Ultrastructural characteristics of spasm in intracerebral arterioles

WOLFGANG ROGGENDORF, JORGE CERVÓS-NAVARRO

From the Institut für Neuropathologie, Klinikum Steglitz der Freien Universität Berlin (West)

SUMMARY  Following craniotomy, three groups of cats were subjected to three different stimuli: group A hyperventilation, group B electroshock, and group C direct electric current. During electric stimuli, pial vessels were observed through a cranial window. Immediately after electric current application, some arterial vessels showed segmental spastic constriction. Tissue samples for electron-microscopy were taken from the parietal lobe and nucleus caudatus. In all three groups of animals, different types of constriction of blood vessels were observed. The respiratory alkalosis achieved by hyperventilation led to physiological constriction of the arterioles. The electric stimuli led to spastic constriction of the meningeal and intracerebral arteries and arterioles in group B and C; the entire vessel wall was greatly deformed and the vessel lumen was almost obstructed. Electroshock resulted in only moderate structural changes of the smooth muscle cells. Direct current, however, caused an extreme and bizarre smooth muscle deformation. The results show that spastic constrictions of arterioles can be clearly distinguished from physiological, that is non-spastic constriction, by morphological parameters. Electric stimulation of cerebral vessels could be an experimental condition for further investigation of intracerebral vasospasm.

Spastic constriction occurs in larger intracranial arteries and may influence cerebral circulation, especially after aneurysmal rupture followed by subarachnoid haemorrhage, as shown by clinical and experimental investigations by Echlin, Wilkens et al., and Peterson et al.¹⁻² Ultrastructural studies by Matakas et al.³ and Cervós-Navarro et al.⁴ have revealed spastic constrictions also in intracerebral arterioles under various experimental conditions. The purposes of this investigation were (1) definition of morphological criteria for identification of contraction of smooth muscle cells in blood vessel walls under physiological conditions, and (2) to determine whether spastic contraction produced by various electrical stimuli was accompanied by specific morphological changes.

Material and methods

For this study we used 21 adult cats (2-3 kg), which were anaesthetised with intramuscular sodium pentobarbital (Nembutal 25 mg/kg bw). A catheter was inserted into the aorta and into the inferior vena cava through the femoral artery and vein, respectively. Arterial blood pressure was monitored and remained at normal levels throughout the experiments. After relaxation with succinyl-bis-choline (2 mg) and intubation, the animals were artificially ventilated. Blood gases were kept normal (arterial pressure of CO₂ 35-45 mm Hg, pH 7-35, arterial pressure of O₂ 90-110 mm Hg).

The cats were divided into 3 groups:

Group A  Respiratory alkalosis in nine cats was achieved by hyperventilation for 20 min (82 ml, frequency 28-33/min) (Cervós-Navarro et al.⁴).

Group B  Electroconvulsive treatment was applied in six cats after craniotomy. Two metal electrodes, each with a diameter of 10 mm, were attached to the temporal muscle on each side. An alternating current of 220 V 50 Hz was applied for 500 ms at a time, two to five times within 5 minutes. After 20 minutes, the animals were killed by injecting 50 ml of India ink suspension within 10 s into the inferior vena cava.

Group C  For electric stimulation by direct current (DC) in six cats after craniotomy, one electrode (10 mm diameter) was attached to the right temporal muscle. The left electrode was an Agar-gel silver electrode with a plastic tube of inner tip diameter of about 0-20 mm. DC with 20-100 V for 2 to 30 s (20-150 ma) was applied. In two animals, two assessments were made. In four animals, three were made.
In group B and C, the animals were killed 20 min after electrical stimulation by injecting 50 ml of India ink suspension rapidly into the inferior vena cava.

A control group of 12 cats was used. Six of these cats were only normally ventilated. The remaining six cats were treated as group B and C, but the cerebral vessels were not subjected to electric stimulation. After treatment of 20 min (group A) or immediately after injection of India ink (group B and C), 5 mm³ blocks of parietal cortex and subcortical white matter and of the caudate nucleus were excised quickly for electron-microscopy. The brain was then removed and fixed in formalin. The tissue for electronmicroscopy was fixed for 2 hours in chilled 5% buffered glutaraldehyde, washed in buffered saccharose solution, postfixed in 1.0% osmium tetroxide, dehydrated and embedded in epon. The brain specimens in this study were fixed by immersion in order to prevent reduction of the spasm which might occur with fixation by perfusion under pressure. Immersion fixation was also necessary for the groups of experimental animals which were perfused with India ink in order to observe the no reflow phenomenon and the spastic constriction of arterioles along large segments.

