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Vasodilator Responses to Cholinergic and Adrenergic Stimulants in Spontaneously Hypertensive (SHR) and Wistar–Kyoto (WKY) Normotensive Rats (41545)

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Abstract. The cardiovascular effects of isoprenaline, methacholine, and sodium nitroprusside were studied in spontaneously hypertensive (SHR) and in Wistar–Kyoto normotensive (WKY) rats. In conscious rats with chronic indwelling arterial cannulae, methacholine caused a much greater fall in mean arterial blood pressure (MAP) in SHR rats, abolishing the initial 30–40 mm Hg difference in MAP at a dose of 2.5 μg/kg. The hypotensive response to methacholine was accompanied by reflex tachycardia in WKY rats but by a dose-related bradycardia in SHR rats. Isoprenaline increased heart rate and reduced blood pressure to a similar extent in rats of both strains. Administration of sodium nitroprusside resulted in a hypotensive response that was of greater duration in SHR rats. In addition, the reflex tachycardia was more marked and of greater duration in WKY compared to SHR rats. This demonstrates that the baroreflex response to vasodilation is suppressed in SHR rats and that this may contribute to the increased vasodepressor response of these animals to methacholine. However, when baroreflexes but not initial MAP were suppressed with pentobarbital, methacholine caused a similar degree of bradycardia in SHR and WKY rats but a greater vasodepressor response in SHR rats. With isoprenaline, the MAP difference between strains was maintained at all doses up to 200 ng/kg. Although it is not yet clear what is responsible for the increased vascular constriction of SHR rats, our findings suggest that it can be removed by muscarinic receptor activation with methacholine but not by stimulation of β-adrenoceptors.

Spontaneously hypertensive (SHR) rats, originally developed by Okamoto and Aoki (1), are widely regarded as an appropriate animal model for essential hypertension in humans (2, 3). In both SHR rats and in some hypertensive patients, it has been suggested that the development of hypertension may be associated with increased sympathetic tone (4, 5). Such increases in sympathetic nervous system activity could bring about a reduction in the sensitivity of vascular β-adrenoceptors which mediate vasodilation to catecholamines, leaving the vasoconstrictor effects unopposed (6). This suggestion was supported by *in vitro* data which demonstrated that the sensitivity of aortic strip preparations from SHR rats to the relaxant effects of isoprenaline was significantly less than for normotensive controls (7, 8).

The present study was undertaken to compare the effects of isoprenaline, methacholine, and sodium nitroprusside on the cardiovascular system of 8-month-old conscious SHR and Wistar–Kyoto normotensive (WKY) rats. By 8 months of age, SHR rats have sustained elevations in blood pressure as well as several complications of hypertension, including left ventricular hypertrophy, impaired renal function, and structural changes in the vasculature (2, 3). Thus, any differences between strains in their cardiovascular responses to drugs must be viewed as resulting from genetic differences between SHR and WKY rats and from the numerous complications of established hypertension observed for SHR rats. It has been demonstrated that the usual indirect procedure for measuring arterial blood pressure in conscious rats (tail-cuff method) causes increases in plasma catecholamines, particularly in SHR rats, because of the restraining and heating procedures (9). Since these effects would obviously obscure or distort the effects of isoprenaline and methacholine, blood pressure was measured directly by means of a permanent indwelling tail artery cannula while rats remained undisturbed in their home cages. Under such conditions, plasma catecholamines remain low and are similar in SHR and WKY rats (5, 8, 10).

