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THE VASORELAXANT EFFECTS OF AMRINONE ON NEONATAL RABBIT THORACIC AORTIC STRIPS

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INDEXING WORDS

amrinone ; aorta ; neonatal ; rabbit ; cAMP

SYNOPSIS

The present study was designed to compare the *in vitro* vasorelaxant responses to the bipyridine compound amrinone of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) precontracted aortic strips isolated from newborn (2 to 3 days old) and nonpregnant adult rabbits. Changes in forces were recorded isometrically. The effects of isoproterenol (β-adrenergic receptor stimulator), forskolin (direct activator of adenylate cyclase) and theophylline (non-specific phosphodiesterase inhibitor) were also studied simultaneously. The vasodilator responses to amrinone, forskolin and theophylline in aortic strips from neonates were comparable to those of strips from adults. In contrast, the maximum relaxations to isoproterenol were significantly reduced in those from neonates as compared with those from adults, suggesting that β-adrenergic receptor

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mediated responses are not fully developed in the neonate. The mean value for the ED_{50} concentration (half the maximum response) for amrinone, isoproterenol and theophylline was $1.10 \pm 0.10 \times 10^{-4}$ M, $3.47 \pm 1.31 \times 10^{-8}$ M and $1.53 \pm 0.10 \times 10^{-4}$ M, respectively, in the neonatal group and $1.17 \pm 0.19 \times 10^{-4}$ M, $3.75 \pm 1.11 \times 10^{-8}$ M and $1.75 \pm 0.06 \times 10^{-4}$ M, respectively, in the adult group; there were no significant differences between the neonatal and adult preparations. We concluded that the vasorelaxation produced by phosphodiesterase inhibitors demonstrated no maturation-related changes probably due to a stable cyclic AMP turnover through an unaltered phosphodiesterase activity in newborn and adult arteries.

INTRODUCTION

Amrinone is a selective inhibitor of cyclic GMP inhibited cyclic nucleotide phosphodiesterase(PDE) with positive inotropic and vasodilator properties. It is widely used for the treatment of heart failure in the adult. [1,16] Amrinone is increasingly used in critically ill infants and children. [3,15] The vasodilator effect of amrinone is apparently mediated through selective inhibition of peak III PDE isozyme and is thereby associated with subsequent increase in cyclic AMP (cAMP) concentration. [7,17,33,10] Vascular relaxations in response to amrinone have been documented by several investigators [18,31,9] using a variety of adult animals *in vitro* .

A previous study in rat aortic strips revealed a high correlation between the relaxant potencies of a number of cardiotonic vasodilators including amrinone with the inhibition by these agents of an isozyme of cyclic nucleotide PDE located in the sarcoplasmic reticulum (SR) of cardiac muscle (SR-PDE). The observation suggested that the vasorelaxation produced by these agents is

related to their capacity to inhibit vascular enzyme similar to SR-PDE.[12]

The majority of laboratory investigations have involved mature animals. However, recently a number of studies have been reported regarding the cardiac effects of amrinone on newborn mammals. These studies have led to the conclusion that the effects of amrinone on cardiac contractility change with growth and development.[4,14] Functional, biochemical and morphological changes also occur in the vascular wall in most of the arteries during the early postnatal period.[34] Therefore, the present experiments were conducted to compare the *in vitro* effect of amrinone in neonatal and adult rabbit aortic strips. In addition to those of amrinone, the vascular effects of isoproterenol, a β-adrenergic receptor agonist, forskolin, a direct activator of adenylate cyclase and theophylline, a nonspecific PDE inhibitor, were also examined.

METHODS

Adult (2 to 3 months old) and newborn (2 to 3 days old) Japanese White rabbits of either sex were used in our experiments. All animals were killed by bleeding from the carotid arteries after administration of 30 mg/kg sodium pentobarbital either intraperitoneally into the newborn or intravenously into the adult rabbits. In the case of the neonates up to three strip segments were sampled from each artery. We harvested a total of 72 artery segments from 36 newborn rabbits and 90 artery segments from 30 adult rabbits. When a single protocol was repeated with multiple segments from the same rabbits, we averaged the results into a single value before statistical analysis. All reported values of "n" refer to the number of arterial segments.

