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REPONSES OF SMALL INTRAPULMONARY ARTERIES TO VASOACTIVE AGENTS IN THE FETAL AND NEONATAL LAMB

A Thesis

Presented for the

Master of Science

Degree

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Julie A. Dunn

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ABSTRACT

The transition of the pulmonary circulation from fetal to newborn life is not fully understood. To characterize this process further, responses of third and fourth generation pulmonary arteries were assessed to the vasoconstrictors epinephrine, norepinephrine, serotonin and potassium chloride and the vasodilators nitroprusside, tolazoline, histamine and dopamine. Vessel segments were obtained from fetal and neonatal lambs at 7 days preterm and at 1, 7 and 21 days of age and mounted on a myograph to measure isometric tension. Responses to potassium chloride were measured as mg per mm2 of tissue (where mm2 = length x circumference). Responses to epinephrine, norepinephrine and serotonin were calculated as a percentage of the maximal KCl response. Responses to the vasodilators nitroprusside, tolazoline, histamine and dopamine were performed after a preconstricting dose of serotonin (10^{-5} M) had been administered. Percent dilation compared to serotonin constriction was calculated.

Fourth generation vessels were more responsive to KCl than third generation vessels. Additionally, the response of fourth generation vessels to KCl increased with age. No significant differences were noted to age for third generation vessels. Fourth generation vessels were significantly less responsive to epinephrine and

norepinephrine than third generation vessels. Also, both generation vessels showed a significant decrease in sensitivity to these compounds at days 1 and 7 compared to preterm and 21 days of age. Serotonin was a potent vasoconstrictor in both third and fourth generation vessels, exceeding the maximal contractile response obtained to KCl. No differences were detected between generations to serotonin, however, like epinephrine and norepinephrine, sensitivity decreased at day 1 compared to 7 and 21 days.

No differences in maximum relaxation to nitroprusside and tolazoline among ages or between generations were demonstrated. Fourth generation vessels were more sensitive to tolazoline than third generation vessels. Nitroprusside was a substantially more potent vasodilator than tolazoline. Histamine was a vasodilator in both third and fourth generation vessels at lower doses and a slight constrictor at higher doses in third generation vessels only. No differences were detected to age. Dopamine was a dilator in fourth generation vessels at 1 day of age. At high doses, a constrictor response was observed primarily of the third generation vessels.

Third generation vessels are functionally mature at birth as exhibited by their response to KCl. Fourth generation vessels are still undergoing maturation at birth through three weeks of age. The differential

responses to both vasoconstrictors and vasodilators between age and generation are further evidence of the striking heterogeneity that exists in vascular smooth muscle both as a function of age and level of the pulmonary arterial tree. Furthermore, the decreased sensitivity of 1 and 7 day vessels to norepinephrine and epinephrine and of day 1 vessels to serotonin may be an important regulatory mechanism to ensure a drop in pulmonary vascular resistance necessary at birth for normal adaptation.

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I. INTRODUCTION

BACKGROUND

Until the 1950's the physiology of the fetal and neonatal pulmonary circulation was largely unstudied. Investigations using cineradiography demonstrated large differences in volume and velocity of blood flow through the lungs before and after birth (Adran et al., 1952; Reynolds et al., 1954). The high pulmonary vascular resistance present in the fetal state and the subsequent dramatic decrease at birth were unexplained. Reynolds (1956) stated "Clearly, some sudden morphological change incident to pulmonary ventilation facilitates the rapid and voluminous flow of blood through the lung" and he postulated congestion of the fetal pulmonary arteries and arterioles was due to packing of erythrocytes in twisted and convoluted vessels. Dawes and Mott (1962) suggested a different phenomenon for the high resistance to blood flow in the lung: tonic contraction of the smooth muscle in the vascular wall.

Numerous factors affect the tonic contraction of pulmonary vascular smooth muscle in the fetus and newborn. These factors include ventilation, oxygenation, autonomic input and the presence of circulating neurohumoral and vasoactive substances. However, despite extensive study of these factors and their effect on the pulmonary

vasculature in the fetus and neonate, the basic mechanisms of the transitional circulatory process remain elusive.

THE FETAL AND NEONATAL CIRCULATION

In utero, fetal gas exchange occurs via the placenta. Oxygen-depleted blood flows to the placenta through the umbilical arteries and leaves oxygenated through the umbilical vein. The umbilical circulation accepts approximately 65% of the fetal cardiac output and is the primary determinant of fetal systemic vascular resistance (Assali, 1968). Oxygenated blood leaves the placenta through the umbilical veins. The umbilical veins approach the liver, becoming the ductus venosus which joins the inferior vena cava. Mixed venous and oxygenated blood enter the right atrium. The umbilical and hepatic beds, as well as the ductus venosus comprise a system relatively unreactive to stimuli (Dawes, 1969). From the right atrium, blood passes through either the foramen ovale into the left atrium or enters the right ventricle. The blood diverted to the left atrium serves to provide the left ventricle with the volume and pressure necessary to develop the left ventricle for its role after birth and to maintain adequate fetal systemic pressure (Assali, 1968). Blood that enters the right ventricle passes into the pulmonary artery and is shunted into the aorta through the ductus arteriosus and thus circumvents the

lung. In contrast to the umbilico-placental circulation, the cardiopulmonary circulation responds readily to a variety of stimuli (Dawes, 1969; Assali, 1968).

At birth, the umbilico-placental circulation is removed. As a consequence, systemic vascular resistance increases. In addition, lung expansion and alveolar oxygenation contribute to a decrease in pulmonary vascular resistance. Fetal shunt pathways functionally close within twelve hours after birth and establishment of the adult circulation is initiated (Dawes, 1968).

DEVELOPMENT OF THE PULMONARY VASCULATURE

The arteries of the pulmonary vasculature have been classified by wall structure: elastic, muscular and mixed (partially muscular and nonmuscular, Hislop and Reid, 1972, 1973; Reid, 1977). The arterial and vascular smooth muscle structure in the human fetus and neonate differ markedly from the adult. Vascular smooth muscle at all stages of fetal life is greater than that of the adult (Naeye, 1961; Hislop and Reid, 1973; Levin et al., 1976). The total number of pre-alveolar arteries is established by the 16th week of gestation. After 16 weeks, changes occur in length and diameter only. Within the alveolar region, the total number of nonmuscular arteries increases with fetal age. As vessels decrease in diameter, there is a rapid rise in percentage wall thickness (vascular

smooth muscle), especially in vessels less than 250 um (Hislop and Reid, 1973; Levin, 1976; Reid, 1977). These vessels are postulated as the site of greatest resistance to blood flow in the fetal lung (Reid, 1977; Levin et al., 1976; Rudolph, 1977).

At birth, intra-alveolar vessels rapidly multiply to keep pace with alveolar development. Extension of vascular smooth muscle occurs into the alveolar region, but is not complete until 19 years of age in humans (Hislop and Reid, 1973). The vessels hypothesized to control pulmonary vascular resistance in the fetus (< 250 um) undergo dramatic changes and by the third postnatal day, wall thickness falls to adult levels (Hislop and Reid, 1973; Larroche et al., 1959; Rendas, 1978). The wall thickness of arteries between 250 and 1000 um remains comparable to fetal ages until 2-4 months postnatally (Hislop and Reid, 1973; Rendas, 1978). The rapid change in vessels less than 250 um is likely due to dilatation, while the changes in vessels greater than 250 um is thought to be growth related (Hislop & Reid, 1973). The mechanisms for these changes are not clear, but Hislop and Reid (1973) postulate differential muscle types, one responsive to humoral agents resulting in rapid dilatation and the other responsive to a pressure changes resulting in a drop in cell multiplication.

DEVELOPMENT OF THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system develops early during fetal life and continues to develop for some time after birth (Lebowitz et al., 1972; Champlain et al., 1970; Lee et al. 1970, Silva and Ikeda, 1971). In the fetal lamb, the pulmonary vascular bed possesses cholinergic receptors which respond to infusion of acetylcholine (Dawes and Mott, 1962) and electrical stimulation of the vagus nerve (Colebatch et al., 1965; Cassin et al., 1964a, b). Furthermore, responses to cholinergic stimulation and to the administration of acetylcholine increase with fetal age (Woods, 1977). Nuwayhid et al., (1975) found that the sensitivity remained constant even though response increased with age. Therefore, a maturation of the pulmonary arteriolar effector system occurs rather than a change in the receptor system.

Despite the presence of cholinergic receptors, bilateral vagotomy, ganglionic cholinergic and peripheral cholinergic blockade do not alter pulmonary vascular tone (Colebatch et al., 1965; Nuwayhid et al., 1975). Thus, although the parasympathetic system is present and capable of exerting influence over the fetal circulation, the cholinergic neurotransmitter is not tonically released in the unstimulated state. As the fetus matures, minimal vagal tone develops, primarily exerting control over heart rate (Nuwayhid et al., 1975).

