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Research Article

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Angiotensin-converting Enzyme Labeled with [³H]Captopril

Tissue Localizations and Changes in Different Models of Hypertension in the Rat

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Abstract

In vitro autoradiography with [³H]captopril was used to localize and quantitate angiotensin-converting enzyme (ACE) in various tissues in two-kidney, one-clip (2K-1C) hypertension, one-kidney, one-clip (1K-1C) hypertension, desoxycorticosterone acetate (DOCA)-salt hypertension, and a normotensive control group. There were no significant differences in mean systolic blood pressure among the hypertensive groups. Plasma renin activity (PRA) was highest in the 2K-1C group (6.20±2.17 ng/ml per h), intermediate in the 1K-1C group (2.19±0.62 ng/ml per h) and control group (3.20±0.53 ng/ml per h), and lowest in the DOCA-salt group (0.07±0.06 ng/ml per h). In the lungs, aorta, mesenteric arteries, and adrenal medulla, ACE labeling was highest in the 2K-1C group, intermediate in the 1K-1C and control groups, and lowest in the DOCA-salt group. ACE levels in these tissues correlated positively with PRA. In the kidney, anterior pituitary, testis, and choroid plexus of the brain, ACE levels correlated negatively with PRA, with lowest ACE levels in the 2K-1C group and highest levels in the DOCA-salt group. In the epididymis, posterior pituitary, and other regions of the brain, ACE levels did not differ significantly among the groups.

Introduction

Angiotensin-converting enzyme (ACE)¹ plays a role in blood pressure regulation as a component of the plasma renin-angiotensin system (RAS) by converting angiotensin I (ANG I) to the potent vasoconstrictor angiotensin II (ANG II) and degrading the vasodilator bradykinin (1). In the plasma RAS, ACE conversion of ANG I occurs primarily in the lungs, producing changes in circulating ANG II levels that vary in accordance with plasma renin levels (2). ACE also influences the local production of ANG II in various tissues. In blood vessel

walls, for example, ACE may generate ANG II locally as part of the vascular RAS (3–7). ACE and other components of this vascular system may be involved in maintaining certain forms of hypertension, perhaps independently of the plasma RAS (3–5). ACE is also responsible for local ANG II production in some regions of the brain (8, 9). ACE occurs in other tissues, such as adrenal, kidney, and pituitary gland (10), where it may also generate ANG II, but its endogenous substrate is not clearly established.

The intent of the present study was to examine the relationship of ACE to changes in the plasma RAS in hypertension. To do so, we studied three models of hypertension associated with high, normal, or low plasma renin levels. ACE was labeled by in vitro autoradiography with [³H]captopril, which binds exclusively to ACE both in tissue homogenates and sections (11–15), permitting the localization of particulate ACE in tissue as well as quantitation of ACE levels by densitometry (16, 17).

Methods

Male Wistar rats with initial weights of 200–220 g were obtained from Charles River Laboratories, Inc., Wilmington, MA. For each rat a baseline systolic blood pressure was recorded before surgery. Baseline blood pressures and all subsequent pressures were taken by the tail cuff method (18), during which rats were lightly anesthetized with ether. Pressure tracings were recorded on a physiograph (Narco Biosystems, Houston, TX). Rats were then divided into four experimental groups.

Group 1: two-kidney, one-clip (2K-1C) hypertension. After rats were anesthetized with sodium pentobarbital (40 mg/kg body wt, i.p.), an abdominal incision was made and a 0.2-mm silver clip placed on the left renal artery. The right kidney and renal artery remained untouched. After surgery, blood pressures were taken twice weekly (tail cuff method) for 4 wk. Animals were given free access to tap water and rat chow.

Group 2: one-kidney, one-clip (1K-1C) hypertension. In this group a 0.2-mm silver clip was placed on the left renal artery and the right kidney was removed. Blood pressures were recorded twice weekly, and the rats received tap water and normal rat chow.

Group 3: desoxycorticosterone acetate (DOCA)-salt hypertension. Animals in this group underwent excision of the right kidney; then silicone rubber molds containing DOCA (Sigma Chemical Co., St. Louis, MO; 200 mg/kg body wt) were implanted subcutaneously (19). This group was given 1% NaCl (instead of tap water) and normal rat chow. Blood pressures were recorded twice weekly.

Group 4: nonhypertensive (control) group. Sham operations were performed in which the left renal artery was manipulated (but not clipped) and the right kidney was left intact. Animals were given tap water and normal rat chow, and blood pressures were recorded twice weekly.

4 wk after surgery all animals were sacrificed by decapitation. ~ 2 ml arterial blood from each rat was collected in chilled tubes containing 0.2 ml EDTA for determination of plasma renin activity (PRA); all

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1. *Abbreviations used in this paper:* ACE, angiotensin-converting enzyme; ANG I and ANG II, angiotensin I and II; DOCA, desoxycorticosterone acetate; 1K-1C, one-kidney, one-clip hypertension; 2K-1C, two-kidney, one-clip hypertension; PRA, plasma renin activity; RAS, renin-angiotensin system.

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samples were collected in one morning to avoid diurnal variation in renin levels (20). Serum was frozen at -20°C , and at a later time PRA was determined by a modification of the microradioimmunoassay method of Husain and Jones (21). Sections of lung, aorta, mesentery, kidney, adrenal gland, testis, brain, and pituitary gland were rapidly removed, embedded in Tissue-Tek (Miles Laboratories Inc., Naperville, IL) or brain paste (except lung), and quickly frozen in liquid nitrogen. Tissue sections ($8\ \mu\text{m}$) were cut on a cryostat, mounted on gelatin-coated slides, and stored at -20°C .

For in vitro autoradiographic studies, (prolyl-3, 4- ^3H)-S-acetyl-captopril (New England Nuclear, Boston, MA; 50 Ci/mmol) was converted to ^3H captopril with 0.1 M NaOH for 20 min at 23°C as previously described (15). Slide-mounted tissue was first incubated in a buffer of 50 mM Tris-HCl, pH 7.9 (4°C), and 100 mM NaCl at 4°C for 5 min, then transferred to a solution of ^3H captopril (8 nM) in the same buffer for 40 min at 4°C . Slides were given two 1-min washes in buffer, briefly dipped in distilled water, and dried in a stream of cool air. To assess nonspecific binding, slide-mounted tissue was treated as described above except that the incubation solution contained $1\ \mu\text{M}$ unlabeled captopril or enalaprilat.