Vessel preparation. Thoracic aortae were removed from each animal and immediately placed in Krebs-Ringer solution with the following composition: 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 1.18 mM

KH_2PO_4, 1.19 mM $MgSO_4$ and 11 mM glucose. After being cleaned of adhering fat and connective tissue, the isolated arteries were cut into helical strips approximately 2 mm wide by 25 mm long in the case of the adult and 1.5 mm by 15 mm in the neonatal preparations. The strips were suspended in a 20 ml tissue bath containing Krebs-Ringer solution at 37° C bubbled with 95% O_2 and 5% CO_2. Freshly mounted arteries were stretched slowly and repeatedly until the optimum resting tensions (Table I) remained stable for at least 30 min. This tension was defined as the precontracted tension that allowed maximal isometric contraction to 40 mm KCl as determined in a pilot study.[19] The cross- sectional areas of the strips were estimated from the wet wt/length at the resting tension, a calculation in which a tissue density of 1 was assumed.[27,28]

The vascular strips were allowed to equilibrate for 90 min. before the start of the experiments. During the equilibration period, the bath solution was replaced every 15 min. Maximum contraction was then induced by application of 40 mM KCl, after which the preparations were repeatedly washed and reequilibrated in control media. Dose response curves for $PGF_{2\alpha}$ were made to obtain the concentration of the drug calculated to induce approximately contraction equal to 80% of the maximum contraction. Changes in muscle tension were recorded through force-displacement transducers (NEC SAN-EI TYPE 45196A) which allowed the contraction to be registered on a polygraph (SAN-EI REC TI HORIZ-8K).

Vasorelaxant experiments. The artery segments were contracted with 40 mM of KCl, washed, allowed to stand until rest, and again contracted with $PGF_{2\alpha}$ (1-5 μM) sufficient to induce contraction approximately equal to 80% of maximal contraction as previously determined. Stable contraction could be attained with $PGF_{2\alpha}$ treatment over a sustained period. Tachyphylaxis was not observed. After the establishment of stable contractile tone concentration-

relaxation curves with amrinone were obtained by addition of the drug in a cumulatively increasing fashion. Using an identical protocol, we also used isoproterenol, forskolin and theophylline for relaxation of aortic strips constricted by $PGF_{2\alpha}$. Isoproterenol responses were obtained in the presence of phentolamine (1 μM), an α-receptor blocking agent.[11] This procedure was necessary to prevent the stimulating effects of isoproterenol on α-receptors. Each strip was tested with each agent in a random order. After the dose-response curves were obtained, the strips were washed at least five times. At the end of the experiments, the strips were treated with papaverine (10^{-4} M) to induce maximum relaxation. This relaxation was taken as 100% for calculation of relaxation responses.

Statistics. All of the results are presented as mean values ± standard error of mean (SEM). Statistical comparisons were performed by using Student's *t* test or analysis of variance (ANOVA) between paired or unpaired data for strips from rabbits at a given age. One-way ANOVA was used to compare intergroup differences. The ED_{50} was calculated for individual curves, and the mean of these values was reported as the molar (M) concentration. The differences between values were considered statistically significant when the *p* value was less than 0.05.

Drugs. The following drugs were used: amrinone (Yamanouchi, Tokyo, Japan), isoproterenol and theophylline (Nacalai, Kyoto, Japan), forskolin and papaverine HCl (Sigma Chemical CO., St. Louis, MO), $PGF_{2\alpha}$ (Upjohn, Tokyo, Japan) and phentolamine mesylate (Ciba-Geigy, CO., Summit, NJ). All drug concentrations are expressed as the final M- concentrations in the organ bath solution.

