald E 1997 Pathophysiology of the heart failure. In: Braunwald E (ed) Heart Disease. WB Saunders, Philadelphia, pp 394–420
19. Baumgart D, Ehring T, Krajcar M, Skyschally A, Heusch G 1994 Characterization of the inotropic and arrhythmogenic action of the sodium channel activator BDF 9148: in comparison to its S-enantiomer BDF 9196, to its congener DPI 201-106, to norepinephrine, and to ouabain. Basic Res Cardiol 89:61–79
20. Raap A, Armah B, Mest HJ, Stenzel W, Schloos J, Blechacz W 1997 Investigations of the mechanism of the positive inotropic action of BDF 9148; comparison with DPI 201-106 and the enantiomers. J Cardiovasc Pharmacol 29:164–173
21. Ravens U, Wettwer E, Pfeifer T, Himmel H, Armah B 1991 Characterization of the effects of the new inotropic agent BDF 9148 in isolated papillary muscles and myocytes of the guinea-pig heart. Br J Pharmacol 104:1019–1023
22. Flesch M, Schwinger RH, Pütz F, Frank K, Südkamp M, Kuhn-Regnier F, Arnold G, Böhm M 1996 Evidence for a functional relevance of an enhanced expression of the Na^+-Ca^{2+}-exchanger in the failing human myocardium. Circulation 94:992–1002
23. Konishi T, Apstein CS 1991 Deleterious effects of digitalis on newborn rabbit myocardium after simulated cardiac surgery. J Thorac Cardiovasc Surg 101:331–341
24. Lorell BH, Isoyama S, Grice WN, Weinberg EO, Apstein CS 1988 Effects of ouabain and isoproterenol on left ventricular diastolic function during low-flow ischemia in isolated, blood-perfused rabbit hearts. Circ Res 63:457–467
25. Hasenfuβ G, Mulieri LA, Allen PD, Just H, Alpert NR 1996 Influence of isoproterenol and ouabain on excitation-contraction coupling, cross-bridge function, and energetics in failing human myocardium. Circulation 94:3155–3160
26. Boerth RC 1975 Decreased sensitivity of newborn myocardium to the inotropic effects of ouabain. In: Morselli PL, Garatinni S, Sereni F (eds) Basic and Therapeutic Aspects of Perinatal Pharmacology. Raven Press, New York, pp 191–199
27. Marsh JD, Allen PD 1989 Developmental regulation of cardiac calcium channels and contractile sensitivity to $[Ca]_o$. Am J Physiol 256:H179–H185
28. Nakanishi T, Shimizu T, Uemura S, Jarmakani JM 1984 Ouabain effect on myocardial mechanical function and sodium pump in the fetus. Am J Physiol 246:H213–H221
29. Park MK 1981 Ouabain-induced inotropism of isolated newborn and adult rabbit myocardium. Dev Pharamcol Ther 2:201–214
30. Driscoll DJ 1987 Use of inotropic and chronotropic agents in neonates. Clin Perinatol 14:931–949
31. Pridjian AK, vanMeter CH, McFadden PM, Chung KC, Touchard CI, Ochsner JL 1996 Repletion of high energy phosphates in the postischemic neonatal heart. Am Surg 62:494–498
32. Hasenfuβ G, Holubarsch CH, Heiss WH, Meinertz T, Bonzel T, Wais U, Lehmann M, Just H 1989 Myocardial energetics in patients with dilated cardiomyopathy. Influence of nitroprusside and enoximone. Circulation 80:51–64
33. Schranz D, Huth R, Dahm M, Stein I, Hein E, Stopfkuchen H, Jüngst BK 1989 Acute hemodynamic response to intravenous enoximone: an animal study and preliminary report in infants after cardiac surgery. J Cardiovasc Pharmacol 14(suppl 1):62–68
34. Hasenfuss G, Holubarsch C, Heiss WH, Bonzel T, Meinertz T, Just H 1987 Influence of phosphodiesterase inhibition on myocardial energetics in dilative cardiomyopathy. Basic Res Cardiol 82(suppl 2):403–409
35. Heiss HW, Hasenfuβ G, Holubarsch C, Meinertz T, Just H 1987 Cardiac energetics after intravenous enoximone in idiopathic dilated cardiomyopathy. Am J Cardiol 60:53C–56C
36. Martin AF, Ball K, Gao LZ, Kumar P, Solaro RJ 1991 Identification and functional significance of troponin I isoforms in neonatal rat heart myofibrils. Circ Res 69:1244–1252
37. Viquerat CE, Kereiakes D, Morris L, Daly PA, Wexman M, Frank P, Parmley WW, Chatterjee K 1985 Alterations in left ventricular function, coronary hemodynamics and myocardial catecholamine balance with MDL 17043, a new inotropic vasodilator agent, in patients with severe heart failure. J Am Coll Cardiol 5:326–332
38. Holubarsch C, Hasenfuβ G, Just H, Blanchard E, Mulieri LA, Alpert NR 1989 Influence of enoximone on mechanics and energetics of right ventricular guinea pig papillary muscles. J Cardiovasc Pharmacol 14(suppl 1):S24–S28
39. Ross-Ascuitto NT, Ascuitto RJ, Ramage D, McDonough KH 1991 The effects of milrinone in the neonatal pig heart. Cardiovasc Drugs Ther 5:1011–1019

Copyright © by International Pediatric Research Foundation, Inc. Unauthorized reproduction of this article is prohibited.