FORMATION OF CEREBROVASCULAR ANOMALIES IN THE AGEING RAT IS DELAYED BY CHRONIC NIMODIPINE APPLICATION

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SUMMARY

At the ultrastructural level two main categories of microvascular anomalies can be distinguished in the aged rat brain. These categories comprise [1] membranous inclusions within the basement membrane and [2] microvascular deposits, which include microvascular fibrosis and thickening of the basement membrane (BMT). In this study we examined the percentage of microvessels displaying ageing-related malformations in the frontoparietal motor cortex of rats aged 16, 24, 30 and 32 months. The percentage microvessels with membranous inclusions and microvascular deposits both gradually increased until the age of 30 months, after which no further increase was observed. The percentage fibrotic microvessels, however, increased until the age of 30 months, but was decreased at 32 months. This decrease of fibrotic microvessels at 32 months coincided with a proportional increase of cerebral microvessels provided with a thickened basement membrane. Combined with qualitative observations these data suggest that in a very late stage of the ageing process collagen fibrils in microvascular fibrotic plaques are depolymerized and degraded. By this mechanism it appears that microvascular fibrosis is transformed into basement membrane thickening. Long-term application of the calcium entry blocker nimodipine did not influence the amount of microvessels with membranous inclusions within the basement membrane, but in contrast resulted in a prominent reduction of ageing-related microvascular deposits when administered from 24 to 30 months. The effect of a prolonged nimodipine treatment from 24 to 32 months on the amount of microvascular deposits was still significant, however, much less conspicuous. We now conclude that chronic administration of nimodipine delays the
formation of microvascular deposits up to the age of 30 months. Furthermore, the beneficial effect of nimodipine treatment from 24 to 30 months on microvascular integrity is not accompanied by a reduced systolic blood pressure.

Key words: Ageing; Cerebral microvasculature; Ultrastructure; Dihydropyridine; Nimodipine

INTRODUCTION

With advancing age a variety of alterations occur within the mammalian central nervous system (CNS). Besides numerous functional changes, morphological anomalies of neuronal, glial and (micro)vascular systems are frequently encountered in the aged CNS. At the ultrastructural level several aberrations of cerebral microvessels have been described in aged mammals [1,9,10,15]. In our previous work we distinguished two main categories of ageing-related microvascular anomalies in the rat brain, the first of which is represented by membranous inclusions within the basement membrane [10]. The second category was defined as microvascular deposits [10], which include microvascular fibrosis [9,10,15] and thickening of the basement membrane [8,26].

Since chronic administration of the calcium entry blocker nimodipine significantly delayed the occurrence of ageing-related motor deficits [5,24,25], we studied the effects of such a long-term nimodipine treatment on ageing-related microvascular anomalies in the frontoparietal motor cortex of Wistar rats. Nimodipine (Bay E 9736) is a calcium entry blocker of the 1,4-dihydropyridine type, which readily permeates the membranes of the blood brain barrier [14]. Therefore nimodipine preferentially acts via an improvement of cerebral microvascular function [13] and/or by a direct effect on the nervous system [20].

Previously we have compared the incidence of microvascular aberrations in aged Wistar rats (30 months of age) and in animals chronically treated with nimodipine from 24 up to 30 months of age and found that such a chronic application of nimodipine significantly reduces the percentage microvessels with deposition products in the aged rat forebrain [10]. The main purpose of the present study was to investigate the effects of a prolonged treatment of nimodipine (24–32 months) on microvascular aberrations in the cortex. Moreover we investigated the development of the distinguished microvascular anomalies in non-treated animals during the ageing process from 16 until 32 months, which enables us to visualize the exact development of microvascular alterations in the second half of the life-span of Wistar rats. Furthermore we considered of crucial importance to define the microvascular condition at the age on which the nimodipine treatment started (i.e. 24 months).