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Methods. Adult male SHR and WKY rats, 8 months of age and weighing 375–450 g, were obtained from Taconic Farms, Germantown, New York, and housed in groups of 4 or 5 for at least one week prior to use. Food and water were available continuously and the vivarium was maintained on a 12-hr light:12-hr dark photoperiod (lights on at 0600 hr). A PE 50 cannula was inserted into the ventral caudal artery of each SHR and WKY rat while under pentobarbital anesthesia (9). In some experiments, a cannula of silastic tubing was also inserted into the left jugular vein, led out at the back of the neck and taped to the spring wire covering the arterial cannula. Rats were then housed singly in plastic cages and the cannulae were flushed twice daily with 0.5 ml of 0.9% saline containing 500 U/ml of sodium heparin. For experiments on anesthetized rats, animals were injected with pentobarbital (40–50 mg/kg, ip) and PE 50 cannulae were inserted into the ventral caudal arteries. Drug studies were then performed using this acute preparation. An additional dose of pentobarbital (10 mg/kg) was given approximately one hour after the first to maintain a stable level of anesthesia.

Blood pressure was measured by connecting the end of the arterial cannula directly to a Statham pressure transducer (P23AC) with tracings made on a Grass polygraph. Heart rate was measured with a Grass tachometer that was triggered by fluctuations in arterial blood pressure. Using this technique, direct measures of mean arterial blood pressure and heart rate can be obtained from conscious rats that are undisturbed in their home cages. Drugs were injected into the arterial cannula by using a three-way stopcock or were injected directly into the venous cannula. All drug injections were given in a volume of 0.2 ml and were flushed in with 0.2 ml of 0.9% saline. Three to five doses of either isoprenaline or methacholine were injected intraarterially twice into each rat in ascending order of magnitude at intervals of not less than 5 min to avoid tachyphylaxis. Only one drug was administered to each of eight pairs of SHR and WKY rats. Sodium nitroprusside (2 μg/kg) was injected intravenously into conscious SHR and WKY rats. Control injections of 0.4 ml of saline were also performed and were not observed to have significant effects on MAP or heart rate. The maximum changes in mean arterial pressure and heart rate were recorded for a given dose of each drug. In addition, the duration of action of isoprenaline and sodium nitroprusside was estimated for each animal by measuring the time from drug injection until the mean arterial pressure and heart rate returned to preinjection values.

Drugs were obtained from the following sources: l-isoprenaline sulfate from Sterling Winthrop, Rensselaer, New York; methacholine chloride from Aldrich Chemical Company, Milwaukee, Wisconsin; and sodium nitroprusside from Roche Laboratories, Nutley, New Jersey. Solutions of isoprenaline (including ascorbic acid, 10 μg/ml, as an antioxidant), methacholine, and sodium nitroprusside were prepared freshly in 0.9% saline before each experiment and stored on ice until use. All doses are expressed as nanograms or micrograms per kilogram of the salt. Student's t test was used to compare the mean values for MAP and heart rate of SHR and WKY rats.

Results. The average resting MAP ± SE of all conscious SHR rats in this study was 153 ± 5 mm Hg compared to a value of 116 ± 3 mm Hg for conscious WKY rats ($P < 0.01$). The mean heart rate of SHR rats (336 ± 6 beats/min) was also higher than that of WKY rats (306 ± 7 beats/min) ($P < 0.05$).

Isoprenaline increased heart rate by similar amounts and for similar durations in conscious SHR and WKY rats at each of three doses (Fig. 1 and Table I). MAP was also reduced in SHR and WKY rats by the same

TABLE I. DURATION OF CHANGES IN MEAN ARTERIAL PRESSURE (MAP, mm Hg) AND HEART RATE (HR, beats/min) OF CONSCIOUS SHR AND WKY RATS FOLLOWING ADMINISTRATION OF ISOPRENALINE[a]

Dose of isoprenaline (ng/kg)	Duration of change (sec)			
	MAP		HR	
	SHR	WKY	SHR	WKY
50	69 ± 10*	30 ± 6	118 ± 9	89 ± 16
100	110 ± 11*	56 ± 9	165 ± 18	133 ± 12
200	196 ± 18**	89 ± 12	225 ± 11	213 ± 21

[a] Values are means ± SEM for eight rats per strain.
* $P < 0.005$.
** $P < 0.001$, two-tailed t test.

amount at each dose level so that the initial strain difference in MAP of 30 mm Hg was maintained (Fig. 2). However, the duration of the fall in MAP was significantly longer (P's < 0.005) for SHR rats at each of the three doses of isoprenaline (Table I).

