

# Vascular responses to 5-hydroxytryptamine in genetic and renal hypertensive rats

D. D. MCGREGOR AND F. H. SMIRK

*Wellcome Medical Research Institute, Department of Medicine, Otago University Medical School, Dunedin, New Zealand*

MCGREGOR, D. D., AND F. H. SMIRK. *Vascular responses to 5-hydroxytryptamine in genetic and renal hypertensive rats.* Am. J. Physiol. 219(3): 687-690. 1970.—Cardiovascular responses to 5-hydroxytryptamine (5-HT) were measured in rats from a colony bred to develop hypertension spontaneously, rats with experimental renal hypertension, and normotensive controls. The effects of intravenous injections of 5-HT on blood pressure were complex. The rise in blood pressure immediately after the initial fall was generally much greater in the genetic hypertensives than in controls. In a second group of experiments, mesenteric arteries were perfused with physiological saline. Vasoconstrictor responses to 5-HT in controls were greatly exceeded by the responses of arteries isolated from genetic hypertensive rats (ratio 1:6) and from renal hypertensives (ratio 1:4). In our earlier experiments the vasoconstrictor responses to norepinephrine and to angiotensin in genetic hypertensive and renal hypertensive rats exceeded those of controls, but the ratio of control to hypertensive responses rarely exceeded 1:1.5. The explanation for this result is considered to be an enhanced response to 5-HT of smooth muscle of mesenteric arteries from genetic and renal hypertensive rats.

New Zealand genetic hypertensive rat colony; vascular reactivity; smooth muscle; pathology of arteries in hypertension; pathogenesis of hypertension; mesenteric arteries; hexamethonium; xylamidinc; norepinephrine; angiotensin

COMPARISONS by different authors of the responses of the blood pressure of hypertensive and control animals to pressor agents have yielded divergent results.

In contrast almost all comparisons, clinical and experimental, of the responses of blood vessels to intra-arterially administered vasoconstrictor agents are reported as showing larger responses from the arterial or arteriolar blood vessels of hypertensives than of the vessels of normotensive controls (20).

Explanations offered for the enhanced responses of the resistance blood vessels of hypertensive include intimal thickening (1), hypertrophy of arteriolar muscle (3, 4, 9, 19) with increased wall-to-lumen ratio, as well as inherent increase in the responsiveness of the vascular smooth muscle (8). The first two are thought to act by providing a mechanical advantage, which we suppose should operate equally to magnify the responses of any vasoconstrictor stimuli which might be applied to the hypertensive blood vessels.

Genetic hypertensive rats from the colony which has been developed here over the past 13 yr by Smirk and colleagues (17, 21, 22) and rats with Goldblatt hypertension were used in the experiments described in this paper.

The responses of hypertensive and control resistance blood vessels to 5-hydroxytryptamine (5-HT) appear to throw some light on mechanisms which may be responsible for the enhanced responsiveness of the hypertensive vessels.

## METHODS

Three groups of male albino rats 260-340 g were used: 1) rats from the New Zealand colony which spontaneously develop hypertension (genetic hypertensive rats) (17, 21); 2) rats with renal hypertension produced by applying a silver clip 0.0095 inch in diameter (23) to the right renal arteries of rats (200-250 g) from the Otago stock colony; 7-12 weeks later the animals were used for experiments; and 3) normal Wistar rats from the Otago stock colony that were used as controls.

Systolic blood pressures were measured prior to operation by a tailcuff method based on that described by Gallagher and Grimwood (6) and incorporating our more recent improvements (17). Genetic hypertensive rats and rats with renal hypertension were matched for weight and age with normotensive controls.

*Blood pressure experiments.* In the study of the effects of 5-HT (50  $\mu$ g/kg) on blood pressure, the rats were anesthetized with ether, and a femoral vein was cannulated. Anesthesia was then continued with chloralose (60 mg/kg). A femoral artery was cannulated and blood pressure recorded kymographically. Drugs dissolved in normal saline were injected via the femoral vein cannula.

