Assiut Vet. Med. J. Vol. 43 No. 85, April 2000

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NATRIURETIC EFFECT OF PROSTAGLANDIN A_1, E_2 AND I_2 IN ADULT MALE ALBINO RATS

(With 2 Tables)

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تأثير البروستاجلاندينات I_2, E_2, A_1 على إخراج الصوديوم في الفئران البيضاء الذكور البالغة

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في هذا البحث تم حقن ٢٠ ميكروجرام من البروستاجلاندين I_2, E_2, A_1 داخل الغشاء البريتوني في الفئران الذكور البالغة . تم جمع البول لمدة ٢٤ ساعة وتم تعيين حجم البول (ميكرولتر/ دقيقة) في المجموعة الضابطة والمجموعات المحقونة بالبروستاجلاندين كما تم تعيين مستوى الصوديوم والبوتاسيوم والكلور في البلازما في المجموعة الضابطة وبعد ١, ٢, ٣, ٤ ساعات من حقن البروستاجلاندين. بفحص عينات البلازما وجد نقص معنوي في مستوى الصوديوم بعد ساعة من حقن البروستاجلاندين بأنواعها الثلاث واستمر النقص حتى ٤ ساعات من الحقن . كما وجد نقص معنوي في مستوى البوتاسيوم بعد ساعة من حقن البروستاجلاندين A_1 , E_2 وبعد ٣ ساعات من حقن البروستاجلاندين I_2 واستمر حتى ٤ ساعات من الحقن. ,اظهر مستوى الكلور نقصا معنويا بعد ١, ٢, ٣, ٤ ساعات من الحقن في جميع المجموعات المحقونة بالبروستاجلاندين بأنواعها الثلاثة. وأظهر معدل إخراج الصوديوم والبوتاسيوم والكلور في البول زيادة معنوية في المجموعات المحقونة بالبروستاجلاندين I_2, E_2, A_1 . وأظهر مستوى الألدوستيرون في البلازما تغيرا غير معنوي في جميع المجموعات المحقونة بالمقارنة بالمجموعة الضابطة. تدل هذه النتائج على أن البروستاجلاندين I_2, E_2, A_1 يزيد من إخراج الصوديوم والبوتاسيوم والكلور فى بول الفئران وجميعها تؤدى إلي زيادة حجم البول . وقد تعزى هذه النتائج إلي التأثير المثبط المباشر على امتصاص الصوديوم والبوتاسيوم والكلور في الفرع الصاعد السميك من لفة هنل بنخاع الكلية حيث يتم امتصاصها معا بواسطة الناقل وليس عن طريق تثبيط هرمون الالدوستيرون. كذلك من خلال التأثير على مرور الدم في نخاع الكلية بتوجيه الدم من القشرة

الخارجية إلي القشرة الداخلية والنخاع وإلغاء التدرج في الضغط الازموزى الذي يؤدي إلـــي نقص امتصاص الصوديوم والكلور والماء في لفة هنل مما يؤدى إلي زيادة إخراجها وأيضـــا التأثير المضاد للهرمون المانع لإدرار البول.

SUMMARY

In this work 20 µg of PGA_1, PGE_2 and PGI_2 were injected intraperitoneally into mature albino male rats. Urine was collected over a period of 24 hours and urine volume in ul/min. was calculated in the control and prostaglandin treated groups. Na^+, K^+ and Cl^- excretion rate in uEq/min. was estimated in all groups. Plasma levels of Na^+, K^+ and Cl^- were estimated in the control group and in the treated groups 1, 2, 3 and 4 hours after prostaglandin injection. A significant decrease in plasma Na^+ and Cl^- were obtained after 1 hour and remained significant up to 4 hours in all treated groups. A significant decrease in plasma K^+ was obtained after 1 hour in PGA_1 and PGE_2 treated groups and after 3 hours in PGI_2 treated group and reamained significant up to 4 hours. Urine volume showed a significant increase in all treated groups injected with the 3 types of prostaglandins. A significant increase in Na^+, K^+ and Cl^- excretion rate was noticed in all treated groups. Plasma aldosterone showed nonsignificant change in all treated groups in comparison with the control group. These results indicate that PGA_1, PGE_2 and PGI_2 have a natriuretic effect on albino male rats. Their natriuretic effect is not through inhibition of aldosterone and is suggested to be due to direct inhibition of active reabsorption of Na^+, K^+ and Cl^- ions in the medullary thick ascending limb of loop of Henle, increased blood flow in the medulla and antagonizing the effect of antidiuretic hormone

Key words: Natriuretic effect of prostaglandin

INTRODUCTION

The function of renal prostaglandin system has received considerable attention over the past several years. As a consequence of this attention, a better understanding of its role in the regulation of renal function is started.

