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Decreased arterial responsiveness to multiple cyclic AMP-generating receptor agonists in spontaneously hypertensive rats

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1 Arterial relaxant responses via β-adrenoceptors are decreased in spontaneously hypertensive rats (SHR) when compared with normotensive Wistar-Kyoto rats (WKY). Recent studies from this laboratory proposed that a reduced function of stimulatory guanosine 5'-triphosphate (GTP)-binding protein (Gs) is responsible for the decreased β-adrenoceptor responsiveness in the SHR femoral artery. Since the Gs is common to all tissues, as opposed to receptors, which are tissue specific, the reduced function of Gs should lead to resistance to multiple receptors that act by activating adenylate cyclase (AC). To test this hypothesis, relaxant responses via β-adrenoceptors, A_2-adenosine, H_2-histamine and D_1-dopamine receptors were compared between arterial strips from 13 week-old WKY and age-matched SHR.

2 The relaxant responses to noradrenaline (NA) via β-adrenoceptors in femoral, mesenteric, renal and carotid arteries were significantly decreased in the SHR, when compared with the respective arteries from WKY.

3 However, under the same conditions arterial relaxant responses to forskolin, an activator of AC, were not significantly different between the WKY and SHR.

4 The relaxant responses due to activation of A_2-adenosine. H_2-histamine and D_1-dopamine receptors were significantly decreased in the SHR arteries.

5 Nitroprusside and nifedipine, agents which are independent of the Gs · AC system, produced similar arterial relaxations in the WKY and SHR.

6 These results support the hypothesis that a reduced function of Gs in the SHR is responsible for the decreased arterial responsiveness to a variety of receptor agonists whose mechanism of action involves AC activation.

Introduction

β-Adrenoceptor-mediated relaxation has been demonstrated to be decreased in a variety of hypertensive animals including spontaneously hypertensive rats (SHR; Amer. 1973; Triner *et al.*, 1975; Cohen & Berkowitz, 1976; Asano *et al.*, 1982). In strips of the SHR femoral artery, the decreased β-adrenoceptor responsiveness is reflected in an enhanced arterial contraction through the activation of α-adrenoceptors (Asano *et al.*, 1982). This decreased β-adrenoceptor responsiveness may contribute to the elevation of total peripheral resistance which occurs in essential hypertension. β-Adrenoceptor-mediated relaxation has been proposed to involve increased cellular adenosine 3':5'-cyclic monophosphate (cyclic AMP) through the activation of adenylate cyclase (AC) and subsequent activation of cyclic AMP-dependent protein kinase in a variety of smooth muscles including vascular smooth muscle (for review, see Anderson & Wilsson, 1977; Kukovetz *et al.*, 1981). According to current models (Gilman, 1987), the activation by β-adrenoceptor agonists of AC-catalyzed cyclic AMP formation involves the interaction of at least three membrane-bound components: β-adrenoceptor, stimulatory guanosine 5'-triphosphate (GTP)-binding protein (Gs) and catalytic component (C) of AC. The interaction of agonists with β-adrenoceptors results in the binding of GTP to Gs, which then activates C, resulting in conversion of adenosine 5'-triphosphate (ATP) to cyclic AMP. The activation of AC can be modified by agents which interact directly with Gs, e.g., cholera toxin (Northup

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et al., 1980) or with C, e.g., forskolin (Seamon & Daly, 1981). Numerous receptors act to stimulate AC, cumulatively leading to an increase in the cellular concentration of cyclic AMP. Stimulation of A_2-adenosine, H_2-histamine and D_1-dopamine receptors usually results in an increase in cyclic AMP concentrations and relaxation of arterial smooth muscle (Goldberg et al., 1978; Bolton, 1979; Daly, 1985; Hilditch & Drew, 1985; Stiles, 1986). However, arterial relaxant responses via these cyclic AMP-generating receptors were not determined in SHR.

