9. C. Williams and C. Ockey, Exp. Cell Res., 63, 365 (1970).
10. G. Wise and D. Prescott, Proc. Natl. Acad. Sci. USA, 70, 714 (1973).

# GLIAL—VASCULAR REACTIONS TO ANGIOTENSIN II IN THE RAT BRAIN

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UDC 615.225.1.015.4:612.82

Reactions of the glia and blood vessels in the sensomotor cortex of adult rats to intraperitoneal injection of angiotensin II were studied electron-microscopically. Repeated injections of the hormone led to edema of increasing severity of the astrocytes, constriction of the lumen of the capillaries, and changes in the structure of their endothelium. It is suggested that these disturbances may be the cause of the cerebrovascular insufficiency and of functional changes in the CNS.

KEY WORDS: brain capillaries; astrocytic glia; angiotensin II.

The structure of the brain capillaries corresponds to the general plan of capillary structure. However, unlike capillaries of other organs, those of the brain have close topographic connections with glial cells. Staining nerve tissue by various methods has shown that processes of astrocytes make contact with the vascular wall [1, 4]. The astrocytic glia is considered to cover 85% of the surface of the brain capillaries [6].

Besides its action on neurons of the CNS, angiotensin II (A-II) also has a direct action on the smooth muscles of blood vessel walls, increasing their tone [5, 7]. It is not yet clear what takes place in the capillary part of the vascular system, in particular, what changes take place in glial—vascular interrelations, which correspond to the level of function of the blood—brain barrier [3].

With these facts in mind, and also considering that a considerable disturbance of functions of the CNS and of visceral regulation persists for a long time after administration of A-II ceases [2], it was decided to study the effect of A-II on the ultrastructural organization of the blood vessels and glia in the cerebral cortex.

## METHODS

Experiments were carried out on 24 male albino rats weighing 180-200 g. A-II (Hypertensin, from Ciba) was injected intraperitoneally in a dose of 0.05 μg/kg body weight daily for 1, 3, 7, 14, and 21 days. Intact animals served as the control. The rats were decapitated 5 and 15 min after injection of the hormone on the 1st day and 15 min after its injection on the other days. Pieces of the sensomotor cortex were fixed in glutaraldehyde, postfixed with $OsO_4$, dehydrated in alcohols of increasing concentration and acetone, and embedded in Araldite. Sections were cut on the LKB Ultrotome, stained with lead citrate by Reynolds' method [8], and studied in the JEM-100B electron microscope. Material from three animals was used at each time. Grids with 6-8 blocks were examined from one animal.

## RESULTS

The capillary reaction is one of the early responses of the brain to injection of A-II. Hyperemia of the vessels could be observed macroscopically in the brain of the rats as early as 5 min after a single intraperitoneal injection of A-II. Dilatation of the capillary net-

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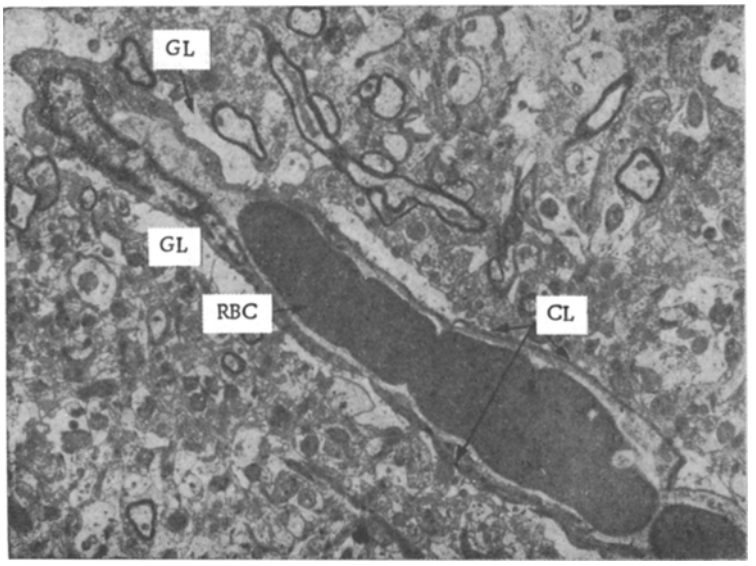


