Effect of Aging on the Response of Guinea Pig Trachea to Isoprenaline

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Abstract—To study the effect of aging on the β-adrenergic receptor potency of isoprenaline, tracheas from 3-, 6-, 10-, 40- and 100-week-old guinea pigs were used as test tissues. The pD₂ values (potency) of isoprenaline increased with age in tracheas treated with corticosterone, but decreased in untreated tracheas. The pA₂ value of propranolol against isoprenaline estimated in the treated tracheas did not change with age. Specific binding of [³H]-dihydroalprenolol to the microsomal fractions from the tracheal muscles from 6-, 10- and 40-week-old guinea pigs was analyzed with Scatchard plots. The capacity of maximum binding sites of [³H]-dihydroalprenolol increased with increasing age, while its dissociation constant did not change. Age-related increase in the potency of isoprenaline in the tracheas treated with corticosterone is considered to be due to the increase in the total amount (density) of β-adrenoceptors. However, the potency of the drug in untreated tracheas decreased with age. These results suggest that extraneuronal uptake plays an important role in the β-adrenergic action of isoprenaline in older (40 and 100 weeks) guinea pigs.

A reduction in airway reactivity during aging is well-documented both in man and animals (1, 2). It was reported that adrenergic control of cardiovascular function declines with age (3). This conclusion was drawn from observations that norepinephrine concentrations in the myocardium decline with increasing age (4–6) and that adrenergic terminal degeneration increase with age (7). We recently reported that the changes in postsynaptic α₁-adrenoceptor mechanisms in vasa deferentia (8) and in aortic strips (9) from rats of different ages and those in the presynaptic α₂-adrenoceptor mechanisms (10) in ileal preparations from guinea pigs of different ages are due to a change in the total amount (density) of receptors, but not to a change of dissociation constant. In this paper, to study the effect of aging on β-adrenoceptor activity in the tracheal preparations, we tested the mechanical response to isoprenaline in tracheal preparations untreated and treated with corticosterone, an extraneuronal uptake inhibitor, and estimated the pA₂ value (the negative logarithm of the dissociation constant) of propranolol against isoprenaline in the treated preparations from guinea pigs of different ages. To study the change in the dissociation constant and maximum binding sites of β-adrenoceptors during aging, we estimated the specific binding of [³H]-dihydroalprenolol to microsomal fractions from the guinea pig trachea of different ages and analyzed it by Scatchard plots.

Materials and Methods

Mechanical response: Male Hartley strain guinea pigs (3 weeks old: 202±7.2 g in body weight, 6 weeks old: 335±8.6 g, 10 weeks old: 540±8.8 g, 40 weeks old: 1049±14.4 g and 100 weeks old: 1106±38.1 g; body weight was expressed as the mean±S.E. of about 25 animals) were killed by a blow on the neck, exsanguinated and airways and lungs were isolated. After removal of the trachea, the tracheal strip preparations were made by the method of Takagi and Takayanagi (11) and suspended in a 20-ml organ bath
filled with a physiological solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂, 2.1 mM MgCl₂, 5.9 mM NaHCO₃ and 2.8 mM glucose) kept at 32°C and gassed with a mixture of 95% O₂ and 5% CO₂. The physiological solution contained ascorbic acid (10⁻³ M), desmethylimipramine (10⁻⁷ M) and pargyline (10⁻⁶ M) to inhibit the oxidation and neuronal uptake of isoprenaline and monoamine oxidase. To block α-adrenoceptors, the solution also contained phenotolamine (10⁻⁶ M).

