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Postnatal Development of Rat Heart during 6-Hydroxydopamine or Propranolol Treatment (42043)

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Abstract. Progressive postbirth development of mammalian heart contractile function is accompanied by augmentations of aerobic metabolic potential and cardiac myofibrillar ATPase activity. The temporal similarity of the above developmental sequences suggested that a single, unifying factor may coordinate myocardial maturation. It was hypothesized that cardiac sympathetic nervous system development might be regulating other aspects of myocardial growth. To test this hypothesis, previously well-defined aspects of heart metabolism and contractile protein ATPase activity were determined in rats which were either sympathectomized with 6-hydroxydopamine (6-OHDA) or subjected to chronic, β-adrenergic blockade (propranolol) throughout the postbirth period from 3 to 6 weeks of age. Neither 6-OHDA treatment nor chronic, β-adrenergic blockade resulted in a significant reduction of any metabolic enzyme specific activity or in myofibrillar ATPase. Myofibrillar creatine phosphokinase (CPK) activity underwent greater enhancement relative to ATPase during normal heart growth. Significant and divergent influences were exerted by 6-OHDA and propranolol drug regimens on myofibrillar CPK/ATPase enzyme activity ratio. These results indicate (a) the potential for independent regulation of myofibrillar CPK and ATPase, and (b) the advisability of evaluating CPK, ATPase, and CPK/ATPase enzymatic activities as myofibrillar correlates of heart contractile function. Nevertheless, the majority of developmentally related processes in the heart are minimally influenced by chemical sympathectomy.

Postbirth augmentation of heart contractile function is a mammalian characteristic. Left ventricular peak systolic pressure values are relatively low in the newborn. Over the intervening weeks to months after birth, heart left ventricular pressure is increased until typical adult values are attained. In the rat heart, for example, left ventricular peak systolic pressure is approximately 70–80 mm Hg in 3-week-old animals (1). A progressive and significant pressure increase takes place until normal, adult values of approximately 110–120 mm Hg are measured in rats 9 weeks old (2). Augmented pressure development capabilities in the growing mammalian left ventricle are accompanied by concomitant elevations in the rate of ventricular pressure development, i.e., dP/dt (3). The latter measurement is frequently accepted as an index of myocardial contractility or inotropy.

In view of the age-related enhancement in heart contractile function, it is not surprising that selected metabolic indices increase in a parallel manner. Estimates of heart metabolic potential during perinatal development have been made by measuring enzymatic activity in left ventricular tissue homogenates. These measurements have shown that glycolytic and anaerobic metabolisms are well established in the heart at birth and undergo little or no postbirth alteration (4). Aerobic metabolism is augmented via the disproportionately greater accumulation of mitochondria with respect to left ventricular tissue accumulation (5). Adenosine triphosphatase (ATPase) activity of cardiac myofibrils also increases during normal growth (4). Thus, progressive postbirth development of mammalian heart contractile function is accompanied by augmentations in aerobic metabolic potential and cardiac myofibrillar ATPase activity.

The temporal similarity of the above developmental sequences suggests that a single, unifying factor may coordinate myocardial development. Sympathetic nervous system regulation of both myocardial contractile function and heart metabolism is well established for the adult heart (6). Interestingly,

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functional enhancement of cardiac sympathetic nervous activity occurs in the neonate with a time course which is similar to the development of heart contractile and metabolic functions (7). Therefore, it seemed reasonable to hypothesize that cardiac sympathetic nervous system development might be regulating other aspects of myocardial growth. To test this hypothesis, previously well-defined aspects of heart metabolism and contractile protein ATPase activity were determined in rats which were either sympathectomized with 6-hydroxydopamine (6-OHDA) or subjected to chronic, β-adrenergic receptor blockade with propranolol throughout the postbirth period from 3 to 6 weeks of age. Neither 6-OHDA treatment nor chronic, β-adrenergic blockade resulted in significant reduction of any metabolic enzyme specific activity or in myofibrillar ATPase activity. Significant and divergent influences were exerted by these drug regimens on myofibrillar creatine phosphokinase (CPK) enzyme activity as well as on the myofibrillar CPK/ATPase enzyme activity ratio.

