the Ag-ion[27]. Gaseous CO alone is able to produce silver blocks in the nucleolar regions of Hela cells[18]. The Ag-ions bound to the carboxyl-groups appear to be reduced very easily by formate ions. This can be demonstrated with Carboxyl-Sephadex which under staining conditions precipitates silver faster than Sephadex G10 or $HSO_3$-Sephadex. Carboxyl-cellulose reacts in a similar fashion.

Another possibility for the preferential silver staining, is that nucleoli contain specific proteins which are able to reduce Ag-ions. To test this, nucleoli were extracted with salt and urea-guanidine hydrochloride solutions to obtain 3 protein fractions. Smears of these fractions did not show any difference in the silver staining ability. By polyacrylamide gel electrophoresis of the 3 protein fractions and subsequent silver staining of the gel, patterns of brown bands were obtained with corresponded with all bands stained with Coomassie blue.

In summary, the investigations show that a preferential silver staining of nucleoli and NORs need not necessarily be caused by a special silver affinity protein. Carboxyl-groups alone, which are active at the pH of the staining conditions, could separate silver. A corresponding accumulation or arrangement of carboxyl-groups of nucleolar proteins could favour the formation of silver nuclei in the nucleoli and NORs and they could therefore cause an equivalent rapid Ag-staining.

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## Autoradiographic localization of $^3$H-angiotensin II in rat adrenal glands

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*Summary.* Autoradiographic studies of rat adrenal gland approximately 1 min after intra-aortic injection of $^3$H-angiotensin II show that radioactivity concentrates in the zona glomerulosa. This concentration is reduced by concomitant administration of excess unlabelled angiotensin II.

The adrenal glands are a well known target tissue for angiotensin II. A stimulatory effect of this hormone on aldosterone secretion from the zona glomerulosa has been reported[1], and angiotensin II has also been shown to stimulate adrenocortical corticosterone release[2] and adrenomedullary catecholamine release[3]. Angiotensin receptors have been studied by biochemical methods in isolated zona glomerulosa cells[4,5] and adrenal homogenates[6–8], but the precise localization of angiotensin II within the adrenal gland after in vivo injection has not been reported. The autoradiographic approach has been used previously to localize specific angiotensin II binding sites within the kidney[9]. Here we have studied the localization of $^3$H-angiotensin II in rat adrenal glands, after its injection in vivo, using 2 different autoradiographic methods.

*Materials and methods.* Adult, male Wistar rats were bilateraly nephrectomized 48 h before the experiments since previous studies[8] have shown that this procedure, as well as eliminating endogenous angiotensin II, also increases adrenal angiotensin binding sites, thus providing optimal conditions for their autoradiographic detection. $^3$H-angiotensin II (2 batches of sp. act. 70 and 46 Ci/mM respectively) was injected under pentobarbital anaesthesia via a cannula placed in the carotid artery, the tip of which just entered the aorta.

*Autoradiographic procedures.* Method I. 1 min after $^3$H-angiotensin II injection (400 ng, 600 ng, 1 µg) adrenals were fixed by intra-aortic perfusion of buffered glutaraldehyde at 2.3%, post-fixed in 2% $OsO_4$ and embedded in epon. 1.5-µm semi-thin sections were mounted on slides and coated with autoradiographic emulsion (stripping film AR.10 Kodak). After 6 weeks exposure at room temperature, autoradiograms were developed, fixed and stained with toluidine blue.

Method II. Rats received an intra-aortic injection of 25, 50 or 100 ng of $^3$H-angiotensin II either alone or in presence of excess unlabelled angiotensin II. 45 sec after the injection, the adrenal glands were removed and quickly frozen in

liquid nitrogen-cooled isopentane. 10-$\mu$m frozen sections were obtained with a cryostat (Cryocut, American Optical) and autoradiograms prepared as previously described[10].

*Quantification.* To determine mean grain density in the zona glomerulosa the number of silver grains located in an area of 2500 $\mu m^2$) of zona glomerulosa in the 4 cardinal points of the section were counted. 2 slides per animal were used and 4 sections per slide were analysed.

Values in the presence of excess unlabelled hormone were compared to those after $^3$H-angiotensin II alone by the Student's t-test.

*Results.* Method I. With all 3 doses of $^3$H-angiotensin II, a high density of silver grains was present in the zona glomerulosa (figure 1). In contrast, the grain density in the zona fasciculata, zona reticularis and the adrenal medulla was very low. Within the zona glomerulosa the density of silver grains was not uniform. In highly labelled areas the radioactivity was principally located at the cell periphery and the majority of cells showed a very low grain density over their nucleus and cytoplasm (figure 1). However, some cells in these regions of the zona glomerulosa, characterized by a small nucleus which stained deeply with toluidine blue, did show a noticeable grain density over their nucleus and cytoplasm.

