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Radioautographic localization of ^{125}I-atrial natriuretic factor (ANF) in rat tissues

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Summary. Rats were injected either with synthetic ^{125}I-Arg 101-Tyr 126 atrial natriuretic factor (ANF) or with ^{125}I-ANF together with an excess of cold Arg 101-Tyr 126 ANF. Binding sites in various tissues were accepted depending on two criteria: displacement of radioactivity by cold ANF and absence of localization of silver grains on putative target cells in the presence of cold ANF. Binding sites were localized on zona glomerulosa cells and on adrenergic and noradrenergic cells of adrenal medulla, on hepatocytes, on the base of mature epithelial cells of villi in the small intestine, on smooth muscle cells of the muscularis layer of the colon and on the base of epithelial cells of the ciliary bodies. In addition, binding sites were localized in the vasculature of kidney, adrenal cortex, lung and liver. Binding sites were particularly numerous on renal glomerular endothelial cells. These results indicate that ANF may have important hemodynamic effects in kidney, lung, liver and adrenal cortex, may regulate water and ion transport in small intestine and ciliary bodies and may have metabolic effects in the liver. The presence of binding sites on the zona glomerulosa is in agreement with the important inhibitory effect of the peptide on aldosterone secretion.

Introduction

Recently a biologically active peptide (atrial natriuretic factor; ANF) has been isolated from rat atria, sequenced, synthetized (Seidah et al. 1984) and localized in atrial cardiocyte secretory granules by immunocytochemistry (Cantin et al. 1984b). This peptide was found to be the C-terminus of a much larger molecule (pre, pro and connecting peptide) made up of 152 amino acids, first by isolation and sequencing of larger molecular weight forms (Thibault et al. 1984; Lazure et al. 1984) and, finally, by cloning of the rat cDNA (Zivin et al. 1984; Yamanaka et al. 1984; Maki et al. 1984). The synthetic peptide (Arg 101–Tyr 126) (the signal peptide has 24 amino acids) was found to possess varied biological effects: diuresis and natriuresis of rapid onset and short duration, with characteristics identical to those of atrial extracts (Seidah et al. 1984; De Bold et al. 1981; Garcia et al. 1982), vasodilatation with inhibition of the arterial contraction produced by norepinephrine and angiotensin II (Garcia et al. 1984), with correction of renal hypertension (Garcia et al. 1985), inhibition of aldosterone (De Lean et al. 1984a; De Lean et al. 1984b; Chartier et al. 1984a, b) and cortisol hypersecretion (De Lean et al. 1984) induced by a variety of stimulatory agents, and stimulation of arginine vasopressin secretion from the isolated posterior lobe of the hypophysis (Januszewicz et al. 1985). This synthetic peptide inhibits the activity of adenylate cyclase in target tissues [arterial wall (Anand-Srivastava et al. 1984), anterior and posterior hypophysis (Anand-Srivastava et al. 1985a), adrenal cortex (Anand-Srivastava et al. 1985b)] and increases the levels of cGMP in blood and urine and renal cortical cells in culture (Hamet et al. 1984). The exact amount of immunoreactive ANF in rat atria (Gutkowska et al. 1984a) and the plasma level of ANF in control, ether-anesthetized rats has now been determined (Gutkowska et al. 1984b). The presence of immunoreactive ANF in plasma indicates that the heart is an endocrine gland (Cantin et al. 1984a). We now report that following an intracarotid injection of ^{125}I-ANF, binding sites for the peptide are found by radioautography in the zona glomerulosa of the adrenal cortex, in the adrenal medulla, on blood vessel walls of adrenal and kidney and in a variety of tissues.

Materials and methods

Preparation of ^{125}I-ANF

^{125}I-ANF was prepared as already described (Gutkowska et al. 1984a) using synthetic Arg 101–Tyr 126[1] ANF with minor modifications of the Chloramine T method (Greenwood et al. 1963). The tracer was purified on a Sepharose 4B anti ANF affinity column. The immunoglobulins from rabbit plasma immunized with synthetic Arg 101–Tyr 126 ANF were partially purified by precipitation with 35% saturated ammonium sulfate at 4° C. This was repeated twice and the final precipitate was dissolved in 0.1 M sodium bicarbonate, pH 8.3 containing 0.5 M NaCl and dialyzed against the same buffer. Antibodies were coupled at pH 8.3 to wet cyanogen bromide activated Sepharose 4B. A small column of Sepharose 4B anti ANF was prepared in disposable Pasteur pipettes (bed volume of about 1 ml). The column was equilibrated with 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.4. The radioactive ^{125}I-ANF (100×10^6 cpm) was deposited on the column which was washed with the equilibration buffer. Then 0.1 M acetic acid was used for elution. One ml fractions were collected and radioactivity in 10 µl aliquots was measured in a gamma counter. Fractions containing radioactivity were pooled. Further purification of the radiolabeled tracer was achieved by HPLC on a Bondapak C_{18}

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[1] Kindly provided by Dr. R. Nutt, Merck Sharp & Research Laboratories

column (0.39 × 30 cm), eluted with a linear gradient of 20% to 50% acetonitrile with 0.1% trifluoroacetic acid with a slope of 0.5% per min and a flow rate of 1 ml/min. One milliliter eluates were collected. Aliquots of 10 µl from each fraction were counted in a gamma counter. The iodinated peptide eluted at about 30% of acetonitrile. The acetonitrile was evaporated with nitrogen at 4° C.

Injections of ^{125}I-ANF. ^{125}I-Arg 101–Tyr 126 ANF (18,9 µCi; ~150 ng) in sodium phosphate buffer 0.1 M, pH 5.5 containing 0.1% BSA was injected in a volume of 0.1 ml through a catheter inserted in the left carotid artery of female, 40 g Sprague-Dawley rats, under pentobarbital anesthesia, so that its tip reached the aortic lumen. In some experiments, the catheters were implanted in a cephalad direction to study the distribution of ^{125}I-ANF binding sites in the head. For displacement analysis, Arg 101–Tyr 126 ANF (25 µg) was mixed with ^{125}I-ANF as above and injected in a single bolus to the rats under the same type of anesthesia.

Injection of ^{125}I-angiotensin II (AII). To compare the renal glomerula localization of binding sites for ^{125}I-ANF, rats of the same sex, breed and body weight were injected in the same way with ^{125}I-AII (67,6 µCi; ~40 ng) (Cantin et al. 1982) either alone (n=4) or after a 30 min infusion (via the jugular vein) with a total of 5 nmoles (1 ml) of either saralasin (Sar^1-Ala^8-AII) (n=4) or angiotensin III inhibitor (des-Asp^1-Ile^8-AII) (n=4). All animals were sacrificed 2 min after the injection of ^{125}I-AII.

Preparation for radioautography

At each interval (2, 5, 10 and 20 min) after ^{125}I-ANF injection, 8 rats were sacrificed by intracardiac perfusion first of Ringer-Locke fluid for exactly one min and then either with glutaraldehyde 2% buffered with cacodylate HCl (0.1 M, pH 7.4) (n=4) or with Bouin fluid (n=4) for 10 min. In both cases, a portion of tissue (Table 1) was used to quantitate radioactivity in a LKB 1270 Rack gamma II counter. After perfusion with glutaraldehyde, the tissues were minced and fixed for a further period of one hour. They were then placed in cacodylate buffer to which 2% sucrose had been added, and embedded in Araldite as already described (Cantin and Benchimol 1975). After perfusion with Bouin fluid, the tissues were further fixed for 24 h and embedded in paraffin. Semi fine sections (1 µm) of tissues embedded in Araldite were done in a Reichert (OMU_2) ultramicrotome while standard sections (5 µm) were prepared from the paraffin-embedded tissues. The deparaffinized sections were stained with hematoxylin and eosin before dipping in Ilford K5 emulsion as already described (Cantin et al. 1979). Unstained semi fine sections were coated with emulsion in the same way. All sections were then exposed for one month and developed as already described (Cantin et al. 1979; Cantin et al. 1981). The semi fine sections were then stained with toluidine blue. At each step of the processing of embedding in Alradite or in paraffin, radioactivity was counted in solutions to evaluate possible losses. It was consistently found that losses occurred mostly during initial fixation in glutaraldehyde or in Bouin fluid and never exceeded 30% of the initial counts of tissues fixed per perfusion.