The formation of microvascular deposits is even more pronounced in the brains
of aged hypertensive rats [15]. For that reason the systolic blood pressure was measured in young adults, aged controls and aged nimodipine treated rats, to find out whether the microvascular effects of nimodipine can be attributed to a reduced blood pressure, or to a direct action of the drug on the microvascular wall itself.

MATERIALS AND METHODS

Animals
In this study we used male rats of an inbred Wistar strain (BOR WISW, Winkelmann, Borchen, FRG). The animals were group housed ($n = 6$) and received food and water ad libitum. Until the age of 24 months the animals were kept at specified pathogen free (SPF) standard laboratory conditions. Thereafter the animals were maintained under similar, however, non-SPF conditions. All rats were regularly checked on their physical health condition.

Experimental design
For the morphometric analysis of the microvasculature four groups (each $n = 6$) aged 16, 24, 30 and 32 months were used. Moreover, we studied two groups of six animals each, which were chronically treated with nimodipine from the age of 24 months on, and sacrificed at the age of 30 and 32 months, respectively. The systolic blood pressure was determined using the tail-cuff method [21] in young adults (3 months, $n = 8$), in aged controls (30 months, $n = 8$) and in aged animals treated with nimodipine from 24–30 months of age (30 months, $n = 9$).

Drug treatment
The animals were either fed with standard food pellets (Sniff, Soest, FRG) or identical pellets to which nimodipine (860 ppm, Bayer, Leverkusen, FRG) was added. This dosage corresponds with a daily dose of approximately 30 mg/kg bodyweight, which was chosen for previously reported optimal pharmacological effects [24,25]. During observations the experimenter was unaware of the kind of treatment given. After statistical analysis the treatment code was broken.

Electron microscopic preparation
At the desired age, all animals were deeply anaesthetized with 60 mg/kg pentobarbital (i.p.) and transcardially perfused with a fixative containing 1% paraformaldehyde, 2% glutaraldehyde and 3% polyvinyl pyrrolidone (PVP) in 0.1 M phosphate buffer, pH 7.38. The brains were removed and cut into 50 μm sections with a vibratome. These vibratome sections were routinely embedded in epon (Serva 812) for ultrastructural examination.

Morphometric procedure
After light microscopic examination of the embedded vibratome sections, selected
Fig. 1. (A) Membranous inclusion within the basement membrane (memb) situated in close proximity to a normal pericyte (p). Within the inclusion a mitochondrion (arrowhead) can be seen. (B) Microvascular fibrosis showing a large amount of collagen fibrils (c) within the basement membrane. In the endothelium a normal tight junction can be observed (arrowhead). (C) Thickening of the basement membrane (*) is heavy and at some places the basement membrane is considerably thicker than the endothelium. Duplication of the basement membrane (arrow) is also shown in this microvessel (as, astrocytic endfeet; e, endothelium; *, basement membrane; scale bar 0.25 μm (A); 1 μm (B and C)).
regions of the frontoparietal motor cortex were collected for semi- and ultrathin sectioning. For all animals the frontoparietal motor cortex area was selected in sections cut in the transverse plane. The areas selected were trimmed en bloc and cut to semithin sections, which were stained with toluidine blue. Guided by these survey sections, the blocs were further trimmed to the desired proportions to match the size of the carrier mesh grids. Ultrathin sections spanning all cortical layers were collected at 200-mesh grids, contrasted with 5% aqueous uranyl acetate and Reynolds lead solution. The ultrastructure of the cortical microvasculature was examined in a Philips 201 electron microscope. Based on previous examinations [10] microvascular aberrations were classified into two major categories: (1) membranous inclusions within the basement membrane and (2) microvascular deposits. The latter category included microvascular fibrosis, basement membrane thickening and a miscellaneous group of more rare deposits within the endothelium. The numbers of microvessels displaying one of the above mentioned aberrations were counted directly from the screen of the electron microscope in layers I, III and V of the frontoparietal motor cortex. Quantification was proceeded until sixty microvessels were encountered. Only completely visible microvessels uncovered by the mesh lines were recorded. This quantification was repeated in three adjacent ultrathin sections in order to compensate for fortuitous distribution patterns as a consequence of using mesh grids. From these quantitative data we calculated the percentage microvessels which displayed either membranous inclusions or microvascular deposits to exclude possible interference of changed numerical density of microvessels.

