Changes in Postjunctional α-Adrenoceptors during Postnatal Growth in Rabbit Arteries

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SUMMARY The pharmacological characteristics of the postjunctional α-adrenoceptors of vascular smooth muscle were studied in ring segments of thoracic aorta, superior mesenteric, and central ear arteries of 4- and 8-week-old, and young adult (12- to 16-week-old) rabbits. Norepinephrine (α1-agonist), phenylephrine (α2-agonist), and UK 14,304-18 (α2-agonist) caused a concentration-dependent contraction of all three arterial segments from 4- and 8-week-old animals. Norepinephrine and phenylephrine but not UK 14,304-18 contracted the young adult thoracic aorta and superior mesenteric artery, whereas all the agonists contracted the central ear artery. The effects of the α1-adrenoceptor antagonists, prazosin and thymoxamine, and the α2-adrenoceptor antagonists, rauwolscine and yohimbine, on these responses were studied. In the 4-week-old rabbits, responses to norepinephrine, phenylephrine, and UK 14,304-18 were reduced by all four antagonists with nominal pA2 values in the range of 7-8.5. The action of the antagonists was competitive. Between 4 and 8 weeks, there was a significant decrease in the pA2 values of rauwolscine against norepinephrine and UK 14,304-18 in the aorta and between rauwolscine and UK 14,304-18 on the superior mesenteric artery. The pA2 values of rauwolscine and yohimbine, but not prazosin and thymoxamine, were lower in the young adult compared with values from the 8-week-old rabbit. In the interpretation of these results, there are two possibilities. Either both α1- and α2-adrenoceptors are found in arteries from 4- and 8-week-old rabbits and there is a loss of α2-mediated contraction in the adult, or there may be undifferentiated α-adrenoceptors present in arteries of 8-week-old rabbits, and subsequent development favors α1 characteristics (Circ Res 58: 867-873, 1986)

SOME of the adrenergic features of vascular tissue vary with age. Park et al. (1976) and Hayashi and Toda (1978) found that β-adrenoceptor responsiveness of the rabbit aorta shows an age-dependent increase from the neonate to the adult. Subsequently, there is a decrease (Fleisch et al., 1978; Hayashi & Toda et al., 1970). However, not all vessels change in this manner—for example, there are no age-related changes to norepinephrine in the baboon mesenteric artery (Hayashi et al., 1984) or in rat renal and femoral arteries and veins (Duckle et al., 1985).

In vivo studies provide considerable evidence that α1- and α2-adrenoceptors are present in blood vessels, and that both mediate vasoconstriction (Drew and Whitting, 1979; Docherty and McGrath, 1980; Langer et al., 1980). There also is abundant in vitro evidence for α1-adrenoceptors in blood vessels (Langer and Hicks, 1984). In contrast, the in vitro demonstration of vascular α2-adrenoceptors has been less successful, except in the case of the dog saphenous vein (De Mey and Vanhouette, 1981; Shepperson and Langer, 1981), the basilar artery (Sakakibara et al., 1981), the feline cerebral artery (Skarby et al., 1983), and rat tail artery (Medgett and Langer, 1984).

This study was designed to determine whether contractions mediated through α-adrenoceptor subtypes present in vascular smooth muscle of three rabbit arteries change during postnatal growth. Our findings suggest that the α-adrenoceptors mediating contraction of arteries from 4- and 8-week-old animals show both α1- and α2-like properties, in contrast to the predominantly α1-adrenoceptor-mediated response in young adult rabbits, 12-16 weeks old. Our studies were conducted in the thoracic aorta, central ear, and superior mesenteric arteries. The thoracic aorta and central ear artery were chosen because they are the most completely studied of vessels in the adult rabbit with respect to their α-adrenoceptors. The α-adrenoceptors of the adult central ear artery have unusual features (Tayo, 1982; Purdy et al., 1983), and this is reflected in some of our results. The superior mesenteric artery supplies the vascular bed most responsible for changes in adrenergic and therefore α-adrenoceptor-mediated vascular resistance.

