

Effect of Ramipril (ACE inhibitor) on Renin Activity Response to Acute Renal Ischemia in the Ovariectomized and Uni-nephrectomized Rats

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Renin-angiotensin system is thought to be modulated by gonadal steroids. However, it has not been well established whether gonadal steroids also modulate the changes in plasma renin activity (PRA) occurring in response to stimuli such as acute renal ischemia and also the effects of angiotensin-converting enzyme (ACE) on PRA. The aim of the present experiment was to study the relationship between sex hormones and plasma renin activity during acute renal ischemia in the Wistar rats. For this purpose, ovariectomy was performed in some rats and the changes in PRA in response to acute renal ischemia were observed. The levels of serum aldosterone and electrolytes were also measured. Ovariectomy not only caused a decrease in PRA but also reduced PRA response to acute renal ischemia. In contrast, ovariectomy had a reverse effect increasing PRA activity when ramipril, an ACE inhibitor, was used to prevent excessive angiotensin II formation due to renal ischemia. In conclusion, it can be said that gonadal steroids may have a role in modulating the PRA response to acute renal ischemia and may lead to changes in the effect of ACE inhibitors.

Key words: Ovariectomy, plasma renin activity, renal ischemia.

Tek taraflı nefrektomi yapılmış olan sıçanlarda ovariektominin böbrek iskemisine karşı renin aktivitesi cevabı üzerine etkileri

Gonadlardan salgılanan steroid hormonlar renin-anjiotensin sistemi üzerinde bir etkiye sahiptirler. Ancak, bu steroidlerin gerek akut iskemiyeye bağlı olarak plazma renin aktivitesinde ve gerekse ACE inhibitörlerinin etkisinde bir değişikliğe yol açıp açmadıkları bilinmemektedir. Bu çalışmanın amacı, akut böbrek iskemisi oluşturulan sıçanlarda cinsiyet hormonları ile plazma renin aktivitesi arasındaki ilişkiyi ortaya koymaktır. Bu amaçla, ovariektomi uygulanan hayvanlarda akut iskemiyeye bağlı olarak plazma renin aktivitesinde meydana gelen değişiklikler araştırıldı. Serum aldosteron ve elektrolitlerinin düzeyleri de ayrıca ölçüldü. Ovariektomili sıçanlarda PRA daha düşük olarak bulundu ve akut böbrek iskemisine cevap olarak normal sıçanlara göre daha fazla azalma gösterdi. Ramipril ile tedavi edilen hayvanlarda ise ovariektomi aksi bir etkiyle plazma renin aktivitesinde daha yüksek artışa yol açtı.

Anahtar kelimeler: Ovariektomi, plazma renin aktivitesi, böbrek iskemisi

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There are several studies indicating that the renin-angiotensin-aldosterone axis may be altered by the female reproductive hormones in many species. Plasma renin activity (PRA) as well as plasma renin concentration (PRC) are reported to be increased during the day of oestrous in rats. This increase in PRA and PRC is not found when rats are ovariectomized (1). The increase in PRA can be attributed in part to an increase in angiotensinogen production, which is known to be caused by oestrogens (2). However, an effect of progesterone on plasma renin concentration also appears to be involved. The combined treatment of ovariectomized rats with oestrogen and progesterone restores the normal increase in PRA and PRC values on the expected day of oestrus (1). The administration of oestrogen (oestrone acetate) in a dose of 10 mg induces a significant increase in plasma renin activity (3). Although the ratio of plasma renin activity to active renin concentration is not dependent on the angiotensinogen in normotensive females, their ratio is correlated with the angiotensinogen concentration in oestrogen-using women (4). Renin substrate rose and renin concentration fell in the ethinyloestradiol-treated women. Progestogen component may contribute to the blood pressure raising effects of oral contraceptives (5). The renin-angiotensin system is known to be activated during renal ischemia (6). However, it is not known whether this response is altered by gonadal steroids.

One of the ways used to treat acute renal failure after ischemia is to form an alteration in the renin - angiotensin system. ACE inhibition effectively prevents kidney structural and functional damage which develops in male rats after unilateral nephrectomy leading to end-stage renal failure (7). The effects of angiotensin can be blocked by administering angiotensin converting enzyme (ACE) inhibitors and thus, renal function can also be preserved (8). Ramipril, given in a dose of 7.5 mg/kg for two days by gastric gavage to inhibit angiotensin converting enzyme activity, attenuated the decrease of renin mRNA levels in the contralaterals to clipped kidneys (9). The inhibition of the renin-angiotensin system by captopril decreased blood pressure and increased

renin secretion (10). However, it has not been well established that the factors such as gonadal steroids may have a role in modulating the effects of the drugs used to prevent acute renal failure.

It has been suggested that hormone replacement therapy causes a decrease in the ACE activity in postmenopausal women and, therefore may have a role in decreasing a risk of coronary artery disease (11).

The aims of the present experiment were to investigate whether the response of PRA to renal ischemia and the effectiveness of ramipril, an ACE inhibitor, in preventing the development of renal failure are altered by absence of ovarian steroids in the rat.

