Effect of Development on Coronary Vasodilator Reserve in the Isolated Guinea Pig Heart

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SUMMARY. Morphological studies have demonstrated an age-related decrease in capillary density and capillary surface area in the developing heart. However, the consequences of these changes on myocardial perfusion are not known. We tested the hypothesis that the decreased capillary density is associated with a reduction in coronary blood flow reserve. To test this hypothesis, we studied coronary responses to adenosine and sodium nitroprusside administration, reactive hyperemia, and autoregulatory capacity. We used a Langendorff-perfused heart preparation from guinea pigs of five different age groups (1 week and 1, 2, 12, and 18 months). Data are expressed as mean ± SEM. Maximal coronary flows (ml/min per g) in response to adenosine (10^{-6} to 10^{-3} M) infusion are: 27 ± 1.3, 18.5 ± 1.4, 12.2 ± 0.4, 10.3 ± 0.3, and 10.6 ± 0.8 at 1 week, 1, 2, 12, and 18 months, respectively, with the flows at 1 week and 1 month significantly higher than those at 2, 12, and 18 months. There is a similar trend for a decreased maximum coronary perfusion in response to sodium nitroprusside (10^{-6} to 10^{-5} M) and following a 45-second occlusion of the coronary inlet flow. Despite the decreased maximal pharmacological and reactive hyperemic flow reserve, autoregulation of flow is not altered with growth. The pressure-flow relationship exhibits autoregulation between 25 and 55 mm Hg perfusion pressure for all but the 1-week age group, which autoregulates within a narrower range of pressures (20—45 mm Hg). Total maximal coronary flow (ml/min) increases during development; this indicates that the growth of vessels continues with development. However, since coronary perfusion, corrected per unit cardiac mass, decreases significantly, we conclude that the vascular growth lags behind that of the parenchyma. (Circ Res 57: 538-544, 1985)

MORPHOLOGICAL changes in the myocardial vasculature occur during growth from neonate to adult. As a result of vascular growth, a gradual decline in fiber to capillary ratio has been reported to occur in several species of experimental animals and in human beings (Wearn, 1940; Roberts and Wearn, 1941; Rakusan et al., 1965; Tomanek, 1970; Olivetti et al., 1980; Tomanek and Hovanec, 1981). The decline continues from prenatal life until adulthood, after which the ratio remains at about 1:1. However, because of hypertrophy of myocardial fibers during development, there is an age-related fall in capillary density and capillary surface area (Roberts and Wearn, 1941; Wearn, 1941; Rakusan et al., 1967; Tomanek, 1970; Henquell et al., 1976; Lund and Tomanek, 1978; Tomanek and Hovanec, 1981; Tomanek et al., 1982).

The consequences of these morphological changes on coronary perfusion have not been studied. In fact, little is known about the influence of development on the maintenance of myocardial perfusion. There are no reports of attempts to correlate the age-related morphological changes in the growing heart with changes in coronary perfusion.

We tested the hypothesis that the decreased capillary density reported to occur early in development is associated with a decreased coronary blood flow reserve. To test this hypothesis, we studied the influence of age on coronary (1) responses to adenosine and sodium nitroprusside administration, (2) reactive hyperemia, and (3) autoregulatory capacity.

**Methods**

Guinea pigs of five different age groups (1 week and 1, 2, 12, and 18 months) were studied. Following anesthesia (sodium pentobarbital: 50 mg/kg, ip), their hearts were rapidly excised and immersed in ice-cold physiological salt solution (PSS) (NaCl, 117 mM; KCl, 4.7 mM; KH2PO4, 1.2 mM; MgSO4, 1.2 mM; NaHCO3, 21 mM; Na2EDTA, 0.2 mM; CaCl2, 2.7 mM; glucose, 8 mM; pyruvate, 2 mM). The aorta was cannulated and the heart was perfused in a retrograde fashion. All hearts were maintained in a non-working state by venting the left ventricle with a catheter through the mitral valve. The hearts were electrically paced at 300 beats/min. The PSS was equilibrated with 95% oxygen + 5% carbon dioxide, continuously filtered through a 3-μm filter, maintained at 37°C and pH 7.4, and not recirculated. Perfusion pressure was maintained constant at 46 mm Hg. Perfusion PO2 was determined by using a Corning blood gas analyzer before drug infusion and periodically throughout the experiment to ensure adequate oxygenation of the perfusate. Coronary flow was determined by means of an electromagnetic flow probe in the aortic line or by timed collection of the coronary venous effluent. Coronary flow was expressed as ml/min per g wet heart weight. We measured heart weight after trimming away the great vessels and fat, and blotting with filter paper.
Protocol

