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Spontaneously hypertensive rat neuroanatomy: applications to pharmacological research

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Summary

Spontaneously hypertensive rats (SHR), which are normotensive at birth and develop sustained hypertension between 3 and 6 months of age, are the model most extensively investigated for evaluating hypertensive brain damage and its treatment. The time-dependent rise of arterial blood pressure and the occurrence of brain atrophy, loss of nerve cells and glial reaction are shared to some extent with what occurs human hypertensive brain. SHR, therefore, can represent a reasonable model of hypertension-related brain damage. Our main studies on cerebrovascular and brain microanatomical changes occurring in SHR and their sensitivity to pharmacological interventions are summarized.

Key words

Hypertension, brain damage, experimental model

Spontaneously hypertensive rats (SHR), which are normotensive at birth and develop in the first 6 months of life a sustained hypertension, currently represent the best model of essential hypertension allowing for the investigation of causes, mechanisms and pathology of hypertension (Amenta et al., 2003).

In SHR brain, ventricular enlargement, accompanied by loss of brain tissue and brain weight, and decrease in volume of grey matter have been reported (Tajima et al., 1993; Lanari et al., 2007). In 6 to 7-month-old SHR, the ventricular volume is twofold greater than in Wistar-Kyoto (WKY) control rats. The volume of the entire brain and of different gray matter areas as well as the thickness of cerebral cortex are 11-25% less in SHR (Fig. 1, A-D) than in wild type animals. The hippocampus, a key area for learning and memory, is smaller in 6-month-old SHR than in normotensive cohorts (Sabbatini et al., 2002), and the same is true for the density of cell nuclei in the dentate gyrus subfield in SHR aged 6-7 months (Tajima et al., 1993) (Fig. 1, E, F).

Cerebrovascular alterations characterized by blood vessel wall hypertrophy or remodeling are closely related to the degree of blood pressure elevation. Hypertrophy consists in thickening of arterial wall with decrease of internal diameter, increase of external diameter and consequent reduction of lumen size. Remodeling consists of smooth muscle rearrangement with decrease of external and internal diameters and of lumen size (Amenta et al., 2003). These changes cause an increase of the wall-to-lumen ratio and consequently of arterial resistance. In the frontal cortex of 24-weeks-old SHR the vascular tree displayed wall hypertrophy and luminal narrowing (Fig. 1, G, H) while in the striatum, the increase of wall area was not accompanied by lumi-

nal narrowing (Amenta et al., 2003). In hippocampal arteries of SHR, luminal narrowing was not accompanied by changes in wall area (Amenta et al., 2003).

Different central nervous system injuries are accompanied by proliferation and/or hypertrophy of astrocytes. An increased expression of glial fibrillary acidic protein (GFAP), a cytoskeletal protein that represents a selective marker for mature astrocytes, was reported in association with aging or disease (Ridet et al., 1997). A significant increase of GFAP mRNA and an increase of GFAP immunoreactivity were noticed in frontal cortex, striatum and hippocampus of 6 months old SHR compared to normotensive WKY animals of the same age (Fig. 1, I-N). The occurrence of astrogliosis in the brain of SHR suggests that hypertension induces a condition of brain suffering sufficient to increase the biosynthesis and expression of GFAP similar to that reported for brain ischemia.

Lowering blood pressure markedly reduces the incidence of stroke. SHR have been used in pharmacotherapy research to assess the effect of different neuroprotective treatments in stroke or hypertensive brain damage. Positive effects of Ca^{2+} channel blockers on hypertension-dependent cerebrovascular and brain changes in SHR were confirmed by comparing lercanidipine, manidipine and nimodipine, and the non-dihydropyridine-type vasodilator agent hydralazine (Sabbatini et al., 2001). Although evidence for brain protection from dihydropyridine-type Ca^{2+} channel blockers in brain ischemia is not conclusive, both nicardipine and lercanidipine effectively countered hypertensive brain damage and cerebrovascular impairment in SHR.