Injection of ^{125}I-AII. The animals injected with ^{125}I-AII were perfused as above, first with Ringer-Locke fluid and then with glutaraldehyde 2% and processed for radioautography after embedding in Araldite. After one month of exposure, the semi fine sections were also stained with toluidine blue.

Results

As can be seen in Table 1, the injection of an excess of ANF together with ^{125}I-ANF resulted in a significant inhibition of the uptake of radioactive ANF by a variety of tissues. In colon, however, the uptake was only decreased by 18% and 29%. No significant displacement could be produced in aorta, thymus, ovary, urinary bladder, stomach (antrum), rectum, uterus and pancreas.

Table 1. Displacement response analysis of radioactive content in various rat tissues 2 min after the injection of 18.9 µCi of ^{125}I-ANF

Tissue	^{125}I-ANF (n=4) (cpm/mg of fixed tissue)	^{125}I-ANF+ cold ANF (n=4) (cpm/mg of fixed tissue)	Inhibition (%)
Heart:			
Right atrium	5,677	819	86
Left atrium	2,893	860	70
Right ventricle	2,343	576	75
Left ventricle	1,547	536	65
Lung	2,036	387	81
Spleen	975	577	41
Adrenal	2,628	1,084	59
Kidney:			
Renal artery	2,801	1,087	61
Cortex	3,460	1,640	53
Outer medulla	1,488	713	56
Inner medulla	1,847	847	54
Ureter	1,760	854	51
Liver	2,270	686	70
Mesenteric artery	1,199	654	45
Duodenum	1,375	712	48
Jejunum	1,764	502	72
Ileum	1,675	490	71
Ascending colon	804	658	18
Descending colon	1,746	1,245	29
Eye	5,751	1,038	81
Aorta	619	574	8
Thymus	384	396	–
Ovary	530	726	–
Urinary bladder	415	573	–
Stomach	1,165	1,418	–
Rectum	625	729	–
Uterus	534	622	–
Pancreas	804	1,143	–

Intraaortic injection of 18.9 µCi of ^{125}I-ANF (~150 ng) was done in both groups. In one group, cold ANF (25 µg) was injected simultaneously. Exactly 2 min after the injection, the rats were perfused through the left cardiac ventricle, first with 40 ml of Ringer-Locke solution and then with 2% glutaraldehyde buffered with cadodylate HCL (0.1 M, pH 7.4) for 10 min

Injection of ^{125}I-ANF

The results obtained with paraffin- or Araldite-embedded sections (which afforded a much better resolution) were essentially similar and will be described together.

Kidney

Cortex. At 2 min after injection, all glomeruli were overlain by dense deposits of silver grains which followed the contour of the endothelium (Figs. 1 and 2). The epithelial cells of Bowman's capsule were not markedly labeled. There was no preferential accumulation of silver grains over mesangial cells. Both endothelial and smooth muscle cells of arteries, arterioles, (including afferent and efferent arterioles), veins and venules were heavily labeled (Figs. 3 and 4). There was no preferential accumulation of silver grains over juxtaglomerular cells and the macula densa was not labeled. Likewise, peritubular endothelial cells were not labeled. A much lesser number of grains was found over the lumen, brush border and periluminal cytoplasm of proximal convoluted

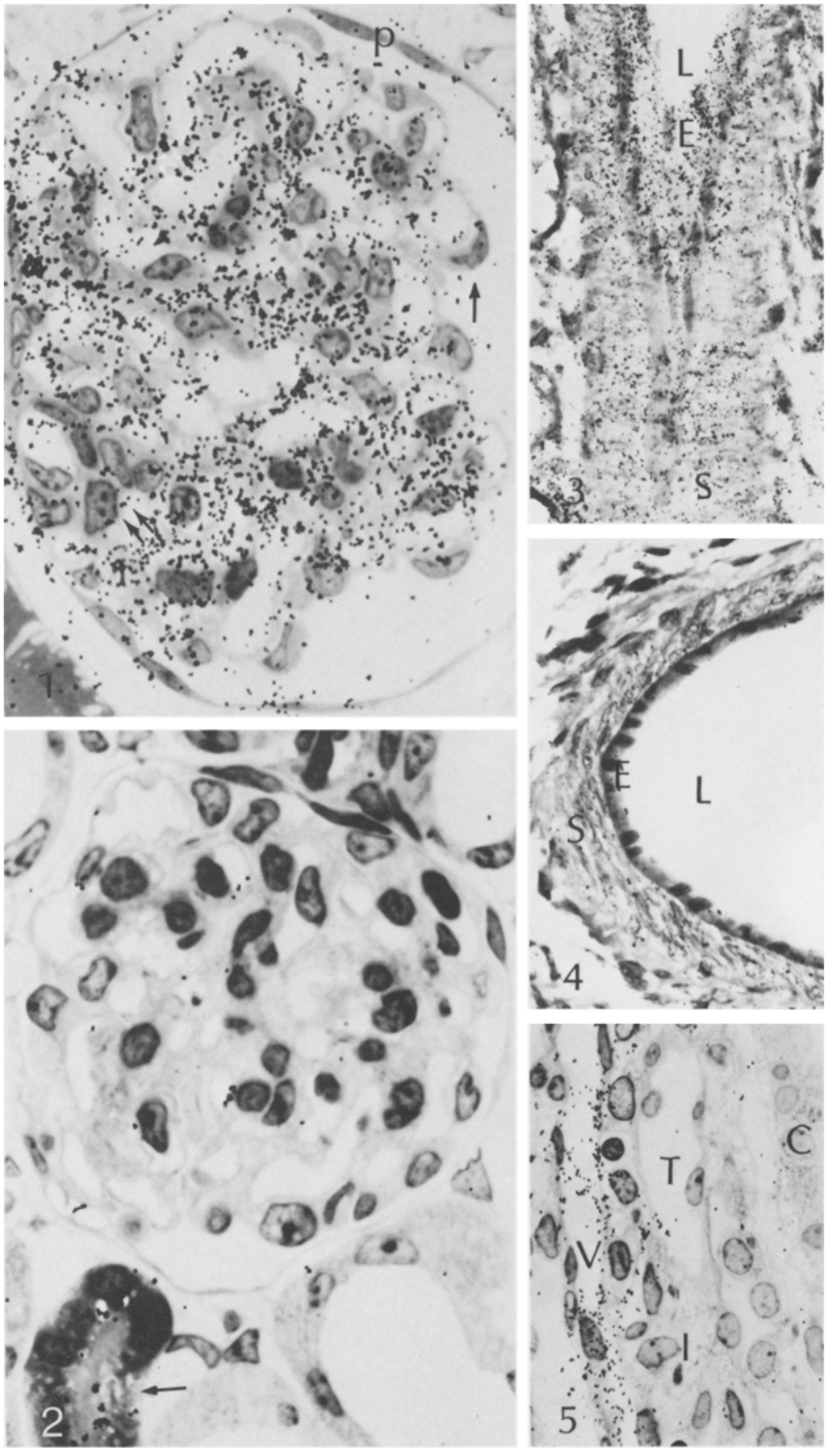


Fig. 1. Semifine section of glomerulus 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are localized over endothelial cells of all capillaries and are absent over visceral epithelial cells (*arrow*), mesangial cells (*double arrow*) and parietal epithelial cells (*P*) (×1,000)

