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AGE-RELATED CHANGES IN THE RESPONSE OF RABBIT ISOLATED AORTAE TO VASOACTIVE AGENTS

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1 In helically-cut strips of aortae from rabbits of different ages (2 to 360 days old), responses to vasoconstrictor and vasodilator agents were compared.

2 The passive tension developed by the same magnitude of stretch was markedly less in aortae from immature rabbits than in aortae from mature rabbits.

3 Contractile responses to noradrenaline and K^+ increased with age in the range from 2 to 90 days. High concentrations of noradrenaline (10^{-5} and 5×10^{-5} M) produced relaxation of aortic strips from immature rabbits (2 to 30 days). The response to histamine was not altered with age. Age-dependent reductions (90 to 360 days) in the response to 5-hydroxytryptamine were observed.

4 Relaxations induced by isoprenaline of aortic strips contracted with prostaglandin $F_{2\alpha}$ increased with age in the range from 2 to 30 days but decreased with age from 30 to 360 days. Similar age-related alterations in the relaxant response to adenosine were observed.

5 It is concluded that aortae from immature rabbits are more distensible than those from mature rabbits; α-adrenoceptors mature during an early postnatal period (2 to 30 days), and 5-hydroxytryptamine receptor mechanisms deteriorate with age (older than 90 days). The β-receptor mechanism does not seem to alter greatly with age.

Introduction

It is widely accepted that cardiovascular responses to drugs differ in animals of different ages. Isolated aortae, arteries or veins from animals at different stages of development respond differently to vasodilator agents. However, the results are not always consistent; Fleisch, Maling & Brodie (1970), Fleisch (1971), Fleisch & Hooker (1976) and Ericsson & Lundholm (1975) demonstrated that vascular relaxations induced by isoprenaline decrease with age, while Park, Diehl & Sunderson (1976) came to the opposite conclusion. On the other hand, little information is available concerning age-related alterations in the response to vasoconstrictor agents.

The present study was undertaken to clarify differences in the responses to vasoconstrictor and vasodilator agents of aortae isolated from rabbits of different ages (2 to 360 days).

Methods

Albino rabbits of either sex and of different ages were used. Under ether anaesthesia, the animals were killed by exsanguination from the common carotid arteries. The thoracic aorta was rapidly removed, cleaned and cut into spiral strips. The specimen was fixed vertically between hooks in the muscle bath containing the nutrient solution, which was maintained at 37 ± 0.5°C and aerated with a mixture of 95% O_2 and 5% CO_2. Hooks anchoring the upper end of the strip were connected to the lever of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo, Japan). The composition of the solution was as follows (mM): Na^+ 162.1, K^+ 5.4, Ca^{2+} 2.2, HCO_3^- 14.9, Cl^- 157.0, and dextrose, 5.6. The pH of the solution was 7.2 to 7.3. Osmotic adjustment was not made when K^+ in concentrations up to 50 mM was added. Before measurements were taken, the preparations were allowed to equilibrate for 60 to 90 min in control media, during which time the solution was replaced every 10 to 15 min.

Neonatal rabbits housed in this laboratory ingested maternal milk and then artificial food for 360 days or longer. Rabbits of 2 ± 1, 5 ± 1, 12 ± 3, 30 ± 3, 90 ± 10, 180 ± 30 and 360 ± 60 days after birth were used. The body weight of these rabbits, the outside

