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Age-dependent differences in the positive inotropic effect of phenylephrine on rat isolated atria

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1 The age-dependent differences in the involvement of α-adrenoceptors in the positive inotropic effect of phenylephrine (Phe) were examined in isolated atria of male Wistar rats 6 weeks (6W), 10 weeks and 7 months (7M) of age.

2 The maximal increase in tension development induced by Phe increased with age, whereas the EC_{50} values for the positive inotropic effect of Phe did not change with age. The inhibitory effect of phentolamine on the response to Phe increased with age. Propranolol caused only slight inhibition of the effect of Phe in both 6W and 7M rats, and the EC_{50} values for Phe in the presence of propranolol did not change significantly with age.

3 The EC_{50} values for isoprenaline and 5-hydroxytryptamine in 7M rats were higher than those in 6W rats.

4 In 7M rats, the duration of the tension development was only slightly affected by Phe in the presence or absence of propranolol, but it was markedly decreased by Phe in the presence of phentolamine.

5 The dose-response curve for Phe was markedly shifted to the left by papaverine in 6W rats, but slightly in 7M rats. The dose-response curve for isoprenaline was markedly shifted to the left by papaverine in both groups.

6 These results are consistent with effects of Phe being mediated by both α- and β-adrenoceptors in both 6W and 7M rats, but there is a shift in the balance from rather more β-receptors in the young animals to more α-receptors in the adults.

Introduction

It is well known that the positive inotropic effect of sympathomimetic amines is mediated by α- as well as β-adrenoceptors in the heart of experimental animals (Govier, 1968; Nakashima, Maeda, Sekiya & Hagino, 1971; Nakashima, Tsuru & Shigei, 1973; Benfey, 1973; Schümann, Endoh & Wagner, 1974; Endoh, Schümann, Krappitz & Hillen, 1976; Kunos, 1981) and humans (Wagner, Schümann, Knorr, Rohn & Reidemeister, 1980). Many factors including hormones (Nakashima *et al.*, 1971, 1973; Kunos, Vermas-Kunos & Nickerson, 1974; Kunos, 1977; Hashimoto & Nakashima, 1978; Hirano, Hashimoto & Nakashima, 1982) and temperature (Endoh, Wagner & Schümann, 1975; Benfey, 1977; Mori, Hashimoto, Hasegawa & Nakashima, 1979) markedly affect the α-adrenoceptor-mediated positive inotropic effect. On the other hand, it has been reported that the responsiveness of the heart to drugs or stress vary with age (Shreiner, Weisfeld & Schock, 1969; Conway, Wheeler & Sannerstedt, 1971; Kronenberg & Drage, 1973; Lakatta, Gerstenblith, Angell, Schock & Weisfeld, 1975; Petrofsky & Lind, 1975; Toda, Fu & Osumi, 1976; Yin, Spurgeon, Raizes, Greene, Weisfeld & Schock, 1976; Gerstenblith, Spurgeon, Froehlich, Weisfeld & Lakatta, 1979; Yin, Spurgeon, Greene, Lakatta & Weisfeld, 1979; Guarnieri, Filburn, Zitnik, Roth & Lakatta, 1980; Toda, 1981). Lakatta *et al.* (1975) and Guarnieri *et al.* (1980) have demonstrated the diminished inotropic response to mature myocardium to catecholamines. The change in the density of cardiac adrenoceptors has been postulated to be a cause of the age-dependent change in the responsiveness to catecholamines (Schocken & Roth, 1977; Baker & Potter, 1980). The present study was undertaken to examine the age-dependent difference in the involvement of α-adrenoceptors in the positive inotropic effect of phenylephrine (Phe) in rat isolated atria.

Methods

Male Wistar rats 6 weeks (6W), 10 weeks (10W) and 7 months (7M) of age were examined. The body weights of these animals were 102–145 g, 245–255 g and 380–535 g, respectively. Animals were killed by a blow on the head, and the heart was removed rapidly. The isolated atrium was suspended in a 10 ml organ bath containing Krebs-Ringer solution of the following composition (mM): NaCl 120, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.0, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 14.0. The bath solution was bubbled with 95% O_2 plus 5% CO_2, and was maintained at a temperature of 30 ± 0.5°C. The left atrium was driven at a frequency of 2.5 Hz through platinum electrodes by a square wave pulse of 3 ms duration and a voltage about twice above threshold. The left atrium was stretched with a resting tension of 0.3 to 0.5 g which was determined to obtain the maximal basal tension development, and isometric contractions were recorded via force-displacement transducers (Nihon Kohden SB-IT-H) on an ink-writing oscillograph (Nihon Kohden, RJG-3002) at paper speeds of 15–200 mm/s. The duration of contraction was also measured at 10% level of tension development. After equilibrating the atrium in Krebs-Ringer solution for about 50 min, the first dose-response curve (DRC) for an agonist was obtained by cumulative application. After the maximal response of an agonist was obtained, the preparation was washed several times. About 60 min later, the 2nd DRC was obtained in the presence of an antagonist. In the study with Phe, a 3rd DRC was obtained about 60 min later in the presence of α- and β-adrenolytic drugs. Antagonists were added 30 min before the application of an agonist. In the absence of antagonists, the three DRCs were almost identical.

