Effects of aging on $\alpha_1$-adrenoeceptor mechanisms in the isolated mouse aortic preparation

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Abstract

The effects of aging on $\alpha_1$-adrenoeceptor ($\alpha_1$-AR)-mediated contractile response in endothelium-removed aortic preparations isolated from 5- to 40-week-old (5, 6, 8, 10, 20-, 40-weeks) mice were studied in the presence of propranolol. The potency of noradrenaline, estimated as its $pD_2$ value, increased with age from 5- to 10-weeks, but decreased thereafter with age from 10- to 40-weeks. However, the affinity of prazosin ($pA_2$ value) did not change with aging. These results suggest that age-related change in noradrenaline potency is not attributable to the change of drug affinity to $\alpha_1$-ARs, but is possibly due to drug affinity-unrelated factors such as change of the reserve of $\alpha_1$-ARs.

Key words: $\alpha_1$-adrenoeceptor, aging, mouse, noradrenaline, thoracic aorta

Introduction

The rat has been used most frequently as a standard experimental animal model for studies of vascular pharmacology from drug receptors to signaling transduction cascades. However, the mouse is recently used increasingly for vascular investigation because a wide variety of gene deletion and overexpression are available in this rodent species. Accordingly, fundamental characteristics regarding vascular contraction and relaxation of normal (wild) mouse are being revealed (Akimoto et al., 2002; Ashton et al., 2000; Chan and Fiscus, 2001; Chruscinski et al., 2001; Hosoda et al., 2005; Pomerleau et al., 1997; Russell and Watts, 2000; Shibano et al., 2002; Yamamoto and Koike, 2001a, 2001b). Distribution of $\alpha_1$-adrenoeceptor ($\alpha_1$-AR) subtypes is one of such issues that are being focused. $\alpha_1$-ARs in native tissues are now classified into three subtypes: $\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$. These three subtypes ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$) exhibit high affinity characteristics for prazosin ($pA_2 > 9$) and thus can be included into $\alpha_{1H}$-AR whereas $\alpha_{1L}$-AR shows low affinity characteristics for prazosin ($pA_2 < 9$) (Muramatsu et al., 1995; Muramatsu et al., 1990; Shibano et al., 2002). In this regard, we have determined principal $\alpha_1$-AR subtypes mediating...
noradrenaline-induced contraction in various regions of mouse vascular beds (Shibano et al., 2002; Yamamoto and Koike, 2001a, 2001b). Our pharmacological analysis using subtype-selective $\alpha_1$-AR antagonists (5-methylurapidil, 2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane hydrochloride (WB 4101)) in normal mouse showed that $\alpha_{1D}$ is a principal contraction mediator in thoracic aorta, upper abdominal aorta and superior mesenteric artery whereas $\alpha_{1A}$ is superior in renal artery, lower abdominal aorta and iliac artery (Shibano et al., 2002; Yamamoto and Koike, 2001a, 2001b). The rationality of this view was strongly substantiated by the analysis of vascular contractions in $\alpha_1$-AR subtype specific knock out mice (Hosoda et al., 2005). This region-specific distribution of $\alpha_1$-AR subtypes might be a crucial determinant to affect mechanical functions of each vascular bed.

Besides vascular region differences, another factor which affects $\alpha_1$-AR-mediated vascular responsiveness is aging. This view is strongly supported by several lines of evidence obtained in the studies employing vascular beds from the rat ( Docherty, 1988; Hyland et al., 1987; McAdams and Waterfall, 1986; Takayanagi et al., 1989) and the rabbit (Hayashi and Toda, 1978). In this regard, Takayanagi et al. provided evidence showing that potency of noradrenaline in eliciting aortic contraction exhibits biphasic changes depending on weekly-age in the rat; $pD_2$ value (an index of agonistic potency) of noradrenaline increases from 3- to 10-weeks whereas it decreases from 10- to 40-weeks (see Table 1) (Takayanagi et al., 1989). Based on the investigations into the changes in the affinities of noradrenaline ($pK_A$ value) and prazosin ($pA_2$ value), the biphasic changes in noradrenaline potency with weekly aging was indicated to be ascribed to a drug affinity-unrelated ingredient (an alteration in receptor reserve). However, little is known about the effects of aging on $\alpha_1$-AR-mediated responsiveness in mouse vascular smooth muscles. In the present study, we investigated possible changes in the potency of noradrenaline for eliciting contractile response in mouse thoracic aorta and will provide evidence showing that contractile potency of noradrenaline changes basically in the same manner as in the rat.

