Cellular Mechanisms of Impaired Adrenergic Responsiveness in Neonatal Dogs

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ABSTRACT The myocardial responsiveness of conscious, instrumented dogs to exogenously administered isoproterenol and norepinephrine was investigated in neonatal, 6-wk-old, and adult animals. Comparable base-line values for peak left ventricular derivative of pressure with respect to time were observed in all age categories. However, when compared with adult responses, the sympathomimetic amine-induced increases in neonatal left ventricular *dP/dt* were significantly blunted at each concentration of adrenergic agonist examined, whereas the 6-wk-old puppies displayed an intermediate inotropic response. To investigate the cellular mechanisms of this blunted neonatal response, we correlated physiologic and biochemical measurements of the myocardial responses to catecholamines in each age category. When compared with adult myocardial membrane preparations, neonatal cardiac membranes were characterized in vitro by an increased density of β-adrenergic binding sites, comparable affinity for adrenergic agonists and antagonists, and an enhanced coupling of adenylate cyclase activation to receptor occupancy. Simultaneous changes in either the serum catecholamine concentration or the membrane content of other intrinsic proteins failed to account for the observed neonatal increase in β-adrenergic receptor density. These findings are most consistent with a compensatory mechanism of the cardiac cell membrane, whereby an inherent depression in the adrenergic responsiveness of the immature myocardium appears to induce the increase in receptor density and activation of adenylate cyclase.

INTRODUCTION

In recent years, increasing attention has been focused upon characterization of the adaptive mechanisms of the immature mammalian cardiovascular system. Despite a thorough examination of the neonatal cardiac responsiveness to adrenergic stimulation (1, 2), the developmental modulation of this autonomic control is still incompletely understood. Early investigators have found both neonatal subsensitivity (3–5) and supersensitivity (6–8) of peripheral vessels to vasoactive drugs in the newborn. Fewer studies have examined the responsiveness of the neonatal heart to catecholamines. Geis et al. (9) observed cardiac supersensitivity to norepinephrine in the neonatal dog whereas, more recently, Buckley et al. (10) found that cardiac responsiveness to isoproterenol was normal, while that to norepinephrine was depressed in anesthetized piglets.

A potential source of confusion in these early attempts at characterization of neonatal autonomic physiology may reside in the use of anesthetized, open chest model systems, or isolated cardiac muscle or vessel preparations. More recently, studies in conscious, chronically instrumented animal models, which obviate the potential, widespread autonomic effects of general anesthesia (11–13), have indicated a subnormal neonatal vascular reactivity to infused adrenergic agonists (14, 15), as well as blunting of the baroreceptor reflex responses to induce carotid sinus hypotension (16). Nevertheless, the cellular developmental events that comprise normal autonomic maturation have been incompletely identified.

Within recent years, alterations in physiologic responses have been explained in terms of the molecular transmembrane modulation of cellular function. In vitro binding studies have enabled the enumeration of β-adrenergic receptor sites within mammalian membrane preparations, the characterization of binding affinities of these receptors for β-adrenergic agonists, and quantitative assessment of the coupling of receptor ligand binding to the activation of membrane adenylate cyclase (17, 18). Utilizing these techniques,

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age-dependent changes in adenylate cyclase hormonal sensitivity have been described in amphibian erythrocytes (19), rat liver (20), and rat myocardium (21). Furthermore, although a rapid increase with age in cerebral binding sites for β-adrenergic ligands has been reported in rats, the concentration of cardiac binding sites appears to change little during neonatal development (22).

There has thus far been no attempt to integrate the physiologic evidence for neonatal cardiac subsensitivity with simultaneously derived, in vitro analyses of cellular membrane function. In this report, we correlate physiologic and biochemical measurements of neonatal and adult adrenergic responsiveness in the instrumented conscious canine model.

METHODS

Physiologic studies. The cardiac responses of conscious animals to exogenously administered isoproterenol were examined in four neonatal (age 1 wk), four immature (age 5–7 wk) and six adult dogs. All animals were treated with sodium thiamylal, 10 mg/kg, administered in intraperitoneal injection. Then, using local anesthesia with lidocaine hydrochloride, a Millar microtip manometer was introduced into the left ventricular cavity through a peripheral vessel. In the puppies the right carotid artery was used, whereas the left femoral artery was used in the adults. In addition a catheter was implanted in the aorta in all animals through the left femoral artery. Continuous measurements of left ventricular (LV)[1] pressure, LV derivative of pressure with respect to time (*dP/dt*), and aortic pressure and heart rate were recorded in all animals on a multichannel oscillograph and tape recorder. When the animals gave no evidence of the effects of sodium thiamylal, i.e., 3–6 h later, responses to isoproterenol or norepinephrine were obtained. Isoproterenol was administered intravenously in bolus doses of 0.1, 0.5, 1.0, 2.0, and 4.0 μg/kg. Norepinephrine was administered in bolus doses of 0.25, 0.50, 1.00, and 2.00 μg/kg.