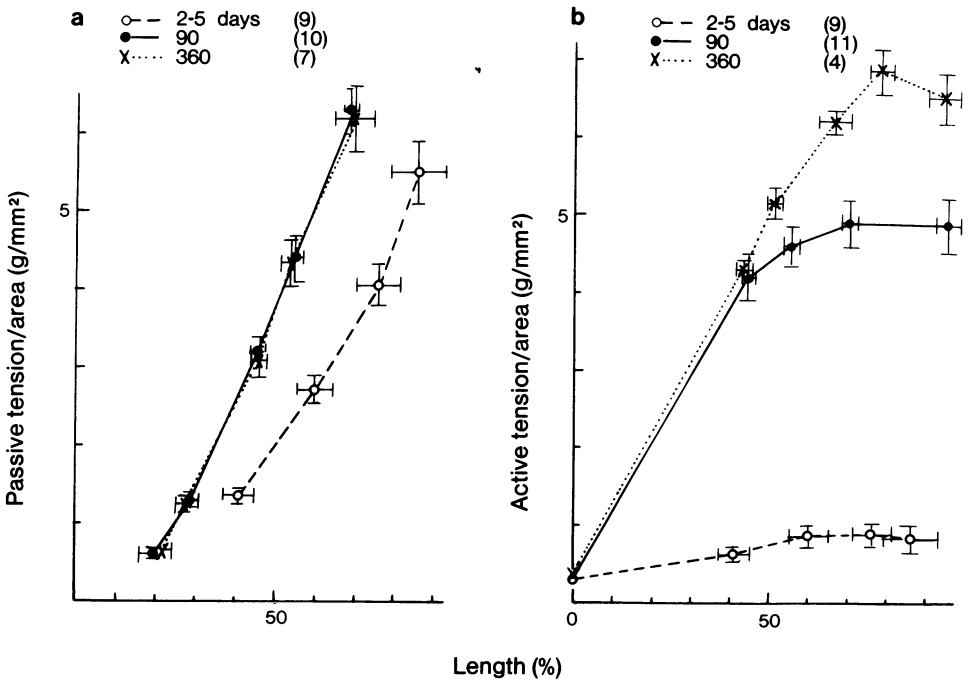

Figure 1 Length-tension relationships of spirally cut strips of aortae isolated from rabbits of different ages. (a) Passive tensions; (b) active tensions developed in response to 2×10^{-4} M histamine. Vertical and horizontal bars represent s.e. means. Figures in parentheses indicate the number of preparations used.

Table 1 Age-related changes in the body weight of rabbits, the outside diameter and wall thickness of aortae, and the length and width of aortic strips

Age (days)	*Body weight (g)*	*Outside diameter (mm)*	*Wall thickness (mm)*	*Length (mm)*	*Width (mm)*
2	68 ± 3 (35)	1.18 ± 0.05 (7)	0.18 ± 0.01 (7)	18.5 ± 0.6 (8)	2.1 ± 0.1 (8)
5	113 ± 6 (26)	1.25 (2)	0.17 (2)	18.9 (2)	2.2 (2)
12	203 ± 15 (28)	1.65 (1)	0.24 (1)	22.6 (1)	2.8 (1)
30	660 ± 45 (18)	2.99 ± 0.09 (6)	0.30 ± 0.01 (6)	26.4 ± 0.8 (6)	4.6 ± 0.1 (6)
90	2280 ± 50 (59)	3.99 ± 0.06 (9)	0.30 ± 0.02 (9)	23.3 ± 0.4 (9)	5.6 ± 0.2 (9)
180	3900 ± 140 (22)	3.98 ± 0.12 (9)	0.32 ± 0.01 (9)	24.9 ± 0.6 (9)	5.2 ± 0.2 (9)
360	4270 ± 140 (11)	3.92 ± 0.12 (6)	0.31 ± 0.01 (6)	23.9 ± 0.1 (7)	5.3 ± 0.3 (7)

Aortae and aortic strips were totally relaxed at room temperature. Figures in parentheses indicate the number of preparations or animals used.