Table I. *Mean values (± SEM) of cross-sectional areas and contractions induced by 40 mM KCl per cross-sectional area of thoracic aortic strips isolated from neonatal and adult rabbits.*

Age	n*	Body wt. (g)	Cross-sectional area (mm^2)	RT † (g)	Contraction/area ‡ (mg/mm^2)
Neonate	11	87.72 ± 4.71	0.565 ± 0.045	1.0	309.1 ± 60.6
Adult	15	2526 ± 186§	1.406 ± 0.166§	2.0	1410.3 ± 205.3§

* Number of strips.

† Resting tension.

‡ KCl (40 mM) - induced contraction/cross-sectional area.

§ Significantly different from neonatal values, $p < 0.05$.

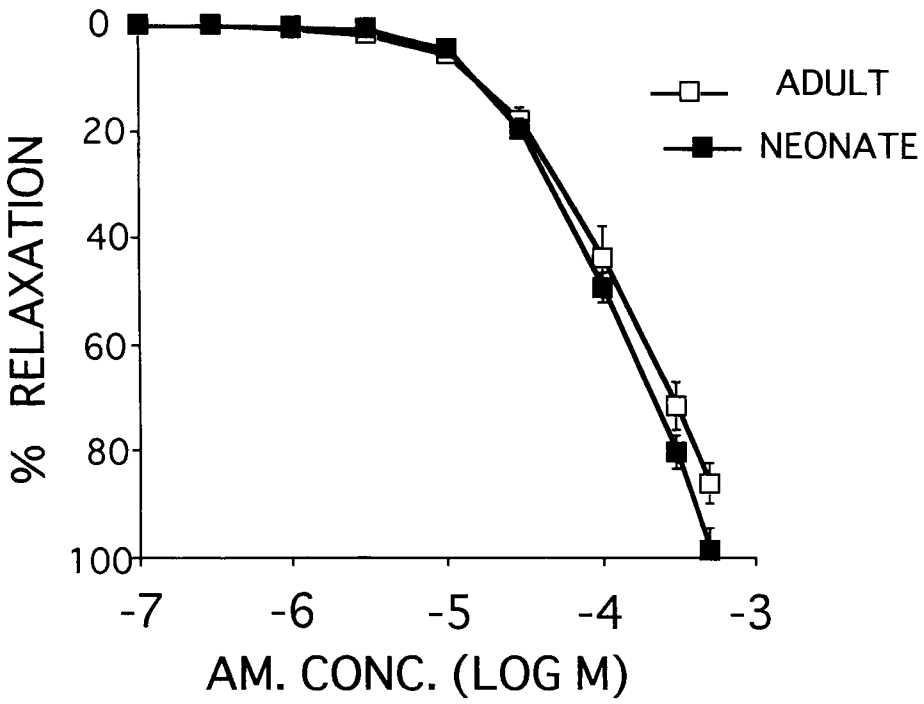

Figure 1. Cumulative concentration-response curves to amrinone (AM) against $PGF_{2\alpha}$ contracted thoracic aortic strips isolated from neonate (n=15) and adult (n=14) rabbits. Papaverine 10^{-4} M induced relaxation is taken as 100%. Values are expressed as means ± SEM.

RESULTS

Effects of amrinone on neonatal and adult rabbit aortic strips. In helical strips isolated from both the neonatal and adult rabbits, the addition of amrinone (3×10^{-6} - 5×10^{-4} M) caused a persistent reproducible relaxation in concentration-dependent manner in $PGF_{2\alpha}$ contracted rabbit thoracic aortic strips. The maximum response obtained in the strips from neonates and from adults was, 98.56 ± 4.04% and 86.08 ± 3.94%, respectively, of the response to 10^{-4} M papaverine induced relaxations (Figure 1). No significant difference was observed between the two groups for the values for maximum relaxations. The mean amrinone ED_{50} concentrations in the aortic strips of newborn and adult rabbits was 1.10 ± 0.10 $\times 10^{-4}$ M and 1.17 ± 0.19 $\times 10^{-4}$ M, respectively (Table II). There were no significant difference between the ED_{50} values for amrinone in the neonatal and adult preparations.