Recently we showed that the effects of long-term nimodipine treatment on microvascular integrity are basically the same for the different cortical layers [11]. Therefore we will show here the percentage aberrant microvessels calculated for the entire cortical thickness.

**Statistical analysis**

The quantitative data of the microvascular morphometric analysis were evaluated using the Mann-Whitney U test. The blood pressure data were tested with the ANOVA-test. When the ANOVA indicated group differences the separate groups were compared with corrected multiple t-tests. Statistical significance was defined as $P < 0.05$.

**RESULTS**

**Qualitative alterations of cerebral microvasculature**

Ageing-related microvascular changes have previously been described [10] and are divided into two major categories. The first category is represented by membranous inclusions within the microvascular basement membrane (Fig. 1A). The inclusions are enclosed by a basement membrane and within the inclusions cytoplasmic elements like, for example, mitochondria can be observed. Mainly due to the
Fig. 2. (A) In the endothelial cytoplasm (e) of the microvessel a short collagen-like fibril (arrowheads) can be observed near the endothelial nucleus (en). (B) This fibrotic microvessel shows collagen fibrils (c) deposited within the basement membrane. In the endothelial cytoplasm (e) an exceedingly large amount of pinocytotic vesicles (arrows) is present (as, astrocytic endfeet; *, basement membrane; scale bar (A and B) 0.5 μm).
ultrastructural position in the microvascular wall these membranous inclusions are generally considered to reflect degenerative stages of pericytes [1,10,23]. The second category are microvascular deposits which include microvascular fibrosis (Fig. 1B), local basement membrane thickenings (BMT, Fig. 1C) and a group of more rarely occurring deposition products within the microvascular wall.

Microvascular fibrosis is characterized by banded fibrils deposited within the basement membrane. The periodicity of 64 nm of the fibrils lead to the identification of collagen as the main constituent of microvascular fibrosis [10]. The origin of these collagen fibrils is not yet known, however, in the endothelium of fibrotic microvessels in the cerebral cortex we observed short fibrils with an identical periodicity of approximately 64 nm within the endothelial cytoplasm (Fig. 2A). This indicates that endothelial cells may contribute to the ageing-related formation of fibrotic cerebral microvessels and may be considered as the source of the fibrotic debris. Moreover the endothelial cytoplasm of most microvessels with deposits possessed a large amount of pinocytotic vesicles (Fig. 2B), suggesting that microvascular deposits influence transport functions of the endothelial cell.

The ultrastructural appearance of collagen fibrils within microvascular fibrosis altered after the age of 30 months. Besides a less clear periodicity of the collagen fibrils (Fig. 3A) several fibrotic plaques in the cerebral microvessels of animals aged 32 months were more electron-dense. Figure 3B shows such a fibrotic plaque in which more vaguely banded collagen fibrils can be seen. Other parts of the collagen deposit have completely lost their fibrillary structure and are covered with fine granular precipitates. These diffuse fibrotic plaques are only present in the cerebral motor cortex of senescent rats at the age of 32 months, which indicates that in a late stage of the ageing process (30–32 months) collagen fibrils within fibrotic plaques can be depolymerized. The more electron-dense appearance and loss of periodicity of these fibrotic plaques lead to the suggestion that these diffuse fibrotic plaques may be an intermediate form in the transition of microvascular fibrosis to basement membrane thickening. This process of a loss of periodicity and a more electron-dense and homogenous thickening of the basement membrane is visualized in Fig. 4.