Changes in some specific neurotransmitter systems that may have functional and behavioral relevance occur in SHR. Impairment of cerebral cholinergic system is documented both in SHR and in stroke prone SHR (SHR-SP). In SHR-SP a decrease in cerebrocortical, hippocampal and cerebrospinal fluid levels of choline and acethylcholine (ACh) was found (Kimura et al., 2000). Changes of ACh levels in cerebral cortex and hippocampus are related to impaired learning and memory (Kimura et al., 2000). More recent studies have assessed the expression of vesicular ACh transporters (VAChT) in 4 and 15-month-old SHR (Hernandez et al., 2003) and in 6-month-old SHR (Tayebati et al., 2008). A slight increase of VAChT expression in the hippocampus of the older age SHR groups investigated was reported (Fig. 1, O, P) (Hernandez et al., 2003; Tayebati et al., 2008). Treatment with the cholinesterase (ChE) inhibitor rivastigmine induced a more pronounced increase in VAChT in SHR than in WKY controls (Tayebati et al., 2008). This suggests a greater than normal sensitivity of the cholinergic system to pharmacological manipulation in this hypertensive strain.

Brain cholinergic system impairment in SHR can be related to altered performance in memory, conditional avoidance and spatial learning tasks (Meneses et al., 1996). SHR exhibit also behavioural abnormalities, such as hyperactivity, lower attention, inhibition deficit and hyper-reactivity to stress, that resemble behavioral abnormalities of human attention-deficit with hyperactivity disorder (ADHD). Because dopamine has been implicated in the pathogenesis of ADHD, possible disorders of this system have been largely investigated in SHR (Russell et al., 2007). In SHR, terminals of mesocortical, mesolimbic and nigrostriatal dopaminergic neurons release less dopamine than in WKY in response to electrical stimulation and/or to depolarization resulting from exposure to high extracellular K^+ concentrations (Russell et al., 2007). Dopamine transporter (DAT) and vesicular storage of dopamine is impaired in SHR. DAT gene expression is transiently reduced in SHR midbrain during the first postnatal month

and increased in adult SHR compared with controls. Alterations in DAT gene expression can affect dopamine uptake and reutilization. The noradrenergic system is also hyperactive in SHR, particularly in response to stress (Russell et al., 2007). This causes an imbalance between noradrenergic hyperfunction and dopaminergic hypofunction.

SHR have also been used to assess the potential protective effects of non-hypotensive pharmacological treatments, including cholinergic neurotransmission enhancers, the such as the cholinergic precursor choline alphoscerate (GPC), ChE inhibitor galantamine (GAL) and the two drugs in association. Although the drugs under discussion did not change arterial blood pressure, treatment of male SHR at the age of 4 months with GPC for 8 weeks countered nerve cell loss and glial reaction primarily in the CA1 subfields and in the dentate gyrus of the hippocampus (Tomassoni et al., 2006). Treatment with GAL and GPC countered nerve cell loss. GPC but not GAL, decreased astrogliosis and restored the expression of the blood-brain barrier marker aquaporin-4 (AQP4). GAL countered phosphorylated neurofilament breakdown to a greater extent than GPC. Simultaneous administration of GAL and GPC resulted in potentiation of treatment effect (Tayebati et al., 2009). This suggests that, similar to what reported for adult-onset dementia disorders of vascular origin, cholinergic neurotransmission enhancing drugs tested may have an activity in countering brain damage associated with cerebrovascular injury.

Conclusion

To sum up, brain alterations in SHR and in hypertensive patients are probably the consequence of structural changes of cerebral arteries, resulting in augmented arterial resistance leading to cerebral hypoperfusion (Sabbatini et al., 2001). This can induce changes in cerebral morphology. These modifications suggest that SHR may represent, from a morphological point of view, a model of vascular brain disorder sensitive to pharmacological treatment.

