inactive at β_2-adrenoceptors, the small (5–10%) relaxation being due to stimulation of β_1-adrenoceptors.

The activities of the (−)-isomers *m*- and *p*-octopamine and *m*- and *p*-synephrine for both β-subtypes are less than that of NA by two orders of magnitude or more, so that any possible neuromodulation by *m*- and *p*-octopamine (or *m*- and *p*-synephrine) of the effect of NA released by noradrenergic neuron stimulation cannot be mediated by β_1- or β_2-adrenoceptors. Our conclusion that enantiomers of *m*- and *p*-octopamine have no significant physiological β-adrenergic activity is in accordance with our earlier observation that racemic *m*- and *p*-octopamine had no detectable effect on the in-vivo β-adrenergic responses of initiation of thirst or increase in tail skin temperature in the rat (Fregly et al 1979).

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Postsynaptic α_1-adrenoceptor mechanisms in rat vas deferens and ageing

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Postsynaptic α_1-adrenoceptor mechanisms in vasa deferentia isolated from 3, 6, 18 and 40 week-old rats were studied by analysis of the concentration-response curve of noradrenaline and the Scatchard plot of specific binding of [^{3}H]prazosin to microsomal fractions. The maximum tension developed by noradrenaline also increased with age from 3 to 18 weeks. The efficacy of noradrenaline and capacity of the maximum binding sites of [^{3}H]prazosin increased with increasing age, while the dissociation constants of noradrenaline (K_A) and prazosin (K_d) were not changed with age. The increase of the maximum tension was proportional to the increase in efficacy. The increase of efficacy for noradrenaline in the vasa deferentia from rats of different ages is due to the increase in the total concentration of postsynaptic α_1-adrenoceptors.

The effects of ageing on responses through β-adrenoceptors have been widely studied (Fleisch 1981) and it has generally been reported that β-adrenoceptor responsiveness is reduced in the elderly. Relatively little is known about the effects of ageing on responses mediated through α_1-adrenoceptors or on their characteristics, partly due to the lack of a constant pattern of change in the available reports (Docherty & Hyland 1986; Docherty 1986; Takayanagi et al 1986).

Therefore, to clarify any change in α_1-adrenoceptor mechanisms with increasing age, we have estimated the affinity and efficacy (Stephenson 1956; Kenakin 1984) for noradrenaline in vasa deferentia from 3 to 40 week-old rats and further calculated the dissociation constant and capacity of maximum binding sites from the [^{3}H]prazosin receptor binding assay using the microsomal fractions of the rat vasa deferentia.

Materials and methods

Wistar rats (3, 6, 18 and 40 weeks old) were killed by a blow on the head and the vasa deferentia isolated. Pieces of the tissue were mounted in glass organ baths containing 20 mL of a physiological solution (composition NaCl 154, KCl 5·6, $CaCl_2$ 2·2, $MgCl_2$ 2·1, $NaHCO_3$ 5·9 and glucose 2·8 mM) kept at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. The solution also contained propranolol (10^{-6} M), desmethylimipramine (10^{-7} M) and normetanephrine (10^{-6} M) to inhibit β-adrenoceptors, and neuronal and extraneuronal

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uptakes, respectively (Minneman et al 1983). Responses to drugs were isometrically recorded under a initial tension of 0·5 g. A concentration-contractile response curve for noradrenaline was obtained cumulatively (van Rossum 1963). To express the developed tension, a cross-sectional area (mm^2) of vas deferens was estimated by wet weight/length, a calculation that assumes a tissue density of 1 (Toda et al 1986).

To estimate the efficacy and dissociation constant (K_A value), the α_1-adrenoceptors were partially blocked by an irreversible antagonist, phenoxybenzamine (Furchgott 1966). After determination of the control concentration-response curves for noradrenaline, the preparations were treated with 3×10^{-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 noradrenaline were determined. The dissociation constant was calculated according to Furchgott (1966).

Efficacy (e) was calculated using this equation:

$$e = \text{antilog}\,(pD_2 - pK_A) + 1$$

where pD_2 and pK_A are the negative logarithms of the concentrations producing 50% of the maximum response to the drug and of K_A, respectively.