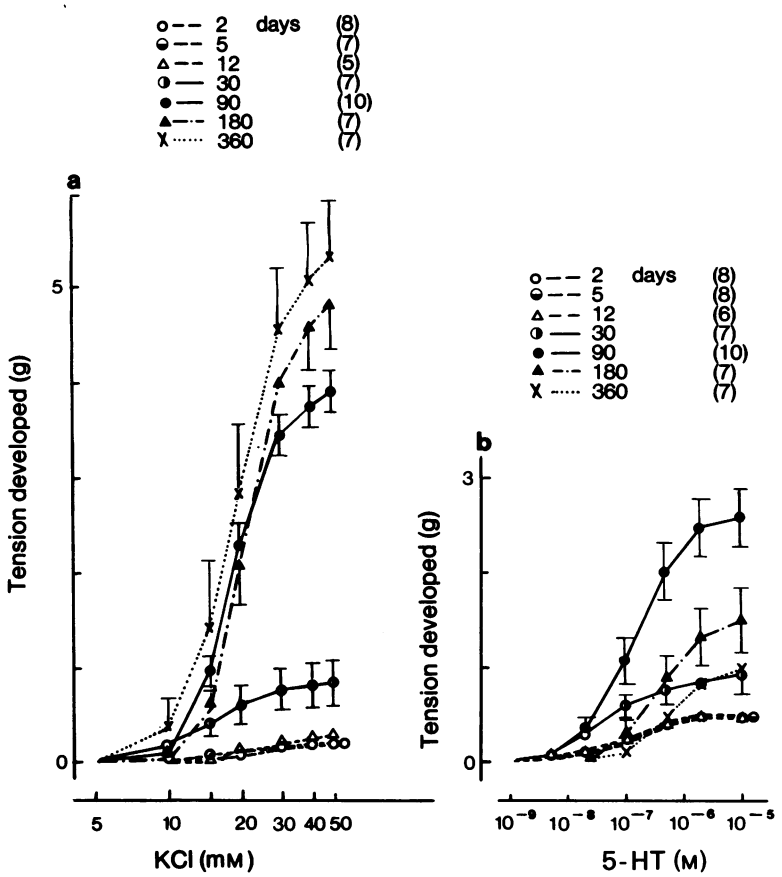

Figure 2 Contractile responses of aortae from rabbits of different ages (a) to K^+ and (b) to 5-hydroxytryptamine. Vertical bars represent s.e. means. Figures in parentheses indicate the number of preparations used.

diameter and wall thickness of aortae, and the length and width of aortic strips are summarized in Table 1. The ratio of the wall thickness to the radius decreased with increasing age up to 90 days; average ratios were 0.31 on day 2, 0.27 on day 5, 0.29 on day 12, 0.20 on day 30, 0.15 on day 90, and 0.16 on days 180 to 360. Resting tensions were adjusted to 1.5 g, 5 g and 7 g for aortic strips from rabbits of 2 to 30 days, 90 days and 180 to 360 days, respectively; at these resting tensions, 76 to 88% the maximum contraction in response to 2×10^{-4} M histamine was obtained (see length-active tension curves in Figure 1).

Length-passive tension relationships were obtained by stretching the strip stepwise in a range from 0 to 2 g for rabbits of 2 to 5 days, to 15 g for 90 days or to 20 g from 180 to 360 days. The length of the strip before and after stretching was measured, and relative increase in the length was estimated. After stretching, the strip was equilibrated for 10 to 20 min in control media and histamine in a concentration of 2×10^{-4} M was added to obtain the active tension. Details of the procedures were described in an earlier paper (Toda, Hatano & Hayashi, 1978).

Isometric contractions and relaxations were recorded on an ink-writing oscillograph (Sanei Sokki Co., Tokyo, Japan). Drugs were added directly to bathing media in cumulative concentrations. Results are expressed as mean values ± standard errors of the means. Statistical analyses were made with Student's *t* test. Drugs used were (±)-noradrenaline hydrochloride, 5-hydroxytryptamine creatinine sulphate, histamine dihydrochloride, (±)-isoprenaline hydrochloride, adenosine, papaverine hydrochloride, (±)-pro-

