applied to an isolated guinea-pig ileum preparation. Kininase inhibition was confirmed by incubating synthetic bradykinin with an aliquot of the acidified extract at pH 7.0 for 20 min at 37 °C and recoverable bradykinin determined by bio-assay. The ability of extracts to liberate kinin in preference to angiotensin was confirmed by 2 methods; firstly, incubates of extract with kininogen were applied to an isolated rat duodenum preparation suspended in DeJalons solution. Secondly, 1×10^{-4} g carboxypeptidase A or 1×10^{-5} g carboxypeptidase B (Worthington) dissolved in 0.16 moles l^{-1} NaCl were incubated for 20 min, pH 7.0 at 37 °C with incubates containing the suspected kinin activity and remaining activity determined by bio-assay.

Results. The pH optima of dog renal kallikrein was found to be pH 9.0. Extracts and substrate possessed no extraneous activity. The acidification procedure produced effective inhibition of renal kininase, 95–100% of added bradykinin was recoverable following incubation with renal extracts. The activity liberated by the extracts had properties characteristic of a kinin[16]. It produced relaxation of an isolated rat duodenum preparation, was destroyed by carboxypeptidase B, but unaffected by carboxypeptidase A. Synthetic bradykinin exhibited the same properties; whereas, synthetic angiotension II (Hypertensin Ciba) was inactivated by carboxypeptidase A and produced an increased tonus of rat duodenum.

Histological examination revealed that the glomerular fraction contained a small amount of arteriolar and tubular tissue, whereas, the tubular fraction was relatively pure. The activity detected in the glomerular, tubular and medullary fractions of 6 dog kidneys is shown in the table. The medulla contained consistently low levels of activity. Both glomerular and tubular fractions were found to contain kallikrein, a significantly greater level of activity being detectable in the glomerular-rich fraction ($p < 0.001$, paired t-test).

Mean kallikrein activity of 3 dog kidney fractions

Dog	Kidney	Glomerular fraction	Tubular fraction	Medulla
1	Left	-	3.73 (2)	0.61 (2)
	Right	8.07 (5)	3.13 (4)	0.91 (4)
2	Left	6.22 (2)	1.35 (3)	0.56 (2)
	Right	9.74 (3)	2.27 (3)	0.28 (2)
3	Left	6.26 (1)	3.04 (1)	0.56 (1)
	Right	5.77 (1)	1.87 (1)	-
Mean ± SEM		7.2 ± 0.74	2.57 ± 0.36	0.98 ± 0.1

Units of activity. µg (10^{-6}) synthetic bradykinin equivalent in potency to the kinin liberated by the extract per mg protein during 20 min incubation. Protein concentration (10^{-6} g ml^{-1}). Glomerular fraction 201 ± 40 (18); tubular fraction 616 ± 85 (19); medulla 772 ± 125 (17); mean ± SEM. Figures in parenthesis indicate the number of determinations performed.

Discussion. Kallikrein was found primarily in the cortex of the dog kidney. The highest level of activity was found in the glomeruli-rich fraction, which contained some juxtaglomerular tissue. Activity was also detected in the tubular fraction. In the rat kidney, kallikrein has been localized to the distal tubule near the juxtaglomerular apparatus[9,10] and glomeruli devoid of juxtaglomerular tissue contain little kallikrein[7]. These observations on dog kidney find significant quantities of kallikrein in glomerular fractions contaminated with juxtaglomerular tissue, supporting the notion that kallikrein may be associated with the juxtaglomerular complex.

1 K. Nustad, Br. J. Pharmac. *39*, 72 (1970).
2 I.F. Carvalho, in: Kidney Hormones, p. 585. Ed. J.W. Fisher. Academic Press, London 1971.
3 K. Nustad, K. Vaaje and J.V. Pierce, Br. J. Pharmac. *53*, 229 (1975).
4 K. Nustad, Br. J. Pharmac. *39*, 87 (1970).
5 A. Scicli, O. Carretero, A. Hampton, P. Cortes and N. Oza, Physiologist *17*, 327 (1974).
6 C. Moriwaki, K. Miyazaki, Y. Matsuda, H. Moriya, Y. Fujimoto and H. Ueki, J. Biochem. *80*, 1277 (1976).
7 A. Scicli, O. Carretero, N. Oza and M. Schork, Proc. Soc. exp. Biol. Med. *151*, 57 (1976).
8 E. Werle and R. Vogel, Archs int. Pharmacodyn. Thér. *126*, 171 (1960).
9 A. Scicli, O. Carretero, A. Hampton, P. Cortes and N. Oza, Am. J. Physiol. *230*, 533 (1976).
10 T. Örstavik, K. Nustad, P. Brandtzaeg and J. Pierce, J. Histochem. Cytochem. *24*, 1037 (1976).
11 K. Mann, R. Geiger and E. Werle, Adv. exp. Med. Biol. *70*, 65 (1976).
12 W. Cook and G. Pickering, Nature, Lond. *182*, 1103 (1958).
13 M. Rocha, E. Silva, W. Beraldo and G. Rosenfield, Am. J. Physiol. *156*, 261 (1951).
14 G. Delory and E. King, Biochem. J. *39*, 245 (1945).
15 O. Lowry, N. Rosebrough, A. Farr and R. Randell, J. biol. Chem. *193*, 265 (1951).
16 M. Schachter, Physiol. Revs. *49*, 509 (1969).