Beside microvascular changes several other ageing-related alterations were detected in the motor cortex of aged rats. Hypertrophy of astrocytic endfeet surrounding capillaries are commonly encountered in aged (30 and 32 months) rats (Fig. 5A). Within the cytoplasm of the astrocytes lipofuscin and glycogen-like deposits were visible. Note that all the microvascular aberrations shown in the previous figures are also surrounded by hypertrophied astrocytes. At the neuronal level degenerated pyramidal cells (Fig. 5B) were frequently observed in layers III and V of the motor cortex of rats aged 32 months. This ultrastructural appearance of neuronal cell death was not quantified in this study, nor was the incidence of hypertrophic astrocytic endfeet.

Quantitative alterations of cerebral microvasculature and effects of chronic nimodipine treatments

Previously we described the incidence of microvascular aberrations in the rat
motor cortex and found an increased amount of microvessels with membranous inclusions and microvascular deposits with advancing ages up to 30 months. The present study is based on examination of the cerebral microvasculature in rats aged from 16 to 32 months. During this life-span a different age-related nature for the distinguished categories of microvascular aberrations is observed.

The percentage microvessels with membranous inclusions within the basement membrane significantly increased with advancing age from 10% in animals aged 16 months, to 37% in animals at the age of 30 months. Thereafter no significant rise in the incidence of membranous inclusions was encountered (Fig. 6A). Nimodipine application from 24 to 30 and from 24 to 32 months did not reveal any significant effect on the percentage microvessels bearing membranous inclusions within the microvascular basement membrane. The formation of microvascular deposits in the frontoparietal motor cortex displays a similar age-dependency (Fig. 6B). Hence, significantly more microvessels with deposits can be observed with increasing age (4% in animals aged 16 months and up to 20% in the animals at the age of 30 months). After the age of 30 months no further significant increase was established. The effects of chronic treatment with nimodipine from 24 to 30 months has previously been reported [10] and consisted of a strong and highly significant reduction in the amount of deposits in the nimodipine treated animals aged 30 months as compared to the age-matched controls ($P < 0.001$). After a prolonged administration of nimodipine from 24–32 months the effects of the drug are less pronounced. Animals treated with nimodipine from 24–32 months still showed significantly less microvessels with deposits than the age-matched controls ($P < 0.01$). However, in senescence the placebo-fed animals showed no significant increase in the amount of microvascular deposits after the age of 30 months ($P < 0.407$), whereas in the same period the frequency of microvascular deposits strongly increased in the nimodipine treated cases ($P < 0.001$).

As described above, the microvascular deposits comprised two major constituents, microvascular fibrosis and local basement membrane thickenings (BMT). The number of fibrotic microvessels significantly increased up to the age of 30 months, from 2% at the age 16 months until 16% in rats aged 30 months. Thereafter, however, the number of fibrotic microvessels significantly declined to 9% (Fig. 7A). Nimodipine application from 24 to 30 months resulted in a prominent and significant reduction of microvascular fibrosis ($P < 0.001$). However, the animals treated with nimodipine from 24 to 32 months did not display a significantly different

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**Fig. 3.** (A) A part of the collagen fibrils (c) in this fibrotic plaque has lost the typical periodicity (arrows), which is characteristic for fibrotic microvessels of animals older than 30 months. Note also the vacuole in the endothelial cytoplasm (arrowhead). (B) In the fibrotic plaque of this microvessel the loss of periodicity is also present. Banded collagen fibrils (c) and non-banded collagen fibrils are covered with fine granular precipitates (arrowheads). The abundant amount of mitochondria (m) in the endothelial cytoplasm is also striking (as, astrocytic endfeet; e, endothelium; *, basement membrane; scale bar (A) 0.25 μm).
Fig. 4. This figure visualizes the possible transition deposits of clearly banded collagen fibrils into basement membrane thickening in which the thickening appears homogeneous and more electron-dense. (A) Parts of the collagen fibrils still show periodicity (arrow). (B) The part of (4A) at higher magnifications, in which the fibrils lost the periodicity. (C) A typical example of a thickening of the basement membrane. (as, astrocytic endfeet; e, endothelium; p, pericyte; *, basement membrane; scale bar (A and C) 0.5 μm; (B) 0.125 μm)
Fig. 5. (A) The astrocytic endfeet (as) surrounding the capillary are hypertrophied and very large with accumulated lipofuscin granules (arrowheads). B shows a degenerated pyramidal neuron in layer V of the FRC. The nucleus (nu) and cytoplasm (cy) are more electron-dense than normal. A large amount of lysosomes (arrows) are concentrated near the Golgi apparatus (g). The neuron is surrounded by hypertrophied neurites (arrowheads). Scale bar (A and B) 1 μm.
Fig. 6. The incidence of membranous inclusions within the basement membrane (A) and microvascular deposits (B) in the frontoparietal motor cortex for animals aged 16, 24, 30 and 32 months of age and for animals treated with nimodipine from 24 to 30 months and 24 to 32 months, the start of the treatment is indicated with an arrow (in percentage ± S.E.M.; **P < 0.01 and ***P < 0.001 compared to the age-matched control).