The preparation was attached via a thin thread to an isotonic transducer under a tension of 0.5 g for isotonic recording of responses. The experiments were started after the preparation had been allowed to develop a spontaneous tone for 90 min. A concentration-response curve of an agonist was obtained at intervals of 60 min. The concentration-response curve of isoprenaline was plotted as a fraction of the response to papaverine (10⁻⁴ M). The agonistic activity of isoprenaline was expressed as the pD₂ value, which is the negative logarithm of the concentration (M) necessary to produce a 50% response. The negative logarithm of dissociation constant of a competitive antagonist was expressed as the pA₂ value. After determination of a control concentration-response curve of the agonist, the preparation was equilibrated with a competitive antagonist for 30 min. A concentration-response curve to isoprenaline was then obtained in the presence of the antagonist and the procedure repeated with a high (either 3-fold or 10-fold) concentration of the antagonist in the same preparation. The pA₂ value was calculated by the method of Arunlakshana and Schild (12) as modified by Tallarida et al. (13). To test the effect of corticosterone on isoprenaline, the tracheal preparation was treated with corticosterone (3×10⁻⁵ M) for 60 min, and the responses were obtained.

After the control concentration-response curves of the agonist were obtained, 2 or 3 successive cumulative curves were determined. Curves were nearly superimposable and changes in sensitivity, sensitization and desensitization were small (data not shown).

Preparation of microsomal fractions: The tracheas isolated from 6-, 10- and 40-week-old guinea pigs were washed with the physiological solution. About 100 guinea pigs were used in each experiment. As the same experiment was repeated 3 times in the binding assay, we used about 300 animals to obtain each result. Tracheal cartilages and connective tissues were cut away until nothing was left except the tracheal smooth muscle (14). The smooth muscles were washed with an ice-cold medium containing 0.25 M sucrose and 10 mM Tris-HCl (pH 7.4 at 4°C). The isolated tissues were minced with scissors and homogenized with a Polytron homogenizer in 20 volumes of 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.4 at 4°C) with the rheostat set at 9 for 5 sec. The homogenate was centrifuged at 2,500×g for 10 min and the supernatant was again centrifuged at 15,000×g for 20 min. Centrifugation of this supernatant at 100,000×g for 60 min yielded the pellet that was used as the microsomal fraction in this study (15).

Protein concentration was determined by the method of Lowry et al. (16) using bovine serum albumin as a standard.

Binding assay: Microsomal fractions were incubated with various concentrations of [³H]-dihydroalprenolol in a total volume of 150 μl of incubation buffer (50 mM Tris-HCl, pH 7.4 at 35°C) for 15 min. The incubation mixture was diluted with 1 ml of 50 mM Tris-HCl buffer (pH 7.4) and the supernatant was then filtered through Whatman GF/B glass fiber filters. The filters were washed 3 times with 3 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.4). After the passage, the filters were dried, and radioactivity was determined in a toluene base scintillator using a liquid scintillation spectrometer.

Nonspecific binding was determined as the radioactivity bound to microsomal fractions which is not displaced by 5×10⁻⁶ M propranolol. Specific binding was determined as the difference between the total binding and nonspecific binding (17).

The binding to the microsomal fractions was analyzed using Scatchard analysis (18) to determine the affinity and number of binding sites.

Statistical analysis: Numerical results are expressed as means±S.E., and Duncan's new multiple range test was used to calculate
statistical significance where appropriate. A P-value less than 0.05 was considered a significant difference.

Drugs: L-Isoprenaline hydrochloride (Sigma), desmethylimipramine hydrochloride (Sigma), pargyline (Sigma), phentolamine mesylate (Ciba Geigy), corticosterone (Sigma), D,L-propranolol hydrochloride (Sigma), indomethacin (Sigma) and papaverine hydrochloride (Sigma), all in powder form. [3H]-Dihydroalprenolol (specific activity: 92.2 Ci/mmol) was obtained from New England Nuclear. Other chemicals used were of analytical grade.

