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In vitro evidence for local generation of renin and angiotensin II/III immunoreactivity by the human adrenal gland

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Abstract. The adrenal gland of various mammalian species has been shown to contain all the components of a functional renin-angiotensin system. We investigated the existence of this local system in human adrenal tissues surgically obtained. Eight normal adrenals (cortex and medulla) and 6 aldosterone-producing adenomas (aldosteronomas) were examined. Minced tissues were superfused over 270 min, and 15-min fractions were collected. In the perfusates, active renin was measured by immunoradiometric assay with human anti-renin monoclonal antibodies; immunoreactive angiotensin II/III and aldosterone were measured by radioimmunoassay. Adrenal tissues, either normal or pathological, were found concomitantly to release renin, angiotensin II/III and aldosterone. The pattern of this spontaneous release exhibited a pulsatile character. The total amount of renin and angiotensin II/III secreted during superfusion clearly exceeded the tissue content (determined by extraction). Addition of the angiotensin-converting enzyme inhibitor quinaprilat (4×10^{-6} mol/l) in the superfusion caused a concomitant decrease of angiotensin II/III and aldosterone secretion by 3 normal tissues, and no change in 2 aldosteronomas. These data provide evidence that the human adrenal gland in vitro generates and releases both renin and angiotensin II/III, and support the hypothesis that locally formed angiotensin II/III may play a role as a paracrine regulator of physiological aldosterone secretion.

The renin-angiotensin system has traditionally been regarded as a circulating endocrine system. Increasing evidence now indicates the existence of local angiotensin-generating systems in several tissues including the brain, kidney, reproductive organs, and blood vessels. Such systems may regulate cell-to-cell communication and function by autocrine and paracrine mechanisms (1,2). All the components of the renin-angiotensin system have been demonstrated in the adrenal gland of various mammalian species (3-7). The majority of adrenal renin and angiotensin II appear primarily located in the zona glomerulosa, raising the possibility of a role of angiotensin II as a paracrine regulator of aldosterone secretion (8,9). Several studies have documented that dietary salt intake regulates the expression of renin and angiotensinogen gene (10,11) as well as adrenal renin and angiotensin contents (12-15). Changes in potassium balance, nephrectomy, and angiotensin II or ACTH administration result in changes in adrenal renin in rats (16-18). Renin and angiotensin II release from cultured animal adrenal capsules and their response to various in vitro manipulations have been shown, providing evidence that adrenal synthesis of these peptides is likely under the control of a regulatory process (19-23). Scarce data are available on the presence of an adrenal renin-angiotensin system in man. Renin-like activity was first reported by Ganten et al. in the human adrenal gland (24). Immunoreactive renin has later been identified not only in the normal human adrenal gland but also in adrenal aldosterone-producing adenoma (aldosteronoma) from patients with primary aldosteronism

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(25-27). Binding sites for angiotensin-converting enzyme (ACE) have been found both in zona glomerulosa and the medulla of normal human adrenal (28). Human adrenal tissue adjacent to aldosteronoma has been shown to contain angiotensin II and angiotensin III (29). Other studies report that angiotensin analogues which block angiotensin receptors exert an inhibitory effect on aldosterone synthesis in human adrenal tissue slices, giving indirect support to the hypothesis that locally produced angiotensin contributes to the basal production of adrenal steroid hormones (30).

The purposes of this study were two-fold: First, to investigate the presence of renin and angiotensin II in human adrenal tissue, either normal or pathological, and to evaluate whether angiotensin II is locally produced. Second, to assess the potential role of adrenal angiotensin II in aldosterone production from the human adrenal gland by using pharmacological agents which inhibit ACE activity.

Materials and Methods

Eight normal adrenals, including cortex and medulla portions, were obtained surgically from patients undergoing unilateral expanded nephrectomy for kidney cancer. The adrenal tissue specimen contained no tumour at histological examination. All patients (6 males and 2 females, aged 48-66 years) were normotensive, and none had clinical symptoms of adrenal dysfunction. Aldosteronomas were surgically obtained from 6 patients (3 males and 3 females, aged 42-56 years) with primary aldosteronism who were diagnosed as having adrenal adenoma by preoperative localization procedures. Both groups of patients were on a diet containing 120 to 150 mmol sodium and 60 mmol potassium daily, and were off any drug for at least two weeks before operation. The day before surgery, blood samples were drawn to determine plasma renin, angiotensin II and aldosterone levels. Samples were taken at 09.00 h in the supine position, after overnight fasting. Informed consent was obtained.

Tissue extraction

After removal of normal or tumourous adrenal tissue, a portion was frozen in liquid nitrogen and stored at −70°C until processing. The time between removal and freezing in liquid nitrogen was approximately 2 min. At the time of the extraction two separate fragments (100-200 mg wet weight each) were used for renin and angiotensin II process and assay.

For measuring renin tissue content, tissue was homogenized by a Polytron (Brinkmann Instruments, Westbury, NJ) in 9 vol (v/w) of ice-cold 0.05 mol/l TRIS-HCl, pH 7.4, containing 10 mmol/l Na_2-ethylene-diaminetetraacetate (EDTA), 2 mmol/l phenylmethanesulphonylfluoride (PMSF) and 0.1 mmol/l captopril (kindly provided by E. R. Squibb, Princeton, NJ). The homogenate was centrifuged for 30 min at 14 000 × g, and the clear supernatant used for renin assay. The recovery of Medical Research Council (MRC) human renin standard (1 pg = 1.6 × 10^{-6} Goldblatt Units, WHO International Reference Preparation 68/356) added to adrenal tissue was 86.4±5.8% (mean ± SEM, N=4) for 50 pg, and 88.2±6.1% for 100 pg (N=4), respectively, after the extraction procedure.

For measuring tissue angiotensin II content, tissue was first homogenized by Polytron in 100 µl of 8 mol/l urea. The dissolved samples were further homogenized and extracted with 9 vol (v/w) of 80% methanol containing 10 mmol/l sodium acetate and 0.5% trifluoracetic acid (TFA), pH 5.6. The homogenate was centrifuged for 30 min at 14 000 × g, and then the supernatant transferred to a polyethylene test tube containing 50 µl of 50% glycerol. The supernatant was then evaporated in a vacuum centrifuge (Speed-Vac, Savant, Hicksville, NY) and the residue was stored at −20°C until angiotensin II assay. The recovery of a standard amount of exogenous angiotensin II (Peninsula Labs, Belmont, CA) added to the tissue was 92.2±3.8% (N=4) for 20 pg, and 93.4±4.1% (N=4) for 80 pg, respectively.

