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Age-related changes in the sensitivity to verapamil and sodium nitroprusside of vascular smooth muscle of rabbit aorta

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1 Age-related changes in the sensitivity to verapamil and sodium nitroprusside were examined in isolated aortic strips of the rabbit.

2 In the aortae of newborn rabbits within 10 days of birth, the resting tone of the muscle was strongly reduced by sodium nitroprusside but not by either Ca-deficient solution or by verapamil. High K-induced contraction and noradrenaline-induced contraction were both inhibited by verapamil or sodium nitroprusside.

3 In the aortae of 24 day-old rabbits, resting tension was slightly reduced by sodium nitroprusside but not by verapamil. High K-induced contraction was less sensitive to sodium nitroprusside than to verapamil whereas noradrenaline-induced contraction was less sensitive to verapamil than to sodium nitroprusside.

4 In the aortae isolated from 60 day-old or older rabbits, resting tension was not affected by either sodium nitroprusside or verapamil. High K-induced contraction was inhibited by verapamil whereas sodium nitroprusside showed only a weak inhibitory effect. Noradrenaline-induced contraction was inhibited by sodium nitroprusside although verapamil had only a slight inhibitory effect.

5 In the aortae of 1 day-old and also in adult rabbits, noradrenaline induced an additional increase in muscle tension when applied during the sustained contraction induced by high K.

6 It is suggested that, in the newborn rabbit aorta, the voltage-dependent Ca channel is sensitive to both verapamil and sodium nitroprusside and the sensitivity to sodium nitroprusside gradually decreases during maturation whereas the receptor-linked Ca channel is also sensitive to both of the inhibitors at birth but the sensitivity to verapamil gradually decreases with age.

Introduction

In smooth muscle, there are two distinct types of Ca channels, a voltage-dependent channel and a receptor-linked channel. The former is activated by decrease in membrane potential and the latter is regulated by drug-receptor interactions (Weiss, 1977; Bolton, 1979; van Breemen *et al.*, 1979). In previous experiments (Karaki & Weiss, 1980; Karaki *et al.* 1984a), it was found in rabbit aorta that high K-induced contraction is due to Ca influx through a voltage-dependent Ca channel which is relatively specifically inhibited by verapamil and that noradrenaline-induced contractions are due to Ca influx through receptor-linked Ca channels which are relatively specifically inhibited by sodium nitroprusside. Such specific correlation between two types of Ca channels and two different inhibitors is observed only in aortae of rabbits and of some rat strains (see Karaki & Weiss, 1984). In many other vascular smooth muscles, not only K-induced contractions but also the contractions induced by noradrenaline and other receptor-agonists are inhibited in varying degree by verapamil (see Triggle, 1981; Flaim, 1982; Cauvin *et al.*, 1983). In these vascular smooth muscles, sodium nitroprusside also inhibits to a varying degree the contractions induced by either high K or receptor activation (see Karaki & Weiss, 1984). From these results, it has been proposed that there are some differences in the characteristics of the Ca channels in the aortae of rabbits and some rat strains (Karaki *et al.*, 1984a; Karaki & Weiss, 1984). In the present experiments, age-related changes in the sensitivity of rabbit aorta to verapamil and sodium nitroprusside were examined in order to discover whether such specific characteristics of rabbit aorta develop during aging of the rabbit.

Methods

Tissue preparations

New Zealand rabbits of either sex were killed under anaesthesia with either ether or sodium pentobarbitone at 1, 10, 24, 60 or more than 180 days (adult) after birth. The thoracic aorta was removed and cut into a spiral strip.

Solutions

The normal bathing solution (pH 7.4, 37°C) contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8 and glucose 5.5 (Karaki *et al.*, 1981). High K solution was made by substituting 60 mM NaCl in the normal solution with equimolar KCl. Ca-deficient solution was made by omitting $CaCl_2$ and adding 0.5 mM ethyleneglycol *bis* (β-aminoethylether)N,N,N′,N′-tetra acetic acid (EGTA). The solutions were aerated with a 95% O_2 and 5% CO_2 mixture.