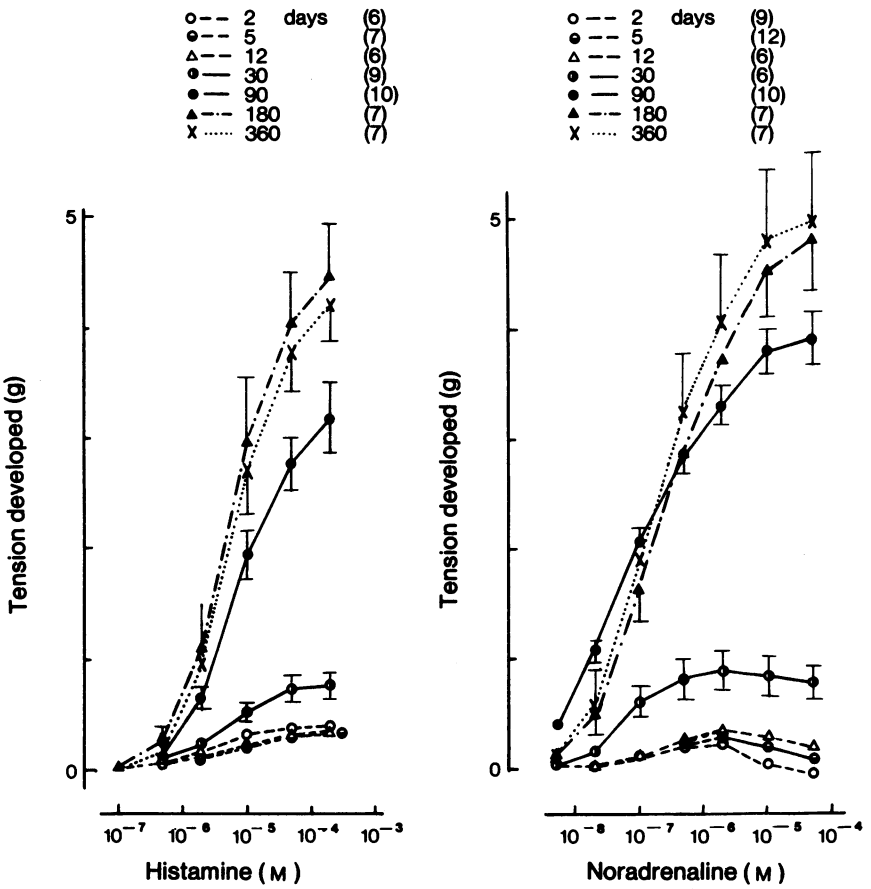

Figure 3 Contractile responses of aortae from rabbits of different ages to histamine and noradrenaline.

pranolol hydrochloride, phentolamine mesylate, cinanserin, chlorpheniramine maleate and prostaglandin $F_{2\alpha}$.

Results

Length-tension relationships

The length-passive tension curve obtained in helically cut strips of aortae from rabbits of 2 to 5 days was appreciably different from the curves at 90 days and 360 days, but the latter curves were practically identical (Figure 1a). Active tensions developed in response to 2×10^{-4} M histamine increased with increasing stretch of the aortic strips: maximum active tensions were attained when aortic strips were stretched 75.8 ± 5.4% ($n = 9$) at 2 to 5 days, 69.7 ± 2.2% ($n = 11$) at 90 days and 78.0 ± 3.4% ($n = 4$) at 360 days (Figure 1b).

Responses to vasoconstrictor agents

The addition of K^+ (5 to 50 mM), noradrenaline (5×10^{-9} to 2×10^{-6} M), 5-hydroxytryptamine (5-HT, 10^{-9} to 10^{-5} M), and histamine (10^{-7} to 2×10^{-4} M) caused a dose-related contraction in helical strips of aortae from rabbits of different ages (Figures 2 and 3). The maximum tension developed by K^+, noradrenaline and histamine was related directly to age (2 to 360 days). However, 5-HT-induced contractions varied directly with increasing age from 2 to 90 days but inversely with age from 90 to 360 days (Figure 2b). Contractile responses of aortae from mature and immature rabbits to noradrenaline, 5-HT and histamine were suppressed by 10^{-6} M phentola-

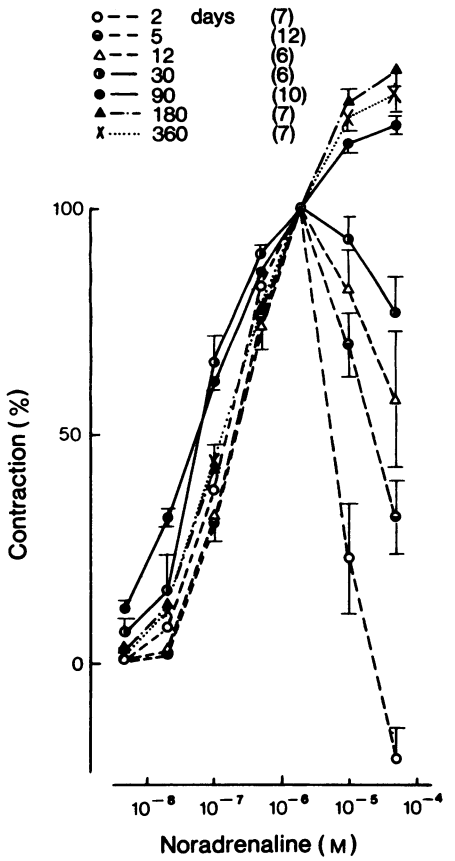

Figure 4 Dose-response curves to noradrenaline in aortae from rabbits of different ages. Contractions induced by 2×10^{-6} M noradrenaline were taken as 100%; mean absolute values from aortae of rabbits aged 2, 5, 12, 30, 90, 180 and 360 days were 214 ± 14 mg (n = 7), 297 ± 38 mg (n = 12), 342 ± 41 mg (n = 6), 902 ± 190 mg (n = 6), 3324 ± 185 mg (n = 10), 3743 ± 378 mg (n = 7) and 4077 ± 606 mg (n = 7), respectively.

mine, 10^{-6} M cinanserin and 10^{-6} M chlorpheniramine, respectively.