The effect of pretreatment with papaverine was examined by adding the drug at a final concentration of 1×10^{-5} M, 30 min after the first DRC; 1 h after the addition of papaverine the 2nd DRC was determined.

When the DRC for Ca^{2+} was determined, the Krebs-Ringer solution was changed after the equilibration period to the Ca^{2+}-free solution which had the same composition as the Krebs-Ringer solution except that $CaCl_2$ was omitted. After the contraction disappeared (usually within 10 min), $CaCl_2$ was added cumulatively.

The drugs used were as follows: (−)-phenylephrine hydrochloride (Sigma Chemical Company); phentolamine mesylate (Regitin, Ciba-Geigy); propranolol hydrochloride (Inderal, ICI); (−)-isoprenaline hydrochloride (Proternol-L inj., Nikken Chemicals); 5-hydroxytryptamine creatinine sulphate (Merck); methysergide dimaleate (Sandaz); papaverine hydrochloride (Wako Pure Chemicals).

All results were expressed as mean ± s.e.mean. The analysis of data for significance was performed by means of Student's *t* test.

Results

The positive inotropic effect of phenylephrine and other agonists

The positive inotropic effect of Phe is shown in Figure 1. The basal tension developments in 6W rats, 10W rats and 7M rats were 290.2 ± 25.2 mg ($n = 17$), 360.7 ± 20.3 mg ($n = 6$), and 388.8 ± 30.5 mg ($n = 12$, $P < 0.05$ vs 6W rats), respectively. The increase in the tension development (TDI) induced by Phe was larger in 10W rats and greatest in 7M rats. Although the basal tension developments in 10W rats and 7M rats were comparable, the TDI was significantly larger in the latter. The EC_{50} values for the positive inotropic effect of Phe are shown in Table 1. The EC_{50} values in the three groups were not significantly different.

Figure 2a shows the positive inotropic effect of isoprenaline in 6W and 7M rats. The TDI induced by isoprenaline was larger in 7M rats at concentrations higher than 1×10^{-9} M, while at 3×10^{-10} M the TDI was similar in the two groups. The EC_{50} value for the positive inotropic effect of isoprenaline in 6W rats was significantly lower than that in 7M rats (Table 2). The positive inotropic effect of 5-hydroxytryptamine (5-HT) is shown in Figure 2b. The TDI induced by 5-HT was larger in 7M rats than in 6W rats at a concentration higher than 1×10^{-6} M. However, the EC_{50} value was significantly lower in 6W rats than in

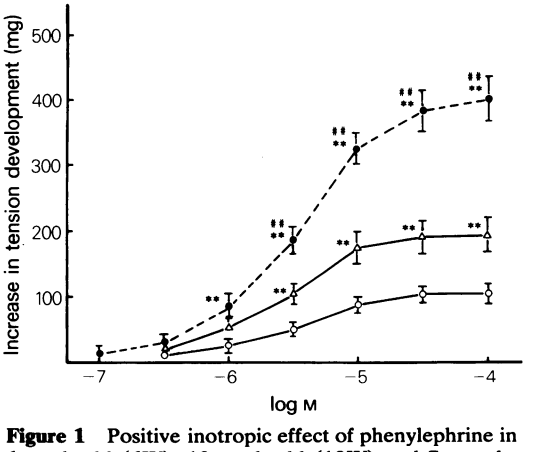

Figure 1 Positive inotropic effect of phenylephrine in 6-week-old (6W), 10-week-old (10W) and 7-month-old (7M) rats. (○) 6W rats, $n = 14$; (△) 10W rats, $n = 6$; (●) 7M rats, $n = 12$. $^{**}P < 0.01$ vs 6W rats. $^{\#\#}P < 0.01$ vs 10W rats.