Relaxant effects of isoproterenol , forskolin and theophylline. In the presence of phentolamine (1 μM), isoproterenol produced concentration-dependent relaxation (10^{-9} - 10^{-6} M) in $PGF_{2\alpha}$ contracted vessels from both newborn and adult rabbits. The mean maximum relaxation was 19.97 ± 2.64% and 58.68 ± 4.07% in the respective groups. There was significantly reduced ($p<0.001$) maximum relaxation induced by isoproterenol in the neonatal aortic strips (Figure 2). In all preparations isoproterenol at a concentration of 3 μmol/l and more produced sustained contraction. The mean ED_{50} concentration for isoproterenol was 3.47 ± 1.31 $\times 10^{-8}$ M and 3.75 ± 1.11 $\times 10^{-8}$ M, respectively, in the neonatal and adult preparations, with no significant difference between the two groups, thereby demonstrating that the sensitivity to the drug was equivalent in the arteries isolated from rabbits in the two age groups (Table II).

Forskolin (3×10^{-8} - 10^{-5} M) produced dose-dependent relaxations in all of the vascular preparations, and no significant differences were observed

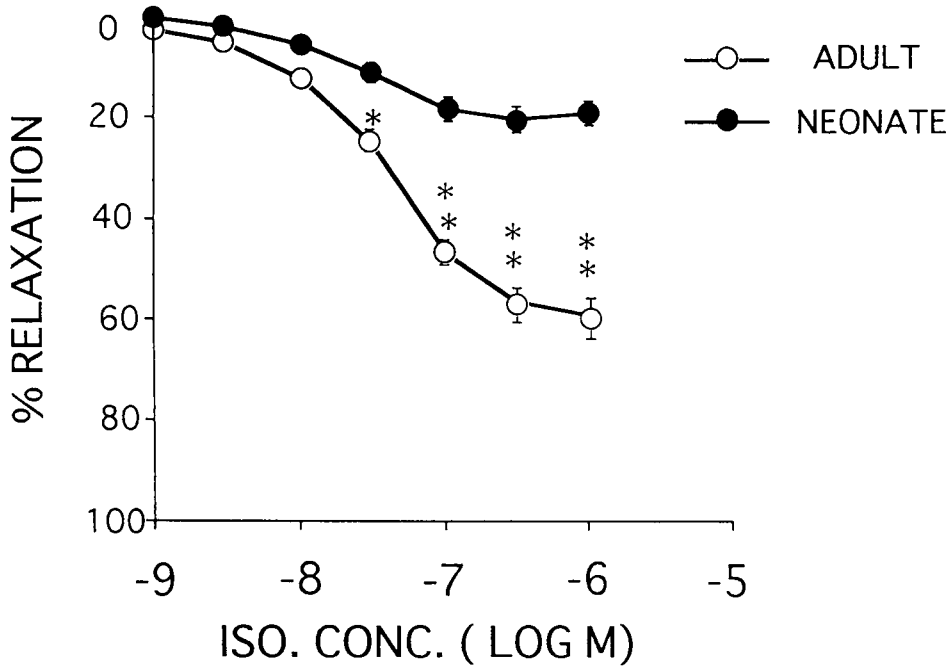

Figure 2. Cumulative concentration-response curves to isoproterenol (ISO) against $PGF_{2\alpha}$ contracted thoracic aortic strips isolated from neonate (n=10) and adult (n=8) rabbits. Phentolamine (4×10^{-6} M) was present to prevent the α-receptor stimulating effects of isoproterenol. Papaverine 10^{-4} M induced relaxation is taken as 100%. $^{*}P < 0.01$ and $^{**}P < 0.001$. Values are expressed as means ± SEM.