Control flow was measured after a 40-minute equilibration period. The maximum reactive hyperemic response was measured following a 45-second occlusion of the inlet flow into each heart of the five age groups. In the first two sets of experiments, the dose-response relationship between coronary flow and adenosine or sodium nitroprusside was established for the five age groups. Drugs were infused into the aortic cannula at a rate of 0.1 ml/min. This was done over a range of 10^-6 to 10^-3 M perfusate concentrations. Maximum flow was measured and the concentration producing the half-maximal response (ED50) was calculated for each drug infused in each age group. Half-maximal flow was calculated in the following way: control flow + 1/2 (maximum flow−control flow). The dose producing this flow (i.e., ED50) was then assessed by interpolation to the dose which produced the half-maximum flow on the dose-response curves. In a third set of experiments, nonpaced hearts from 1 week and 1- and 18-month-old guinea pigs (n = 4) were arrested with a high dose of adenosine (5 x 10^-5 M). Coronary flow was then measured in the absence of extravascular wall compression. In the fourth set of experiments, the autoregulatory capacity in four age groups (1 week and 1, 12, and 18 months old) was assessed by measuring steady state coronary flow following 5 mm Hg step-changes in perfusion pressure within a range of 20-80 mm Hg. The gains for the autoregulatory curves were calculated according to the following formula: G = (ΔF/F + ΔP/P) - 1 (Moff and Granger, 1983). Where G = gain, F = coronary flow, P = perfusion pressure, and ΔF and ΔP represent the difference between the initial and final flow and pressure, respectively.

Statistical Analysis

Results are expressed as mean ± SEM. We used one-way, between-group analysis of variance and Student-Neuman-Keuls tests for multiple comparisons between mean data (Linton and Gallo, 1975) to compare values for maximum coronary flow, ED50, body weight, heart weight, heart weight-to-body weight ratio, and myocardial water content in the five age groups. The same tests were used to compare coronary flows or gains among the different age groups at various perfusion pressures. One-way, within-group analysis of variance and Student-Neuman-Keuls tests were applied to the pressure-flow or pressure-gain data to determine the autoregulatory range for each age group. Differences between mean data were considered significant at P < 0.05.

Results

Relationship between Body Weight and Heart Weight

The data pertaining to the heart weight, body weight, and myocardial water content for each of the five age groups are summarized in Table 1. Heart weight and body weight almost doubled between 1 week and 1 month, 1 and 2 months, and 2 and 12 months. The weights did not change after 12 months. The heart weight-to-body weight ratio was similar between 1-week and 1-month-old guinea pigs. However, the ratio in these two groups was significantly lower than in the other three age groups. The ratio was not different between 12- and 18-month-old guinea pigs; however, both were significantly lower than the 2-month-old group. The differences in heart weight were not due to differences in water content.

Reactivity of the Coronary Bed to Pharmacological Stimuli

The dose-response relationship of coronary flow (ml/min per g) to adenosine infusion is presented in Figure 1. Maximum coronary flows were achieved at concentrations of 10^-6 to 10^-3 M in all age groups. The maximum coronary flow per gram (Table 2) at 1 week was significantly higher than at 1 month of age. In addition, the maximum flow in these two groups was significantly higher than at 2, 12, and 18 months. No significant differences were observed among the 2-, 12-, and 18-month-old groups.

To test whether the decreased vasodilator capacity is specific to adenosine, the effect of sodium nitroprusside was studied in another set of experiments (Fig. 2). A similar trend for an age-related decline in maximum coronary flow was observed in response to sodium nitroprusside (Table 2).

Despite the decreased responsiveness to adenosine and sodium nitroprusside in 2-, 12-, and 18-month hearts, there were no significant differences in the ED50 values for these two drugs among the five age groups (Table 2).

To test whether the age-related decrease in vasodilator reserve is specific to pharmacological stimuli,
we studied the effect of development on coronary retractive hyperemic responses. As in the pharmacological hyperemic responses, a similar age-related decline in vasodilator capacity was observed with retractive hyperemic responses (Table 3).