*Mesenteric artery perfusion.* The preparation of mesenteric arteries for perfusion has been described by McGregor (12). The superior mesenteric artery was perfused through a cannula inserted into the artery at its origin from the aorta. The intestine was severed from the mesentery by cutting close to the intestinal border of the mesentery. Thus, only arteries of the mesentery itself were perfused so that there was no confusion between the activity of vascular and intestinal smooth muscle. In each preparation of mesenteric arteries, the perfusion involved only the four main arterial branches from the superior mesenteric artery trunk running to the ileum. All other ileal branches and the caecal, ileocolic, colic, and pancreaticoduodenal branches from the superior mesenteric artery were tied off. The mesentery was isolated from the rat and placed ready for perfusion in a water-jacketed Perspex box maintained at 38 C. The perfusion rate was constant at 2 ml/min. Thus, the perfusion pressure which was recorded kymographically became a measure of the degree of vasocon-

striction. The physiological saline had the following composition (mM): NaCl, 154; KCl, 5.6; CaCl<sub>2</sub>, 2.1; NaHCO<sub>3</sub>, 2.0; glucose, 5.5. The solution was bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

Serotonin creatinine sulfate (5-HT) was dissolved in the physiological saline solution, and the specified doses were injected, in 0.05 ml of solution, into the perfusion stream close to the superior mesenteric artery cannula.

Mean values have been given  $\pm$  standard errors of the means (SE). Differences between means have been tested for significance with the Student *t* test.

## RESULTS

*Effect on blood pressure.* As has been reported by others (5, 7, 11, 16), the effects of intravenous injections of 5-HT on blood pressure are complex. There was a sharp fall in the blood pressure, then a sharp rise which was sometimes bifid, followed by a fall of blood pressure of longer duration, returning gradually to the original level (Fig. 1). Such changes occurred in all of 20 genetic hypertensive and 21 out of 22 control rats, but the magnitude of the blood pressure rises and falls varied.

Figure 1 illustrates the most usual difference between the responses of genetic hypertensives and controls. There was no consistent difference between hypertensives and controls in the extent of the initial sharp fall in the blood pressure. The mean fall in the genetic hypertensives was  $84 \pm 8$  mm Hg compared with  $72 \pm 4$  mm Hg in the controls. However, the rise in blood pressure immediately after the fall was generally much greater in the genetic hypertensives than in the controls;  $92 \pm 3$  mm Hg for the genetic hypertensives compared with  $47 \pm 5$  mm Hg for the controls. This difference between the response of the genetic hypertensives and controls was significant ( $P < 0.001$ ). The rise took blood pressure above the initial blood pressure in 12 out of 20 of the hypertensives but only in 4 out of 22 controls. It is of interest that in six of the eight hypertensives in which the rise did not carry the blood pressure to above the original level the antecedent fall was above the average magnitude. The terminal fall in blood pressure was somewhat similar in the hypertensives and controls, but, as an average, of slightly longer duration in the hypertensives.

After maximal doses of hexamethonium bromide (20 mg/kg) were given intravenously to five genetic hypertensives and four controls, the initial sharp fall in blood pressure after 5-HT administration was abolished in all the rats, and the rise of blood pressure above the hexamethonium floor level was greater in the genetic hypertensives than in the controls (Fig. 1). When xylamide tosylate (1 mg/kg), a 5-HT antagonist (2), is administered intravenously before giving the 5-HT, the sharp rise of blood pressure is diminished or abolished in both genetic hypertensives and controls (Fig. 2). This action of xylamide was noted in all of 9 hypertensives and all of 11 controls.

As the pressor action of 5-HT was stronger in genetic hypertensives than in controls, a study was made of the effect of 5-HT on the perfused mesenteric artery preparation (12).