Superfusion experiments

After removal of the adrenal gland, a portion of normal or tumour tissues was quickly placed in ice-cold superfusion medium. Two 1-ml volume superfusion chambers made from the barrel of 5-ml plastic syringe were set up and run simultaneously as control (blank channel without tissue) or experimental preparation, with Bio-Gel P-2 (Bio Rad, Richmond, CA) as a support matrix in each chamber. Tissue (0.3-0.5 g wet weight) was rinsed several times with superfusion medium, minced into approximately 1-mm^3 fragments with fine curved scissors, and placed in one of the chambers. The medium for superfusion was constantly gassed with 95% O_2-5% CO_2 and consisted of Medium 199 (Gibco Co, Grand Island, NY) containing 3.6 mmol/l K, 25 mmol/l $NaHCO_3$, and 0.1% bovine serum albumin (BSA, Ria Grade, Sigma Chemicals Co, St. Louis, MO), and the pH was adjusted to 7.3-7.4. BSA was heat inactivated, so that it contained no detectable renin. The tissue remained at the bottom of the chamber, suspended on a nylon filter (10 µm, Henry Simon Limited, Chesire, England) and continuously bathed in superfusion fluid. The superfusion chamber rested in a water bath at 37°C, and the medium was delivered to each chamber by a constant speed peristaltic pump (Gilson Medical Electronics, Villiers-le-Bel, France) at a flow rate of 0.5 ml/min. After an initial 30-min stabilization period, the superfusate effluent was automatically collected for 270 min during 3-min successive intervals in pre-chilled tubes with 5 mmol/l EDTA and 0.1 mmol/l captopril added as inhibitor solution. The 3-min fractions were im-

mediately frozen in dry ice and pooled by five consecutive additions (total volume 7.5 ml) in a 15-ml polyethylene test tube, lyophylized under vacuum, and the residue stored at −20°C until the assay for renin, angiotensin II and aldosterone. Outside the water bath, the teflon channel connecting the chamber with the test tubes was kept on ice and the time of passing from the chamber to the tubes was approximately 60 seconds. The first set of experiments, including tissue extraction coupled with superfusion studies, was carried out using 5 normal adrenals and 4 aldosteronoma tissues. In all patients from whom these tissues were removed, plasma renin, angiotensin II and aldosterone were also measured (see below). Four pilot experiments with human adrenal tissue were previously carried out to find the correct timing/pooling of samples.

In a second set of experiments, a similar amount of adrenal tissue was placed into two chambers and superfused according to the same protocol. After 90 min of baseline collection period, one of the two chambers was superfused with the ACE inhibitor quinaprilat at a concentration of 4×10^{-6} mol/l by changing to a second reservoir of medium. Collection of the fractions then continued for 180 min in both chambers, one being considered as control. Three experiments with normal adrenal and two experiments with aldosteronoma tissues, respectively, were carried out.

The active diacid form of quinapril, quinaprilat (CI 928) was kindly donated by Parke-Davis, Warner-Lambert Co, Ann-Arbor, MI.

To follow the nonspecific release of cell components as a result of cell damage and leakage, in each experiment superfusate fractions were assayed for lactic dehydrogenase (LDH) activity at different time points, using a Sigma test kit.

Assays

Before assays, the residue of either extract or superfusate samples was redissolved in 2 ml of buffer consisting of 0.05 mol/l K_2HPO_4, 0.003 mol/l Na_2-EDTA, 0.02% NaN_3, 0.01% Triton X-100, pH 7.4, and 2.5 g/l radioimmunoassay grade BSA. To inactivate the peptidase contaminating the BSA, the buffer was first heated at 56°C for 30 min.

The concentration of immunoreactive active renin was measured by an immunoradiometric assay kit (Diagnostics Pasteur, Marnes La Coquette, France) using two monoclonal human anti-renin antibodies 3E8 and 4G1, as previously described (31,32). Briefly, 3E8 was selected to be the first antibody immobilized on Magnogel (Diagnostics Pasteur) to trap both active and inactive renin, 4G1 was iodinated and used as the second antibody to be retained selectively by active renin immobilized by 3E8. Results of immunoradiometric assay were expressed as pg of renin per litre and were derived from a standard curve using monkey serum renin, calibrated from MRC:1000

Table 1.
Renin and angiotensin II/III concentrations in normal human adrenal and aldosteronoma tissues, and in plasma of patients studied. Tissue content of renin and angiotensin II/III was measured after extraction as described in Methods. Ratio tissue/plasma was expressed assuming that 1 g of tissue is equal to 1 ml of plasma.

	Renin		Ratio	Angiotensin II/III		Ratio
	tissue pg/g	plasma pg/ml	tissue/ plasma g/ml	tissue pg/g	plasma pg/ml	tissue/ plasma g/ml
Normal adrenals						
1	720	16.2	44	2401	10.9	220
2	1120	10.1	112	2839	37.5	76
3	1460	8.6	170	2128	21.2	100
4	880	12.2	72	1812	30.0	67
5	1220	15.8	77	2164	31.6	68
Mean	1080	12.5	95	2268	26.2	104
±SEM	129	1.5		170	4.6	
Aldosteronomas						
1	516	6.4	80	1118	5.3	211
2	820	5.8	141	824	6.8	121
3	842	5.2	161	968	6.4	151
4	612	6.2	099	1406	6.0	68
Mean	697	5.9	120	1079	6.1	137
±SEM	79	0.2		124	0.3	