Fig. 1. Blood capillary from sensomotor cortex of rat 5 min after a single injection of angiotensin II. Capillary lumen (CL) dilated and packed with red blood cells (RBC). Capillary wall reduced in thickness. Pericapillary glial processes (GL) unchanged (6000 ×).

work was observed in the brain sections. The lumen of the capillaries was packed with blood cells and plasma (Fig. 1). Under the electron microscope, changes in the endothelial cells were observed in the structure of these capillaries. The cytoplasm of the endothelial cells was flattened. In some areas of the endothelial cells the cytoplasm was no bigger than the basement membrane, and sometimes it was even smaller. The nuclei of the endothelial cells were flattened and elongated, and the chromatin was concentrated near the nuclear membrane. Considerable dilatation of the capillary network 5 min after injection of A-II caused no significant changes in the glia surrounding the capillaries, although it did modify the structure of the endothelium.

The lumen of the capillaries 15 min after a single injection of A-II was relatively empty, few blood cells could be seen in the lumen, the walls of the capillaries were collapsed, and the lumen itself reduced in size.

The structure of the endothelial cells remained similar to that of the endothelium observed 5 min after injection of A-II. Meanwhile glial processes surrounding the capillaries were swollen and widened, considerably so in some places. These expansions of the astrocytic glia gave a picture of marked perivascular edema. Repeated injections of A-II led to a further increase in pericapillary edema of the glia, spreading to the bodies of the astrocytes.

After seven injections of A-II the increasing edema of the glia led to compression of the structures of the neuropil adjacent to the capillaries and of the capillary walls. Many closed and half-closed capillaries could be seen in the animals, with unevenly twisted walls and with a lumen of varied width (Fig. 2). One cause of this irregular compression of the vessel walls was the varied degree of edema of the pericapillary glial processes adjacent to the same capillaries. This also led to irregularity of the diameter of the capillary lumen. As injections of A-II continued the number of capillaries compressed by glial processes increased. The bodies of the astrocytes became edematous and greatly enlarged, whereas other types of glia, oligodendrogliocytes, and microgliocytes did not change significantly. After 14 and 21 injections of A-II capillaries with edema of the endothelial cells appeared. Inclusions with a lamellar structure (Fig. 3) were found in the cytoplasm of the endothelial cells. This phenomenon was evidently connected either with destruction of the intracellular organelles and phagocytosomes or with disturbance of metabolism of the components of the cell membranes.

The continuing process of accumulation of fluid in the pericapillary glia during repeated injections of A-II is evidence that diffusion of plasma from the blood stream took place more rapidly than the opposite process of its absorption; consequently liquid accumulated in the pericapillary glia.