Muscle tension

Muscle tension was recorded isometrically with a force-displacement transducer connected to a Nihon Kohden polygraph. Resting tension of 0.5 g was applied to the aortae isolated from rabbits 1, 10 and 24 days of age and 1.0 g to the aortae from 60 day-old and adult rabbits. High K solution was applied in the presence of phentolamine 10^{-6} M in order to avoid the possible effect of endogenous catecholamines released by high K solution on muscle contraction (Karaki & Urakawa, 1977; Karaki *et al.* 1984b). Concentration-response curves for noradrenaline were constructed by cumulative application of noradrenaline and EC_{50} values (concentration of noradrenaline to induce half-maximum contraction) were calculated from these curves. Inhibitors were added cumulatively when the contractile tension induced by either high K or 10^{-6} M noradrenaline reached a steady level (usually 20 min after the addition of a stimulant). The tensions achieved during these contractions are listed in Table 1 and the results of the experiments are expressed in terms of these contractile tension levels except where otherwise stated. IC_{50} values (concentration of inhibitors required to induce a 50% inhibition of contraction) were calculated from the cumulative concentration-inhibition curves.

Statistics

Results of the experiments are expressed as mean ± s.e.mean. Student's *t* test was used for statistical analysis of the results and a *P* value less than 0.05 was taken as significant. In the experiment to examine the inhibitory effect of sodium nitroprusside on agonist-induced contractions, the mean decrease in basal tension induced by sodium nitroprusside was subtracted from the mean decrease in the agonist-induced contraction brought about by this inhibitor (see Figure 3a inset). Standard errors for the mean decrease in the agonist-induced contraction were calculated as:

$$\text{s.e.} = \{[\text{s.e.(stim)}]^2 + [\text{s.e.(rest)}]^2\}^{1/2}$$

where s.e.(stim) is standard error of the mean decrease in the agonist-induced contraction and the s.e.(rest) is standard error of the mean decrease in resting tension induced by sodium nitroprusside (Nachshen & Blaustein, 1979).

Drugs

Verapamil (donated by Eisai), sodium nitroprusside (Wako Pure Chemical Industries), noradrenaline bitartarate (Wako), phentolamine mesylate (Ciba-Geigy) and EGTA (Sigma) were used.

Table 1 Responses of rabbit aortic strips to high K and noradrenaline

		Days after birth				
		1	10	24	60	>180
Muscle tension induced by 65.4 mM K	(g)	0.31 ± 0.03 (9)	0.42 ± 0.02 (8)	0.66 ± 0.03 (5)	0.93 ± 0.03 (12)	2.35 ± 0.12 (8)
Muscle tension induced by 10^{-6} M noradrenaline	(g)	0.23 ± 0.01** (9)	0.41 ± 0.02 (8)	0.84 ± 0.04** (5)	1.05 ± 0.04* (12)	2.82 ± 0.12* (8)
EC_{50} for noradrenaline	(M)	2.95 ± 0.33 × 10^{-8} (4)	4.58 ± 0.09 × 10^{-8} (4)	3.05 ± 0.59 × 10^{-8} (8)	3.60 ± 0.11 × 10^{-8} (6)	4.00 ± 0.10 × 10^{-8} (8)

Number in parentheses indicates number of experiments; values are mean ± s.e.mean.
* and ** Significantly different from muscle tension induced by 65.4 mM K with $P < 0.05$ and $P < 0.01$, respectively.

Results

Response to high K and noradrenaline

Changes in the responses of aortic strips to high K and noradrenaline during aging are summarized in Table 1. Sustained contractions induced by noradrenaline 10^{-6} M were smaller than those induced by high K in the aortae isolated from rabbits 1 day after birth, almost the same in the aortae of 10 day-old rabbits, and greater in the aortae of 24 day- and older rabbits. EC_{50} values for noradrenaline were within the range of 2.95×10^{-8} M (in 1 day-old rabbit aorta) to 4.58×10^{-8} M (in 10 day-old rabbit aorta) and the small variations in the sensitivity of aortae to noradrenaline did not seem to correlate with age of the rabbits.