Fig. 2. Semifine section of glomerulus 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are almost completely absent over glomerulus but persist in the lumen of proximal convoluted tubules (*arrow*) (×1,000)

Fig. 3. Paraffin section of renal artery 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are present over endothelial cells (*E*) and smooth muscle cells (*S*). Lumen (*L*). (×400)

Fig. 4. Paraffin section of renal artery 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are almost completely absent over endothelium (*E*) and smooth muscle cells (*S*). Lumen (*L*). (×400)

Fig. 5. Semifine section of inner medulla 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are localized over endothelial cells of some vasa recta (*V*). Silver grains are absent over collecting duct cells (*C*), interstitial cells (*I*) and thin limbs of Henle's loop (*T*). (×400)

tubular cells. Distal tubular cells and cortical collecting duct cells were not labeled. The pattern was identical at later time intervals except that glomeruli at 10 and 20 min were less intensely labeled. No silver grain was found over proximal convoluted tubular cells at 5, 10 and 20 min after injection.

Outer medulla. The endothelial cells of several but not all vasa recta of both outer and inner stripes were heavily labeled so that in favorable sections these labeled capillaries could be followed for a considerable distance in the medulla. No tubular structure was ever found to be labeled. This pattern of labeling did not change with time.

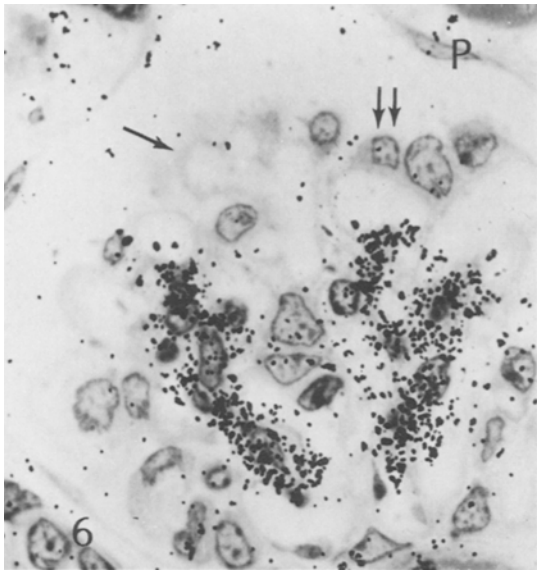


Fig. 6. Semifine section of glomerulus 2 min after injection of ^{125}I-Angiotensin II. Numerous silver grains are localized over mesangial cells but not over endothelial cells (*arrow*), visceral epithelial cells (*double arrow*) or parietal epithelial cells (*P*). (×1,000)

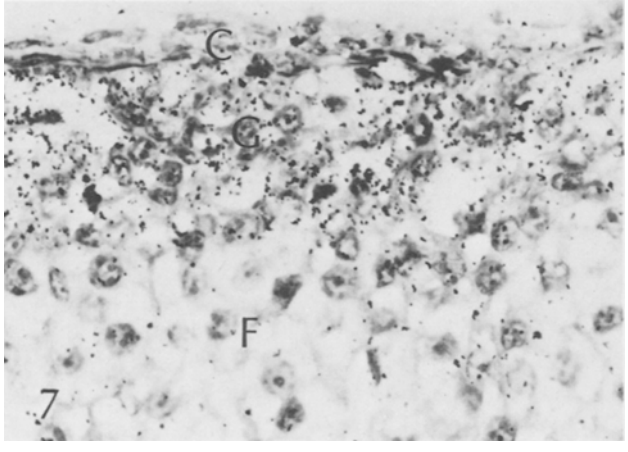


Fig. 7. Paraffin section of adrenal cortex 2 min after injection of ^{125}I-atrial natriuretic factor. Capsule (*C*). Silver grains are localized over zona glomerulosa cells (*G*) but are absent over zona fasciculata cells (*F*) (×400)

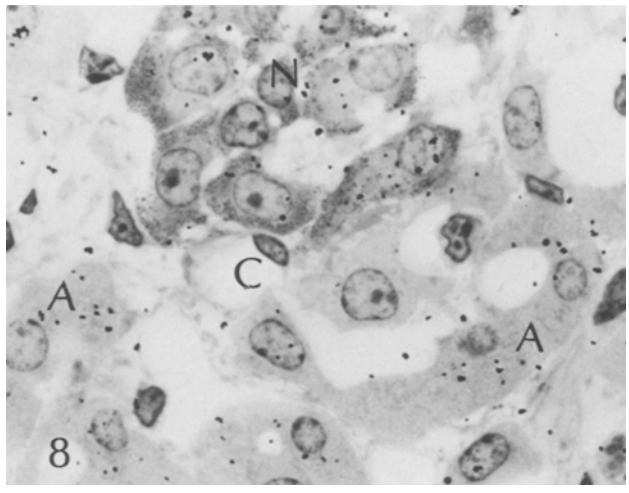


Fig. 8. Semifine section of adrenal medulla 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are present over both adrenergic (*A*) and noradrenergic (*N*) cells but are not present over capillary endothelial cells (*C*). (×1,000)

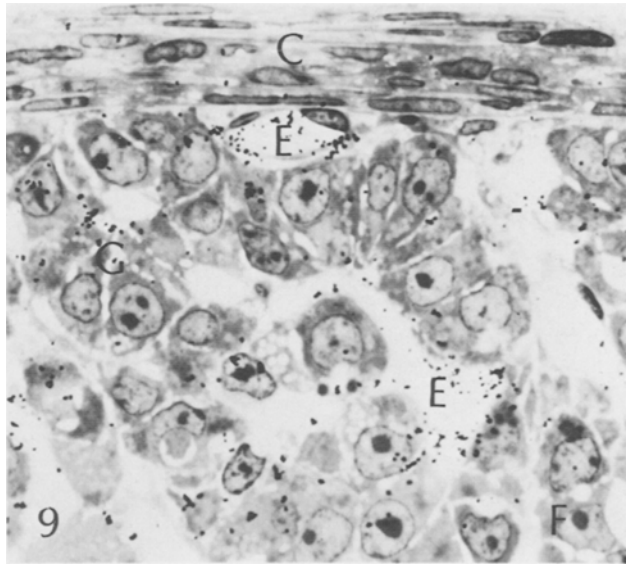


Fig. 9. Semifine section of adrenal cortex 20 min after injection of ^{125}I-atrial natriuretic factor. Capsule (*C*). Silver grains are present over zona glomerulosa cells (*G*) but not over zona fasciculata cells (*F*). Silver grains are numerous over capillary endothelial cells (*E*). (×630)