In previous studies, we have examined the possible mechanism responsible for the decreased β-adrenoceptor responsiveness in the femoral artery of SHR using several agents which interact with different processes of the β-adrenoceptor · AC system, and have obtained evidence suggesting that a reduced function of Gs is responsible for the decreased responsiveness in the SHR (Asano et al., 1988a,b). Since the Gs and AC are common to the cyclic AMP-generating receptors, the reduced function of Gs could lead to resistance to the stimulation of multiple receptors that link to AC. Moreover, the reduced β-adrenoceptor responsiveness would occur in all the blood vessels from SHR. Therefore, the aim of the present study was two fold: (1) to determine whether the reduced β-adrenoceptor responses can be observed in other arteries than the femoral artery, and (2) to compare relaxant responses due to activation of A_2-adenosine, H_2-histamine and D_1-dopamine receptors in the arteries from normotensive Wistar-Kyoto rats (WKY) and SHR.

Methods

Preparation of arterial strips for recordings of mechanical activity

Male SHR, 13 weeks of age, and age-matched male WKY were used (Asano et al., 1988a). The rats were stunned and exsanguinated. The femoral artery (0.7–0.9 mm outside diameter), distal portion of the superior mesenteric artery (0.7–0.9 mm o.d.), renal artery (0.4–0.6 mm o.d.) and carotid artery (0.9–1.1 mm o.d.) were quickly dissected and placed in the Krebs-bicarbonate solution of the following composition (in mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and dextrose 10.0. Helical strips (0.8 mm in width and 7 mm in length) of these arteries were prepared according to the method described previously (Asano et al., 1988a). The size of the strips of renal arteries was somewhat smaller (0.6 mm × 5 mm). To avoid the possible effects of the endothelium-derived relaxing factor, the endothelium of the strip was removed (Asano et al., 1988a).

Arterial strips were mounted vertically between hooks in water-jacketed (37 ± 0.5°C) muscle baths containing 20 ml of the Krebs-bicarbonate solution. Muscle bath solutions were maintained at 37 ± 0.5°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The hook anchoring the upper end of the strip was connected to the lever of a force-displacement transducer (TB-612T, Nihon Kohden Kogyo Co., Tokyo, Japan) for isometric tension recording. The strips were stretched passively to optimal length by imposing the optimal resting tension (femoral, 0.6 g; mesenteric, 0.5 g; renal, 0.3 g; carotid, 0.4 g). The length-passive tension studies failed to demonstrate differences in the resting tension between the strips from WKY and SHR.

After the 90 min equilibration period, the submaximally effective concentration of K^+ (30 mM) was administered two or three times at 40 min intervals until the responses were reproducible. At the final response, 60 mM K^+ was cumulatively added to obtain the maximum contraction of the strip.

Relaxation of arterial strips

To compare agonists that relax blood vessels, arterial strips were contracted with K^+ to an equivalent magnitude of tension (85% of the maximum contraction developed by 60 mM K^+) before challenge with the agonists (Asano et al., 1988a). These contractions induced by ED_{85} K^+ were well sustained for at least 3 h. Cumulative dose-response curves for the arterial relaxant responses to agonists were determined by producing a stepwise increase in the concentration of the agonist as soon as a stable response to the preceding dose had been obtained. At the end of experiments, 10^{-4} M papaverine was added to obtain the maximum relaxation of the strip. In some experiments, strips were contracted to 70% of the maximum contraction developed by 60 mM K^+ before challenge with the agonists.

All the experiments were conducted in phenoxybenzamine (Pbz)-treated strips to eliminate possible α-adrenoceptor responses. Strips were treated with 2×10^{-6} M Pbz during the first 60 min of the 90 min equilibration period. Using these Pbz-treated strips, relaxant responses to NA via β-adrenoceptors could be consistently demonstrated (Asano et al., 1982; 1988a).

Statistical analysis

When assessing the ED_{50} value, responses to agonists were calculated as % of the maximum response obtained with each agonist. The ED_{50} value was obtained visually from a plot of % response vs log

concentration of the agonist and expressed as a negative log (pD_2 value).

