EFFECT OF EXPERIMENTAL SUBARACHNOID HEMORRHAGE ON THE SYMPATHETIC NERVES OF CEREBRAL ARTERIES, INVESTIGATED WITH WGA-HRP ANTEROGRADE TRACING IN THE RAT

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SUMMARY :

The effect of subarachnoidal hemorhage (SAH) on sympathetic fibers running on the cerebral vessels have been studied by histochemical staining methods which were unable to clarify the functional status of nerve fibers. WGA-HRP, a neurotracer which can be transported anterogradely and retrogradely gives functional status as well as anatomical details of the nerve fibers. In this study WGA-HRP was used to clarify the effect of SAH on the function and anatomy of sympathetic fibers on the cerebral vessels. SAH was produced by introducing 1 microliter/kg homologous fresh arterial blood to the cisterna magna of rats. The following day WGA-HRP was injected into the superior cervical ganglion. Three days later the rats were sacrificed, their circle of Willis and Pineal body were dissected. After TMB reaction the whole mount circle of Willis and Pineal body were examined under the light microscope. The results demonstrated that SAH affected the release and maybe reuptake of neuronal transmitters rather than anatomical damage and axonal transportation.

KEY WORDS :

Subarachnoid Hemorrhage, Sympathetic system, WGA-HRP

INTRODUCTION

Cerebral vasospasm is one of the most important complications after subarachnoid hemorrahage (SAH) from an intracranial aneurysm. The role of catecholaminergic perivascular nerve fibers of cerebral arteries in the pathogenesis of SAH is still obscure. There are few studies concerning the effect of SAH on the activity of sympathetic nerve fibers which might help to explain this role. In these studies, it has been demonstrated that SAH transiently decreases the histochemical fluorescence of the adrenergic nerve plexus courses on the cerebral arteries (2, 3, 4, 11, 12, 13, 14, 17). The decrease in immuno reactive perivascular nerve fibers at the initial stage after SAH may be due to damage to the immuno reactive neurons, arrest of axonal transportation, excessive release or/and block of re-uptake of the neuropeptides or their decreased production (17). Since the studies were all performed by the histochemical immunostaining procedure, it is not possible to have confirmatory evidence for these speculations. On the other hand, wheat germ agglutinin combined with horseradish peroxidase (WGA-HRP) has been used as a highly sensitive neurotracer which is able to label the entire course of the axon, neuron to its terminal (15). It can be transported transneuronally, anterogradely and retrogradely (1, 7). This axonal transportation reflects the physiological activity of the neurons and their synaptic connections (6, 11, 12).

In the present study, we achieved anterograde labelling of the sympathetic nerve fibers originating from the superior cervical ganglion (SCG) in rats with SAH and were able to clarify the effect of experimental SAH on the activation of sympathetic nerves on the cerebral arteries and the pineal body.

MATERIAL AND METHOD

Seventeen male albino rats of the Spraque-Dawley strain weighing 350-450g were anesthetized with intraperitoneal sodium pentobarbital (35 mg/kg body weight). In nine rats, SAH was produced as follows; 1ml/kg body weight fresh autologous blood was obtained from the femoral artery, and introduced into the cisterna magna through a cannula which was inserted several days before through a midline burr hole anterior to the interparietal occipital suture. In seven other rats a sham operation was performed. The rats were treated in the same way as those undergoing SAH except that they received a single

injection of 0.9 % saline (1ml/kg body weight) into the cisterna magna.

Twenty-four hours later the rats were re-anesthetized and supinely mounted in a stereotactic apparatus. The unilateral SCG received an injection of 1 microliter of 3 % WGA-HRP (Toyobo, Tokyo, Japan) in 1 M KCI solution colored with brilliant blue, through a glass micropipette (the outer diameter of the tip was 50 micrometer) using the pressure of N2 gas cylinder (10). This procedure was performed under an operation microscope. Four rats (two Sham operated the others with SAH) were allowed to survice 24 hours. The other 13 rats were kept alive for 48 hours after the WGA-HRP injection. At the time of sacrifice they were deeply anesthetized with an overdose of pentobarbital and perfused transcardially with 300 ml of 9 % saline and consequently with a fixative containing 2 % paraformaldahyde, 0.5 % glutaraldehyde and 0.1M phosphate buffer (pH 7.4) for 3 minutes.

After perfusion the circle of Willis and pineal body were dissected out under the operation microscope, soaked in a fixative solution for an hour, and then immersed in sucrose buffer solution overnight. The whole mount circle of Willis and pineal body were reacted according to the tetramethylbenzidine protocol of Mesulam (9). The circles of Willis were mounted on gelatin-coated slides, and after being air dried covered with microglasses. The pineal bodies were observed under the microscope just after the reaction.

The intensity of labelling and density (the number of labelled fibers per square micrometer) of the sympathetic fibers and their course on the vessels and pineal body were observed.

RESULTS

In all the rats injected with fresh autologous arterial blood into the cisterna magna and allowed to survive 2 and 4 days, SAH was confirmed by gross inspection at the time of sacrifice by the presence of subarachnoid clot and/or xantochromic staining of the arachnoid and pial membranes.

In the sham operated rats, well labelled perivascular nerve fibers were seen on the circle of Willis and pineal body. These were substantially the same as those in the rats which were injected WGA-HRP for the intracranial trajectories of these fibers in previous studies (Fig. 1).

Fig.1 : Distribution of the labelled sympathetic fibers on the internal cerebral artery in sham operated rat. (12X10 mag.)