Effects of inhibitors on resting tension

Neither treatment of the muscle with Ca-deficient solution nor addition of verapamil in concentrations from 10^{-8} M to 10^{-5} M affected the resting tension of aortae isolated from rabbits aged from 1 day to more than 180 days. In contrast, sodium nitroprusside strongly decreased the resting tension of the aorta isolated from a 1 day-old rabbit. The inhibitory effect of sodium nitroprusside on resting tension gradually decreased in the first few weeks of life and the relaxant effect was not observed in the aortae isolated from rabbits 60 days after birth (Figure 1).

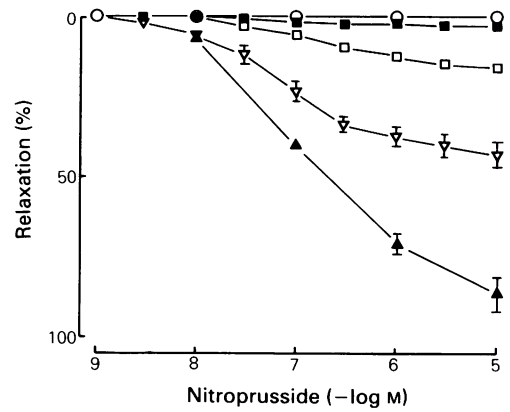

Figure 1 Effects of sodium nitroprusside on resting tension of rabbit aorta. 0% represents resting tension level and 100% represents maximum relaxation level (without resting tension) in 1 day- (▲), 10 day- (▽), 24 day- (□), 60 day- (■) and adult rabbit aortae (○). Resting tension level was 0.5 g for aortae isolated from rabbits 1, 10 and 24 days of age and 1.0 g for aortae from 60 day-old and adult rabbits. Mean of 4 to 12 experiments are shown; s.e.mean is indicated by an error bar when it is greater than the symbol.

Effects of verapamil on high K- and noradrenaline-induced contractions

As shown in Table 2 and Figure 2, verapamil inhibited high K-induced contractions in aortae with IC_{50} values ranging from 0.27×10^{-7} M (10 day-old rabbit aorta) to 1.50×10^{-7} M (1 day-old rabbit aorta; not significantly different). Verapamil also inhibited contractions induced by noradrenaline in aortae isolated from 1 and 10 day-old rabbits at concentrations higher than those needed to inhibit high K-induced contraction. The inhibitory effect of verapamil on noradrenaline-induced contraction then decreased with increasing age and the IC_{50} value was more than 2 log units greater in aortae of adult rabbits compared to those of 10 day-old rabbits.

Table 2 IC_{50} values for verapamil and sodium nitroprusside on high K- and noradrenaline-induced contraction in rabbit aorta

	Days after birth				
Verapamil	1	10	24	60	180
65.4 mM K	$1.50 \pm 0.63 \times 10^{-7}$(10)	$0.27 \pm 0.12 \times 10^{-7}$ (4)	$1.10 \pm 0.21 \times 10^{-7}$ (6)	$0.78 \pm 0.03 \times 10^{-7}$ (6)	$1.47 \pm 0.09 \times 10^{-7}$ (5)
10^{-6} M Noradrenaline	$7.63 \pm 1.58^{*} \times 10^{-7}$ (8)	$1.67 \pm 0.31^{*} \times 10^{-7}$ (4)	$3.80 \pm 0.09^{*\dagger} \times 10^{-6}$ (5)	$9.03 \pm 1.11^{*\dagger} \times 10^{-6}$ (6)	$> 10^{-5*\dagger}$ (6)
Sodium nitroprusside					
65.4 mM K	$0.89 \pm 0.15 \times 10^{-6}$ (9)	$1.68 \pm 0.26\dagger \times 10^{-6}$ (4)	$6.68 \pm 0.62\dagger \times 10^{-6}$ (6)	$9.50 \pm 0.68\dagger \times 10^{-6}$ (5)	$> 10^{-5}\dagger$ (6)
10^{-6} M Noradrenaline	$2.30 \pm 0.50^{**} \times 10^{-8}$ (9)	$1.60 \pm 0.26^{**} \times 10^{-8}$ (4)	$5.40 \pm 0.61^{**\dagger} \times 10^{-8}$ (5)	$9.35 \pm 0.64^{**\dagger} \times 10^{-8}$ (6)	$1.30 \pm 0.16^{**\dagger} \times 10^{-7}$ (5)

Number in parentheses indicates number of experiments.
* and ** Significantly higher and lower, respectively, than the IC_{50}s on 65.4 mM K-induced contraction ($P < 0.01$).
† Significantly higher ($P < 0.01$) than the value in the 1 day-old rabbit aorta.