*Effect on perfused mesenteric arteries.* When mesenteric ar-

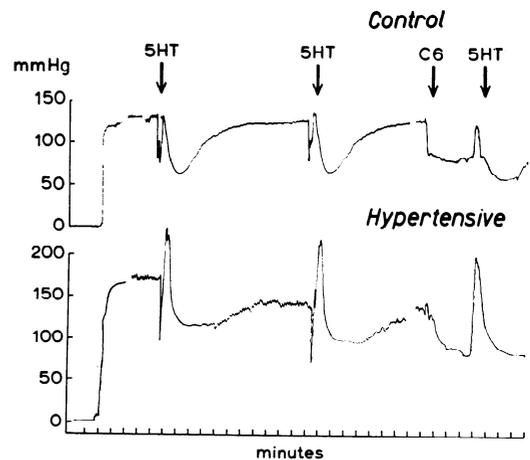


FIG. 1. Comparison of pressor responses of rats with genetic hypertension and controls to intravenous injections of 5-HT (50  $\mu$ g/kg) before and after intravenous injection of 20 mg/kg of hexamethonium (C<sub>6</sub>).

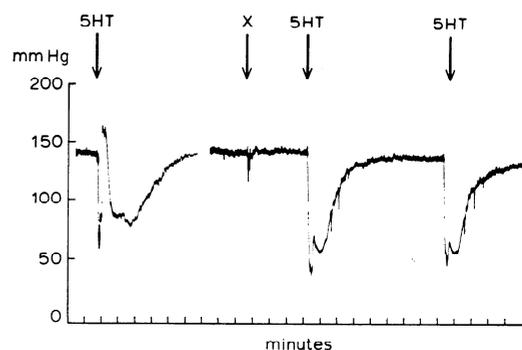


FIG. 2. Comparison of pressor responses of a genetic hypertensive rat to 5-HT (50  $\mu$ g/kg) before and after injection of a 5-HT antagonist: 1 mg/kg of xylamide tosylate.

teries were perfused at constant flow with a physiological saline solution, the rise of perfusion pressure following close injection of 5-HT was greater in isolated mesenteric arteries removed from genetic hypertensive rats than from control rats (Table 1). Mesenteric arteries isolated from rats with experimental renal hypertension also showed much larger responses to 5-HT than did controls (Table 2). Perfusion pressure base lines in the genetic hypertensive and renal hypertensive groups were not significantly different from those in the normotensive groups (Tables 1 and 2). This result contrasts with that we obtained in earlier experiments (13) in which perfusion pressure base lines were significantly higher in mesenteric arteries from genetic and renal hypertensive rats than in similar preparations from weight-matched normotensive control rats; however, we did over 3 times as many experiments in the significant series, and the rats used in the present series were slightly younger.

## DISCUSSION

It was shown by McGregor and Smirk (13) that, when perfused with saline, mesenteric arteries removed from rats bred to develop hypertension spontaneously, and also mesenteric arteries from rats with experimental renal hypertension, differed from mesenteric arteries from normotensive control rats in that both norepinephrine and angiotensin,

TABLE 1. Vasoconstrictor responses to 5-hydroxytryptamine in mesenteric arteries isolated from genetic hypertensive and normotensive control rats

Group of Rats	5-HT Dose, $\mu\text{g}$	Mean Vasoconstrictor Response, mm Hg
Genetic hypertensive	1	32.0 $\pm$ 2.6
Normotensive control	1	5.8 $\pm$ 0.4*
Genetic hypertensive	10	111.9 $\pm$ 10.0
Normotensive control	10	15.3 $\pm$ 2.4*

Vasoconstrictor response values are given as means  $\pm$  SEM. Nine weight-matched pairs of rats were used for comparison. Mean systolic blood pressures before operation: genetic hypertensives, 174  $\pm$  8 mm Hg; normotensive controls, 118  $\pm$  3 mm Hg. Mean perfusion pressure base lines during the experiments were: genetic hypertensives, 24.4  $\pm$  1.9 mm Hg; normotensive controls, 23.2  $\pm$  2.5 mm Hg. The difference between the mean perfusion pressure base lines was not significant ( $t = 0.38$ ;  $P < 0.8$ ). \* Vasoconstrictor responses in hypertensives and normotensives differed significantly;  $P < 0.001$ .