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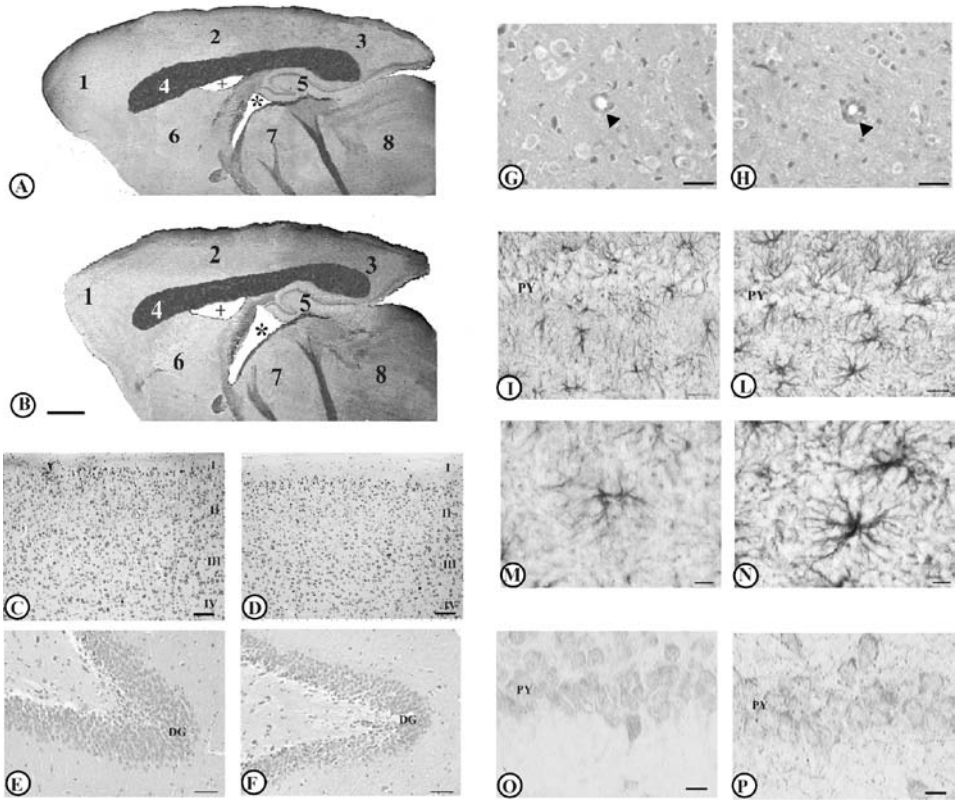
Figure



A,B: Parasagittal sections of brain from a 6-month-old male WKY rat (A) and an age-matched SHR (B) stained with Luxol fast blue. Note in the SHR the enlargement of the lateral ventricle (+) and of the third ventricle (*), a decreased thickness of cerebral cortex (2) and a reduced size of corpus callosum (4). 1:frontal cortex;3: occipital cortex;5: hippocampus; 6: striatum; 7:dienchephalon; 8: midbrain (modified from Lanari et al., 2007). Calibration bar 1.5 mm.

C-F: Sections of frontal cortex (C,D) and dentate gyrus (E,F) of WKY rats (C,E) and SHR (D,F) stained with Nissl's technique. Note in SHR the reduced number of neurons in zone II, III and IV of frontal cortex and in dentate gyrus (DG) (E and F modified from Tomassoni et al., 2006). Calibration bar 50 μm (C,D), 25 μm (E, F).

G-H: Pictures of small sized (diameter range 25-10 μm) intracerebral arterioles of a WKY rat (G) and SHR (H) stained with Masson's trichrome. Note in SHR the increased thickness of the arterial wall (arrow heads) and the decrease in lumen area. Calibration bar 25μm.

I-N: GFAP immunohistochemistry in the CA1 subfiels of a 8-month-old WKY rat (I,N) and an aged-matched SHR (L,N). GFAP-immunoreactive astrocytes appeared as dark-brown cells. Note in the SHR the higher number of GFAP-immunoreactive astrocytes in the white matter and the increase of the size of single astrocyte. Calibration bar 25μm (I, L), 10 μm (M, N).

O-P: VAChT immunohistochemistry of a 6-month-old WKY rat (O) and an aged-matched SHR (P) in the CA1 subfield of hippocampus. Note, in the SHR, the increase in immunoreaction product in cholinergic fibers that supply the pyramidal neurons (PY). Calibration bar: 10 μm.