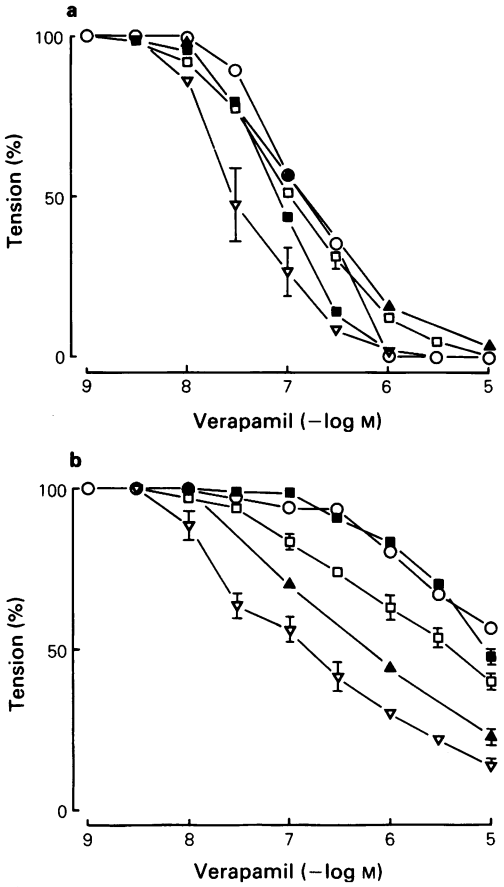

Figure 2 Effects of verapamil on 65.4 mM K- (a) and 10^{-6} M noradrenaline-induced contractions (b) in rabbit aortae isolated from 1 day- (▲), 10 day- (▽), 24 day- (□), 60 day- (■) and adult (○) rabbits. 100% represents the sustained tension level induced by each agonist and 0% represents resting tension level. The actual contractile tension levels representing 100% are shown in Table 1. Mean of 4 to 10 experiments are shown; s.e.mean shown by an error bar when it is greater than the symbol.