Table 1 EC_{50} values for the positive inotropic effect of phenylephrine (Phe) in the presence or the absence of adrenolytic drugs on the isolated atria of 6-week, 10-week and 7-month-old rats

	6-week-old	*10-week-old*	*7-month-old*
Phe (a) ($\times 10^{-6}$ M)	3.21 ± 0.34 (17)	2.49 ± 0.22 (6)	3.58 ± 0.42 (12)
Phe with phentolamine† (b) ($\times 10^{-6}$ M)	4.82 ± 0.62 (9)	5.23 ± 0.36 (6)	13.50 ± 2.10** (7)
Phe with propranolol† ($\times 10^{-6}$ M)	3.94 ± 0.50 (5)	—	3.75 ± 0.41 (8)
Phe with phentolamine and propranolol ($\times 10^{-4}$ M)	1.34 ± 0.29 (9)	0.80 ± 0.14 (6)	1.18 ± 1.71 (7)
$\log \frac{b}{a}$	0.150 ± 0.051 (9)	0.325 ± 0.028* (6)	0.534 ± 0.026** (7)

Means ± s.e.mean are given. Numbers in parentheses are the numbers of experiments. *$P<0.05$; **$P<0.01$ vs 6-week-old rats. †3×10^{-7} M.

7M rats (Table 2). The DRC for 5-HT in 6W rats was shifted to the right by 3×10^{-7} M methysergide by 0.82 ± 0.11 log units ($n = 5$).

Figure 3 shows the positive inotropic effect of Ca^{2+}. The TDI induced by 1 mM Ca^{2+} was approximately of the same degree in 6W and 7M rats. However, with Ca^{2+} at concentrations higher than 2 mM the TDI was larger in 7M rats than in 6W rats. When the DRC was represented in terms of % of the maximal response, the DRC in 7M rats lies at the right of the DRC in 6W rats. The EC_{50} value in 7M rats was significantly higher than that in 6W rats (Table 2). The tension development with 2 mM Ca^{2+}, which is the same concentration as in the Krebs-Ringer solution, was 70.0 ± 1.3% ($n = 6$) and 48.9 ± 2.1% ($n = 6$) of the maximal tension development in 6W and 7M rats, respectively.

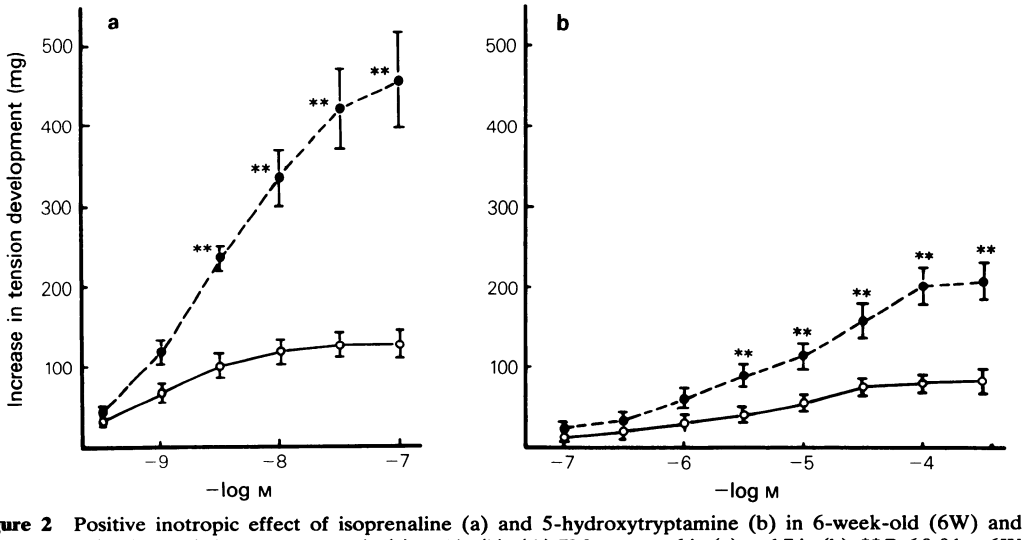

Figure 2 Positive inotropic effect of isoprenaline (a) and 5-hydroxytryptamine (b) in 6-week-old (6W) and 7-month-old (7M) rats. (○) 6W rats, $n = 8$ in (a) and in (b); (●) 7M rats, $n = 6$ in (a) and 7 in (b). **$P<0.01$ vs 6W rats.