Unless specified, results shown in the text and figures are expressed as the mean value ± s.e. (n = number of preparations). Statistical analysis of the data was done by Student's t test for unpaired data, or by completely randomized design, one-way analysis of variance followed by Newman-Keuls test for a significant F ratio ($P < 0.05$), depending on which test was statistically appropriate. Two groups of data were considered to be significantly different when $P < 0.05$.

Drugs and chemicals

The following drugs were used: (−)-noradrenaline bitartrate (NA; Sigma Chemical Co., St. Louis, MO), adenosine (Sigma), 5'-N-ethylcarboxamide adenosine (NECA; Sigma), histamine dihydrochloride (Wako Pure Chemical Industries, Osaka, Japan), dopamine hydrochloride (Sigma), forskolin (Nippon Kayaku Co., Tokyo, Japan), phenoxybenzamine hydrochloride (Pbz; Nakarai Chemicals, Kyoto, Japan), timolol maleate (Banyu Pharmaceutical Co., Tokyo, Japan), cimetidine (Fujisawa Pharmaceutical Industries, Osaka, Japan), pyrilamine maleate (Sigma), SCH 23390 maleate ((R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol; Schering Corp., Kenilworth, NJ), sodium nitroprusside dihydrate (Wako), nifedipine (Bayer Yakuhin Ltd., Osaka, Japan) and papaverine hydrochloride (Wako).

Stock solutions of Pbz (10^{-3} M) and forskolin (10^{-3} M) were prepared using 50% ethanol with further dilution in distilled water before use. Nifedipine was dissolved in 99.5% ethanol to make a stock solution of 10^{-3} M with further dilution in distilled water before use. NA, histamine and dopamine were prepared daily in Krebs-bicarbonate solution and kept on ice during the course of the experiment. Aqueous stock solutions were prepared for other drugs. Concentrations of drugs are expressed as final molar concentrations in the muscle bath.

Results

Relaxation of arterial strips induced by NA and forskolin

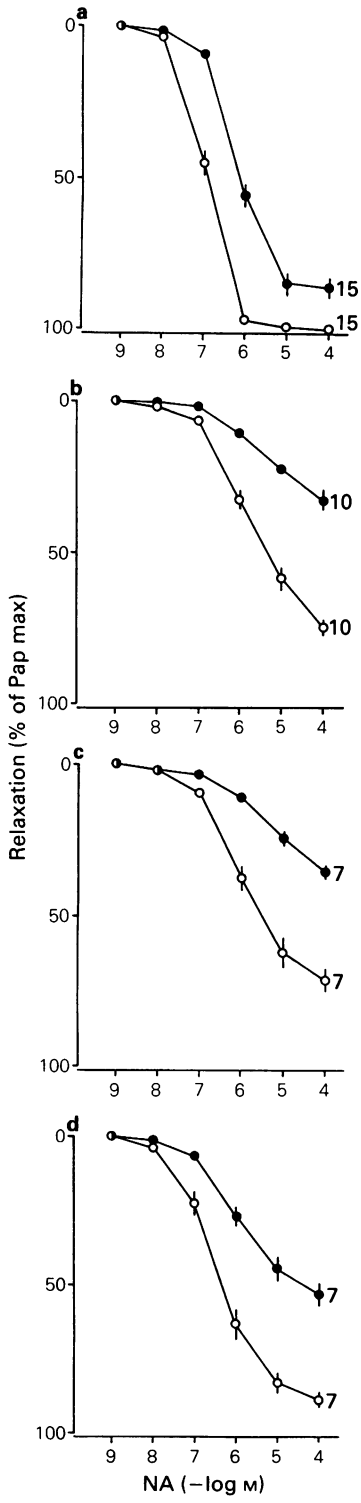
Relaxant responses to NA were first compared between the Pbz-treated, K^+-contracted strips of femoral arteries from WKY and SHR. The addition of 17.5 ± 0.6 mM K^+ ($n = 15$) to a strip of the WKY femoral artery caused a sustained contraction which was approximately 85% of the maximum contraction developed by 60 mM K^+ (Figure 1a). On the other hand, for an equivalent contraction to be observed in a SHR strip, 12.7 ± 0.8 mM K^+ ($n = 15$) was required (Figure 1a). The addition of NA (10^{-9}–10^{-4} M) produced a dose-dependent relaxation in Pbz-treated, ED_{85} K^+-contracted strips of femoral arteries from both strains (Figure 1a). These relaxations were dose-dependently antagonized by 5×10^{-8} and 5×10^{-7} M timolol, confirming that the relaxation induced by NA is due to the activation of β-adrenoceptors. The relaxant responses of femoral arteries to NA were significantly weaker in the SHR than in the WKY (Figure 1a). When the relaxant responses to NA were determined in Pbz-treated, ED_{85} K^+-contracted strips of mesenteric, renal and carotid arteries, there was a decreased responsiveness to NA in the three arteries from SHR when compared with the respective arteries from WKY (Figure 1b,c,d). The relaxant responses to NA in the mesenteric, renal and carotid arteries were significantly weaker than the responses in the femoral arteries. The pD_2 value for NA-induced relaxation in the WKY femoral artery (6.87 ± 0.06, $n = 15$) was significantly different from the pD_2 value in other WKY arteries. The maximum relaxant response to NA in the WKY femoral artery (99.2 ± 0.2%, $n = 15$) was significantly greater than the maximum response in other WKY arteries. Similar results were also obtained in the SHR arteries (Figure 1).

