ences between the vascular reactions observed in the GC and in the organs. Supposing that the resistance vessels of the tumor have a low alfa constrictor and a significant beta dilator tone as compared to the organs, one would expect vasoconstriction provoked either reflexly or by phenylephrine or propranolol. It is possible that a secondary so called passive vasoconstriction could ensue following decrease in the arterial blood pressure during phenoxybenzamine or isoproterenol. The peculiar effect of isoproterenol in larger dose - no change in arterial pressure accompanied by an increase in the resistance of tumor vessels - cannot be explained even on the basis of the previous assumptions. Differences in vascular reactions of the organs and tumors might have some practical implications.

1 Present address: National Korányi Institute for TBC and Pulmonology, Budapest 1529, Hungary.
2 L.A. Sapirstein, Am. J. Physiol. *193*, 161 (1958).
3 L.A. Sapirstein and G.E. Hanusek, Am. J. Physiol. *193*, 272 (1958).
4 L. Takács, K. Kállay and A. Karai, Acta physiol. hung. *25*, 389 (1964).
5 L. Takács, L.A. Debreczeni and Cs. Farsang, J. appl. Physiol. *38*, 699 (1975).
6 P.M. Gullino and F.H. Grantham, J. nat. Cancer Inst. *27*, 679 (1961).
7 R.F. Edlich, W. Rogers, Jr, C.V. DeShazo and J.B. Aust, Cancer Res. *29*, 1420 (1966).
8 J.H.G. Rankin, R. Jirtle and T.M. Phernetton, Circulation Res. *41*, 496 (1977).

Nuclear localization of aldosterone in rat brain cells assessed by autoradiography[1]

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Summary. Autoradiographic studies with ^{3}H aldosterone demonstrate nuclear concentration of hormone in neurons of the hippocampus, septum, allocortical regions and brain stem reticular formation and motor nuclei of cranial nerves and in the meninges. The results suggest that mineralocorticoids have wide ranging effects on different parts of the central nervous system.

It is now well established that cell nuclei of the central nervous system are capable of binding steroid hormones and that the sites of localization vary, depending on the molecular structure of the steroid[2,3]. 2 examples may be cited. 1. Dihydrotestosterone, an androgenic metabolite of testosterone incapable of aromatizing to estradiol, binds to nuclei of motor neurons in contrast to estradiol for which sensory neurons are target sites[2,4,5]. 2. Nuclear binding of androgen and estradiol to cells contained in the hippocampal formation, while distinct, is less pronounced than the binding to cell nuclei of specific areas in the hypothalamus[6,7]; in contrast, corticosterone, an adrenal steroid hormone classified as a glucocorticoid because it primarily effects carbohydrate metabolism, localizes in cell nuclei of the hippocampal formation, but no such nuclear localization within any of the cell aggregates of the hypothalamus has as yet become evident by radioautographic techniques[2,8,9]. We have studied the nuclear localization of aldosterone, the most potent representative of the 2nd group of adrenocortical steroid hormones, classified as mineralocorticoids because they alter electrolyte and water balance and conclude that it also has a characteristic distribution pattern, differing from that of estradiol but overlapping in part with that of dihydrotestosterone and resembling that of corticosterone, although with aldosterone a more intense localization in rudimentary as compared to postcommissural hippocampal structures and a marked concentration in cell nuclei of the arachnoid are noted.

Materials and methods. 5 weanling female Wistar rats were adrenalectomized and maintained on 0.9% saline and food *ad libitum* for 3 days prior to i.v. administration (1 µg/100 g b.wt) of 1, 2, 6, 7-^{3}H aldosterone (N.E.N., sp. act. 90 Ci/mmole). 2 animals were injected with radioinert aldosterone (100 µg/100 g b.wt) 5 min before administration of the tritiated compound. 1 h after injection of radioactive steroid the rats were decapitated and the brains frozen in liquid propane[10]. Sections of 4 µm were thaw-mounted onto slides coated with photographic emulsion, Kodak NTB 3, then exposed for 2 months prior to development in Kodak D19 and staining with methylgreen pyronin. The relative concentrations of radioactive material in