Methacholine caused a pronounced reflex tachycardia in WKY rats while in SHR rats only a dose-related bradycardia was observed (Fig. 1). In addition, methacholine caused a much greater fall in MAP in SHR rats. Thus, at a dose of 2.5–5 μg/kg of methacholine, the MAP reached the same level in the two strains of rats in spite of an initial difference between strains of 42 mm Hg (Fig. 2).

Pentobarbital anesthesia did not significantly lower the resting MAP of either strain of rats, nor did it raise the heart rate of SHR rats. However, the heart rate of WKY rats was increased significantly following pentobarbital anesthesia from 306 ± 7 to 335 ± 9 beats/min ($P < 0.05$). The increase in heart rate induced by isoprenaline was similar in anesthetized rats compared to conscious rats, and was of similar magnitude in the two strains at each of five dose levels. On the other hand, pentobarbital anesthesia abolished the reflex tachycardia of WKY rats induced by methacholine, converting it to a dose-related bradycardia similar to that seen in SHR rats (Fig. 3). Isoprenaline lowered the MAP of anesthetized SHR and WKY rats by similar

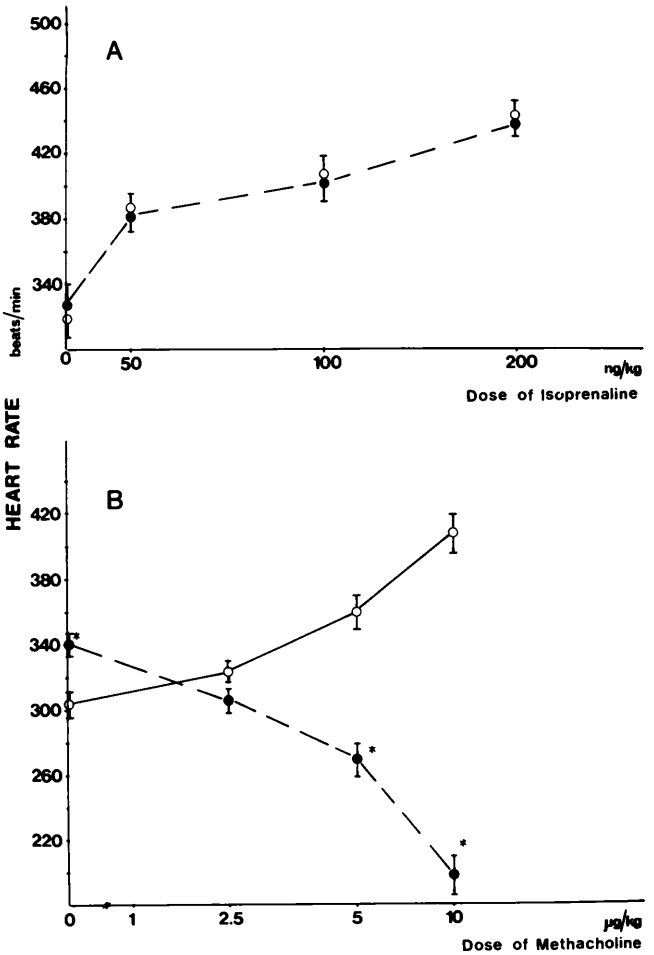

FIG. 1. Effects of isoprenaline (A) and methacholine (B) on heart rate in conscious SHR and WKY rats. ○, WKY rats; ●, SHR rats. * denotes significant from WKY rats ($P < 0.05$).