TABLE 2. Vasoconstrictor responses to 5-hydroxytryptamine in mesenteric arteries isolated from renal hypertensive and normotensive control rats

Group of Rats	5-HT Dose, $\mu\text{g}$	Mean Vasoconstrictor Response, mm Hg
Renal hypertensive	1	33.7 $\pm$ 2.5
Normotensive control	1	7.4 $\pm$ 1.5*
Renal hypertensive	10	94.4 $\pm$ 5.6
Normotensive control	10	22.3 $\pm$ 2.6*

Vasoconstrictor response values are given as means  $\pm$  SEM. Thirteen weight-matched pairs of rats were used for comparison. Mean systolic blood pressures before operation: renal hypertensives, 190  $\pm$  5 mm Hg; normotensive controls, 112  $\pm$  3 mm Hg. Mean perfusion base lines during the experiments: renal hypertensives, 28.5  $\pm$  2.2 mm Hg; normotensive controls, 30.2  $\pm$  2.5 mm Hg. The difference between the mean perfusion pressure base lines was not significant ( $t = 0.52$ ;  $P < 0.6$ ). \* Vasoconstrictor responses in hypertensives and normotensives differed significantly;  $P < 0.001$ .

when injected into the perfusion fluid, caused a greater degree of vasoconstriction in the hypertensives than in the normotensive controls.

It was decided to repeat the above study but using serotonin (5-hydroxytryptamine-5-HT) as the vasoconstrictor agent, because when 5-HT was administered intravenously the resulting changes of blood pressure were strikingly different in the genetic hypertensives and normotensive controls—notably in the pressor phase of the complex response (Fig. 1) which was of greater magnitude in the genetic hypertensive.

The complexity of the response of the whole rat to 5-HT may be partly explained by the responses to xylamidine and hexamethonium, together with the perfusion experiments. The administration of xylamidine stops or greatly reduces the pressor response to 5-HT, leaving a comparatively simple depressor response. The complex pattern before xylamidine administration appears to be due to the depressor trough being interrupted by a short duration pressor peak, which as the perfusion experiments show is partly due to vasoconstriction.

When the blood pressure has been reduced to the hexa-

methonium floor, there is no longer a sharp initial fall of blood pressure. The 5-HT now causes a rise in pressure sometimes succeeded by a slow fall to below the hexamethonium floor level.

The complexity of the pattern of response to 5-HT is largely eliminated by either hexamethonium or by xylamidine.

It has been shown in the present experiments that the responses to injected 5-HT of isolated perfused arteries from genetic and renal hypertensive rats are much larger than those of arteries from normotensive rats. The magnitude of the difference between the preparations from hypertensives and normotensives in response to 5-HT is particularly noteworthy, because it is much greater than the difference between the responses of preparations from hypertensive and normotensive rats following injections of either norepinephrine or angiotensin which we described in an earlier publication (13) using identical techniques, the results of which are reproduced for comparison in Table 3.

There has been much discussion as to whether the stronger vasoconstrictor responses of perfused blood vessels from hypertensives as compared with controls might be the result of a structural change, such as thickening of the intima or hypertrophy of the vascular muscle in the hypertensives, rather than a more specific increase in the responsiveness of arterial smooth muscle (1, 3, 4, 8-10, 13-15, 18, 19).

The essence of the hypothesis that a structural factor is responsible for the enhanced responsiveness of blood vessels from hypertensives is the assumption that the smooth muscle behaves in the same way in hypertensives and controls. The structural factor or factors are thought to act by rendering smooth muscle contraction more effective and to modify the response of the blood vessels to any vasoconstrictor agent in essentially the same way.