Methods

General

Three age groups of New Zealand white male rabbits were studied, 4 weeks (body weight 0.4-0.5 kg), 8 weeks (body weight 0.8-1.2 kg), and 12-16 weeks, the latter designated as young adults, with body weights 2.2-2.6 kg. The animals were stunned and exsanguinated, and the central ear artery (CEA), superior mesenteric artery (SMA), and thoracic aorta (TA) were quickly removed and placed...
in a physiological salt solution (PSS) of the following composition (mmol/liter). Na⁺, 144.2; K⁺, 4.9; Ca²⁺, 1.6; Mg²⁺, 1.2; Cl⁻, 126.7; HCO₃⁻, 25.0; SO₄⁻, 1.19; glucose, 11.1; EDTA, 0.024; and ascorbic acid, 0.11. The PSS was gassed with 95% O₂:5% CO₂. The vessels were freed of adhering tissues under a dissecting microscope. Three-millimeter ring segments were mounted in 50-ml organ baths containing PSS at 37°C for isometric tension measurements, according to the method of Bevan and Osher (1972). The pH of the solution was maintained at 7.40 ± 0.06. The vessels were equilibrated for 90 minutes with a solution change every 15 minutes. At the end of the equilibration time, the arteries were subjected to an optimal resting tension. This was the tension at which a standard concentration of NE in the region of the ED₅₀ caused maximum contraction. This value was determined for each age group each week from the active length-tension relationship obtained with norepinephrine (10⁻⁷ M). In preliminary experiments, we determined that the optimum resting tension for α₁- and α₂-adrenoceptor-mediated responses in arteries from 4- and 8-week-old rabbits were not different. The experiment was continued only after two separate contractions to norepinephrine (NE 10⁻⁵ M) that were within 10% of each other had been obtained, and after the tissue had been washed several times and then rested for a further 60 minutes. One concentration-response curve per tissue was obtained for analysis.

Receptor Characterization

Responses were obtained to increasing concentrations of the agonists added cumulatively once the response to the previous concentration had plateaued. At the end of the first concentration-response determination, the tissue was washed at 10-minute intervals over 40 minutes to ensure maximum washout of drugs and to enhance the possibility of receptor desensitization (Tayo, 1979; Ruffolo et al., 1979). In all experiments, one vascular segment was set up as a control and run in parallel with adjacent experimental tissues. Control arteries received agonists but no antagonist, and they were used to correct for any time-dependent change in agonist sensitivity (Furchgott, 1972). Antagonists were added to the bath 60 minutes before the agonists for adequate tissue equilibration. pA₂ values were measured according to the method of Arulalakshana and Schild (1959). A minimum of three, but usually more, antagonist concentrations were utilized within the range shown in Results. A Schild slope different from unity was taken as evidence of noncompetitive antagonism.

All experiments were performed in the presence of desipramine (10⁻⁵ M), deoxycorticosterone acetate (DOCA) (10⁻⁵ M), and propranolol (10⁻⁷ M) to block neuronal uptake, extraneuronal uptake, and β-adrenoceptors, respectively (Furchgott, 1972).

Statistical Analysis

Results are expressed as means ± SEM. The difference between two means was analyzed by Student's unpaired t-test. P < 0.05 was the accepted level of significance. Linear regressions were drawn, and these were tested, when applicable, for deviations from linearity by analysis of variance (ANOVA) in regression (Sokal and Rohlf, 1969). The slopes of regression lines were tested for significance by an F-test (Woolf, 1968).

Results

The results selected for citation in the text are used to support the hypothesis that the α₁-subtype is the dominant adrenoceptor involved in blood vessels of young adults leading to contraction, but that arteries from younger rabbits show both α₁ and α₂ characteristics. Whereas this seems to be clearly the case for the thoracic aorta and superior mesenteric artery, the results with the central ear artery are, only in part, consistent with this hypothesis. Although data for all agonists and antagonists are presented, only those values that fulfill criteria for competitive antagonism will be used to support our proposal. To simplify the text, statements of similarity imply no significant difference at 0.05 level.