Methods. *Animal selection and treatment.* Pregnant, Sprague–Dawley rats were obtained from TIMCO, Inc., Houston, Texas, and gave birth in our animal quarters. At 4 days postbirth, litters of eight male rats were made up at random from animals born on the same day. At 21 days of age, rats were selected to receive one of four experimental treatments. One group of rats, designated the weanling group, was studied at 21 days of age. The remaining rats were weaned, placed in groups of four, and experimental treatments were initiated. One group of rats, designated the 6-hydroxydopamine (6-OHDA) group, underwent sustained chemical sympathectomy. Intravenous injections of 6-OHDA (100 mg/kg) were given at weekly intervals starting at 3 weeks of age and continuing until the animals were 6 weeks old. The drug was freshly prepared in saline to which 1 mg/ml ascorbic acid had been added. Drug concentration was adjusted so that a 0.25-ml volume was injected per each animal in the 6-OHDA group. A second group of rats, designated the propranolol group, underwent chronic β-adrenergic receptor blockade. Propranolol (20 mg/kg) was given twice daily, 7 days per week by intraperitoneal injection. The drug was freshly prepared in saline to which 1 mg/ml ascorbic acid had been added. Drug concentration was adjusted so that a 0.25-ml volume was injected per each animal in the propranolol group. Propranolol injections were made in the early morning and, again, in the late afternoon. In addition, 0.5 mg/ml propranolol was added to the drinking water of all rats comprising the propranolol group. Propranolol treatments were initiated at 3 weeks of age and were continued until the animals were 6 weeks of age. A final group of rats, designated the adult group, served as controls for the drug treatment procedures. Rats in the adult group received twice-daily, intraperitoneal injections of saline (0.25 ml per injection) for 7 days per week starting at 3 weeks of age and continuing until the rats were 6 weeks old. All rats were weighed at 3 weeks of age and, again, at weekly intervals thereafter until used for experimentation.

Hemodynamic evaluations. At either 3 weeks of age (weanling group) or 6 weeks of age (6-OHDA, propranolol, and adult groups), animals were weighed and then anesthetized with pentobarbital (50 mg/kg, ip). Positive pressure ventilation with room air was initiated. Rats were then prepared for hemodynamic evaluation using an open-chest, *in situ* method previously described in detail (8). Briefly, a jugular vein cannula was placed to allow intravenous administration of tyramine and isoproterenol (see below). A cannula was positioned in the abdominal aorta for arterial blood pressure measurements. After a midline thoracotomy had been performed, left ventricular peak systolic pressure measurements were made by puncturing the ventricle with a 1-in., 20-gauge needle attached directly to a Statham P37B miniature pressure transducer. The rate of left ventricular pressure development (dP/dt) was derived by using an analog differentiator. This measurement served as an index of heart contractile function, i.e., contractility. All hemodynamic measurements were recorded simultaneously on a Beckman Type R dynograph.

After control hemodynamic measurements had been recorded, the efficacy of 6-OHDA treatment or β-adrenergic receptor blockade was evaluated by bolus injections of tyramine

and isoproterenol, respectively. Tyramine was freshly prepared in saline to which 1 mg/ml sodium meta-bisulfite had been added. Drug concentration was adjusted so that 200 μg/kg of tyramine was contained in a 0.2-ml volume. This tyramine concentration elicited near maximal hemodynamic responses in control rats (14). Drug was injected into the dead space of the intravenous cannula and then flushed into the rat over a 5-sec period with an additional 0.3 ml of saline. Peak responses to tyramine were then determined and the animal was allowed to recover. Responses to isoproterenol injection were then determined in the same manner. Isoproterenol was freshly prepared in saline containing sodium meta-bisulfite. Drug concentration was adjusted so that 1 μg/kg of isoproterenol was contained in a 0.2-ml volume. Isoproterenol was injected and flushed into the rat as described above for tyramine. Peak responses to isoproterenol were determined and the animal was allowed to recover.