Similar experiments were performed on the relaxant responses to forskolin in the femoral, mesenteric, renal and carotid arteries. The addition of forskolin (3×10^{-9}–1×10^{-6} M) produced a dose-dependent relaxation in the Pbz-treated, ED_{85} K^+-contracted strips of these arteries (Figure 2). Under the same experimental conditions as those used to obtain NA-induced relaxation, the relaxant responses to forskolin were not significantly different between the WKY and SHR (Figure 2). Moreover, the relaxant responses to forskolin were not significantly different between the four arteries (Figure 2).

Relaxation of arterial strips due to activation of A_2-adenosine, H_2-histamine and D_1-dopamine receptors

Relaxant responses to either adenosine or NECA were compared between the Pbz-treated, ED_{70} K^+-contracted strips of femoral arteries from WKY and SHR (Figure 3a,b). For an equivalent 75% contraction to be observed in the femoral arteries, 15.2 ± 0.5 ($n = 6$) and 10.8 ± 0.7 ($n = 6$) mM K^+ were required for WKY and SHR, respectively. Both agonists produced a dose-dependent relaxation in these strips. However, the relaxant responses to the two agonists were significantly weaker in the SHR than in the WKY (Figure 3a,b).

Relaxant responses to histamine were determined in the presence of 1×10^{-6} M pyrilamine and



5×10^{-7} M timolol in Pbz-treated, ED_{70} K^+-contracted strips of femoral arteries (Figure 3c). Histamine produced a dose-dependent relaxation in these strips. The maximum relaxation was obtained when 1×10^{-4} M histamine was added, and a higher concentration of this agonist (3×10^{-4} M) produced a significant contraction. These relaxations were diminished by 1×10^{-4} M cimetidine, an H_2-histamine receptor antagonist, suggesting that histamine produces the relaxation through the activation of H_2-histamine receptors. The extent of relaxation induced by histamine was significantly weaker in the SHR than in the WKY (Figure 3c).

Relaxant responses to dopamine were determined in Pbz-treated, ED_{70} K^+-contracted strips of renal arteries, because renal arteries have been demonstrated to possess vasodilator D_1-dopamine receptors (Goldberg *et al.*, 1978; 1984; Alkahdi *et al.*, 1986). The relaxant responses to dopamine were significantly weaker in the SHR than in the WKY (Figure 3d). These relaxations were diminished by 1×10^{-7} M SCH 23390, a selective D_1-dopamine receptor antagonist (Iorio *et al.*, 1983; Goldberg *et al.*, 1984; Hilditch & Drew, 1985), suggesting that dopamine produces the relaxation through activation of D_1-dopamine receptors.