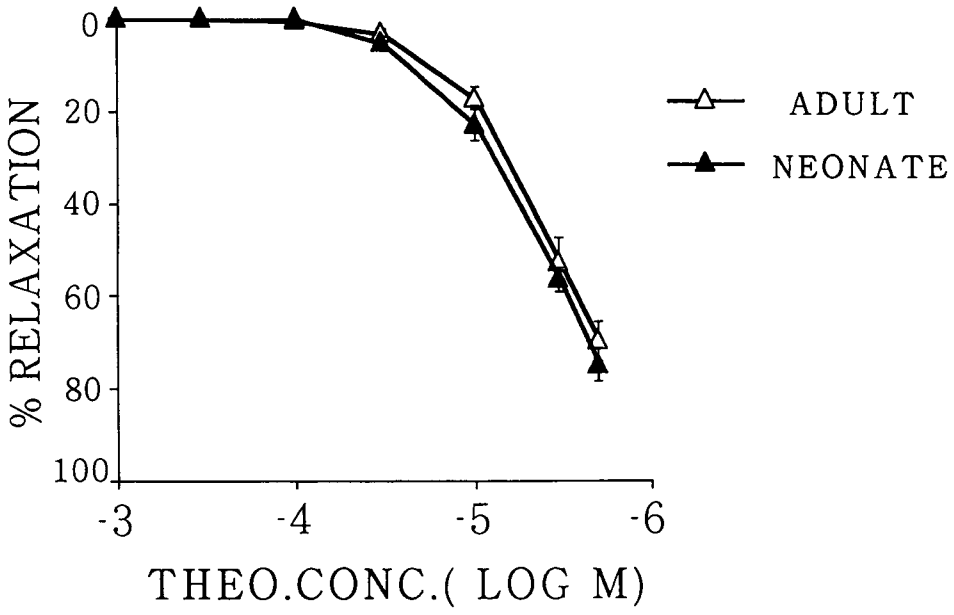

Figure 3. Cumulative concentration-response curves to theophylline (THEO) against $PGF_{2\alpha}$ contracted thoracic aortic strips isolated from neonate (n=10) and adult (n=8) rabbits. Papaverine 10^{-4} M induced relaxation is taken as 100%. Values are expressed as means ± SEM.

Table II. *Effects of Amrinone, Isoproterenol and Theophylline on* $PGF_{2\alpha}$ *Contracted Thoracic Aortic strips.*

	Neonate		Adult	
	ED_{50}	n*	ED_{50}	n*
Amrinone	$1.10 \pm 0.10 \times 10^{-4}$ M	13	$1.17 \pm 0.19 \times 10^{-4}$ M	11
Isoproterenol	$3.47 \pm 1.31 \times 10^{-8}$ M	14	$3.75 \pm 1.11 \times 10^{-8}$ M	10
Theophylline	$1.53 \pm 0.10 \times 10^{-4}$ M	10	$1.75 \pm 0.06 \times 10^{-4}$ M	10

* Number of strips

Values are expressed as mean ± SEM

No significant difference between neonate and adult groups was observed

between the two groups (data not shown). Cumulative application of theophylline (10^{-5} - 5×10^{-4} M) also elicited relaxation of the aortic strips from the two groups in a similar way (Figure 3). In the neonate and adult preparations, respectively, the maximum relaxation was $74.30 \pm 3.64\%$ and $69.64 \pm 4.15\%$ and the mean ED_{50} value was $1.53 \pm 0.10 \times 10^{-4}$ M and $1.75 \pm 0.06 \times 10^{-4}$ M, with no significant intergroup differences (Table 2).

DISCUSSION

The present study demonstrated that amrinone relaxes the thoracic aortic preparations isolated from neonatal rabbits similarly as it relaxes the adult vascular preparation. The two groups showed no significant differences for the maximal relaxation elicited by and sensitivity to the drug.

Investigators have reported that when amrinone is injected into the right pulmonary artery in newborn lambs, it causes a dose-related decrease in pulmonary arteriolar resistance with a simultaneous decline in systemic vascular resistance which was not significant.[6] Furthermore, in a recent study, amrinone showed selective vasodilator properties depending on the magnitude of pulmonary arterial pressure and resistance. In infants with elevated pulmonary arterial pressure, amrinone reduced pulmonary arteriolar resistance more than it reduced systemic vascular resistance.[25] The above findings suggest the existence of a vasodilator response to amrinone in the neonate and infant. Our results corroborated these findings.