To test the influence of extravascular compression on coronary vasodilator reserve, a high dose of adenosine (5 × 10^{-5} M) was infused into non-paced hearts. This dose of adenosine simultaneously arrests the heart probably by causing atrioventricular block (Bellardinelli et al., 1982) and a maximum vasodilator response. Coronary flows were 35.9 ± 0.7, 24.2 ± 0.4, and 16.8 ± 0.5 ml/min per g for 1-week, and 1- and 18-month-old hearts, respectively. Thus, the age-related decrease in maximum coronary flow between 1 week vs. 1 month and 1 vs. 18 months is still demonstrated (P < 0.05), despite the elimination of extravascular compression.

The maximum total coronary flows (ml/min) of each of the five age groups were compared in Figure 3. Total flows in 12- and 18-month-old hearts were similar, and both are significantly different from the other three groups. Total flow at 1 week of age is significantly lower than at 1 and 2 months of age. Despite the decreased coronary flow per unit mass in the 2-month-old group vs. 1-month group (Figs. 1 and 2), no significant differences were observed in the total flows for the two age groups (Fig. 3).

**Reactivity of Coronary Bed to Changes in Perfusion Pressure**

The results of experiments in which coronary perfusion pressure was systematically altered between 20 and 80 mm Hg are shown in Figure 4. Under steady state conditions, the pressure–flow relationships exhibit autoregulation between 25 and 55 mm Hg for all but the 1-week age group, which regulates within a narrower range of pressures (20–45 mm Hg). The pressure–flow curves were not significantly different among the 1-, 12-, and 18-

<table>
<thead>
<tr>
<th>Age</th>
<th>Maximum Coronary Flow (ml/min per g)</th>
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<th>Maximum Coronary Flow (ml/min per g)</th>
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<tr>
<td>1 wk</td>
<td>27.0 ± 1.3*</td>
<td>18.5 ± 1.4†</td>
<td>12.2 ± 0.4</td>
<td>10.3 ± 0.3</td>
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<tr>
<td>1 mo</td>
<td>1.7 × 10^{-4}</td>
<td>2.6 × 10^{-7}</td>
<td>4.5 × 10^{-8}</td>
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<td>2 mo</td>
<td>±6.9 × 10^{-9}</td>
<td>±7.9 × 10^{-9}</td>
<td>±1.5 × 10^{-9}</td>
<td>±2.6 × 10^{-9}</td>
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<tr>
<td>12 mo</td>
<td>22.5 ± 1.0*</td>
<td>14.0 ± 0.7†</td>
<td>11.1 ± 0.5</td>
<td>9.3 ± 0.6</td>
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<tr>
<td>18 mo</td>
<td>2.0 × 10^{-7}</td>
<td>4.8 × 10^{-7}</td>
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<tr>
<th>Age</th>
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<tr>
<td>1 wk</td>
<td>1.7 × 10^{-4}</td>
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<td>1 mo</td>
<td>2.6 × 10^{-7}</td>
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<td>2 mo</td>
<td>4.5 × 10^{-8}</td>
<td>±1.5 × 10^{-9}</td>
<td>2.5 × 10^{-7}</td>
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<tr>
<td>12 mo</td>
<td>2.0 × 10^{-7}</td>
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<td>9.3 × 10^{-7}</td>
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<td>18 mo</td>
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<td>±2.3 × 10^{-7}</td>
<td>±4.7 × 10^{-7}</td>
<td>±7.4 × 10^{-7}</td>
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Values are mean ±SEM (n = 6). Mean ED50 values for adenosine and sodium nitroprusside in different age groups are not significantly different.

* P < 0.05 vs. 1-, 2-, 12-, and 18-month-old group.
† P < 0.05 vs. 2-, 12-, and 18-month-old group.
month age groups at any given point. However, in the 1-week group, coronary flow was significantly higher than in the other three groups at any given point. In order to demonstrate more clearly the effect of development on the autoregulatory capacity, we calculated the gains for the curves. Figure 5 shows plots of gain vs. pressure for the four age groups. The negative values for gain indicate autoregulation; positive values indicate a dominance of passive elasticity over autoregulation. The autoregulatory gains for the 1-, 12-, and 18-month age groups were not significantly different at any given pressure; however, they were significantly different from the 1-week age group at 35, 50, 55, 60, and 65 mm Hg. Furthermore, the 1-week-old group apparently autoregulates over a narrower range; at higher perfusion pressures, they don’t autoregulate as well as the other three groups.

Discussion

We investigated the effects of myocardial growth and development on coronary flow reserve in guinea pig hearts. The major findings in this study can be summarized as follows:

1. Maximum coronary flow per gram heart weight was highest in the neonate, and declined with heart growth until sexual maturity (2 months of age).
2. Total coronary flow increased with development, but not in proportion to heart weight until sexual maturity was achieved (Fig. 3).
3. The autoregulatory capacity of the coronary bed was not altered (Figs. 4 and 5).