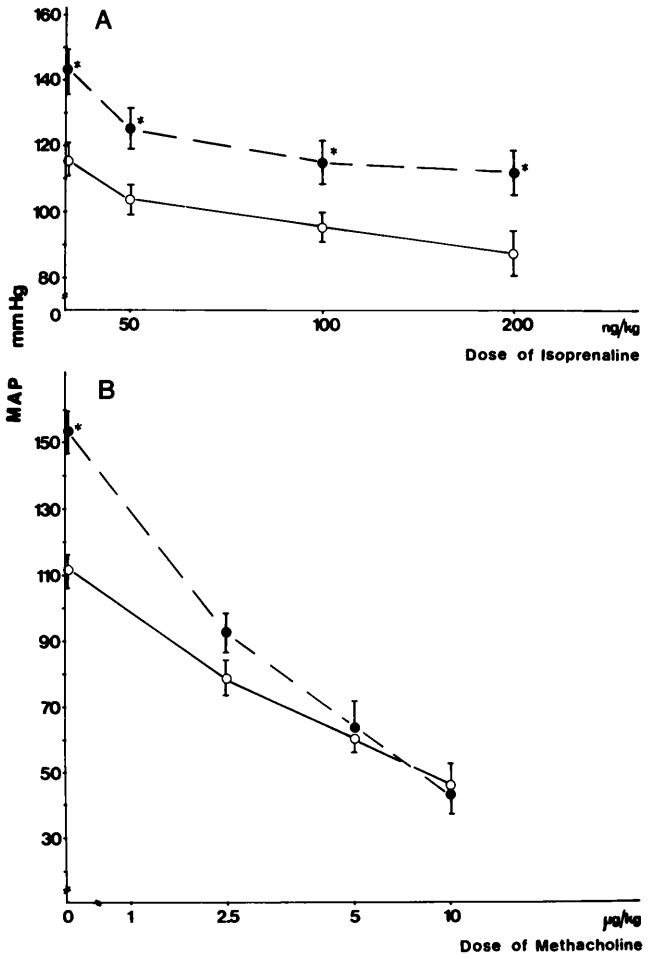

FIG. 2. Effects of isoprenaline (A) and methacholine (B) on MAP in conscious SHR and WKY rats. ○, WKY rats; ●, SHR rats. * denotes significant difference from WKY rats ($P < 0.05$).

amounts and for similar durations, so that at each dose level the difference between strains in MAP remained between 26 and 35 mm Hg (Fig. 4 and Table II). In anesthetized rats, isoprenaline caused a significant reduction of MAP at lower doses when compared to conscious rats of the same strain. For example, in SHR rats a dose of 50 ng/kg of isoprenaline produced a fall in MAP of 12 ± 1 mm Hg in conscious animals and 29 ± 3 mm Hg in anesthetized animals ($P < 0.01$). Similarly, in WKY rats a dose of 50 ng/kg of isoprenaline produced a fall in MAP of 16 ± 2 mm Hg in conscious animals and 30 ± 4 mm Hg in anesthetized animals ($P < 0.05$) (compare Figs. 2 and 4).

Anesthetized SHR and WKY rats were also more sensitive to the vasodepressor effects of methacholine than were conscious rats, especially at doses of 1 μg/kg and less. In addition, methacholine had a more marked hypotensive effect in SHR than WKY rats. For example, a dose of 1 μg/kg of methacholine produced a fall in MAP of 83 ± 5 mm Hg in anesthesized SHR rats and 41 ± 6 mm Hg in anesthesized WKY rats ($P < 0.01$). Thus, the initial strain difference in MAP of 43 mm Hg in anesthesized rats was abolished by a dose of 1 μg/kg of methacholine (Fig. 4).