Autoradiograms were produced by placing either Ultrafilm or emulsion-coated coverslips over the slide-mounted tissue for 12 d at 4°C . Density of ^3H captopril labeling on Ultrafilm was quantitated by microdensitometry and converted to femtomoles of ^3H captopril bound per milligram protein using ^3H standards obtained from Amersham (Arlington Heights, IL) (16). Tissue was stained with toluidine blue after autoradiography. ^3H captopril binding in homogenates of the lungs was assayed as described previously (14).

The drug and peptide specificity of ^3H captopril binding in homogenates and brain slides used for autoradiography are the same in brain, choroid plexus, lung, pituitary, adrenal, testis, and epididymis (11–15). In preliminary experiments we observed a similar specificity in mesenteric arteries, kidney, and aorta (data not shown).

Densitometric studies of unperfused mesenteric arteries could not be performed because the vessels were collapsed and convoluted. Therefore four additional experimental groups (2K-1C, 1K-1C, DOCA-salt, and nonhypertensive controls) were set up as described above but were sacrificed by vascular perfusion rather than decapitation. PRAs were not determined in this set of animals owing to the effects of anesthesia on PRA (22). Rats were anesthetized with sodium pentobarbital (40 mg/kg body wt, i.p.), and mesenteric arteries were perfused through the aorta with a solution of 1.0% NaCl and 5.5% dextrose in 0.1 M phosphate buffer. Longitudinal sections of mesenteric arteries were mounted on microtome chucks and quickly frozen in liquid nitrogen; then $8\text{-}\mu\text{m}$ sections were cut and mounted on slides. Slides were incubated in ^3H captopril as described above, covered with emulsion-coated coverslips, and exposed for 2 wk at 4°C .

Slides of mesenteric arteries were examined in a model 3 photomicroscope (Carl Zeiss, Inc., Thornwood, NY), and vessel segments were photographed first with transmitted light, then under darkfield conditions with identical exposure times. Darkfield negatives were enlarged three times and prints were used to quantitate grain density over the endothelium by microdensitometry (16, 17).

To perform statistical tests, means and standard errors for blood pressure, PRA, and specific ^3H captopril binding in various tissues were calculated for each rat, then for each group of rats. Comparisons among groups were performed by analysis of variance, and differences between paired groups were compared by the Walker-Duncan adaptive procedure (23). Correlation coefficients for all variables were calculated by the Spearman rank order method and the level of significance determined by a two-tailed test (23).

Results

Baseline mean systolic blood pressures (SBP) \pm SEM for the four groups were 100.3 ± 1.4 mmHg for the 2K-1C group ($n = 8$), 105.4 ± 2.8 mmHg for the 1K-1C group ($n = 5$),

Table 1. Specific ^3H Captopril Binding in Various Tissues

Groups	Lungs	Aorta	Mesenteric arteries	Kidney		Anterior pituitary	Posterior pituitary	Choroid plexus	Supra-optic nucleus	Paraventricular nucleus	6.2-mm corpus		8.7-mm striatum		Testis	Epididymis
				C	UC						intra-auricular level*	intra-auricular level*	intra-auricular level*	intra-auricular level*		
2K-1C	898 \pm 28 (n = 8)	513 \pm 15 (n = 6)	507 \pm 20 (n = 5)	192 \pm 11 (n = 8)	389 \pm 67 (n = 8)	827 \pm 58 (n = 7)	2705 \pm 32 (n = 7)	10720 \pm 494 (n = 7)	483 \pm 31 (n = 6)	494 \pm 41 (n = 4)	317 \pm 38 (n = 7)	878 \pm 43 (n = 4)	584 \pm 33 (n = 7)	1391 \pm 105 (n = 7)		
1K-1C	726 \pm 12 (n = 5)	321 \pm 14 (n = 5)	229 \pm 23 (n = 5)	131 \pm 8 (n = 5)	478 \pm 36 (n = 5)	905 \pm 76 (n = 5)	2719 \pm 94 (n = 5)	12000 \pm 454 (n = 5)	480 \pm 32 (n = 4)	424 \pm 10 (n = 3)	364 \pm 67 (n = 5)	1010 \pm 96 (n = 3)	664 \pm 66 (n = 5)	1450 \pm 218 (n = 5)		
DOCA-salt	615 \pm 51 (n = 6)	319 \pm 22 (n = 6)	120 \pm 35 (n = 5)	62 \pm 26 (n = 6)	845 \pm 72 (n = 6)	1404 \pm 77 (n = 6)	2705 \pm 51 (n = 6)	13880 \pm 578 (n = 6)	512 \pm 27 (n = 6)	383 \pm 50 (n = 3)	360 \pm 50 (n = 5)	956 \pm 121 (n = 6)	758 \pm 44 (n = 6)	1463 \pm 81 (n = 6)		
Control	676 \pm 57 (n = 5)	348 \pm 22 (n = 4)	264 \pm 27 (n = 5)	123 \pm 20 (n = 5)	446 \pm 29 (n = 5)	930 \pm 90 (n = 5)	2526 \pm 126 (n = 5)	11820 \pm 319 (n = 5)	490 \pm 24 (n = 5)	377 \pm 7 (n = 3)	277 \pm 31 (n = 5)	938 \pm 85 (n = 3)	660 \pm 51 (n = 5)	1633 \pm 127 (n = 5)		
ANOVA	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01^{\dagger}$	$P < 0.01$	NS	$P < 0.01$	NS	NS	NS	NS	NS	NS		
Walker-Duncan (paired groups, $P < 0.05$)	1 vs. 3 1 vs. 4 2 vs. 3	1 vs. 2 1 vs. 3 1 vs. 4	1 vs. 2 1 vs. 3 1 vs. 4 2 vs. 3 3 vs. 4	1 vs. 2 1 vs. 3 1 vs. 4 2 vs. 3 3 vs. 4	1 vs. 3 2 vs. 3 3 vs. 4	1 vs. 3 2 vs. 3 3 vs. 4										

Mean \pm SEM expressed as fmol/mg protein except in the lungs, expressed as fmol/2.5 mg tissue. ^3H Captopril binding was assayed in lung homogenates and quantified by in vitro autoradiographic densitometry in tissue sections in all other organs. UC, unclipped kidney. C, clipped kidney. * Reference 24. † C in 2K-1C, 1K-1C groups; UC in DOCA-salt, control groups. \ddagger C in 1K-1C group; UC in all other groups.