α-Adrenoceptor Agonists

Alpha 1

All arterial segments at all ages responded to norepinephrine and phenylephrine (Fig. 1). The SMA was less sensitive than the other two vessels (Fig. 1; Table 1). This is in agreement with the results obtained by Bevan et al. (1983).

Alpha 2

All arterial segments from the 4- and 8-week-old rabbits responded to UK 14,304-18 (Fig. 1). In the young adult, the CEA, but not the TA and SMA, responded to this drug (Table 1; Fig. 1). Sensitivity of all arteries to norepinephrine, phenylephrine, and UK 14,304-18 was unchanged for the period examined.

α-Adrenoceptor Antagonists

Alpha 1

Prazosin. At all three age groups, this α₁-adrenoceptor antagonist (Cambridge et al., 1977; Doxey et al., 1977) in the concentration range (10⁻⁸ M to 10⁻⁶ M) was competitive for the same receptor as norepinephrine and phenylephrine in the TA with pA₂ values in the range 7.5–8.5. However, as the Schild slope was significantly different from 1.0, prazosin did not fulfill the requirements for such antagonism in the SMA and CEA at any of the ages studied (Fig. 2).

Thymoxamine. This predominantly α₁-antagonist (Drew, 1976) in the 4-week, 8-week, and young adult rabbit in the dose range 10⁻⁸ to 10⁻⁶ M was a
competitive antagonist of norepinephrine and phenylephrine in the TA, SMA, and CEA, with pA₂ values within the range 7.50 and 8.1 (Fig. 2).

Alpha 2

Yohimbine. In the young adult rabbit high concentrations of yohimbine (10⁻⁶ M to 10⁻⁵ M) antagonized the responses of the TA to norepinephrine and phenylephrine [pA₂ 4.75 and 4.35, respectively (Fig. 2)]. The SMA and CEA responses to norepinephrine and phenylephrine also required high concentrations for antagonism (pA₂ 5.1-5.5). Lower concentrations of yohimbine (10⁻⁷ M to 10⁻⁸ M) caused contraction. In every case, the antagonism was competitive (see Fig. 2). In the CEA, the contraction due to UK 14,304-18 was competitively antagonized by yohimbine (10⁻⁸ M to 10⁻⁷ M), with a mean pA₂ value that was higher than those for other agonists (Fig. 2).

In arteries from the 4- and 8-week rabbits, yohimbine was a competitive antagonist of norepinephrine, phenylephrine, and UK 14,304-18 at much lower concentrations than in the young adult (10⁻⁶ M to 10⁻⁷ M) corresponding to a pA₂ range of 8.7-6.7 in all vessels (Fig. 2). The only exception was phenylephrine on CEA, where yohimbine enhanced the agonist effect. In all instances in which antagonism was competitive and fulfilled the necessary criteria, pA₂ values were less in the young adults compared with values from either 4- or 8-week-old animals (see Fig. 2). This trend toward a requirement for a higher concentration of yohimbine with maturation was seen at 8 weeks for UK 14,304-18.

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**Figure 1.** Log (molar) concentration-response curves of norepinephrine (NE) (●), phenylephrine (PE) (■), and UK 14,304-18 (▲) on segments of thoracic aorta and superior mesenteric and central ear arteries from 4- and 8-week-old and young adult rabbits. Note that young adult arteries (except the CEA) did not contract to UK 14,304-18. Each point is the mean ± se of at least six determinations for norepinephrine and at least four determinations each for phenylephrine and UK 14,304-18.
TABLE 1
—Log EC50 Values of Agonists in the Thoracic Aorta, Superior Mesenteric and Central Ear Arteries of 4-Week, 8-Week, and 12- to 16-Week-Old Rabbits