Though the kidney might be capable of producing all types of prostaglandins (at least under certain conditions) the medullary prostaglandin E_2 (PGE_2) is the most likely responsible for the majority of the physiologic effect. The major active members of renal prostaglandins are PGE_2, PGI_2 and thromboxane A_2 (Stokes, 1981).

Recently, interest has focused on the role of endogenously produced prostaglandins in the renal regulation of sodium and water excretion. Varying results have been obtained and the exact physiologic role of prostaglandins and the mechanism of action is not yet well established.

The administration of prostaglandins of the A, E and I series to experimental animals or in isolated perfused renal tubules produced natriuresis (Haas et al., 1984; Hamed et al., 1987; Breyer and Ando, 1994 and Herbert et al., 1995). Also, PGE_2 inhibited arginin vasotocin dependent sodium transport (Rytved et al., 1996). In addition indomethacin (a prostaglandin synthesis inhibitor) diminished natriuresis in normal volunteers given an intravenous saline infusion (Stokes et al., 1997). On the other hand inhibition of prostaglandin synthesis caused natruiresis in the conscious dog undergoing a water diuresis suggesting that endogenous prostaglandins may decrease sodium excretion (Kirschenbaum and Stein, 1974). While Kirschenbaum and Aserros (1980) and Kokko (1981) reported that PGI_2 and $PGF_{2\alpha}$ have little or no natriuretic effect. Also a newly synthesized prostaglandin analogue, increased renal blood flow without increasing sodium excretion (Haas et al., 1984).

Prostaglandins of the A, E and I series administrated to experimental animals increased urine volume (Bolger et al., 1978; Haas et al., 1984 and Hamed et al., 1987). In addition acute administration of indomethacin to rats, dogs or man reduced urine volume (Altsheler et al., 1978 and Stokes et al., 1997). On the other hand PGE_2 stimulated water transport in microperfused cortical collecting duct (CCD) (Noland et al., 1992).

Several mechanisms have been proposed by different investigators to explain the natriuretic effect of prostaglandins but the exact mechanism is still not well established.

The aim of this work is to study the natriuretic effect of PGA_1, PGE_2 and PGI_2 in adult male rat and to throw light on the mechanism of action of these prostaglandins.

MATERIALS and METHODS

In this study 24 Sprague-Dawley adult male albino rats, with 200 g average weight were used to investigate the effect of PGA_1, PGE_2 and PGI_2 on the volume of urine and the excretion rate of sodium, potassium and chloride and the possible mechanism of action.

Four groups six, animals each were used. Animals of the first group were kept in metabolic cages and urine was collected over a period of 24 hours for each rat and urine volume was calculated in ul/ minute. Half ml of blood was obtained from orbital sinus and plasma was separated by centrifugation and kept in deep freeze for future use. This group was considered as a control group.

Groups 2, 3 and 4 were injected intraperitoneally (ip) with 20 µg/0.2 ml (0.1 mg/kg body weight) of PGA_1, PGE_2 and PGI_2 (Sigma, dissolved in 95% ethanol at concentration of 0.1 mg/ ml) respectively. Half ml of blood was obtained from the orbital sinus 1, 2, 3 and 4 hours after injection. Plasma was separated by centrifugation and kept in deep freeze for future use. The animals were put in metabolic cages for 24 hours after injection of prostaglandins and urine was collected and urinary excretion rate was calculated as in the control group.

Sodium and potassium levels (mEq/L) were estimated in the plasma of the control group and in the plasma of prostaglandin treated groups 1, 2, 3 and 4 hours after injection using flame photometer (Corning 400) and plasma chloride was estimated using chloride analyzer (Model 925). Also sodium, potassium and chloride excretion rate was calculated in uEq/m (Gross and Bartner, 1973).

Plasma aldosterone was determined by RIA according to the method by Sufi et al. (1984) using commercial RIA kits (SORN-Biomedica- ALDOCTK-2).

RESULTS

Intraperitoneal injection of PGA_1 in a dose of 20 µg into mature male rates resulted in a significant decrease in the plasma level of Na, 1h ($P<0.01$), 2h, 3h ($P<0.001$) and 4h ($P<0.01$) following injection. The maximum decrease was reached 2 h after injection and begin to increase again 3 hours following injection. Plasma level of K^+ showed a significant decrease after 1h ($P<0.05$), 2h ($P<0.01$), 3h ($P<0.001$) and 4h

($P<0.01$) after injection and the maximum decrease was reached 3h after injection. Plasma level of chloride showed also a significant decrease 1h, 2h, 3h ($P<0.001$) and 4h ($P<0.01$) after injection (Table 1). Also the same dose of PGA_1 resulted in a significant increase in the volume of urine (urinary excretion rate in ul/m) after injection. At the same time there was a significant increase ($P<0.001$) in Na^+, K^+ and Cl^- excretion rate (Table 2).