105.7±1.7 mmHg for the DOCA-salt group ($n = 6$), and 101.0±2.3 mmHg for the nonhypertensive control group ($n = 5$) (no statistically significant differences among the groups). During the week of sacrifice, SBPs for the four groups were 151.7±3.7 mmHg (2K-1C), 156.8±5.3 mmHg (1K-1C), 157.3±1.8 mmHg (DOCA-salt), and 109.2±2.2 mmHg (control). Paired comparisons showed no statistically significant differences among the three hypertensive groups, but mean SBPs in each of the hypertensive groups were significantly higher than those of the control group ($P < 0.01$ for all comparisons).

PRA for the four groups were 6.20±2.17 ng/ml per h (2K-1C, $n = 8$), 2.19±0.62 ng/ml per h (1K-1C, $n = 5$), 0.07±0.06 ng/ml per h (DOCA-salt, $n = 6$), and 3.20±0.53 ng/ml per h (control, $n = 5$). Differences among the groups were statistically significant by analysis of variance ($P < 0.05$). Mean PRA values of the 2K-1C group were significantly greater than those of the 1K-1C group ($P < 0.05$), DOCA-salt group ($P < 0.01$), and controls ($P < 0.05$). PRAs for the 1K-1C and control groups were significantly greater than those of the DOCA-salt group ($P < 0.01$ for both comparisons).

Mean densities for specific [³H]captopril binding to ACE in various tissues are presented in Table I. In Table II, Spearman's rank order correlation coefficients are listed for PRA, blood pressure, and tissues for which statistically significant differences exist.

In the lungs [³H]captopril binding in homogenates was significantly different among the groups, with highest levels in the 2K-1C group, intermediate levels in the 1K-1C group and control group, and lowest binding in the DOCA-salt group (Table 1). Pulmonary [³H]captopril binding correlated positively with PRA and [³H]captopril binding in the aorta and adrenal medulla, but negatively with binding levels in the testis and choroid plexus (Table II).

In the aorta, labeling for ACE was densest in the endothelium and adventitia. Minimal labeling was observed in the tunica media (Fig. 1). The density of endothelial but not adventitial labeling differed among the groups. Grain densities over the aortic endothelium (Table I) were greater in 2K-1C rats than in controls ($P < 0.05$), whereas in DOCA-salt rats densities were lower than those in controls (NS). Endothelial densities in 1K-1C rats were slightly higher than those of DOCA-salt animals but lower than those of controls (NS). Aortic endothelial ACE levels correlated positively with those in the lung and adrenal medulla and with PRA, but correlated negatively with ACE levels in the testis and choroid plexus (Table II).

In the perfused mesenteric arteries, as in the aorta, dense labeling of ACE was observed over the endothelial layer, with patchier and less dense labeling in the perivascular and interstitial tissue (Fig. 2). Endothelial labeling in the mesenteric vessels differed significantly among the groups, with greatest

Table II. Spearman's Rank Order Correlation Coefficients for PRA, Blood Pressure, and Specific [³H]Captopril Binding in Various Tissues

	PRA	Lung	Aorta	Adrenal medulla	Kidney*	Anterior pituitary	Testis	Choroid plexus	Blood pressure [†]
PRA	—	0.45 ($P < 0.05$)	0.44 ($P < 0.05$)	0.62 ($P < 0.01$)	-0.58 ($P < 0.01$)	-0.49 ($P < 0.05$)	-0.47 ($P < 0.05$)	-0.68 ($P < 0.01$)	-0.18 (NS)
Lung	—	—	0.43 ($P < 0.05$)	0.76 ($P < 0.01$)	-0.30 (NS)	-0.30 (NS)	-0.43 ($P < 0.05$)	-0.47 ($P < 0.05$)	0.06 (NS)
Aorta	—	—	—	0.47 ($P < 0.05$)	-0.56 ($P < 0.01$)	-0.12 (NS)	-0.32 (NS)	-0.66 ($P < 0.01$)	-0.20 (NS)
Adrenal medulla	—	—	—	—	-0.49 ($P < 0.05$)	-0.78 ($P < 0.01$)	-0.80 ($P < 0.01$)	-0.94 ($P < 0.01$)	-0.16 (NS)
Kidney	—	—	—	—	—	0.28 (NS)	0.27 (NS)	0.15 (NS)	0.18 (NS)
Anterior pituitary	—	—	—	—	—	—	0.83 ($P < 0.01$)	0.30 (NS)	0.33 (NS)
Testis	—	—	—	—	—	—	—	0.42 ($P < 0.05$)	0.33 (NS)
Choroid plexus	—	—	—	—	—	—	—	—	0.15 (NS)
Blood pressure	—	—	—	—	—	—	—	—	—

Number of pairs, 22-24. * Left (clipped) kidney in 2K-1C and 1K-1C groups and left kidney in all other groups. † During the week of sacrifice.