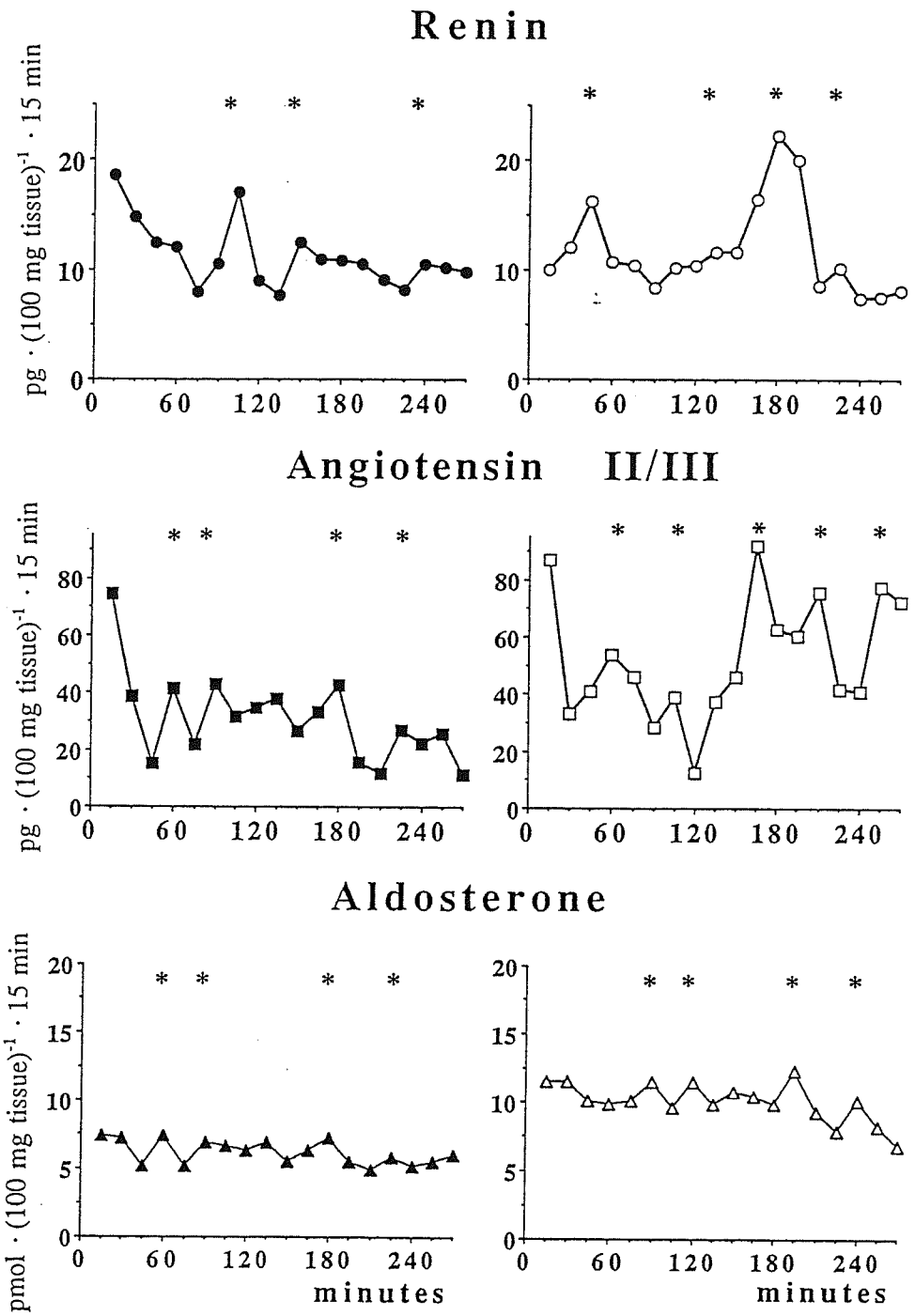

Fig. 1.
Renin, angiotensin II/III and aldosterone concentrations during superfusion in vitro of one normal human adrenal gland (left panel) and one aldosteronoma (right panel). Asterisks denote significant pulses detected by a modified version of Santen & Bardin analysis (see Methods).

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Goldblatt units per mg; 250 µl in duplicate was used for the assay. In the patients, the results were given as pg/ml plasma. The limit of detection was 5 ng/l, the intra-assay coefficient of variation (CV) 6% (N=10), and the inter-assay CV 10% (N=20). Normal plasma values are 5-20 ng/l (i.e. pg/ml), in the supine position. Since the exact molecular weight of active renin is unknown, traditional weight units instead of SI units are used.

Angiotensin II was assayed using a double-antibody RIA system. The first antibody (Arnel Products Co, Inc, New York, NY) raised in rabbits had 100% cross-reactivity with angiotensin III and all other C-terminal fragments, and less than 0.1% with angiotensin I. The second one was a donkey anti-rabbit antibody coupled to magnetic beads (Amerlex M separation reagents, Amersham International plc, Buckinghamshire, England). Briefly, 0.4 ml of both serially diluted standard and samples were incubated with 0.1 ml antiserum (final dilution of 1:25 000) and 0.1 ml ^{125}I-angiotensin II (equivalent to about 8000 cpm, Amersham; specific activity, 2000 µCi/µg) for 24 h at 4°C. Then, 250 µl of the magnetic phase bearing second antibody was added to each standard or sample to bind the first antibody-hormone complex. After 30 min, the tubes were placed in magnetic racks for 10 min. The supernatant containing free ^{125}I-angiotensin was removed, and ^{125}I-angiotensin bound to the solid phase was counted for radioactivity, after additional washing with buffer to eliminate residual droplets. The lowest concentration of angiotensin II detected was 1 pg/tube, and the 50% intercept was at 96±6 pg/tube (N=12). Intra-assay CV was 7% (N=12), inter-assay CV 10% (N=18). For plasma measurements 10 ml whole blood samples were collected into prechilled polypropylene tubes which contained 0.5 ml of an inhibitor solution (0.125 mmol/l Na_2-EDTA, 1 mmol/l captopril, 2 g/l neomycin sulphate). Two ml of plasma were then applied to C_{18}OH cartridges (Incstar, Stillwater, MN) previously activated by 5 ml of methanol/TFA 0.1% followed by 5 ml of water/TFA 0.1%. Cartridges were washed with 2 ml of methanol 80%/water 19.9%/TFA 0.1%. The extract was dried with a Speed-Vac evaporator, and then redissolved in 1 ml buffer for RIA. Recovery of synthetic angiotensin II added to the cartridge was 93.4±4.1% (N=12). Normal plasma values are 10-40 ng/l (i.e. pg/ml) in the supine position. Since the antibody against angiotensin II shows 100% cross-reactivity with angiotensin III, it is not possible to use a single molecular weight for molar transformation.

Aldosterone was measured by RIA with a commercial kit (ALDOK-^{3}H) by Sorin, Italy. Cross-reactivity of antiserum with cortisol and corticosterone is less than 0.1%. Detectability is 20 pmol/l, intra-assay CV 5% (N=10), inter-assay CV 8% (N=20). Normal plasma values range from 55 to 415 pmol/l in the supine position.

