A Difference in Alpha-Adrenoceptor Mechanisms in Vasa Deferentia Isolated from Young and Old Rats

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Abstract—A difference in alpha-adrenoceptor mechanisms in vasa deferentia isolated from 3 weeks old and 40 weeks old rats were studied by analysis of the concentration-response curve of norepinephrine and Scatchard plot of specific $[^3$H]-$prazosin binding to microsomal fractions. The efficacy of norepinephrine and capacity of the maximum binding sites of $[^3$H]-$prazosin estimated in 40 weeks old rats were larger than those in the 3 weeks old rats, while there was no difference in the affinity of norepinephrine estimated in the 3 weeks old rats and in the 40 weeks old rats. The increase of efficacy for norepinephrine in the vas deferens from the 40 weeks old rats is due to the increase in the total concentration of alpha adrenoceptors.

Little is known about the effects of ageing on responses mediated through alpha-adrenoceptors and also on characteristics of alpha-adrenoceptors. The results from the few studies tend to be contradictory. The binding characteristics of alpha-adrenoceptors in intact human platelets were not changed (1), while Brodde et al. (2) reported that a number of alpha-adrenoceptors in human platelets decreased age-dependently. A contractile response to norepinephrine was increased with age in helically cut strips of aortae from rabbits (3).

The pharmacological effects of drugs should in many cases be considered as results of an interaction of the drugs with their receptors. In such cases, two essential parameters should be distinguished: the affinity of the drug to its receptor and the efficacy of the active drug. Therefore, in order to clarify any change in characteristics of alpha-adrenoceptors with increased age, we estimated the affinity and efficacy for norepinephrine with analysis of concentration-contractile response curves of norepinephrine in vasa deferentia from young (3 weeks old) and aged (40 weeks old) rats, and we also calculated the dissociation constant and capacity of maximum binding sites from the $[^3$H]-$prazosin-receptor binding assay using the microsomal fractions of the vasa deferentia.

Vasa deferentia were isolated from young Wistar rats (3 weeks old: 65–80 g in body weight) and from aged Wistar rats (40 weeks old: 400–500 g in body weight). Pieces of vas deferens were mounted in glass organ baths containing 20 ml of a physiological solution kept at 32°C and gassed with a mixture of 95% O$_2$ and 5% CO$_2$. The composition of the physiological solution was NaCl, 154; KCl, 5.6; CaCl$_2$, 2.2; MgCl$_2$, 2.1; NaHCO$_3$, 5.9; and glucose, 2.8 mM. Responses to drugs were isotonically recorded under a tension of 0.5 g. A concentration-contractile response curve for norepinephrine was obtained cumulatively (4).

In order to estimate the efficacy (5) and dissociation constant ($K_a$-value) the alpha-adrenoceptors were partially blocked by an irreversible antagonist, phenoxybenzamine (5). After the determination of the control concentration-response curves for norepinephrine, the preparations were treated with $3 \times 10^{-7}$ M of phenoxybenzamine for 10 min. The preparations were then allowed to
equilibrate for 60 min with repeated washing every 10 min and second concentration response curves for norepinephrine were determined. The dissociation constant ($K_A$) of norepinephrine was calculated from the following equation (1):

$$\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q[A']}$$  \hspace{1cm} (1)

where $[A]$ and $[A']$ are corresponding equilibrium concentrations of norepinephrine before and after irreversible blockade of a fraction of receptors with phenoxybenzamine, respectively, and $q$ is the remaining fraction of active receptors after phenoxybenzamine treatment. The reciprocal values were plotted as $1/[A]$ vs $1/[A']$. A straight line was fitted to the data by linear regression analysis. The dissociation constant, $K_A$-value, was obtained by the following equation (2):

$$K_A = \text{slope} - \frac{1}{\text{intercept}}$$  \hspace{1cm} (2)

The efficacy was calculated from equation 3:

$$antilog(pD_2 - pK_A) + 1$$  \hspace{1cm} (3)

where $pD_2$ and $pK_A$ were the negative logarithm of the concentration (M) which produced 50% of the maximum response to the drug and the negative logarithm of $K_A$.

The isolated vasa deferentia were minced and homogenized twice 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 setting 9 for 5 sec. The supernatant was centrifuged at 10,000 x g for 10 min. The supernatant was again centrifuged at 15,000 x g for 20 min. Centrifugation of the supernatant at 100,000xg for 60 min resulted in a pellet which was used as microsomal fractions in this study. All the procedures were carried out at 5°C.

Protein concentrations were determined by the method of Lowry et al. (7) using bovine serum albumin as a standard.

The microsomal fractions were incubated with various concentrations (0.2–5 nM) of $[^3H]$-prazosin in a volume of 0.6 ml of incubation buffer (50 mM Tris/HCl, pH 7.4) at 25°C for 60 min. The incubation mixture was rapidly filtered through Whatman GF/C glass fiber filter. The filters were washed 3 times with 3 ml of ice-cold 50 mM Tris/HCl. The filters were then dried and radioactivity was determined as in toluene base scintillator (Aloka LSC-900). Specific binding was determined as radioactivity binding to each microsomal fraction, which was displaced by phentolamine (10 μM).

Drugs used: $[^3H]$-Prazosin (specific activity=80.9 Ci/mmol) was obtained from New England Nuclear. Other drugs were norepinephrine hydrochloride (Wako-Junyaku), phentolamine methanesulfonate (Ciba) and phenoxybenzamine hydrochloride (Tokyo-Kasei), all in powder form. All the drugs were used as solutions in distilled water. Other chemicals used were of analytical grade.