Administration of PGE_2 (20 µg) by the same route resulted in a significant decrease in the plasma level of Na^+ 1h ($P<0.01$), 2h, 3h and 4h ($P<0.001$) following its injection. The maximum decrease was reached 4h after injection. Plasma level of K^+ showed also a significant decrease after 1h, 2h ($P<0.01$), 3h ($P<0.05$) and 4h ($P<0.01$) when compared with the control level. Chloride plasma level showed a significant decrease 1h, 2h, 3h and 4h ($P<0.001$) after injection (Table 1). The volume of urine was significantly increased ($P<0.05$) by the same dose of PGE_2. At the same time there was a significant increase in Na^+, K^+ ($P<0.001$) and Cl^- ($P<0.01$) excretion rate (Table 2).

PGI_2 injected intraperitoneally resulted in a significant decrease in plasma level of Na^+, 1h ($P<0.01$), 2, 3 and 4h ($P<0.001$) after injection and the maximum decrease was reached 4h after injection. Plasma level of K^+ showed a significant decrease 3h ($P<0.05$) and 4h ($P<0.001$) after injection. Plasma level of Cl^- showed a significant decrease 1,2,3 and 4h ($P<0.01$) after injection (Table 1).

The same dose of PGI_2 resulted in a highly significant ($P<0.001$) increase in the volume of urine and in the urinary Na^+, K^+ and Cl^- excretion rate (Table2).

Plasma aldosterone in PGA_1, PGE_2 and PGI_2 treated groups showed non significant change in comparison with the control group (Table 1)

DISCUSSION

Injection of PGA_1, PGE_2 and PGI_2 in a single dose of 20µg into mature male rats increased the volume of 24 hour urine, increased sodium, potassium and chloride excretion rate and decreased plasma levels of Na^+, K^+ and Cl^- estimated at hourly intervals after injection.

The natriuretic effect of PGA_1, PGE_2 and PGI_2 observed in this study is in agreement with the results of Stokes (1981); Haas et al. (1984); Hamed et al., (1987) and Breyer and Ando (1994). In addition, the natriuretic effect of prostaglandins observed in this study was supported by many investigators. Hilchey and Bell-Qualley (1995) reported that PGI_2 participate in the natriuretic action of angiotensin (1-7) and Stokes et al. (1997) found that indomethacin (a prostaglandin inhibitor) diminished natriuresis in normal volunteers given an intravenous saline infusion. While our results are inconsistent with that of Els and Helman (1981) and Fine and Kirschenbaum (1981) who reported that PGE_2 either stimulated or had no effect on sodium transport and that PGI_2 has little or no natruiretic effect. Contradictory results were also stated by Kirchenbaum and Stein (1974) who found that inhibition of prostaglandin synthesis causes natriuresis in conscious dog undergoing water diuresis suggesting that prostaglandin may decrease sodium excretion.

Several mechanisms have been proposed by different investigators to explain the natriuretic effect of prostaglandins. The site of altered sodium chloride and fluid reabsorption following prostaglandin treatment must be either the loop of Henle, the collecting ducts (Moore, 1985 and Noland et al., 1992) or the renal distal convoluted tubules, (Stokes et al., 1997) but not the proximal convoluted tubules (Fulgraff and Meiforth, 1971).

For studying the possible mechanism by which prostaglandins induce natriuresis, plasma aldosterone level was measured. From our data aldosterone is not involved in the natriuresis and diuresis induced by the administered prostaglandins as plasma aldosterone showed non significant changes in all treated groups in comparison with the control group. The results of direct infusion of prostaglandin on aldosterone secretion recorded in man were contradictory (Fishman et al., 1972 and Carr, 1973). However, prostaglandin E_2 and prostaglandin precursor, arachidonic acid and $PGF_{2\alpha}$ had no effect on aldosterone production by isolated rat granulosa cells (Matsuka et al., 1980 and Enyedi et al., 1981). In addition, prostaglandin E_2 was without effect on the production rate of aldosterone when infused into the adrenal artery of sheep (Blair et al., 1971). Also lack of any significant effect by the nonsteroidal antiinflamatory drugs (inhibitors of prostaglandin synthesis) on basal aldosterone production rate indicates the absence of any essential

prostaglandin effect in the non-stimulated state (Enyedi et al., 1981). In addition, Scherzer et al. (1992) found that the Na^+ retaining effect of indomethacin in the cortical collecting duct is aldosterone-independent. Moreover, indomethacin did not modify the change in plasma aldosterone associated with volume expansion (Stokes et al., 1997). However observations in man (Norbiato et al., 1978) and in conscious rats (Spät et al., 1979 and Suzuki et al., 1981) using prostaglandin synthetase inhibitors and PGE_2, and in slices of rabbit renal cortex using arachidonic acid metabolites (Haas et al., 1984) suggested that prostaglandins were involved in the control of aldosterone synthesis. But this was under certain condition or with high dose. The dose of PGE_2 in the work of Suzuki et al. (1981) was 1mg/kg.