Cardiac membrane preparation. Healthy neonatal, immature, and adult dogs were anesthetized with pentobarbital. The hearts were immediately excised and placed into iced Krebs-Ringer's solution. All subsequent procedures were carried out at 4°C. Epicardium and endocardium were removed and left ventricular myocardium was coarsely minced in buffer (Tris 0.25 M, $MgCl_2$ 1 mM, EGTA 5 mM), and homogenized in a PT-10ST Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.) tissue disruptor. The homogenate was filtered through two thicknesses of Japanese silk screen, size 12, and centrifuged in a Sorvall RC-2 DuPont Instruments, DuPont Co., at 3,000 *g* for 10 min. The supernate was respun at 18,000 *g* for 15 min and the resulting pellet was resuspended in buffer using a Teflon pestle in a Potter-Elvehjem homogenizer.

Binding studies. All studies were performed in triplicate in the presence of Tris-$MgCl_2$-EGTA buffer. For the determination of β-adrenergic receptor binding saturation, 100 μl of the cardiac membrane preparation (1–2 mg protein/ml) were incubated at 37°C for 10 min with increasing concentrations (0.5–10 nM) of [^{3}H](−)dihydroalprenolol (New England Nuclear, Boston, Mass., specific activity 58.6 Ci/mmol), with or without unlabeled (−)-propranolol (1 μM), in a final reaction volume of 150 μl. For the assessment of competitive binding inhibition, all incubations were performed with [^{3}H](−)dihydroalprenolol (4.2 nM) in the presence of 0.1 mM guanosine triphosphate (GTP) and varying concentrations of (−)-isoproterenol. GTP was included in the assays of competitive binding inhibition to eliminate any heterogeneity of agonist binding to different receptor states and to ensure that determination of the dissociation constant (K_D) for agonist binding and for adenylate cyclase activation could be achieved under comparable reaction conditions. Isoproterenol solutions were prepared in the presence of 2 μg/ml of sodium metabisulfite immediately before use. Following incubation, 100 μl of the reaction mixture were rapidly filtered under vacuum onto Whatman GF-C glass fiber filters (Whatman Inc., Clifton, N. J.). The filters were heated for 30 min at 60°C with 0.5 ml of Protosol (New England Nuclear), followed by the addition of glacial acetic acid. The filters were counted in 10 ml of Aquasol-2 (New England Nuclear) in a Packard Tri-Carb (Packard Instrument Co., Inc., Downers Grove, Ill.), with a counting efficiency of 40%.

Catechol-O-methyl transferase (COMT) assay. 500 μl of the membrane preparation were added to 500 μM S-adenosyl-L-methionine chloride, 2.5 mM $MgCl_2$, and a 0.1 mM solution of catecholamine containing 300,000 to 1,100,000 cpm of [^{3}H]-epinephrine (New England Nuclear). The reaction mixture was removed from ice and incubated at 37°C for 15 min to 1 h. The reaction was terminated by the addition of 2 ml of 0.5 M potassium borate, pH 10.0. The 3-*O*-methyl derivatives were extracted into 5 ml of toluene:isoamyl alcohol (3:2) by vortexing at the highest speed for 15 s. The organic supernatant phase was added to 15 ml of Bray's solution and counted. The control reaction, for the determination of background activity, lacked S-adenosyl methionine. Under these conditions, enzymatic activity was linear for 1 h, expressed as picomoles [^{3}H]metanephrine per milligram protein per hour.

The adenylate cyclase assay was performed as previously described (18). Reaction conditions were identical to those used for binding studies. Recovery of added ^{3}H-cyclic 3′-5′ AMP was 40–80%.

The protein concentrations for each membrane assay were determined by the Lowry method (23). Analysis of saturation binding assays was performed according to the method of Scatchard (24). The K_D for unlabeled (−)-isoproterenol binding to the cardiac β-adrenergic receptor was derived from analysis of the competitive inhibition studies according to the method of Cheng and Prusoff (25). Plasma catecholamine concentrations were measured with a sensitive radioenzymatic method (26).