TABLE 3. Responses of mesenteric arteries from genetic hypertensive (GH) and renal hypertensive (RH) rats, and from controls (C) to vasoconstrictor agents

Drug	Dose, $\mu\text{g}$	Mean Perfusion Pressure Increases, mm Hg		GH as % of C	P*
		GH	C		
NE	0.5	44.2 $\pm$ 1.9	34.7 $\pm$ 1.1	127	<0.001
NE	1.0	79.1 $\pm$ 2.7	54.8 $\pm$ 2.1	144	<0.001
A	2.5	47.8 $\pm$ 1.6	35.8 $\pm$ 1.3	134	<0.001
A	5.0	51.6 $\pm$ 2.1	43.4 $\pm$ 1.7	119	<0.005
5-HT	1.0	32.0 $\pm$ 2.6	5.8 $\pm$ 0.4	552	<0.001
5-HT	10.0	111.9 $\pm$ 10.0	15.3 $\pm$ 2.4	731	<0.001
		RH	C	RH as % of C	
NE	0.5	49.9 $\pm$ 3.2	34.5 $\pm$ 1.5	145	<0.001
NE	1.0	83.0 $\pm$ 3.6	56.0 $\pm$ 2.1	148	<0.001
5-HT	1.0	33.7 $\pm$ 2.5	7.4 $\pm$ 1.5	455	<0.001
5-HT	10.0	94.4 $\pm$ 5.6	22.3 $\pm$ 2.6	423	<0.001

Mesenteric arteries of rats were perfused at a constant rate with physiological saline. The changes in perfusion pressure brought about by the vasoconstrictors norepinephrine (NE), angiotensin (A), and serotonin (5-HT) are set out above. The responses of GH and RH rats are also shown as percentages of the responses of C rats. Data for NE and A are from McGregor and Smirk (13). \* P = significance of the difference between the mean perfusion pressure increases in the hypertensive and control rats.

The results we obtained, however, are insufficiently explained by the structural hypothesis.

It will be seen from Table 3 that 0.5  $\mu\text{g}$  of norepinephrine caused a mean rise of perfusion pressure of 34.7 mm Hg in the mesenteric artery preparations using blood vessels from normotensive rats, and even so large a dose as 10  $\mu\text{g}$  of serotonin only raises the perfusion pressure by 15.3 mm Hg in the same preparation. Were structural factors alone responsible for the larger than normal responses of the mesenteric arteries from hypertensives, one would expect that preparations made from the mesenteric arteries of either genetic or a renal hypertensive rat would also show larger responses to 0.5  $\mu\text{g}$  of norepinephrine than to 10  $\mu\text{g}$  of serotonin. This, however, was not the result obtained. Using blood vessels from genetic hypertensive rats, the mean response of the perfusion pressure to 0.5  $\mu\text{g}$  of norepinephrine was 44.2 mm Hg and to 10  $\mu\text{g}$  serotonin was 111.9 mm Hg. Using blood vessels from renal hypertensive rats, the mean response to 0.5  $\mu\text{g}$  norepinephrine was 49.9 mm Hg, and to 10  $\mu\text{g}$  serotonin was 94.4.

We find it difficult to imagine any structural difference between mesenteric arteries of hypertensive and normotensive rats which could explain the fact that the mean responses of hypertensives are raised above the control level by

no more than 50% when norepinephrine or angiotensin are used as vasoconstrictor agents, yet are raised to as much as 420–730% of the control level when 5-HT is used. No structural difference which has yet been conceived between hypertensive and normotensive arteries could account for so crude a difference between the reactions to norepinephrine or angiotensin on the one hand and 5-HT on the other. It is difficult, indeed, to explain the above results other than by an increase above normal of the response of the mesenteric arteries of genetic and renal hypertensive rats, brought about by enhanced reactivity to serotonin of the smooth arterial muscle of the hypertensives.

At the same time we do not deny the possible participation of structural factors such as hypertrophy of smooth muscle which might amplify the effect of smooth muscle contraction on vasoconstrictor responses of hypertensive blood vessels.

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