Adrenergic receptors are present in lambs as early as 60 days of gestation (Nuwayhid et al., 1975, Colebatch et al., 1965; Barrett et al., 1972; Dawes et al., 1956). Like the cholinergic system, responsiveness to sympathetic stimulation in the lung increases with gestational age, although is less responsive than the adult. Several factors can account for this difference from the adult: 1) the presence of fetal shunt pathways and 2) presence of the low-resistance placental circulation which has no neural control (Assali, 1968; Barcroft, 1947; Dawes, 1968). In addition, sympathetic nerves do not exert a significant tonic effect on resting fetal pulmonary circulation (Lewis et al., 1976). Whereas the pulmonary vasculature of the fetal and neonatal lung is sensitive to both parasympathetic and sympathetic agonists, no evidence exists for tonic release of either neurotransmitter in the resting state (Downing and Lee, 1980).

Adrenal release of catecholamines is thought to be a more important mechanism for sympathetic response to fetal stress than autonomic adrenergic pathways (Barrett et al., 1972; Iwamoto et al., 1983). Parer et al., (1978) found that abrupt exposure of hypoxic fetuses to alpha and beta blockade resulted in death, whereas fetuses chemically sympathectomized did not die (Iwamoto, 1983) . Adrenal catecholamine release also plays an important role in

increasing cardiac output and heart rate required during the first week of life (Baylen et al., 1987) and is responsible for controlling regional blood flow during this period (Padbury et al., 1987).

The site and role of adrenergic effects in the neonatal lung remains to be clarified. The distribution of nerve fibers is most intense in the larger elastic vessels, less in the muscular arteries and absent in vessels less than 30 um (Downing and Lee, 1980). Sympathetic control of vascular tone may be restricted to large arteries and may not regulate pulmonary vascular resistance, but rather might alter wall stiffness of the large pulmonary arteries, subsequently decreasing pulmonary vascular compliance (Su, 1978; Goetzman and Bennett, 1986). Information regarding the site of action of adrenergic and cholinergic compounds within the lung would provide insight into the regulation of the pulmonary vascular resistance by these compounds in the fetus and neonate.

LUNG EXPANSION AND OXYGENATION

Initiation of ventilation (lung expansion) and changes in gas tensions in the lungs of the newborn contribute to the reduction of pulmonary vascular resistance and increased blood flow (Dawes, 1968). However, quantification of expansion versus oxygenation

and expansion/oxygenation interactions has been difficult. Dawes et al., (1953) found an increase in pulmonary blood flow and decrease in blood pressure upon rhythmic ventilation. This occurred whether ventilation was accomplished with air, oxygen or nitrogen. These same effects did not occur when the lungs were expanded with saline. These investigators concluded that the drop in pulmonary vascular resistance was a direct mechanical effect independent of the autonomic nervous system. Colebatch et al., (1965) and Lauer et al., (1965) confirmed that the drop in pulmonary vascular resistance upon ventilation was independent of the autonomic nervous system using vagotomy, sympathectomy and cholinergic and adrenergic blockade.

Changes in arterial and alveolar PO_2 also affect pulmonary vascular resistance. Cook et al., (1963) pointed out that ventilation with nitrogen may lower PCO_2 and result in vasodilation. Pulmonary vasodilation occurred in the absence of lung expansion when arterial PO_2 was increased (Dawes and Mott, 1962; Cassin, 1964b). Local changes in PO_2 that do not alter arterial PO_2 may also produce a drop in pulmonary vascular resistance (Lauer et al., 1965). Thus, mechanical forces alone do not account for the drop in pulmonary vascular resistance at birth.

The relative contributions of ventilation versus oxygenation is still a topic of debate. Assali (1968) emphasized that the mechanical effect of expanding the alveoli plays the major role. An increase in alveolar and pulmonary venous PO2 is stressed the primary determinant for the decrease in pulmonary vascular resistance at birth by Dawes (1969) and Rudolph (1977). Rudolph et al. (1986) reassessed the effect of ventilation versus oxygenation and concluded that ventilation in the absence of changes in fetal blood gases resulted in a 30-fold decrease in pulmonary vascular resistance and only a further small increase with oxygen. S. Cassin has questioned this result, stating that the experiment was not done under rigorously controlled conditions (personal communication).

> Quantitative comparison between changes in fetal pulmonary circulation produced by lung expansion and those produced by oxygen in the unexpanded lungs is difficult for various reasons. Among these are the complex hemodynamic changes that occur after initiation of breathing, the technical difficulties in measuring continuously and accurately all the parameters that take part in these changes, the variation in the degree of lung expansion, and the uncertainty about pulmonary blood PO2 at the time of measurement. (Assali, 1968)

HUMORAL SUBSTANCES

Because of the rapid drop in pulmonary vascular resistance at birth, complete dilatation of the smallest

vessels by the third postnatal day and the lack of nervous control over these changes, attention has focused on humoral substances as potential rapid mediators of the drop in pulmonary vascular resistance. Also, exogenous and humoral substances produce profound changes in pulmonary artery pressure and resistance (Lyrene, 1984). It is important to understand how and where these compounds act on the pulmonary circulation in order to 1) delineate normal physiological mechanisms of the transitional circulation and 2) provide a means of control over the transitional circulation in cases of maladaptation. Specifically, the pulmonary effects of bradykinin, serotonin, histamine, dopamine, tolazoline and nitroprusside will be discussed.

Bradykinin

In earlier experiments on the fetal and neonatal lamb, spontaneous pulmonary vasodilation was observed during the course of the experiments (Cassin et al., 1964a; Colebatch et al., 1965; Campbell et al., 1967a, b; Lauer, et al, 1965). Campbell (1968) demonstrated that upon exposure of fetal whole blood to glass, a potent vasodilator is released. He verified that this substance was bradykinin (BK). Other studies demonstrated that BK constricted the umbilical artery *in vitro* (Eltherington et al., 1968), constricted the ductus arteriosus *in vitro*

(Kovalcik, 1963) and dilated the pulmonary bed (Melmon et al., 1968; Rudolph, 1970). Melmon et al., (1968) also showed that the level of BK increased at birth and noted that the kinen generation system of the neonate was functional. A BK precursor, kinogen, occurs in the fetal lamb as early as 61 days of gestation, and increases during fetal maturation (Heymann et al., 1969). In addition, activation of the BK-generating system occurs with increases in PO_2 but not in the presence of mechanical ventilation alone. Upon delivery and continued ventilation, within one hour, a rapid drop in BK concentrations occurs, although pulmonary vasodilation is maintained. Thus, BK may have some role in establishing adequate circulation after birth.

Serotonin

Serotonin, a potent pulmonary vasoconstrictor, is found in high concentration in blood platelets. Because of its powerful vasoconstrictor effect, it was considered as a potential mediator of the hypoxic pulmonary response. However, depletion of serotonin from nerve endings did not diminish the pulmonary hypoxic response. Also, there was not a significant increase in circulating serotonin during hypoxia (Nayar, 1972). Thus, serotonin has been eliminated as the chemical mediator of hypoxia and its role in the neonate remains uncertain (Lyrene, 1984).

Histamine

Histamine, an endogenous amine, exhibits complex effects based upon age, dose, vascular tone, receptor distribution and type. There are two types of histamine receptors: H1 which mediate vasoconstriction in the adult and H2 which mediates vasodilation (Barer et al., 1978; Tucker et al., 1975). However, the vasculature of the newborn dramatically differs from that of the adult. The main pulmonary artery of the puppy constricts to histamine via H1 receptors in an age-dependent fashion (Newman, 1979). Pulmonary vasoconstriction occurs in response to histamine infusion in the normoxic, newborn lamb (Woods, et al. 1976; Lock et al., 1980) and vasodilation occurs in the fetus (Dawes and Mott 1962). However, in hypoxic lambs with increased pulmonary vascular resistance, low dose histamine acts as a dilator via H1 and H2 receptors (Woods et al., 1976; Goetzman and Milstein, 1980; Goetzman and Bennett, 1987). The H1 mediated vasodilation converted to vasoconstriction by the fourth postnatal week in one study (Woods et al., 1976) but not in the study by Goetzman and Milstein (1980).

The opposite actions of histamine mediated through the same receptor is a biologic paradox. This paradox may be the effect of oxygen concentration or may be related to the status of the pulmonary vascular tone, regardless

of the stimulus. Clearly, the vasculature of the lung in the newborn differs from the adult and is highly heterogeneous.

Dopamine

The endogenous catecholamine, dopamine is used as an inotropic agent for a variety of conditions in the newborn, including shock (Perkins and Levin, 1982; Lang et al., 1980), asphyxia (DiSessa et al., 1981) and refractory hypoxemia secondary to persistent fetal circulation (Hegyi and Hiatt, 1980). At low doses, specific dopamine receptors mediate vasodilation (Drummond et al., 1981b; Goetzman and Bennett, 1986). At high doses, dopamine acts on alpha receptors resulting in systemic vasoconstriction. Lesser vasoconstrictor activity to dopamine occurs in the pulmonary circulation (Reid and Thompson, 1975). Studies performed to clarify the differential effects of dopamine on the systemic versus the pulmonary circulation led to the discovery of a previously unrecognized dopaminergic mechanism in the pulmonary bed.

In the lamb, alpha-blockade blocks the rise in systemic arterial pressure due to dopamine, but does not block the increase in pulmonary arterial pressure (Williams and Drummond, 1983). Fenoldopam, a highly selective dopamine agonist, increased pulmonary artery pressure and resistance, but did not cause any change in

systemic pressure. Furthermore, SCH-23390, a specific dopamine blocker, blocks the effects of fenoldopam (Polack and Drummond, 1986). At lower doses of fenoldopam and dopamine in the anesthetized neonatal lamb, there is an increase in pulmonary vascular compliance, but no change in resistance (Goetzman and Bennett, 1987).