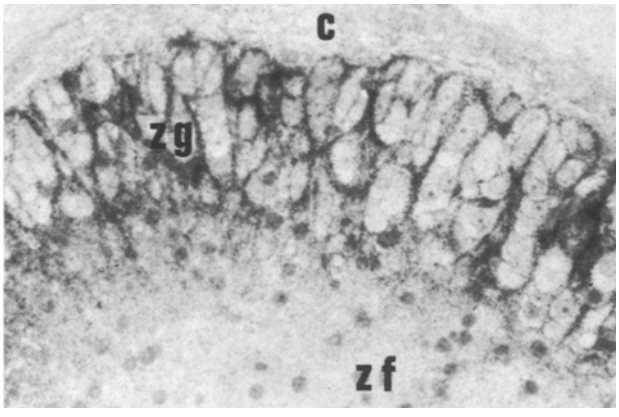


Fig. 1. Autoradiograms of epon-embedded adrenals. c: capsule, zg: zona glomerulosa, zf: zona fasciculata.

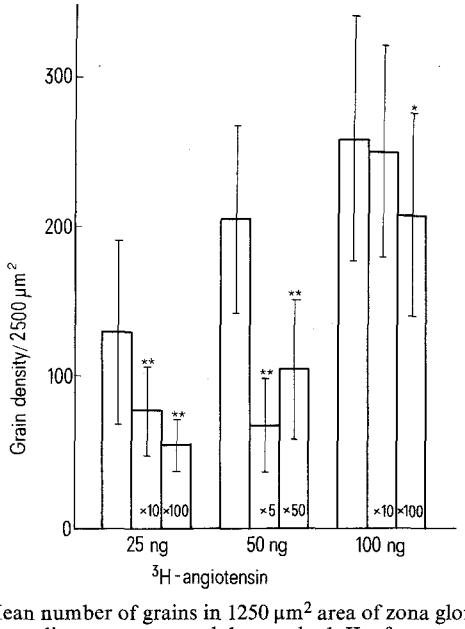


Fig. 2. Mean number of grains in 1250 $\mu m^2$ area of zona glomerulosa of autoradiograms prepared by method II after competition experiments. For each dose of $^3$H-angiotensin II given, the 1st column represents the mean value $\pm$ SD after injection of tracer alone and the 2nd and 3rd columns represent mean values $\pm$ SD after injection of tracer with concomitant excesses of unlabelled angiotensin (**$p < 0.001$, *$p < 0.05$).

Method II. With this method radioactivity was also localized exclusively in the zona glomerulosa. Again, the density of silver grains throughout this zone was variable, and in highly labelled regions the radioactivity appeared to be localized principally at the cell periphery. The greater the dose of $^3$H-angiotensin, the higher the grain density over the zona glomerulosa. Concomitant injection of excess unlabelled angiotensin II markedly reduced zona glomerulosa grain density at doses of 25 and 50 ng and to a lesser extent at the 100-ng dose (figure 2).

*Discussion.* Our autoradiographic studies show, by 2 different methods, that after $^3$H-angiotensin II injection radioactivity concentrates in the zona glomerulosa and that very small amounts are present in the rest of the adrenal gland. Competition experiments suggest that these zona glomerulosa sites binding radioactivity after $^3$H-angiotensin administration are saturable. In addition the fact that adrenals were fixed or frozen very shortly after injection (1 min) suggests that the radioactivity probably represents intact $^3$H-angiotensin II or one of its early metabolites, e.g. $^3$H-heptapeptide ($^3$H-angiotensin III). These results are in agreement with the presence of angiotensin receptors in the zona glomerulosa, mediating the stimulatory action on aldosterone release.

Both autoradiographic methods showed the radioactivity to be present at the periphery of the majority of zona glomerulosa cells, a result which agrees with the hypothesis of an initial effect of the hormone on the plasma membrane[11].

Although doses of $^3$H-angiotensin II were high in method I (to favour autoradiographic detection after perfusion of fixative), those employed in method II were sub-maximal for angiotensin II-mediated aldosterone release in the rat according to Campbell et al.[12].

Despite previous reports of the action of angiotensin II on the zona fasciculata and reticularis[2] and on the adrenal medulla[3], this method did not provide evidence for a concentration of radioactivity in these zones. This may be due to differences in affinity of the receptor sites in various zones of the adrenal, or may reflect regional differences in accessibility to the hormone due to the irrigation of the gland, or to local degradation of the hormone.

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