Effect of Ketanserin on Pressor Response to Vasoactive Substances in Early Phase of One-Kidney, One Clip Renal Artery Stenosis in Rats and Rabbits

MASAO FUJIE, M.D., SHUICHI ICHIKAWA, M.D., MASAMI KOGURE, M.D.
KUNIYA HATAKEYAMA, M.D., SHOUICHIROU KAWAJIRI, M.D.
AND KAZUHIKO MURATA, M.D.

The effect of ketanserin (KET), a specific 5-hydroxytryptamine₂ (5-HT₂) receptor blockade, on pressor response to vasoactive substances was examined in rats with one-kidney, one clip renal artery stenosis of 2 days' duration (2-day clipped rat) and in rabbits with renal artery stenosis of 3 days' duration (3-day clipped rabbits). The 2-day clipped rats showed hyperresponsiveness to norepinephrine (NE), arginine vasopressin (AVP) and 5-HT. All hyper-responsiveness were attenuated by a subdepressor dose of KET. The infusion of KET, 10 µg/kg/min for 30 minutes, decreased mean arterial pressure of the 3-day clipped rabbits; the dose did not alter blood pressure of the normal controls. Exaggerated pressor response to NE was observed in the 3-day clipped rabbits and was abolished by a subdepressor dose of KET, 2.5 µg/kg/min. These results suggest that 5-HT may be involved in the enhanced pressor response to vasoconstrictor substances in the 2-day clipped rats and 3-day clipped rabbits, and that it may also play an important role in maintaining blood pressure in the 3-day clipped rabbits.

ALTERED pressor response to vasoactive substances could be a factor responsible for the development and/or maintenance of hypertension. Previous studies showed exaggerated pressor response to norepinephrine and vasopressin in rabbits with one-kidney, one clip renal artery stenosis of 3 days' duration (3-day clipped rabbit)¹⁻³. Although the results revealed an important role of the renin-angiotensin system in the pressor hyperresponsiveness, many other humoral and neurogenic mechanisms may be involved, because pressor or vascular reactivity could be modulated by various factors.³⁻⁵

It has been reported that ketanserin (KET), a

specific 5-hydroxytryptamine₂ (5-HT₂) blockade, affects the vascular reactivity to norepinephrine and angiotensin II in tissue preparations⁵⁻⁷ and decreases blood pressure both in clinical and experimental hypertension⁸⁻¹². Therefore, 5-HT could also play a role in the enhanced pressor response to vasoconstrictor substances in hypertension.

The present study examined the effect of KET on pressor response to vasoactive substances in rats with one-kidney, one clip renal artery stenosis of 2 days' duration (2-day clipped rat) and in the 3-day clipped rabbit.

MATERIAL AND METHOD

Experiments in Rats

Forty-four male Wistar rats weighing 200 to 250 grams were used. The rats were fed with a commercial laboratory diet (Oriental Rat Chow)

Key Words:

Serotonin
5-hydroxytryptamine
5-HT
Norepinephrine
Vasopressin

The Second Department of Internal Medicine, Gunma University School of Medicine, Maebashi, Japan

Mailing address: Shuichi Ichikawa, M.D., The Second Department of Internal Medicine, Gunma University School of Medicine, 3-39-22, Showa, Maebashi 371, Japan

containing 91 mEq of sodium per kg of food. The rats for renal artery stenosis received left nephrectomy and was placed a silver clip of 0.43 mm inner diameter on a right renal artery under ether anesthesia. Sham-operated rats received left nephrectomy and right renal artery was only exposed without placing a clip. Experiments were performed 2 days after these surgical procedures. On the day of the experiments, the rats were intubated with PE-10 and PE-50 polyethylene tubing (CLAY ADAMS) into femoral artery and vein, respectively, under light ether anesthesia and allowed to recover from the anesthesia at least 6 hours. The experiments were carried out on conscious rats. Mean arterial pressure (MAP) and heart rate were measured through the arterial catheter with a pressure transducer (P-50, Statham).