Dopamine receptors are present in the pulmonary vascular bed of the neonatal lamb. These receptors appear to mediate both vasoconstriction and vasodilation under different conditions and may act to modulate the vasomotor tone in the neonatal animal.

Tolazoline

Tolazoline is widely used in neonatal intensive care units to vasodilate the pulmonary vasculature under conditions which result in persistent pulmonary hypertension (V. Lorch, personal communication). Tolazoline has a variety of actions: alpha blockade (Chess and Yonkman, 1946), cholinergic effects (Yonkman et al., 1946), direct vasodilator effects (Lock et al., 1979) and histamine-like effects (Goetzman and Milstein, 1979). Despite its prevalent use, tolazoline is neither selective nor a potent pulmonary vasodilator (Drummond and Lock, 1984). In addition, there is a high complication rate associated with tolazoline with side effects such as systemic hypotension, shock and hemorrhage. Furthermore,

there has been no correlation with survival in infants treated with tolazoline (Stevenson et al., 1979). The actions of tolazoline last less than 5 minutes (Lock et al., 1979), despite the fact that serum half life exceeds 1 hour, with disposition entirely dependent on renal clearance (Ward et al., 1982). Despite the above contraindications, tolazoline is still used primarily due to the lack of any other more effective agent (Drummond and Lock, 1984).

Nitroprusside

Nitroprusside is a potent direct pulmonary and systemic vasodilator of unknown mechanism (Drummond and Lock, 1984). In adults nitroprusside inhibits the hypoxic pulmonary vasoconstrictor response (Hill et al., 1979). In the newborn, little experimental data is available. Beverly (1979) reported on the early use of nitroprusside in an infant with severe respiratory distress syndrome with good results. However, other factors such as pH, ventilation and oxygenation were manipulated simultaneously, thus obscuring nitroprusside's role in the course of events. The comparative danger in using this drug because of its potent and nonselective nature limits its clinical usefulness (Roberton, 1979; Silverman, 1979).

Statement of Purpose

This research project was undertaken to examine the responses of third generation pulmonary arteries (500-1500 um diameter and fourth generation pulmonary arteries (< 500 um diameter) to a number of vasoactive compounds in order to determine differences among levels of the pulmonary arterial tree and assess the differences among these vessels with age during adaptation to extrauterine life occurs. A wealth of information exists on larger pulmonary arteries, however, differences in structure and function between large and small arteries make extrapolation of data inappropriate. Due to the lack of data on fourth generation vessels, this study is intended as a survey of vascular responsiveness of large and small pulmonary blood vessels to a number of physiologically active compounds; epinephrine, norepinephrine, serotonin, nitroprusside, tolazoline, histamine and dopamine.

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II. RESPONSES OF SMALL INTRAPULMONARY ARTERIES TO VASOACTIVE COMPOUNDS IN THE FETAL AND NEONATAL LAMB: NOREPINEPHRINE, EPINEPHRINE, SEROTONIN AND POTASSIUM CHLORIDE

ABSTRACT

To characterize the transition of the pulmonary circulation from fetal to newborn life, we compared the responses of third and fourth generation pulmonary arteries to norepinephrine, epinephrine, serotonin and potassium chloride from lambs 7 days preterm and one, 7 and 21 days of age. Preterm vessels were significantly smaller in internal diameter than all other aged vessels. Fourth generation vessel response to KCl increased with age. Third generation vessel response to KCl did not change with age. There were no differences in maximal contractile response to norepinephrine and epinephrine at the ages tested, but third generation response to these compounds was significantly greater than fourth generation vessel response. Third and fourth generation vessels had the same maximal contractile response to serotonin regardless of age or generation. Third generation vessels were significantly reduced in sensitivity at day 1 and 7 to norepinephrine and epinephrine, and at day 1 to serotonin for both third and fourth generation vessels. A similar, nonsignificant trend occurred in third generation vessels to epinephrine at day 1 and 7. Greater than 75%

of fourth generation vessels did not respond to norepinephrine and epinephrine, therefore EC_{50} values were not calculated. The small intrapulmonary arteries of the neonate are undergoing dynamic changes which are dependent on both age and generation.

INTRODUCTION

At birth, the neonatal lung is still undergoing maturation and development: multiplication of intra-alveolar vessels, remodeling of vessels under 250 um, and extension of smooth muscle into the alveolar region occur (Hislop and Reid, 1973; Levin, 1976; Rendas 1978; Meyrick and Reid, 1982; Inselman and Mellins 1981; Reid, 1977). Physiologically, the lungs adapt to extrauterine life with a progressive decrease in pulmonary vascular resistance and increase in blood flow to perform the vital function of gas exchange. However, despite considerable study, the mechanisms involved in the neonatal transitional circulation remain obscure.

A wealth of information exists regarding the contractility and reactivity of large diameter vessels (>1 mm) in the neonatal lung (Su et al., 1977; Newman et al., 1979; Green and Leffler, 1984). However, arteries with a diameter greater than 500 um are unimportant in controlling resistance (Andersson et al., 1985). Furthermore, because of tremendous regional variation in

both structure and function throughout the vasculature, extrapolation of data from larger vessels to those less than 500 um is inappropriate (Bevan and Bevan, 1984; Su et al., 1977; Somlyo 1965; Muller-Schweinitzer and Weidmann 1977; Buckner, 1982; Holl et al., 1980, Kong and Stephens, 1984). An assessment of blood vessel response at levels potentially important in controlling pulmonary vascular resistance and flow may further clarify the mechanisms of the transitional circulation.

In this study, we examined the reactivity and contractility of vessels less than 400 um internal diameter and vessels of 1000-1500 um internal diameter to the vasoactive compounds norepinephrine (NE), epinephrine (EPI), serotonin (5HT), and potassium chloride (KCl) in the lamb over a period of adaptation to extrauterine life.

MATERIALS AND METHODS

Fetal and neonatal purebred lambs were used. Preterm lambs of approximately 136-140 days of gestation were delivered by C-section and a saline-filled bag placed over the nose to prevent breathing. Lambs of 1, 7 and 21 days of age were delivered by natural birth without intervention. For all ages, a euthanizing dose (45 mg/kg) of sodium pentobarbital (Elkins-Sinn, Inc.) was injected intracardiac. In preterm animals, the cord was then clamped and cut. The lungs and heart were rapidly removed

en bloc via a median sternotomy. The pulmonary vascular bed was injected with cold (4°C), oxygenated (95% O_2-5%CO_2) Krebs bicarbonate solution (composition in mM: NaCl 118.3, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 11.1) through an incision in the right ventricle. The main pulmonary artery was dissected free and cut longitudinally down the right and left branches to the base of both lungs and down third generation arteries. Blocks of lung tissue containing third and fourth generation arteries were excised, placed in cold Krebs, and segments dissected free and cleaned of surrounding parenchyma. Vessel segments threaded onto two 40 um stainless steel wires (California Fine Wire Co.) and secured to mounting supports. The mounting supports were attached on side to a displacement micrometer (Starret) and on the other side to a force transducer (Kistler-Morse DSC-6BE4-110) to record isometric tension. Responses were amplified (Beckman RB Dynograph) and recorded on a 2-channel rectilinear chart recorder (Kipp and Zonen Model BD 41).

The vessels equilibrated for 90 minutes while temperature was brought up to 37°C. At the end of the equilibration period, a length-tension curve was performed by stretching in increments and stimulating the vessels with iso-osmotic 125 mM KCl solution until an optimal contractile response was obtained.

Standard cumulative half-log dose response curves were generated to norepinephrine (Winthrop-Breon) epinephrine (Parke-Davis) and serotonin (Sigma Chemical Co.) by the method of Van Rossum (1963). Fresh stock solutions of all drugs were made and used within 8 hours.

Responses to KCl were expressed as mg/mm2 (vessel circumference x length) and then standardized as a function of the KCl response where:

$$\text{Response (\%)} = \frac{\text{mg/mm2 constrictor}}{\text{mg/mm2 KCl}} \times 100$$

The sensitivity to each compound was assessed by determining the concentration which produced 50% of the maximal response (EC_{50}) extrapolated from a plot of log concentration versus percent of the maximal response. The EC_{50} responses are expressed as a negative logarithm (-log molar EC_{50}). Repeated measures (dose) 2-way analysis of variance and Tukey's HSD test were used to compare differences of main treatment effects.

RESULTS

Internal Diameter. Table 1* shows the internal diameters in micrometers of third and fourth generation vessels as a function of age. Third generation vessels were significantly larger than fourth generation vessels

*All tables and figures may be found at the end of this section.

in internal diameter ($P < .0001$). Preterm vessels were significantly smaller than vessels at all other ages ($P < .005$). In addition, a significant statistical interaction occurred between age and generation ($P < .05$).

Response to KCl. The response of third and fourth generation vessels to KCl are shown as a function of age (Table 2). Day 21 response was significantly greater than preterm vessel response ($P < .005$). Third and fourth generation vessels were similar in their response to KCl. A statistically significant interaction was determined between age and generation ($P < .01$).