The isolated, non-working, non-blood-perfused heart preparation used in this study has been well characterized by our laboratory (DeWitt et al., 1983;
Wangler et al., 1984). It has several distinct advantages. We are interested in age-related changes in the coronary resistance vessels, unobscured by the effects of nerves or circulating vasoactive sub-
stances. The purpose of this study was to examine maximum coronary conductance during development; for this purpose, the Langendorff preparation exhibits the pertinent features of an intact, blood-perfused heart. Spontaneous heart rate and coronary flow were stable. Hyperemic responses were reproducible for up to 3 hours; this exceeds the time of experimentation. The preparation exhibits autoregulation over a range of perfusion pressures similar to that found by other laboratories (Bunger et al., 1975, Schrader et al., 1977). It has a basal coronary flow, a maximum flow, and reactive hyperemic responses which are similar to blood-perfused rodent hearts (Wangler et al., 1982; Peters et al., 1984). Based on these observations, we also believe that delivery of O₂ to these hearts is adequate for their O₂ consumption.

The remainder of this discussion will focus on three areas: the effect of myocardial development on the pharmacological and physiological vasodilator reserve, the effect of development on the autoregulatory capacity of the coronary bed, and the factors involved with alterations in vasodilator re-
serve.

Effect of Growth on Pharmacological and Reactive Hyperemic Responses

The different pharmacological interventions (adenosine and sodium nitroprusside) and transient coronary occlusion resulted in different maximum coronary flow responses (Table 3). This variation could be the result of: (1) different degrees of relaxation of vascular smooth muscle, (2) different sites of action of the three stimuli (i.e., small vs. large vessels), and/or (3) differences in ventricular compression. We cannot distinguish among these options. However, it is important to recognize that regardless of the quantities of maximum flow, the qualitative conclusion stands: maximum coronary flow fell with maturation. The dilator reserve demonstrated in this study is similar to that seen when myocardial blood flow is measured (microspheres) before and during maximum dilation (dipyridamole) in conscious rats (Wangler et al., 1982). The magnitude of the reactive hyperemic responses reported in this study is also similar to that elicited from intact, blood-perfused rat hearts (Peters et al., 1984) and Langendorff-perfused guinea pig hearts (Schrader et al., 1977). This study demonstrated an age-related decrement in the pharmacological as well as reactive hyperemic responses in the guinea pig coronary bed (Table 3). Thus, it appears that the previously observed morphological decrease in vascular density of the developing heart from the neonatal life to adulthood in humans, rabbits, and rats (Weamin, 1941; Rakusan, et al., 1967; Tomanek et al., 1971) is associated with diminishing maximum coronary flow response in the guinea pigs reported in this study.

The maximum total coronary flow not corrected for cardiac mass can be viewed as an index of the
total vascular conductance (Mueller et al., 1978). Between the ages of 1 week and 1 month, total flow increases significantly (Fig. 3), cardiac mass doubles (Table 1), and myocardial perfusion (defined here as flow/g) decreases significantly (Figs. 1 and 2; Table 3). At 1 and 2 months, total coronary flow is similar, in spite of a doubling of cardiac mass, and again perfusion declines significantly. These results indicate that the growth of coronary bed lags behind parenchymal growth. Total coronary flow at 12 or 18 months significantly exceeds that at 2 months. However, if flow is corrected for heart mass, the perfusion does not differ among these three ages, in spite of a doubling of cardiac mass between 2 and 12 or 18 months. These data indicate that a relative slowing of the parenchymal growth rate between 2 and 12 months enabled the proliferating vasculature to keep pace.

We conclude that vascular proliferation continues with growth of the heart; initially, however, it is not proportional to the parenchymal growth rate.