Heart weight determinations. After hemodynamic evaluations were completed, the heart was excised and placed in a beaker in ice. Right and left atria were dissected from the heart, pooled into a single sample, weighed, and discarded. Extraneous tissue and great vessels were then removed. The remaining portion of the heart was separated into right ventricular and left ventricular (including septum) samples and weighed. Left ventricular tissue samples were frozen and remained frozen until used for chemical and enzymatic analyses as outlined below.

Chemical and enzymatic analyses: Metabolic enzymes. Left ventricular tissue from one complete series of experimental animals was used to prepare a 5% (w/v) homogenate using a Brinkman Polytron at setting 5 for a single 10-sec period. Homogenates were prepared in 100 m*M* potassium-phosphate buffer, pH 7.4, to which 5 m*M* reduced glutathione had been added. An aliquot of the left ventricular homogenate was taken for protein determination by the biuret reaction (9). Phosphofructokinase (PFK) activity was measured using the methods described by Mansour *et al.* (10). Lactate dehydrogenase (LDH) activity was measured with pyruvate as enzyme substrate (11). Substrate concentrations were adjusted so that LDH activities were assayed in the presence of 10, 1, or 0.33 m*M* pyruvate. Malate dehydrogenase (MDH) enzyme activity was determined using the procedures of Shonk and Boxer (12). Total MDH enzyme activity was measured in one untreated homogenate sample. An additional homogenate sample was treated with 100% ethanol to inactivate the cytoplasmic form of MDH. From these determinations, it was possible to quantitatively differentiate mitochondrial and cytoplasmic forms of MDH enzyme activities present in whole heart homogenate. Measurement of 3-hydroxyacyl-CoA dehydrogenase (3-HADH) enzyme activity was made in whole heart homogenate. Nonionic detergent treatment with Triton X-100 fully disrupted mitochondria within the homogenate and assured optimum 3-HADH enzyme activity. Enzymatic measurements were then conducted using the "backward" reaction (oxidation of NADH) described by Fong and Schulz (13).

Cardiac myofibrillar protein. In a separate series of experiments, animals were selected and treated identically as described above. However, left ventricular tissue was utilized for the preparation of purified cardiac myofibrils as previously described (14). Myofibrillar adenosine triphosphatase (ATPase) activity was measured in a reaction mixture containing (m*M*): 1 $MgSO_4$, 0.1 $CaCl_2$, 1 Na_2ATP, 20 Tris, pH 7.4, and 1.2 mg myofibrillar protein in a final volume of 4.0 ml. Sodium azide (2 m*M* final concentration) was routinely added. Other incubation conditions, procedures, and ATPase enzymatic determinations were as previously described (1). Concurrent myofibrillar protein samples were taken for measurement of creatine phosphokinase (CPK) enzyme activity. The coupled, enzymatic method of Rosalki (15) was employed for the kinetic measurement of myofibrillar CPK at 30°C.

Statistical analyses. One-way analysis of variance was used throughout these experiments for multigroup comparisons (16). The Scheffe post hoc test identified significant group differences (17). A probability level of 0.05 or less was considered significant for all statistical procedures.

Results. *Body weight–heart weight.* The protocol used for the present study resulted in the selection of animals having nearly

identical body weights prior to imposition of experimental treatments. During the intervening time between 3 weeks of age and 6 weeks of age, animals in the adult group exhibited a progressive and significant increase in body weight (Fig. 1). Both of the drug treatments employed allowed augmentation in body weight; however, growth was significantly attenuated when 6-OHDA and propranolol groups were compared with the adult group (Fig. 1).