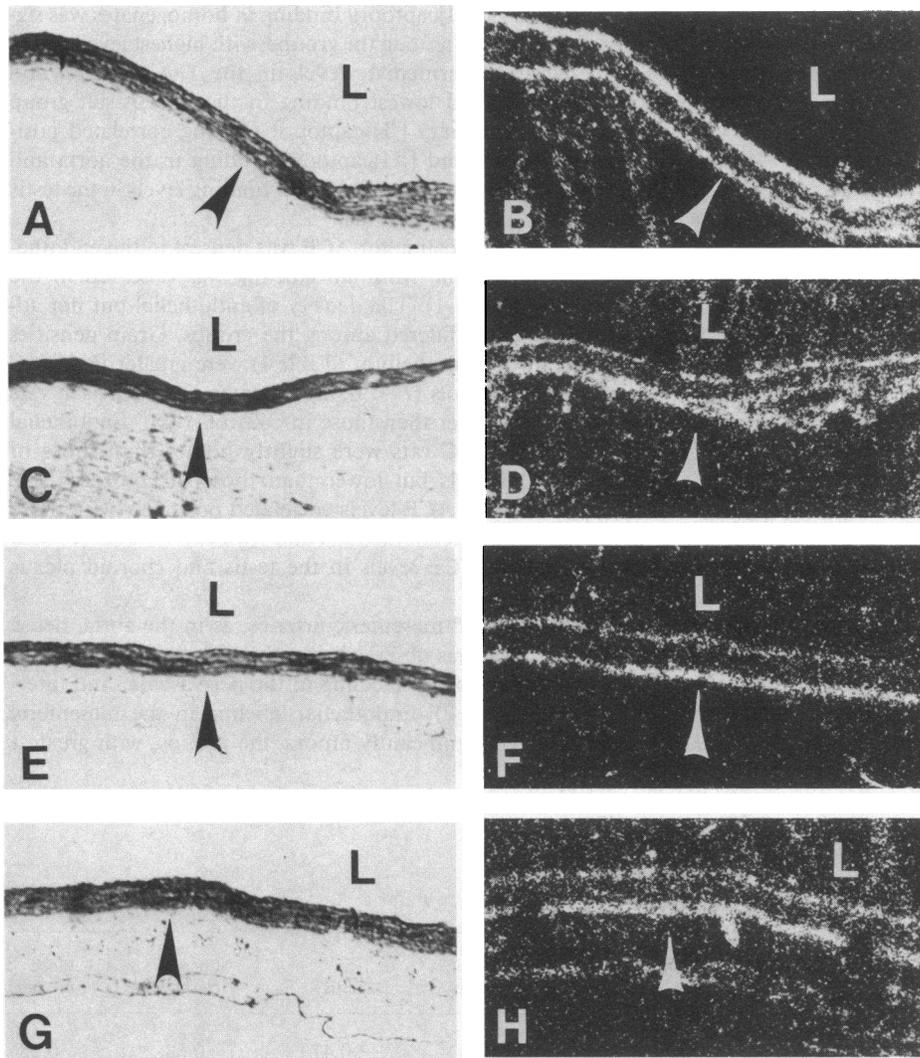


Figure 1. Light micrographs and corresponding darkfield micrographs of [^3H]captopril binding in aortas. (A and B) 2K-1C rat. (C and D) 1K-1C rat. (E and F) DOCA-salt rat. (G and H) Control (normotensive) rat. (L) vessel lumen; (arrows) adventitial layer. The density of endothelial labeling (adjacent to vessel lumen) is highest in B, intermediate in D and H, the lowest in F. Labeling is minimal in the medial layer, and the density of adventitial labeling does not vary substantially among the groups. 15 \times .

densities occurring in the 2K-1C group, intermediate values in the 1K-1C groups and control group, and lowest levels in the DOCA-salt rats (Fig. 2, Table I).

Labeling in the adrenal gland was localized to the medulla and to a thin, less dense capsular or pericortical rim; minimal labeling was observed in the cortex (Fig. 3). Labeling densities in the medulla were significantly different among the groups (Table I). Highest levels of [^3H]captopril binding were found in the 2K-1C animals, intermediate levels in the 1K-1C and control animals, and lowest levels in the DOCA-salt rats (Fig. 3, Table I). Significant positive correlations were found between adrenal medullary densities and PRA, lung densities, and aortic densities. Significant negative correlations were observed between labeling densities in the adrenal medulla and those in the anterior pituitary, kidney, testis, and choroid plexus (Table II).

In the kidney, high-density labeling for ACE in all groups was localized over tubules in the inner cortex and juxtamedullary regions, with minimal or low-density labeling occurring in the medulla and outer cortex (Fig. 4). Small areas of infarction were occasionally seen in the clipped kidneys of the 2K-1C and

1K-1C groups; these showed no labeling for ACE and were not included in densitometry studies. Labeling densities were lowest in the clipped kidneys of the 2K-1C group, intermediate in the kidneys of 1K-1C and control groups, and highest in the kidneys of the DOCA-salt group; these differences were statistically significant (Table I). ACE binding densities in the left kidneys of all groups correlated negatively with PRA and densities in the aorta and adrenal medulla (Table II).

ACE labeling in the pituitary gland was patchy in the anterior lobe and more dense and homogeneous in the posterior lobe. No labeling was observed in the intermediate lobe (Fig. 5). In the anterior lobe [^3H]captopril binding was significantly different among the groups, with binding levels lowest in the 2K-1C group, intermediate in the 1K-1C and control groups, and highest in the DOCA-salt rats (Table I). Densities in the anterior pituitary were positively correlated with those in the testis and negatively correlated with those in the adrenal medulla and PRA (Table II). Densities in the posterior pituitary were slightly elevated in all three hypertensive groups compared with control groups but there were no significant differences among the groups (Table I). There were also no statisti-

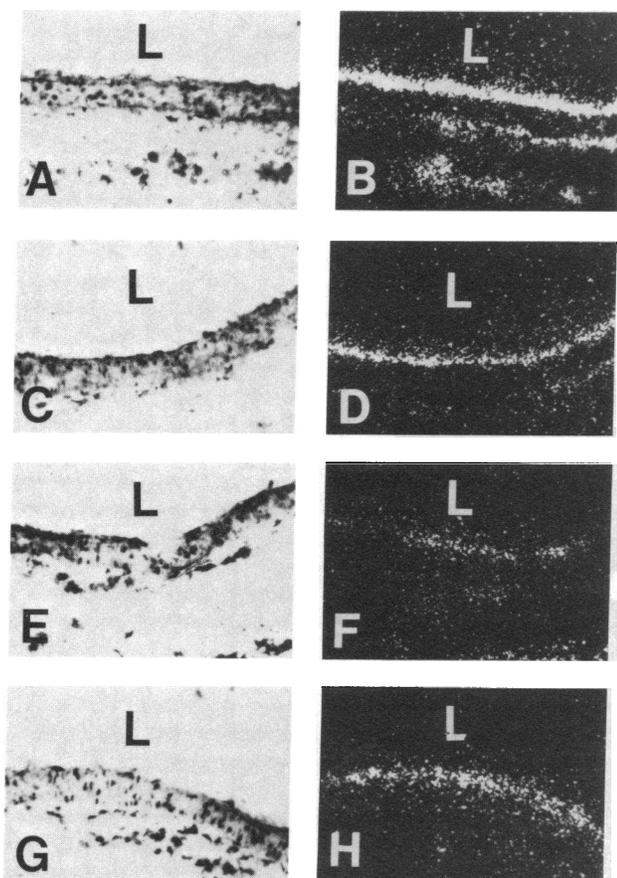


Figure 2. Light micrographs and corresponding darkfield micrographs of [³H]captopril binding in small arteries of the mesentery. (A and B) 2K-1C rat. (C and D) 1K-1C rat. (E and F) DOCA-salt rat. (G and H) Control (normotensive) rat. (L) Vessel lumen. The density of endothelial labeling (adjacent to the vessel lumen) is highest in B, intermediate in D and H, the lowest in F. Patchy, less dense labeling is seen in the perivascular and interstitial tissue. 40×.

cally significant correlations between ACE labeling in the posterior pituitary and labeling in other tissues, PRA, or blood pressure.