Experiment I: Effect of KET on Pressor Response to Norepinephrine

Seven sham-operated rats and 7 clipped rats received the infusion of norepinephrine (NE), 400 ng/kg/min for 5 min via a venous catheter. After recording pressor response and heart rate, subdepressor dose of KET, 10 μ g/kg/min, was infused for 30 min. At the 30th min of KET infusion, NE, 400 ng/kg/min, was infused again concurrently with KET, and the pressor response and heart rate were recorded. The blockade effect of KET was assessed in each rat by the pressor response to injection of 5-HT, 5 μ g/kg, before and after KET infusion.

Experiment II: Effect of KET on Pressor Response to Vasopressin

An infusion of arginine-vasopressin (AVP) with a dose of 1 mU/kg/min for 5 min was performed on 6 sham-operated rats and 6 2-day clipped rats. The pressor response, heart rate and the blockade effect of KET was determined with the same method in Experiment I.

Experiment III: Effect of KET on Plasma Renin Activity

Nine sham-operated rats and 9 clipped rats were used for this experiment. One milliliter sample of blood was obtained before and 30 min after the infusion of KET for the determination of plasma renin activity (PRA).

Experiments in Rabbits: Effect of KET on Pressor Response to NE and PRA

Male New Zealand white rabbits weighing 2.0

to 2.5 kg were used. All rabbits were fed with a commercial laboratory diet (Oriental Rabbit Chow) containing 91 mEq of sodium per kg of food. Six rabbits were subjected to renal artery stenosis of 3 days' duration (3-day clipped rabbit) and 6 normal were used as controls. Renal artery stenosis was produced as follows. Under gas anesthesia (halothane with nitrous oxide and oxygen) the rabbit received right nephrectomy and a silver clip of 1.1 mm inner diameter was placed on the left renal artery through dorsal incision; in sham-operated rabbits right nephrectomy was done and the left renal artery was only exposed without placing a clip. Experiments were performed 3 days after these surgical procedures. On the day of the experiment, the rabbits were intubated with a polyvinyl catheter into the femoral artery and vein (Fr.5 and Fr.4, respectively) under the gas anesthesia. The marginal ear vein was also cannulated with polyethylene tubing (PE-50). All experiments were carried out on conscious rabbits at least 6 hours after recovering from anesthesia. At the beginning of the experiments, one milliliter sample of blood was taken for the determination of PRA. MAP and heart rate were measured via a arterial catheter. After obtaining the pressor response to NE 800 ng/kg/min for 5 min through a venous catheter, the rabbit received infusion of KET in doses of 1.25, 2.5, 5 and 10 μ g/min per kg. Each dose of KET was infused for 30 min and the concurrent infusion of NE was repeated. The MAP was continuously recorded. One milliliter sample of blood for PRA measurement was obtained at the 30th min of KET infusion in each dose before infusing NE.

PRA was determined by radioimmunoassay utilizing a commercially available angiotensin I radioimmunoassay kit (CIS, France).

STATISTICS

All values were expressed as means $\pm$ standard error of the mean. For comparison of the values within a group, Student's *t* test for paired observation or analysis of variance followed by Tukey's test were used. For comparison the values between groups, Student's *t* test for unpaired observation was used. $p < 0.05$ was considered statistically significant.

RESULTS

Experiments in Rats

The initial values for all rats are summarized in Table I. The MAP of the 2-day clipped rats was significantly ($p < 0.01$) elevated compared to the sham operated (control) rats, while the heart rate in the 2-day clipped rats was significantly ($p < 0.01$) decreased when compared with the control rats. Because the basal MAP of 2-day clipped rats was higher than that of the control rats, the pressor response to NE was expressed as percent increase of MAP; (maximal MAP–basal MAP)/basal MAP. As shown in the left panel of

TABLE I INITIAL VALUES FOR MEAN ARTERIAL PRESSURE (MAP) AND HEART RATE (HR) IN ALL RATS

	MAP (mmHg)	HR (beat/min)
Sham operated rats (n = 22)	111 ± 2	427 ± 9
2-day clipped rats (n = 22)	118 ± 2 [†]	395 ± 11 [‡]

Values are mean ± SEM. [†] = $p < 0.01$ when compared between sham operated rats and 2-day clipped rats by Student's *t* test for unpaired observation.