The ageing-related nature of BMT during the animal’s life span is completely different when compared to microvascular fibrosis. Figure 7B shows that virtually no amount of fibrotic microvessels compared to the untreated animals of 32 months (P < 0.155).

Fig. 7. The percentage microvessels with microvascular fibrosis (A) and BMT (B) for animals aged 16, 24, 30 and 32 months and for animals treated with nimodipine from 24 to 30 and 24 to 32 months of age, the start of the treatment is indicated with an arrow (in percentage ± S.E.M.; ***P < 0.001 compared to the age-matched control).
TABLE I

THE SYSTOLIC BLOOD PRESSURE OF YOUNG ADULTS, AGED CONTROLS AND AGED NIMODIPINE TREATED (24–30) MONTHS ANIMALS IN MEAN ± S.E.M.

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>n</th>
<th>Blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Aged</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
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*Significantly different from young control $P < 0.05$.

BMT's are encountered in cerebral microvessels of animals at the various ages up to 30 months. In rats aged 32 months, however, 8% of all microvessels showed local thickening of the basement membrane. Nimodipine treatment from 24 to 32 months revealed a small but not significant reduction of microvessels with a thickened basement membrane ($P < 0.149$).

**Effect of nimodipine treatment on blood pressure in aged rats**

The systolic blood pressure was measured in young adults (3 months), in aged control animals (30 months) and in aged rats treated with nimodipine from 24 to 30 months (Table I). Both aged groups showed a significantly increased blood pressure as compared to the young controls. This ageing-related increase of the systolic blood pressure was, however, not significantly influenced after chronic nimodipine application from 24 to 30 months.

**DISCUSSION**

The cerebral microvasculature is comprised of three cell types, the first of which is the specialized endothelial cell. The endothelial cell forms the actual blood-brain barrier (BBB) and is surrounded by a basement membrane. Furthermore, pericytes are embedded within the microvascular basement membrane, while astrocytic end-feet cover the microvascular wall. Proper functioning of these cell types is of crucial importance for an appropriate functioning of the BBB on the one hand and for an efficient nutrient and gas exchange on the other [7].

With advancing age morphological and morphometric alterations of the cerebral microvasculature occur in a variety of mammalian brain regions. Recently, Topple et al. [26] showed in the rat fascia dentate that capillaries are much more affected by the ageing process than larger vessels. We present here an increasing frequency of ultrastructural anomalies of the microvascular wall throughout the second half of the life-span of Wistar rats. We distinguished membranous inclusions within the microvascular basement membrane and microvascular deposits, the latter category being subdivided into microvascular fibrosis and basement membrane thickening.
Membranous inclusions within the basement membrane have previously been described in the aged rat brain [1,10,15]. We now demonstrated a clear ageing-related increase of the incidence of these microvascular anomalies. The formation of membranous inclusions has in earlier studies been attributed to pericyte degeneration, mainly based on the fact that these inclusions are embedded within the microvascular basement membrane, as are pericytes [4]. Moreover cellular cytoplasm and debris can be observed within the inclusions. Current studies are underway to identify the chemical nature of the inclusions with a histochemical marker for pericytes.