RESULTS

Physiologic studies. Base-line hemodynamic values for the adults, newborn, and intermediate age puppies are shown in Table I. In both groups of puppies base-line values for LV systolic and mean aortic pressure were significantly lower ($P < 0.01$) while heart rates were significantly higher ($P < 0.01$) than observed in adults. However, values for peak LV *dP/dt* were not significantly different among the three groups. The effects of bolus injections of (±)-isoproterenol on LV function are illustrated for a representative newborn and adult in Fig. 1, whereas dose-response curves for all the animals are illustrated in Fig. 2. The average increase in the neonatal LV *dP/dt* was significantly

[1] *Abbreviations used in this paper*: COMT, catechol-*O*-methyl transferase; *dP/dt*, derivative of pressure with respect to time; LV, left ventricular.

TABLE I
Base Line Hemodynamics

	Adults	Puppies	
		1 wk old	5–7 wks old
LV systolic pressure (*mm Hg*)	119.7±1.6	99.0±2.2*	103.9±2.8*
LV *dP/dt* (*mm Hg/s*)	3,341±106	3,057±198	3,180±282
Mean arterial pressure (*mm Hg*)	94.4±1.6	66.1±4.5*	75.6±2.1*
Heart rate (*beats/min*)	93.4±3.0	237.9±4.4*	193.3±9.1*

* Significantly different from adult ($P < 0.01$).

blunted at each concentration of isoproterenol examined, while the 6-wk-old puppies displayed an inotropic response intermediate to the other age categories. It is particularly noteworthy that the disparity in the immature inotropic response became increasingly pronounced as the concentration of infused adrenergic agonist was increased. In fact, administration of four times the dose per kilogram of agonist to the immature dogs, i.e., a dose of 4.0 μg/kg, produced a smaller rise in LV *dP/dt* than 1.0 μg/kg of isoproterenol did in the adults (Table II, Fig. 1). Increases in cardiac rate with isoproterenol were also blunted in the newborns (Table II). However, in contrast to the inotropic responses, depressed chronotropic responses in the newborns could be explained on the basis of altered base line. The decreases in mean arterial pressure with isoproterenol were less in the newborns, as has been shown previously from this laboratory (14).

The effects of bolus injections of norepinephrine on LV function are illustrated for a representative newborn and adult in Fig. 3, whereas dose-response curves for all the animals are illustrated in Fig. 4. The average increase in neonatal LV *dP/dt* was significantly blunted at each concentration of norepinephrine examined, while the 6-wk-old puppies displayed an inotropic response intermediate to the other age categories. Increases in mean arterial pressure with norepinephrine were less in the newborns (Table III) as

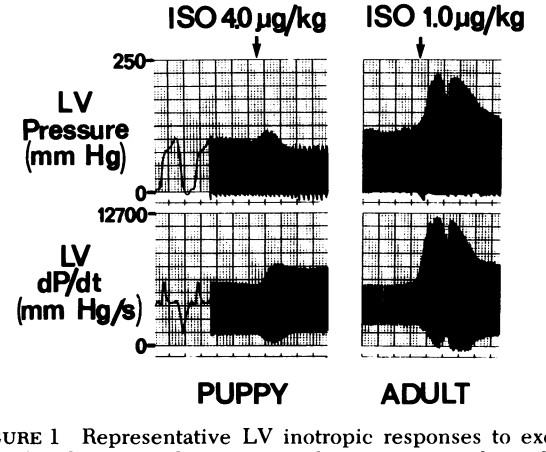

FIGURE 1 Representative LV inotropic responses to exogenously administered isoproterenol (ISO) in a newborn (left panel) and adult dog (right panel). Continuous measurements of LV pressure and LV *dP/dt* were recorded in conscious dogs with an intracavity Millar microtip manometer. Basal values for LV *dP/dt* were similar in each age category. In this example, the adult received a dose of 1 μg/kg i.v. and the newborn received 4 μg/kg of isoproterenol i.v. The newborn displayed a subnormal inotropic response despite a fourfold increment in the dose.

