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KEYWORDS: cerebral vasospasm, noradrenaline, subarachnoid hemorrhage.

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MORPHOLOGICAL STUDY ON EXPERIMENTAL CEREBRAL VASOSPASM
II. FLUORESCENCE MICROSCOPIC EXAMINATION ON EXPERIMENTAL CEREBRAL VASOSPASM

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Abstract. Basilar arteries with experimental vasospasm were studied histochemically using a catecholamine fluorescence technique in 15 cats. Fluorescence microscopy of normal vessels revealed abundant catecholamine fluorescence in the adventitia and in close contact with the media, but none within the media. Experimental subarachnoid hemorrhage resulted in depletion of catecholamine fluorescence in the adventitia and in its appearance in the media. In vessels with vasospasm which lasted for 2 or 5 h and responded to vasodilators, fluorescence was scarce in the adventitia, but abundant in the media. In vessels with vasospasm unresponsive to spasmolytic agents, fluorescence was observed neither in the adventitia nor in the media. The results suggest that noradrenaline in the media of spastic arteries plays an important role in the development of cerebral vasospasm.

Key words: cerebral vasospasm, noradrenaline, subarachnoid hemorrhage.

The presence of noradrenergic fiber plexus in major intracranial vessels has been confirmed by morphological studies, utilizing a catecholamine fluorescence technique and electron microscopy (1–5). Catecholamine fluorescence in the noradrenergic fiber plexus of vessel walls disappeared after subarachnoid hemorrhage (SAH) in experimental models (4, 5). High plasma levels of catecholamine have been observed in clinical cases with SAH (6, 7). From these results, denervation supersensitivity of vessels after SAH was proposed as the pathogenesis of late vasospasm (8, 9).

However, it has been reported that dopamine-β-hydroxylase activity and the amount of noradrenaline in cerebral vessel walls increased 3 days after intracisternal injection of fresh autogenous arterial blood, when marked vasospasm was present angiographically (10).

Part 1 of this study demonstrated that granules and vesicles ranging from 500 to 2,000 Å in diameter appeared in the media of vessel walls with prolonged

vasospasm, and that some of them (500 Å in diameter) were morphologically similar to the vesicles in adrenergic nerve terminals (11).

This study was designed to clarify the topographical and quantitative changes of noradrenaline in vessel walls with experimental vasospasm using 'Falck-Hillarp's highly sensitive histochemical method'.

MATERIALS AND METHODS

Fifteen adult cats, weighing 3.0–3.5 kg were used for the experiments. Prolonged vasospasm was produced by topical application of a solution containing serotonin (100 μg/ml) on the exposed basilar arteries. The responsiveness of these arteries to vasodilating agents, such as methylprednisolone and L-ascorbic acid, was examined in 10 cats according to the method previously reported (11).

Reversible vessels, in which vasospasm continued for 2 or 5 h but was relieved by topical application of methylprednisolone (10 mg/ml) or L-ascorbic acid (25 mg/ml), and irreversible vessels in which vasospasm lasted for 10 h and was not relieved by such agents, were examined histochemically using a catecholamine fluorescence technique.

Experimental subarachnoid hemorrhage was produced by injection of 2 to 3 ml of fresh autogenous arterial blood into the cisterna magna following evacuation of an equal volume of cerebrospinal fluid in 3 cats. In these cats, catheterization of the femoral artery was performed 3 days after SAH and vasospasm of the basilar artery was confirmed by angiography before the cat was killed. Basilar arteries with experimental SAH were also studied histochemically, and were compared with vessels with vasospasm due to serotonin.

Cannulation of the descending aorta was performed after thoracotomy. The superior vena cava was divided. Vascular perfusion was done through the cannula with isotonic saline at 0°C. A basilar artery was immediately excised after craniectomy in a cold room at 4°C. The specimen was frozen in iso-pentane, cooled by dry ice-acetone mixture and kept at −80°C until dehydration. The specimen was dehydrated in a vacuum at −35°C for the first 4 days, −20°C for the next day, +35°C for 2 h and +50°C for 1 h. After thorough drying, the specimen was exposed to formaldehyde gas at +80°C for one and a half h, then embedded in paraffin at +60°C. Sections (10–12 μ) were placed on non-fluorescent slide glasses and deparaffinized by the addition of xylene to the glass.

Sections were examined under a fluorescence microscope (Olympus) equipped with a dark-field condensor, and photographs were taken with Kodak Ektachrome 200. The exciting light was provided by an Osram HOB 200 high pressure mercury lamp and was filtered through Schott BG 12 and Zeiss 50.

RESULTS

Normal basilar artery. The vessel walls of normal basilar arteries were studied by fluorescence microscopy in 2 cats.