Maximum Response to Vasoactive Compounds. Figures 1 through 3 show the responses of third and fourth generation blood vessels expressed as a percentage of the KCl response. Third generation vessels had a significantly greater maximal response to NE ($P < .0001$) and EPI ($P < .0005$) than fourth generation vessels at all ages (Figure 1a,b and 2a,b). No differences were detected between ages. Third generation vessels were not different between ages or generation to 5HT (Figure 3a and b).

Sensitivity to Vasoactive Compounds. The -log molar EC_{50} values to EPI, NE and 5HT are shown in Table 3. Because of lack of responsiveness (>75%) to NE and EPI in fourth generation vessels, EC_{50} values were not

less sensitive to NE at days 1 and 7 compared to preterm and day 21 vessels ($P < .005$). This same trend, although not significant, also occurred at day 1 and 7 to to EPI. Both third and fourth generation vessels were significantly less sensitive to 5HT at day 1 compared to days 7 and 21 ($P < .0001$). In addition, fourth generation vessels were significantly more sensitive to 5HT than third generation vessels ($P < .0001$). Preterm vessel response to 5HT was not performed.

DISCUSSION

The significantly smaller internal diameters of preterm vessels compared to all other ages is consistent with the drop in pulmonary vascular resistance at birth. Growth alone cannot account for these diameter differences. Pulmonary vascular remodeling occurs throughout the the postnatal ages tested, yet day 1, 7 and 21 vessels were not different. Both lung expansion and alveolar oxygenation play a role in decreasing the pulmonary vascular resistance at birth, however, the relative contribution of these factors is an area of some debate (Assali et al., 1968; Cassin et al., 1964; Rudolph et al., 1986; Cassin, personal communication). In this study, fetal and neonatal vessels were removed and placed in Krebs perfused with 95% oxygen. Also, the fetal lung never underwent lung expansion. Thus, while oxygen may be

an important mediator of pulmonary vasodilation at birth, it may not be an initiating or sustaining factor. In addition, oxygen may require an intact pulmonary bed to mediate the vasodilatory process at birth.

A statistically significant interaction was encountered for internal diameter between age and generation. A comparison of third and fourth generation vessel internal diameter shows that these vessels do not change in a parallel fashion with age. The third generation vessel internal diameter progressively increases with age. The fourth generation vessel internal diameter increases at 1 day of age and then progressively decreases at days 7 and 21. This may reflect the dramatic remodeling of the small vessels and extension of vascular smooth muscle (Hislop and Reid, 1973). The significant increase in vessel response to KCl with age also indicates smooth muscle extension and/or maturation, especially of the fourth generation vessels.

Third generation maximal response was similar between ages to NE and EPI and between ages and generation to 5HT. Thus, there are no changes in receptor quantity in these vessels (Van Rossum, 1963; Newman et al., 1979). However, there is a relative paucity of receptors to EPI and NE in fourth generation vessels compared to third generation vessels as indicated by the significantly smaller maximal

response to these compounds. Decreases in adrenergic activity with decreases in vessel diameter have also been reported in the rabbit lung (Su et al., 1978). The significance of this finding was not discussed, but may reflect the relative lack of vasomotor tone in the adult lung (Bevan and Bevan, 1984).

Unlike maximal contractile response, the sensitivity of vessels to EPI and NE decreased at 1 and 7 days in third generation vessels. Also, both third and fourth generation vessels were least sensitive to 5HT at 1 day of age. The decreased sensitivity at these ages may be due to the degree of neuronal and extraneuronal uptake (Buckner, 1982). Furthermore, this decrease in sensitivity may be an important mechanism in the neonate to adjust to the stress of birth. During the birth process, high levels of catecholamines are released into the circulation (Agata et al., 1986; Padbury et al., 1987). The pulmonary vascular bed may exhibit selective desensitization of the third generation vessels and a paucity of adrenergic receptors of the fourth generation vessels. Thus, the dramatic drop in pulmonary vascular resistance necessary at birth is maintained, despite the presence of high circulating vasoconstrictor agents.

A high concentration of oxygen (95%), a potent pulmonary vasodilator, was perfused throughout our studies. Oxygen may selectively exert effects that either

decrease or eliminate the responses to NE and EPI. This possibility and the possibility of increased adrenergic uptake require further study.

Serotonin produced a marked vasoconstrictor response that exceeded the maximal response to KCl in both third and fourth generation vessels. Because of its ability to produce a marked vasoconstrictor response in the absence of effects to EPI and NE of the fourth generation vessels, indicates that the serotonin response was not mediated by alpha receptors. Work using specific serotonergic agonists and antagonists would be helpful in clarifying this response.

In conclusion, the neonatal pulmonary vascular bed is undergoing dynamic changes characterized by a developing vascular smooth muscle effector system of the fourth generation vessels and by changes in agonist affinity of both third and fourth generation vessels. These changes are dependent upon age and generation. This study verifies that third and fourth generation vessels are quite different in their patterns of responsiveness. Thus, responsiveness of large large arteries does not reflect the reactivity of arteries and arterioles important in controlling pulmonary vascular hemodynamics of the fetus and newborn.

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Table 1. Internal Diameters (Mean ± S.E.M.) of Third and Fourth Generation Blood Vessels

GENERATION	AGE			
	Preterm(a) (n=5)	Day 1 (n=12)	Day 7 (n=11)	Day 21 (n=11)
Third(b)	530 ± 40	8801± 70	965 ± 110	1000 ± 60
Fourth	175 ± 15	330 ± 40	275 ± 25	245 ± 20

Units: micrometers

(a) Preterm vessels significantly smaller than all other ages ($P<.01$)

(b) Third generation vessels significantly larger than fourth generation vessels at all ages ($P<.001$)

Table 2. Response (Mean ± S.E.M.) of Third and Fourth Generation Vessels to 125 mM KCl

	AGE			
GENERATION	Preterm(a) (n=5)	Day 1 (n=12)	Day 7 (n=11)	Day 21 (n=11)
Third	136 ± 15	189 ± 32	103 ± 12	158 ± 18
Fourth	94 ± 14	165 ± 22	183 ± 16	259 ± 31

Units: mg/mm^2

(a) Preterm vessels significantly different from 21 day vessels, fourth generation ($P<.005$)

Table 3. -Log Molar EC_{50} Values (Mean ± S.E.M.) for Third and Fourth Generation Vessels to Norepinephrine (NE), Epinephrine (EPI) and Serotonin (5HT)

		AGE			
DRUG	GENERATION	Preterm (n=4)	Day 1 (n=8)	Day 7 (n=6)	Day 21 (n=6)
NE	Third	6.48 ± .10	5.89 ± .12(a)	5.90 ± .21(a)	6.50 ± .03
	Fourth	---	---	---	---
EPI	Third	6.20 ± .03	5.77 ± .11	5.64 ± .24	6.20 ± .25
	Fourth	---	---	---	---
5HT	Third	---	5.04 ± .17(b)	5.77 ± .13	5.82 ± .24
	Fourth(c)	---	5.61 ± .10	6.36 ± .12	6.50 ± .15

Units: Molar

(a) Day 1 and 7 vessels significantly less sensitive to norepinephrine than preterm and day 21 vessels ($P < .005$)

(b) Day 1 vessels significantly less sensitive to serotonin than day 7 and 21 aged vessels ($P < .0001$)

(c) Fourth generation vessels significantly mores sensitive to serotonin than third generation vessels at all ages ($P < .0001$)