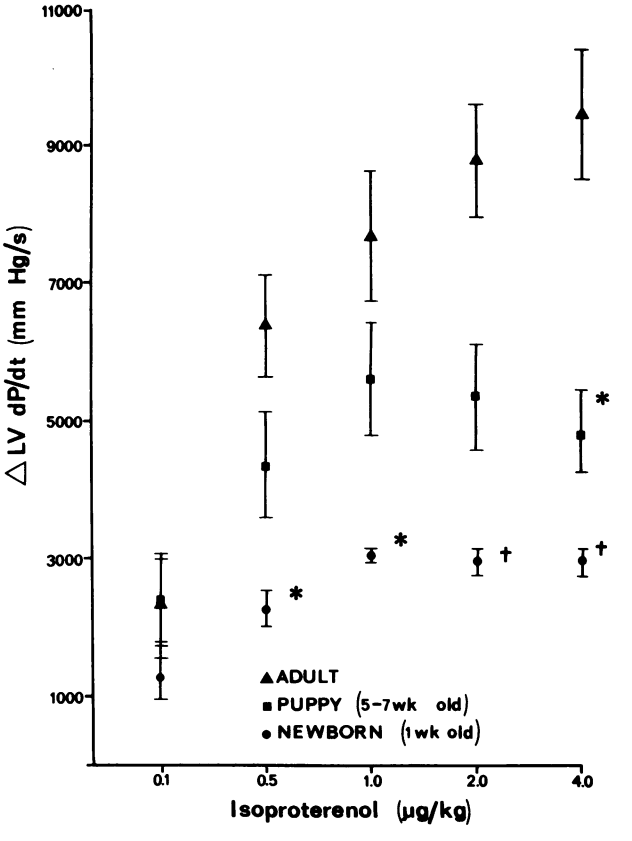

FIGURE 2 Inotropic dose response curves is isoproterenol for adults, 1-wk-old puppies and 5–7-wk-old puppies. The mean inotropic response of newborn animals was blunted throughout the dose-response range, and the maximal stimulation was achieved at a much lower dose of isoproterenol at this stage of development. Responses significantly different from adults are noted by * ($P < 0.05$) and + ($P < 0.01$).

TABLE II
Cardiovascular Response to Isoproterenol

	Isoproterenol (μg/kg)				
	0.1	0.5	1.0	2.0	4.0
Mean arterial pressure (Δ *mm Hg*)					
Newborn	−0.7±0.7*	−4.0±3.1‡	−8.7±3.3‡	−9.3±2.9‡	−10.0±3.1‡
Adult	−17.2±1.9	−19.8±3.6	−20.0±2.2	−20.5±1.8	−21.3±1.3
Heart rate (Δ *beats/min*)					
Newborn	6.3±3.4*	28.3±3.5*	36.8±6.6‡	43.5±6.7‡	47.0±4.0*
Adult	52.7±9.6	103.8±11.8	106.0±17.0	113.2±20.2	125.5±17.5
LV *dP/dt* (Δ *mm Hg/s*)					
Newborn	1,273±294	2,276±255‡	3,031±85‡	2,988±181*	2,943±129*
Adult	2,647±584	6,359±708	7,664±872	8,624±808	9,397±940

* Newborn significantly different from adult, $P < 0.01$.
‡ Newborn significantly different from adult, $P < 0.05$.

has been shown previously from this laboratory (14). The decreases in cardiac rate were not significantly less in the newborns than in the adults with norepinephrine. However, it is important to point out that the pressure rise, i.e., the stimulus to the baroreceptors was greater in the adults than in the newborns (Table III).

Myocardial β-adrenergic binding studies. Specific binding of [^{3}H]dihydroalprenolol to the myocardial β-adrenergic receptor was plotted as a function of the radioligand concentration and replotted by the method of Scatchard. As previously reported (18), binding of labeled adrenergic antagonists to these preparations is saturable, yielding a single component, linear Scatchard relationship. Antagonist binding to representative neonatal and adult membrane preparations is depicted in Fig. 5. In this experiment, neonatal and adult receptors displayed comparable affinity for dihydroalprenolol (K_D = 3.5 nM), but the density of binding

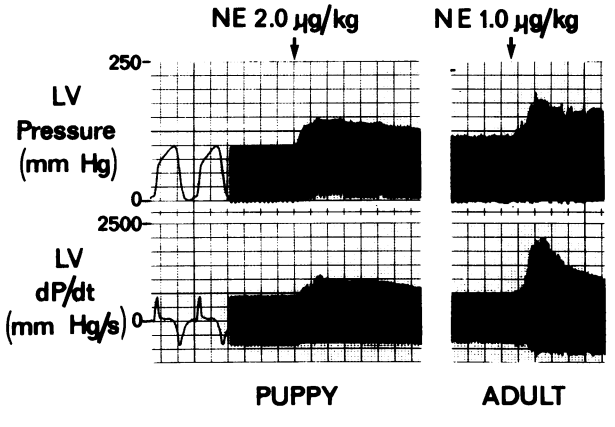

FIGURE 3 Representative LV inotropic responses to exogenously administered norepinephrine (NE) in a newborn (left panel) and adult dog (right panel). Continuous measurements of LV pressure and LV *dP/dt* were recorded in conscious dogs with an intracavity Millar microtip manometer. Basal values for LV *dP/dt* were similar in each age category. In this example, the adult received an intravenous dose of 1 μg/kg and the newborn received 2 μg/kg of norepinephrine i.v. The newborn displayed a subnormal inotropic response despite a twofold increment in the dose.