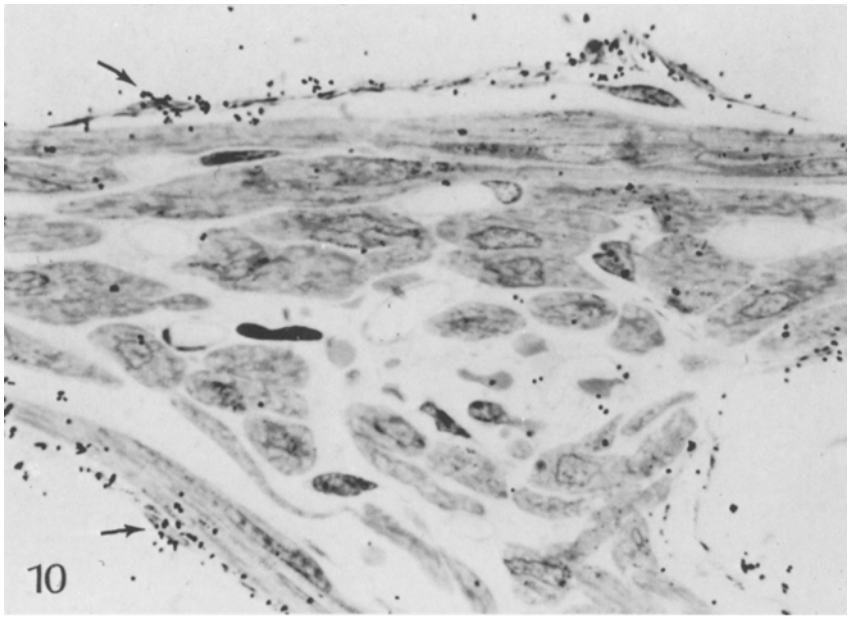
Inner medulla. Here again, several but not all capillaries were labeled and could sometimes be followed for a considerable distance in between totally unlabeled tubular and interstitial cells (Fig. 5). Here again, the labeling pattern did not change with time.

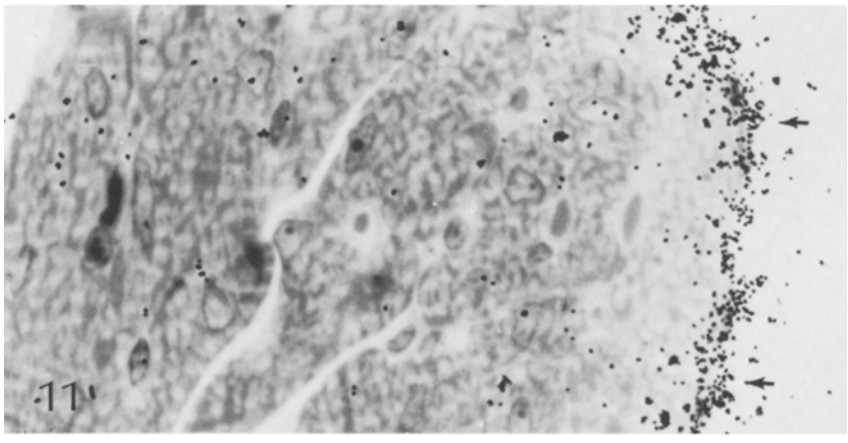
Injection of ^{125}I-AII

As can be seen in Fig. 6, binding sites for AII were mostly localized, as already described (Skorecki et al. 1983), over the mesangial cells of glomeruli.

Adrenal

At 2 min after injection of ^{125}I-ANF, silver grains were localized in a narrow subcapsular zone corresponding to the zona glomerulosa (Fig. 7). Most of the silver grains were localized at the periphery of parenchymal cells. No silver grains could be found in either zona fasciculata or reticularis cells but they were present, although in much lesser amount, over both adrenergic and noradrenergic cells of the adrenal medulla (Fig. 8). The endothelial and smooth muscle cells of subcapsular arterioles and arterioles of the zona glomerulosa and arterioles in zona fasciculata as well as endothelial cells of capillaries in zona glomerulosa were more heavily labeled than zona glomerulosa cells themselves. The sinusoidal cells of the zona fasciculata and the capillaries of the medulla were not labeled. The pattern of labeling in all adrenal layers remained essentially the same with time except that the number of grains over zona glomerulosa and adrenal medulla decreased slightly while





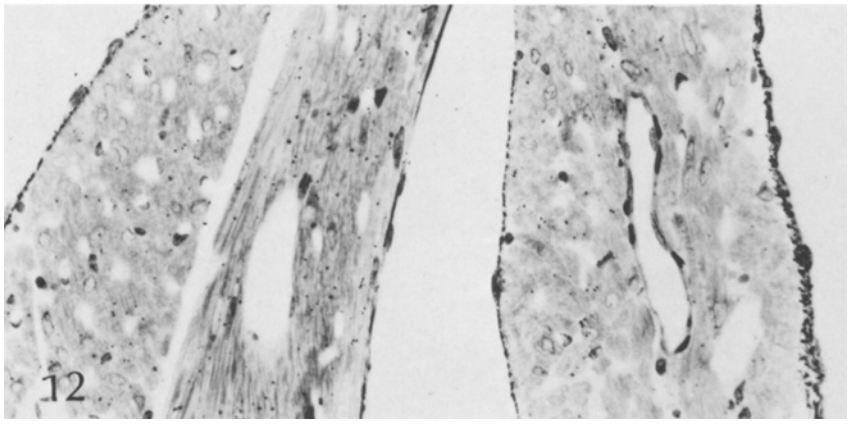


Fig. 10. Semifine section of free atrial wall 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are localized over endothelial cells (*arrow*) of endocardium ($\times$1,000)

Fig. 11. Semifine section of portion of right cardiac ventricle 2 min after injection of ^{125}I-atrial natriuretic factor. Numerous silver grains are localized over endothelial cells of endocardium (*arrow*). ($\times$1,000)

Fig. 12. Semifine section of atrial free wall 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Note complete absence of silver grains over endocardium ($\times$400)

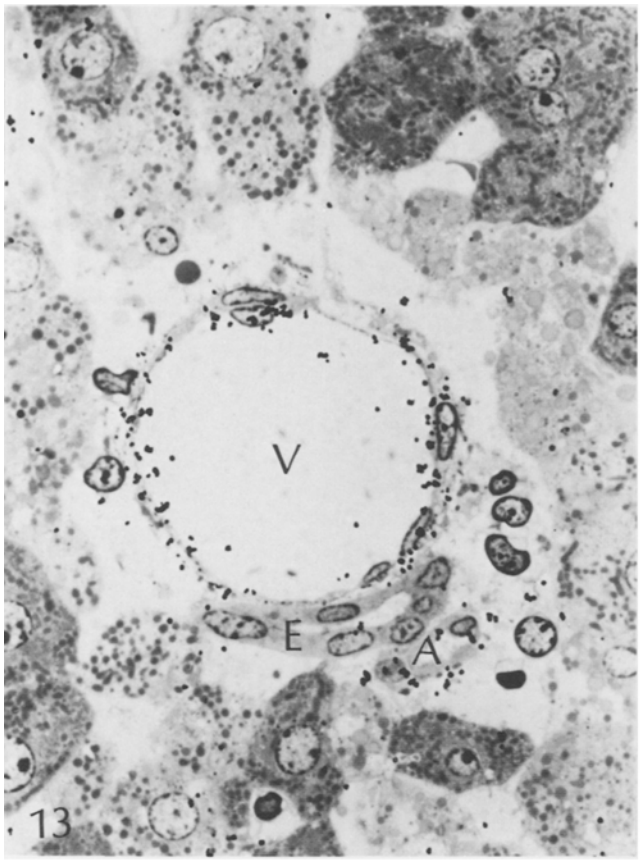
there was no appreciable decrease of labeling in blood vessel walls, particularly in capillaries of zona glomerulosa (Fig. 9). The grains over zona glomerulosa cells became more and more localized near nuclei as time went on.

Heart

A large number of grains was found exclusively associated with the endothelial cells of the endocardium of all four heart chambers (Figs. 10–12). There was no binding to cardiocytes and the blood vessels were not labeled. There was a slight decrease with time in the number of silver grains associated with the endocardium.