**Effect of Age on Coronary Autoregulation**

The intrinsic ability of the isolated heart preparation to regulate coronary flow over a wide range of perfusion pressures has been demonstrated in several studies (Bunger et al., 1975; Schrader et al., 1977; Bardenheuer and Schrader, 1983). The pressure-flow relationship in this study demonstrated autoregulation between 25 and 55 mm Hg in 1-, 12-, and 18-month-old guinea pig hearts (Fig. 4); this range compares favorably with the previously cited studies. However, the 1-week-old guinea pig hearts do not appear to autoregulate as well at higher perfusion pressures. These observations were confirmed after calculating the gains for the pressure-flow responses (Fig. 5). It appears that autoregulatory capacity in the 1-week-old hearts is impaired; this is demonstrated by values of gain at pressures of 35, 50, 55, 60, and 65 mm Hg, which are significantly different from the three older groups in the positive direction. As perfusion pressure increases, autoregulatory capacity is exhausted earlier, and coronary flow is modulated predominantly by passive coronary elasticity. On the other hand, lowering perfusion pressure to 20 mm Hg resulted in similar coronary flow responses for 1-, 12-, and 18-month age groups. This indicates that, despite previously reported age-related decreases in capillary density (Rakusan, 1967; Tomanek et al., 1982) and our observation of decreased pharmacological and reactive hyperemic responses, the developmental process does not affect the usable autoregulatory reserve of the coronary bed. Infusion of adenosine at various points on the descending limb of the autoregulation curve of dog hearts results in a further decrease in coronary vascular resistance (Sparks et al., 1984), demonstrating that resistance vessels do not maximally dilate in response to lowered perfusion pressure. If reduced perfusion pressure does not elicit maximum vasodilation in isolated guinea pig heart, this could explain the apparent discrepancy between the pharmacological and autoregulatory responses.

**Factors Involved in the Decreased Vasodilator Reserve**

There are several explanations that could account for the age-related decrement in coronary flow reserve. One possibility is an increase in extravascular pressure secondary to an age-related increase in myocardial stiffness. This possibility is unlikely, because increased myocardial stiffness is associated with senescence (Yin et al., 1980), and we have demonstrated a decrease in the dilator reserve as early as 1 month of age. In fact, the data obtained from arrested hearts demonstrate that differences in flow remained despite the elimination of intramyocardial compressive effects. Decreased coronary dilator capacity could result from increased stiffness of vessel wall with age due to increased connective tissue deposition which limits the ability of the coronary vessel to dilate. This is unlikely, because coronary vessels of 1-week, 1-, 12-, and 18-month hearts show comparable degrees of passive elasticity, judging from the increase in flow in response to high perfusion pressures (Fig. 5). The diminished responsiveness to vasodilation is not the result of down-regulation of a specific receptor, because adenosine, sodium nitroprusside, and reactive hyperemia all are decreased. It is possible that the decreased flow reserve is due to impaired relaxation of vascular smooth muscle. The decreased relaxation of aortic strips in response to isoproterenol (Fleisch et al., 1970; Fleisch and Hooker, 1976) dopamine (Cohen and Berkowitz, 1975), and cyclic adenosine monophosphate (cAMP) (Cohen and Berkowitz, 1974) has been related to impaired relaxation mechanism during aging. Numerous studies have shown an age-related increase in stiffness of the myocardium (Temelton et al., 1979; Yin et al., 1980; Spurgeon et al., 1983). These changes have been attributed to impaired sarcoplasmic reticular handling of Ca**+ ions (Froehlich et al., 1978). It is possible that a similar age-related impairment in relaxation of coronary vascular smooth muscle could account for the decreased pharmacological dilator reserve reported in this study. Finally, the decreased reserve could be the result of decreased vascular density. Several studies on cardiac hypertrophy suggest that the reduction in coronary vasodilator reserve (reflected by increased minimum coronary vascular resistance) is at least partially the result of failure of total cross-sectional area of the coronary bed to increase in proportion to increased cardiac mass (Mueller et al., 1978; Murray and Vatner, 1981; Wangler et al., 1982; Peters et al., 1984). The age-related decrement in coronary flow reserve found in this study appears to be associated closely with the reported decrement in capillary density and capillary surface area during cardiac development of humans, rabbits, and rats (Wearn, 1941; Rakusan et al., 1967;
Tomanek et al., 1982). Currently, available information is not adequate to determine whether the increased resistance is the result of decreased capillary density or a parallel decrease in another vascular segment, such as the arterioles. Adenosine and nitrates have a preferential dilator action on small and large coronary arteries, respectively (Schnaar and Sparks, 1972). As shown in Figure 3, the difference between total maximum coronary flow in response to adenosine vs. nitroprusside is significantly different at 1 week, 1 month, and 2 months. Therefore, we speculate that there is a tendency for development to affect the small resistance vessels preferentially to larger arteries.

In summary, the maturational process is associated with a decreased coronary flow reserve in the guinea pig heart, although it does not seem to affect its capacity for autoregulation. Although the possibility of an impaired relaxation mechanism as a cause of this decrement has not been ruled out, we think that failure of the coronary bed to keep pace with myocardial growth is the most likely reason for the decline in flow reserve.

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