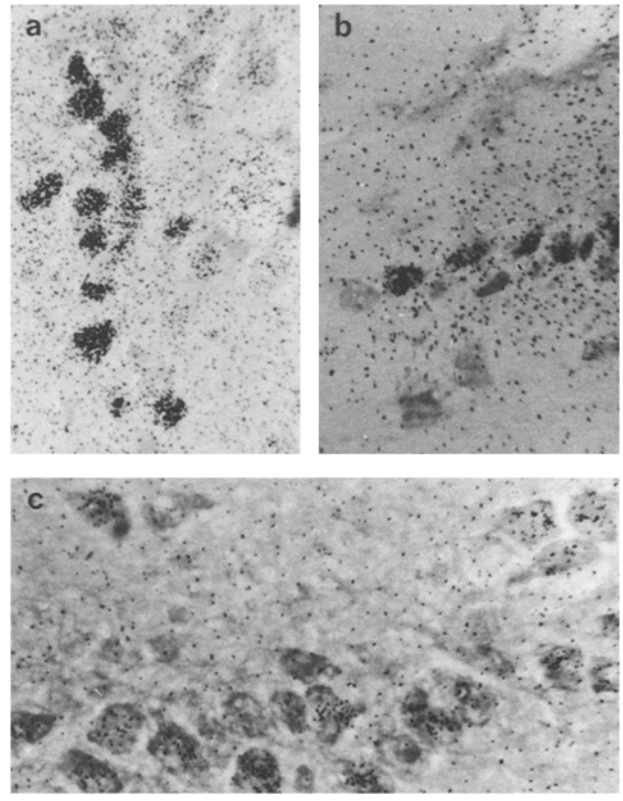

Fig. 1. Autoradiogram showing nuclear localization of radioactivity, after i.v. injection of tritiated aldosterone, in neurons of the hippocampus anterior (a), indusium griseum (b), and postcommissural hippocampus, area CA 1 (c). Exposure time 60 days, magnification × 560.

cells exhibiting nuclear localization were compared by positioning a reticule square comprising an area of 72 μm^2 over the cell nucleus and counting under the microscope at a magnification of $\times 580$ all the silver-grains contained in and touching 2 sides of the square. The dimensions of the square were chosen because they usually corresponded to the area occupied by the silver grains associated with the nuclei. In all instances adjacent areas outside the cells, occupied solely by neuropil were counted as well for comparison and the results are displayed since these counts always greatly exceeded the background count for the emulsion which was negligible (less than 1 silver grain per 1000 cm^2).

Results and discussion. As seen from a comparison of photomicrographs depicted in figure 1 and of counts of silver grains expressed per 100 μm^2 area in figure 2, the greatest concentration of silver grains occurred over the cell nuclei of the indusium griseum and hippocampus anterior, the arachnoid, and over motor components of the cranial nerves. Distinct, although less intense nuclear localization was noted in the post-commissural hippocampus and dentate gyrus, the anterior olfactory nucleus and in the reticular formation. No nuclear accumulation of silver grains was observed in any of the regions exposed to a 100-fold excess of unlabelled aldosterone before administration of the tritiated compound (range of counts 0–3 grains/1000 μm^2).

In the preference for motor over sensory components of the cranial nerves aldosterone resembles both the androgen steroid hormones and corticosterone but differs from estradiol[3]. It further differs from the sex hormones in its failure to localize in the area postrema and locus ceruleus. Our distribution studies extend the recent findings of Ermisch and Rühle[11] and provide quantitative evaluation of regional differences in the intensity of nuclear localization.

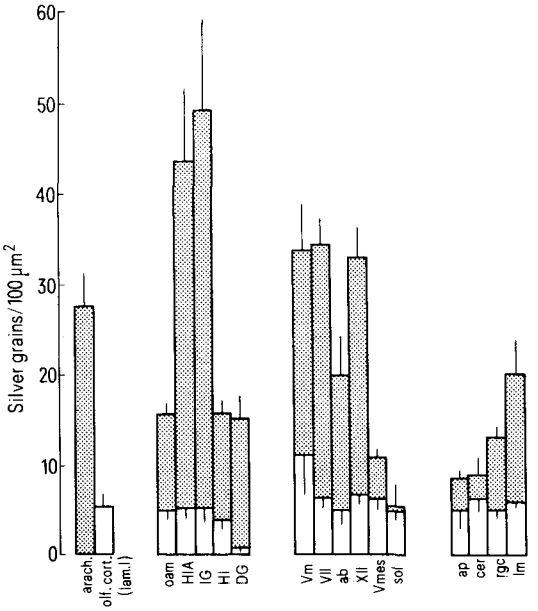

Fig. 2. Concentration of radioactivity in different brain regions, expressed per 100 μm^2 area and assessed by counting the silver grains in a 72 μm^2 grid positioned over the cell nucleus (stippled bars) or over adjacent neuropil (open bars). Vertical lines denote SEM (N = 5–10). arach = arachnoid over olfactory cortex (olf. cort.): oam = nucleus olfactorius anterior; HIA = hippocampus anterior; IG = indusium griseum; HI = hippocampus; DG = dentate gyrus; Vm, VII, ab, XII = motor nuclei of the 5th, 7th, 9th and 12th cranial nerves; Vmes, sol = sensory nuclei of the 5th and 10th cranial nerves; ap = area postrema, cer = locus ceruleus; rgc = nucleus reticularis gigantocellularis; lm = nucleus reticularis lateralis magnocellularis.