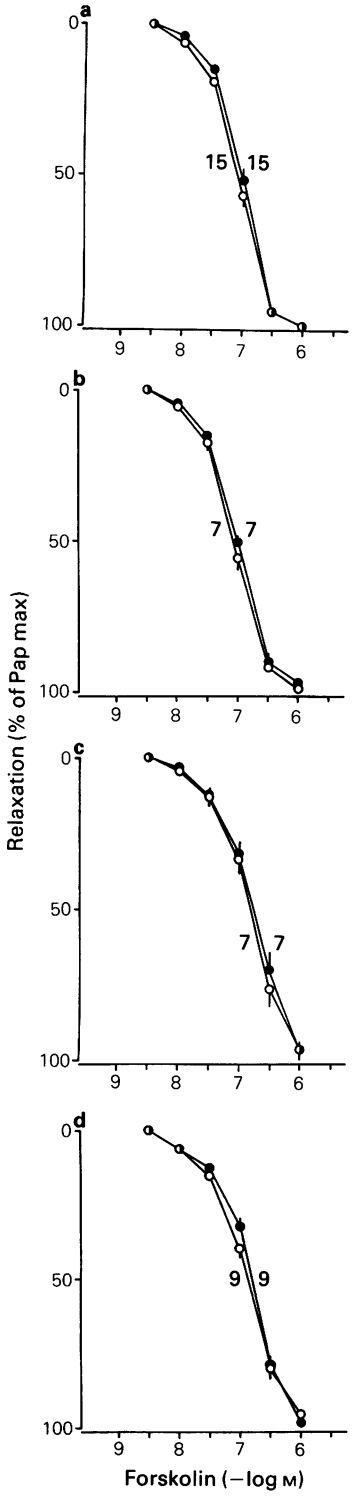
Relaxation of arterial strips induced by nitroprusside and nifedipine

The addition of either nitroprusside or nifedipine also produced a dose-dependent relaxation in the Pbz-treated, ED_{85} K^+-contracted strips of femoral arteries from WKY and SHR. Dose-response curves for the relaxation induced by either nitroprusside or nifedipine were not significantly different between the two strains (Figure 4).

Discussion

The present study evaluated the relaxant responses due to activation of a cyclic AMP-generating system in strips of several peripheral arteries isolated from

Figure 1 Dose-response curves for the relaxant responses to noradrenaline (NA) determined after precontraction with ED_{85} K^+ in phenoxybenzamine-treated strips of femoral (a), mesenteric (b), renal (c) and carotid (d) arteries isolated from 13 week-old WKY (○) and age-matched SHR (●). ED_{85} K^+ = 17.5 and 12.7 mM in (a), 18.2 and 20.3 mM in (b), 22.2 and 21.6 mM in (c), and 19.2 and 16.0 mM in (d) for WKY and SHR, respectively. Relaxation induced by 10^{-4} M papaverine (Pap) was taken as 100%. Vertical lines represent s.e. Numbers beside the dose-response curves indicate the number of preparations used.



WKY and SHR. β-Adrenoceptor-mediated relaxations were decreased in SHR femoral, mesenteric, renal and carotid arteries when compared with the respective WKY arteries. The relaxant responses due to activation of A_2-adenosine, H_2-histamine and D_1-dopamine receptors were also decreased in SHR arteries. However, forskolin-induced relaxations did not differ between these arteries from WKY and SHR.

A consistent loss in responsiveness of arterial smooth muscle to β-adrenoceptor agonists has been demonstrated in SHR (Amer, 1973; Triner *et al.*, 1975; Klenerova *et al.*, 1975; Cohen & Berkowitz 1976; Sands *et al.*, 1976). However, the data were limited to the aorta. Decreased β-adrenoceptor responses were observed in all the arterial strips used in the present study. Thus, the β-adrenoceptor · AC system appears to be systemically impaired in SHR. Judging from the NA-induced relaxations (Figure 1), β-adrenoceptor activities were quite different between these arteries. On the other hand, the relaxant responses of these arteries to forskolin, an activator of AC, were not decreased in the SHR, suggesting that components of the β-adrenoceptor · AC system distal to and including AC are not responsible for the decreased responsiveness to multiple cyclic AMP-generating vasodilators. This suggestion is also supported by observations from our previous study (Asano *et al.*, 1988a) that the relaxant responses of femoral arteries to dibutyryl cyclic AMP were the same in WKY and SHR. Thus, the abnormality in SHR arteries is assumed to occur at the level of the β-adrenoceptor or Gs. Judging from the forskolin-induced relaxations (Figure 2), the activity of AC appears to be similar in the four arteries used.