Fig. 1, the pressor response to NE in the 2-day clipped rats was significantly ($p < 0.01$) greater than that of the control rats (2-day clipped; $25.9 \pm 1.8\%$ vs. control; $13.2 \pm 2.1\%$). The heart rate was not altered by the infusion of a subdepressor dose of KET either in the 2-day clipped or the control rats. The pressor response to NE after KET infusion was significantly ($p < 0.01$) reduced to $12.6 \pm 2.5\%$ in the 2-day clipped rats, while in the control rats, the pressor response was not decreased ($9.3 \pm 0.8\%$). The middle panel of Fig. 1 shows the pressor response to AVP. The response in the 2-day clipped rats was $18.6 \pm 2.1\%$ which was significantly ($p < 0.01$) higher than that of the control rats ($9.6 \pm 1\%$). After KET infusion it was significantly ($p < 0.01$) reduced to $7.9 \pm 2.1\%$ in the 2-day clipped rats, while in the normal rats it was $8.0 \pm 1.3\%$, which was not different from the initial response before KET administration. In addition, as shown in the right panel of Fig. 1 the pressor response to the test dose of 5-HT before KET infusion in the 2-day clipped rats was $30.3 \pm 2.3\%$ which was significantly ($p < 0.01$) higher than that in the control rats ($13.0 \pm 1.5\%$). By the infusion of KET,

Fig. 1. Changes of pressor responsiveness to norepinephrine (NE), arginine vasopressin (AVP) and 5-hydroxytryptamine (5-HT) in rats with one-kidney, one clip renal artery stenosis for 2 days' duration (CLIP) and sham-operated rats (SHAM) by the treatment with ketanserin (KET).

Pressor responsiveness was expressed as % increase of mean arterial pressure. † = $p < 0.01$ when compared between sham-operated and 2-day clipped rats by Student's *t* test for unpaired observation. § = $p < 0.01$ when compared before and after administration of ketanserin by Student's *t* test for paired observation.

the pressor response to 5-HT was $11.5 \pm 2.4\%$ in the 2-day clipped rats and was $6.2 \pm 1.3\%$ in the control rats. The response in the two groups was significantly ($p < 0.05$) reduced by KET infusion and the difference of pressor response between the two groups was diminished. The effect of KET infusion on PRA is summarized in Table II. The control values for PRA were not different

between the 2-day clipped rats and the control rats. After KET infusion, the PRA increased significantly ($p < 0.01$) in both groups.

Experiments in Rabbits

The initial value for MAP and heart rate in all twelve rabbits are presented in Table III. The MAP and heart rate in the 3-day clipped rabbits

TABLE II PLASMA RENIN ACTIVITY (PRA) IN RATS BEFORE AND AFTER KETANSERIN ADMINISTRATION

	PRA (ng/ml/hr)	
	Before ketanserin	After ketanserin
Sham operated rats (n = 9)	6.1 ± 1.3	$8.6 \pm 1.3^\dagger$
2-day clipped rats (n = 9)	7.6 ± 1.6	$12.8 \pm 1.8^\dagger$

Values are mean $\pm$ SEM. $^\dagger = p < 0.05$ when compared between sham operated rats and 2-day clipped rats by Student's *t* test for paired observation.