[³H]Captopril binding to ACE was quantitated in various regions of the brain, including the choroid plexus, supraoptic nucleus, paraventricular nucleus, and corpus striatum (rostral and caudal regions [24]). In the choroid plexus, [³H]captopril labeling was denser than in other regions and differed significantly among the groups, with lowest densities in the 2K-1C group, intermediate densities in the 1K-1C and control groups, and highest densities in the DOCA-salt group (Fig. 6, Table I). ACE levels in the choroid plexus correlated positively with those in the testis and negatively with those in the adrenal medulla, aorta, lung, and with PRA (Table II). Other regions of the brain showed no statistically significant differences among the groups and no significant correlations with binding levels in other tissues.

ACE labeling in the testis was diffuse and somewhat patchy, while that in the head of the epididymis was more dense. Grain densities were lowest in the 2K-1C group, intermediate in the 1K-1C and control groups, and highest in the

DOCA-salt group, but these differences were not statistically significant (Table I). Testicular ACE labeling densities correlated positively with those in the anterior pituitary and choroid plexus, and negatively with those in the adrenal medulla, aorta, and with PRA (Table II). No statistically significant differences were observed among the groups for ACE labeling in the epididymis, and there were no significant correlations between densities in the epididymis and those in other tissues.

Discussion

In the present study tissue and vascular ACE levels show distinct patterns of variation in different models of hypertension. In the lungs, aorta, small mesenteric arteries, and adrenal medulla, ACE levels vary in accordance with levels of PRA. ACE levels in these tissues are highest in the high-renin model (2K-1C hypertension), intermediate in the normal renin model (1K-1C hypertension) and normotensive controls, and lowest in the low-renin model (DOCA-salt hypertension). In contrast, ACE in the anterior pituitary, kidney, testis, and choroid plexus of the brain varies inversely with plasma renin values. In these regions ACE densities are lowest in the high-renin model and highest in the low-renin model. Finally, ACE levels in sites such as the posterior pituitary, epididymis, and most regions of the brain do not differ significantly among the different models of hypertension. These findings are of particular interest in assessing the relationship between circulating renin levels (or the plasma RAS) and more localized renin-angiotensin systems in the vessels, brain, and other tissues.

In the three models of hypertension examined here, high blood pressure involves distinct pathogenetic mechanisms associated with different levels of PRA (25, 26). PRAs in this study correspond with those previously reported (25), with activities being highest in the 2K-1C group, intermediate in the 1K-1C and control groups, and very low in the DOCA-salt group. However, the degree of hypertension is similar in the three experimental groups, facilitating comparison of PRA with tissue and vascular ACE levels.

In the lung ACE levels vary among the hypertensive groups and correlate positively with PRA. Our results agree with a recent report of increased pulmonary ACE activity in 2K-1C rats at 2–16 wk after renal artery clipping (other models of hypertension were not studied) (27). The lungs, highly vascular organs, are probably the principal sites of conversion of plasma ANG I to ANG II (2), and our data suggest that pulmonary ACE levels are regulated in coordination with the plasma RAS.

Our observations of dense ACE labeling over the endothelium of the aorta and small mesenteric arteries fits with immunohistochemical localization of ACE (28, 29), and endothelial ACE has been shown to play an essential role in the vascular conversion of ANG I to ANG II (30). We also detect ACE in the aortic adventitia and perivascular tissue of mesenteric arteries, but here it does not appear to be regulated in the same fashion as endothelial ACE. The absence of substantial [³H]captopril binding in the tunica media of the aorta differs from an earlier report which suggested that ACE activity occurs in the aortic media of rats (31).

Endothelial ACE levels in the aorta and mesenteric arteries vary among the three hypertensive groups in parallel with PRA