The functional significance of these target sites in the brain for aldosterone is not known nor does the autoradiographic evidence to date permit definite conclusions regarding receptor specificity. While similarities in the regional distribution of aldosterone and corticosterone are suggestive of common binding sites, the profiles for variations in regional intensities did not coincide and the possibility of separate specific receptors, located within the same cell or in adjacent cells, capable of discriminating between steroid configurations imparting glucocorticoid and mineralocorticoid properties to the molecule is not ruled out. Ongoing comparative autoradiographic studies on the localization of aldosterone and corticosterone following the administration of competing steroids of either category should shed light on this problem. While aldosterone is quantitatively only a minor secretory product in the rat, the structurally related mineralocorticoid 18-hydroxydeoxycorticosterone which also contains an oxygen function at carbon 18 can be secreted by this species in quantities exceeding those of corticosterone. In contrast to corticosterone the C-18 oxygenated adrenocortical steroids have only a minimal affinity for the corticosteroid binding globulin in plasma, a feature that might render them more accessible to target organs. Aldosterone is capable of inhibiting the stress-induced rise of plasma corticosterone in the rat, a property usually associated with glucocorticoids, at $^1/_{10}$ the concentration at which corticosterone itself is effective[12,13]. This might implicate primitive allocortical structures which appear to be even more conspicuous targets for aldosterone than for corticosterone as relevant sites for negative feedback action, a consideration rendered attractive by their projection to the hypothalamus. It is noteworthy that nuclear localization of aldosterone, and also of corticosterone occurred in the reticular formation which is considered an anatomical focus for arousal behaviour. Radiochemical evidence indicates that deoxycorticosterone, another potent natural mineralocorticoid capable of suppressing stress-induced elevation of corticosterone levels in the rat[14], locates in the reticular formation as well[15]. The concentration of aldosterone in cell nuclei of the arachnoid may be of significance in its therapeutic effect on cerebral edema[16]. The implications of the radioactivity associated with the neuropil also remain to be assessed.

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2 W.E. Stumpf and L.D. Grant, eds. Anatomical Neuroendocrinology. Karger, Basel 1975.
3 W.E. Stumpf and M. Sar, J. Steroid Biochem. *7*, 1163 (1976).
4 M. Sar and W.E. Stumpf, Science *197*, 77 (1977).
5 W.E. Stumpf and M. Sar, Proc. Vth int. Cong. Endocr., p. 18. Excerpta Medica, Amsterdam 1976.
6 M. Sar and W.E. Stumpf, J. steroid Biochem. *8*, 1131 (1977).
7 M. Sar and W.E. Stumpf, Experientia *28*, 1364 (1972).
8 J.L. Gerlach and B.S. McEwen, Science *175*, 1133 (1972).
9 M. Warembourg, Lille Med. *20*, 541 (1975).
10 W.E. Stumpf, Am. Zool. *11*, 725 (1971).
11 A. Ermisch and H.-J. Rühle, Brain Res. *147*, 154 (1978).
12 M.K. Birmingham, I. Kraulis and H. Traikov, in: Oral Contaceptives and High Blood Pressure, p. 315. Ed. M.J. Fregly and M.S. Fregly. Dolphin Press, Gainesville 1974.
13 M.K. Birmingham, I. Kraulis, H. Traikov, A. Bartova, M.P. Li, J.T. Oliver and G. Possanza, J. steroid Biochem. *5*, 789 (1974).
14 I. Kraulis, H. Traikov, M.P. Li and M.K. Birmingham, J. steroid Biochem. *4*, 129 (1973).
15 I. Kraulis, G. Foldes, H. Traikov, B. Dubrovsky and M.K. Birmingham, Brain Res. *88*, 1 (1975).
16 C.D. Withrow and D.M. Woodbury, in: Steroids and Brain Edema, p. 41. Ed. H.J. Reulen and K. Schürmann. Springer-Verlag, New York 1972.