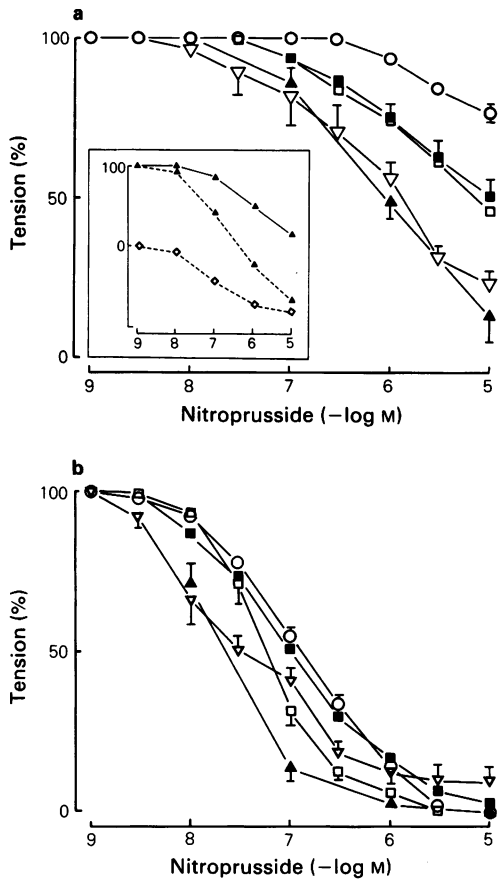

Figure 3 Effects of sodium nitroprusside on 65.4 mM K- (a) and 10^{-6} M noradrenaline-induced contractions (b) in rabbit aortae isolated from 1 day- (▲), 10 day- (▽), 24 day- (□), 60 day- (■) and adult (○) rabbits. Since sodium nitroprusside decreases resting tension of aortae as shown in Figure 1, the difference between the decrease in the stimulated tension (upper dotted line in inset) and the decrease in the resting tension (lower dotted line in inset) were calculated and a concentration-inhibition curve (continuous line in inset) was obtained to show the effect of sodium nitroprusside on the stimulated tension. 100% represents the sustained tension level induced by each agonist and 0% represents resting tension level. The actual tension levels representing 100% are shown in Table 1. S.e.mean was calculated as described in Methods. Mean of 4 to 9 experiments are shown; s.e.mean is shown by an error bar when it is greater than the symbol.

Effects of sodium nitroprusside on high K- and noradrenaline-induced contractions

Sodium nitroprusside inhibited high K-induced contractions in aortae isolated from 1 day-old rabbits. Higher concentrations of sodium nitroprusside (10^{-6} M or higher) decreased the muscle tension below resting tension level, suggesting that a portion of the inhibitory effect on high K-induced contraction reflects the inhibition of resting tension. Therefore, the results in Figure 3 and Table 2 were corrected for the changes in resting tension (see Figure 3a, inset). The inhibitory effect of sodium nitroprusside on high K-induced contraction became less with increasing age; IC_{50} significantly increased from 8.9×10^{-7} M in the aortae of 1 day-old rabbits to much higher than 10^{-5} M in the aortae of adult rabbits. Sodium nitroprusside

showed a potent inhibitory effect on noradrenaline-induced contraction in the aorta. IC_{50} values significantly increased with increasing age from 1.6×10^{-8} M (in 10 day-old rabbit aorta) to 1.30×10^{-7} M (adult rabbit aorta).

Additive effect of high K and noradrenaline on muscle tension

Addition of 10^{-6} M noradrenaline during the sustained contraction induced by high K further increased the muscle tension in both 1 day-old and adult rabbit aortae. Mean additional increase was 0.19 ± 0.01 g ($n = 4$) in the 1 day-old rabbit aorta (61.3% increase) and 1.23 ± 0.05 g in the adult rabbit aorta (52.8% increase).

Discussion

Sensitivity to α-adrenoceptor stimulation of various vascular tissues has been reported either not to change or to decrease after birth (see Duckles & Banner, 1984). In the present experiments, it was found that the EC_{50} for noradrenaline in rabbit aorta did not change after birth. Similar results have been obtained in sheep carotid and ear arteries, guinea-pig and rabbit renal arteries and rat portal vein (see Duckles & Banner, 1984). Compared to the maximum contraction induced by a non-specific contractile stimulus (65.4 mM K), the contraction induced by 10^{-6} M noradrenaline was smaller in the aortae isolated from 1 day-old rabbits, almost the same in 10 day-old rabbit, and greater in rabbits 24 day-old and older. This change may be at least partly explained by the fact that noradrenaline stimulates both α- and β-adrenoceptors and that, in rabbit aorta, relaxation resulting from β-adrenoceptor stimulation declines with advancing age (Fleisch *et al.*, 1970; Fleisch & Hooker, 1976).

The resting tension of rabbit aorta was not affected by verapamil irrespective of the age of the rabbits. However, sodium nitroprusside strongly decreased the resting tension in aortae from 1 day-old rabbits. The inhibitory effect of sodium nitroprusside gradually decreased with increasing age and was not observed in the 60 day-old rabbit aorta. Since Ca-depleted solution had little inhibitory effect on resting tension of newborn as well as adult rabbit aortae, Ca influx does not seem to play an important role in maintaining resting tension. It has been suggested that sodium nitroprusside inhibits smooth muscle contraction by inhibiting Ca influx through Ca channels (Karaki *et al.*, 1984a) and also by hyperpolarizing the membrane (Haeusler & Thorens, 1976; Ito *et al.*, 1978; Kreye, 1981). Although agonist-induced contractions in the aorta seem to be inhibited by the former mechanism (Karaki *et al.*, 1984a), the resting tension of aortae isolated from immature rabbits (1 to 24 day-old) may be inhibited by the latter mechanism. In contrast, resting tension in adult rabbit aorta might be due to passive stretch of the tissue.