Intravenous injection of sodium nitroprusside (2 μg/kg) into conscious rats resulted in a pronounced fall in MAP of greater intensity

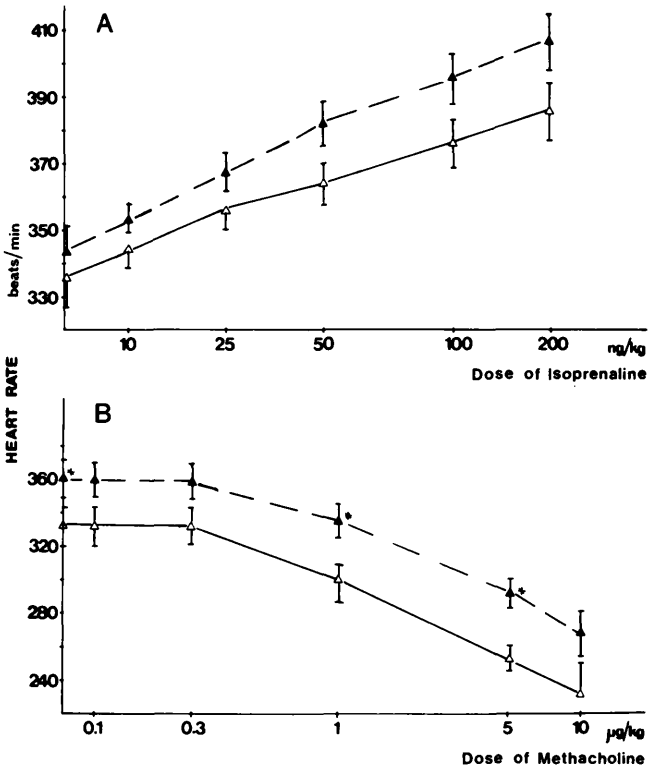

FIG. 3. Effects of isoprenaline (A) and methacholine (B) on heart rate in pentobarbital-anesthetized SHR and WKY rats. △, WKY rats; ▲, SHR rats. * denotes significant difference from WKY rats ($P < 0.05$).

but of much shorter duration than after the highest dose of isoprenaline (Table III). The duration of the hypotensive effect of sodium nitroprusside was approximately 44% greater in SHR rats ($P < 0.01$). Although the final heart rate reached after this dose of nitroprusside was not different in the two groups of rats the increase in heart rate of 130 ± 11 beats/min was more marked ($P < 0.05$) and of greater duration ($P < 0.01$) in WKY than SHR rats.

Discussion. The direct effects of isoprenaline on the cardiovascular system which result from activation of β-adrenoceptors are: (a) stimulation of the SA node to produce tachycardia, (b) stimulation of cardiac muscle to increase contractile force, and (c) dilation of peripheral blood vessels to produce hypotension. Methacholine, acting on muscarinic cholinergic receptors, causes vasodilation and has negative inotropic and chronotropic effects on the heart. In contrast, nitroprusside produces vasodilation by a direct action on vascular smooth muscle. These actions also result in a number of indirect effects, which include activation of baroreceptor reflex mechanisms as a result of a lowering of pressure in the aortic arch and carotid sinus. This reflex response stimulates sympathetic nervous system activity, inhibits vagal influences on the heart and inhibits sympathetic cholinergic vasodilator fibers in skeletal muscle to cause tachycardia and vasoconstriction (11). These indirect actions of the drugs would be expected to hasten the termination of the hypotensive response and restore blood pressure to its control level.

The present findings in conscious SHR and WKY rats demonstrate the considerable influence of baroreceptor reflex activity on the vasodilator responses to isoprenaline, methacholine, and nitroprusside. In addition, this activity was found to be impaired in SHR rats. Previous studies using vasoconstrictor agents have also shown that baroreceptor function is deficient in hypertensive rats (12–