In our in vitro experiments, the results are given as pg × (100 mg tissue)$^{-1}$ for extraction samples, and as pg or pmol (for aldosterone) × (100 mg tissue)$^{-1}$ × 15 min fraction for superfusate samples, respectively.

All samples from an individual experiment were analysed in the same assay, and were run in dupliate.

Statistics

In each superfusion experiment, pulsatile patterns of renin, angiotensin II/III, and aldosterone secretion were analysed by the threshold method of Santen & Bardin (33) as modified by Ross et al. (34), where a peak (pulse) is defined as an increase in hormone concentration from nadir to peak exceeding three times the dose-dependent CV (see above) of the assay. For each experiment, the relationship between the variations of angiotensin II and aldosterone was investigated by calculating the nonparametric Spearman's rank correlation coefficient between all pairs of concentration profiles over the superfusion time (i.e. 9 comparisons of 2 series of 18 values per experiment). When statistical difference was assessed, the Mann-Whitney *U*-test was applied for comparison between groups. Significance was defined as a p value of less than 0.05.

The response of renin, angiotensin II/III and aldosterone production to administered quinaprilat in the superfusion experiments was expressed as the change from baseline, with baseline consisting of the mean of the first 6 consecutive 15-min fractions.

All results are expressed in the text and figures as the mean ± SEM.

Table 2.
Pulse frequency (per 270 min) data for renin, angiotensin II/III and aldosterone in the single superfusion experiments of normal human adrenals and aldosteronomas. Pulses were detected using modified version of Santen and Bardin analysis (see Methods).

	Pulse frequency (per 270 min)		
	Renin	Angiotensin II/III	Aldosterone
Normal adrenals			
1	3	5	4
2	4	4	3
3	3	3	5
4	3	4	3
5	3	4	4
Aldosteronomas			
1	3	3	3
2	4	5	4
3	4	3	4
4	3	5	3

Results

Studies in plasma and adrenal extracts

Table 1 summarizes the concentrations of renin and angiotensin II/III found in plasma and in the adrenal tissue extracts of patients. Levels of both renin and angiotensin II/III were higher in normal adrenals than in aldosteronoma tissues. Assuming 1 ml of plasma is equal to 1 g of tissue, tissue renin and angiotensin II/III were several-fold higher than their respective concentrations in the blood. The ratio tissue/plasma was slightly greater for aldosteronomas than for normal adrenals.

Plasma aldosterone levels were significantly higher in patients with an aldosterone-producing adenoma than in normal subjects (690.7±94.3 vs 277.4±19.4 pmol/l, $p<0.01$).

Release of renin, angiotensin II/III and aldosterone from superfused adrenal tissue

During superfusion, each of the 5 normal adrenals and each of the 4 aldosteronomas spontaneously released renin, angiotensin II/III and aldosterone in a pulsatile fashion (see example in Fig. 1). No measurable levels of these substances were found in the control channels. Number of pulses of renin, angiotensin II/III and aldosterone in the single experiments are shown in Table 2. While pulse frequency was similar, pulse intervals were different in each experiment, so that the pulsatile pattern was lost when the release was examined as a mean of the experiments (not represented). Mean renin release ranged from 10.5±0.3 to 18.3±1.3 pg × (100 mg tissue)$^{-1}$ × 15 min in the 5 normal adrenals, and from 11.0±3.5 to 19.9±1.2 pg × (100 mg

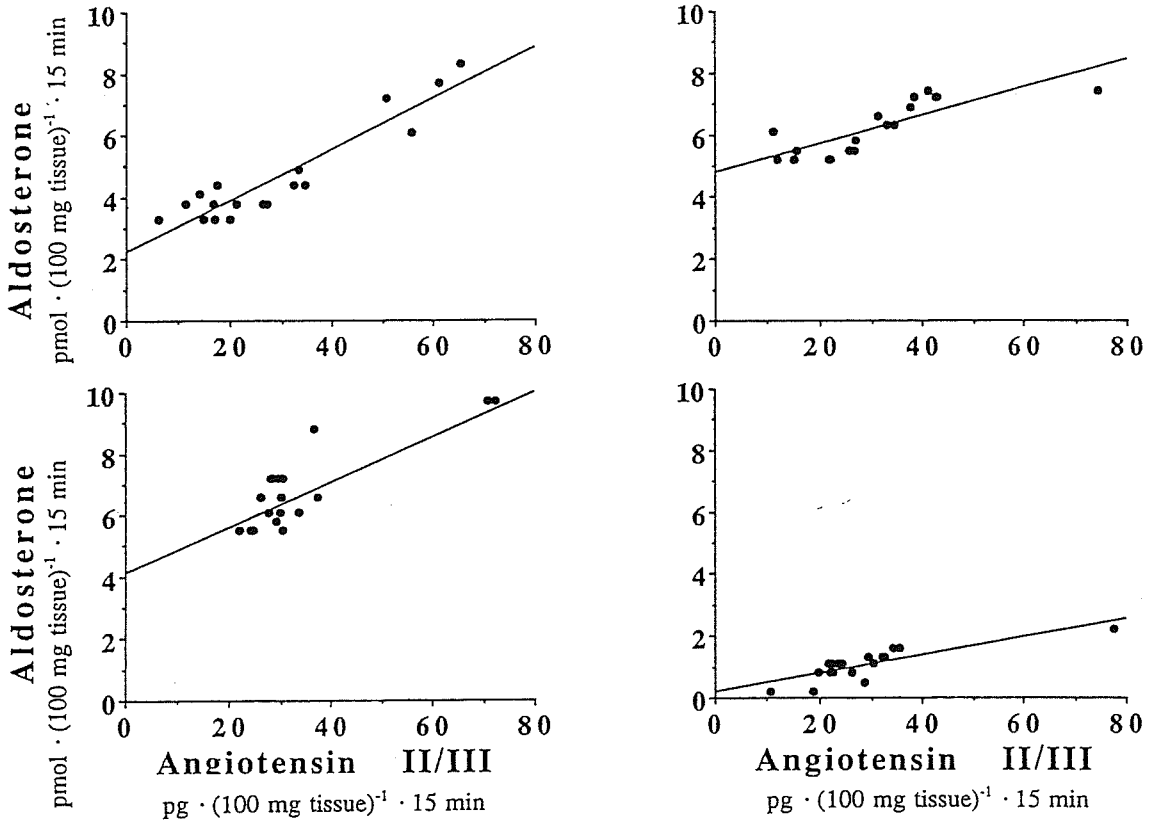
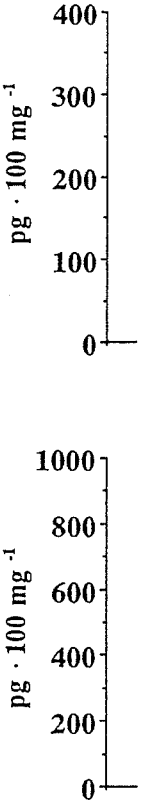


Fig. 2.
Scatter plots showing the correlations between angiotensin II/III and aldosterone levels measured in the perfusates of 4 superfusion experiments using normal adrenal tissues.