MATERIAL and METHODS

The animals

The experiment was performed on female Wistar rats with body weights of 180 to 200 g. The animals were housed in the cages in a cycle of 12h light/dark and had free access to a diet and water. The rats which exhibited at least three 4-day oestrous cycles were used in this study.

Surgical procedures and experimental protocol

Bilateral ovariectomy and right nephrectomy were performed through dorso-lateral incisions under ketalar 60 mg/kg and xylazine 5 mg/kg body wt i.p. general anesthesia. Left renal ischemia was induced by placing a clip on the left renal arteries for 45 minutes. Sham-operated animals were exposed to dissection of left renal pedicle. Ramipril (7.5 mg/kg) was given for two days by gastric gavage. The rats were allocated to the following experimental groups.

- Group 1. Sham-operated; bilateral ovariectomy and right nephrectomy
- Group 2. Bilateral ovariectomy, right nephrectomy and left renal ischemia
- Group 3. Bilateral ovariectomy, right nephrectomy, renal ischemia and ramipril
- Group 4. Sham-operated; only right nephrectomy
- Group 5. Right nephrectomy and left renal ischemia

Group 6. Right nephrectomy, renal ischemia and ramipril

Blood sampling

The animals were decapitated 48 hours after acute renal ischemia. Blood was collected into tubes containing sodium EDTA. Plasma was removed and stored at -20 °C until assayed for PRA and aldosterone. Serum was also obtained for measuring the electrolytes, sodium and potassium.

Measurement of plasma renin activity

Plasma renin activity was determined by radioimmunoassay as follows: In a ice-cooled bath, 1 ml plasma was pipetted into uncoated glass or plastic tube marked with the identifying number and the suffix 37 and was added 10 µl of the phenylmethylsulfonyl fluoride (PMSF, Incstar Corp.-Stillwater, Minnesota, U.S.A) and 100 µl of the maleate generation buffer (pH:6, containing sodium EDTA, neomycin sulfate and inert blue coloring with 0.1% sodium azides a preservative) (Incstar Corp.) and was mixed and placed in ice bath. 0.5 ml aliquot of mixture was transferred into chilled tube marked with the suffix 4. 37 series tubes were incubated for 90 min at 37 C water bath, while the 4 series of tubes were incubated for 90 min at an ice bath. At the end of the generation period, 37 series and 4 series of tubes were transferred into ice bath. Pre-existent angiotensin I and that generated at 37 °C were measured by a modification of the radioimmunoassay (RIA) of Haber, using an antiserum raised in rabbits against angiotensin I (Incstar Corp.) coupled with bovine serum albumin (Incstar Corp.). PRA was expressed as ng angiotensin I per ml plasma and per hour of incubation (ng angiotensin I /ml /h).

Measurement of serum aldosterone level

Levels of aldosterone in serum was measured by radioimmunoassay with the DSL-8600 active aldosterone coated-tube radioimmunoassay kit of Diagnostic System Laboratories (Diagnostic System Laboratories, Texas, USA).

Measurement of serum electrolytes

The levels of sodium and potassium were measured by ISE method with Technicon RA-XT autoanalyzer

Statistics

Results were reported as mean ± standart error. Differences between means of the groups were determined by Mann Whitney U test, 2 tailed, with a 95 % confidence interval. An analysis of variance (Kruskal Wallis) and Tukey's honestly significant difference between means test were used to compare more than two means.

RESULTS

The mean values of the groups are shown in Table 1. There were significant differences between PRAs of all three ovariectomized groups. Ramipril-treated group, Group 3, had higher PRA than the other two groups (p<0.001). PRA was higher in the rats exposed to renal ischemia (Group 2) than the sham-operated rats (Group 1).

The ovariectomized groups, Group 1 and 2, had lower PRAs than the non-ovariectomized (intact) groups, Group 4 and 5 (p (0.05 and p (0.01). Ramipril-treated ovariectomized rats, Group 3, had higher PRA than the intact rats,

Table 1. Plasma renin activity (PRA), serum aldosterone and electrolytes in the ovariectomized (O) and intact rats. Values are mean ± S.E.M. The numbers of samples assayed are indicated in parentheses.

	UNx+O Group 1	P' 1 - 2	UNx+O+ ARI Group 2	P' 2 - 3	UNx+O+ARI Ramipril Group 3	P' 1 - 3	Unx Group 4	P' 4 - 5	UNx+ARI Group 5	P' 5 - 6	UNx+ARI Ramipril Group 6	P' 4 - 6
PRA ng/ml/h	7,2±0,5* n:12	<0.001	4,06±0,5** n:10	<0.001	18,15±1,14* n:10	<0.001	15±1,31 n:6	<0.05	9,89±0,9 n:10	N.S	13,58±1,2 n:10	N.S
Ald pg/ml	203±13,2* n:12	<0.05	171±5,2* n:10	N.S	194±9,74 n:10	N.S	86±8,411 n:6	N.S	97±8,01 n:10	<0.001	170±5,9 n:10	<0.001
Na mEq/L	144±2,22* n:12	N.S	145±3,80 n:10		149±2,08 n:10	N.S	153±4,63 n:6	<0.05	139±3,2 n:10	N.S	145±0,58 n:10	N.S
K mEq/L	8,5±0,87 n:12	N.S	7,1±0,32* n:10		6,7±0,34 n:10	N.S	6,6±0,43 n:6	N.S	5,4±0,30 n:10	N.S	7,2±0,46 n:10	<0.05

*P: Analysis of variance(Kruskal-Wallis test and the Tukey's honestly test) between means test to compare more than two means.