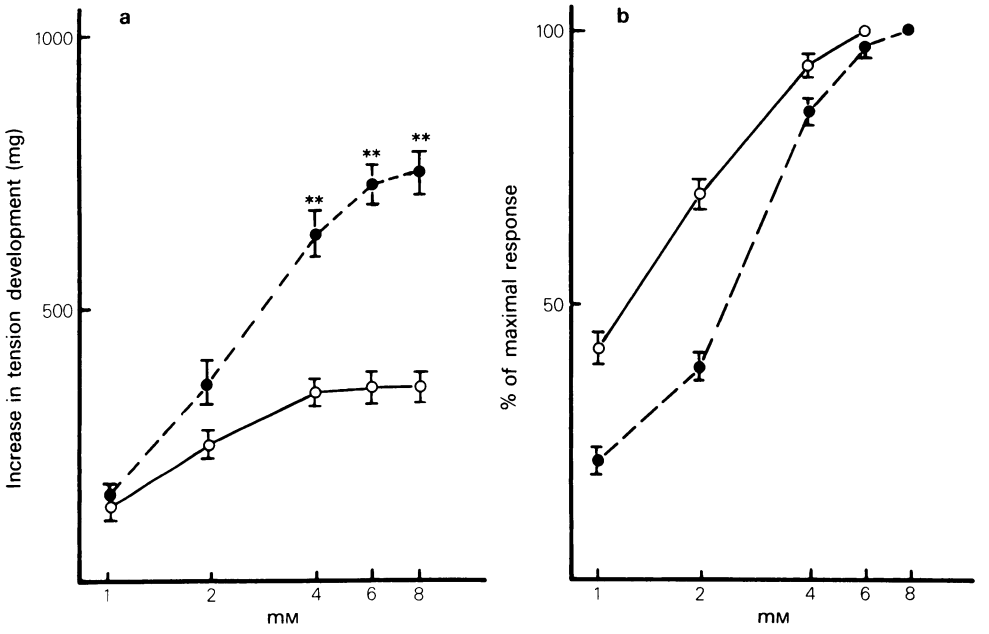

Figure 3 Positive inotropic effect of Ca^{2+} in 6-week-old (6W) and 7-month-old (7M) rats. (○) 6W rats, $n = 6$; (●) 7M rats, $n = 6$. The positive inotropic effect is given in terms of the increase in tension development in (a) and as a % of maximal response in (b). $^{**}P < 0.01$ vs 6W rats.

Effect of adrenolytic drugs on the positive inotropic effect of phenylephrine

When the positive inotropic effect of Phe was represented in terms of % of the maximal response, the DRC in 6W and 7M rats was not significantly different (Figures 4 and 5). Phentolamine at 3×10^{-7} M did not significantly change the DRC for Phe in 6W rats, but in 7M rats it shifted the DRC to the right. The degree of the rightward shift of the DRC was 0.15 log units in 6W rats, 0.32 log units in 10W rats and 0.53 log units in 7M rats (Table 1). The rightward shift of the DRC for Phe with phentolamine plus propranolol 3×10^{-7} M was approximately of the same extent in the three groups.

Figure 5 shows the influence of propranolol 3×10^{-7} M on the effect of Phe. Propranolol showed a slight inhibition of the effect of higher concentrations

Table 2 EC_{50} values for the positive inotropic effect of isoprenaline, 5-hydroxytryptamine (5-HT) and Ca^{2+} on the isolated atria of 6-week and 7-month-old rats

	6-week-old	*7-month-old*
Isoprenaline	0.91 ± 0.20	2.62 ± 0.63*
($\times 10^{-9}$ M)	(8)	(7)
5-HT	0.22 ± 0.04	1.06 ± 0.30**
($\times 10^{-6}$ M)	(8)	(7)
Ca^{2+}	1.20 ± 0.04	2.04 ± 0.08**
($\times 10^{-3}$ M)	(6)	(6)

Means ± s.e.mean are given. Numbers in parentheses are the numbers of experiments. $^{*}P < 0.05$; $^{**}P < 0.01$ vs 6-week-old rats.

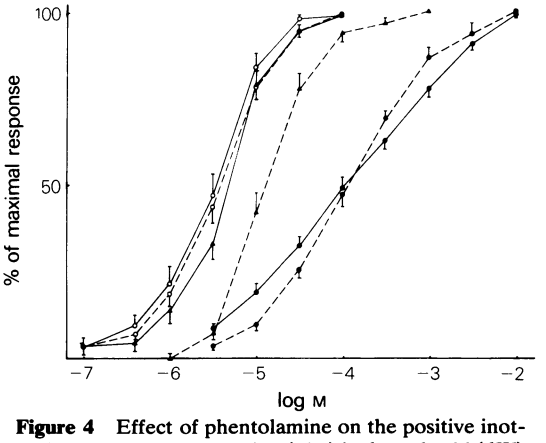

Figure 4 Effect of phentolamine on the positive inotropic effect of phenylephrine (Phe) in 6-week-old (6W) and 7-month-old (7M) rats. (○) Phe; (▲) Phe in the presence of 3×10^{-7} M phentolamine; (●) Phe in the presence of phentolamine plus 3×10^{-7} M propranolol. Solid line = 6W rats ($n = 9$); dashed line = 7M rats ($n = 7$).