Deposition of collagen fibrils within the microvascular basement membrane has been reported before in the aged mammalian brain. In our own studies and those of others [9,10,15] collagen deposits in the basement membrane were encountered between the endothelium and pericyte as well as in the outer basement membrane. Together with the identification of short collagen-like fibrils in the endothelial cytoplasm it may be concluded that the endothelial cell is involved in the formation of collagen fibrils within fibrotic plaques and may be the source of perivascular fibrotic deposits. We also observed numerous thin basement membranes with a heavy investment of collagen fibrils extending into astrocytic endfeet (data not shown). This indicates that also astrocytes may participate in the formation of fibrosis.

In previous reports microvascular fibrosis has been encountered in cerebral arterioles and capillaries of rats older than 24 months [9,10,15]. We demonstrate here an increased incidence up to the age of 30 months, after which, however, the occurrence of microvessels with fibrosis significantly decreases. This may possibly be indicative for a lower survival rate of animals with a relatively high number of microvessels with fibrotic deposits. However chronic nimodipine treatment did not increase the average life-span expectancy of Wistar rats. On the other hand the lower percentage of fibrotic microvessels coincided with ultrastructural alterations in the appearance of microvascular fibrosis in rats aged 32 months. Instead of a clearly banded fibril type, there is an apparent transition into a more homogenous structure with a diminished periodicity and a fine granular precipitate covering the deposit. Similar precipitates were reported by Castejon [2] in the basement membrane of cortical microvessels in patients with perifocal brain edema and were attributed to degradation of basement membrane proteins. Therefore we interpret the combined qualitative and quantitative alterations of microvascular fibrosis after the age of 30 months as the degradation and depolymerization of collagen fibrils.

Many morphometric studies described an increased capillary basement membrane thickness in the brain of aged mammals [8–10,15,18,22,26]. We quantified local thickenings of the basement membrane (BMT) and found a low frequency until the age of 30 months. Between the age of 30 and 32 months, however, approximately 10% of all cortical microvessels developed BMT. In exactly the same period the number of microvessels showing microvascular fibrosis decreased with 10%.
Therefore the increased frequency of basement membrane thickening (BMT) after the age of 30 months coincided with and was proportional to the decreased incidence of microvascular fibrosis. Together with the qualitative observation of intermediate stages between microvascular fibrosis and BMT (as depicted in Fig. 3) we suggest that degradation of the collagen fibrils leads to the appearance of local thickenings of the basement membrane. Taken into account the combined qualitative and quantitative data, the transformation of microvascular fibrosis into BMT seems the most acceptable explanation for the ageing-related development of microvascular aberrations we presented here. Microvessels in the brains of Alzheimer patients displayed BMT only in the outer basement membrane which indicated a major role for astrocytes in the formation of BMT [22]. However, we observed BMT in basement membrane between endothelium and pericytes as well as in the outer basement membrane. This indicates that in the aged rat brain both endothelial cells and pericytes are involved in the formation of BMT. BMT formation may serve as a response to an altered barrier function [26] and has been described to coincide with increased vesicular transport through the endothelium. As described above and illustrated in Fig. 2B a large amount of pinocytotic vesicles in the endothelial cells, indicative for a disturbed transport function, was also encountered in the microvessels which possessed deposits.

Hypertrophied astrocytes, also observed in the brain of Alzheimer patients [19], surround aberrant capillaries in the aged rat motor cortex thus implying an involvement of astrocytes in the deterioration of the microvascular wall or indicating a response to a diminished microvascular function.

Nimodipine is a calcium entry blocker of the 1,4-dihydropyridine type [13] and therefore specifically blocks the (slowly inactivating, L-type) voltage-sensitive calcium channels [20]. Due to its membrane permeability nimodipine readily crosses the blood-brain barrier [14]. In the CNS nimodipine exerts its action directly on neurons and on the cerebrovascular system [13,20].