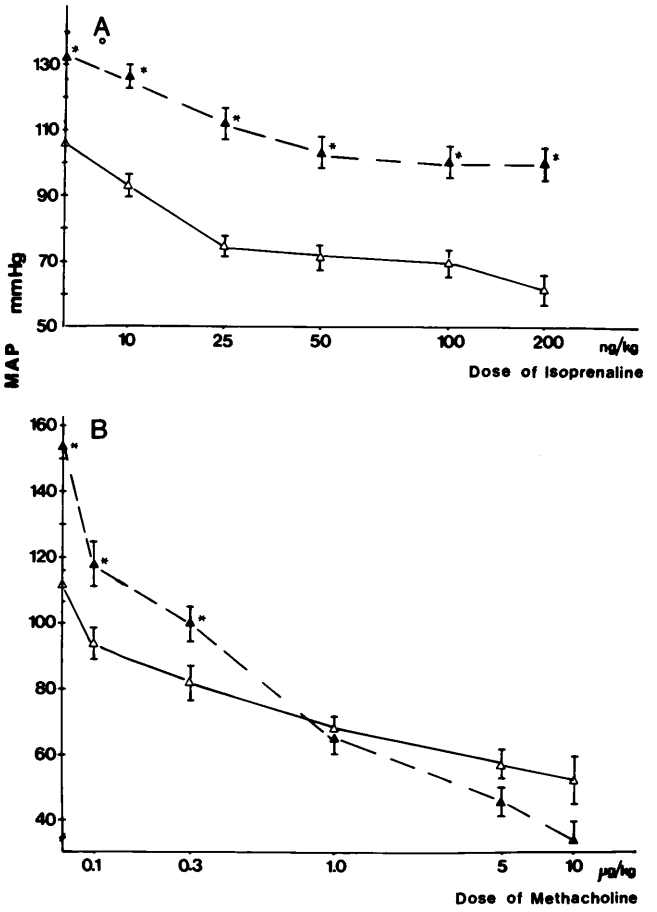

FIG. 4. Effects of isoprenaline (A) and methacholine (B) on MAP in pentobarbital-anesthetized SHR and WKY rats. △, WKY rats; ▲, SHR rats. * denotes significant difference from WKY rats ($P < 0.05$).

TABLE II. DURATION OF CHANGES IN MEAN ARTERIAL PRESSURE (MAP, mm Hg) AND HEART RATE (HR, beats/min) OF PENTOBARBITAL-ANESTHETIZED SHR AND WKY RATS FOLLOWING ADMINISTRATION OF ISOPRENALINE[a]

Dose of isoprenaline (ng/kg)	Duration of change (sec)			
	MAP		HR	
	SHR	WKY	SHR	WKY
10	26 ± 5	54 ± 14	36 ± 13	66 ± 27
25	85 ± 6	100 ± 15	88 ± 15	144 ± 37
50	119 ± 11	121 ± 3	174 ± 31	203 ± 39
100	143 ± 8	153 ± 18	224 ± 16	216 ± 40
200	205 ± 24	205 ± 48	281 ± 40	305 ± 54

[a] Values are means ± SEM for groups of four to seven rats per strain.

14). Thus, while the vasodepressor response to methacholine resulted in dose-related tachycardia in WKY rats, only marked bradycardia occurred in SHR rats. There was no significant difference in the tachycardia induced by isoprenaline in the two rat strains.

The hypotensive effect of methacholine (5 and 10 μg/kg) in SHR rats was almost double that in WKY rats. The greater hypotensive response of SHR rats to methacholine is similar to the observation that hypertensive humans may react to methacholine with a significant fall in blood pressure (15). In contrast, isoprenaline and nitroprusside caused similar decreases in MAP in the two strains of rats. It is possible that the exaggerated depressor response of SHR rats to methacholine

TABLE III. EFFECTS OF SODIUM NITROPRUSSIDE (2 μg/kg, iv) ON MEAN ARTERIAL PRESSURE (mm Hg) AND HEART RATE (beats/min) IN CONSCIOUS SHR AND WKY RATS[a]

	Mean arterial pressure		Heart rate	
	SHR	WKY	SHR	WKY
Before drug	145 ± 5**	110 ± 3	326 ± 8	307 ± 14
Change after drug	−53 ± 5	−42 ± 5	97 ± 8*	130 ± 11
Duration of response (sec)	64 ± 6**	44 ± 2	62 ± 4**	92 ± 10

[a] Values are means ± SEM for seven rats per strain.
* $P < 0.05$.
** $P < 0.01$ compared to WKY rats.

may have resulted from a lack of reflex sympathetic stimulation and vagal inhibition and not from any difference in the action of this drug on blood vessels.