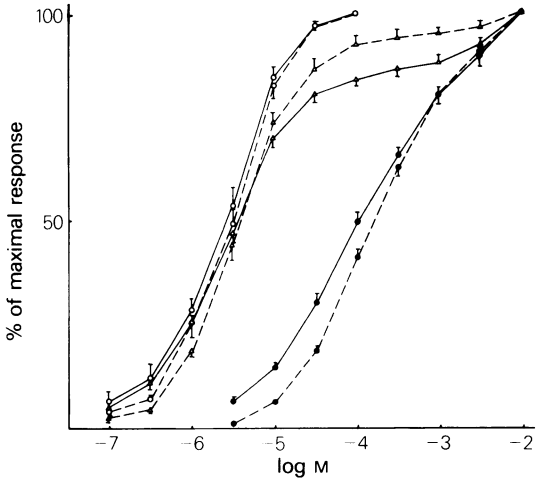

Figure 5 Effect of propranolol on the dose-response curves for phenylephrine (Phe) in 6-week-old (6W) and 7-month-old (7M) rats. (○) Phe; (△) Phe in the presence of 3×10^{-7} M propranolol; (●) Phe in the presence of 3×10^{-7} M phentolamine plus propranolol. Solid line = 6W rats ($n = 9$); dashed line = 7M rats ($n = 5$).

of Phe. The effect of Phe at concentrations less than 1×10^{-6} M was not significantly inhibited by propranolol in either 6W or 7M rats. Thus, the DRC for Phe in the presence of propranolol was almost identical in the two groups except at higher concentrations of Phe, where the degree of inhibition by propranolol was slightly greater in 6W rats. The EC_{50} values for Phe in the presence of propranolol were not significantly different between the two groups. The effect of Phe in the presence of propranolol was markedly inhibited by 3×10^{-7} M phentolamine in both groups.

Effects of phenylephrine on the duration of the contraction in the presence or absence of adrenolytic drugs

The DRC for the effect of Phe on the duration of the contraction is shown in Figure 6. The basal value in 6W rats (84.1 ± 0.8 ms, $n = 9$) was significantly less than that in 7M rats (94.3 ± 1.6 ms, $n = 10$, $P < 0.01$). In 6W rats, Phe significantly decreased the duration of the contraction. Phe in the presence of propranolol showed a tendency to increase the duration, but the change was not statistically significant. The effect of Phe in the presence of phentolamine was not significantly different from that of Phe alone. In 7M rats, Phe in the presence or absence of propranolol produced a tendency to increase or decrease the duration of the contraction, respectively, but the changes were not statistically significant. Phe in the presence of phentolamine decreased the duration of the contraction.

The effect of papaverine on the positive inotropic effects of phenylephrine and isoprenaline

Papaverine initially caused a positive inotropic effect which disappeared during the incubation. Thus, the basal tension development just before the determination of the DRC was not significantly different between the first and the 2nd DRC. As shown in Figure 7a, the DRC for Phe in atria from 6W rats was shifted to the left by papaverine by 0.96 ± 0.12 log units ($n = 8$), whereas the DRC in 7M rats was shifted by 0.40 ± 0.04 log units ($n = 6$, $P < 0.01$ vs 6W rats). The DRC for isoprenaline was also markedly shifted to the left by papaverine in both 6W and 7M rats to the same extent (Figure 7b). The shifts in the DRC for isoprenaline induced by papaverine were 0.98 ± 0.08 log units ($n = 6$) in 6W rats and 0.94 ± 0.06 log units ($n = 5$) in 7M rats.

Discussion

Many authors have reported that the responsiveness of the heart to drugs or stress changes with age (Halloran, Schimoff, Nicholas & Talner, 1970; Rogers, Willerson, Goldblatt & Smith, 1972; Hayes, Butler & Cersony, 1973; Lakatta *et al.*, 1975; Kelliher & Roberts, 1976; Guarnieri *et al.*, 1980; Toda, 1981; Hougen & Friedman, 1982). In the present study, the age-dependent differences in the positive inotropic effect of Phe have been observed in rat isolated atria. The maximal TDI induced by Phe increased with age. A similar age-dependent increase in maximal TDI was observed with isoprenaline, 5-HT and Ca^{2+}, suggesting that some nonspecific mechanism other than changes in receptors are involved in this age-dependent difference. Increased size of the atrium with age may not fully explain this age-dependent difference, because the age-dependent difference in the maximal TDI induced by Phe was not parallel to the age-related increase in the basal tension development. When the DRC for the positive inotropic effect of Ca^{2+} was represented in terms of % of the maximal response, the effect of 2 mM Ca^{2+} was approx. 49% and 70% of the maximal response in 7M and 6W rats, respectively, suggesting that agonists can increase the tension development in 7M rats to a greater extent than in 6W rats. In other words, the age-dependent increases in the maximal TDI induced by Phe as well as other agonists are partially due to the age-dependent change in the responsiveness to Ca^{2+}.