Neuronal binding sites for dihydropyridines are most frequent in neuron-rich areas such as the hippocampus and the cerebral and cerebellar cortex [14]. Neuronal calcium homeostasis alters during ageing, tentatively because of an increased calcium influx through L-type calcium channels [16]. Landfield and coworkers [17] showed that a blockade of these calcium channels by nimodipine prevents the increased calcium influx in aged hippocampal pyramidal cells.

Besides a direct neuronal action, nimodipine also influences the cerebrovascular system. Dihydropyridine (dhp) binding sites on cerebral microvessels have similar, if not identical, pharmacological properties to neuronal dhp binding sites [3]. Nimodipine exerts a vasodilatory action by a blockade of vascular L-type calcium channels, hereby increasing cerebral blood flow [13], known to be impaired during ageing [6]. Our studies indicate a direct action of nimodipine on endothelial cells and/or adjacent astrocytic endfeet, clearly delaying the ageing-related deterioration of the microvascular integrity.
When nimodipine was chronically administered to Wistar rats a delay in ageing-related decline in cognitive, social and motor functioning occurred [24]. In animals selected for impaired motor functioning long-term oral nimodipine treatment initially even improved motor functioning, indicating a therapeutic drug effect [5]. However, when the treatment was continued nimodipine became less effective in preventing ageing-related deterioration of motor activity [5]. In the present study we found similar nimodipine effects on the development of cerebrovascular alterations in the course of the ageing process. During ageing the amount of microvascular deposits increased and chronic administration of nimodipine for a long time delayed, but ultimately did not prevent the ageing-related deterioration of microvascular structure. The strong resemblance of the longitudinal effects of nimodipine application on motor performance and microvascular ultrastructure in the motor cortex indicates a close relationship between behavioral performance and microvascular integrity.

Besides the ageing factor, an increased blood pressure induces the formation of aberrant microvessels [15]. Especially the thickening of the basement membrane and microvascular fibrosis showed an accelerated occurrence in aged spontaneously hypertensive rats [15]. The beneficial effect of nimodipine on the incidence of microvascular deposits however, can not be attributed to a hypotensive action of nimodipine, since nimodipine administration from 24 to 30 months did not influence the increased blood pressure that occurs in the ageing animals. In earlier experiments it was found that nimodipine applied to spontaneously hypertensive animals of the stroke-prone type (SHR-SP) increases the life-span of these animals but without any hypotensive action of this drug [12]. A preventive action of nimodipine on the occurrence of vasospasm in these SHR-SP has previously been reported [12]. Whether the beneficial effect of nimodipine can also be related to an ameliorated microvascular integrity, besides this prevention of vasospasm and a possible direct neuronal action, is currently under study.

In summary, the current quantitative data showed a gradual increase in the percentage microvessels with membranous inclusions and deposits up to the age of 30 months, whereafter no significant increase was encountered. At the age of 32 months approximately 10% of the cerebral microvessels displayed local thickenings of the basement membrane (BMT), whereas the incidence of microvascular fibrosis was decreased with about 10%. Furthermore, qualitative observations revealed that after the age of 30 months intermediate forms of fibrosis and BMT became apparent. Therefore we propose that after the age of 30 months a transformation of microvascular fibrosis into BMT occurs in the aged rat brain. Chronic administration of nimodipine from 24 to 30 and from 24 to 32 months did not influence the degree of pericyte degeneration. The suppressive effect of nimodipine on the development of microvascular deposits was most prominent when the drug was administered from 24 to 30 months. A prolonged treatment with nimodipine from 24 to 32 months was much less potent in preventing the formation of microvascular
deposits. In animals treated with nimodipine from the age of 24 to 30 months the beneficial effect on microvascular integrity could not be related to a hypotensive action of this drug, since the blood pressure of these treated animals was not significantly different from the age-matched controls.

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