Group 6.

All the ovariectomized groups had similar aldosterone values. The two ovariectomized groups, Group 1 and 2, had higher aldosterone values than Group 4 ($p < 0.05$) and 5 ($p < 0.01$), respectively. Aldosterone values did not show significant changes between the two ramipril-treated groups, Group 3 and 6. The ramipril-treated intact rats, Group 6, had higher aldosterone values than the other two intact groups ($p < 0.001$).

Na and K values did not show any significant changes between the ovariectomized groups. The ovariectomized group, Group 1, had lower Na values than the intact group, Group 4 ($p < 0.05$). Na values was higher in Group 4 than

Group 5 ($p < 0.05$). The ramipril-treated intact group, Group 6, had higher K values than Group 5 ($p < 0.05$). The ovariectomized rats, Group 2, had higher K values than the intact group, Group 5 ($p < 0.05$).

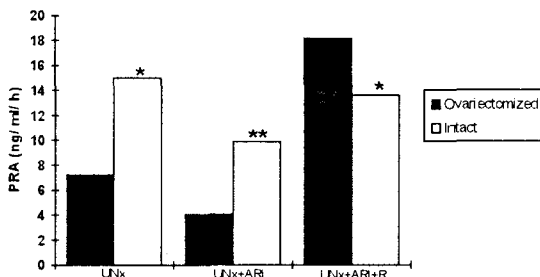


Figure 1. The levels of PRA in the unilateral nephrectomized (UNx), acute renal ischemic (UNx+ARI) and ramipril-treated (UNx+ARI+R) groups. (* $P < 0.05$, ** $P < 0.01$)

DISCUSSION

Our results demonstrates an effect of ovariectomy on PRA as shown in Figure 1. PRA is decreased in the ovariectomized groups except ramipril-treated group. The significant decrease in PRA following ovariectomy is in agreement with previous findings that PRA decreases after ovariectomy (12).

PRA response to renal ischemia was similar in the ovariectomized and intact groups, being lower in these two groups. However, the decrease in PRA of the ovariectomized group was significantly higher than the intact group. The decrease in PRA following renal ischemia may be due to an increase in the production of angiotensin II because it is known to have an inhibitory effect on renin secretion (6). A much higher decrease in the ovariectomized group may result from the absence of ovarian steroids, which have been shown to increase PRA (1).

Our results demonstrates that ramipril, an ACE inhibitor, has an increasing effect on PRA, which is consistent with the other studies (13). The increase in PRA in the ramipril-treated groups is likely to be related to the lack of the inhibitory feedback effect of angiotensin II on renin secretion. When the production of angiotensin II is prevented by ACE inhibitor, renin secretion is no longer under a negative feedback effect of angiotensin and thus renin is produced more, forming hyperreninemia.

The finding that the ramipril-treated ovariectomized rats has a significantly higher PRA than the intact ramipril-treated group shows a possible effect of ovariectomy and therefore ovarian steroids on PRA response to ACE inhibitor. This study demonstrates that ramipril become more potent in increasing PRA in the absence of ovarian steroids. This result may be related to the effect of gonadal steroids on ACE activity. In one study (11), it has been shown that ACE activity is changed by gonadal steroids.

According to our results, serum aldosterone levels do not seem to be affected by renal ischemia while they were significantly increased by ovariectomy. The reason that serum aldosterone levels increased in the ovariectomized groups with compared to intact groups may be due to a low sodium and a high potassium level, which are known to induce aldosterone secretion.

The finding that ramipril-treated intact group had higher aldosterone levels may result from a high serum potassium level in this group the reason of which remains to be determined. Ovariectomy did not lead to any significant

change in the serum aldosterone levels in the ovariectomized and intact ramipril treated groups.

Thus, the differences observed in the serum aldosterone levels between the groups can be attributed to the changes in the serum electrolytes rather than the production of angiotensin II, which is one of the main stimulators for aldosterone secretion. So, it can be said that aldosterone secretion is more sensitive to the changes in the serum electrolytes during renal ischemia and, its rate is mainly determined by the levels of electrolytes.

In summary, we have found that renin-angiotensin system is activated during acute renal failure caused by renal ischemia. Ovariectomy reduces PRA response to renal ischemia while it has a reverse effect when ramipril, an ACE inhibitor, is used. The data suggest that PRA is modulated by gonadal steroids in the rat. The mechanism by which gonadal steroids exert their effects need further studies.

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