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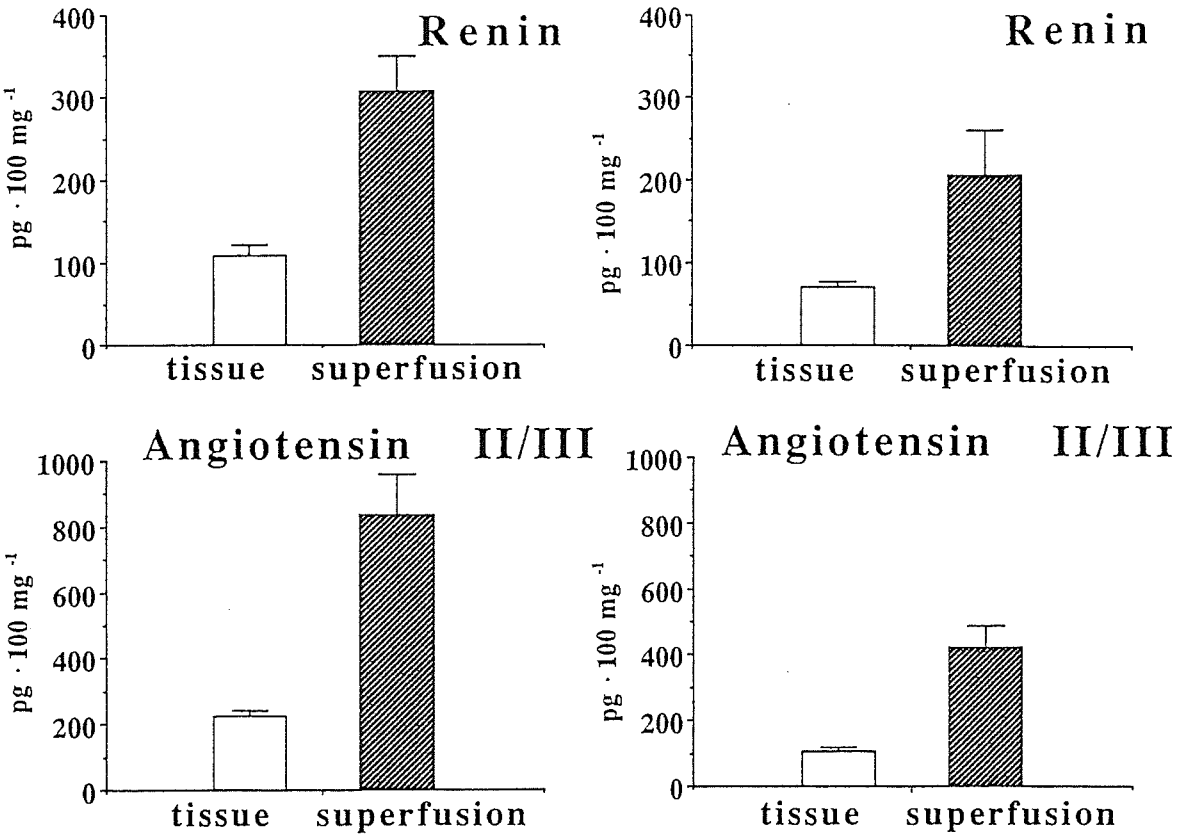


Fig. 3.
Total amount of renin and angiotensin II/III secreted by 5 normal human adrenals (left panel) and 4 aldosteronomas (right panel) during in vitro superfusion compared with the amount (determined by extraction) pre-existing in the tissue. The superfusion amount represents the sum of 18 15-min fractions over a time period of 270 min. Data are given as mean ± SEM.

tissue)$^{-1}$ × 15 min in the 4 aldosteronomas. Release of angiotensin II/III ranged from 20.3±1.9 to 47.9±3.5 pg × (100 mg tissue)$^{-1}$ × 15 min, and from 30.0±5.6 to 57.8±3.8 pg × (100 mg tissue)$^{-1}$ × 15 min, and from 30.0±5.6 to 57.8±3.8 pg × (100 mg tissue)$^{-1}$ × 15 min in the same tissues, respectively. Release of aldosterone ranged from 4.7±1.3 to 6.9±0.8 pmol × (100 mg tissue)$^{-1}$ × 15 min, and from 11.9±4.4 to 22.7±7.7 pmol × (100 mg tissue)$^{-1}$ × 15 min, respectively.

In four out of five superfusion experiments using normal adrenal tissue, the rate of angiotensin II/III release was directly related to the rate of aldosterone release (Fig. 2) (R=0.614, R=0.823, R=0.883, R=0.805, $p<0.01$). No correlation between angiotensin II/III and aldosterone was found in the superfusates of aldosteronomas. No trend to a decline for renin, angiotensin II/III and aldosterone release was seen up the 270 min of superfusion either for normal or pathological adrenal tissues. The cumulative amount of renin and angiotensin II/III released by normal adrenals over the time of the superfusion was higher than the amount released by aldosteronomas (306±41 vs 208±53, and 836±124 vs 421±68 pg × (100 mg tissue)$^{-1}$ × 270 min, respectively), given the same weight of tissue. On the contrary, aldosterone release was higher in aldosteronomas than in normal adrenal tissues (301.8±70.7 vs 100.1±20.5 pmol × (100 mg tissue)$^{-1}$ × 270 min, data not shown). The total amount of renin and angiotensin II/III released by adrenal tissue, either for normal gland or for aldosteronoma, exceeded three to four-fold the amount pre-existing in the tissue, determined by extraction (Fig. 3).