Fluorescence microscopy revealed the presence of green fluorescence of various densities representing adrenergic transmitter in sympathetic nerves of

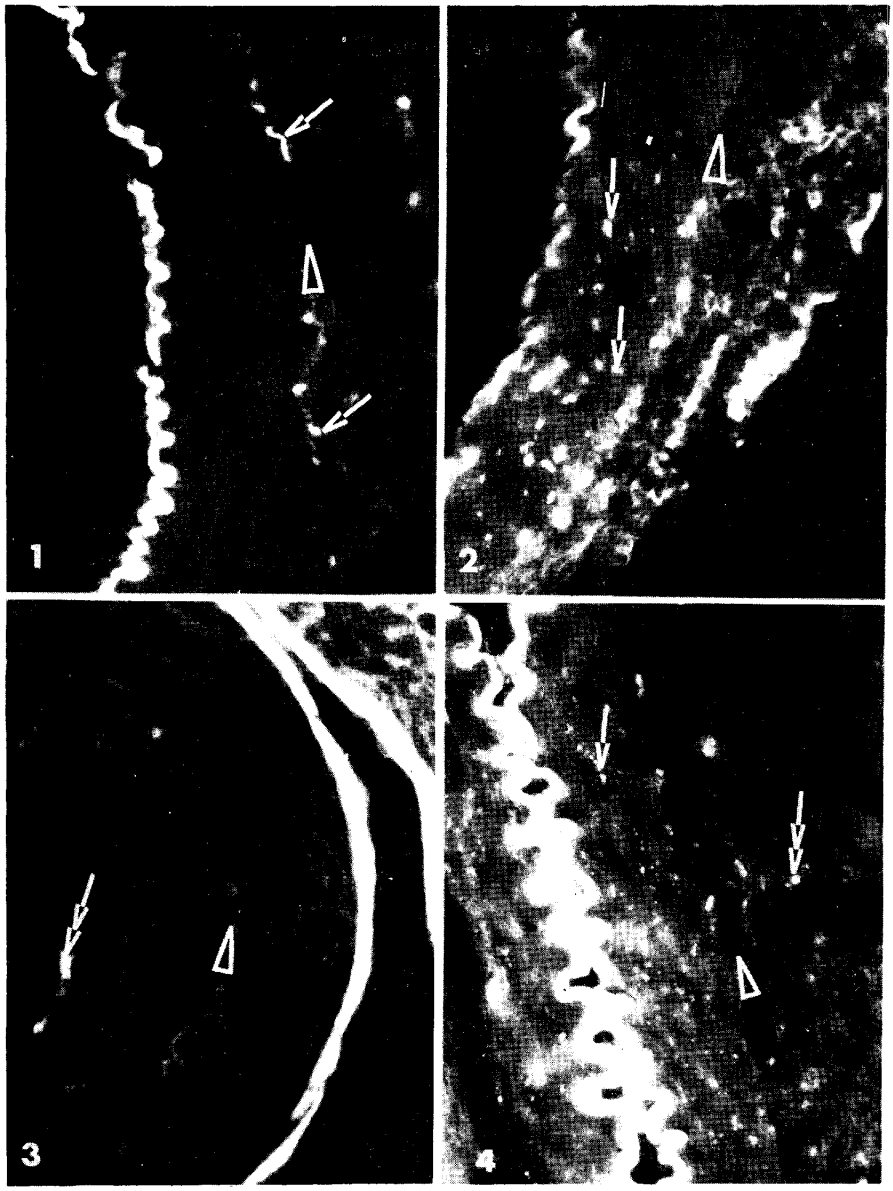

Fig. 1. Fluorescence micrograph of normal feline basilar artery. Varicose lines and spots representing noradrenaline (—>) are confined to the adventitial-medial border (▷). The internal elastic lamina shows a corrugating autofluorescence. ×400.

Fig. 2. Fluorescence micrograph of basilar artery in spasm (reversible state). Note accumulation of catecholamine in the media (—>) and decrease of catecholamine fluorescence in the adventitia. Adventitial-medial border (▷). ×400.

Fig. 3. Fluorescence micrograph of basilar artery in spasm (irreversible state). Catecholamine fluorescence is not observed in the vessel wall. Orange fluorescence arising from serotonin in platelets is observed in the adventitia (—>>). Adventitial-medial border (▷). ×400.

Fig. 4. Fluorescence micrograph of basilar artery with experimental subarachnoid hemorrhage. Catecholamine fluorescence is abundant in the medial layer (—>) but scarcefound in the adventitia (—>>). Adventitial-medial border (▷). ×400.

the vessel wall. The fluorescence appearing as varicose green lines and spots was present in the adventitia and at the adventitial-medial border, but not within the medial layer.

In the adventitia, orange fluorescence was also observed. This was thought to arise from serotonin in platelets. The internal elastic lamina exhibited a regularly corrugating autofluorescence (Fig. 1).

Basilar artery with spasm in the reversible state. Specimens were obtained from basilar arteries with vasospasm lasting for 2 h in 4 cats and for 5 h in another 4 cats.

Green catecholamine fluorescence was scarce in the adventitia of reversible vessels. However, abundant green fluorescent lines and spots were observed in the media of all cats examined. In a few vascular preparations, these fluorescent lines and spots were localized beneath the internal elastic lamina, but they were diffusely existent throughout the media in most vascular preparations. (Fig. 2)

There was no difference in these findings between vessels with vasospasm lasting for 2 h and for 5 h.

