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Frequency of Juxtaglomerular Granulated Cells in Diuretic (Frusemide) Treated Mice

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ABSTRACT. The present study was carried out to elucidate the effects of frusemide on the degree of granularity of the JGCs.

Fifteen male young-adult albino mice were given 0.3 mg/ml frusemide in their drinking water. Each experimental mouse was found to have drunk 10-12 ml of water containing frusemide, while control animals drunk 2-3 ml of drinking water. After three weeks, all animals were sacrified and kidneys were fixed in 10% buffered formalin and processed for light microscopy. Sections were stained for juxtaglomerular cells (JGCs) demonstration by alkaline crystal violet (Harada 1971) and by Bowie's method (Bowie 1936). The juxtaglomerular index (JGI) and the percentage of granulated nephron (%GN) were calculated according to the method of Hartroft and Hartroft (Hartroft & Hartroft 1953).

Kidneys from experimental animals showed an increase in both JGI and %GN following treatment with frusemide indicating hypertrophy, hyperactivity of the JGCs and increasing rate of renin release.

It is now generally accepted that renin is produced by and stored in the granular myoepithelial cells of the affrent arteriole in the juxtaglomerular apparatus (JGA). A close relationship between granule content of the JGCs and pressor activity of the kidney has been shown by many authors (Pitcock *et al.* 1959, Tobian *et al.* 1959). The juxtaglomerular index (JGI) indicates the frequency and degree of granularity of th JGCs in the JGA. The JGI was found to be changed under various pathological conditions. Latta *et al.* 1975 demonstrated a parallel relationship between renal renin level and the degree of granulation of the JGCs in rats with renal hypertension. Cain and Craus (1976) reported an increased JGI in the kidneys of patients with malignant hypertension. A significant relationship was found between serum renin concentration and JGI in hypertensive patients

(Saruta *et al.* 1969) and in normal and experimental hypertensive rats (Dauda 1975).

Frusemide is a diuretic drug of great clinical value and is indicated especially in the treatment of sever edema and in chronic renal failure. It has been shown to increase the rate of renin release (Baile *et al.* 1973). Until now there has been no published work on the effect of frusemide on the granularity of the JGCs.

Materials and Methods

Fifteen male young-adult albino mice weighting between 20-25 gm were each given 0.3 mg/ml frusemide in their drinking water. Each experimental mouse was found to have drunk 10-12 ml of water containing frusemide. Accordingly each animal received an average of 3.3 mg of frusemide per day. Fifteen control mice received drinking water only. Each control animal was found to have drunk 2-3 ml of water. Both groups were fed a standard laboratory diet. After three weeks, all the animals were sacrified and transverse kidney slices were fixed in 10% buffered formalin and processed for light microscopy. Sections were stained for JGCs demonstration by alkaline crystal violet (Harada 1971) and by Bowie's method (Bowie 1936). The JGI and the percentage of granulated nephrons (%GN) were calculated according to the method of Hartroft and Hartroft (Hartroft & Hartroft 1953).

Results

Distribution of juxtaglomerular granulation of control and experimental animal are given in Table 1. Mean values of JGI and %GN of control and frusemide treated mice are shown in Table 1.

Experimental animals showed a marked increase in both JGI and %GN Fig. 1.

Frusemide treated mice increased their water intake to (10-12) ml/day, and no change was found in their body weight.

Kidneys from experimental animals showed an increase in both JGI and %GN following treatment with frusemide indicating hypertrophy, hyperactivity of the JGCs and increasing rate of renin release.

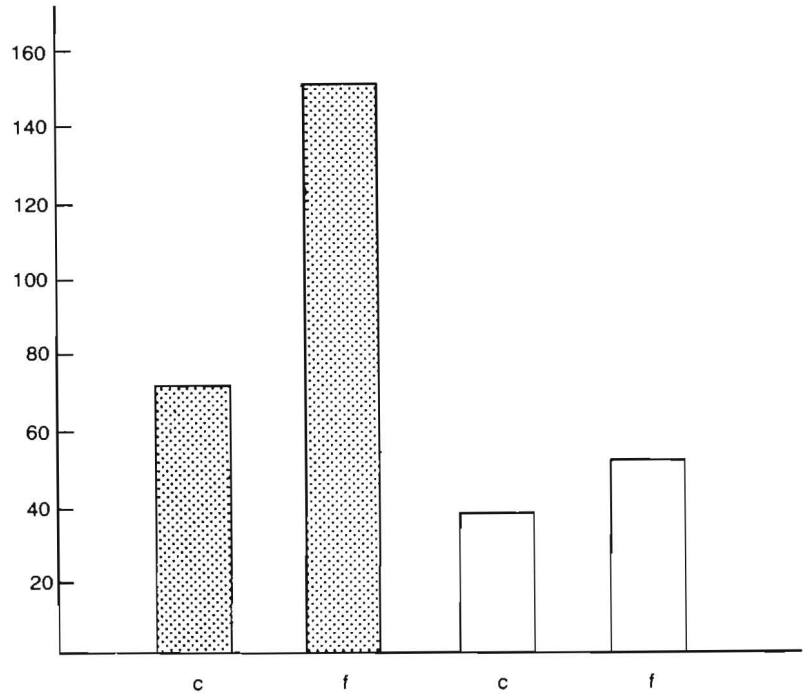

Fig. 1. The JGI ▨ and the %GN □ of control (c) and frusemide (f) treated mice.