Although EC_{50} values for the positive inotropic effect of Phe did not change with age, the inhibitory effect of phentolamine increased with age. In 6W rats, neither phentolamine nor propranolol inhibited the effect of Phe, if given separately. However, phen-

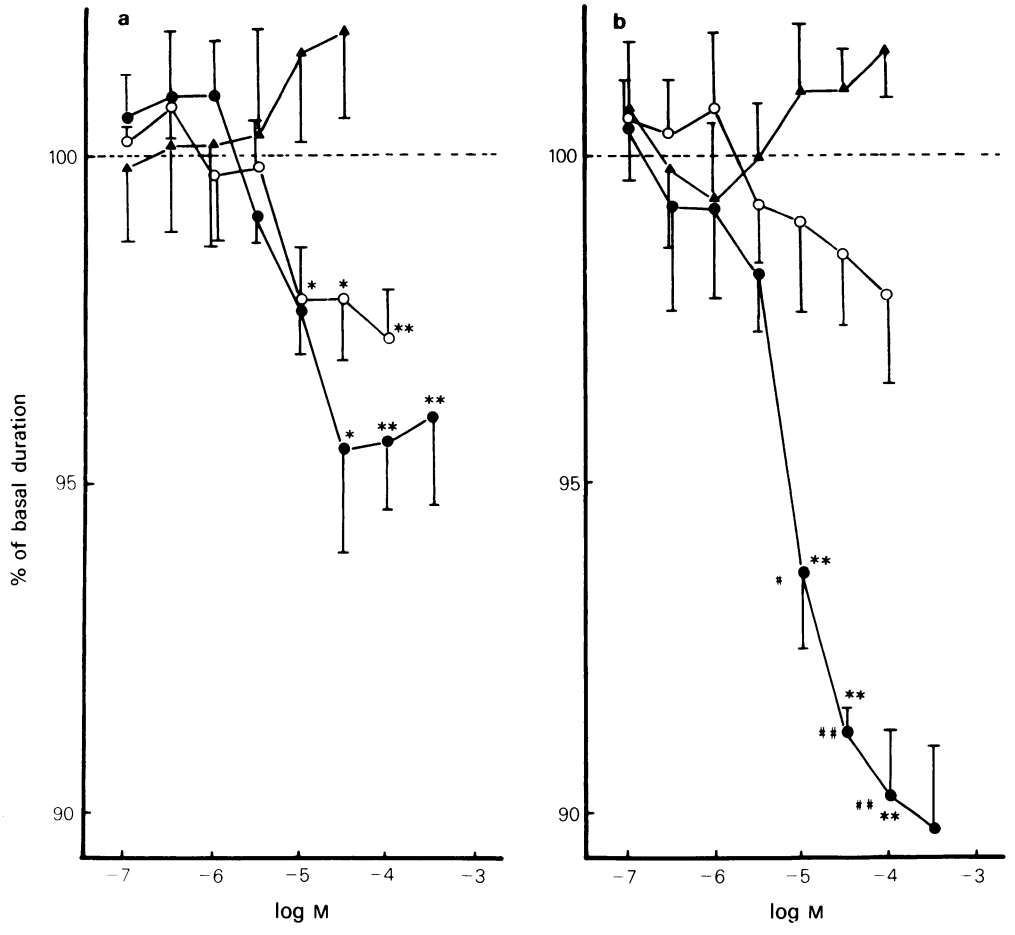

Figure 6 Effect of phenylephrine (Phe) on the duration of contraction in the presence or absence of adrenolytic drugs in (a) 6-week-old (6W, $n = 8$) and (b) 7-month-old (7M, $n = 7$ or 10) rats. (○) Phe; (●) Phe in the presence of 3×10^{-7}M phentolamine; (▲) Phe in the presence of 3×10^{-7}M propranolol. $^{*}P < 0.05$; $^{**}P < 0.01$ vs basal value. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ vs Phe.