<table>
<thead>
<tr>
<th></th>
<th>4-Wk</th>
<th>8-Wk</th>
<th>Young adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thoracic aorta</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>7.80 ± 0.60 (10)</td>
<td>7.60 ± 0.22 (18)</td>
<td>7.50 ± 0.15 (15)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>7.50 ± 0.38 (6)</td>
<td>7.60 ± 0.42 (10)</td>
<td>7.80 ± 0.65 (10)</td>
</tr>
<tr>
<td>UK 14,304-18</td>
<td>6.70 ± 0.30 (6)</td>
<td>7.20 ± 0.55 (6)</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Superior mesenteric artery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>6.30 ± 0.20 (6)</td>
<td>6.0 ± 0.30 (8)</td>
<td>5.80 ± 0.3 (10)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>5.50 ± 0.30 (4)</td>
<td>5.50 ± 0.50 (6)</td>
<td>5.80 ± 0.4 (6)</td>
</tr>
<tr>
<td>UK 14,304-18</td>
<td>5.30 ± 0.20 (4)</td>
<td>5.60 ± 0.20 (4)</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Central ear artery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>7.0 ± 0.30 (10)</td>
<td>7.52 ± 0.35 (15)</td>
<td>7.65 ± 0.22 (15)</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>7.10 ± 0.50 (6)</td>
<td>7.50 ± 0.15 (8)</td>
<td>7.55 ± 0.10 (10)</td>
</tr>
<tr>
<td>UK 14,304-18</td>
<td>6.70 ± 0.10 (4)</td>
<td>6.50 ± 0.20 (4)</td>
<td>6.0 ± 0.10 (6)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE Numbers in parentheses = number of observations

Rauwolscine. In the young adult, only high concentrations of rauwolscine (10^-5 M and above) antagonized the action of norepinephrine and phenylephrine on TA, CEA, and SMA. In the CEA, as with yohimbine, lower concentrations (10^-9 M to 10^-8 M) of rauwolscine caused contraction and enhanced the effect of phenylephrine. In 4- and 8-week-old arteries, yohimbine produced somewhat similar results. Lower concentrations (10^-9 M to 10^-8 M) were effective in antagonizing all agonists in all vessels with pA2 values of 7.0-8.5 (see Fig 2). In the TA and SMA, the pA2 values against norepinephrine at 8 weeks were lower than at 4 weeks; a similar change was obtained with UK 14,304-18 on the TA. In all instances, there was a significant difference between results obtained with 8-week-old and young adult rabbits.

Discussion

The present study was undertaken to test the hypothesis that changes take place in the characteristics of the α-adrenoceptor (mediating contraction) of arterial smooth muscle during postnatal development. That both subtypes of α-adrenoceptors are present in the artery wall at 4 and 8 weeks of age is supported by our findings that (1) not only norepinephrine, but UK 14,304-18, a potent and selective α1-agonist (Cambridge, 1981), contracted arterial segments from 4- and 8-week-old rabbits. The pA2 values obtained are in the range usually cited in the literature for action at α1-adrenoceptors (Drew, 1976; Doxey et al., 1977; Agrawal et al., 1984). (4) α1 pA2 values do not, whereas α2 pA2 values do, change with increasing age. Thus, the pA2 of prazosin and thymoxamine remained essentially constant, and the values for yohimbine and rauwolscine decreased significantly between 8-week and young adult rabbits.

Evidence that α2-adrenoceptors are not involved in the contractions in the young adult animal is as follows: (1) The α2-agonist, UK 14,304-18, did not contract young adult arteries, with the exception of CEA, suggesting that α2-adrenoceptors may be absent postjunctionally, at least in the TA and SMA at this age. (2) There was no difference in the pA2 values for α1-antagonists in the 8-week-old and young adult rabbit, whereas, in all cases, the values for α2-antagonists were different for these two ages. It is doubtful whether our results could be explained by increasing α2-adrenoceptor desensitization with age, in that α2-agonists did not contract adult thoracic aorta and superior mesenteric artery. One would expect an initial contractile response and then desensitization. This was not the case. In all age groups, responses to the agonists can be elicited repeatedly without an appreciable decrease, once the tissue has stabilized.

Whereas the above evidence supports our hypothesis, the results with phenylephrine on the CEA are not consistent with it. Thus, although α1-antagonists reduced the effect of phenylephrine in the 4- and 8-week-old CEA, both α2-antagonists either had no inhibitory effect, or enhanced its action. They did antagonize contraction in the adult.