The control brains of regularly ventilated cats were also fixed by immersion. Arterioles in these brains showed only a slight contraction evidenced by undulation of the basal lamina. Marked constriction or even spasm of arterioles was not observed in any of the control brains. There were no differences between normal cat brain arterioles fixed by immersion or by perfusion with regard to the appearance of the cellular organelles. In particular pinocytotic activity and filamentous components in endothelial cells were identical.

The gross and microscopic aspects of groups B and C after India ink injection have been described in detail by Matakas et al.² (we will give only a short summary). In some cortical areas, patches without blackening of India ink were observed, indicating that cerebral circulation was disturbed. In all cases, light-microscopically large arterioles and arteries varied in diameter and showed some segments completely spastic. The spastic segments were exclusively observed in the grey substance of the brain and in either the cortex or basal ganglia.

In the following description, we refer to spastic contraction when marked deformation of the entire vessel wall leads to a completely obstructed vessel in contrast to physiological contraction produced by alkalosis, in which the basic configuration of the vessel is retained.

Results

1 ENDOTHELIUM AND SUBENDOTHELIAL SPACE

In groups A, B, and C, oval to round endothelial cell nuclei covered by a thin rim of cytoplasm protruded far into the vessel lumen (fig 1). In the endothelial cell body away from the nuclear zone, slender, digital cytoplasmic extensions projected towards the vessel lumen (figs 1, 2).

Slightly increased endothelial pinocytosis was evident only in the first experimental group (physiological constriction after respiratory alkalosis). In both of the experimental groups of animals exposed to electric stimulation, no changes in endothelial pinocytotic activity were observed, possibly because of the short duration of the experiments. The endothelial tight junctions in dilated vessels ran diagonally and overlapped. In contracted arterioles (group A), the junctions usually ran perpendicular to the basal membrane (fig 2). Spicular cytoplasmic extensions containing clusters of 7 nm filaments were also seen, arising abluminally from the endothelial cells of
Fig 3 Microfilaments of the endothelium are especially close to the basal lamina and increase near myoendothelial junctions (→) (group A). Fixation by immersion. × 10285.

Fig 4 Spastic arteriole of the cortex (group B): cleft-like vessel lumen with particles of India ink (→). Widened perivascular space. India ink perfusion. Fixation by immersion. × 2640.

contrasted arterioles (fig 3). The basal lamina was widened. In the arterioles strongly or spastically contracted by electroshock and direct current (group B, C), the basal membrane appeared more homogeneous and less electron-dense than in non-contracted or simply contracted arterioles (figs 2, 5). A detailed description of the media and of the perivascular space will be given separately.

2 MEDIA
The media differed conspicuously in the three different groups. The muscle cells of a 1-2-layer media of intracerebral arterioles of cats of group A which showed simple contraction were characterised by: moderate deformation of the smooth muscle cell, with compression and indentation of the nucleus, the cell membrane was extremely scalloped and showed deformation of the prominent attachment devices, their broad bases were situated in the cell membrane and extended into the cell like a supporting belt. The cytoplasm with the pinocytotic vesicles lined up along the cell membrane bulged out between the attachment devices towards the abluminal side (figs 2, 7). Here the diameter of the media wall remained almost constant. Moreover, spastically contracted arterioles occurred occasionally with an almost completely obstructed vessel lumen. In animals of group A we occasionally found obstructed blood vessels in which deformation of endothelium, subendothelial space and smooth muscle cells followed the pattern defined above as spastic contraction. In arterioles of group B, the lumen was almost completely occluded while endothelial cells, subendothelial space and smooth muscle cells were only slightly scalloped. The nucleus of the smooth muscle cell appeared moderately scalloped. The abluminal membrane of smooth muscle cells was flattened in large segments. The triangular attachment devices were situated on the cell membrane without leading to the deformation characteristic of simple contraction. Occasionally there was a narrow patch of
Ultrastructural characteristics of spasm in intracerebral arterioles

Table

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Next to spastically contracted vessel sections with completely occluded lumina, one also found sections, where the vessel wall was only moderately infolded. The endothelium was flattened, the subendothelial space appeared only moderately undulated, and the media showed very slight deformation. The width of the vessel wall was nearly constant. The interconnection of the muscle cells by myomyal tight junctions did not differ from those in physiological contraction.