Influence of age on the renal renin response to a high salt intake in the rat

P. Karen, I. Pohlová and J. Jelínek

Institute of Physiology, Czechoslovak Academy of Sciences, Budějovická 1083, CS-14220 Prague 4 (Czechoslovakia), 17 October 1977

Summary. Saline drinking combined with DOCA-treatment was found to decrease renal renin in weanlings at a higher rate than in adult rats, with a comparable saline consumption level. The decrease was not potentiated by uninephrectomy.

Renin-angiotensin system (RAS) activity decreases during postnatal ontogeny in the rat, presumably because of maturation of water, salt and circulatory homeostasis[1]. There is evidence that, in suckling rats, increasing body sodium does not influence RAS activity[2]. However, no data are available about RAS reactivity in the weaning period. Kidney function is more developed at this period than in suckling rats, but is still not fully mature[3]. Rats of this age are more sensitive than adults to the hypertensogenic effects of a chronically increased salt intake[4], particularly

when combined with deoxicorticosterone-acetate (DOCA) administration[5]. These regimens decrease renal renin activity (RRA) in adult rats[6] and the age-dependent difference in this reaction might be related to the difference in the hypertensive response. We therefore compared the RRA reaction to both these regimens in weanling and adult rats. Since the response was reported to be increased by uninephrectomy[7], both intact and uninephrectomized animals were used.

Methods. Male Wistar strain rats, either intact or uninephrectomized 1 week previously, were offered 1% saline as the only drinking fluid, at the age of 25 (young) or 87 (adult) days. Saline was given either alone (saline group) or combined with 3.5 mg DOCA in microcrystalline suspension/100 g b.wt, i.m., twice weekly (DOCA-saline group). Intact and uninephrectomized animals of the same age, which drank water, served as controls. All animals were fed a pellet diet containing 180 mEq Na^+/kg. After 7 and 21 days systolic blood pressure (BP) was measured indirectly on the hind extremity[8], the animals were sacrificed and RRA estimated as described previously[1]. Saline consumption in each animal (mean of the 7 last daily values before sacrifice) was expressed in ml/100 g body surface area (i.e. g b.wt 0.67×0.1). The results were statistically evaluated using Duncan's test. Spearman's rank correlation coefficients were also used.

Results. RRA values (table 1) in control groups did not change between day 7 and 21 of the experiment and were pooled. In uninephrectomized animals of this group, the RRA was lower, the difference being significant in adults only. RRA was decreased in the saline groups and more so in DOCA-saline groups. This decrease, however, was not potentiated by uninephrectomy. After a 21-day exposure to both regimens, when the young rats were already sexually mature and physiologically similar to adults, the RRA was suppressed comparably in both age groups. After a 7-day exposure, however, the decrease of RRA was much more pronounced in the young than in the adult DOCA-saline group. In the saline group a similar trend was present. The more pronounced RRA decrease in the DOCA-saline than the saline groups was accompanied by higher saline intake and BP levels (table 2). Neither of these variables in the young was higher than in the adult group after a 7-day exposure to DOCA-saline regimen, in spite of the 50% difference in RRA. After a 21-day exposure moderate hypertension developed in the DOCA-saline groups, the mean BP value being non-significantly higher in the younger group.