Results

Mechanical response: Spontaneous contraction of all the preparations were relaxed by indomethacin (10^{-5} M). All preparations from guinea pigs of different ages responded to isoprenaline with concentration-dependent relaxation (Figs. 1 and 2), and the maximum responses of all of them were equal to those in response to papaverine. The concentration-
response curve of isoprenaline shifted to a lower concentration range with increasing age from 6 to 100 weeks in the presence of corticosterone (3×10⁻⁵ M) (Fig. 1), but to a higher concentration range in the absence of corticosterone (Fig. 2). Therefore, the pD₂ values of isoprenaline in the presence of corticosterone (3×10⁻⁵ M) significantly increased with age from 6 to 100 weeks, whereas those in the absence of corticosterone significantly decreased (Table 1). The pD₂ values of isoprenaline obtained in the preparations from 40- and 100-week-old guinea pigs were significantly increased by corticosterone (Table 1 and Fig. 3), while in the preparation from 40-week-old guinea pig, the concentration-response curve of papaverine was not influenced by corticosterone (data not shown). Propranolol was used as a competitive antagonist against isoprenaline. The concentration-response curves of isoprenaline were shifted by propranolol in a parallel manner in all preparations from guinea pigs of different ages (Fig. 4). Schild plots of these results yielded straight lines with a slope of 1 (Table 1). The pA₂ values of propranolol estimated by Schild plot analysis of the data were not significantly different.

Table 1. The pD₂ values of isoprenaline in the presence and absence of corticosterone, the pA₂ value of propranolol against isoprenaline and the slope of Schild plots in the presence of corticosterone

<table>
<thead>
<tr>
<th>Drug</th>
<th>Isoprenaline</th>
<th>Propranolol</th>
</tr>
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<tbody>
<tr>
<td>Corticosterone</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>pD₂</td>
<td>pD₂</td>
<td>pA₂</td>
</tr>
<tr>
<td>3 weeks</td>
<td>8.81±0.10 (5)</td>
<td>8.93±0.07 (6)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>8.84±0.07 (6)</td>
<td>8.84±0.05 (9)</td>
</tr>
<tr>
<td>10 weeks</td>
<td>8.90±0.07 (10)</td>
<td>8.78±0.07 (5)</td>
</tr>
<tr>
<td>40 weeks</td>
<td>9.26±0.03* (12)</td>
<td>8.64±0.10* (5)</td>
</tr>
<tr>
<td>100 weeks</td>
<td>9.15±0.03* (8)</td>
<td>8.57±0.06* (4)</td>
</tr>
</tbody>
</table>

Values are presented as means±S.E. and number of experiments are indicated in parentheses. + and −: in the presence and absence of corticosterone (3×10⁻⁵ M). *: significantly different from the corresponding value in the tissue from 3-week-old guinea pig (P<0.01). #: significantly different from the corresponding value in the absence of corticosterone (P<0.001).

Fig. 3. Effect of corticosterone (3×10⁻⁵ M) on concentration-response curves of isoprenaline in the tracheal preparations from 40- (left) and 100- (right) week-old guinea pigs. Ordinate: relaxation (%) expressed as a fraction of the relaxation by papaverine (10⁻⁴ M). Abscissa: logarithm of molar concentration of isoprenaline. Each value is presented as a mean±S.E. of 4 to 5 experiments. O: with corticosterone and ●: without corticosterone.
from one another (Table 1).

**Binding assay**: The specific bindings of \(^{[3}H\)-dihydralprenolol to the microsomal fractions of the tracheal muscle were saturable (Fig. 5). Scatchard plots of the specific bindings of \(^{[3}H\)-dihydralprenolol yielded straight lines, three of which were practically parallel (Fig. 6). The dissociation constants (\(K_D\)) and maximum binding sites (\(B_{\text{max}}\)) are summarized in Table 2. An age-dependent increase in the maximum number of binding sites was observed, although the dissociation constant of dihydralprenolol did not change with age. The effects of a 60-min treatment with corticosterone (\(3 \times 10^{-6}\) M) on the dissociation constants and maximum binding

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**Fig. 4.** Antagonism between isoprenaline and propranolol in the tracheal preparations from 3- (left) and 100- (right) week-old guinea pigs. Ordinate: relaxation (%) expressed as a fraction of the relaxation by papaverine (\(10^{-4}\) M). Abscissa: logarithm of molar concentration of isoprenaline. Each value is presented as a mean±S.E. of 5 (for 3 weeks old) and 12 (for 100 weeks old) experiments. •, ○, △ and △: isoprenaline alone, with propranolol \(3 \times 10^{-9}\), \(10^{-8}\) and \(3 \times 10^{-9}\) M, respectively.