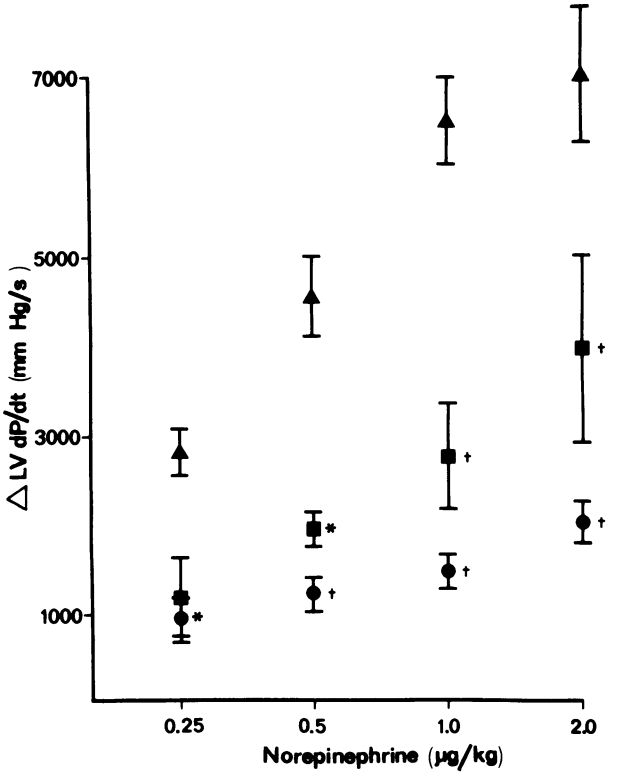

FIGURE 4 Intropic dose-response curves to norepinephrine for adults (▲), 1-wk-old puppies (●) and 5–7-wk old puppies (■). The mean intropic response of newborn animals was blunted throughout the dose response range, and the maximal stimulation was achieved at a much lower dose of norepinephrine at this stage of development. Responses significantly different from adults are noted by * ($P < 0.05$) and + ($P < 0.01$).

TABLE III
Cardiovascular Response to Norepinephrine

	Norepinephrine (μg/kg)			
	0.25	0.5	1.0	2.0
Mean arterial pressure (Δ *mm Hg*)				
Newborn	13.3±2.9	16.0±2.0	17.3±2.7*	31.3±4.4*
Adult	16.7±3.6	29.0±3.8	53.3±7.6	65.7±7.5
Heart rate (Δ *mm Hg*)				
Newborn	−12.0±6.4	−13.0±7.9	−11.5±8.4	13.5±6.7‡
Adult	−16.8±5.3	−27.3±7.2	−37.8±9.9	−42.2±8.1
LV *dP/dt* (Δ *mm Hg/s*)				
Newborn	980±211*	1,206±161‡	1,473±192*	2,007±238*
Adult	2,820±262	4,570±411	6,499±483	6.965±755

* Newborn significantly different from adult, $P < 0.05$.
‡ Newborn significantly different from adult, $P < 0.01$.

sites, as determined by the abscissa of the Scatchard relationship, was substantially greater in the neonatal cardiac preparation than in the adult membranes (110 and 30 fmol per mg protein, respectively). Repeated, independent enumeration of myocardial β-adrenergic receptors confirmed a significantly higher density of these binding sites in the neonatal tissues; in contrast, there was no difference in the observed dihydroalprenolol binding affinity (Table IV). The observed differences in β-adrenergic receptor density could not be explained by age-related differences in serum catecholamine concentrations (Table IV): epinephrine concentrations were comparable in all three categories, and norepinephrine concentrations demonstrated a tendency to decline with increasing age. These data are inconsistent with the possibility of receptor down-regulation as an explanation for the decreasing receptor density observed during maturation. Membrane preparations from older developing animals displayed an intermediate number of alprenolol binding sites; however, the receptor density, binding affinity, and catecholamine concentrations did not differ significantly from the adult preparations.