Differences in the response to noradrenaline of aortic strips from rabbits of different ages were compared by taking contractions induced by 2×10^{-6} M noradrenaline as 100%. The results are shown in Figure 4. Relative contractions induced by noradrenaline in concentrations ranging from 5×10^{-9} to 2×10^{-6} M were only slightly different in these aortic strips. However, the addition of noradrenaline in concentrations higher than 2×10^{-6} M elicited a dose-related relaxation in aortae from rabbits of 2 to 30 days, the relaxation being related inversely to age. Following treatment for 20 min with 10^{-6} M propranolol, the relaxant response of aortae from immature rabbits (2 to 12 days) to high concentrations of noradrenaline (10^{-5} and 5×10^{-5} M) was significantly attenuated (Figure 5a). The relaxation induced by 10^{-5} M noradrenaline was reversed to a contraction. In aortae from 90 day rabbits, propranolol potentiated the contractile response to high concentrations of noradrenaline (Figure 5b).

Age-dependent differences in the maximum contraction induced by K^+, noradrenaline, 5-HT and histamine are presented in Figure 6. In the same preparations, the greatest contraction was elicited by 5-HT on aortae from 2 to 30 day old rabbits and by K^+ or noradrenaline on aortae from 90 to 360 day old rabbits; the greatest tension developed was taken as 100%. The relative responses to 5-HT were greatly attenuated in aortae after day 90, whereas the responses to noradrenaline and K^+ increased with increasing age from 2 to 90 days. Histamine-induced contractions were not appreciably altered with age. Median effective concentrations of K^+, noradrenaline and histamine were not appreciably different in aortae at different ages, while the value for 5-HT greatly increased with increasing age from 90 to 360 days (Table 2).

Responses to vasodilator agents

The addition of isoprenaline (10^{-9} to 10^{-5} M) and adenosine (10^{-7} to 10^{-4} M) caused a dose-dependent relaxation when the strips had been contracted with prostaglandin $F_{2\alpha}$ (10^{-6} to 3×10^{-6} M) (Figure 7). Such concentrations of prostaglandin produced a 40 to 65% the maximum contraction, which persisted for 20 min or longer. Maximum relaxations induced by isoprenaline relative to those by papaverine (10^{-4} M) increased with increasing age in aortae isolated from rabbits of 2 to 30 days old, but the relaxations were related inversely to age in rabbits older than 30 days. Similar age-dependent differences were observed in the response to adenosine (Figure 7b). The relaxant effect of 10^{-4} M papaverine did not significantly differ in aortae from rabbits of different ages. Mean values of median effective concentrations of isoprenaline and adenosine are summarized in Table 2. Lowest values were obtained in aortae from rabbits of 30 days old; at this age, the greatest relaxation was attained in response to 10^{-5} M isoprenaline or 10^{-4} M adenosine (Figure 7).

Discussion

The passive tension developed by the same magnitude of stretch was markedly less in spiral strips from aortae of neonatal rabbits (2 to 5 days) than in those