Both the smooth muscle preparations from the young and aged rats responded to norepinephrine with concentration-dependent contractions. The $pD_2$-values for norepinephrine were 6.36±0.21 for the aged rats and 6.23±0.18 for the young rats (Table 1). The $pD_2$-values estimated were practically equal to each other. The preparations lost

| Table 1 | The $pD_2$-, $pK_A$- and $pK_t$-values, efficacy and B$_{max}$ estimated in the vasa deferentia from the young (3 weeks old) and aged (40 weeks old) rats, respectively |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | from mechanical response (N=5)                                                                                                           | from binding assay (N=4)                                                                                     |
|         | $pD_2$        | $pK_A$       | efficacy     | $B_{max}$ | $K_t$ (pM) |
| Young (3 weeks old rats) | 6.23 (0.18) | 6.29 (0.14) | 1.87 (0.27) | 80.6 (17) | 238 (85) |
| Aged (40 weeks old rats) | 6.36 (0.21) | 5.90 (0.15) | 5.59* (0.97) | 194* (28) | 299 (51) |

Each value is presented as a mean±S.E. in parentheses. N: number of experiments. B$_{max}$: fmol/mg protein. *: significant difference from the value for the young rats.
about 50 to 70% of the maximum response to norepinephrine by phenoxybenzamine (3×10^{-7} M) (Fig. 1). The negative logarithm of the dissociation constant (pKₐ) of norepinephrine to its receptor, calculated according to the method of Furchgott (6), was summarized in Table 1. The pKₐ-values estimated in both the preparations isolated from the young and aged rats were not significantly different from each other. The efficacy of norepinephrine in the aged rats was significantly larger than that in the young rats (Table 1).

The specific bindings of [³H]-prazosin to the microsomal fractions of the vasa deferentia from the young and aged rats were saturable, respectively. The Scatchard plots of the specific bindings of [³H]-prazosin yielded straight lines for the young and aged rats, respectively, and the dissociation constants (Kd-values) and maximum binding sites (BmaX) were 299±51 pM and 194±28 fmol/mg protein for the aged rats, and 238±65 pM and 80.6±17 fmol/mg protein for the young rats, respectively (Table 1). The maximum binding site (Bmax) for the aged rats was significantly different from that for the young rats, while the Kd-value of [³H]-prazosin to the alpha-adrenoceptors was not independent on age.

The concentration-contractile response curves for norepinephrine obtained in the vasa deferentia from the young (3 weeks old) and aged (40 weeks old) rats were practically same (Fig. 1). Therefore, the pD₂-value of norepinephrine estimated in the young rats was almost equal to that in the aged rats (Table 1). However, the efficacy (5) for norepinephrine was increased with age (Table 1). According to Stephenson (5), the biological stimulus, S, to induce the contractile response is expressed as S=ey, e being efficacy and y being the proportion of the receptors occupied by the drug. As it is well known that there is no spare receptor in the vas deferens from the adult rat (8), the pD₂-value is unchanged by a decrease of efficacy. Therefore, the shape of the concentration-response curve of norepinephrine estimated the maximum response or the maximum tension developed as 100% was unchanged with age as shown in Fig. 1. The present results suggest that the tension developed by norepinephrine was greater in the vasa deferens from the aged rat than in that from the young rat. Our results coincide with the findings of Hayashi and Toda (3) who reported that the tension of the helically cut aorta preparation of rabbit developed by norepinephrine was increased with age in the range from 2 to 90 days.

The results that the dissociation constant (Kd-value) for [³H]-prazosin estimated from the Scatchard plot was unchanged with age (Table 1) coincides with the findings on the pD₂- and pKₐ-values in this study. The maximum binding sites (Bmax) for [³H]-prazosin were increased with age, indicating the increase in the total concentration of alpha-adrenoceptors. As defined by Stephenson (5), efficacy (e) was a drug and tissue dependent term. Furchgott (6) modified this model to differentiate the drug and the tissue factors of efficacy by defining intrinsic efficacy (ε):

$$e = ε[R_t]$$  \hspace{1cm} (4)

where $[R_t]$ refers to the total concentration of receptors. In the present results, the ratio of the efficacy for norepinephrine in the aged

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**Fig. 1.** Concentration-contractile response curves of norepinephrine before and after the 10 min treatment of the rat vas deferens with phenoxybenzamine (3×10^{-7} M). Left: young (3 weeks old) rats. Right: aged (40 weeks old) rats. Each value is presented as a mean with S.E. (bar) of 5 experiments. • and △: before and after the treatment with phenoxybenzamine, respectively. Ordinate: contraction (%). Abscissa: negative log concentration (M) of norepinephrine.
rats against that in the young rats was 2.96, which is almost equal to the ratio (2.44) calculated from the maximum binding sites, suggesting the possibility that the intrinsic efficacy is the same in both the preparations. These indicate that the increase of efficacy with age is due to the increase of the total concentration of receptors. However, further studies are necessary to clarify effects of age on alpha-adrenoceptor mechanisms.

In conclusion, we have clarified in the vasa deferentia from the young (3 weeks old) and aged (40 weeks old) rats that the capacity of alpha-adrenoceptors was increased with age, though the affinity of drug was not influenced. However, differences between the present results and the findings in the platelets (1, 2) may be attributed to differences in species, tissues and age.

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