Adenosine receptors are linked to the AC system in various tissues (for review, see Daly, 1985; Stiles, 1986). The studies of Burnstock and his colleagues demonstrated that the effects of adenosine and adenine nucleotides were mediated by two distinct purine receptors termed P_1 and P_2 (Burnstock, 1979). By using adenosine and its derivatives, Londos & Wolff (1977) obtained evidence supporting the existence of two distinct receptors that

Figure 2 Dose-response curves for the relaxant responses to forskolin determined after precontraction with ED_{85} K^+ in phenoxybenzamine-treated strips of femoral (a), mesenteric (b), renal (c) and carotid (d) arteries isolated from 13 week-old WKY (○) and age-matched SHR (●). ED_{85} K^+ = 18.1 and 12.9 mM in (a), 17.9 and 20.0 mM in (b), 22.0 and 21.3 mM in (c), and 20.1 and 15.4 mM in (d) for WKY and SHR, respectively. Relaxation induced by 10^{-4} M papaverine (Pap) was taken as 100%. Vertical lines represent s.e. Numbers beside the dose-response curves indicate the number of preparations used.

altered the activity of AC. Depending upon the type of cells, cellular effects of adenosine are mediated by two separate subtypes of P_1-receptor, termed A_1 (or Ri) and A_2 (or Ra) (Van Calker *et al.*, 1979; Londos *et al.*, 1980). By interacting with one of these two receptor subtypes, adenosine can initiate a transmembrane signal to either inhibit (A_1) or stimulate (A_2) the AC activity. Adenosine receptor subtypes can be defined by a unique agonist potency series of adenosine derivatives such that the A_2-adenosine receptors have a potency series of NECA > adenosine > N^6-phenylisopropyladenosine (PIA) while the reverse is true for the A_1-receptors (Brown & Collis, 1982; Mustafa & Askar, 1985; Stiles, 1986). NECA is 10 to 100 times more potent than adenosine at A_2-adenosine receptors (Brown & Collis, 1982; Edvinsson & Fredholm, 1983; Mustafa & Askar, 1985). In the present study of femoral arteries, NECA was approximately 30 times more potent than adenosine in producing the arterial relaxation, suggesting that the two agonists produce relaxation through activation of A_2-adenosine receptors.

Both H_1- and H_2-histamine receptors are located on smooth muscle of resistance vessels. In various smooth muscles, relaxations induced by histamine through the activation of H_2-histamine receptors are associated with a rise in cyclic AMP. In some smooth muscles, however, the H_2-receptor responses were mediated by the release of catecholamines from