In the rats with SAH, the intensity and density of labelled sympathetic nerve fibers was significantly reduced (Fig. 2). Especially these findings were more prominent on the basilar artery where subaracnoidal clots were frequently observed. Although the appearance of labelling was weaker than in the sham operated cases, it was possible to observe the terminal branches of these sympathetic fibers in all cases (Fig. 3). On the other hand, well labelled sympathetic fibers were observed on the pineal body and there was no difference in appearance compared to the sham operated cases.

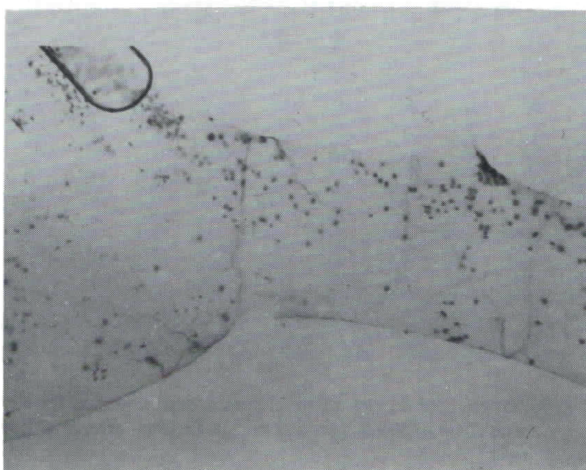

Fig.2 : Intensity and density of labelled sympathetic fibers on the internal cerebral artery after SAH. (12X10 mag.)

Only in one case with SAH, was labelling of the contralateral sympathetic fibers on the anterior part

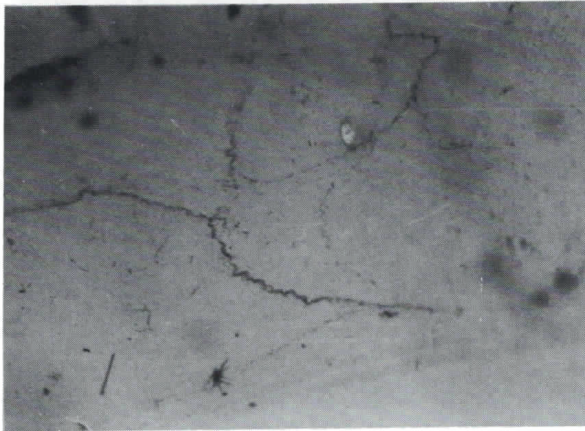

Fig. 3 : The terminal branches of sympathetic fibers on the middle cerebral artery in case of SAH. (12X20 mag.)

of circle of Willis as far as the left middle cerebral artery observed (Fig. 4).

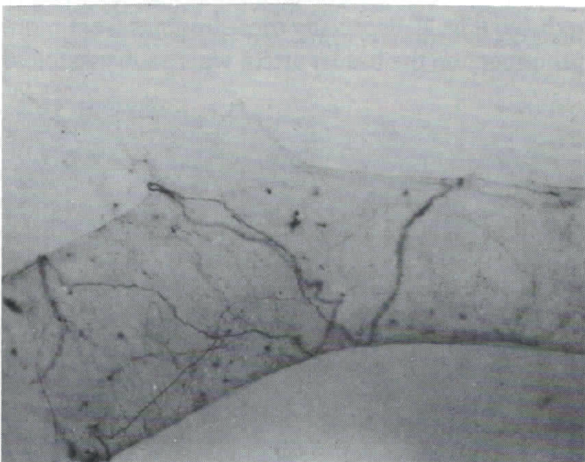

Fig. 4 : Labelling of the contralateral sympathetic fibers on the left side circle of Willis near the middle cerebral artery. (12X10 mag.)

DISCUSSION

In the present study, the new neurotracer agent WGA-HRP was used to examine the physiological activity of sympathetic nerve fibers on the vessels of circle of Willis and pineal body in the post-SAH condition. In previous studies concerning the effect of SAH on the sympathetic nerves on the cerebral arteries, immunohistochemical staining techniques were used for the histopathological examination (4, 5, 11, 12, 13, 17). Lobato et al used another index to show the adrenergic innervation during the SAH by examining the ability of cerebral arteries to take up and retain tritiated noradrenaline content and dopamine beta-hydroxylase activity. All these studies clearly showed that SAH induced a transient adrenergic denervation and suppressed the immuno reactivity of perivascular nerve fibers. Although confirmatory evidence was lacking, it was suggested that the most probable cause of this decreased activity was the excessive release or/and block of re-uptake of the neuropeptides at perivascular axon terminals (6). Our findings also showed that SAH induced an incomplete adrenergic denervation of the cerebral arteries, furthermore, we clearly demostrated that there was no arrest of axonal transportation or anatomical damage of sympathetic fibers, because it was possible to trace the sympathetic nerve fibers as far as their terminal brach, 24 hours after WGA-HRP injection in two rats with SAH. The 24-hour interval was enough to obtain well labelled terminal fibers in the sham operated cases.

Reduced intensity and density of labelling of the sympathetic fibers might be explained by extensive release of dopamine and incomplete block of re-uptake due to decreased activity of dopamine beta hidroxylase(11). Although it is difficult to explain the contralateral labelling of the circle of Willis which was seen only in one rat, we suggested that the block of re-uptake of WGA-HRP was not prominent, so big amounts of WGA-HRP could be re-uptaken by contralateral sympathetic fibers in this case.

The reason why almost the same density and intensity of labelled sympathetic nerve fibers were seen on the pineal body, might be the course of the sympathetic fibers which approach the pineal body through the tentorium where they can not competely contact the subarachnoid space.

In conclusion, using WGA-HRP which can reflect the physiological activity of sympathetic nerve fibers on the brain arteries, it was demonstrated that SAH mainly affects the release and may be the re-uptake activity of sympathetic fibers rather than axonal transportation or anatomical damage.

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