TABLE III INITIAL VALUES FOR MEAN ARTERIAL PRESSURE (MAP) AND HEART RATE (HR) IN ALL RABBITS

	MAP (mmHg)	HR (beat/min)
Sham operated rabbits (n = 6)	96 ± 2	264 ± 11
3-day clipped rabbits (n = 6)	102 ± 2	245 ± 6

Values are mean $\pm$ SEM.

Fig. 2. Changes of mean arterial pressure (MAP) and heart rate (HR) in rabbits with one-kidney, one clip renal artery stenosis for 3 days' duration (CLIP) and sham-operated rabbits (SHAM) by the treatment with ketanserin (KET).

* = $p < 0.05$ when compared by analysis of variance followed by Tukey's method.

Fig. 3. Changes of pressor responsiveness to norepinephrine in rabbits with one-kidney, one clip renal artery stenosis for 3 day's duration (CLIP) and sham-operated rabbits (SHAM) by the treatment with ketanserin (KET).

† = $p < 0.01$ when compared between 3-day clipped rabbits and sham operated rabbits by Student's *t* test for unpaired observation. ‡ = $p < 0.05$ and § = $p < 0.01$ when compared by analysis of variance followed by Tukey's method.

TABLE IV PLASMA RENIN ACTIVITY (PRA) IN RABBITS BEFORE AND AFTER KETANSERIN ADMINISTRATION

		PRA (ng/ml/hr)			
	Control	Ketanserin (μg/kg/min)			
		1.25	2.5	5.0	10.0
Sham operated rabbits (n = 6)	3.0 ± 0.6	2.8 ± 0.7	2.5 ± 1.0	4.9 ± 1.3	4.3 ± 0.7
3-day clipped rabbits (n = 6)	4.5 ± 0.7	6.2 ± 1.8	6.5 ± 2.1	6.8 ± 2.7	6.5 ± 1.8

Values are mean ± SEM.

were not different from those of the sham-operated (control) rabbits. The infusion of KET in doses of 1.25, 2.5 and 5 $\mu\text{g/kg/min}$ did not

change the MAP, either in the 3-day clipped or the control rabbits; however the maximum dose of KET infusion, 10 $\mu\text{g/kg/min}$, decreased the MAP significantly ($p < 0.05$) in the 3-day clipped rabbits to 94 ± 2 mmHg, while this dose did not alter the MAP in the control rabbits (the left panel of Fig. 2). No change in the heart rate was observed in any of the groups (the right panel of Fig. 2). Because the control MAP was not different between the 3-day clipped rabbits and the control rabbits, the pressor response was expressed as an absolute value; maximal MAP minus basal MAP. The effect of KET on the pressor response to NE is shown in Fig. 3. The control response in the 3-day clipped rabbits was 17 ± 1 mmHg, which was significantly ($p < 0.01$) greater than that of the control rabbits (11 ± 1 mmHg). The minimum dose of KET infusion, 1.25 $\mu\text{g/kg/min}$, reduced significantly ($p < 0.05$) the pressor

response in the 3-day clipped rabbits to 12 ± 2 mmHg, while this dose did not decrease it in the control rabbits. The pressor response to NE infusion in the 3-day clipped rabbits concurrently infused with the maximum dose of KET was 10 ± 2 mmHg, which was not different from that in the control rabbits. The control values for PRA were not significantly different between the 3-day clipped rabbits and the normal rabbits. The changes of the PRA from the control values by any dose of KET were not significant in either of the groups (Table IV).