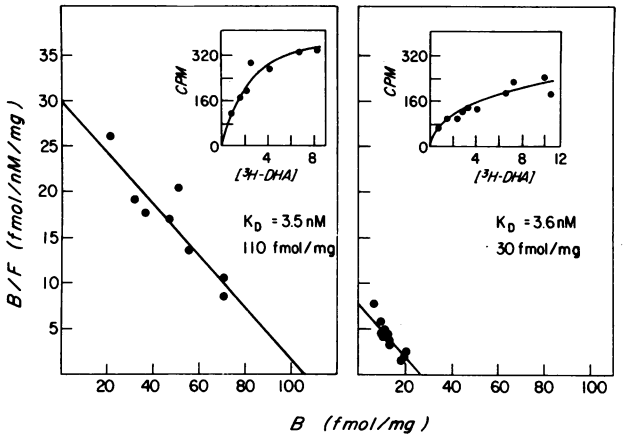

FIGURE 5 Representative saturation binding of [³H]dihydroalprenolol ([³H]DHA) to newborn (left) and adult (right) cardiac membrane preparations. The insets represent the specifically bound [³H]DHA plotted as a function of total [³H]DHA. Scatchard analysis of the binding data revealed a comparable K_D for binding, but demonstrated an increased density of binding sites in the newborn preparation. B, bound; F, free.

Enzymatic analysis of cardiac membranes. To ascertain that the apparent developmental decrease in membrane receptor density did not reflect an artifactual difference in protein content induced by the preparation of cardiac microsomes, the membranes were simultaneously assayed for their content of other membrane-associated proteins uninvolved in receptor ligand binding. When examined in this fashion, the membrane-associated activity of COMT was depressed in the neonate, but did not significantly differ from adults in the 6-wk-old dogs (Table V). In contrast to the dramatic decrease in receptor density in the postnatal period, membrane COMT activity showed a marked tendency to increase with age. In addition, the maximal stimulation of cyclic AMP production by isoproterenol, a quantitative reflection of the total membrane content of adenylate cyclase catalytic activity, showed little difference among the age categories examined. Despite a somewhat higher cyclase content in the intermediate-aged membranes, there was no significant difference between neonatal and adult preparations.

Coupling of β-adrenergic receptor binding to the activation of adenylate cyclase. To investigate the functional correlates of an increased density of ligand binding sites in immature animals, the binding affinity of the β-adrenergic receptor for (−)-isoproterenol was compared to the receptor-mediated stimulation of adenylate cyclase by this agonist. In a representative experiment (Fig. 6), competition for dihydroalprenolol binding sites demonstrated a 50% displacement of

TABLE IV
Scatchard Analysis of Dihydroalprenolol Binding to Canine Myocardial Membranes

	β-adrenergic Receptor density	K_D	Plasma norepinephrine	Plasma epinephrine
	*fmol mg protein**	*nM**	*pg/ml**	
Newborn (n = 5)	147±27‡	4.8±1.9§	831±31§	414±28§
Immature (n = 3)	83±28§	4.0±1.1§	477±86§	460±52§
Adult (n = 7)	60±19	3.7±1.7	461±221	394±248

* Mean±SD.
‡ $P < 0.001$, compared to adult value.
§ NS.

bound radioligand with an identical concentration of (−)-isoproterenol in neonates and adults (0.3 μM). However, simultaneous examination of the isoproterenol stimulation of membrane adenylate cyclase activity revealed a striking disparity in the agonist concentration that effected a 50% stimulation of the enzyme ($K_{D\ activation}$); thus, in this experiment, the same degree of enzyme stimulation occurred at a substantially lower isoproterenol concentration in the neonate (0.01 μM) than in the adult (0.1 μM). The averaged results of several experiments are illustrated in Fig. 7. The dissociation constants for isoproterenol binding were derived from the results of competitive inhibition studies (22). Despite the comparable receptor binding affinity for agonist in newborns and adults, the neonatal cardiac membranes in repeated experiments were characterized by a significantly lower ($P < 0.05$) mean K_D for the activation of cyclase (0.02±0.02 μM) than the adult preparations (0.15±0.09 μM). Furthermore, in the neonatal membranes, the K_D for isoproterenol activation of adenylate cyclase was significantly lower than the simultaneous K_D for binding (Fig. 4). In contrast, there was no significant difference between the K_D for binding and activation in adult membranes.