Developmentally related increases in weight for the several portions of the heart are shown in Fig. 2. Atrial and right ventricular weights approximately doubled over the 3-week postbirth period studied. Neither chemical sympathectomy nor β-adrenergic blockade exerted any significant influence on atrial and right ventricular growth patterns. Left ventricular weight was also augmented approximately two-fold during the transition from weanling to adult (Fig. 2). 6-OHDA treatment reduced left ventricular weight by a significant amount. Although not statistically significant, propranolol treatment also reduced left ventricular weight by approximately 10–15%. The relative weight of the left ventricle with respect to body weight (LV/BW) for all groups of animals is shown in the inset to Fig. 2. As the control animals progressed from weanling to adult, augmented body weight outstripped the increasing left ventricular weight, resulting in a significant

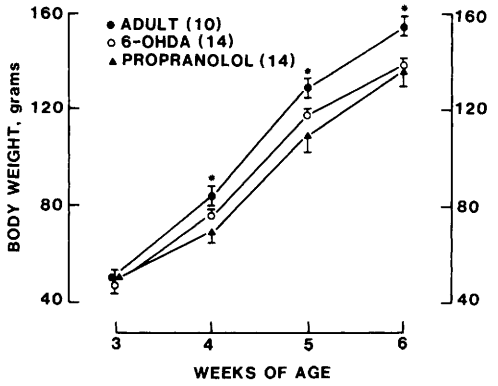

FIG. 1. Overall growth patterns of control, 6-hydroxydopamine (6-OHDA), and propranolol-treated rats. Values shown are means ± SE. Number of animals in each group is given in parentheses. *$P < 0.05$ versus 6-OHDA and propranolol groups.

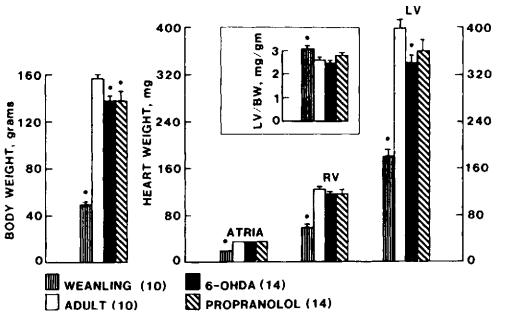

FIG. 2. Body weight, heart weights, and left ventricular/body weight (LV/BW) results at the time of sacrifice. Values shown are means ± SE. Number of animals in each group is given in parentheses. RV = right ventricle, LV = left ventricle. *$P < 0.05$ versus adult, control group.

reduction in the left ventricular/body weight ratio. Sustained sympathectomy and chronic propranolol treatment influenced both body weight and left ventricular weight. Overall growth and heart growth were equally retarded, however, as indicated by the maintenance of normal, adult LV/BW values in these experimental groups.

Hemodynamic measurements. Hemodynamic consequences of postbirth development are shown in Fig. 3. Under control conditions, mean arterial blood pressure, heart rate, and *dP/dt* values are all significantly lower in weanling animals than in adult animals. Adult animals which had experienced chronic β-adrenergic blockade had significantly lower values for pressure, heart rate, and *dP/dt*. Detectable, but statistically nonsignificant reductions in these same measurements were observed in the 6-OHDA group. When tyramine was injected, both the weanling and the adult groups responded by significant increases in each hemodynamic parameter recorded. Lower values for weanling animals were still evident after tyramine injection. Animals in the 6-OHDA and propranolol groups had no significant elevations in any of the hemodynamic measurements taken in response to tyramine. Significant and directionally appropriate hemodynamic responses were elicited by isoproterenol injection in weanling and adult groups. As before, weanling animals had significantly lower values than adults following drug treatment. Isoproterenol administration to 6-