Liver

At 2 min after injection (Figs. 13–16), the endothelial cells of all vascular beds of the liver were labeled with an intensity proportional to the size of the vessel: density of labeling decreased from lobular to segmental to interlobular veins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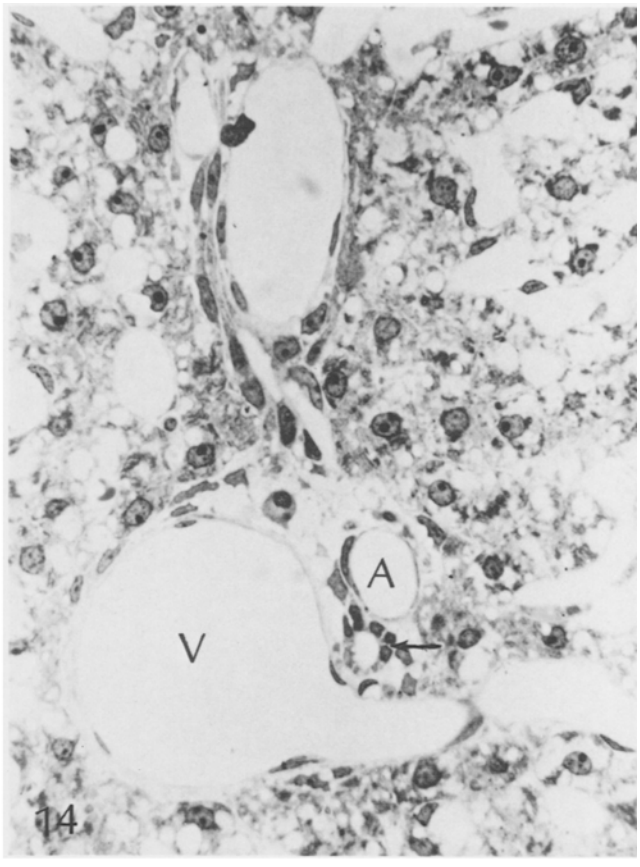
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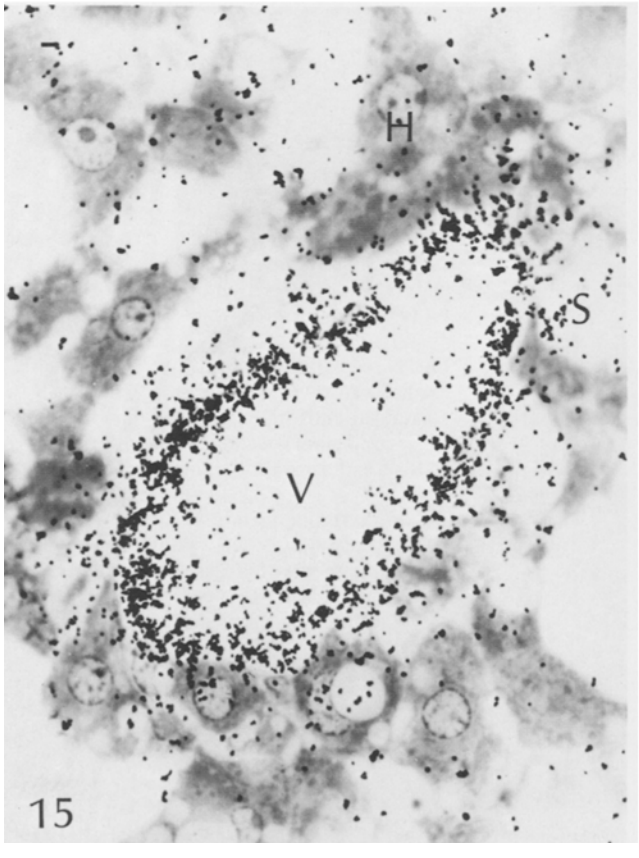
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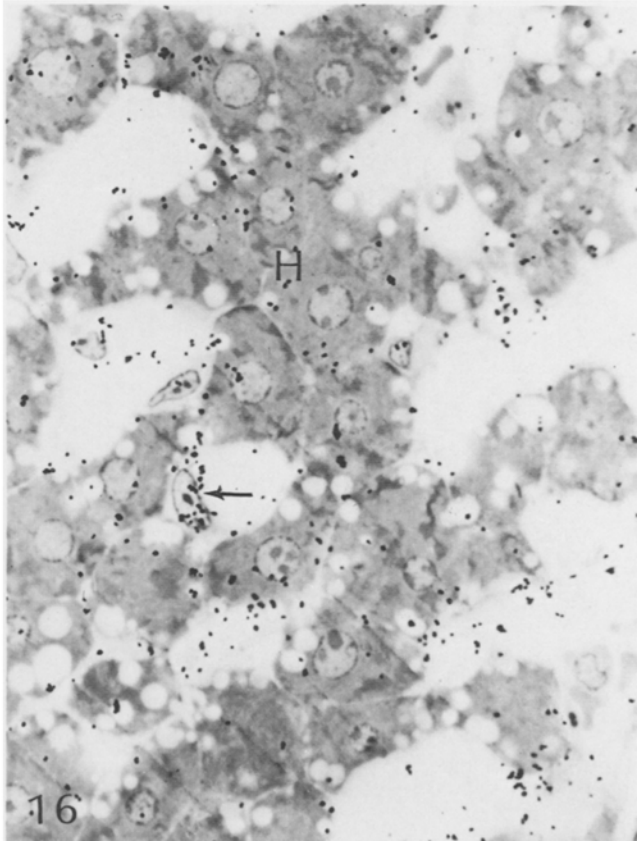
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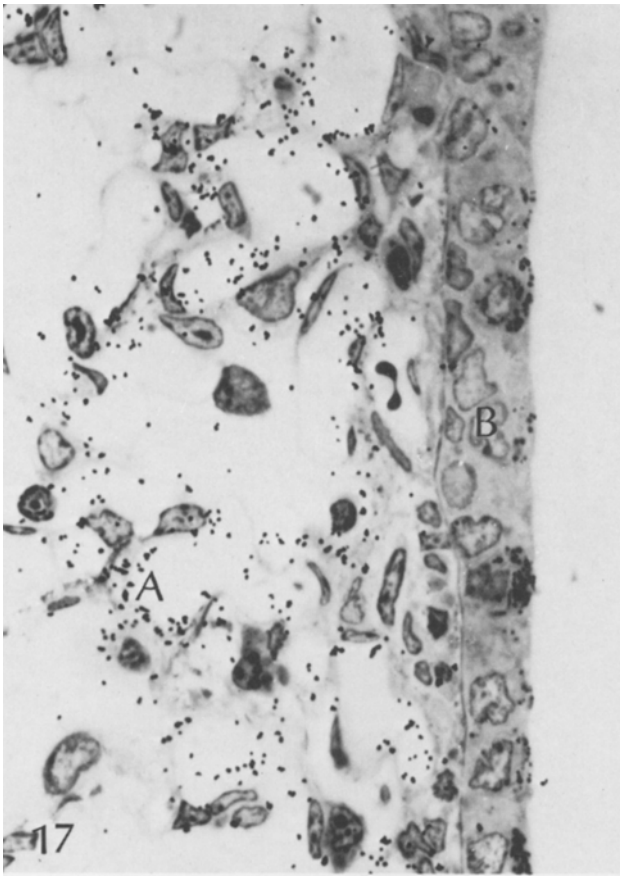


Fig. 17. Semifine section of lung 2 min after injection of ^{125}I-atrial natriuretic factor. The epithelium of a bronchiole (*B*) is not overlain by silver grains which are abundant over bordering cells of alveoli (*A*) (× 1,000)