TABLE V
Enzymatic Analysis of Canine Myocardial Membranes

		Isoproterenol-stimulated adenylate cyclase	
	COMT	Basal	Maximal*‡
	pmol [³H]metanephrine/mg per h‡	*pmol/mg per min*	*%*
Newborn (n = 3)	0.1±0.08§	75±10‖	116±51‖
Immature (n = 3)	0.5±0.07‖	60±34‖	176±68§
Adult (n = 4)	2.2±1.3	98±86	76±14

* Maximal isoproterenol stimulated adenylate cyclase activity, expressed as [(maximal − basal/basal) × 100].
‡ Mean±SD.
§ $P < 0.05$, compared to adult value.
‖ NS.

DISCUSSION

Base-line levels of LV *dP/dt* were not significantly different in the three groups studied. This finding of normal baseline inotropic function in newborn animals differs with results of studies in isolated cardiac muscle preparations, where myocardial contractility was found to be depressed in the newborn (1, 2). Recently Berman and Musselman (27) found the LV *dP/dt* was significantly higher in conscious newborn lambs than adult sheep. The levels of LV *dP/dt* found by Berman and Musselman (27) in newborn lambs (27) correspond to the values observed in newborn and adult animals in the present investigation. However, the levels of LV *dP/dt* in conscious adult dogs appear to be higher than those for LV *dP/dt* in adult sheep as reported by Berman and Musselman (27).

Observations on the effect of adrenergic agonists on myocardial function in newborn and adult animals have been controversial. On the one hand, Friedman (1) and

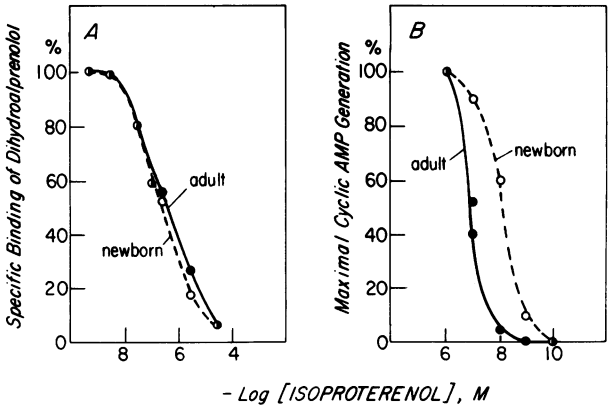

FIGURE 6 Isoproterenol interaction with cardiac membranes in the representative experiment. (A) Competitive inhibition of [³H]DHA binding to newborn and adult cardiac preparations with increasing concentrations of (−)-isoproterenol demonstrated a comparable apparent affinity for the agonist in each case. (B) Simultaneous assay of the in vitro adenylate cyclase dose response curves revealed a disparity in the K_D for activation (adult, 0.1 μM; newborn, 0.01 μM).

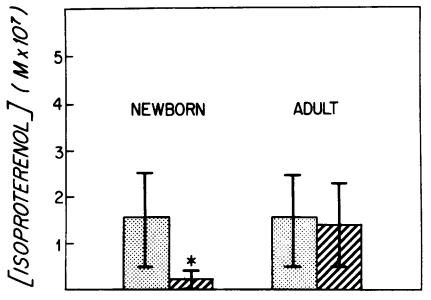

FIGURE 7 Comparison of isoproterenol binding and adenylate cyclase activation in newborn and adult cardiac membranes. The mean K_D for binding (stippled bar) was 0.15±0.01 μM in both age categories, but the mean K_D for activation (hatched bar) in the newborn (0.02±0.02 μM) was significantly lower than in the adult (0.15±0.09 μM). Vertical bars denoted the SD and the asterisk denotes a statistically significant difference ($P < 0.05$).

Geis et al. (9) felt that the newborn heart was supersensitive to effects of isoproterenol and norepinephrine. More recently Buckley et al. (10), studying open chest anesthetized piglets, found that the response to norepinephrine, but not isoproterenol, was depressed in the newborn. Therefore, our results, demonstrating marked depression of the inotropic response to both isoproterenol and norepinephrine in newborn dogs, differ from what has been shown previously for LV function in anesthetized animals (1, 9, 10), but is in agreement with what has been shown for peripheral vascular effects to these adrenergic agonists in conscious animals (14). It is possible that many of these differences may be explained by the presence or absence of general anesthesia and the open chest model. It is also important to note that depression of inotropic response was observed both for an adrenergic agonist that increased heart rate (isoproterenol) and one that reduced heart rate (norepinephrine). The fact that heart rate fell less with norepinephrine in newborns when equivalent increases in arterial pressure were compared, confirms the immaturity of the arterial baroreceptor reflex in the neonatal animals (16).