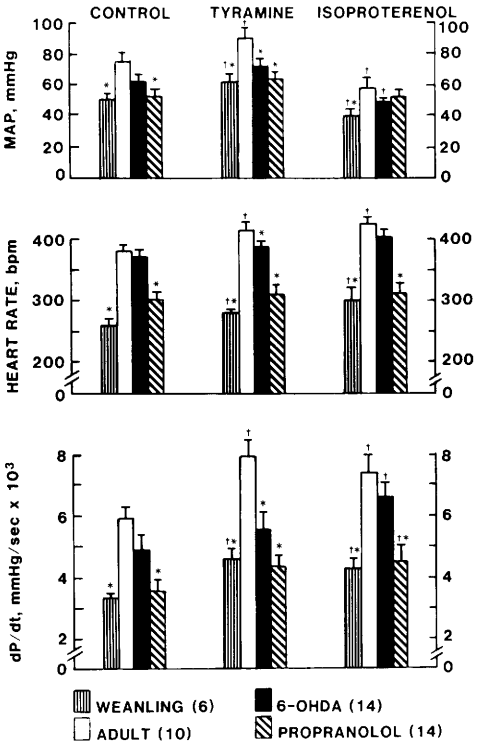

FIG. 3. Hemodynamic measurements under control conditions and in response to tyramine and isoproterenol. Values shown are means ± SE. Number of animals in each group is given in parentheses. MAP = mean arterial blood pressure, *dP/dt* = rate of left ventricular pressure development. **P* < 0.05 versus adult, control group. †*P* < 0.05 versus control conditions.

OHDA-treated rats resulted in a significant reduction in mean arterial blood pressure compared with that in control conditions. Heart rate and *dP/dt* were elevated by isoproterenol; however, the magnitude of the heart rate response in the 6-OHDA group did not achieve statistical significance. In the propranolol group, isoproterenol injection failed to significantly alter either mean arterial pressure or heart rate; however, *dP/dt* values were significantly increased.

The efficacy of chemical sympathectomy and β-adrenergic receptor blockade can be more easily evaluated from the relative hemodynamic responses to tyramine and isoproterenol as shown in Fig. 4. Despite marked differences between weanling and adult animals with respect to the actual hemodynamic values observed, the relative hemodynamic responsiveness to tyramine and isoproterenol was nearly identical in these two groups of animals. In keeping with the known pharmacological effects of tyramine, both 6-OHDA-treated animals and propranolol-treated animals had attenuated heart rate and *dP/dt* responses. Mean arterial pressure responses were either reduced or normal in 6-OHDA and propranolol groups, respectively. With the exception of *dP/dt,* the direction and magnitude of hemodynamic responses to isoproterenol in 6-OHDA-treated animals were comparable to the adult, control group. Relative *dP/dt* responsiveness of 6-OHDA-treated animals was significantly greater than in adult animals. Propranolol treatment effectively prevented mean arterial pressure and heart rate responses to isoproterenol injection. In contrast, similar *dP/dt* respon-

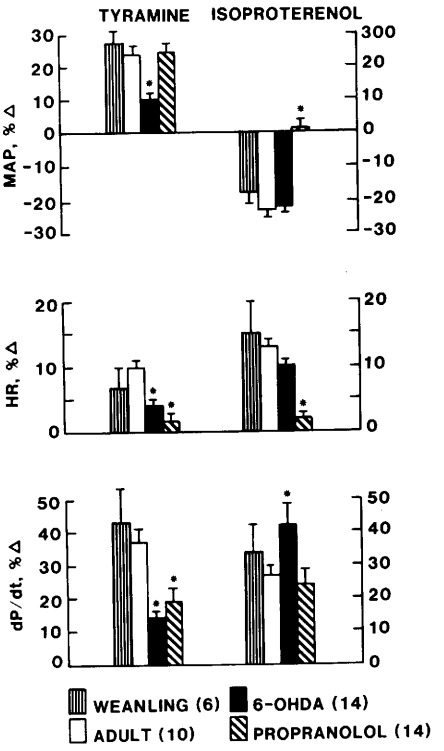

FIG. 4. Relative hemodynamic responses to tyramine and isoproterenol. Individual responses were calculated as percentage change from control. Values shown are means ± SE. Number of animals in each group is given in parentheses. MAP = mean arterial blood pressure, HR = heart rate, *dP/dt* = rate of left ventricular pressure development. **P* < 0.05 versus adult, control group.

siveness was observed in adult and propranolol groups.

Chemical analyses: Protein. Normal, left ventricular growth was accompanied by approximately a three-fold, net accumulation of protein in the adult animal group (67 ± 4 mg; Mean ± SE) compared to that in weanlings (24 ± 3 mg). Protein accumulation in the left ventricle was significantly retarded by 6-OHDA and propranolol treatments; however, protein concentration in the left ventricle (approximately 170 mg/g) was not influenced by the drug treatments employed.