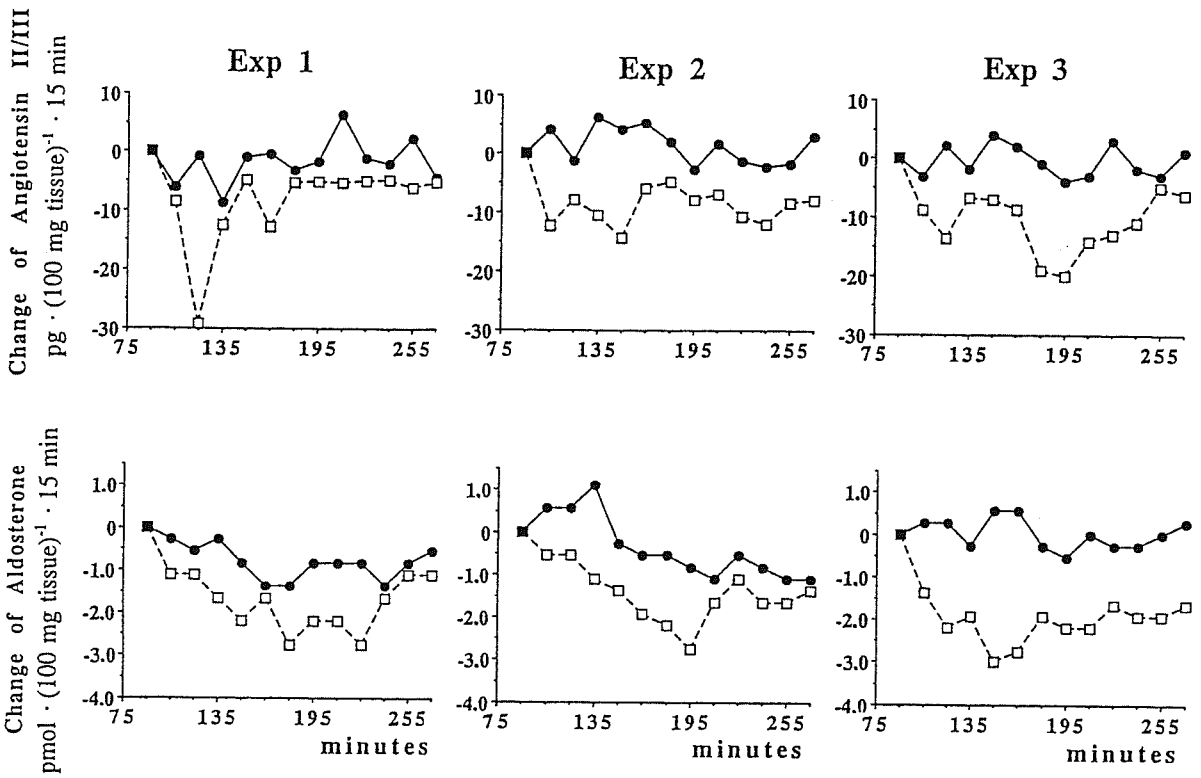


Fig. 4.
Response of angiotensin II/III and aldosterone to 4×10^{-6} mol/l quinaprilat (solid symbols), in comparison with control (open symbols), during in vitro superfusion of 3 normal adrenals. In each experiment, the response is depicted as the change from the mean of the first 6 consecutive 15-min collection fractions. After this 90-min period, twelve 15-min fractions were collected from superfusion of tissue in two parallel chambers, one with medium containing quinaprilat and one with medium alone (i.e. control).

Effect of quinaprilat on release of renin, angiotensin II/III and aldosterone

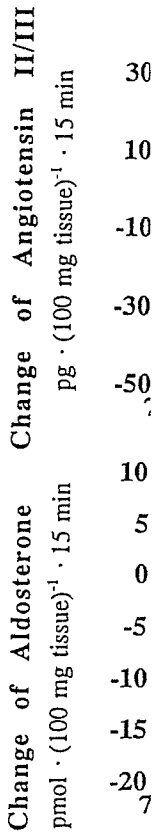
In comparison with control, addition of ACE inhibitor quinaprilat (4×10^{-6} mol/l) for 180 min in the superfusion medium, caused a concomitant decrease of angiotensin II/III and aldosterone secretion by 3 normal adrenal tissues (Fig. 4). Reciprocally, renin production was greater during quinaprilat than during control in the normal tissues (data not shown). No difference between quinaprilat and control superfusion channel for renin (not shown), angiotensin II/III, and aldosterone production was found in the 2 aldosterone-producing adenomas (Fig. 5).

Lactic dehydrogenase measurement

LDH accumulated in the superfusate fractions did not show significant variations over the time of superfusions, ranging from 0.45±0.08 to 0.50±0.10 nkat × (100 mg tissue)$^{-1}$ × 15 min (NS), N=13. Further, no change was observed during superfusion with quinaprilat, from 0.36±0.08 to 0.41±0.1 nkat × (100 mg tissue)$^{-1}$ × 15 min (NS), N=4.

Discussion

Our data document the presence of immunoreactive renin and angiotensin II/III in the human adrenal gland, and their release during superfusion in vitro of this tissue. Naruse et al. have first demonstrated biochemically and immunohistochemically the existence of renin in the human adrenal, either in the cortical or in the medullary portion of the gland (25). Mizuno et al. further characterized the adrenal renin in extracts from aldosteronomas, and the renin was revealed to possess biochemical properties similar to those of human kidney renin