Discussion

The renin content of myoepithelial cells has been confirmed by immunocytochemistry using antibodies to pure renin in human (Lindop & Downie 1984) and experimental animals (Lacasse *et al.* 1985). The present study has shown that treatment with frusemide produces a marked increase in both JGI and %GN. Frusemide is a natruritic diuretic and causes diuresis by increasing the permeability of cell membrane to sodium ions allowing sodium to leak from the peritubular fluid (Robson & Lambie 1973).

It is not clear why there was a considerable increase in the juxtaglomerular granulation obtained from kidneys of frusemide treated mice. It is possible that frusemide acts by producing sodium depletion, sodium depletion was found to produce an increase of juxtaglomerular granulation (Hartroft & Hartroft 1953). The decrease in the sodium or chloride load on the macula densa increases renin

Table 1. Frequency of juxtaglomerular granulation in control and frusemide treated mice

	Control JGI	Frusemide JGI	Control %GN	Frusemide %GN
	81	218	50	70
	65	170	30	56
	73	115	32	53
	60	125	40	45
	92	129	50	48
	102	155	55	50
	55	163	26	54
	70	141	38	47
	66	111	40	53
	68	183	30	38
	59	108	39	65
	71	133	42	52
	62	265	40	75
	80	195	50	70
	61	167	27	55
Mean	71	158.6	39.6	55.3
	±	±	±	±
S.D.	12.6	43.5	8.7	10.3
	P<0.0001		P<0.0005	

secretion (Barajas 1979). Friedberg (1965) showed a significant rise in JGI in the kidney of salt depleted mice. The degree of granulation has also been increased by a sodium deficient diet, while loading with sodium over a long time leads to a decrease in the granulation of the JGCs (Tobian 1964).

It is also possible that frusemide affects the JGCs activity *via* prostaglandins. Studies utilizing prostaglandin infusion and prostaglandin synthetase inhibitors suggest that prostaglandins play an important role in renin release in man and experimental animals. Yun *et al.* 1977 reported that infusion of PGE1 or PGE2 into dog renal artery caused an elevation in renin secretion. Infusion of PGA1 in human volunteers was also found to increase plasma renin activity "PRA" (Golub 1976). Indomethacin has been found to reduce PRA and renin secretion in dog (Yun *et al.* 1979) and in man (Frolich 1976). The mechanism by which prostaglandins stimulate renin release was related to the marked diuresis and natriuresis produced by infusion of PGE and PGA (Lee *et al.* 1976). Prostaglandins might directly stimulate the JGA to release renin since renin release was

increased in a cortical cell suspension incubated with PGE2 and PGI2 (Wharton *et al.* 1977). Treatment of dogs (Beckmann & Leovey 1976), rats (Suzuki *et al.* 1981) and human volunteers (Frolich 1976) with indomethacin inhibits the elevation on PRA normally induced by diuretics, insulin-induced hypoglycemia, isoproterenol, sodium free diet, or arachidonic acid. The effects of aspirin on the prostaglandin producing interstitial cells and on the JGA granulation has been studied in the mouse (Al-Ani 1980). Here, it was suggested that the decrease in the JGI was related to the suppression of prostaglandins produced by the destruction of the interstitial cells.

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تأثير المدرر على الخلايا المجاورة للكبيبة في الفئران

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الغاية من هذا البحث هو دراسة تأثير المدرر على الخلايا المجاورة للكبيبة في الفئران. ومن أجل ذلك أُخِذ خمسة عشر فأراً ذكراً يزن كل منها حوالي (٢٠ ـ ٥٠) غرام، ومن ثمَّ تمَّ إعطاء المدرر بنسبة ٠,٣ ميليغرام لكل ميليلتر مكعب من ماء الشرب. فلوحظ بأن كل فأر تحت التجربة قد شرب يومياً (١٠ ـ ١٢) ميليلتر مكعب من الماء الحاوي على المدرر، أي أنه حصل على حوالي ٣,٣ ميليغرام من المدرر في اليوم.

وفي نفس الوقت أخذِ خمسة عشر فأراً آخر للمقارنة، ووُضِع لها ماء للشرب خالٍ من المدرر. فلوحظ بأن كل فأر قد شَرِب (٢ ـ ٣) ميليلتر مكعب في اليوم.

لقد دامت هذه التجربة ثلاثة أسابيع، قُتِلت بعدها جميع الفئران: تلك التي شربت ماءً فيه المدرر، وتلك التي شربت ماءً خالٍ من المدرر (فئران المقارنة). وبعد قتلها ثبّتت كِلَى هذه الفئران وحُضّرت بطرق المجهر الضوئي الإعتيادية. ثمَّ تمَّ صبغ الشرائح المحضَّرة بصبغات خاصة لصبغ حبيبات الخلايا المجاورة للكبيبة وذلك باستخدام طريقة البللورات البنفسجية وطريقة باو. وبعد ذلك حُسِب مُعدَّل التحبُّب في الخلايا المجاورة للكبيبة (J G I)، وحُسِبت كذلك نسبة الوحدات الكلوية المحببة GN % وذلك باستخدام طريقة هارتروفت.

وقد وُجِد بنتيجة الدراسة أنَّه يوجد زيادة في مُعدَّل التحبب للخلايا المجاورة للكبيبة وفي نسبة الوحدات الكلوية المحبّبة في الفئران التي شربت ماءً فيه المدرر، وذلك بمقارنتها بالفئران التي شربت ماءً خالٍ من المدرر. ممَّا يدل على زيادة في عدد وحجم الخلايا الفارزة للأنفحة. نذكر أخيراً بأن تفسير النتائج المحصول عليها قد تمَّ بناءً على فكرة التوازن المائي الملحي وعلى تأثير البروستا كلاندين.