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 a rheostat setting of 9 for 5 s. The homogenate was centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 15000*g* for 20 min. Centrifugation of the supernatant at 100000*g* for 60 min resulted in a pellet which was used as microsomal fraction in this study. All the procedures were carried out at 5 °C. Protein concentrations were determined by the method of Lowry et al (1951) using bovine serum albumin as a standard.

The microsomal fractions were incubated with various concentrations ($0{\cdot}2 - 5 \times 10^{-6}$ M) of [^{3}H]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 a Whatman GF/C glass fibre filter. They were washed 3 times with 3 mL of ice-cold 50 mM Tris-HCl. The filters were then dried and radioactivity was determined in toluene base scintillator with a liquid scintillation spectrometer (Aloka LSC-900). Specific binding was determined as radioactivity bound to each microsomal fraction, which was displaced by phentolamine (10^{-5} M).

Statistical analyses were made using Student's *t*-test. Drugs used were noradrenaline hydrochloride (Wako-Junyaku), phentolamine methanesulphonate (Ciba) and phenoxybenzamine hydrochloride (Tokyo-Kasei), all in powder form. [^{3}H]Prazosin whose specific activity was 80·9 Ci $mmol^{-1}$, was obtained from New England Nuclear. All the drugs were used as solutions in distilled water. Other chemicals used were of analytical grade.

Results

All the smooth muscle preparations from the rats of different ages responded to noradrenaline with concentration-dependent contraction (Fig. 1). The pD_2 value of noradrenaline significantly ($P < 0{\cdot}05$) increased with age in the range from 3 to 18 weeks (Table 1). All the

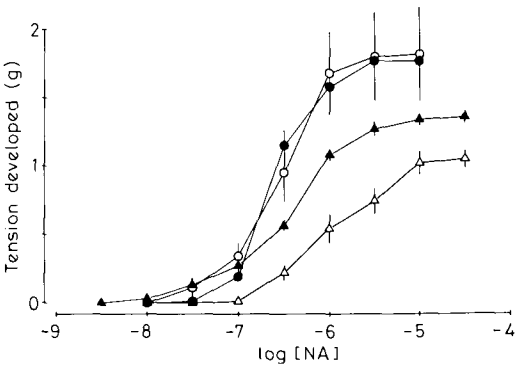

FIG. 1. Concentration-response curves of noradrenaline in vasa deferentia from rats of different ages. △, ▲, ○ and ●: 3, 6, 18 and 40 week-old rats, respectively. Each value is presented as a mean ± s.e. of 4 to 5 experiments.

Table 1. The pD_2 and pK_A values, efficacy of noradrenaline and maximum tension developed by noradrenaline in vasa deferentia from rats of different ages.

Age (weeks)	Noradrenaline			Change in tension			n
	pD_2	pK_A	Efficacy	g	g mm^{-2}	g g^{-1} tissue	
3	6·17 (0·05)	6·30 (0·15)	1·86 (0·29)	1·05 (0·06)	3·37 (0·01)	125 (3·5)	4
6	6·40 (0·04)	6·17 (0·09)	2·92* (0·47)	1·36 (0·04)	3·04 (0·19)	97·3 (6·9)	4
18	6·57 (0·57)	5·89 (0·13)	6·53* (1·30)	1·82 (0·34)	1·27 (0·22)	34·2 (8·7)	5
40	6·52 (0·09)	5·91 (0·13)	5·55* (0·95)	1·77 (0·18)	0·90 (0·07)	22·9 (1·1)	5

Each value is presented as a mean ± s.e. in parentheses. n: number of experiments. g: developed tension. g mm^{-2}: developed tension/area. g g^{-1} tissue: developed tension/wet tissue weight. * Significant difference from the value obtained in the vasa deferentia of rats of 3 weeks old at $P < 0{\cdot}05$.

preparations lost 40 to 70% of maximum response to the drug by the 10 min treatment with phenoxybenzamine (3×10^{-7} M). The dissociation constant (K_A) of noradrenaline to α_1-adrenoceptors, calculated according to the method of Furchgott (1966) is summarized in Table 1. The pK_A values estimated in the preparations from rats of different ages were not significantly different from one another. The efficacy of noradrenaline and the maximum tension developed by noradrenaline were related directly to age in the range from 3 to 18 weeks but tended to decrease with age from 18 to 40 weeks (Table 1, Fig. 1).