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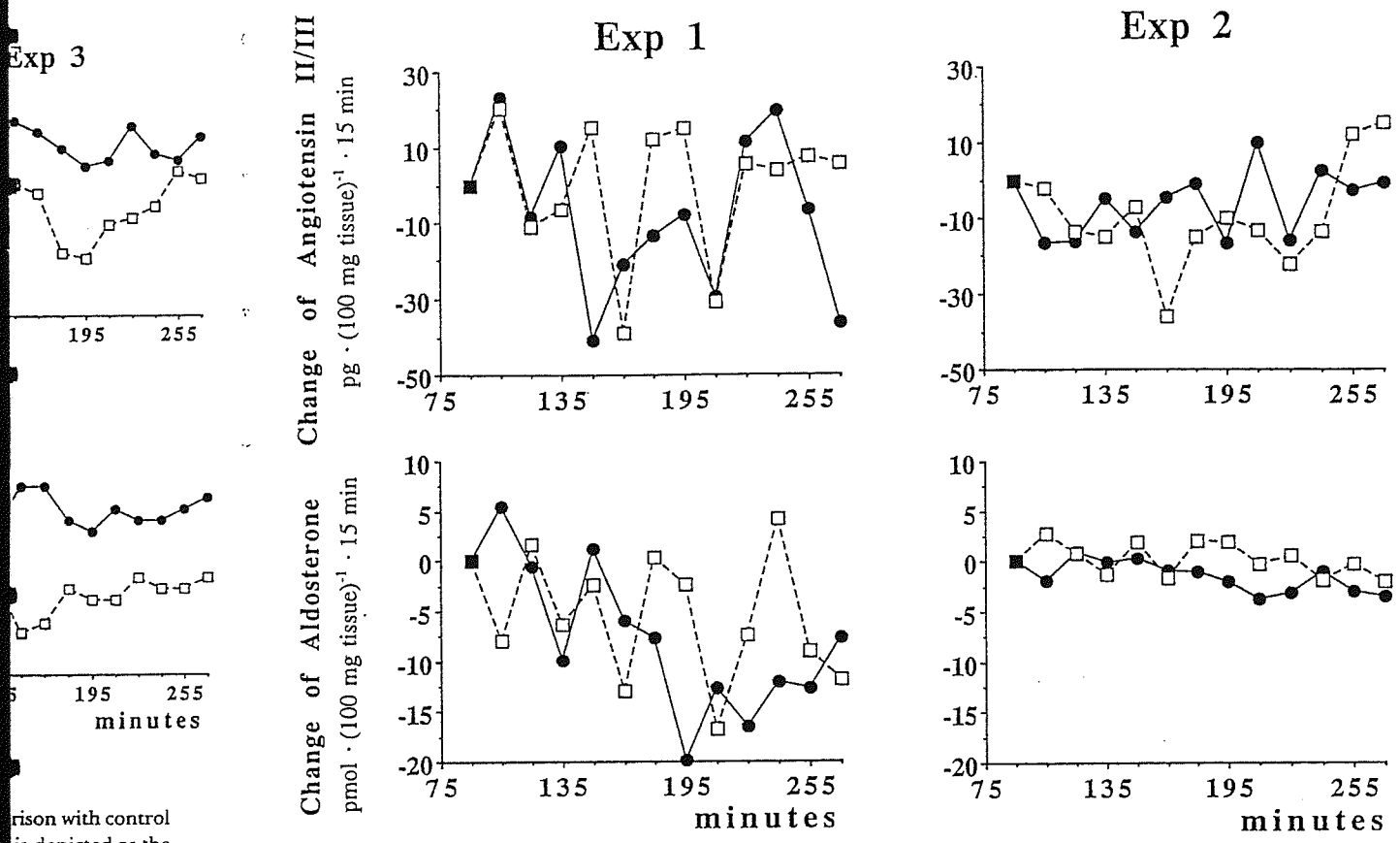

Fig. 5.
Response of angiotensin II/III and aldosterone to 4×10^{-6} mol/l quinaprilat (solid symbols), in comparison with control (open symbols), during in vitro superfusion of 2 aldosteronomas. The response is depicted as the change from the mean of the first 6 consecutive 15-min collection fractions (see explanation in legend to Fig. 4).

(27). Though they used the method of assessing angiotensin I-generating activity for their renin assay and we used direct measurement of active renin immunoreactivity, we can confirm their results. Our study does not permit to assess the distribution of renin in the different anatomical portions of normal adrenal. Renin content extracted in the whole gland as well as the amount of renin accumulated during the superfusion were clearly higher than in aldosteronomas. Since the glomerulosa portion is less than 15% of the whole normal gland, this can at least suggest that the number and/or the activity of renin-secreting cells is greater in normal than in tumourous glomerulosa. To our knowledge, only one report refers to the presence of angiotensin II and III in the normal human adrenal (29). Our results show sizable quantities of angiotensin II/III immunoreactivity either in the whole normal adrenal or in aldosteronomas, in a proportion similar to that found for renin. Coexistence of renin and angiotensin II in animal adrenal cell lysate, and their concomitant release from cultured adrenal explants or cells have been been considered an indication of local biosynthesis of angiotensin II through renin produced by the gland (19,21). In the human adrenal, renin is in fact localized in the primary mitochondrial fraction of the tissue rather than the soluble fraction, suggesting that a sequestration of renin from plasma is unlikely (25). On the other hand, other authors reported that the angiotensin II immunoreactivity detected in the rat adrenal cortex is mainly derived from receptor-mediated internalization of circulating angiotensin II from its receptor (14). The results of our study provide many issues in favour of the idea of local production of both renin and angiotensin II/III. Firstly, adrenal concentrations of both renin and angiotensin II/III were much

higher than their respective concentrations in the blood, either in normal or in aldosteronoma tissue. It is noteworthy that renin and angiotensin II/III immunoreactivities were present in high concentrations in the aldosterone-producing adenoma tissue, while circulating levels of these peptides in the patients were negligible. Secondly, we were able to detect both immunoreactive renin and angiotensin II/III in the perfusate during superfusion of human adrenals. The total amount of either renin and angiotensin II/III accumulated in the perfusate in each experiment clearly exceeded the tissue content, determined by extraction. This is not in support of a release of preformed, endocytozed, or stored renin and angiotensin II/III, and indicates an active biosynthesis of these peptides in the tissue followed by a secretion. We did not use an isolated adrenal cell preparation, and our data cannot allow to distinguish between an intracellular and an extracellular generation of angiotensin II/III (35). Since ACE is localized not only on the cell surface (36), but also in the subcellular compartment (37), generation of angiotensin II/III could actually occur at both levels. Furthermore, chromatographic separation of angiotensin II from its immunoreactive C-terminal fragments has not been attempted. Degradation of angiotensin II to peptide fragments during the extraction procedures is, however, unlikely since there was no evidence of degradation in recovery experiments with exogenously added angiotensin II.

An active secretory process of renin and angiotensin II/III from our normal and pathological adrenal tissue, rather than a mere discharge of stored forms, was also indicated by the pulsatile mode of release during superfusion. A pulsatile pattern of secretion using in vitro perifusion systems has been shown for growth hormone and prolactin from primate pituitary (38), and for luteinizing hormone from human fetal pituitary (39). The source of an intra-adrenal intrinsic pulse generating mechanism is unclear. As in many other similar enzyme systems (40), the end product, angiotensin II, is known to exert negative feedback on the early reaction, i.e. renin-substrate, of its biosynthetic pathway (41). Blockade of angiotensin II generation leads to an increase in renin formation, which in turn triggers the reaction. The pulse of angiotensin II/III and renin could indeed reflect the periodic course of this phenomenon within the adrenal tissue. The pattern of aldosterone release exhibited the same pulsatile character, suggesting a relationship between locally produced angiotensin II/III and aldosterone production. The positive correlation between the amount of angiotensin II/III and the amount of aldosterone released during the superfusion gave a statistical support to this concept in normal adrenal tissues.