Our findings with nitroprusside support the view that SHR rats have a diminished baroreceptor reflex response to vasodilation. Thus, for an equal drop in mean arterial pressure, WKY rats had a greater increase in heart rate and blood pressure returned to baseline levels more quickly than for SHR rats. To our knowledge, this is one of the few studies on baroreceptor reflexes in SHR rats that have employed vasodilators. In a recent report (16), prehypertensive and hypertensive Dahl genetically salt-sensitive rats were shown to have an impaired baroreceptor reflex response to vasoconstriction with phenylephrine but not to vasodilation with nitroglycerin. Taken together, these results indicate that the disturbances in baroreceptor reflexes may differ in the various animal models of hypertension.

Although baroreceptor reflexes can be abolished by a combination of nicotinic and muscarinic receptor blockade of ganglionic transmission (10) or by spinalization, the resulting low blood pressure would make it impossible to study quantitatively the vasodepressor responses to isoprenaline or methacholine. However, pentobarbital anesthesia has been shown to suppress cardiovascular reflexes and this was accomplished without a significant decrease in blood pressure (11, 18), as was also illustrated in the present study. The inhibitory effect of pentobarbital on parasympathetic influences (11) was also seen in the significant tachycardia it induced in WKY rats. The suppression of baroreceptor reflex activity by pentobarbital was further demonstrated in the present study by the findings that methacholine failed to cause tachycardia in anesthetized WKY rats but only a dose-related bradycardia of similar magnitude to that seen in SHR rats. Thus, in pentobarbital-anesthetized rats, the direct effects of isoprenaline and methacholine predominate.

Although we did not measure cardiac output in our rats, it is likely that the effect of isoprenaline on the heart was similar in SHR and WKY rats, as heart rate was increased by the same amount in both strains at each dose level following induction of anesthesia. Similarly, methacholine caused the same degree of dose-related bradycardia in anesthetized SHR and WKY rats. This suggests that any differences in the vasodepressor responses of SHR and WKY rats to isoprenaline or methacholine probably do not result from a differential effect of the drugs on cardiac output.

Methacholine (1 μg/kg) was able to bring the blood pressure to the same level (68 mm Hg) in SHR and WKY rats in spite of the considerable strain difference in initial MAP. This suggests that SHR rats are more sensitive to the direct effects of methacholine on blood vessels, which, together with some baroreflex sensitivity impairment, results in the greater overall vasodepressor response to this agent. In contrast, several doses of isoprenaline and a single dose of nitroprusside failed to abolish the difference in blood pressure between the two strains, thereby demonstrating a differential sensitivity of blood vessels of SHR to different vasodilator agents.

Previous studies have shown that vascular relaxation mechanisms appear to be impaired in SHR rats (8). Vasodilation is believed to result from the sequestering of calcium by microsomes via an ATP-dependent mechanism (19). Recent research suggests that this mech-

anism is deficient in microsomes from blood vessels of SHR rats (20). The results from the present study suggest that stimulation of muscarinic cholinergic receptors can overcome any initial difference in the contractility of the blood vessels of SHR and WKY rats. This leveling action does not appear to be shared by the β-adrenoceptor agonist, isoprenaline, suggesting that β-adrenoceptor activation and cyclic AMP production cannot abolish the increased vascular constriction of SHR rats. It would be of interest to determine if methacholine can normalize the calcium sequestering activity and calcium mobilizing ability of microsomes in blood vessels of SHR rats such that the initial strain differences in calcium movement are minimized.

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