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**Fig. 5.** Specific bindings of \(^{[3}H\)-dihydralprenolol with increasing concentrations of \(^{[3}H\)-dihydralprenolol to microsomal fractions from the tracheal smooth muscles of 6-, 10- and 40-week-old guinea pigs. Ordinate: \(^{[3}H\)-dihydralprenolol (bound) in femtomoles per milligram of protein. Abscissa: concentration (nM) of \(^{[3}H\)-dihydralprenolol. Each value is presented as a mean±S.E. of 3 experiments. Left, middle and right: 6, 10 and 40 weeks, respectively. •, ○ and △: total, non-specific and specific bindings, respectively.
sites were tested in the microsomal fractions from the tracheal muscle of the 6 week-old guinea pigs. The $K_D$ and $B_{max}$ values and the Hill coefficient estimated in the microsomal fractions treated with corticosterone for 60 min were $0.651 \pm 0.05$ (nM), $80.3 \pm 6.15$ (fmol/mg protein) and $1.01 \pm 0.15$ (a mean±S.E. of 3 experiments), respectively. These values are not significantly different from those estimated in the untreated fractions (Table 2).

### Table 2. Maximum binding ($B_{max}$), apparent dissociation constant ($K_D$-value) and Hill coefficient obtained from specific binding of $[^3H]$-dihydroalprenolol to microsomal fractions derived from the tracheal smooth muscles of 6-, 10- and 40-week-old guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>$B_{max}$ (fmol/mg protein)</th>
<th>$K_D$ (nM)</th>
<th>Hill coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 weeks</td>
<td>79.5±6.6</td>
<td>0.61±0.07</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td>10 weeks</td>
<td>96.4±3.4</td>
<td>0.51±0.10</td>
<td>0.94±0.08</td>
</tr>
<tr>
<td>40 weeks</td>
<td>239.0±13.1*</td>
<td>0.50±0.03</td>
<td>1.13±0.07</td>
</tr>
</tbody>
</table>

Values are presented as means±S.E. of 3 experiments. *: significantly different from the others ($P<0.001$).

**Fig. 6.** Typical Scatchard plots of the data in Fig. 5. Ordinate: the ratio of bound to free $[^3H]$-dihydroalprenolol found in the incubation medium. •, ○ and △: 6, 10 and 40 weeks, respectively.

Discussion

The spontaneous contraction of tracheal preparations was inhibited by indomethacin, a cyclooxygenase inhibitor. This fact suggests the spontaneous contraction is due to endogenous prostaglandins. The amplitude of a spontaneous contraction is considered to be due to the amount of endogenous prostaglandins. Therefore, the relaxation by isoprenaline was expressed as a fraction of the relaxation by papaverine ($10^{-4}$ M), a nonspecific smooth muscle relaxant. In the present study, the pD$_2$ values of isoprenaline estimated in the tracheal preparations isolated from 3- to 100-week-old guinea pigs increased with age in the presence of corticosterone, but decreased in the absence of corticosterone. However, Aberg et al. (19) also reported that there was an age-related reduction in the sensitivity of isolated rat and guinea pig tracheal preparations to isoprenaline. Hayashi and Toda (20) also reported similar results that the relaxing response to isoprenaline of rabbit tracheal strips contracted with acetylcholine was inversely related to age. These findings were in agreement with our results without corticosterone, but not with those with corticosterone.

Isoprenaline is a relatively poor substrate for sympathetic neurons to the same extent as norepinephrine (21). However, in the present study, the extraneuronal uptake inhibitor corticosterone increased the pD$_2$ value of isoprenaline in tracheal preparations from 10-, 40- and 100-week-old guinea pigs; and in the preparations from 40- and 100-week-old guinea pigs, these increases were highly significant ($P<0.001$). In the preparations from 40-week-old guinea pigs, corticosterone did not influence the concentration-response curve of papaverine, suggesting that relaxation mechanisms are not influenced by corticosterone. The present results suggest that extraneuronal uptake increases with age and that tissue sensitivity to isoprenaline is modified by extraneuronal uptake. The age-related increase in the pD$_2$ value of isoprenaline in the presence of corticosterone probably reflects the results of drug-receptor in-
teraction. However, a difference between $pD_2$ values in the presence and absence of corticosterone was not observed in the preparations from 3- and 6-week-old guinea pigs, suggesting that extraneuronal uptake is poor in the 3- and 6-week-old guinea pigs.