To further address the role of adrenal angiotensin II/III as regulator of aldosterone secretion, we evaluated the effect of the ACE inhibitor quinaprilat on the angiotensin II/III and aldosterone release during superfusion of human adrenals. Both angiotensin II/III and aldosterone levels were consistently lowered by quinaprilat in the perfusate of normal tissues. Furthermore, the angiotensin II/III decrease was associated with a reciprocal renin increase, confirming that all the components of a functional local renin angiotensin system were present. At variance with normal, we observed that renin, angiotensin II/III and aldosterone levels did not vary after addition of quinaprilat in the superfusion medium of aldosteronomas. Lack of these changes could be due to an insufficient penetration of quinaprilat into pathological tissue, characterized by a much higher lipid content than normal (42), preventing its pharmacological action (43). Alternative routes for angiotensin I conversion to angiotensin II by-passing converting enzyme, also cannot be excluded (44,45).

In summary, our results provide the first evidence, to our knowledge, that the human adrenal gland, either normal or pathological (aldosteronoma), in vitro generates and secretes both renin and angiotensin II/III. This secretion exhibits pulsatile patterns. Locally formed angiotensin II/III may play an important role as a paracrine regulator of the physiological aldosterone secretion, and the possibility that this mechanism operates in vivo needs to be explored. It has been speculated that an abnormal activity of local renin-angiotensin system is involved in the pathogenesis of aldosterone overproduction owing to idiopathic adrenal hyperplasia known to occur in humans (46). ACE inhibitors are able to decrease aldosterone production in this disorder (47,48), suggesting that these drugs could act as blockers of tissue angiotensin II/III generation.

Acknowledgment

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ERRATUM

In vitro evidence for local generation of renin and angiotensin II/III immunoreactivity by the human adrenal gland

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The legends of Figs. 4 and 5 were incorrect. The figures and their correct legends are reproduced here.

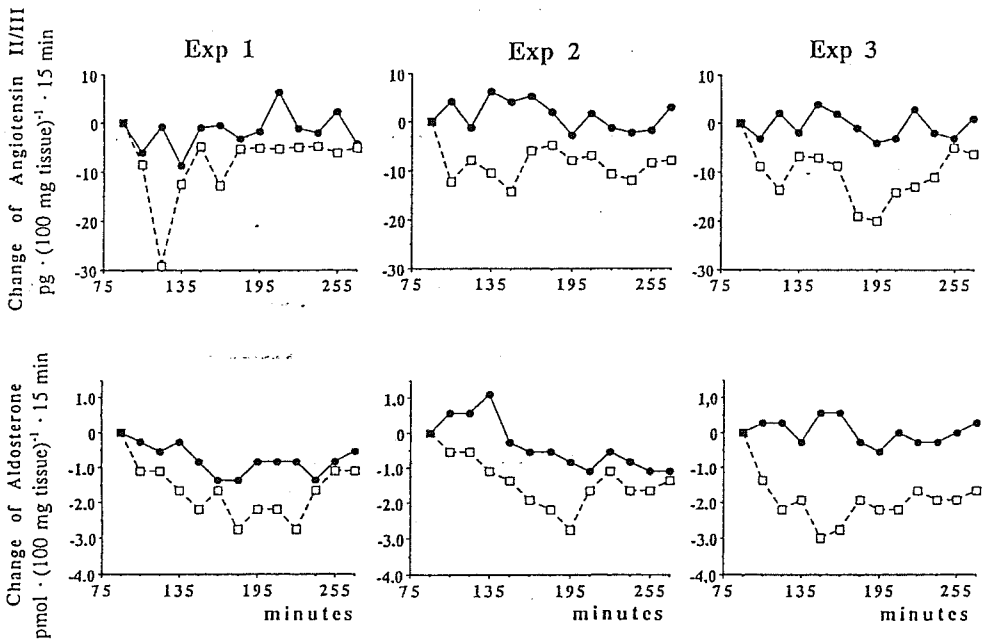

Fig. 4. Response of angiotensin II/III and aldosterone to 4×10^{-6} mol/l quinaprilat (open symbols), in comparison with control (solid symbols), during "in vitro" superfusion of three normal adrenals. In each experiment, the response is depicted as the change from the mean of the first six consecutive 15-min collection fractions. After this 90-min period, twelve 15-min fractions were collected from superfusion of tissue in two parallel chambers, one with medium containing quinaprilat and one with medium alone (i.e. control).

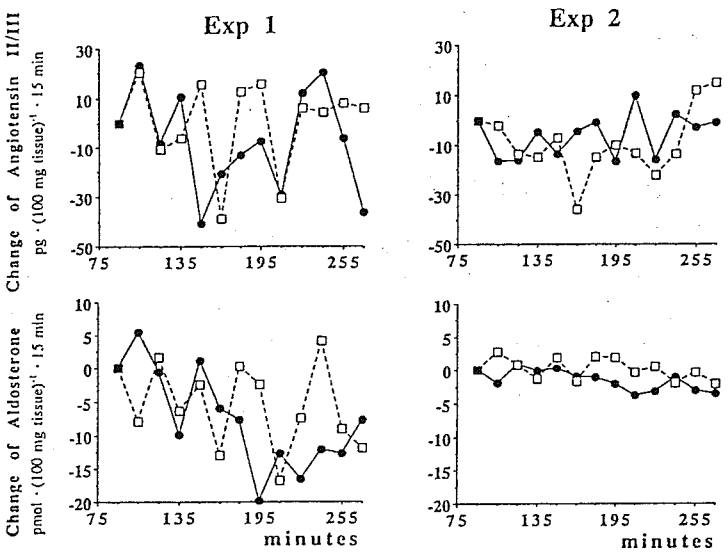

Fig. 5. Response of angiotensin II/III and aldosterone to 4×10^{-6} mol/l quinaprilat (open symbols), in comparison with control (solid symbols), during "in vitro" superfusion of two aldosteronomas. The response is depicted as the change from the mean of the first six consecutive 15-min collection fractions (see explanation in Fig. 4).