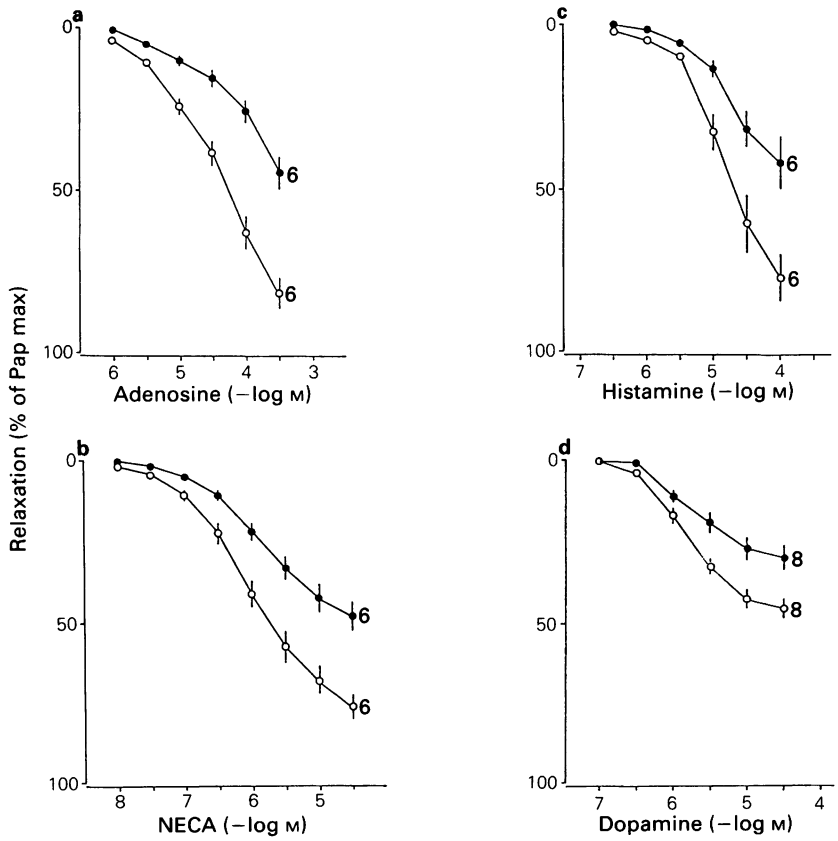


Figure 3 Dose-response curves for the relaxant responses to adenosine (a), 5'-N-ethylcarboxamide adenosine (NECA, b), histamine (c) and dopamine (d) in phenoxybenzamine-treated, ED_{70} K^+-contracted strips of femoral (a, b, c) and renal (d) arteries isolated from 13 week-old WKY (○) and age-matched SHR (●). The relaxant responses to histamine were determined in the presence of 1×10^{-6} M pyrilamine and 5×10^{-7} M timolol after precontraction with ED_{70} K^+. ED_{70} K^+ = 15.2 and 10.8 mM in (a), 14.6 and 10.2 mM in (b), 14.2 and 10.0 mM in (c) and 20.1 and 17.5 mM in (d) for WKY and SHR, respectively. Relaxation induced by 10^{-4} M papaverine (Pap) was taken as 100%. Vertical lines represent s.e. Numbers beside the dose-response curves indicate the number of preparations used.

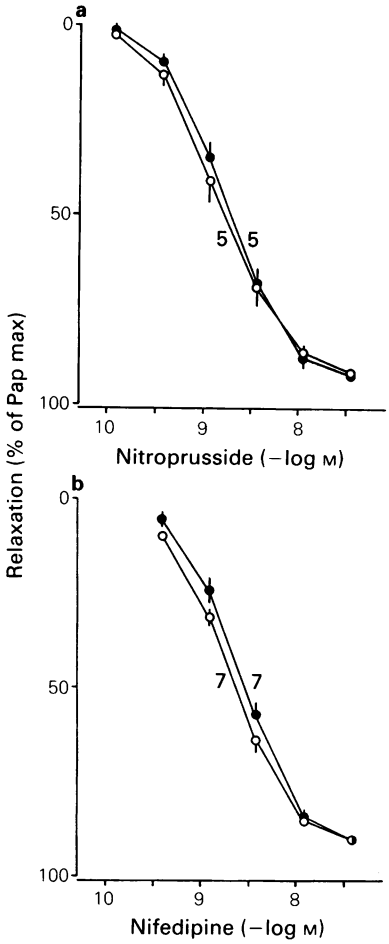


Figure 4 Dose-response curves for the relaxant responses to nitroprusside (a) and nifedipine (b) in phenoxybenzamine-treated, ED_{85} K^+-contracted strips of femoral arteries isolated from 13 week-old WKY (○) and age-matched SHR (●). ED_{85} K^+ = 18.0 and 12.7 mM in (a), and 18.2 and 13.0 mM in (b) for WKY and SHR, respectively. Relaxation induced by 10^{-4} M papaverine (Pap) was taken as 100%. Vertical lines represent s.e. Numbers beside the dose-response curves indicate the number of preparations used.

sympathetic nerve endings (for review, see Bolton, 1979). Moreover, histamine has also been demonstrated to produce arterial relaxation through the activation of H_1-histamine receptors in the endothelium (Toda, 1986). Therefore, to exclude these possibilities, the relaxant responses to histamine in the present study were conducted in Pbz-treated, endothelium-removed strips of femoral arteries in the presence of timolol and pyrilamine. Under these conditions, relaxant responses through the activation of smooth muscle H_2-histamine receptors could be consistently demonstrated.