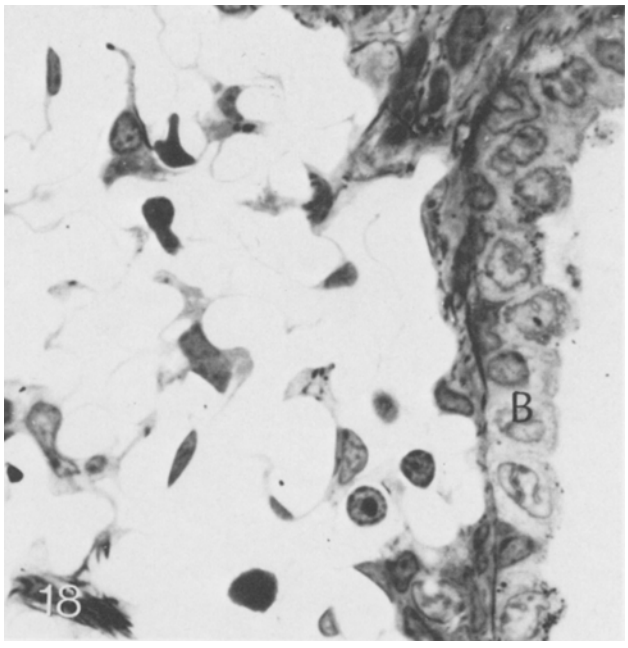


Fig. 18. Semifine section of lung 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Bronchiole (*B*). Silver grains are not present over bordering cells of alveoli. (× 1,000)

to inlet veins to portal veins. The endothelial cells of the sinusoids were less labeled as were capillaries in portal spaces. The endothelial cells of arterioles in portal areas were as intensely labeled as sinusoids. Several grains were present on each parenchymal cell. No labeling was seen on cells of bile canaliculi in portal spaces. At later time intervals, labeling was seen more and more on smooth muscle cells lining veins and arteries as well as on parenchymal cells.

Lung

The endothelial and smooth muscle cells of arteries and arterioles as well as of veins were consistently labeled at 2 min after injection. Label was also found associated with the endothelial cells of all alveoli throughout the lung (Figs. 17 and 18). Bronchi and bronchioles were not labeled. There was a slight decrease in the number of silver grains over the endothelium at 5, 10 and 20 min after the injection and a proportional increase over smooth muscle cells.

Small intestine

In the duodenum, jejunum and ileum, label was found exclusively at the base of the mature columnar epithelium of villi (Figs. 19 and 20). The immature epithelial cells of the crypts, where mitoses are numerous, were not labeled. At 2 min after injection, about 50% of label was found over the basal cytoplasm of mature epithelial cells and 50% over the connective tissue surrounding them. Progressive internalization of label into the epithelial cells was found at 5, 10 and 20 min after injection.

Colon

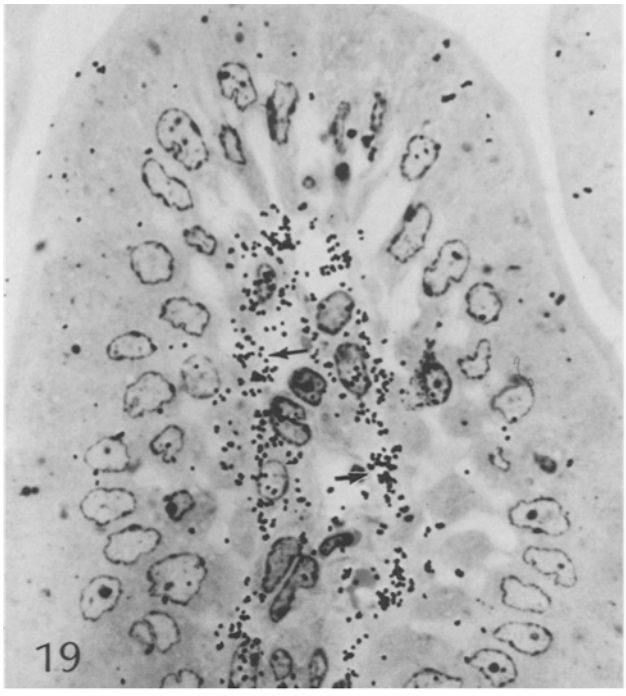
In the ascending (Figs. 21 and 22) and descending colon, label was exclusively associated with the smooth muscle cells of the muscularis layer. The smooth muscle cells of the muscularis mucosae were not labeled. A slight decrease in the amount of silver grains over the labeled structures became evident with time.

Fig. 13. Semifine section of hepatic portal space 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are present over endothelial cells of portal arteriole (*A*) and venule (*V*). Silver grains are not present over epithelial cells of bile canaliculus (*E*). (× 1,000)

Fig. 14. Semifine section of hepatic portal space 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are absent over portal arteriole (*A*), portal venule (*V*) and bile canaliculus (arrow). (× 400)

Fig. 15. Semifine section of liver 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are extremely numerous over an interlobular vein (*V*), less over sinusoidal walls (*S*) and less over hepatocytes (*H*). (× 1,000)

Fig. 16. Semifine section of liver 10 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are abundant over cells (*arrow*) of sinusoids and over hepatocytes (*H*). (× 1,000)



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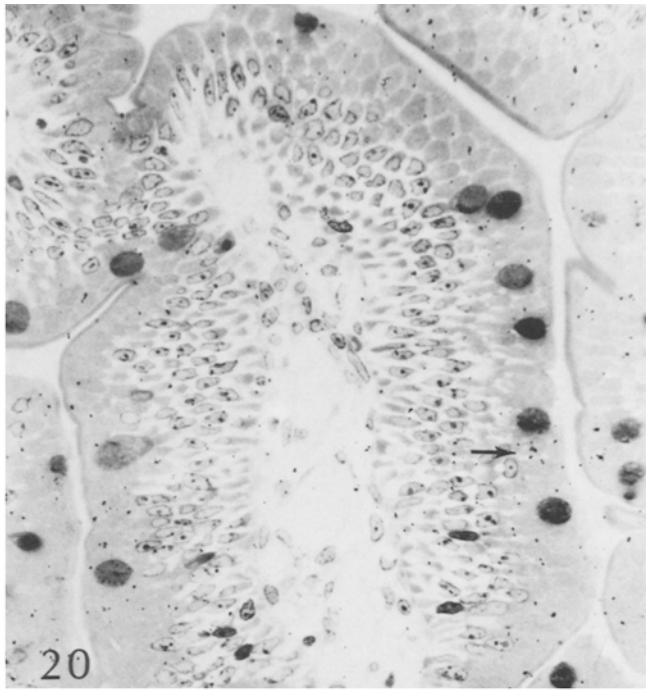

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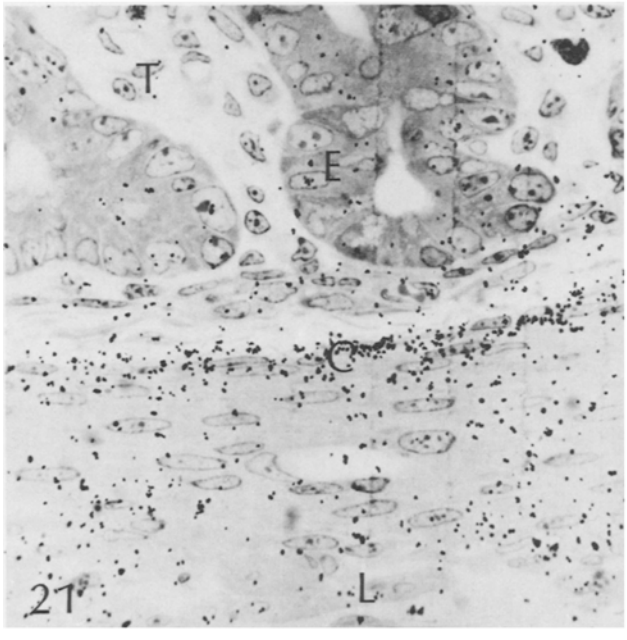