Based on the present subclassification of α-adrenoceptors (McGrath, 1982), our results suggest the presence of both α1- and α2-adrenoceptors in the
TA and SMA of the 4- and 8-week-old rabbits. In young adult vessels, only the $\alpha_1$ type seems to be involved in contraction. The very low $pA_2$ values for $\alpha_2$-antagonism on contraction in the young adult vessels (4.60–5.8) suggest that this may be due to their relatively lower affinity for the $\alpha_1$-subtype adrenoceptor (Weitzell et al., 1979; Starke, 1981; Doxey et al., 1984). The $pA_2$ values obtained for prazosin, rauwolscine, thymoxamine, and yohimbine are similar to those obtained by Drew (1976), Doxey et al. (1977), Tayo (1979), and Weitzell et al. (1979).

The difference seen between $pA_2$ values for $\alpha_1$-adrenoceptor antagonists between the 4-week and young adult vessels is reflected in several changes seen between 4- and 8-week-old arteries; for rauwolscine against NE and UK 14,304-18 in the aorta, and rauwolscine and UK 14,304-18 on the SMA. In the present study, the antagonist action of prazosin, except in the TA, was noncompetitive. This contrasts with many reports of competitive antagonism for this drug. However, the data agree with the results of Agrawal et al. (1984) who tested prazosin against phenylephrine and found a $pA_2$ of 8.75 in the canine mesenteric artery. Yohimbine competitively reduced the effect of phenylephrine in the same preparation (with a $pA_2$ of 7.13), suggesting the presence of the two subtypes. Randrianaroa et al. (1981) obtained a Schild plot slope significantly different from unity for prazosin antagonism of phenylephrine and clonidine in the rat aorta. This may indicate that more than one subtype of $\alpha$-adrenoceptor is activated by high doses of the agonists, and prazosin blocks only one of these sites (Furchgott, 1972; Kenakin, 1982). Other known examples of Schild slopes less than 1 for prazosin are in the rat renal vein (Cohen et al., 1979), cat hepatic vein (Greenway, 1979), and canine cutaneous veins (Rusch et al., 1980).

UK 14,304-18 contracted the young adult CEA, but not the TA and SMA, an action blocked competitively by yohimbine ($pA_2$ 6.5 similar to 6.68 obtained for yohimbine-guanfacine ($\alpha_2$-agonist) interaction in the rat aorta (Digges and Summers, 1983]) and noncompetitively by prazosin ($pA_2$ 7.10).
This value is rather low for yohimbine \( \alpha_2 \)-antagonism (literature values, 7–8). In addition, the resistance of phenylephrine to yohimbine antagonism in the 4- and 8-week-old CEA is also unique. These two results call for caution in interpreting results obtained with the CEA. This artery is different in its behavior toward some adrenoceptor antagonists. For instance, yohimbine in low concentrations constricts the perfused adult CEA, an action blocked by phentolamine with pA\(_2\) values similar to those against norepinephrine and serotonin (Tayo, 1982). Labetalol, an \( \alpha \) - and \( \beta \)-adrenoceptor antagonist (Farmer et al., 1972), contracted ring segments of adult CEA but blocked norepinephrine-evoked contractions of the rabbit aorta (Purdy et al., 1983).

In conclusion, our findings suggest one of two possibilities: (1) There are both \( \alpha_1 \) - and \( \alpha_2 \)-adrenoceptors located postjunctionally in the 4-week-old rabbit artery segments whose occupation leads to contraction, and that some aspect of the \( \alpha_2 \) feature decreases over the next few weeks. This could be the receptor number or some other characteristic of the receptor or of the postsynaptic events that are specific to it. The other possibility is (2) that the \( \alpha \)-adrenoceptors mediating the action of agonists and antagonists in these vessels are not functionally differentiated into \( \alpha_1 \)- and \( \alpha_2 \)-subtypes in the younger animals, and differentiation takes place later in life in favor of \( \alpha_1 \)-adrenoceptor characteristics. Our data do not allow us to distinguish between these possibilities.

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