Peripheral dopamine receptors are subdivided into D_1 and D_2 subtypes, primarily on the basis of agonist and antagonist profiles of action (Goldberg *et al.*, 1978; 1984; Hilditch & Drew, 1985). The D_1-dopamine receptors produce smooth muscle relaxation including vasodilatation in the renal, mesenteric, coronary and cerebral vascular beds, and these relaxations are selectively antagonized by SCH 23390. The activation of D_1-receptors in vascular blood vessels leads to an increase in the AC activity (Alkadhi *et al.*, 1986). On the other hand, D_2-receptors inhibit the release of NA from sympathetic nerve endings, and these effects are selectively antagonized by domperidone. In the present study of renal arteries, the dopamine-induced relaxations were antagonized by SCH 23390, suggesting that dopamine was acting on D_1-receptors.

The relaxant responses to the stimulation of cyclic AMP-generating receptors, including β-adrenoceptors, A_2-adenosine, H_2-histamine and D_1-dopamine receptors, were decreased in SHR arteries. On the other hand, relaxant responses to nitroprusside and nifedipine, agents which are independent of the Gs · AC system, were not decreased in SHR, suggesting that the decreased responsiveness of SHR arteries is specific for the receptor agonists whose mechanism of action involves AC activation.

Recent studies from this laboratory (Asano *et al.*, 1988b) have provided evidence suggesting that a reduced function of Gs is involved in the decreased β-adrenoceptor responses in the SHR femoral artery. In these studies (Asano *et al.*, 1988b) the inhibitory effects of cholera toxin, an activator of Gs (Northup *et al.*, 1980), on the contractile responses to α-adrenoceptor stimulation with NA were compared with the contractile responses to NA determined in the absence of β-adrenoceptor antagonists. When the contractile responses of femoral arteries to NA were determined in the absence of timolol, we observed that the responses consisted of α-adrenoceptor-mediated contractions and β-adrenoceptor-mediated relaxations. The interaction between α- and β-adrenoceptors was quite different between WKY and SHR. On the other hand, the ability of cholera toxin to antagonize the α-adrenoceptor-mediated contractions was weaker in SHR than in WKY (Asano *et al.*, 1988b). The dose-response curve for NA determined in the absence of timolol was in good agreement with the dose-response curve for α-adrenoceptor-mediated contractions determined after the pretreatment with cholera toxin in either WKY or SHR. These results strongly suggest that the difference at the level of Gs between WKY and SHR reflected the difference at the level of β-adrenoceptors. If both Gs and β-adrenoceptors are

assumed to be impaired, such agreement of the dose-response curves cannot be expected, because the activation by NA of β-adrenoceptors contains two components and the activation by cholera toxin of Gs contains one component. For these reasons, we proposed that a reduced function of Gs is responsible for the decreased β-adrenoceptor responsitveness in the SHR femoral artery (Asano *et al.*, 1988b). Since the Gs and AC are common to multiple cyclic AMP-generating receptors, the reduced function of Gs could lead to resistance to the activation of A_2-adenosine, H_2-histamine and D_1-dopamine receptors. The results of the present study support this hypothesis.

Although radioligand bindings for each receptor were not actually measured, it is concluded that a reduced function of Gs is responsible for the decreased arterial responsiveness to multiple cyclic AMP-generating receptor agonists in SHR. The present pharmacological approach to the Gs·AC system in SHR arteries needs to be strengthened by the biochemical analysis of Gs and receptor binding studies in the same tissues, and this is the subject of our current investigations.

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