Methods

Animals

Male albino ddY mice (5-, 6-, 8-, 10-, 20- and 40-weeks old) (Murai Experimental Animals, Saitama, Japan) were used in the present study. Mice were housed under laboratory standard conditions on a 12-h light/dark cycle (lights on 8:00 a.m.; lights off 8:00 p.m.) in the rooms in which temperature (20-22°C) and relative air humidity (50 ± 5%) were strictly regulated. Food and water were available ad libitum to all animals. This study was conducted in accordance with the Guideline for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Toho University School of Pharmaceutical Sciences (accredited by The Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan).

Preparation of thoracic aorta

Mice were stunned and killed by cervical dislocation and exsanguinated. A section of the
thoracic aorta between aortic arch and diaphragm was removed and placed in Krebs solution (mM: NaCl, 118; KCl, 4.75; CaCl₂, 2.54; MgSO₄, 1.20; KH₂PO₄, 1.19; NaHCO₃, 25.0 and D-(+)-glucose, 11.0), which was entirely gassed with a mixture of 95% O₂-5% CO₂. The aorta was cleaned of loosely adhering fat and connective tissue under a dissecting microscope. The aorta was then helically cut into a section approximately 15 mm in length and 1 mm in width. Only one helical strip preparation was obtained from each aorta. The endothelium was removed by gentle rubbing of the intimal surface with a moistened filter paper.

Measurement of tension changes

The spiral segments were mounted under the optimal resting tension of 0.1–0.2 g in an organ bath containing 20-ml Krebs solution. Tension changes of the muscle preparation were isometrically recorded with a force-displacement transducer (TB-612T, Nihon Kohden, Tokyo, Japan) connected to a carrier amplifier (AP-621G, Nihon Kohden). The aortic preparations were equilibrated for 60–90 min prior contractions by successive 2–3 times applications of noradrenaline (10⁻⁸ M) with 30-min washout interval. In the last time of this muscle contraction by noradrenaline, the lack of relaxation by acetylcholine (ACh, 10⁻⁵ M) was confirmed to assure the absence of endothelial cells. Noradrenaline-induced contraction experiments were started further after a 30-min equilibration period. Krebs solution was continuously gassed with 95% O₂-5% CO₂, and kept at 36.5 ± 0.5°C (pH=7.4). The following drugs were present in the bath solution throughout the experiments: propranolol (10⁻⁶ M, a β₁-/β₂-AR antagonist), yohimbine (3 × 10⁻⁷ M, an α₂-AR antagonist), desipramine (3 × 10⁻⁷ M, a neural uptake inhibitor), deoxycorticosterone acetate (10⁻⁵ M, a non-neural uptake inhibitor), indomethacin (3 × 10⁻⁶ M, a cyclo-oxygenase inhibitor), L-ascorbic acid (10⁻⁵ M, an antioxidant for noradrenaline).

Construction of the concentration-response curve for noradrenaline in the absence and presence of prazosin

The concentration-response curves to noradrenaline were constructed in the absence and presence of prazosin. For this purpose, noradrenaline (10⁻¹⁰ – 3 × 10⁻⁷ M) was added cumulatively in the absence of prazosin to obtain control noradrenaline concentration-response curve. This procedure was repeated at least two times until almost the same concentration-response curves were obtained consecutively, and the last concentration-response curve was regarded as the control response. When the maximum contractile response to noradrenaline was obtained, the bath solution was exchanged to a fresh medium for several times. After a 30-min equilibration time with a 15-min wash out interval, 10⁻⁹ M prazosin was applied and the preparation was incubated for further 30-min. After this period, noradrenaline concentration-response curve was obtained in the presence of 10⁻⁹ M prazosin. These procedures were repeated in the presence of prazosin at 3 × 10⁻⁹ M and 10⁻⁸ M.

Drugs

The following drugs were used in the present study: (R)-(−)-noradrenaline hydrogen tartrate, yohimbine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan); prazosin hydrochloride, (±)-propranolol hydrochloride, desipramine hydrochloride, deoxycorticosterone
acetate, indomethacin, L-ascorbic acid (Sigma-Aldrich, St. Louis, Mo., USA). The other chemicals used were of analytical grade. Deoxycticotinesterone and indomethacin were dissolved in 100% ethanol as a stock solution of $10^{-2}$ M. Distilled water was used for dissolution and dilution of all other drugs. Drug concentrations were expressed as mol/L (M) order.