tolamine plus propranolol markedly inhibited the effect of Phe. A similar observation was made concerning the positive inotropic effect of dopamine in the rabbit papillary muscle when phentolamine and pindolol were used as adrenolytic drugs (Endoh *et al.*, 1976; Motomura, Brodde & Schümann, 1978). The authors suggested that dopamine produces its positive inotropic effect on the rabbit papillary muscle by stimulation of α- as well as β-adrenoceptors to about the same degree. A similar explanation may be applied in the case of the positive inotropic effect of Phe on atria of 6W rats. On the other hand, the effect of Phe in 7M rats was inhibited by phentolamine but not by propranolol, and propranolol was effective on the Phe in the presence of phentolamine. Therefore, it seems that the positive inotropic effect of Phe in atria is mediated by both α- and β-adrenoceptors in 7M rats as well as 6W rats, but that the balance shifted to more α-receptors in 7M rats. The EC_{50} values both for Phe in the presence of phentolamine and for isoprenaline increased with age (Tables 1 and 2), whereas the EC_{50} values for Phe in the presence of propranolol remained unchanged (Table 1). Thus the EC_{50} value for the α-adrenoceptor-mediated effect of Phe was lower than that for the β-adrenoceptor mediated effect in 7M rats, resulting in the predominant contribution of α-adrenoceptors to the effect of Phe in 7M rats.

In 7M rats, Phe in the presence of propranolol produced a slight increase in the duration of the contraction, although the changes were not statistically significant because of rather large variations in the data. Ledda *et al.* (1975) found that the increase in the duration of the contraction in guinea-pig ven-

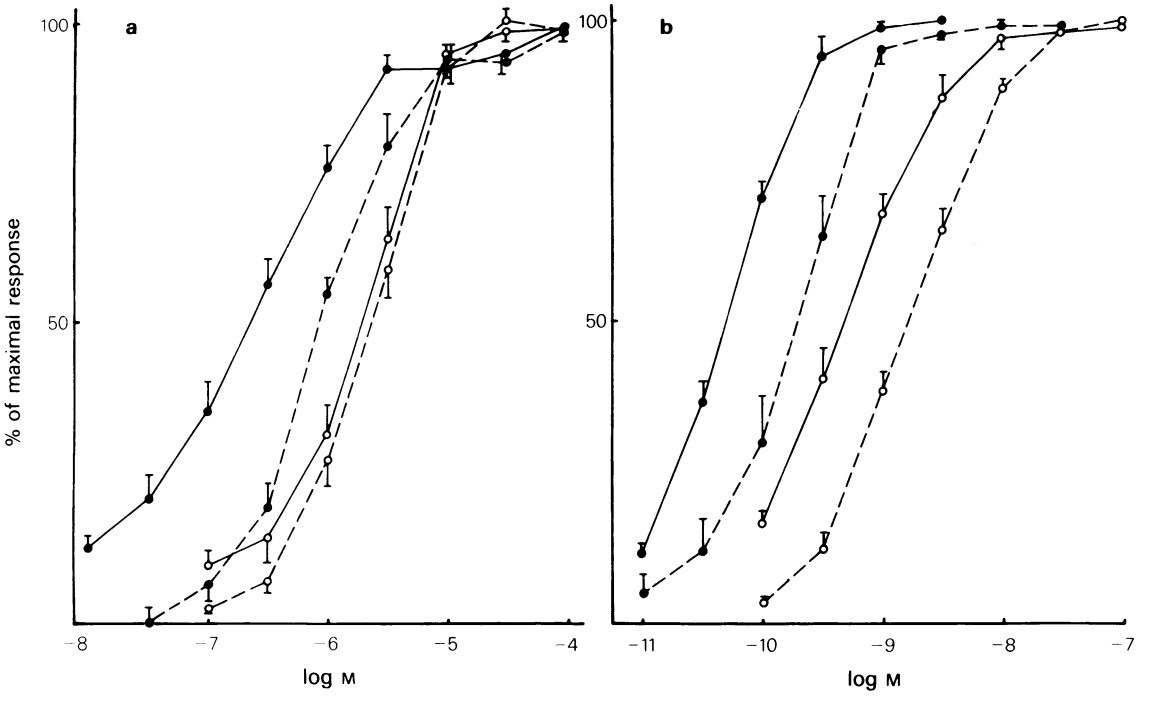

Figure 7 Effects of papaverine on the dose-response curve for phenylephrine (Phe, a) or isoprenaline (b) in 6-week-old (6W) and 7-month-old (7M) rats. (○) Phe or isoprenaline; (●) Phe or isoprenaline with papaverine. Solid line = 6W rats; dashed line = 7M rats. Numbers of the experiments are 8 for 6W rats and 6 for 7M rats in (a), and 6 for 6W rats and 5 for 7M rats in (b).