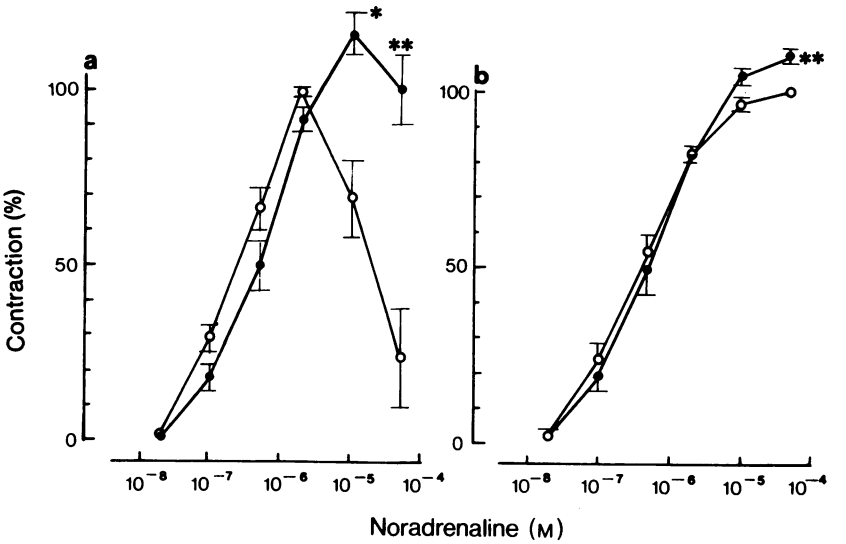

Figure 5 Alterations induced by propranolol (10^{-6} M) in the dose-response curve to noradrenaline (a) in aortae from immature (2 to 12 days) and (b) mature (90 days) rabbits. (○) Control; (●) with propranolol. Maximum contractions induced by noradrenaline in control media were taken as 100%; mean absolute values in immature and mature rabbit aortae were 250 ± 42 mg (n = 10) and 3466 ± 355 mg (n = 11), respectively. Significantly different from controls, $^{**}P < 0.001$; $^{*}P < 0.01$.

from mature rabbits (90 and 360 days), suggesting that neonatal rabbit aortae are more elastic and distensible. Similar age-dependent alterations in the distensibility of arterial segments from litters of puppies and their mother dogs have recently been reported (Cox, Jones & Swain, 1976).

In helical strips of aortae from mature rabbits (90 to 360 days) the maximum contraction induced by

Table 2 Median effective concentrations of vasoactive agents in aortae isolated from rabbits of different ages

Agent	*Age (days)* 2	5	12	30	90	180	360
Noradrenaline ($\times 10^{-7}$ M)	1.5 ± 0.4 (9)	2.2 ± 0.3 (12)	2.6 ± 0.7 (6)	0.7 ± 0.2 (6)	1.0 ± 0.1 (10)	2.9 ± 0.7 (7)	1.9 ± 0.3 (7)
5-Hydroxytryptamine ($\times 10^{-8}$ M)	9.7 ± 1.8 (8)	11.8 ± 2.2 (8)	11.2 ± 2.8 (6)	5.0 ± 0.8[c] (7)	17.5 ± 2.7[c] (10)	44.3 ± 10.0[a] (7)	121 ± 40[a] (6)
Histamine ($\times 10^{-6}$ M)	3.7 ± 0.6 (6)	6.7 ± 1.3 (7)	7.0 ± 1.8 (6)	4.6 ± 1.2 (7)	7.3 ± 1.2[c] (10)	7.4 ± 2.6 (7)	7.1 ± 1.9 (7)
K^+ (mM)	20.5 ± 2.1 (8)	26.0 ± 2.3 (7)	26.2 ± 1.8 (5)	15.5 ± 1.5 (7)	18.9 ± 0.8 (10)	22.0 ± 1.4 (7)	19.7 ± 1.6 (7)
Isoprenaline ($\times 10^{-8}$ M)	20.6 ± 6.1 (8)	21.0 ± 5.7 (7)	22.6 ± 10.0 (10)	0.5 ± 0.1[b] (7)	4.7 ± 0.7[c] (15)	28.8 ± 12.6 (11)	24.2 ± 5.2 (9)
Adenosine ($\times 10^{-7}$ M)	28.5 ± 8.0 (9)	7.3 ± 1.5[c] (8)	6.8 ± 1.9[c] (6)	3.8 ± 0.5[c] (6)	14.6 ± 2.9 (13)	20.0 ± 3.9 (13)	144 ± 48.2 (7)

Significantly different from values at day 2: $^{a}P < 0.01$; $^{b}P < 0.02$; $^{c}P < 0.05$. Figures in parentheses indicate the number of preparations used.