It is now well recognized that the agonistic effects of adrenergic ligands upon catecholamine-sensitive tissues are mediated through transmembrane modulation of intracellular events. In order to identify a possible cellular mechanism for the apparent neonatal subsensitivity to exogenous catecholamines, we have attempted to compare the subcellular responsiveness of neonatal and adult cardiac membrane preparations to adrenergic ligand binding and adenylate cyclase stimulation. In the absence of measurable, age-related differences in the binding affinity of these receptors for agonists and antagonists, the neonatal cardiac membrane is characterized by a substantial increase in the density of such binding sites. It is unlikely that this disparity could be explained by alterations in the endogenous serum catecholamine concentrations, which did not correlate inversely with receptor density. The absolute values for catecholamine concentrations compare favorably with previously reported values (28). Furthermore, unlike the adults, neonatal membranes display 50% of maximal adenylate cyclase stimulation by isoproterenol at a concentration of agonist that corresponds to much less than 50% receptor occupancy. These findings can be interpreted to represent a stage of closer coupling of receptor binding to adenylate cyclase activation in newborns when compared to normal adults.

In both the physiologic and biochemical approaches to a study of the maturation of adrenergic control, the chief difficulty resides in the construction of comparable experimental conditions at each stage of development. The identification of a disparate inotropic responsiveness to exogenous catecholamines in neonates could potentially be ascribed to the established larger volume of distribution for these drugs in the newborn animal, when expressed as a function of lean body mass (29). However, in the current investigation, even with very large doses of adrenergic agonists in neonates, an inotropic effect comparable to the adult response cannot be elicited. In addition, examination of comparative dose-response curves (Figs. 2, 4) indicates that neonatal responsiveness was comparatively blunted throughout the dose-response range. The newborn, however, achieves its maximal response to isoproterenol at a lower dose than observed in the adult, a phenomenon that can be interpreted to correspond to the lower neonatal K_D for adenylate cyclase activation. The comparative observations of in vitro membrane characteristics might likewise be obscured by noncomparable protein composition of microsomes prepared from the canine left ventricle at different stages of development. To ascertain that the observed developmental decrease in membrane receptor density did not represent an artifactually induced difference in protein content, the membrane preparations were assayed for their content of other intrinsic protein constituents. Through the quantitation of adenylate cyclase and COMT content, the specificity of differences in receptor density was confirmed by the documentation of two other, distinct patterns of change in specific membrane proteins with increasing age. The disproportionately high adenylate cyclase content of the 6-wk-old dogs is currently unexplained, but may reflect an intermediate pattern of adaptation to incomplete adrenergic development.

The observation of enhanced β-adrenergic receptor density in neonatal dogs is of interest in several respects. Although the observation of an enhanced β-adrenergic receptor density in the neonatal subjects

might in itself suggest a "spare receptor" phenomenon, the correlative changes in adenylate cyclase and end-organ responsiveness to catecholamine stimulation suggest, rather, that the pronounced increase in neonatal receptor density is a specific, adaptive change to a blunted effector mechanism in adrenergic modulation. The exact nature of the cellular impairment of immature animals remains speculative, but appears to follow the stimulation of membrane adenylate cyclase in the cellular activation sequence. Unlike most of the previously studied acquired changes in membrane adrenergic density (30–32), the neonatal cardiac response bears little relation to changes in the endogenous concentration of receptor ligands (Table II). Thus, in addition to the previously described phenomenon of down-regulation in response to increased agonist concentration (30–32), these studies would seem to indicate yet another important mechanism for target cell regulation of receptor concentration. Although the K_D for isoproterenol activation of adenylate cyclase in the neonate is an order of magnitude less than that in the adult, the increase in the neonatal receptor density results in a comparable number of occupied receptor sites when the enzyme is half-maximally activated. This increase in receptor concentration in the neonate appears to represent one mechanism whereby it can compensate for an inherent depression in adrenergic responsiveness. Further investigation of these phenomena must involve attempts to probe the development of intracellular response mechanisms to cyclic AMP (33, 34). Quantitation of age-dependent changes in the inotropic modulation of intracellular cyclic nucleotides, the cyclic AMP-dependent activation of protein kinase, the specific phosphorylation of cytosolic proteins, and contractile protein response should yield greater insight into the developmental nature of cardiac adrenergic control.

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