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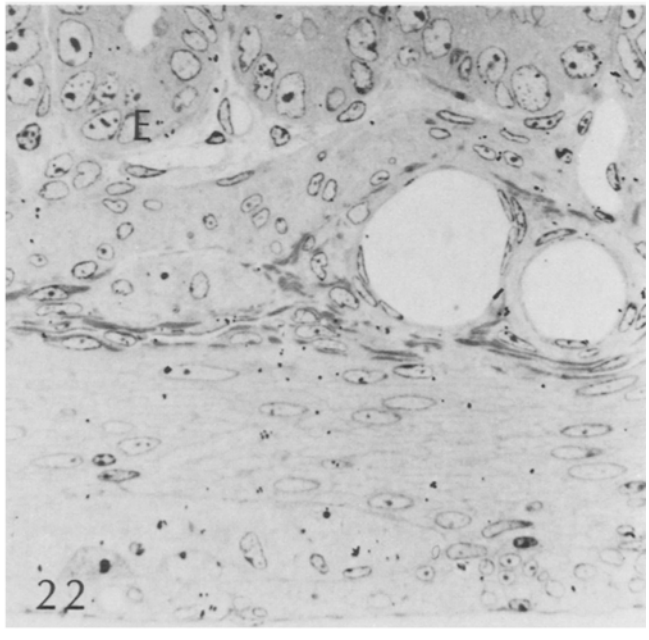

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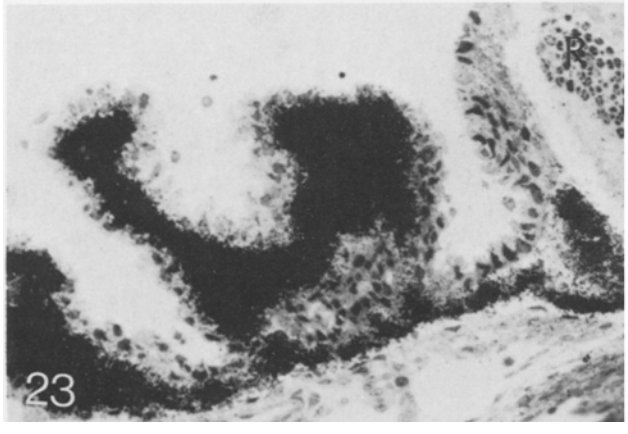

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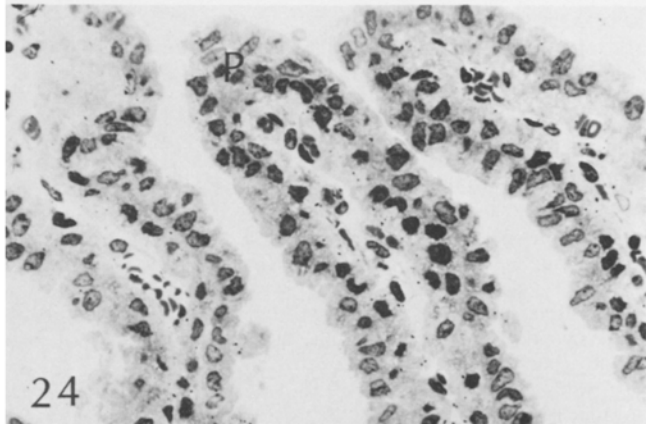

24

Eye

In experiments with cephalad injection of ^{125}I-ANF, a dense deposit of silver grains was found associated with the base of the epithelium of the ciliary bodies (Figs. 23 and 24). No other ocular structure, including the retina, was labeled. Here again, progressive internalization of label was found in the epithelial cells with time. The localization of binding sites in pituitary and brain will be the subject of another communication.

Other tissues

Despite the fact that receptors for ANF are known to exist in vascular tissues (Napier et al. 1984), no binding sites could be localized in aorta and mesenteric artery. No silver grains could be found in any meaningful number in these structures after fixation either with Bouin fluid or glutaraldehyde. In other tissues such as thymus, ovary, urinary bladder, stomach, rectum, uterus and pancreas, radioactivity was not displaceable and the few silver grains present were distributed at random even after concomitant injections of cold ANF. Although radioactivity was displaced in spleen and ureter, no meaningful localization of silver grain could be found in these tissues.

Discussion

The inhibition of uptake of ^{125}I-ANF by simultaneous injection of an excess of cold ANF and the virtual absence of silver grains over putative target cells in the same conditions indicate that the silver grains over labeled structures after injection of ^{125}I-ANF alone represent real binding sites.

The results obtained in the present study as regard the localization of receptors in the adrenal are in agreement with previous findings: synthetic ANF (Arg 101–Tyr 126) significantly inhibits the hypersecretion of both aldosterone from the zona glomerulosa and of cortisol from the zona fasciculata in primary cultures of beef adrenal cortical cells (De Lean et al. 1984; De Lean et al. 1985). Inhibition of aldosterone hypersecretion from the rat zona glomerulosa by synthetic Arg 101–Tyr 126 ANF has also been shown in vitro and in vivo (Chartier et al. 1984a, b) and atrial extracts have the same inhibitory effect (Atarashi et al. 1984). The absence of binding sites in rat zona fasciculata cells confirms the fact that ANF has no effect on corticosterone secretion in this species (Atarashi et al. 1984). A similar situation exists as regards stimulation of cortisol and corticosterone secretion from beef and rat zona fasciculata by angiotensin II. While the latter peptide stimulates the secretion of cortisol from beef zona fasciculata, it has no such effect on the secretion of corticosterone from rat zona fasciculata (Capponi et al. 1981). The presence of a small number of receptor sites on catecholamine-secreting cells of the adrenal medulla is also consistent with previous results: synthetic Arg 101–Tyr 126 ANF has a weak inhibitory effect at high (10 nM) concentration on the secretion of catecholamines stimulated by nicotine (De Lean et al. 1984). The presence of silver grains over arterioles and capillaries of the zona glomerulosa and arterioles of zona fasciculata may indicate that ANF acts on the vasculature of the adrenal as it does in aorta and renal artery (Garcia et al. 1984).

A possible role for the atria in the regulation of extracellular fluid volume and electrolyte concentration has been revealed by the induction of sodium and water excretion in response to changes in intraatrial pressure and stretch of the atrial wall (Goetz et al. 1975). Crude extracts (De Bold et al. 1981) and isolated secretory granules (Garcia et al. 1982) of rat atria have been shown to be potent diuretic and natriuretic agents. The diuretic and natriuretic response is rapid (within 1–2 min) and short lived ($\pm$20 min) and is accompanied by the simultaneous though lesser excretion of potassium, calcium, magnesium and phosphate (Keeler and Azzarolo 1983). It is now well established that synthetic (C-terminal) peptides identical in sequence to the ones extracted from atria have a direct effect on the kidney (Oshima et al. 1984; Camargo et al. 1984). It is not yet clear whether the effects of atrial extracts or their synthetic copy on diuresis and electrolyte excretion are a consequence of renal tubular and/or hemodynamic action.