In this work, prostaglandin injection produced a significant increase in Na^+, K^+ and Cl^- excretion rate and a significant increase in the volume of 24 hours urine. The natriuretic effect of prostaglandin can be explained by direct inhibition of active reabsorption of Na^+, K^+ and Cl^- ions in the medullary thick ascending limb of loop of Henle (Moore, 1985 and Noland et al., 1992) where they are actively transported by Na-K-2Cl co-transporter (Patrick et al., 1998). In rat kidney the greatest density of prostaglandin E_2 receptors in normal animals was found in the medullary thick ascending limb of loop of Henle. Other investigators attributed the natriuretic effect of PGE_2 to increased renal interstitial hydrostatic pressure (Haas et al., 1984). However, Moore (1985) suggested that prostaglandins may affect sodium chloride and water excretion by an influence on medullary blood flow by directing blood from the outer and towards the inner cortex and medulla and increase blood flow through the vasa recta. This leading to washout of the osmotic gradient as a result of which, sodium chloride and water reabsorption in the loop of Henle is reduced resulting in natriuresis.

The increase in the volume of urine in this experiment can be attributed to increased rate of sodium, potassium and chloride excretion and antagonizing the fluid retaining effect of vasopressin in the collecting tubules by inhibiting cAMP (Noland et al., 1992).

It can be concluded that the natriuretic effect of prostaglandin is not through inhibition of aldosterone hormone but can be attributed to direct effect on tubular sodium reabsorption or increased blood flow in the medulla.

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Table (1): Plasma levels of sodium, potassium, chloride (mEq/L) and aldosterone (Pg/ml) in adult male rats injected with PGA_1, PGE_2 and PGI_2

	Control	PGA_1				PGE_2				PGI_2			
		1h	2h	3h	4h	1h	2h	3h	4h	1h	2h	3h	4h
Sodium	149.67 ± 2.62	132.40** ± 1.07	118.40*** ± 1.70	127.38*** ± 1.55	133.40** ± 1.83	128.80** ± 2.41	125.00*** ± 2.11	122.27*** ± 1.87	119.9*** ±1.97	132.8 ** ± 1.45	128.33*** ± 1.34	124.30 *** ± 1.71	121.87*** ± 1.38
Potassium	6.57 ± 0.17	6.03* ± 0.07	5.40** ± 0.09	4.87 *** ± 0.13	5.82** ± 0.06	5.58** ± 0.20	5.57** ± 0.09	5.83* ± 0.13	5.55** ± 0.13	N.S 6.48 ± 0.13	N.S 6.53 ± 0.11	6.08* ± 0.09	5.48 *** ± 0.10
Chloride	119.05 ± 1.66	97.17*** ± 1.68	95.17 *** ± 2.79	101.33 *** ± 1.58	105.33 ± 2.29	95.33*** ±1.96	85.17 *** ± 2.76	101.67*** ± 1.65	101.17 ** ± 1.14	102.67 *** ± 1.05	104.00 *** ± 1.13	101.17 *** ± 1.08	100.83 *** ± 1.25
Aldosterone	88.27 ± 3.82	N.S. 89.38 ± 4.60	N.S. 92.55 ± 4.76	N.S. 91.53 ± 3.91	N.S. 96.10 ± 4.79	N.S. 86.63 ± 3.96	N.S.88.83 ± 4.10	N.S. 89.11 ± 4.09	N.S. 87.50 ± 3.62	N.S. 82.12 ± 2.99	N.S. 82.52 ± 3.75	N.S. 88.05 ± 5.05	N.S 83.30 ± 3.93

Data represent mean ± standard error

* P< 0.05 ** P<0.01 *** P < 0.001 N.S. = Non significant

Table (2): Urine volume (ul/min), Sodium, Potassium and Chloride excretion rate (u Eq/ min) in the control and prostaglandin treated mature male rats

Treatment	Urine volume ul / min	Urinary Na^+ u Eq / min	Urinary K^+ u Eq/ min	Urinary Cl^- u Eq/ min
control	4.74 ± 1.22	0.43 ± 0.27	0.37 ± 0.019	0.46 ± 0.035
PGA_1	10.30 ± 0.85 **	1.27 ± 0.055 ***	0.94 ± 0.037 ***	1.44 ± 0.099 ***
PGE_2	7.17 ± 0.58 *	0.85 ± 0.033 ***	0.66 ± 0.024 ***	0.83 ± 0.061 **
PGI_2	12.97 ± 0.91 ***	1.25 ± 0.072***	1.07 ± 0.051 ***	1.13 ± 0.086 ***

Data represent mean ± standard error

* P< 0.05

** P<0.01

*** P < 0.001