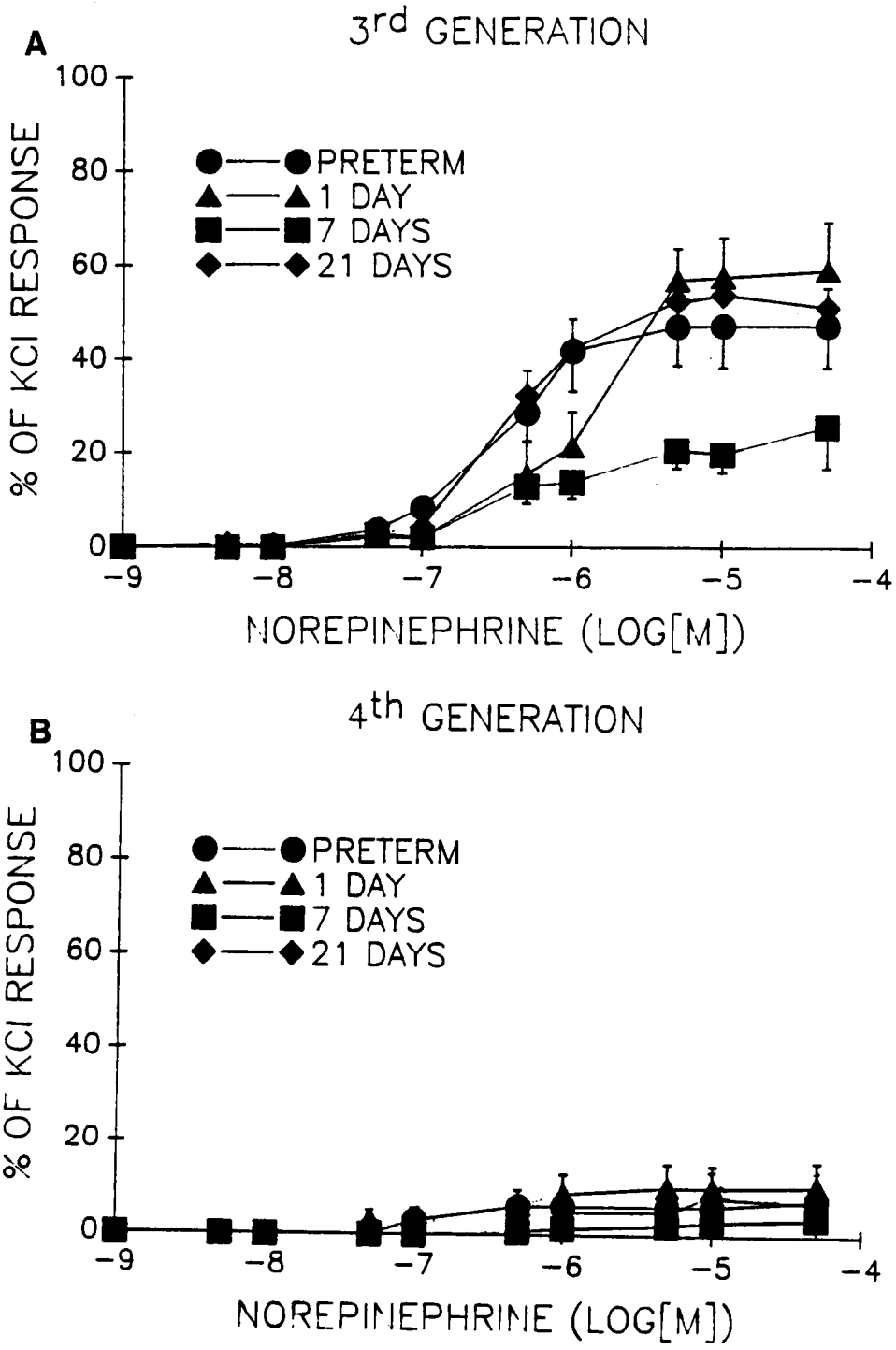

Figure 1. Response of Third and Fourth Generation Vessels to Norepinephrine at Preterm and Days 1, 7 and 21 as a Percentage of the KCl Response.

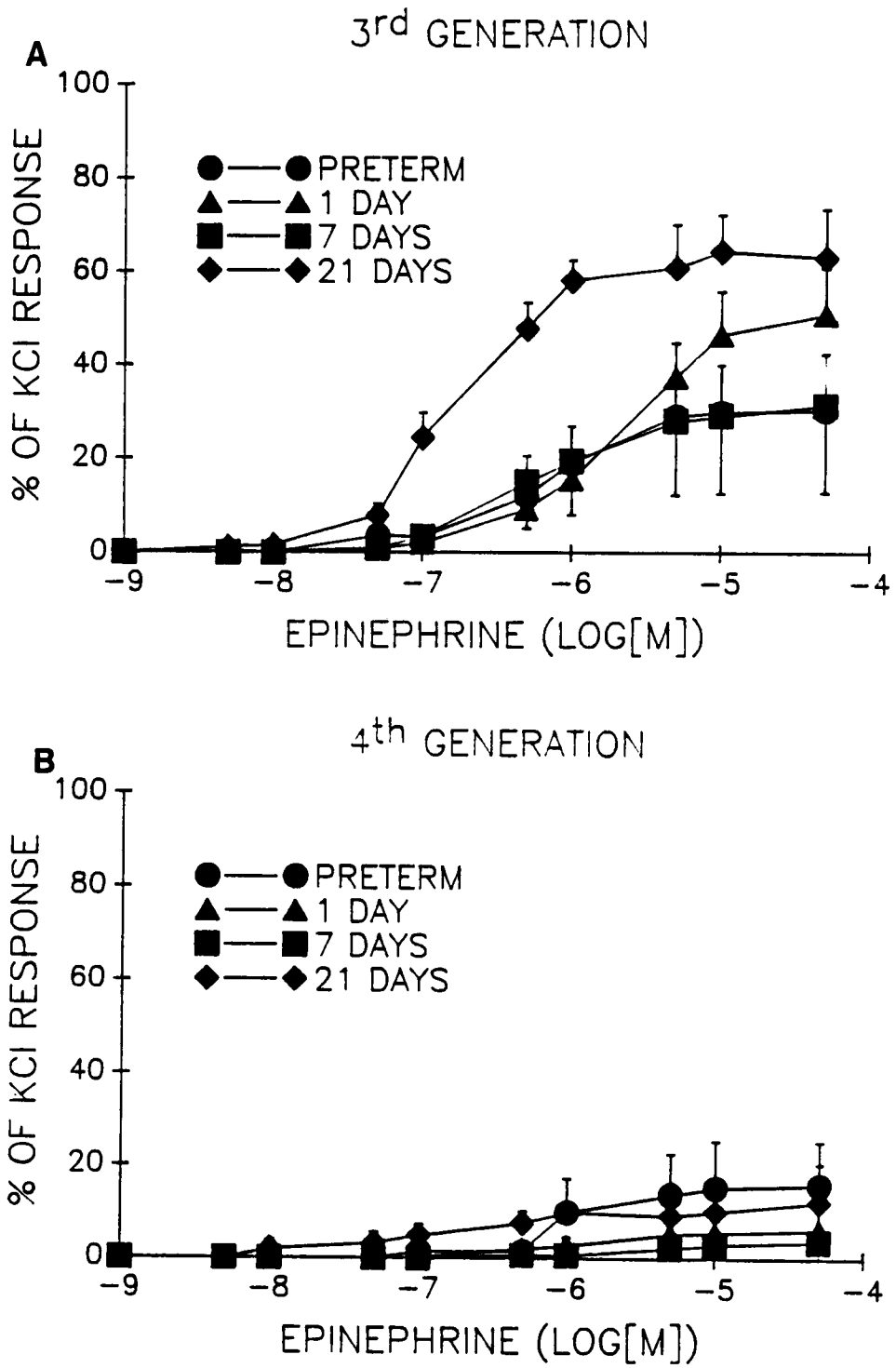

Figure 2. Response of Third and Fourth Generation Vessels to Epinephrine at Preterm and Days 1, 7 and 21 as a Percentage of the KCl Response.

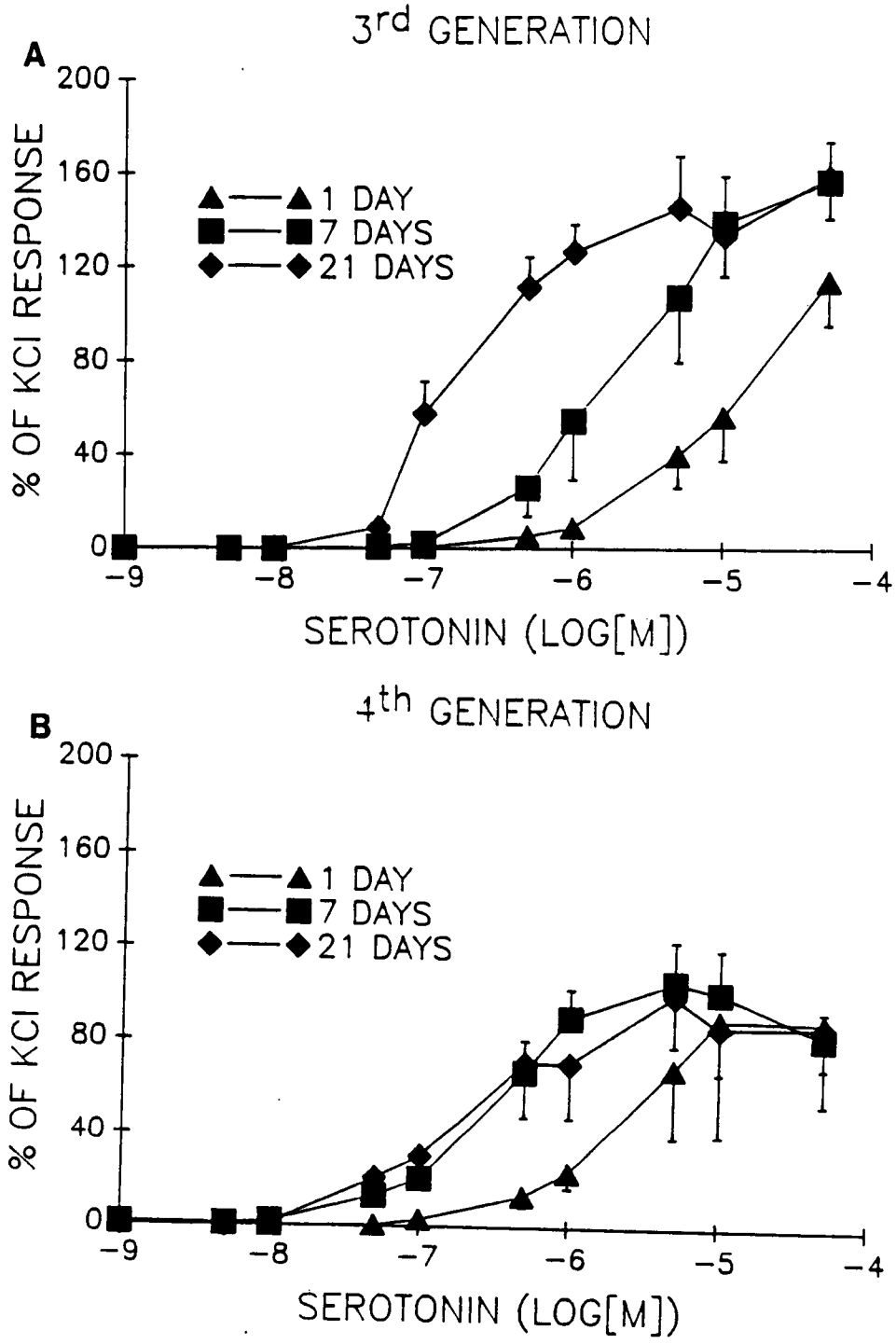

Figure 3. Response of Third and Fourth Generation Vessels to Serotonin at Days 1, 7, and 21 as a Percentage of the KCl Response.