Metabolic enzymes. Phosphofructokinase enzyme results are shown in Table I. None of the observed differences were statistically significant.

Lactate dehydrogenase (LDH) enzyme activity in heart homogenates is shown in Table I. In these experiments, optimum enzymatic activity was observed at 0.33 m*M* pyruvate concentration. At all substrate concentrations measured, an increase in LDH enzyme activity occurred as normal heart development took place. These increases were approximately uniform at all substrate concentrations, thus, no growth-related differences were apparent in the 0.33/10 m*M* pyruvate ratio (approximately 2.60 ± 0.10). Neither 6-OHDA nor propranolol treatments exerted a significant influence on optimum LDH enzyme activity or the 0.33/10 m*M* pyruvate ratio. Thus, it is possible to conclude that no large changes in LDH isoenzyme distribution occurred.

Heart homogenate enzyme activity measurements of 3-hydroxyacyl-CoA dehydrogenase are shown in Table I. A consistent activity level for 3-HADH was observed as normal growth and development occurred. 6-OHDA or propranolol treatments had no significant effect on 3-HADH enzyme activity levels.

TABLE I. METABOLIC ENZYME ACTIVITIES

	PFK	LDH	3-HADH
Weanling (5)	25 ± 1	345 ± 8*	87 ± 5
Adult (5)	27 ± 3	412 ± 9	87 ± 3
6-OHDA (7)	32 ± 1	398 ± 6	91 ± 4
Propranolol (7)	34 ± 3	374 ± 12	93 ± 5

Note. Values are means ± SE. Number of animals in each group is given in parentheses. All enzyme activities are μmole/g tissue/min. PFK = phosphofructokinase, LDH = lactate dehydrogenase at 0.33 m*M* pyruvate concentration, 3-HADH = 3-hydroxyacyl-CoA dehydrogenase, 6-OHDA = 6-hydroxydopamine. *$P < 0.05$ versus adult, control group.

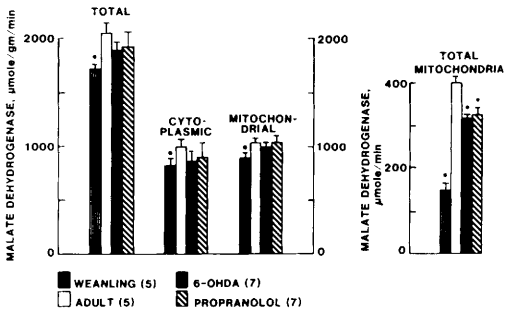

FIG. 5. Malate dehydrogenase enzyme activities in tissue homogenates. Total, cytoplasmic, and mitochondrial enzyme activities were differentiated by ethanol inactivation procedures. Total mitochondria (μmole/min) were estimated by multiplying mitochondrial enzyme activity in tissue homogenate by left ventricular tissue weight. Values shown are means ± SE. Number of animals in each group is given in parentheses. *$P < 0.05$ versus adult, control group.

Malate dehydrogenase (MDH) enzyme assays in whole homogenate determined both the cytoplasmic and the mitochondrial forms of the enzyme. The total quantity of MDH enzyme, i.e., sum of cytoplasmic and mitochondrial forms, increased approximately 18% more than left ventricular weight over the intervening 3-week period of normal heart growth in view of the "total activity" results shown in Fig. 5. Significant and comparable magnitudes of enzyme activity increase in both cytoplasmic and mitochondrial forms of MDH contributed to the adult animal response. Neither 6-OHDA nor propranolol treatments had any significant effect on MDH enzyme activity measurements in heart homogenates. MDH results (Fig. 5, right panel) show that mitochondrial mass increased approximately two-fold from 3 weeks postbirth until 6 weeks postbirth. Although mitochondrial accumulation was significantly attenuated by both drug regimens employed, mitochondria accumulated in proportion to left ventricular tissue accumulation as indicated by the consistent mitochondrial MDH activity levels among all of the 6-week-old animal groups studied.