Discussion. The present results indicate that, in the rat, the RRA is more responsive to an increased salt intake during the weaning period than in adults. RRA bears an indirect relation to salt intake[6] and arterial BP level[9]. However, neither of these factors can explain the accelerated RRA decrease found in the young DOCA-saline group after a 7-day exposure satisfactorily. In rats of corresponding age, a lesser efficiency of the diuretic and natriuretic response was found to volume expansion[10]. Thus, an increase in body fluid volumes due to a high salt intake might play a role. In

Table 1. Renal renin activity (in ng of val^5 angiotensin II - amide equivalents per 1 mg of renal protein after 10 min incubation of renal renin extract with renin substrate) in intact and uninephrectomized rats exposed to a saline or a DOCA-saline regimen

Exposure (days)	Regimen	Age group	n	Intact	n	Uninephrectomized
7+21	Control	young	27	53.7±3.24	14	43.8±3.32
		adult	23	51.2±3.28	15	38.3±3.10[a]
7	Saline	young	14	27.6±3.36[b]	7	20.4±3.54[b]
		adult	12	32.6±2.62[b]	7	27.4±0.89[b]
	DOCA-saline	young	14	9.4±0.87[cd]	7	7.9±1.12[cd]
		adult	12	18.8±1.96[c]	8	18.2±1.82[c]
21	Saline	young	10	18.6±3.55[b]	8	16.6±4.41[b]
		adult	8	19.9±3.90[b]	8	24.9±4.72[b]
	DOCA-saline	young	10	0.4±0.26[c]	9	3.1±0.91[c]
		adult	8	0.7±0.42[c]	9	1.6±0.51[c]

Means ± SEM; n = number of values in each group. In the control group the values found on day 7 and 21 of the experiment are pooled.

Significant difference between groups of the same exposure: [a] uninephrectomized vs intact (of the same age group and regimen, $p<0.05$); [b] saline vs control (of the same age group, $p<0.01$); [c] DOCA-saline vs saline (of the same age group, $p<0.02$); [d] young vs adult (of the same regimen, $p<0.01$).

Table 2. Saline consumption (ml/100 cm^2 body surface area per day) and blood pressure (mm Hg) in rats exposed to saline or DOCA-saline regimens

Exposure (days)	Regimen	Age group	n	Blood pressure	n	Saline consumption		R
7	Saline	young	14	95±3.5[b]	14	14.2±0.67[b]	(21)	−0.444
		adult	13	126±3.1	13	11.8±0.88	(19)	+0.035
	DOCA-saline	young	14	104±5.1[b]	14	17.6±1.50	(21)	−0.264
		adult	14	139±3.2[a]	14	16.1±0.62[a]	(20)	−0.312
21	Saline	young	13	129±2.7	10	15.6±0.60	(15)	−0.814
		adult	12	126±4.4	9	15.0±1.22	(13)	+0.207
	DOCA-saline	young	14	171±4.8[a]	11	28.2±2.37[a]	(16)	+0.191
		adult	13	159±5.3[a]	10	31.3±2.39[a]	(14)	−0.358

R = Spearman's rank correlation coefficients between renal renin activity (RRA) and saline consumption. Intact and uninephrectomized animals are pooled. Number of correlated pairs is given in brackets. In intact animals RRA values from both kidneys were used for evaluation. Statistically significant coefficients are underlined. Other symbols as in table 1.
Significant differences between groups of the same exposure ($p<0.05$):
[a] DOCA-saline vs saline (of the same age group); [b] young vs adult (of the same regimen).

agreement with this, the accelerated RRA decrease in the young saline group with a lower saline intake level was only indicated, but the different reactivity manifested itself by an inverse relation between saline intake and RRA, which was absent in the adults (table 2). This presumably reflects a higher RRA responsiveness to salt excess in immature rats. There is evidence that RRA plays a role in regulation of glomerular blood flow[11,12] and some data indicate that the vasculature of immature rats is more prone to develop hypertensive lesions[13]. It may be speculated that, in connection with the accelerated RRA decrease, renal glomeruli in weanling rats developing DOCA-saline hypertension are exposed to an elevated perfusion pressure in the period of higher vulnerability. A vicious circle mechanism[14] might thus be started more easily and lead to a more pronounced hypertensive response indicated here, which becomes more evident after a prolonged exposure to the DOCA-saline regimen[5].