III. RESPONSE OF SMALL INTRAPULMONARY ARTERIES TO VASOACTIVE COMPOUNDS IN THE NEONATAL LAMB: NITROPRUSSIDE, TOLAZOLINE, HISTAMINE AND DOPAMINE

ABSTRACT

To assess the effects of vasodilators during the transitional circulatory process, we compared the responses of third and fourth generation pulmonary arteries to nitroprusside, tolazoline, histamine and dopamine from lambs 1, 7 and 21 days of age. Nitroprusside was the most potent vasodilator. The maximal vasodilatory response was independent of age or generation. However, vessels were less sensitive to nitroprusside at 21 days of age ($P < .05$). Tolazoline was only a weak pulmonary vasodilator in vessels at all ages. Fourth generation vessels were significantly more sensitive to tolazoline than third generation vessels. However, only 50% (7/14) of fourth generation vessels responded to tolazoline and 70% (10/14) of third generation vessels responded. No differences were detected between age or generation to histamine, which produced a maximum vasodilatory response of 30-50%. In general, dopamine produced vasodilation at doses less than $5x10^{-6}$ M and vasoconstriction at higher doses. Day 1 vessels demonstrated a significantly greater vasodilation at doses of 10^{-7} M to $5x10^{-6}$ M compared to day 7 vessels

($P < .05$). The small intrapulmonary arteries of the neonate undergo dynamic alterations in function which are dependent upon age and generation.

INTRODUCTION

The factors that effect the pulmonary circulation during adaptation to extrauterine life are not entirely understood. Studies on pulmonary vascular effects of vasodilators have been done in the intact neonatal animal (Lock et al., 1980; Goetzman and Milstein, 1980; Drummond et al., 1981) and on isolated large pulmonary arterial strips (Green and Leffler, 1984; Su et al., 1977; Su et al., 1978). In the intact animal, the mechanism of the vasoactive response can be obscured by such variables as changes in left atrial pressure, blood volume, intrapleural and intratracheal pressures (Rudolph et al., 1959; Lock et al., 1980). Differences in the anatomy (Hislop and Reid, 1973; Levin et al. 1976) and function (Su et al., 1978; Buckner et al., 1982; Holl et al., 1980; Kong and Stephens, 1984) of large arteries versus small intrapulmonary arteries and arterioles, indicates extrapolation of findings from one level of the vascular tree to another is invalid.

In a previous study, we documented differences between third and fourth generation pulmonary arteries to the vasoconstrictors epinephrine and norepinephrine (Dunn

et al., 1986). Third and fourth generation vessels may also respond differently to vasodilators. The purpose of the present study is to evaluate third and fourth generation vessel responses to nitroprusside, tolazoline, histamine and dopamine as the neonate adapts to extrauterine life.

MATERIALS AND METHODS

Purebred neonatal lambs were delivered by natural birth without intervention. A bolus dose (45 mg/kg) of sodium pentobarbital (Elkins-Sinn, Inc.), was injected intracardiac and the lungs and heart were rapidly removed en bloc via a median sternotomy. The pulmonary vascular bed was injected with cold, oxygenated (95% O_2- 5% CO_2) Krebs bicarbonate solution (composition in mM: NaCl 118.3, KCl 4.7, KH_2PO4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 11.1) through an incision in the right ventricle. The main pulmonary artery was dissected free and cut longitudinally down the right and left branches to the base of both lungs and down third generation arteries. Blocks of lung tissue containing third and fourth generation arteries were excised, placed in cold Krebs, and segments dissected free and cleaned of surrounding parenchyma. Vessel segments were threaded onto two 40 um stainless steel wires (California Fine Wire Co.) and secured to mounting supports. A displacement device

(Starret) was attached to one mounting support and a Kistler-Morse transducer (DSC-6BE4-110) to the other support for recording of isometric tension. Responses were amplified (Beckman RB Dynograph) and recorded on a 2-channel rectilinear chart recorder (Kipp & Zonen Model BD 41).

The vessels equilibrated for 90 minutes as temperature was brought up to 37°C. At the end of the equilibration period, a length-tension curve was performed by stretching in increments and stimulating the vessels with iso-osmotic 125 mM KCl solution until an optimal contractile response was obtained.

Standard cumulative dose-response curves were generated in vessels preconstricted with serotonin to sodium nitroprusside (Sigma), tolazoline (Sigma), histamine phosphate (Lilly) and dopamine (Sigma). Fresh stock solutions of all drugs were made and used within 8 hours. Serotonin was used as a preconstricting agent because of its ability to produce and maintain a contraction in both third and fourth generation vessels and because of its excellent reproducible response.

Responses were expressed as a percentage of the serotonin preconstricting dose as follows:

$$\% \text{ Response} = \frac{\text{5HT response + drug response}}{\text{5HT response}} \times 100$$

Values greater than 100% were vasoconstrictor responses and those less than 100% were vasodilatory responses.

The concentration which produced 50% of the maximal response (EC_{50}) was extrapolated from a plot of log concentration vs. percent of the maximal response obtained to the drug tested. The EC_{50} responses are expressed as a negative logarithm (-log molar EC_{50}). Repeated measures (dose) 2-way analysis of variance and Tukey's HSD test were used to compare differences of main treatment effects.

RESULTS

Nitroprusside. Maximum vasodilatory response to nitroprusside was similar between age and generation (Figure 4a and b*). Day 1 vessels were significantly more sensitive to nitroprusside than vessels at day 21 ($P<.05$).

Tolazoline. The maximum vasodilator response to tolazoline did not differ between age or generation (Figure 5a and b). Fourth generation vessels were significantly more sensitive to tolazoline than third generation vessels ($P < .05$, Table 4). However, only 50% (7/14) of fourth generation vessels responded to tolazoline and 70% (10/14) third generation vessels responded to tolazoline. No differences in sensitivity to tolazoline were detected between ages.

*All tables and figures may be found at the end of this section.

Histamine. Histamine was a vasodilator at the doses tested (10^{-9} to 10^{-5}) and caused a 30 to 50% vasodilation (Figure 6a and b). No differences in maximal vasodilatory response or sensitivity were detected between age or generation.

Dopamine. Responses to dopamine were biphasic. The EC_{50} value was not calculated to dopamine because of the complex response pattern. Also, the constrictor response did not reach a plateau, hence there was uncertainty as to whether a maximal response was achieved. There were no differences to dopamine between generations. However, at doses between 10^{-7} M and $5x10^{-6}$ M 1 day old vessels exhibited a significant vasodilation response compared to 7 day old vessels ($P < .01$).

DISCUSSION

Sodium nitroprusside has potent vasodilator effects on the pulmonary and systemic vascular beds. The vasodilator response to nitroprusside is not mediated through receptors and its mechanism of action remains unknown (Drummond and Lock, 1984). The differences in sensitivity between 1 and 21 day vessels may be due to well-documented anatomical changes (Hislop and Reid, 1973; Levin, 1976) with concomitant functional changes as a result. Despite the consistent and potent vasodilatory effect on our vessels, nitroprusside is neither

efficacious or safe (Roberton, 1979; Silverman, 1979) due to its nonselective nature.

Tolazoline is commonly used in neonatal intensive care units to vasodilate the pulmonary vasculature. Tolazoline vasodilates through several modes of actions: alpha blockade (Chess & Yonkman, 1946), cholinergic effects (Yonkman et al., 1946), direct vasodilatory effects on the smooth muscle and histamine-like effects (Goetzman and Milstein, 1979). In our study, tolazoline was only a mild vasodilator. No differences in the maximum vasodilatory response to tolazoline were detected between age or generation. Furthermore, fourth generation vessels, though more sensitive, responded to tolazoline only 50% of the time (7/14) and third generation vessels only 70% of the time (10/14). The vasodilator response to tolazoline of the fourth generation vessels is probably not alpha mediated since fourth generation vessels exhibit little or no alpha activity when challenged with epinephrine and norepinephrine (Dunn et al., 1986). Despite its weak vasodilator properties, no other compound currently exists that efficiently lowers pulmonary vascular resistance in cases of persistent fetal circulation and persistent pulmonary hypertension (Drummond and Lock, 1984).