Duncan and Douglas (22) demonstrated that inhibition of extraneuronal uptake by hydrocorticosterone in bronchial and tracheal preparations from immature (<1-week-old) and mature (>12-week-old) guinea pigs affected neither the potency nor the extent of relaxation induced by isoprenaline in histamine-contracted preparations, and they concluded that extraneuronal uptake does not modify tissue sensitivity to $\beta$-adrenoceptor stimulants. In the present study, corticosterone only slightly increased the $pD_2$ values in the preparations from 3- to 10-week-old guinea pigs, but greatly influenced those in the tracheal preparations from 40- and 100-week-old animals. The discrepancy between the results of Duncan and Douglas (23) and the present findings can be attributed to differences in age of the guinea pigs used.

The maximum binding sites ($B_{\text{max}}$) estimated in the microsomal fractions treated with corticosterone ($3 \times 10^{-5}$ M) from 60 min was not different from those in the untreated fractions, suggesting that the amount of total receptors is not influenced by the 60 min treatment with corticosterone ($3 \times 10^{-5}$ M). The response to drugs in the presence of corticosterone are considered to reflect the results of drug-receptor interaction. The antagonism between isoprenaline and propranolol was tested in the tracheal preparations treated with corticosterone. The $pA_2$ value of propranolol did not change with age, suggesting the possibility that the affinity of propranolol to $\beta$-adrenoceptors in the tracheal preparations is constant at all ages. The result that the dissociation constant ($K_D$) of $[^3H]$-dihydroalprenolol, estimated from the Scatchard plot did not change with age coincides with the findings on $pA_2$ values of propranolol against isoprenaline. Age-dependent increase in maximum binding sites was observed (Fig. 6, Table 2). It is well-documented that the $pD_2$ value of an agonist is larger in a tissue where there is a large amount of receptors than in a tissue where the amount of receptors is small (10, 24, 25). Therefore, the age-related increase in the $pD_2$ value of isoprenaline found here in the presence of corticosterone is considered to be due to the increase in the total amount of $\beta$-adrenoceptors, as reported for post $\alpha_2$- and pre $\alpha_2$-adrenoceptors (8-10).

Duncan et al. (23) tried to relate the pharmacological responses of tracheal preparations to changes in affinity and density of $\beta$-adrenoceptors using the radioligand $[^{125}]$-hydroxybenzylpindolol. The specific binding of $[^{125}]$-hydroxybenzylpindolol was detected in tracheal tissues from middle aged (417 g) and older (757 g) guinea pigs, but was not measurable in tracheal preparations from young (118 g) animals. They suggested that receptor affinity was identical at any age, while receptor density was significantly less in preparations from young animals than in those from mature or older animals, and also showed that the potency of isoprenaline in relaxing bronchial muscle was reduced during development. They suggested from their data that the reduced sensitivity of airway muscle to catecholamine during development may be, in part, due to increased density of $\beta$-adrenoceptors which are not involved in eliciting the response.

The conclusions in the present study are as follows: In guinea pigs aged between 3 and 100 weeks used as test animals, the response to isoprenaline in the trachea treated with corticosterone increased with age. Age-related increase in the maximum bindings of $[^3H]$-dihydroalprenolol was also observed, although its dissociation constant did not change with age. This age-related increase in the sensitivity of the corticosterone-treated trachea to isoprenaline is therefore considered to be due to the density of $\beta$-adrenoceptors. On the other hand, age-related decrease in the response to isoprenaline was observed in the trachea untreated with corticosterone. These observations suggest that extraneuronal uptake plays an important role in the response to isoprenaline in the tracheal preparations from older (more than 40 weeks old) guinea pigs.

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References