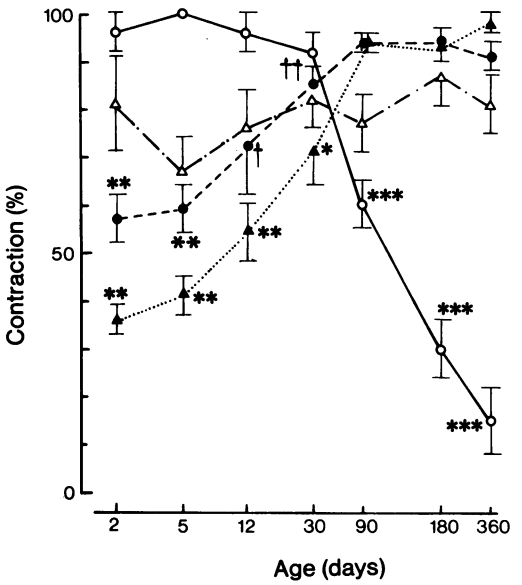

Figure 6 Age-related changes in the maximum contraction induced by K^+ (▲), noradrenaline (●), 5-hydroxytryptamine (○) and histamine (△). The greatest contraction induced by one of these agonists in same preparations was taken as 100%; mean absolute values for aortae from rabbits aged 2, 5, 12, 30, 90, 180 and 360 days were 483 ± 65 mg (n = 7), 490 ± 27 mg (n = 6), 488 ± 38 mg (n = 5), 911 ± 183 mg (n = 7), 4160 ± 239 mg (n = 10), 5200 ± 516 mg (n = 7) and 5466 ± 597 mg (n = 7), respectively. Significantly different from values at day 90, $^{**}P < 0.001$; $^{*}P < 0.01$; $^{\dagger}P < 0.02$; $^{\dagger\dagger}P < 0.05$. Significantly different from values at day 2, $^{***}P < 0.001$.

vasoconstrictor agents, including noradrenaline, 5-HT and histamine did not exceed the maximum contraction induced by K^+, whereas in aortae from neonatal and young rabbits (2 to 30 days) the contraction induced by K^+ was significantly less than that induced by 5-HT. Contractile responses to K^+ appear to develop during an early postnatal period. Excess K^+ may depolarize the membrane of vascular smooth muscle cells to a lesser extent in immature rabbit aortae than in mature aortae, or the influx of Ca^{2+} across cell membranes and the mobilization of Ca^{2+} from intracellular stores may be induced by excess K^+ less effectively in immature rabbit aortae.

5-HT produced a greater contraction of aortae from neonatal rabbits, as compared with contractions induced by other vasoconstrictor agents used. However, 5-HT-induced contractions relative to those induced by K^+ or noradrenaline markedly decreased with increasing age. The 5-HT-induced contraction of mature and immature rabbit aortae was specifically antagonized by a 5-HT antagonist, cinanserin. Therefore, it appears that the quantity or sensitivity of 5-HT receptors in smooth muscles of rabbit aortae decreases with increasing age. On the other hand, in mature (3 months old) and old rats (12 months), age-related reductions in the contractile response of isolated aortic strips to 5-HT have been demonstrated; however, such reductions are also observed in the responses to noradrenaline and K^+ (Cohen & Berkowitz, 1976).

Contractile responses to noradrenaline relative to the maximum contraction induced by 5-HT were related directly to age in a range from 2 to 90 days (Figure 6). The noradrenaline-induced contraction of immature rabbit aortae was specifically attenuated by phentolamine, as was the contraction of mature aortae. The present study revealed that the relaxation induced by β-receptor stimulation was not greater in neonatal rabbit aortae than in mature aortae. The content of noradrenaline and the quantity of catecholamine fluorescence in the rabbit heart increase with age from 2 to 30 days after birth (Friedman, Pool, Jacobowitz, Seagren & Braunwald, 1968; Toda, Fu & Osumi, 1976), suggesting that adrenergic innervation develops during an early postnatal period. The ability to accumulate [^{3}H]-noradrenaline in different tissues of the developing rat, including the heart, spleen, salivary gland and intestine, develops parallel to endogenous noradrenaline (Iversen, DeChamplain, Glowinski & Axelrod, 1967). Shibata, Hattori & Sakurai (1971) have demonstrated that cocaine-induced potentiation in the response to noradrenaline is not different in aortae isolated from young (35 to 42 days old) and old rabbits (10 months). The possibility that reuptake of noradrenaline by adrenergic nerve terminals is greater in immature rabbit aortae may thus be excluded. It seems likely that an α-adrenoceptor mechanism is immature in aortae from neonatal rabbits and develops during the first 30 postnatal days. A similar conclusion has been drawn for rat portal veins (Ljung & Stage, 1975) and chick mesenteric arteries (Knight & McGregor, 1974).

In immature rabbit aortae (2 to 30 days), noradrenaline in concentrations higher than 2×10^{-6} M produced a dose-related relaxation, which was greater in aortae isolated from neonatal rabbits (Figure 4). This relaxation was partially antagonized by treatment with propranolol, thus being at least in part due to β-receptor activation. However, in the present study the relaxation induced by isoprenaline was not inversely related to age in immature rabbits. The tension developed by noradrenaline is considered to be a balance between the contraction and relaxation induced by stimulation of α- and β-receptors, respectively. These findings lead us to conclude that a greater