Data analysis and statistics

To construct concentration-response curves for noradrenaline, percentage of the contractile response was calculated considering the tension level before application of noradrenaline as 0% and the maximum contraction induced by $3 \times 10^{-7}$ M noradrenaline in the absence of prazosin as 100%.

Data were plotted as a function of noradrenaline concentration and fitted to the equation:

$$E = \frac{E_{\text{max}} \times A^{n_H}}{EC_{50} + A^{n_H}}$$

where $E$ is % contraction at a given noradrenaline concentration, $E_{\text{max}}$ is the maximum contraction at $3 \times 10^{-7}$ M in the absence of prazosin, $A$ is the concentration of noradrenaline, $n_H$ is the slope function and $EC_{50}$ is the effective noradrenaline concentration that produce a 50% contractile response. The curve fitting was carried out using GraphPad Prism™ (Version 4.00) (GraphPad Software, Inc., San Diego, Calif., USA). The EC$_{50}$ values were converted to logarithmic values (pD$_2$, -logEC$_{50}$) for statistical analysis.

The competitive antagonistic potency of prazosin was expressed as \( pA_2 \) value, which was calculated according to the method originally reported by Arunlakshana and Schild (Arunlakshana and Schild, 1959).

Data are presented as means ± S.E.M. or mean values with 95% confidence intervals and $n$ refers to the number of experiments. The significance of the difference between mean values was evaluated with GraphPad Prism™ by one-way analysis of variance (one-way ANOVA) followed by Tukey’s multiple comparison test. A $P$ value less than 0.05 was considered statistically significant.

Results

All aortic preparations from mice of different ages responded to noradrenaline with concentration-dependent contraction. However, sensitivity of thoracic aorta to noradrenaline was not consistent in all ages tested (5- to 40-weeks). pD$_2$ value of noradrenaline increased from 5-weeks to 10-weeks, and the difference was statistically significant ($P<0.01$) between 5-weeks and 10-weeks. The pD$_2$ value of noradrenaline decreased thereafter from 10- to 40-weeks, although the pD$_2$ for 20-weeks was still significantly higher than the 5-week’s value (Fig. 1 and Table 1).

Concentration-response curves to noradrenaline were shifted in a parallel fashion by an \( \alpha \)-AR-selective antagonist, prazosin ($10^{-9}$ – $10^{-8}$ M), in all mouse preparations from different ages. Figure 2A shows the results from 6-week old mice as a typical one. Schild plot yielded a straight line, the slope of which was not significantly different from of 1.0 (Fig. 2B). pA$_2$ values of
Fig. 1. Changes in pD₂ value of noradrenaline in thoracic aorta from mice of different ages (5- to 40-weeks). Each value is presented as means ± S.E.M. of 5 to 13 preparations (one preparation per one animal). a) Significantly different from 5-week old mice (P<0.01); b) significantly different from 10-week old mice (P<0.05 for 20-weeks or P<0.01 for 5, 6, 8, 40-weeks).

Table 1  pD₂ values of noradrenaline and pA₂ values of prazosin vs. noradrenaline in thoracic aorta from different ages of mouse and rat

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Mouse (the present study)</th>
<th>Rat</th>
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<tbody>
<tr>
<td></td>
<td>Noradrenaline pD₂</td>
<td>Noradrenaline pD₂</td>
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<td></td>
<td>n</td>
<td>pA₂ Slope</td>
</tr>
<tr>
<td>3</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>5</td>
<td>8.20 ± 0.04 e</td>
<td>10.20</td>
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<tr>
<td>6</td>
<td>8.22 ± 0.05 d</td>
<td>10.22</td>
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<tr>
<td>8</td>
<td>8.31 ± 0.04 d</td>
<td>10.07</td>
</tr>
<tr>
<td>10</td>
<td>8.64 ± 0.04 d</td>
<td>10.04</td>
</tr>
<tr>
<td>18</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>20</td>
<td>8.44 ± 0.06 d</td>
<td>10.10</td>
</tr>
<tr>
<td>40</td>
<td>8.05 ± 0.08 b</td>
<td>9.84</td>
</tr>
</tbody>
</table>

Each value is presented as means ± S.E.M. or means with 95% confidence intervals shown in parentheses from n numbers of experiments. The pD₂ value (an index of agonist potency) of noradrenaline increased with age from 5- to 10-weeks, but decreased thereafter with age from 10- to 40-weeks. The pA₂ value (an index of antagonist affinity) of prazosin did not alter with aging.


bSlope of Schild plot for antagonism between noradrenaline and prazosin.