In aortae isolated from adult rabbits, high K-induced contractions were inhibited relatively specifically by verapamil and noradrenaline-induced contractions by sodium nitroprusside, as reported earlier (Karaki *et al.*, 1984a). In contrast, both high K- and noradrenaline-induced contractions were inhibited by verapamil or by sodium nitroprusside in aortae isolated from immature rabbits. These characteristics of immature rabbit aortae are similar to those of a wide variety of vascular smooth muscle including rat aorta (see Karaki & Weiss, 1984). Even in the vascular tissues isolated from adult rabbit, high K- and noradrenaline-induced contractions in ear, pulmonary, carotid, renal and saphenous arteries were inhibited rather nonspecifically by verapamil and by sodium nitroprusside (see Karaki & Weiss, 1984).

The difference between the characteristics of aortae isolated from adult and newborn rabbits may be explained as follows: (1) in the newborn rabbit aorta, there is only one type of Ca channel which is activated by membrane depolarization and is inhibited by verapamil and also by sodium nitroprusside. Noradrenaline depolarizes the cell membrane, as high K does, and opens this Ca channel. With maturation of the rabbit, this Ca channel loses its sensitivity to sodium nitroprusside and another type of Ca channel (receptor-linked) also begins to function. Thus, in the adult rabbit aorta, noradrenaline opens a receptor-linked channel (which is inhibited by sodium nitroprusside) without changing or with slight depolarization of cell membrane whereas high K depolarizes cell membrane and opens a voltage-dependent channel (which is sensitive to verapamil). Alternatively, (2) even in newborn rabbit aorta, there are two types of Ca channels (voltage-dependent and receptor-linked ones) although the selectivities for verapamil and sodium nitroprusside of these channels are not as great as in the adult rabbit aorta. The sensitivities of the receptor-linked Ca channel to verapamil and of the voltage-dependent Ca channel to sodium nitroprusside are gradually lost with increasing age of rabbits.

There are no available data to show whether noradrenaline depolarizes cell membranes in the newborn rabbit aorta. Even if it does, the first explanation is not likely because the following results suggest that there are two different types of Ca channel in the newborn rabbit aorta: (1) although verapamil inhibited both high K- and noradrenaline-induced contractions in the newborn rabbit aorta, the K-induced contraction was more sensitive than the latter. Similarly, sodium nitroprusside inhibited noradrenaline-induced contraction at a much lower concentration than

that needed to inhibit the high K-induced contraction. A similar result has been obtained in the rat aorta (Karaki *et al.* 1984a; Karaki & Weiss, 1984). In most other vascular smooth muscle, organic Ca antagonists like verapamil inhibit high K-induced contraction at a lower concentration than that required to inhibit noradrenaline-induced contraction (Triggle, 1981; Flaim, 1982; Cauvin *et al.*, 1983). (2) In adult rabbit aorta, as well as rat aorta, high K increased muscle tension and ^{89}Sr uptake in Ca-free, Sr-substituted solution whereas noradrenaline induced only slight changes, suggesting that Sr passes through a voltage-dependent Ca channel but not a receptor-linked Ca channel (Karaki *et al.*, 1984c). In the newborn rabbit aorta, Sr also supported high K-induced contractions but not those induced by noradrenaline (Nakagawa, Karaki & Urakawa, unpublished). (3) In the adult rabbit aorta, noradrenaline induced an additional contraction, as well as an additional increase in ^{45}Ca influx, in the muscle depolarized by high K in which the voltage-dependent Ca channel might have been expected to be fully activated (Karaki & Weiss, 1980; Meisheri *et al.*, 1981). In newborn rabbit aorta depolarized by high K, noradrenaline also induced an additive contraction.

It is concluded that the newborn rabbit aorta may have two types of Ca channels both of which are inhibited by verapamil and also by sodium nitroprusside. These characteristics of newborn rabbit aorta are similar to those of most of other vascular smooth muscle. With increasing age, the voltage-dependent Ca channel seems to lose sensitivity to sodium nitroprusside and receptor-linked channels to verapamil and thus establish the unique characteristics of the adult rabbit aorta.

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