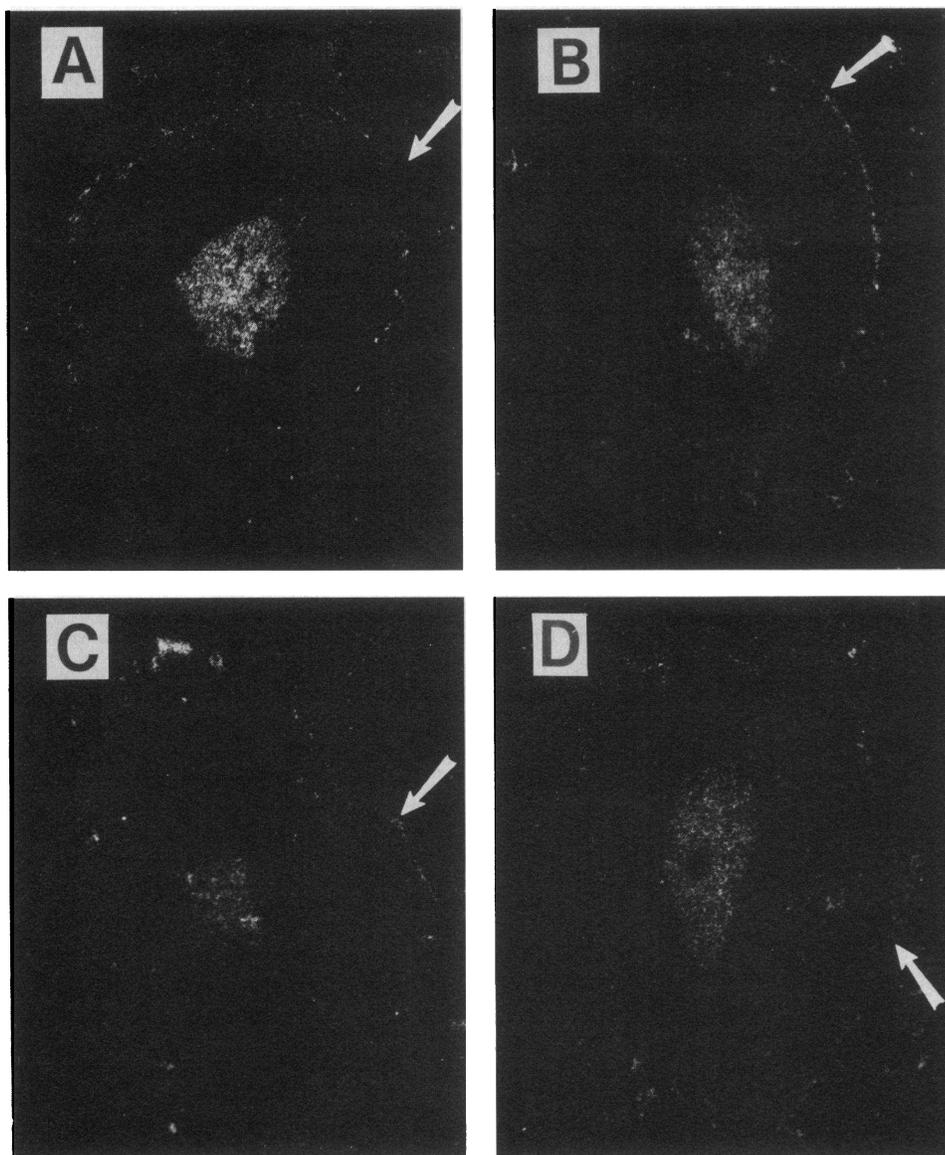


Figure 3. [³H]Captopril binding in adrenal glands. (A) 2K-1C rat. (B) 1K-1C rat. (C) DOCA-salt rat. (D) Control (normotensive) rat. The density of labeling in the adrenal medulla is highest in A, intermediate in B and D, the lowest in C. Faint pericortical labeling (arrows) can be seen but there is minimal labeling of the cortex itself. 16X.

and pulmonary ACE, with variations even more prominent in mesenteric vessels than the aorta. An earlier report detected higher ACE activity in homogenates of rat aorta in 2K-1C rats compared with 1K-1C rats and controls (the DOCA-salt model was not studied) (32). In another study (27), ACE activity was also significantly higher in the aorta and mesenteric artery in 2K-1C rats, but only after 16 wk of hypertension, not as early as 4 wk as we have observed.

As demonstrated in hindquarter arteries of the rat (33, 34), ACE and the vascular RAS probably regulate vasoconstriction by local production of ANG II. Moreover, vascular ACE may be rate limiting in the formation of ANG II in vessel walls (35). ACE and the vascular RAS probably play a role in maintaining some forms of hypertension (4, 5, 7), and the antihypertensive effects of the ACE inhibitors captopril and enalapril may be mediated by inhibiting the vascular RAS (3, 36–40). The high-density labeling of ACE that we find in vascular endothe-

lium supports a major physiological role for the vascular RAS. However, the positive correlation between vascular ACE and PRA indicates a close link between plasma and vascular renin-angiotensin systems, and it seems unlikely that vascular ACE is regulated independently of the plasma RAS in hypertension. We find no evidence of increased vascular pools of ACE in hypertensive rats with normal plasma renin levels (1K-1C group) as some investigators have suggested (31, 37).

The high densities of ACE in the adrenal medulla observed here and in previous studies (11, 41) may reflect a role for ANG II in adrenal catecholamine release (11, 42), although medullary catecholamines are not thought to regulate blood pressure on a long-term basis (43). Elevated plasma catecholamine levels have been reported in 2K-1C and DOCA-salt hypertension, but these probably reflect increased sympathetic activity and not necessarily ANG II-stimulated release of adrenal catecholamines (43). Nonetheless, the marked alterations