Cardiac myofibrillar protein. Myofibrillar protein yield was 50 ± 3 mg/g (n = 5) from left ventricular tissue of 3-week postbirth rats and was not significantly different in any other groups studied. As shown in Fig. 6, myofibrillar ATPase enzyme activity was at relatively low levels in the weanling group, but activity increased significantly in the adult animals. Propranolol treatment was without significant effect on the normal ATPase augmentation. 6-OHDA injections during the developmental period facilitated the increase in myofibrillar ATPase. In keeping with the ATPase results, myofibrillar CPK activity (Fig. 6) was significantly augmented as normal heart development proceeded. Greater enhancement in CPK relative to ATPase accounts for the significant increase in the adult CPK/ATPase ratio versus values observed in weanling animals. Enhancement of myofibrillar CPK was significantly retarded or significantly augmented by 6-OHDA and propranolol treatments, respectively. Enhanced ATPase activity, in concert with reduced CPK activity, in the 6-OHDA group resulted in a substantial reduction in the CPK/ATPase ratio. In propranolol-treated animals, the relative magnitudes of increase for ATPase and CPK enzyme activities did not achieve statistically significant levels when the CPK/ATPase ratio was considered.

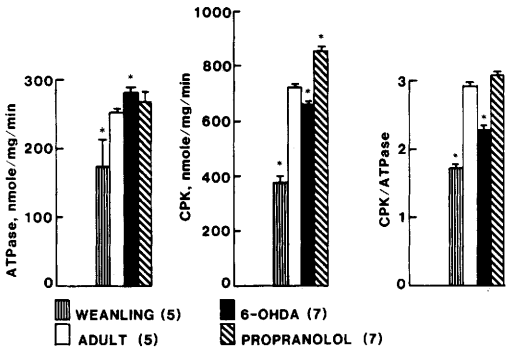

FIG. 6. Enzymatic activities in left ventricular myofibrillar protein of weanling, adult, 6-hydroxydopamine (6-OHDA), and propranolol-treated rats. Adenosine triphosphatase (ATPase), creatine phosphokinase (CPK), and the enzyme activity ratio (CPK/ATPase) were determined concurrently. Values shown are means ± SE. Number of animals in each group is given in parentheses. *$P < 0.05$ versus adult, control group.

Discussion. Metabolic and contractile functions are coordinately enhanced during rat heart postnatal growth. Because the maturational sequence is related temporally it appeared as if a single, unifying factor might be controlling myocardial development. The sympathetic nervous system is known to exert a profound influence on myocardial metabolism and heart contractile performance (6). In the perinatal heart, indices of cardiac sympathetic activity are enhanced with a time course which is similar to the augmented heart contractile and metabolic functions (7). Therefore, it was hypothesized that cardiac sympathetic nervous system function coordinates myocardial maturation. The hypothesis was tested by measuring properties previously shown to have maturationally related augmentation. Chemically induced adrenergic nerve terminal ablation or chronic, β-adrenergic receptor blockade were employed to reduce sympathetic nervous system influences on postnatal heart development encompassing the 3- to 6-week postbirth time period previously described (4).

The modes of action by which 6-OHDA and propranolol treatments elicit chemical sympathectomy of the heart require consideration. Adrenergic nerve terminals are destroyed by 6-OHDA. The β-adrenergic antagonist, propranolol, blocks the effector organ receptor while nerve terminals remain intact. Sustained occupancy of cardiac β receptors may lead to alterations in receptor density and/or receptor–adenylyl cyclase coupling may be changed. Moreover, "excess" or "spare" β receptors may exist in cardiac tissue. Growth, in general, and left ventricular growth, in particular, were retarded by both drug treatments; however, left ventricular growth was influenced in proportion to overall growth as shown by normal, adult LV/BW ratios. Comparable left ventricular protein concentration values in control, 6-OHDA, and propranolol groups provide evidence that heart protein accumulated normally.

Hemodynamic results obtained in the present study illustrate postnatal functional enhancement and demonstrate the efficacy of chemical sympathectomy. When adult animals were compared with weanlings, mean arterial blood pressure, heart rate, and dP/dt

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