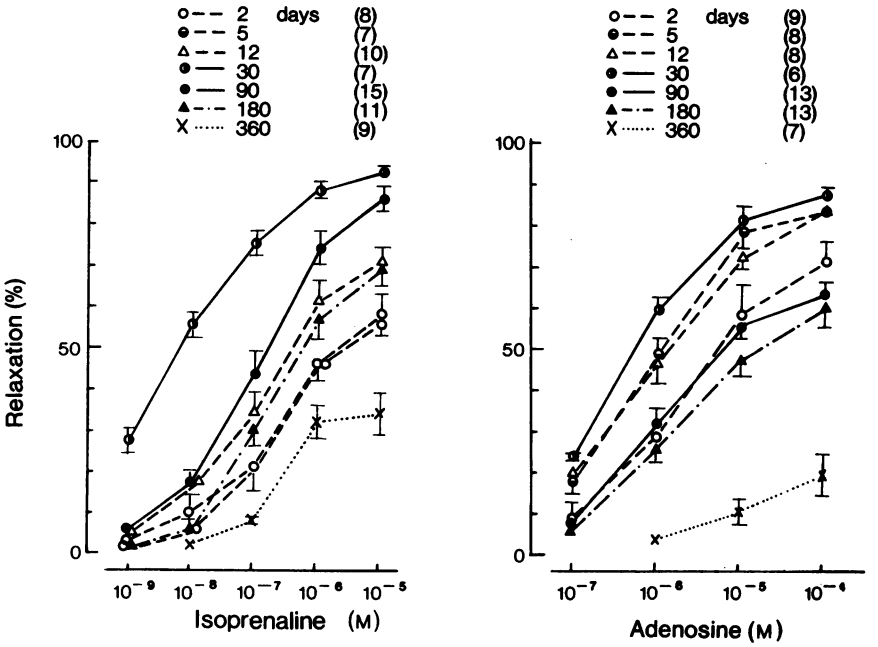

Figure 7 Dose-relaxation curves (a) of isoprenaline and (b) of adenosine in aortae isolated from rabbits of different ages. Preparations were contracted with prostaglandin $F_{2\alpha}$ (10^{-6} to 3×10^{-6} M). Relaxations induced by 10^{-4} M papaverine were taken as 100%; mean absolute values in preparations with isoprenaline on aortae from rabbits aged 2, 5, 12, 30, 90, 180 and 360 days were 544 ± 34 mg (n = 8), 601 ± 57 mg (n = 7), 355 ± 43 mg (n = 10), 824 ± 56 mg (n = 7), 1497 ± 190 mg (n = 15), 1710 ± 233 mg (n = 11) and 1122 ± 153 mg (n = 9), respectively, and those in preparations with adenosine were 490 ± 52 mg (n = 9), 509 ± 50 mg (n = 8), 390 ± 85 mg (n = 8), 933 ± 66 mg (n = 6), 1345 ± 160 mg (n = 13), 1442 ± 184 mg (n = 13) and 964 ± 143 mg (n = 7), respectively.

relaxation seen in neonatal rabbit aortae when high concentrations of noradrenaline are added may not be due to a greater response to an activation of β-receptors but to a lesser degree of contraction by α-receptor activation, because of an immature α-adrenergic mechanism. Greater relaxations are always obtained in partially contracted arteries than in arteries contracted to a maximum level or to a level close to the maximum (unpublished data).

Isoprenaline-induced relaxations of aortae were greater in 30 day old rabbits; age-related increase in relaxations was observed in aortae from 2 to 30 day old rabbits and the opposite was true in aortae from 30 to 360 day rabbits. Similar age-related alterations were also seen in the response to adenosine; thus age-dependent alterations in the action of isoprenaline are not due to changes in the activity of β-receptors. In rat (41 to 255 days after birth) and rabbit (50 to 1835 days) aortae, the response to isoprenaline, but not to $NaNO_2$, is inversely related to age, therefore, an age-dependent decrease in the aortic β-receptor activity has been postulated (Fleisch *et al.*, 1970). In contrast, Park *et al.* (1976) have suggested that a β-adrenoceptor function in the aortae and pulmonary artery of new-born rabbits matures with age because of an age-dependent (1 day to 5 months) increase in the response to isoprenaline. Whether the discrepancy between our findings and those obtained by other investigators is due to differences in the experimental conditions, such as resting tensions (different or same in rabbits of different ages), contractile agents (prostaglandins, histamine, 5-HT, etc.) and the extent of contraction before the addition of relaxant drugs, remains to be ascertained.

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