Not determined.

Significantly different from 10-week old mice (P<0.05 for 20-weeks or P<0.01 for 5, 6, 8, 40-weeks).

Significantly different from 5-week old mice (P<0.01).

prazosin against noradrenaline estimated by Schild plot analysis did not significantly differ in all ages from 5 to 40 weeks (Table 1).

Discussion

α₁-ARs in native tissues are classified pharmacologically into α₁H and α₁L, and α₁H is
subdivided into $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$, the cloned counterparts of which correspond to $\alpha_{1a}$, $\alpha_{1b}$, and $\alpha_{1d}$ (Muramatsu et al., 1995; Muramatsu et al., 1990; Shibano et al., 2002). Among these $\alpha_1$-AR subtypes, the principal $\alpha_1$-AR mediating noradrenaline-induced contraction of mouse thoracic aorta is $\alpha_{1D}$ (Hosoda et al., 2005; Yamamoto and Koike, 2001b). Therefore, $\alpha_1$-AR that is focused in the present study is regarded as $\alpha_{1D}$.

In the present study, we showed that the potency of noradrenaline estimated as its $pD_2$ value changed with aging. However, age-related change in the potency of noradrenaline was not a unidirectional increment or decrement but was biphasic in its characteristic; the noradrenaline potency increased significantly from 5- to 10-weeks whereas it decreased thereafter from 10- to 40-weeks. This biphasic feature of age-related change in the noradrenaline potency is well consistent with the findings in rat thoracic aorta (Takayanagi et al., 1989) and rat vas deferens (Takayanagi et al., 1987), the noradrenaline-induced contractions of which are mediated primarily through $\alpha_1$-AR. These findings in the mouse and the rat suggest that $\alpha_1$-AR-mediated contractile process in thoracic aorta changes with aging.

One of the rationales explaining the present finding is that changes in the potency of noradrenaline are due to changes in the affinity of noradrenaline to $\alpha_1$-ARs ($\alpha_{1D}$). However, this possibility may be ruled out since $\rho A_2$ value of prazosin vs. noradrenaline did not change with aging. In this regard, Takayanagi et al. (Takayanagi et al., 1989) also reported that the $\rho A_2$ value of prazosin vs. noradrenaline was practically the same from 3- to 40-weeks, precluding the possible variations in drug affinity to $\alpha_1$-ARs. They also showed that $pK_A$ value (minus logarithm of dissociation constant) of noradrenaline, an index of the affinity of noradrenaline vs. $\alpha_1$-ARs, was consistent from 3- to 40-weeks, strongly supporting the view that drug affinity to $\alpha_1$-ARs

![Fig. 2. Antagonistic effects of prazosin on the concentration-response curve for noradrenaline in aorta from 6-week-old mice. A: Concentration-response curves for noradrenaline in the absence and presence of prazosin ($10^{-9} - 10^{-8}$ M). Abscissa: logarithm of molar concentrations (M) of noradrenaline. Ordinate: noradrenaline contraction expressed as percentage against the maximum response to noradrenaline ($3 \times 10^{-7}$ M) in the absence of prazosin. Each value is presented as means ± S.E.M. of 7 experiments. B: Schild plot analysis carried out for prazosin against noradrenaline. The data analyzed are from experiments shown in A. CR: concentration ratio for noradrenaline, which was calculated as the ratio of noradrenaline EC50 values in the absence and presence of each concentration of prazosin.](image-url)
remains invariable without being affected by aging (Takayanagi et al., 1989). On the other hand, pD2 values of noradrenaline in different aged rat were found to be proportional to the differences between pD2 and pK_A values (pD2 - pK_A values) (Takayanagi et al., 1989). Since pD2 - pK_A value is an index of adrenoceptor reserve (Kenakin, 1984), changes in ß-AR mechanisms with aging are due to those in receptor reserve rather than drug affinity changes to ß-ARs (Takayanagi et al., 1989). This view seems to be applicable to the phenomenon observed in mouse thoracic aorta presented in this study.

In conclusion, the present study showed for the first time that the potency of noradrenaline in eliciting contraction of thoracic aorta changes with aging, reaching the highest at ~10-weeks in the mouse. As an inducer of these age-related changes in the potency of noradrenaline, alterations in the drug affinity to ß-ARs may be ruled out, but could be accounted for by changes in possible reserve of ß-ARs (ß_R).

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References