The specific bindings of [^{3}H]prazosin to the microsomal fractions of vasa deferentia from variously aged animals were saturable (data not shown). The Scatchard plots of the specific bindings of [^{3}H]prazosin yielded straight lines. Dissociation constants (K_d) and maximum binding sites (B_{max}) are summarized in Table 2. Age-dependent increase in the maximum binding sites was observed (Table 2), though the dissociation constant (K_d) of [^{3}H]prazosin as well as the pK_A value of noradrenaline did not alter with age.

Table 2. The maximum bindings and dissociation constants for [^{3}H]prazosin estimated in microsomal fractions of vasa deferentia from rats of different ages.

Age (weeks)	Maximum binding (B_{max}; fmol mg^{-1} protein)	Dissociation constant (K_d; pM)	n
3	80·6 ± 14·6	240 ± 70	3
6	87·8 ± 4·8	267 ± 90	3
18	190 ± 5·1*	284 ± 32	3
40	184 ± 28*	295 ± 53	3

Each value is presented as a mean ± s.e. n: number of experiments. *Significant difference from the value obtained in the vasa deferentia in rats of 3 weeks old at $P < 0.05$.

Discussion

The change in the pD_2 value of noradrenaline with increasing age was small but significant ($P < 0.05$), while age-dependent change in the pK_A value of noradrenaline was not observed (Table 1). Therefore, the efficacy of noradrenaline, calculated with the pD_2 and pK_A values was directly related to increasing age. The result that the dissociation constant (K_d) of [^{3}H]prazosin, estimated from the Scatchard plot, was unchanged with age (Table 2) coincides with the findings on the pK_A value of noradrenaline in this study. The maximum binding sites (B_{max}) for [^{3}H]prazosin were increased with age indicating the increase in the total concentration of α_1-adrenoceptors. The efficacy (e) is a drug- and tissue-dependent term, as defined by Stephenson (1956). Furchgott (1966) modified this model to differentiate the drug and the tissue factors of efficacy by defining intrinsic efficacy (ε):

$$e = \varepsilon[R_t]$$

where $[R_t]$ refers to the total concentration of receptors. In the present results the efficacy is proportional to the increase in the maximum binding (B_{max}) (Fig. 2) suggesting the possibility that the intrinsic efficacy (ε) is not, or is little, changed with increasing age. These data indicate that the increase of efficacy with age is due to the increase of the total concentration of receptors.

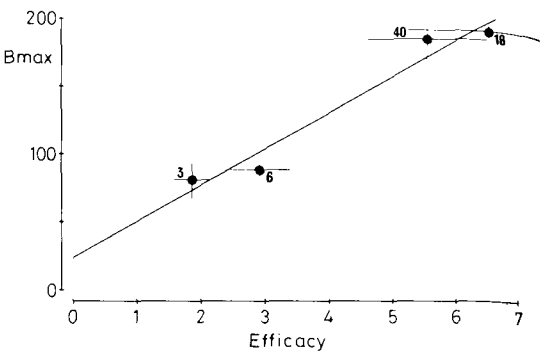

FIG. 2. A relationship between the efficacy of noradrenaline and the specific binding of [^{3}H]prazosin. Ordinate: specific binding of [^{3}H]prazosin (B_{max}; fmol mg^{-1} protein) which is presented as a mean ± s.e. of 3 experiments. Abscissa: efficacy (as defined by Stephenson 1956: see text for detail) presented as a mean with s.e. of 4 to 5 experiments. 3, 6, 18 and 40 are the ages of rats in weeks, respectively. A positive correlation was found ($r = 0.987$, $P < 0.05$). The best fitting line ($y = 26.6x + 23.5$) is shown.

The present results were similar to the findings of Hayashi & Toda (1978) who concluded that tension of the helically cut aorta preparation of rabbit developed by noradrenaline was increased with age in the range from 2 to 90 days. Docherty & O'Malley (1983) also investigated age-related changes in pre- and post-synaptic α_1-adrenoceptors in vasa deferentia from young adult (2–3 months) and 24-month old Sprague-Dawley rats and concluded that there was no age-related change. In our results the maximum amount of α_1-adrenoceptors, efficacy and maximum tension increased with age from 3 weeks to 18 weeks. However, the total amount of α_1-adrenoceptors and maximum tension decreased significantly ($P < 0.05$) with increasing age from 40 weeks to 60 weeks (unpublished observations). The findings of Docherty & O'Malley (1983), who reported that there was no change in α_1-adrenoceptors in vasa deferentia from 2–3- and 24-month old rats, might be due to the advanced age of animals used. The difference between those and our results are attributed to differences in ages of the experimental animals.