tricular muscles was α-receptor-mediated. Phe in the presence of phentolamine significantly decreased the duration in 7M rats, which is consistent with other authors' results (Ledda, Marchetti & Mugelli, 1975; Korth, 1978; Hirano *et al.*, 1982). The fact that Phe alone in 7M rats produced only a slight change in the duration of the contraction supports the idea that the extent of the involvement of α-adrenoceptors in the positive inotropic effect of Phe was large in 7M rats. In 6W rats, the effect of Phe alone on the duration was not significantly different from the effect of Phe in the presence of phentolamine, indicating that the effect of Phe alone is mediated mainly by β-adrenoceptors. Although both α- and β-adrenoceptors are involved in the positive inotropic effect in 6W rats, the β-adrenoceptor-mediated effect is predominant in the effect on the duration in 6W rats, because the stimulation of α-adrenoceptors produced only a small change in the duration. It is uncertain why the maximal decrease in duration of contraction induced by Phe in the presence of phentolamine was less in 6W rats but it may be due to the shorter basal duration in 6W rats.

Papaverine, a phosphodiesterase inhibitor, markedly potentiated the positive inotropic effect of Phe in 6W rats, but only slightly in 7M rats. The effect of isoprenaline was potentiated by papaverine in both groups. Several authors have shown that papaverine potentiated the β-adrenoceptor-mediated effect by the inhibition of phosphodiesterase in the rabbit papillary muscle (Schümann *et al.*, 1974; Korth, 1978). The potentiating effect on the β-adrenoceptor-mediated effect by papaverine was of the same degree in 6W and 7M rats, the effect of isoprenaline being markedly potentiated in both groups. Therefore, the smaller potentiation of the effect of Phe by papaverine in 7M rats suggests a smaller degree of involvement of β-adrenoceptors in the effect of Phe in 7M rats, and supports the idea that the positive inotropic effect of Phe in 7M rats is mediated by α-adrenoceptors to a large extent.

Diminished responsiveness to β-adrenoceptor-stimulation has been observed in the heart (Lakatta *et al.*, 1975; Yin *et al.*, 1976; 1979; Guarnieri *et al.*, 1980) and vascular smooth muscle (Cohen & Berkowitz, 1974; Fleisch & Hooker, 1976; Hayashi & Toda, 1978) of older animals. Lakatta *et al.* (1975) have suggested that the diminished inotropic response of the aged myocardium to catecholamines may result from a decreased ability of catecholamines to increase the intracellular Ca^{2+} available for contraction. Guarnieri *et al.* (1980) have reported that

the factors that limit the positive inotropic effect of catecholamines in the aged myocardium act subsequent to protein kinase activation but proximal to the Ca^{2+}-troponin interaction. On the other hand, the decreased number of cardiac β-adrenoceptors in the mature myocardium has also been reported (Baker & Potter, 1980). In the present study, the EC_{50} values for 5-HT and Ca^{2+} also increased with age, suggesting the involvement of nonspecific mechanisms. Because ATPase activity and the rate of superprecipitation of myosin B are greater in young rats (Heller & Whitehorn, 1972), age-dependent alterations of myocardial contractile proteins may result in nonspecific increase in the EC_{50} values for drugs. However, the age-dependent difference in the EC_{50} value for Ca^{2+} was less than those to isoprenaline. Therefore, the alterations in contractile proteins cannot fully explain the increased EC_{50} values for β-adrenoceptor-mediated effects of Phe or isoprenaline. Age-dependent changes in cardiac membrane which have been described by Cavoto, Kelliher & Roberts (1974) and Baker & Potter (1980) may also contribute to the age-related increase in EC_{50} values for drugs.

In spite of the increased EC_{50} values for isoprenaline, 5-HT and Ca^{2+}, the EC_{50} value for Phe in the presence or absence of propranolol did not change with age. According to Sharma, Sundaresan & Banerjee (1979) and Lai, Maeda, Fujita, Watanabe & Yoshida (1981), the number of α-adrenoceptors increases with age. A similar change in α-adrenoceptors was also observed in rat liver (Hornbrook, 1978). Therefore, it is probable that the expected increase in the EC_{50} value for Phe which may be caused by nonspecific mechanisms such as changes in contractile proteins or cell membranes is cancelled by the increase in the number of α-adrenoceptors.

In conclusion, the present results are consistent with the effects of Phe being mediated by both α- and β-adrenoceptors in both 6W and 7M rats, but there is a shift in the balance from rather more β-receptors in young animals to more α-receptors in adults.

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