DISCUSSION

The exaggerated pressor response to NE and AVP was observed not only in the 3-day clipped rabbits,¹⁻³ but in the 2-day clipped rats in early stage of hypertension. These rats also had hyperresponsiveness to 5-HT. The cause of increased pressor response to vasoactive substances in the rats may be mainly functional, and not caused by structural changes of blood vessels, since 2 days' duration of renal artery stenosis is too short a period to produce organic alternation of the arteries. A subdepressor dose of KET abolished all of the enhanced pressor responses. Apart from the antagonism for 5-HT induced pressor response, the precise mechanisms for attenuating effect of KET on the response to NE and AVP were not completely demonstrated in the present study. It has been reported that, in isolated vessels, 5-HT could potentiate contractile response to vasoconstrictors and the treatment with 5-HT₂ receptor blockade attenuated the potentiating effect.⁵⁻⁷ Therefore, it may be speculated that in the 2-day clipped rats, 5-HT is involved in the hyperresponsiveness to NE and AVP, which was abolished by the infusion of KET. As KET acts as α_1 antagonist,^{6,13} attenuation of the response to NE might be explained in part by the mechanism of α_1 antagonism. However, the effect on AVP is not yet understood, because the response to AVP is not mediated through α_1 adrenoceptor.

The MAP in the 3-day clipped rabbits was not higher than that in the normal controls. The blood pressure decreased in the 3-day clipped rabbits, but not in the sham-operated rabbits with the highest dose of KET infusion, $10 \mu\text{g/kg/min}$, suggesting that 5-HT may be involved in the maintenance of the arterial pressure in the 3-day clipped rabbits. With the reduction of blood pressure by KET infusion, the heart rate in these

rabbits did not increase and reflex tachycardia was not observed. Although KET, by itself, slowed the heart rate in an isolated heart preparation,¹¹ the mechanisms for the absence of the reflex tachycardia have not been clarified in the present study and so further studies are needed. In the 3-day clipped rabbits, pressor hyperresponse to NE was observed, as was reported in previous studies.¹⁻³ The enhanced pressor response was completely attenuated by the smallest amount of KET, $1.25 \mu\text{g/kg/min}$ and was not further decreased by the higher doses. In addition to the role in the maintenance of blood pressure, as mentioned above, 5-HT may participate in the hyperresponsiveness in these rabbits. The mechanisms discussed in the 2-day clipped rats may be also applied to the rabbits.

The PRA in the rats was increased by the infusion of KET, while that in the rabbits tended to be elevated. It was reported that in human studies, KET did not alter PRA,^{14,15} but increased the active renin with a reduction of blood pressure.¹⁶ As far as we know, there has been no report on the effect of KET on PRA in an animal experiment. The present study did not reveal the mechanisms of the increase in PRA by KET; however, the alteration of blood pressure did not play any role, as subdepressor doses of KET were used. Whatever the mechanisms may be, the renin-angiotensin system is not involved in the attenuation of the hyperresponsiveness to vasoconstrictor substances, because angiotensin II potentiates vascular reactivity or pressor response.^{1,3} The PRA in the present study was increased, not decreased by the KET infusion. It should be noted that in the previous reports the exaggerated pressor response in the 3-day clipped rabbits was abolished by angiotensin II antagonists, indicating the importance of the renin-angiotensin system.¹⁻³ Moreover, the hyperresponse was also attenuated by prostaglandin E₂.⁴ These results, including the present study, suggest that the enhanced pressor response to vasoconstrictor substances in the early stage of renal artery stenosis of one-kidney, one clip animals is mediated not by a single, but by various many factors. It is of interest that inhibiting any one of them completely abolishes the hyperresponsiveness.

Acknowledgment

The authors are appreciative of the excellent technical help given by Miss Nagai N. and Miss Iriya S. Ketanserin was kindly supplied by Kyowa Hakko Kogyo Co., Ltd.