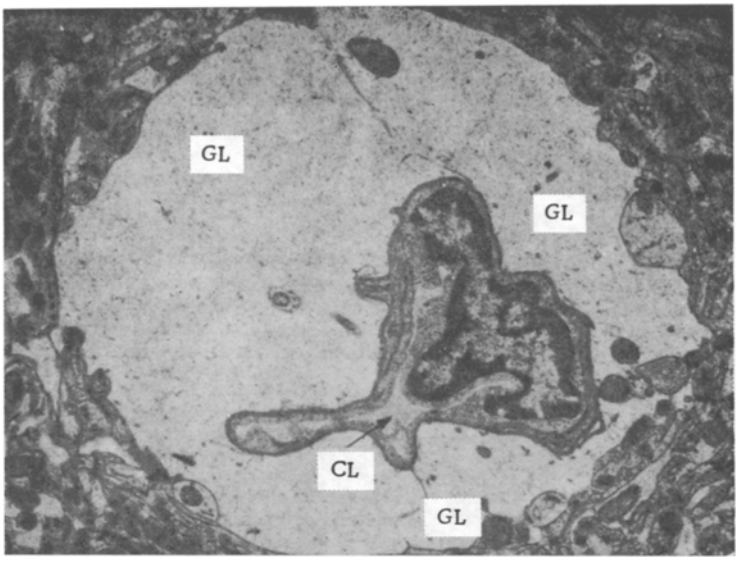


Fig. 2. Blood capillary in rat brain after 14 injections of angiotensin II. Marked edema of pericapillary processes of astrocytes (GL). Compression of walls and constriction of capillary lumen (CL); 6000 ×.

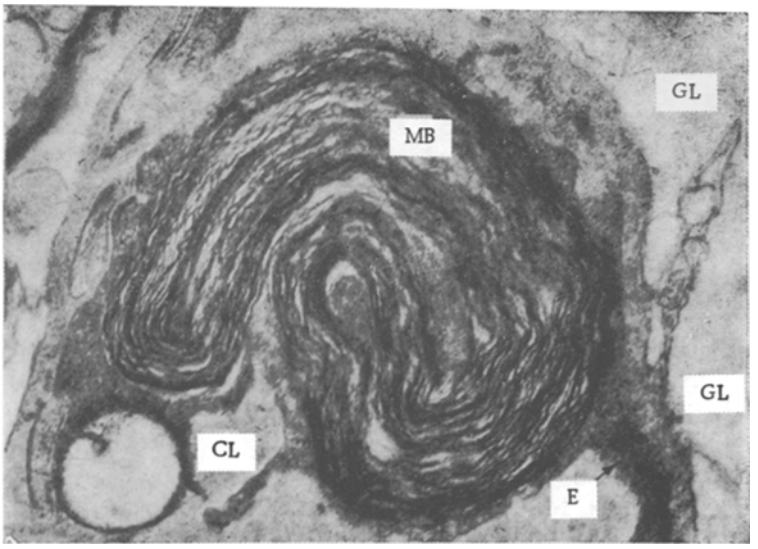


Fig. 3. Endothelial cell (E) of capillary (CL) from sensomotor cortex of rat after 21 injections of angiotensin II. Membranous body (MB) in cytoplasm of endothelial cell. Glial processes (GL) in contact with basal layer of capillary; 30,000 ×.

Near the large vessels, whose walls contain smooth-muscle cells, the glia was unchanged. The lumen of the large vessels, especially 5 min after injection of A-II, was mainly constricted. Many arterioles also remained constricted 15 min after injection of A-II.

Repeated injections of A-II thus led to disturbance of glial—vascular interrelations and to cerebrovascular insufficiency, which was bound to affect glial—neuronal responses. This may perhaps also explain the long-lasting disturbance of functions of the CNS observed in animals under the influence of A-II.

## LITERATURE CITED

1. M. M. Aleksandrovskaya, Vascular Changes in the Brain in Various Pathological States [in Russian], Moscow (1955).

2. I. P. Levshina, K. Hecht, M. Poppei, et al., Zh. Vyssh. Nerv. Deyat., No. 2, 363 (1977).
3. M. Ya. Maizelis, The Blood—Brain Barrier and Its Regulation [in Russian], Moscow (1973).
4. P. E. Snesarev, Theoretical Basis of the Pathological Anatomy of Mental Diseases [in Russian], Moscow (1950).
5. K. V. Sudakov, V. V. Sherstnev, and S. A. Osipovskii, Byull. Éksp. Biol. Med., No. 8, 899 (1976).
6. P. Glees, Dtsch. Z. Nervenhilk., 184, 607 (1964).
7. H. Thurston, Am. J. Med., 61, 768 (1976).
8. E. S. Reynolds, J. Cell Biol., 17, 208 (1963).