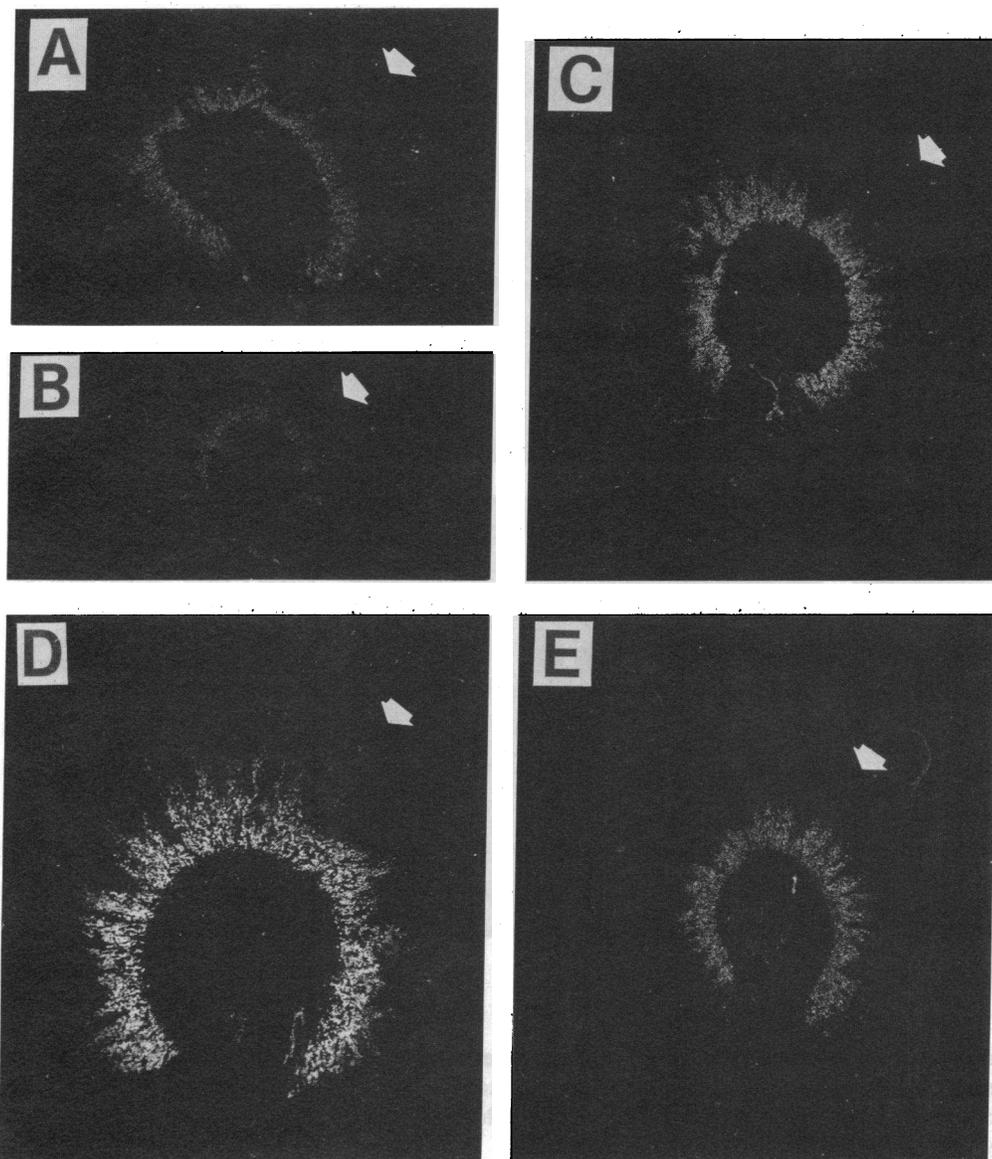


Figure 4. [^3H]Captopril binding in kidneys. (A) Unclipped kidney of 2K-1C rat. (B) Clipped kidney of 2K-1C rat. (C) Clipped kidney of 1K-1C rat. (D) Kidney of DOCA-salt rat. (E) Unclipped kidney of control (normotensive) rat. The density of labeling in the inner renal cortex is lowest in A and B, intermediate in C and E, and highest in D. There is minimal labeling in the medulla and outer cortex. The outer edge of the cortex is designated by arrows. 4 \times .

in adrenal medullary ACE in the hypertensive models studied here suggest a function for the adrenal enzyme in hypertensive pathophysiology.

The ACE localization we observed over inner cortical kidney tubules corresponds to earlier reports of the enzyme associated with proximal tubules (44, 45), especially at brush borders (45). Whether renal ACE generates ANG II, inactivates kinins, or cleaves other substrates is unclear, although ANG II does enhance sodium and fluid reabsorption via direct effects on proximal tubules (46). The negative correlation we observe between renal renin production and tubular ACE levels is not readily explained. The low levels of ACE in the clipped (renin-producing) kidneys of 2K-1C rats cannot be attributed simply to ischemia from renal artery constriction, as kidneys from 1K-1C rats with the same constriction show different ACE responses. Elevated renal ACE in DOCA-salt rats cannot derive solely from renal hypertrophy because the un-

clipped kidneys of 2K-1C rats are also hypertrophied but do not show similar increases in renal ACE. The functional relationship of altered renal ACE to elevated blood pressure in this study is not clear.

The exact function of anterior pituitary ACE is not established. One of its roles may be to inactivate luteinizing hormone-releasing hormone (47). ACE may also generate ANG II locally, and the ANG II-stimulated release of adrenocorticotrophic hormone (48), prolactin (49), and beta-endorphin (50) and ANG II immunoreactivity in gonadotrops and lactotrops (51) implies a physiological role for ACE. The similar response of anterior pituitary and renal ACE in the various forms of hypertension suggests similar regulatory mechanisms in these organs, perhaps reflecting reciprocal regulation of localized ANG II production in response to circulating levels of renin and ANG II. In the posterior pituitary, an organ almost devoid of ANG II receptors (52), we detect no significant changes in

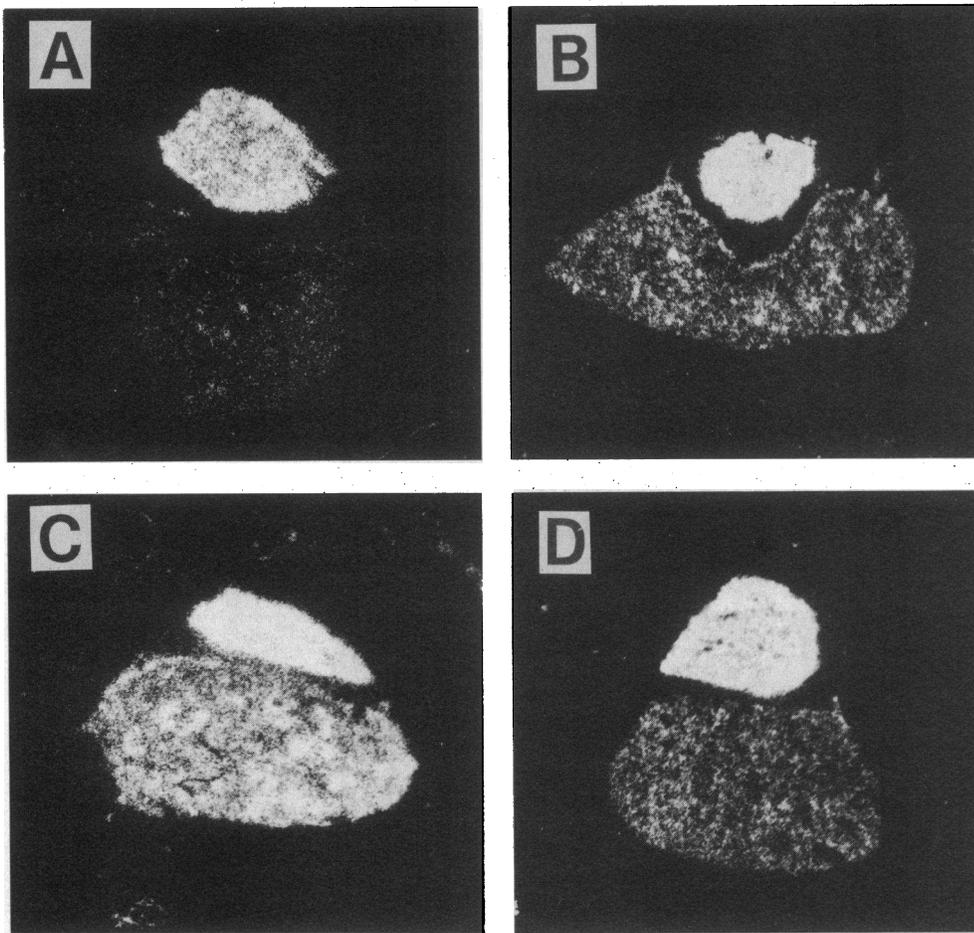


Figure 5. [³H]Captopril binding in pituitary glands. (A) 2K-1C rat. (B) 1K-1C rat. (C) DOCA-salt rat. (D) Control (normotensive) rat. Labeling in the anterior pituitary (bottom of each figure) is patchy, and labeling density is lowest in A, intermediate in B and D, and highest in C. Labeling is dense and homogeneous in the posterior pituitary (top of each figure), with no substantial differences among the groups. 18×.