Basilar artery with spasm in the irreversible state. Basilar arteries with irreversible spasm were examined in 2 cats.

Fluorescence microscopy revealed a complete absence of catecholamine fluorescence in both adventitia and media of vessel with irreversible spasm. (Fig. 3).

Basilar artery with experimental subarachnoid hemorrhage. Intracisternal injection of fresh arterial blood was performed in 3 cats. Vasospasm was angiographically confirmed 3 days after experimental SAH.

These spastic vessels revealed reduction of catecholamine fluorescence in the adventitia, while an abundant catecholamine green fluorescence was observed in the media of these vessel wall. These findings were the same as those of vessels with reversible vasospasm. (Fig. 4)

DISCUSSION

There have been many physiological and morphological investigations of the innervation of intracranial blood vessels using nerve stimulation, light microscopic technique, fluorescence microscopy and electron microscopy (1–5, 12, 13).

Electron microscopy revealed adrenergic nerve endings in close contact with the lateral surface of the media in intracranial vessels treated with 6-hydroxydopamine (14).

Fluorescence microscopy of normal intracranial major vessels has also revealed abundant green fluorescent fiber plexuses representing adrenergic nerves in the adventitia, but none within the media (5, 14). The fluorescence of these

fiber plexuses has been observed to be depleted following subarachnoid hemorrhage, although the preparations were obtained from vessels cut open longitudinally and transverse sections were not examined (4, 5).

In this study, fluorescence microscopy revealed green fluorescence representing noradrenaline in the adventitia of normal feline basilar arteries. The catecholamine fluorescence decreased in the adventitia of vessels with reversible vasospasm and with experimental SAH. However, fluorescence microscopy of transverse sections revealed green catecholamine fluorescence within the media of these vessels.

Morooka (10) reported that the amount of noradrenaline and dopamine-β-hydroxylase activity increased in the vessel wall 3 days after intracisternal injection of fresh autogenous arterial blood, and that these vessels were more sensitive than untreated vessels to noradrenaline. Increased noradrenaline in the vessel wall following experimental SAH, as reported in Morooka's study, might be present in the media but not in the adventitia, and result in the hyperreaction of cerebral vessels to not only noradrenaline but also other spasmogenic substances.

In part 1 of this study (11), the granules and vesicles ranging from 500 to 2,000 Å in diameter were observed in the extracellular space of the medial layer of reversible vessels. Granules and vesicles 500 Å in diameter morphologically resembled the vesicles in adrenergic nerve endings. Although these granules and vesicles were present in vessels with irreversible spasm, catecholamine fluorescence was completely absent. This result suggests that granules and vesicles do not contain noradrenaline.

Noradrenaline is released from nerve endings by nerve stimulation and binds temporarily to the receptors of smooth muscle cells and/or is taken up again into the nerve ending. Blood in the subarachnoid space destroys the ability of adrenergic vesicles in nerve endings to take up free noradrenaline (15). Part of the noradrenaline released from adrenergic nerve endings is taken up by smooth muscle cells, and most of them are readily degraded by catechol-O-methyl transferase (COMT) and/or monoamine oxidase (MAO) in the cytoplasm (16). It has been demonstrated by the use of the fluorescence histochemical technique of Falck and Hillarp that noradrenaline accumulated in smooth muscle cells of various organs loaded with noradrenaline (17, 18). This accumulation of noradrenaline in arterial smooth muscle cells was higher than that in visceral smooth muscle cells regardless of species differences, although there were species differences in the extent of the accumulation of noradrenaline in smooth muscle cells (17).

This study showed that noradrenaline fluorescence accumulated in the media, but decreased in the adventitia of vessels with reversible spasm and

experimental SAH. The noradrenaline fluorescence in the media was thought to be within the smooth muscle cells. Complete depletion of the noradrenaline fluorescence was also observed in the vessel wall with irreversible spasm where myonecrosis was observed by electron microscopy in part 1 of this study (11). To explain the increase of noradrenaline in spastic arterial wall responsive to spasmolytic agents, the following is proposed: Serotonin and blood around vessels of the cerebral base release noradrenaline from adrenergic nerve endings by nerve stimulation. The noradrenaline and other spasmogenic substances liberated from subarachnoid blood cause muscle contraction by stimulation of receptors of smooth muscle cells. Blood in the subarachnoid space also destroys the function of adrenergic vesicles in nerve endings to take up free noradrenaline from the neuromuscular gap. Excessive extracellular noradrenaline may be taken up into smooth muscle cells, and accumulated in the cells when noradrenaline degradation is impaired as a result of marked vascular contraction.

Increase of noradrenaline in the media of vessel walls may lead to hypersensitivity of the vessels to spasmogenic substances and result in sustained vasospasm. Subsequently, myonecrosis due to prolonged vasospasm may cause irreversible changes such that the vessels fail to respond to any kind of pharmacological agent.

Though it is still unclear which part of the media contains noradrenaline or where the noradrenaline comes from, noradrenaline in the media of spastic arteries appears to play an important role in the development and prolongation of cerebral vasospasm.

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