It has been postulated that atrial tissue contains a factor that decreases the tubular reabsorption of sodium (De Bold et al. 1981; Keeler 1982; Sonnenberg et al. 1982; Trippodo et al. 1982). On the other hand, the synthetic peptide also relaxes the renal artery and induces a concentration-dependent renal vasodilation (Camargo et al. 1984) which is not related to prostaglandin or dopamine release (Oshima et al.

Fig. 19. Semifine section of tip of duodenal villus 2 min after injection of ^{125}I-atrial natriuretic factor. Numerous silver grains are localized at the base of epithelial cells (*arrow*). ($\times$1,000)

Fig. 20. Semifine section of villi of jejunum 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are completely absent from the base of epithelium although a few grains are present over the epithelial cells (*arrow*). ($\times$400)

Fig. 21. Semifine section of wall of ascending colon 2 min after injection of ^{125}I-atrial natriuretic factor. Silver grains are most abundant on the inner part of the circular ring (*C*) of smooth muscle cells but they are also present on the outer logitudinal layer (*L*) of the muscularis. A few silver grains are also present on the epithelial cells (*E*) and connective tissue cells (*T*) of the mucosa ($\times$630)

Fig. 22. Semifine section of wall of ascending colon 2 min after injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are almost completely absent from the outer and inner muscular layers but are still present in small number over epithelial cells (*E*) of the mucosa ($\times$630)

Fig. 23. Semifine section of part of the eye after intracarotid, cephalad injection of ^{125}I-atrial natriuretic factor. Extremely numerous silver grains obscure the epithelial layer of the ciliary body. Part of the retina (*R*) may be seen at right. ($\times$250)

Fig. 24. Semifine section of ciliary body after intracarotid, cephalad injection of ^{125}I-atrial natriuretic factor together with an excess of cold atrial natriuretic factor. Silver grains are almost completely absent over epithelial cells (*P*). ($\times$400)

1984). The reported effects of the atrial extracts on glomerular filtration rate (GFR) have varied: some have not been able to show an effect on this parameter (De Bold et al. 1981; Sonnenberg et al. 1982; Keeler 1982) while others have reported one (Camargo et al. 1984; Vaughan et al. 1985; Oshima et al. 1984). In every instance, however, the mean GFR was higher than in the control period although the difference was not statistically significant. In a careful study, Keeler has shown that increase in GFR in vivo occurs during the first five min after injection (Keeler and Azzarolo 1983). Similarly some investigators report that atrial extracts do not affect renal blood flow (Keeler 1982) while others describe an important renal vasodilatation (Camargo et al. 1984; Oshima et al. 1984) with redistribution of renal blood flow from the outer to the inner cortex and increase in the inner medullary blood flow (Borenstein et al. 1983).

The present results indicate that the binding sites for synthetic Arg 101–Tyr 126 ANF in the kidney are exclusively localized on blood vessels. The localization on renal arteries, arterioles, veins and venules are consistent with the vasodilatory effect of the peptide. The presence of receptors on endothelial cells of glomeruli, vasa recta, arteries, arterioles, veins and venules may indicate that some of the renal effects of the peptide may be endothelium-mediated (Furchgott 1983). Our interpretation of the localization of binding sites for ^{125}I-ANF on endothelial cells of glomeruli is strenghtened by the fact that, in strictly comparable experimental conditions, binding sites for ^{125}I-AII are found, as already described (Skorecki et al. 1983), almost exclusively over mesangial cells. Infusion of either saralasin or AIII inhibitor before injection of ^{125}I-AII almost completely inhibited the localization of silver grains over glomerular structures (data not shown).

The present results are also consistent with the lack of effect of ANF on active sodium transport by inhibition of the Na^+K^+ATPase (Thibault et al. 1983; Kohashi et al. 1984; Pollock et al. 1983) or sodium ion flux in the proximal tubules as measured by Na NMR (Napier et al. 1984) and on tubular oxygen consumption (Napier et al. 1984) or oxygen consumption in kidney slices (Kohashi et al. 1984).

The presence of receptors for ANF in rat renal cortical membranes (Napier et al. 1984) must be reinterpreted in the light of the present results. The localization of receptors in cultured tubular cells of the LLC-PK_1 cell line (which exhibits properties consistent with both distal and proximal tubules) (Goldring et al. 1978; Sepulveda and Pearson 1982) may suggest that, apart from its overwhelming effects on the renal vasculature, ANF may also possess some slight direct action on tubular cells. The hypothesis that ANF might undergo proximal secretion like the loop diuretic drugs (Sonnenberg et al. 1981) is not confirmed by the present study. Although silver grains are indeed present over the lumen, brush border and periluminal cytoplasm of proximal tubules at 2 min after injection of ^{125}I-ANF, these cannot be displaced by cold ANF and possibly represent that part of the peptide which crosses the glomerular barrier. These grains disappear at later time intervals. No internalization into the cytoplasm of these cells could be ascertained and they were never found in the lumen or cytoplasm of other types of tubules.

The presence of numerous binding sites on the endothelial cells of the endocardium and on the endothelial lining of vessels in kidney, adrenal, lung and liver may indicate, here again, that some of the effects of ANF on these organs are endothelium-mediated (Furchgott 1983).

The presence of binding sites on the base of mature epithelial cells of the mucosa of the small intestine and on the base of epithelial cells in the ciliary bodies (Sears and Mead 1983; Green and Pederson 1972; Mishima et al. 1982; Nathanson 1980) may indicate that this peptide is involved in the regulation of water and/or electrolyte transport in these tissues. Indeed, blood volume expansion, which seems to be accompanied by increased amounts of ANF in rat plasma and the renal effects of which can be inhibited by ANF antibodies (Cantin et al. 1984a), is followed by a decrease in net sodium and water absorption at the level of the jejunal mucosa (Nizet et al. 1978; Higgins and Blair 1971; Humphreys and Earley 1971; Richet and Hornych (1969). The presence of binding sites in the smooth muscle cells of the colonic muscularis layer is in agreement with the relaxing effect of ANF on the chick rectum preparation (Currie et al. 1983).

The localization of ^{125}I-ANF on smooth muscle cells of arteries, arterioles, veins and venules in both lung and liver may indicate that ANF has important hemodynamic effects on these tissues. Water immersion in man induces a profound redistribution of pulmonary regional blood flow as demonstrated by scintigraphy (Risch et al. 1978): blood channels are preferentially opened in the apical regions of the lungs which in the resting state are underperfused. As a consequence, the lung area with a normal ventilation-perfusion ratio is considerably increased. Whether these effects of water immersion, which are accompanied by distention of the atria (Risch et al. 1975) are induced by ANF mediated changes in the pulmonary vasculature remains to be determined.

The presence of binding sites on parenchymal cells of the liver may indicate that ANF possesses some metabolic role in this organ. Studies in rats during space flights, in which blood is possibly translocated (Hoffler 1971; Grounds 1979), have shown that the activity of many hepatic enzymes is profoundly modified (Abrahams et al. 1983). Whether these effects are related to ANF remains to be determined.

Note added in proof: Since the present paper was submitted for publication, the presence of receptors in the following tissues has been ascertained by various techniques. Stimution of particulate guanylate cyclase by ANF has been shown in the kidney cortex and medulla, adrenal cortex and medulla, aorta, lung, liver, intestinal mucosa and testis (Waldman, S.A. et al. (1984) J. Biol. Chem. 259:14332). In the dog kidney, ANF selectively stimulates particulate guanylate cyclase and elevates cGMP levels in isolated glomeruli, Henle's loops and collecting ducts but not in proximal tubules (Tremblay et al. (1985) FEBS Lett. 181:17). Radioautography in vitro has also revealed the presence of binding sites in the ciliary body (Quirion et al. (1984) Peptides 5:1167.

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