ACE levels. ACE in the posterior pituitary is probably not directly associated with vasopressin release or processing (11), and vasopressin itself is probably not a major factor in the pathogenesis of the models of hypertension we evaluated (53). In contrast to our findings, an earlier study showed that a low-sodium diet (which increases plasma renin levels) increased pituitary ACE activity (54). However, it is not stated whether the increase occurred in the anterior lobe, posterior lobe, or both.

In the brain the choroid plexus is the only region we studied with ACE alterations in hypertension. In the choroid plexus, ACE may be involved in the local production of ANG II, which in turn affects thirst and blood pressure through actions on circumventricular organs (55). In the choroid plexus ACE levels are negatively correlated with PRA, suggesting reciprocal regulation of localized ANG II production. ACE levels in other areas of the brain that lie inside the blood-brain barrier apparently are not regulated by or coordinated with the plasma RAS. At some of these sites ACE is thought to function as part of another peptidergic system (15, 55), and in other areas (e.g., supraoptic and paraventricular nuclei) ACE may generate ANG II locally as part of an independent RAS in the central nervous system (56). Our findings in the brain differ somewhat from earlier reports of increased ACE activity

in the hypothalamus of 2K-1C rats as compared with 1K-1C rats and normotensive controls (27), and low midbrain, striatal, and hypothalamic ACE in sodium-loaded rats (a low-renin state) (54).

Testicular ACE levels are inversely correlated with PRA, with highest densities in the low-renin group and lowest densities in the high-renin animals—a pattern like that of the anterior pituitary, kidneys, and choroid plexus. Testicular ACE is physicochemically and immunologically distinct from ACE in the lungs or other tissues (57). Testicular ACE is localized to spermatid heads and the lumina of seminiferous tubules in stages I–VIII and XII–XIV (12). The function of testicular ACE is not well delineated, but it seems unlikely that it is regulated directly by the plasma RAS owing to the blood-testis barrier (57). ACE levels in the testis may be linked to those in the anterior pituitary, because the pituitary gland is necessary for the maintenance of testicular ACE, and hypophysectomy in mature male rats depletes ACE from the testis (58, 59). We find a strong positive correlation between ACE levels in the anterior pituitary and those in the testis. In the epididymis, however, ACE levels do not vary among the groups, consistent with findings that nonsoluble ACE in the epididymis is not regulated by the anterior pituitary and represents a distinct protein from the testicular enzyme (58).

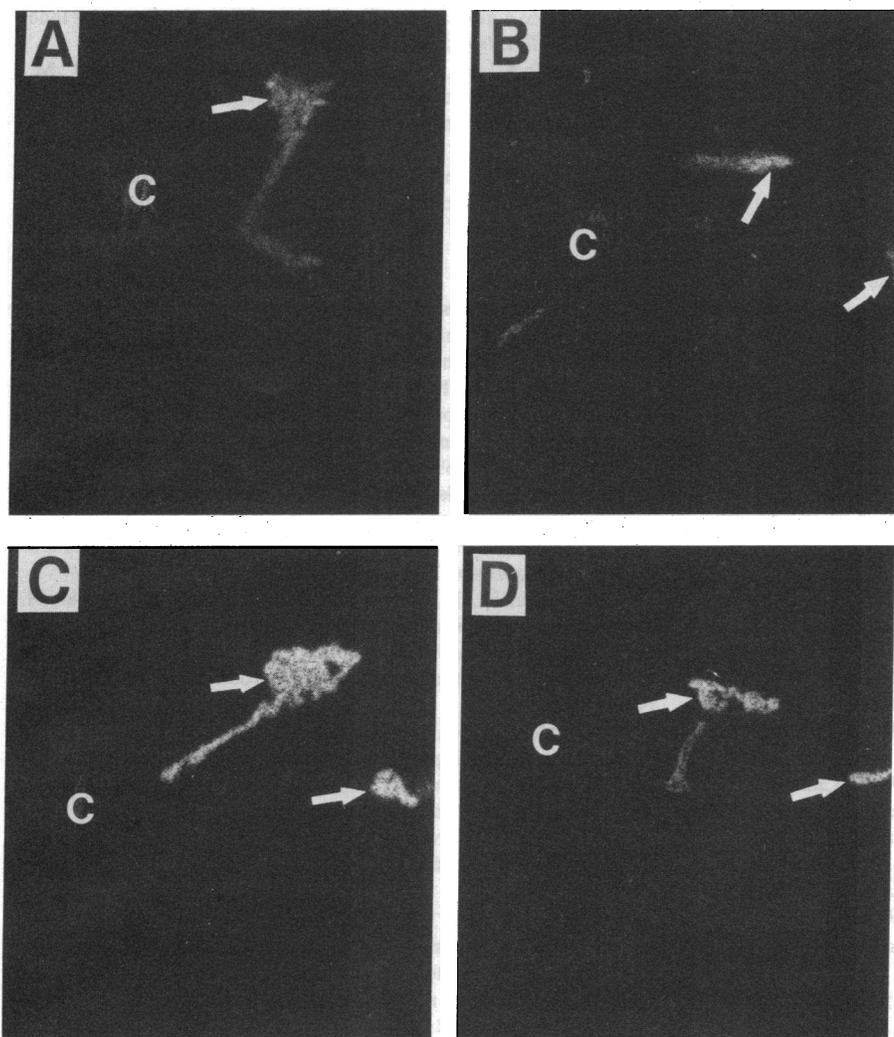


Figure 6. [^3H]Captopril binding in the choroid plexus of brain sections (A) 2K-1C rat. (B) 1K-1C rat. (C) DOCA-salt rat. (D) Control (normotensive) rat. Labeling density in the choroid plexus (arrows) is lowest in A, intermediate in B and D, the highest in C. (C) Caudate-putamen. 10 \times .

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