With regard to the action of amrinone, developmental studies have demonstrated that, in contrast to the adult rabbit, newborn rabbits are relatively insensitive to the positive inotropic effects of amrinone,[21] and a biphasic effect of amrinone on tension development was also observed in newborn

rabbit myocardium .[14] A pronounced negative inotropic action was exhibited by amrinone in neonatal canine and piglet heart .[1,4] These results indicate that, unlike the vascular response, the responses to amrinone differ in immature and mature myocardium. Although the specific mechanisms of action remain to be elucidated, the inotropic effect of amrinone is speculated to be attributable to inhibition of PDE fraction III, thereby increasing intracellular cAMP. There may be two possibilities accounting for this different influence according to maturation and development in vascular and cardiac tissue, one being a possible developmental change in the characteristics and types of PDE isozymes in cardiac smooth muscle which differ from those of the aorta. The second possibility is a developmental change in the cAMP system in cardiac tissue which is quite different from that of vascular tissue. A comparative study of tissue distribution has shown the availability of PDE isozyme III in cardiac and vascular tissue (inhibition of which results in positive inotropism and vascular relaxation), and study regarding subcellular distribution of PDE isoenzymes has shown that the particulate fraction of this isozyme is dominant in cardiac muscle whereas the cytosolic fraction is the responsible factor for PDE activity in vascular tissue .[20] In the rabbit heart muscle a substantial proportion of PDE III is membrane bound, representing the SR fraction ,[30] and the activity of this fraction increases fivefold during maturation .[13] The weaker inotropic response elicited by PDE III inhibitors in newborn hearts might be explained by the immaturity of SR in the neonatal heart .[22,26] The negative inotropic effect of amrinone may be partially caused by a lack of matured t-tubular system in neonatal cardiac muscle .[1,6] One previous study demonstrated that basal adenylate cyclase activity and response increased with maturation in the myocardium of the dog .[29] These findings are not necessarily contradictory to our results, as a different tissue was used in the experiments. In accord with the present experiment, decreased coronary vascular resistance was observed in

response to amrinone in isovolumetrically beating heart collected from piglets and it was comparable to the similar effect in the adult pig. [1]

The existence of maturational changes has been observed previously in different vascular tissues isolated from different species of animals after exposure to various vasodilating and vasoconstricting agents. A number of investigators have already studied the developmental changes in the β-adrenergic receptor-mediated mechanism in the rabbit aorta, rabbit pulmonary artery and canine saphenous vein. [8,23] Consistent with the previous findings, we demonstrated a lesser vasorelaxation elicited by isoproterenol in the neonatal preparations, whereas the sensitivities to the drug of the vessel did not differ in the two groups. However, in our experiment the vasodilatation produced by forskolin due to the direct stimulation of adenylate cyclase was unchanged during maturation, which further corroborates the findings of other investigators, [8] suggesting immaturity of either the receptors or the G-proteins as an underlying cause of the reduced β-adrenoreceptor mediated vasorelaxant response in the neonate.

The vasorelaxant effects of theophylline were identical to those of amrinone, showing no differences in the adult and neonatal vascular preparations. Theophylline is known to increase intracellular cAMP concentration through a nonspecific PDE inhibition but in addition possesses several other subcellular actions. [24,32] The results of the present study suggested that the similar vascular effects of theophylline and amrinone might be due to involvement of the common PDE III isoenzyme. However, the PDE activity should be directly measured before arriving at this conclusion. Furthermore, increased cAMP accumulation by mechanisms other than PDE inhibition, such as A_1 adenosine receptor antagonism and inhibition of G_i function, remains to be investigated.

In this study, we have demonstrated the unaltered vasorelaxant responses

to amrinone and theophylline in aortic strips isolated from neonate and adult rabbits. Both of these drugs are known PDE inhibitors and their vascular effects are thought to be cAMP mediated, as both are associated with an increased intracellular accumulation of cAMP. The intracellular levels of cyclic nucleotides were not measured in the present study, and, from a purely mechanistic perspective, our results do not definitely demonstrate but rather suggest a probability that the capacity of the cAMP pathway to produce vascular relaxation is unaltered through a stable cAMP turnover.

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