The effects of histamine on the pulmonary vascular bed are variable. H1 receptors mediate vasoconstriction of the main pulmonary artery in the neonatal dog (Newman et al., 1979). H1 and H2 receptors mediate vasodilation in the hypoxic newborn lamb (Woods et al., 1976; Goetzman and Milstein, 1980). The H1 mediated vasodilator response converts to a vasoconstrictor response three weeks postnatally (Woods et al., 1976). In our study, histamine produced vasodilation at all ages in third and fourth generation pulmonary vessels. Observations indicate that the differences in response to histamine may be related to the status of the pulmonary vascular tone. However, in our initial studies in the absence of the preconstrictor, serotonin, vasoconstriction did not occur. Histamine may cause contraction of the main pulmonary artery and vasodilation of the smaller arteries by a similar mechanism. Contraction of the main pulmonary artery would decrease compliance while dilation of the smaller arteries would cause a decrease in resistance. Further studies are required to clarify the histamine response and the receptors that mediate the response in third and fourth generation vessels.

In our study, dopamine exerted a biphasic response - minimal vasodilation at low doses and vasoconstriction at higher doses. The vasodilator response was most prominent in the fourth generation vessels at 1 day of age.

Conversely, vasoconstriction was most noticeable in third generation vessels. The vasoconstriction of the fourth generation arteries is not likely alpha-mediated, but may be due to dopamine receptors (Williams and Drummond, 1983; Dunn et al., 1986; Polack and Drummond, 1986). Dopamine is used as a cardiotonic agent in neonates. Its role as a modulator of pulmonary vasomotor tone in the neonate is unknown, thus should be used with care in infants also experiencing difficulties with gas exchange.

The neonatal pulmonary vasculature is undergoing dynamic changes in function upon adaptation to extrauterine life. These changes occur both between age and different levels of the vasculature. Further, these studies were performed on preconstricted vessels perfused with 95% O_2. The pre-existing vasomotor tone plays a role in determining vasodilatory responses (Rudolph et al., 1959; Woods et al., 1976). Oxygen may also alter responses of the pulmonary vasculature in yet an undefined manner.

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Table 4. -Log Molar EC_{50} Values (Mean ± S.E.M.) for Third and Fourth Generation Vessels to Nitroprusside (NP), Tolazoline (TOL) and Histamine (HIS)

		AGE		
		Day 1	Day 7	Day 21
DRUG	GENERATION			
NP (n=5)	Third	6.73 ± .22	6.37 ± .38	5.39 ± .21
	Fourth	6.97 ± .49	6.13 ± .36	6.65 ± .14
TOL (n=5)	Third	6.17 ± .53	5.21 ± .39	5.87 ± .47
	Fourth	6.18 ± .56	7.82 ± .27	6.41 ± .26
HIS (n=5)	Third	6.27 ± .15	6.53 ± .18	6.82 ± .26
	Fourth	6.34 ± .24	6.81 ± .16	6.83 ± .15

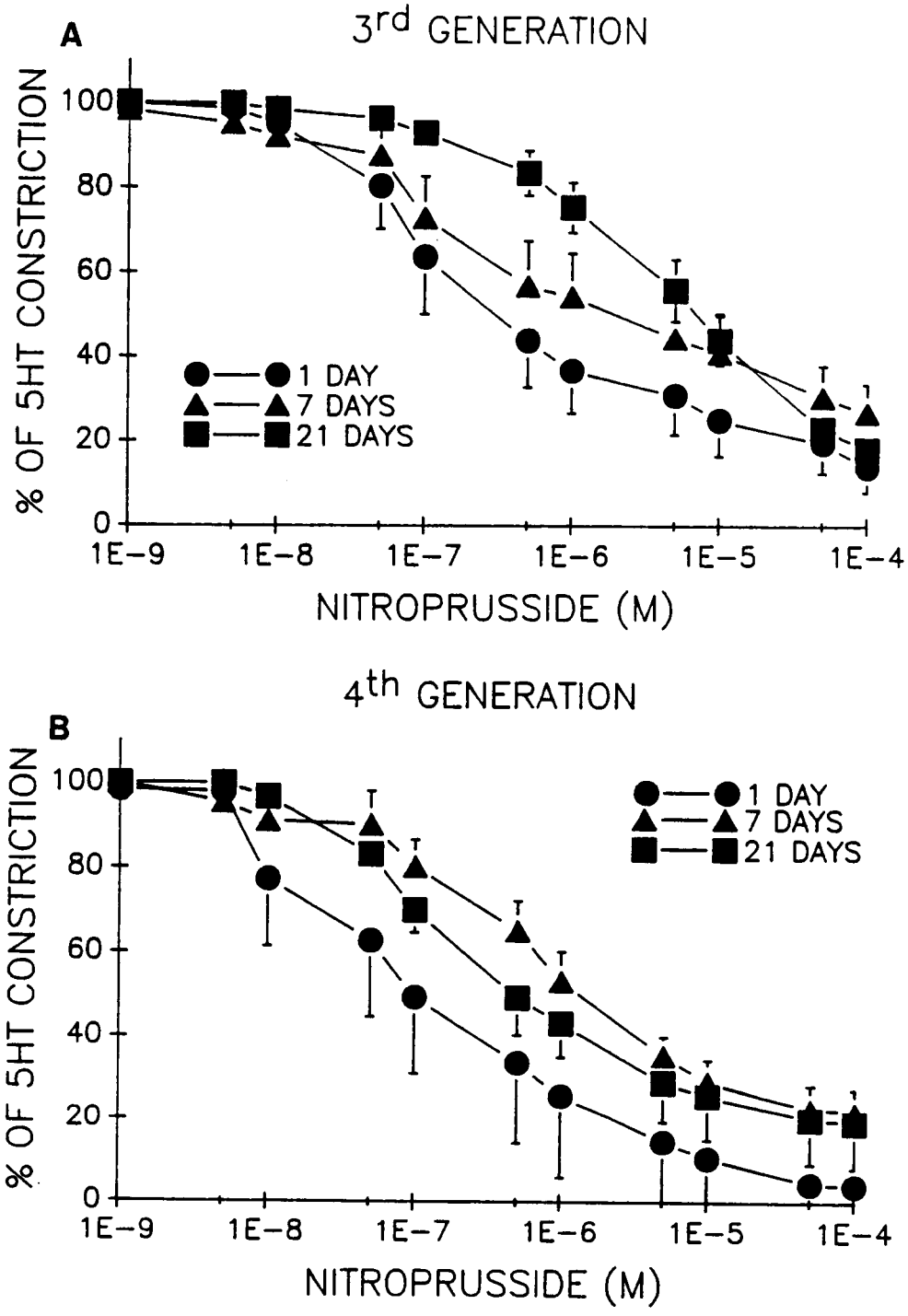

Figure 4. Response of Third and Fourth Generation Vessels to Nitroprusside at Days 1, 7 and 21 as a Percentage of the Serotonin (5HT) Constriction.

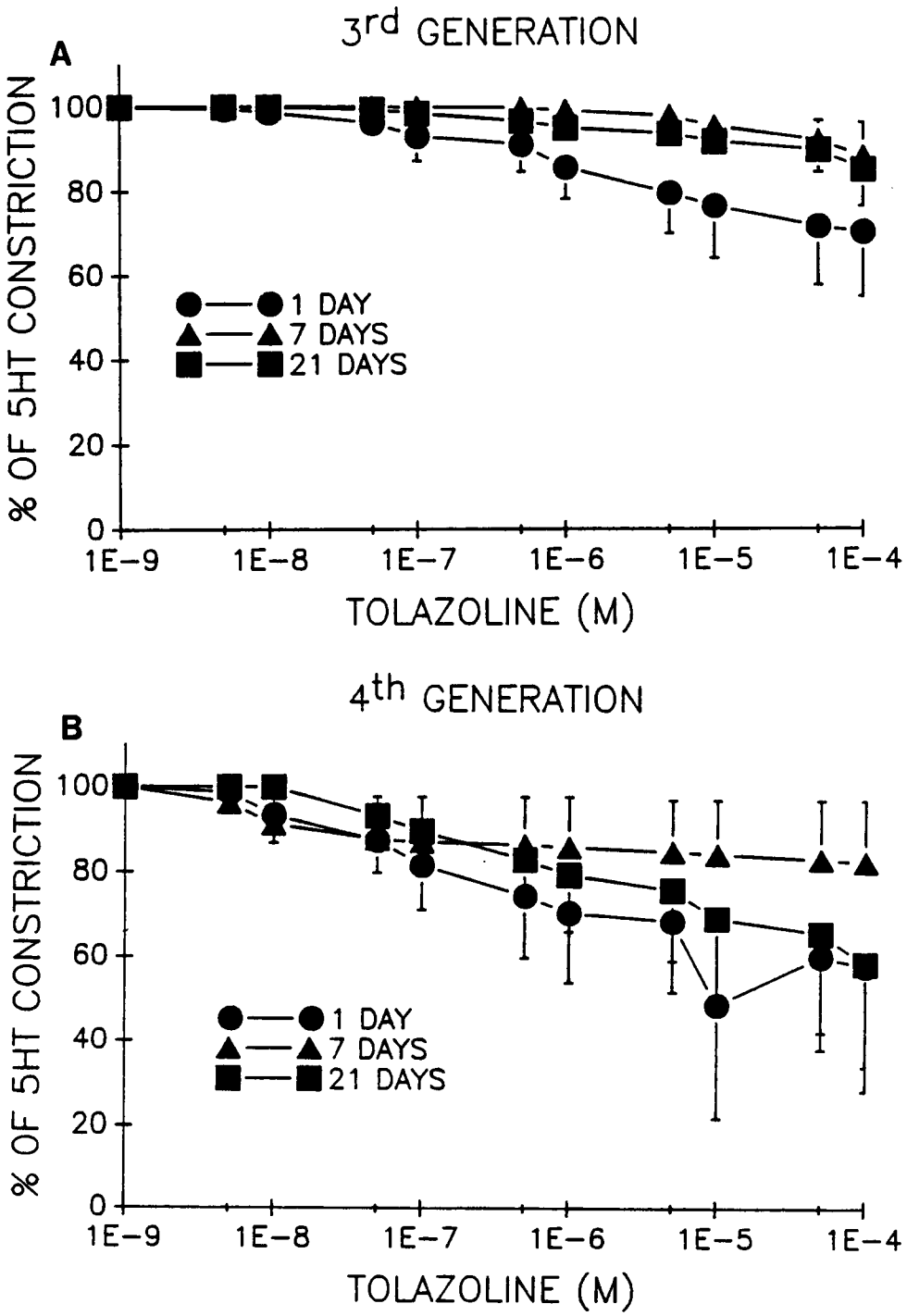

Figure 5. Response of Third and Fourth Generation Vessels to Tolazoline at Days 1, 7 and 21 as a Percentage of the Serotonin (5HT) Constriction.