1 I. Pohlová and J. Jelínek, Pflügers Arch. *351*, 259 (1974).
2 S. Solomon, A. Iaina and H. Eliahou, Proc. Soc. exp. Biol. Med. *153*, 309 (1976).
3 A. Aperia and P. Herin, Am. J. Physiol. *228*, 1319 (1975).
4 L.K. Dahl and E. Schackow, Can. med. Ass. J. *90*, 155 (1964).
5 H. Musilová, J. Jelínek and I. Albrecht, Physiologia bohemoslov. *15*, 525 (1966).
6 F. Gross, H. Brunner and M. Ziegler, Recent Prog. Horm. Res. *21*, 119 (1965).
7 R. Müller-Suur, H.V. Gutschke, K.F. Samwer, W. Oelkers and K. Hierholzer, Pflügers Arch. *359*, 33 (1975).
8 J. Jelínek and V. Krpata, Physiologia bohemoslov. *16*, 182 (1967).
9 L. Tobian, Fedn Proc. *26*, 48 (1967).
10 H.H. Bengele and S. Solomon, Am. J. Physiol. *227*, 364 (1974).
11 K. Thurau, in: Angiotensin, p.480. Ed. I.H. Page and F.M. Bumpus, Springer Verlag, Berlin 1974.
12 J.S. Kaufman, R.J. Hamburger and W. Flamenbaum, Am. J. Physiol. *231*, 744 (1976).
13 W.A.J. Crane and L.P. Dutta, J. Path. Bact. *88*, 291 (1964).
14 F.B. Byrom and L.F. Dodson, Clin. Sci. *8*, 1 (1949).

Effect of sex hormones on plasma cholesterol in castrated and noncastrated male rats[1]

G.M. Fischer and M.L. Swain

Bockus Research Institute and Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia (Pa. 19146, USA), 22 September 1977

Summary. The administration of estradiol to both castrated and noncastrated male rats was associated with significantly increased plasma cholesterol levels as compared to controls, the estradiol in the noncastrated rats overriding the tendency of testosterone to lower plasma cholesterol.

The widespread clinical use of estrogen and contraceptive steroid combinations has given rise to many studies on the effect of these substances on plasma lipids. Results in both humans and experimental animals have been conflicting. Early human clinical studies suggested a hypocholesterolemic effect of estrogen[2-5], whereas more recent studies have indicated that the administration of estrogen or estrogen-progestogen combinations results in an increase in plasma cholesterol[6-8]. Experimental animal studies in the chicken and turkey[9,10] indicated a hypercholesterolemic effect of estrogen, whereas studies in the rat have indicated that estrogen decreases plasma cholesterol concentrations[11,12]. A biphasic, dose-related response to estrogen has also been suggested, high doses decreasing and low doses increasing plasma cholesterol levels[13].

Androgens, on the other hand, have in general been found to lower plasma cholesterol in both humans and experimental animals, although there are some conflicting reports[14]. The data for androgens are not so extensive as those for estrogens.

Little has been done in the way of comparison of estrogen effect in the castrated versus noncastrated male animal, and the following is a report on the effect of testosterone and estradiol on plasma cholesterol in the castrated male rat as well as the effect of estradiol on plasma cholesterol in the intact male rat.

Male rats of approximately 5 weeks of age were obtained from Charles River Breeding Laboratories (CD strain) and divided into 5 groups. Rats in 3 of these groups were castrated, using pentobarbital anesthesia (40 mg/kg i.p.), and all rats were maintained on a diet of Purina rat chow. Weekly i.m. injections were initiated the day after operation and were given to all rats for 3 weeks according to the following schedule. In each case the injection volume was 0.1 ml.

Group I. Castrated. Cottonseed oil.
Group II. Castrated. Depotestosterone cypionate (Upjohn) in cottonseed oil, 1 mg.
Group III. Castrated. Depoestradiol cypionate (Upjohn) in cottonseed oil, 10 µg.
Group IV. Noncastrated. Cottonseed oil.
Group V. Noncastrated. Depoestradiol cypionate (Upjohn) in cottonseed oil, 10 µg.

After 3 weeks of treatment rats were killed instantaneously by cervical dislocation. Blood was drawn immediately from the heart into tubes containing EDTA and plasma was separated by centrifugation and was frozen for storage. Plasma total cholesterol levels were subsequently determined by the method of Pearson et al.[15] as used by Kritchevsky et al.[16].

The mean plasma total cholesterol levels for the 5 groups are given in the table. In both castrated and noncastrated male rats the administration of estradiol was associated

Total cholesterol concentrations in plasma of hormone treated rats

Group	Manipulation and therapy	Number of animals	Total cholesterol mg/dl plasma (Mean + SE)
I	Castrated, cottonseed oil	19	93.8 ± 2.7*
II	Castrated, testosterone	19	89.4 ± 4.1
III	Castrated, estradiol	17	117.0 ± 3.1*
IV	Noncastrated, cottonseed oil	14	92.1 ± 3.5**
V	Noncastrated, estradiol	13	113.1 ± 4.5**

* $p < 0.0005$, ** $p < 0.005$.