The spastically contracted arterioles from cats of

Fig 6(a) Arteriole (group B). The smooth muscle cells (SMC) show no strong deformation of the cell membrane as opposed to (b).
(b) The arteriole of group C, which shows a bizarre deformation of smooth muscle cells and endothelium. Small processes of glia in the perivascular space (—). (a) × 6360 (b) × 5830.

Discussion

Our observations on the intracerebral arterioles show a characteristic group of morphologic changes accompanying physiological contraction. In the endothelial cells, clusters of filaments were seen in the abluminal cytoplasm near tight junctions. The basal lamina was undulated, and smooth muscle cells were
deformed by a scalloped cell membrane and prominent attachment devices. The vessel diameter was markedly decreased. However, the vessel lumen was not obstructed. These findings are in agreement with the observations by light microscopy on small arterioles undergoing physiological constriction and with the ultrastructural findings in mesenteric arterioles by Phelps and Luft. In particular the presence of contractile filaments within the endothelial cells observed by us and others indicates that the endothelium plays an active role in the contractile process. Further, these contractile filaments may determine the typical arrangement in endothelial cells.

The spastic constrictions of arterioles can be clearly distinguished from the physiological, that is non-spastic constriction by morphological parameters. Both DC and electroshock lead to a spastic constriction with occlusion of the vessel lumen. There is no characteristic deformation of smooth muscle cell membrane by attachment devices and no typical undulation of the basal lamina. The perivascular glia participates in the spasm by folding of the astrocytic plasma membrane and the gial basal lamina probably because of a reduction of the total surface of the glial ensheathment.

These morphological changes indicate that it is possible to produce a complete obstruction of the vessel lumen with both electric stimulation methods, DC and electroshock. The effect of these electrical stimuli on the microcirculation is described in more detail by Matakas et al. and Cervós-Navarro et al.

Morphologic alterations occurring with spastic processes are not homogeneous. There are morphologic differences between the spastic vessels in group B and group C. The principal difference involves the reaction of the smooth muscle cell. In cases where the spasm has been induced by direct current (group C), the spastic constriction is characterised by a bizarre deformation of smooth muscle cells. In cases where spasm was produced by electroshock (group B) there is only a moderately scalloped smooth muscle cell layer and no deformation of the smooth muscle cell membrane by attachment devices. We believe that a spastic contraction is not an extreme deformation of the single muscle cell but rather a folding and indentation of the whole vessel wall which leads to occlusion of the lumen.

The question arises as to whether spastic contraction takes place without typical signs of the physiological constriction. We consider that the pattern of contraction depends on the arrangement of the smooth muscle cells of the media. The spiral pattern of the smooth muscle cells found in small arteries and arterioles by Benninghoff by light microscopy has been confirmed by ultrastructural examinations. Roggendorf and Cervós-Navarro showed non-contracted intracerebral arterioles with a nearly circular course with interruptions in the smooth muscle cell layer by segments in which the myofilaments of neighbouring smooth muscle cells are arranged vertically on top of one another in the course of a two layered media. In tangential sections, Rhodin demonstrated that the muscle cells of the outermost layer are spirally arranged in a pitch of 18°. This spiral course of the smooth muscle cell renders possible the extreme deformation and deep indentation of the vessel in the presence of spastic contraction with occlusion of the vessel lumen. Lesions of single cells, for example in the endothelium or in the smooth muscle cells were not found more often in experimental animals than in the control animals. There were no significant changes of organelles, generalised vacuolisation or oedema. These observations are in agreement with the studies of Fein et al. and Tanabe et al. which revealed that there are no changes due to spasm in the first hour after stimulation. The intriguing phenomenon of the segmental arrangement of spasm, which is well documented in meningeal vessels after subarachnoidal haemorrhage cannot be explained by the results of our electron microscopical study. A systematic investigation of the precise mechanism of intracerebral spasm, however, could be based, at least in part, on experimental conditions derived from this investigation with electric stimulation of intracerebral arteries and arterioles.

References

8. Phelps PC, Luft JH. Electron microscopical study of
Ultrastructural characteristics of spasm in intracerebral arterioles