REFERENCES

1. ICHIKAWA S, JOHNSON JA, FOWLER WL Jr, PAYNE CG, KURZ K, KEITZER WF: Pressor responses to norepinephrine in rabbits with 3-day and 30-day renal artery stenosis; the role of angiotensin II. *Circ Res* 43: 437, 1978
2. JOHNSON JA, ICHIKAWA S, KURZ K, FOWLER WL Jr, PAYNE CG: Pressor responses to vasopressin in rabbits with 3-day renal artery stenosis. *Am J Physiol* 240: H862, 1981
3. KOIVUNEN DG, JOHNSON JA, NICHOLAS WK, ZEIGLER DW, SIRIPAISARNPIPAT S, PAYNE CG: Pressor responses to norepinephrine during captopril in renal prehypertensive rabbits. *Hypertension* 5: 159, 1983
4. ICHIKAWA S, KOGURE M, MURATA K: Pressor responses to norepinephrine in rabbits with 3-day renal artery stenosis - the role of prostaglandins - (The 13th conference on the pathogenesis of hypertension). *Jap Circ J* 48: 1282, 1984
5. VAN NEUTEN JM, JANSSEN PAJ, DE RIDDER W, VANHOUTTE PM: Interaction between 5-hydroxytryptamine and other vasoconstrictor substances in the isolated femoral artery of the rabbit; effect of ketanserin (R 41 468). *Europ J Pharmacol* 77: 281, 1982
6. VAN NEUTEN JM, JANSSEN PAJ, VAN BEEK J, XHONNEUX R, VERBEUREN TJ, VANHOUTTE PM: Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT₂ serotonergic receptors. *J Pharmacol Exper Ther* 218: 217, 1981
7. CHARLTON KG, JOHNSON TD, CLARKE DE: Vasoconstrictor and norepinephrine potentiating action of 5-hydroxykynuramine in the isolated perfused rat kidney: involvement of serotonin receptors and alpha₁-adrenoceptors. *Arch Pharmacol* 328: 154, 1984
8. ZABLUDOWSKI JR, ZOCCALI C, ISLES CG, MURRAY GD, ROBERTSON JIS, INGLIS GC, FRASMER R, BALL SG: Effect of the 5-hydroxytryptamine type 2 receptor antagonist, ketanserin, on blood pressure, the renin-angiotensin system and sympatho-adrenal function in patients with essential hypertension. *Br J Clin Pharmacol* 17: 309, 1984
9. WENTING GJ, WOITTIEX AJ, SCHALEKAMP MADH, MAN IN'T VELD AJ: Hemodynamic effects of ketanserin, a selective 5-hydroxytryptamine (serotonin) receptor antagonist, in essential hypertension. *Clinical Science* 63: 435s, 1982
10. COHEN ML, FULLER RW, JURZ KD: Evidence that blood pressure reduction by serotonin antagonists is related to alpha receptor blockade in spontaneously hypertensive rats. *Hypertension* 5: 676, 1983
11. PETTERSON A, PERSSON B, HENNING M, HEDNER T: Antihypertensive effects of chronic 5-hydroxytryptamine (5-HT₂) receptor blockade with ketanserin in the spontaneously hypertensive rat. *Arch Pharmacol* 3r27: 43, 1984
12. PETTERSON A, GRADIN K, HEDNER T, HENNING M, PERSSON B: Chronic serotonergic blockade with ketanserin in the spontaneously hypertensive rats. *J Cardiovascular Pharmacol* 7 (Suppl. 7): S112, 1985
13. FOZARD JR: Mechanism of the hypotensive effect of ketanserin. *J Cardiovasc Pharmacol* 4: 829, 1982
14. DEGLI UBERTI EC, TRASFORINI G, MARGUTTI AR, ROTOLA CA, PANSISNI R: Effects of ketanserin, a new inhibitor of 5-HT₂ receptors, on plasma renin activity and aldosterone levels in normal subjects. *Hormone Res* 16: 244, 1982
15. FAGARD R, CATTART A, LIJNEN P, STAESSEN J, VANHEES L, MOERMAN E, AMERY A: Responses of the systemic circulation and of the renin-angiotensin-aldosterone system to ketanserin at rest and exercise in normal man. *Clin Sci* 66: 17, 1984
16. ZOCCALI C, ZABLUDOWSKI JR, ISLES CG: The effect of a 5-HT antagonist, ketanserin, on blood pressure, the renin-angiotensin system and sympatho-adrenal function in normal man. *Br J Clin Pharmacol* 16: 305, 1983