As expressions of tension, tension developed per area (g mm^{-2}), tension developed per tissue weight (g g^{-1} tissue) and tension developed (g), are generally used. In the present results the increase of efficacy is proportional to the maximum tension developed (g) (Fig. 3), but not to other expressions of tension (Table 1). It is generally considered that the increase of efficacy of a drug causes an intensive response to the drug. If such is the case, it is most suitable simply to express this as tension developed (g). Our results (Fig. 3) coincide with the findings of Hayashi & Toda (1978) who expressed

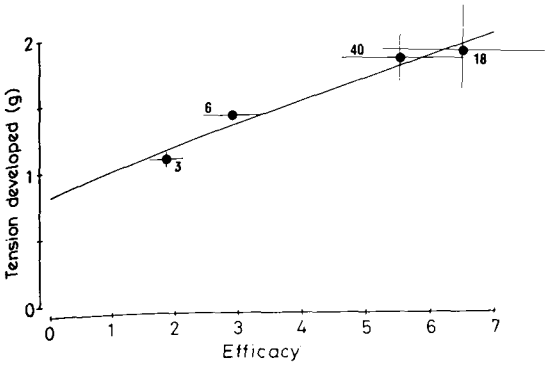

FIG. 3. A relationship between the efficacy of noradrenaline and the maximum tension developed. Ordinate: efficacy (as defined by Stephenson 1956: see text for detail). Abscissa: maximum tension (g) developed by noradrenaline. Each value is presented as a mean ± s.e. of 4 to 5 experiments. 3, 6, 18 and 40 are the ages of rats in weeks, respectively. A positive correlation was found ($r = 0.980$, $P < 0.02$). The best fitting line ($y = 0.163x + 0.811$) is shown.

the response of rabbit aorta to noradrenaline as developed tension (g) and concluded that tension (g) developed by noradrenaline increased with age.

The observations that the efficacy correlates with the maximum tension in g, but not to other expressions of tension, were not explained in that paper. It might be related to the present observation that the number of smooth muscles involved in a cross-sectional area (mm^2) or in a wet tissue weight (g) may decrease with increasing age.

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Effects of chronic treatment with amiodarone on hepatic demethylation and cytochrome P450

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The effect of chronic treatment with amiodarone on hepatic oxidative metabolism using an in-vivo [^{14}C]aminopyrine breath test and on hepatic cytochrome P450 was examined in Wistar rats. Aminopyrine demethylation was significantly impaired but returned to pretreatment values following amiodarone for 4 weeks. In contrast the levels of cytochrome P450 were significantly depressed during treatment and at 4 weeks following treatment. While an inhibitory effect on oxidative metabolism may explain the reported drug interactions with amiodarone, the discrepancy between its in-vivo effects and cytochrome P450 levels may suggest the development of 'compensatory' extra-hepatic site of drug metabolism.

Amiodarone, a benzofuran derivative, is now established in many countries as a highly effective antiarrhythmic agent for supraventricular and ventricular arrhythmias. The more widespread use of this drug has resulted in the identification of several side-effects, which range from corneal deposits and pulmonary toxicity to hepatic and thyroid dysfunction (Heger et al 1984). Case reports suggest possible pharmacokinetic interactions with warfarin, acenocoumarol, quinidine, digoxin, procainamide, phenytoin and aprindine. Only recently, however, has it been suggested that the underlying mechanism of many of these interactions may be inhibition of the hepatic metabolism (McGovern et al 1984; Saal et al 1984; Richard et al 1985).

Demethylation is an important route of metabolism for many drugs and is primarily catalysed by one or more of the forms of hepatic microsomal cytochrome P450. Several studies suggest that aminopyrine *N*-demethylation is a good probe for cytochrome P450 isozymes (Houston et al 1981). Therefore the aim of this study was to examine the effects of chronic doses of amiodarone on hepatic oxidative drug metabolism in

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