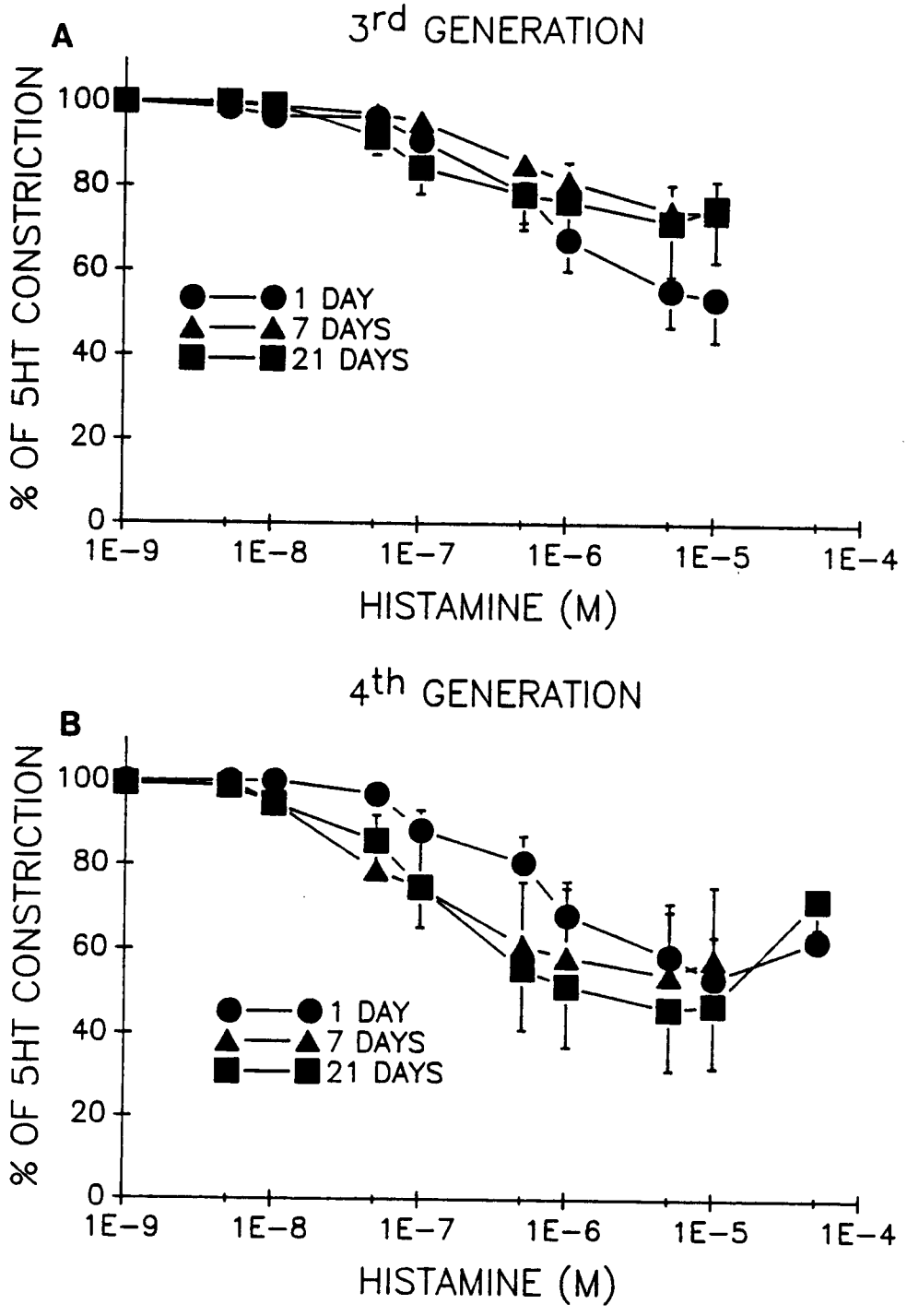

Figure 6. Response of Third and Fourth Generation Vessels to Histamine at Days 1, 7 and 21 as a Percentage of the Serotonin (5HT) Constriction.

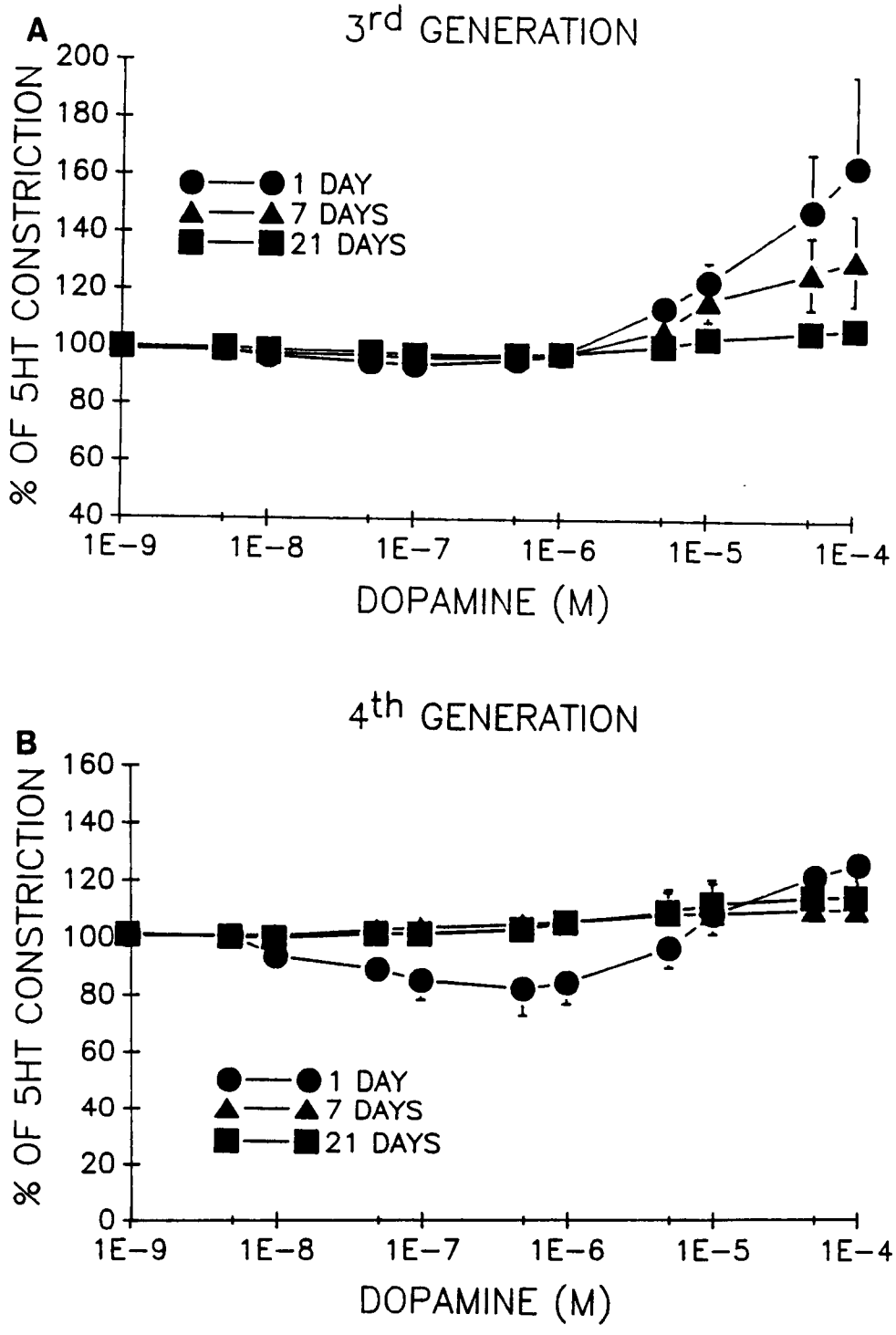

Figure 7. Response of Third and Fourth Generation Vessels to Dopamine at Days 1, 7 and 21 as a Percentage of the Serotonin (5HT) Constriction.

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