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Forebrain and brainstem perivascular neurons projecting to the thalamus (An anatomical explanation of the pathophysiology of fatal familial insomnia)

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Summary

Afferent projections to the mediodorsal and anterior nuclei of the cat thalamus were studied by means of stereotaxic injections of neuronal tracers (horseradish peroxidase, HRP, and fluorochromes). The co-location of HRP and NADPH-diaphorase in neuronal bodies sending projections to the mediodorsal and anterior nuclei of the thalamus was studied.

The differences in connectivity between the mediodorsal nucleus and the anterior nuclei of the thalamus are discussed. The possibility that prion agents responsible for fatal familial insomnia could spread from the mediodorsal and anterior nuclei of the thalamus (mainly the intermediate band of the mediodorsal nucleus and the anteroventral nucleus of the anterior complex) through a retrograde pathway is discussed.

Retrograde-labelled neuronal cell bodies around vessels in the basal prosencephalon and brainstem were mainly observed after tracer injections in the intermediate band of the mediodorsal nucleus and in the anteroventral nucleus of the anterior complex of the thalamus. This finding, along with the observation of the co-location of HRP and NADPH-diaphorase in this type of neurons in the cases of mediodorsal and anterior nuclei tracer injections leads us to propose a possible pathophysiological involvement of nitrergic systems in fatal familial insomnia.

Key Words: Fatal familial insomnia - Thalamus - Perivascular neurons.

Introduction

In fatal familial insomnia (FFI), anatomopathological alterations in the mediodorsal (MD) and anterior (A) nuclei of the thalamus (specially, the intermediate band of MD and the anteroventral nucleus) are consistently observed (Manetto et al., 1992; Gambetti et al., 1995; Dorandeu et al., 1998). These thalamic structures can be considered as a part of the limbic thalamus (Macchi and Bentivoglio, 1994). After some time, other structures, such as the deep layers of the cerebral cortex are also affected (Manetto et al., 1992; Parchi et al., 1995). The most affected area of the cerebral cortex is the cingular cortex, and the least affected is the occipital cortex (Parchi et al., 1995; Cortelli et al., 1997). The alteration of other thalamic structures is not constant, and it should be noted that both corpora geniculata remain unaffected.

Experimentally, after inoculation of infected prion proteins, an accumulation of these has been observed in the thalamus, hypothalamus and brainstem (Casaccia-Bonnetti et al., 1993). An anterograde propagation of prion proteins has been proposed (Borchelt et al., 1994), as has a retrograde one (Scott et al., 1992) and a bidirectional transmission (Beekes et al., 1996). We have previously proposed for FFI a mainly retrograde propagation of the modified proteins from MD and A to several areas of the central nervous system; for example, to nitrergic areas of the basal prosencephalon and brainstem, and in this case, related to MD. Some of these cells are seen in a perivascular location (Velayos et al., 1998).

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Here, we attempted to define in cats the connectivity of neurons whose bodies have a perivascular location and project to the nuclei primarily affected in FFI. Since extrapolation is possible (Heckers et al., 1992) because the connectivity of MD and A in the cat is similar to that seen in primates, we attempt to provide anatomical data which should help to the understanding of the pathophysiology of FFI.

Material and Methods

We studied retrograde labelling after stereotaxic injections of horseradish peroxidase (HRP, HRP-WGA) in thirty adult cats, and after stereotaxic injections of fluorochromes in ten others. The injections of the tracers were performed in the anterior, medial (Figure 1) and intralaminar nuclei of the thalamus and also in the habenula.

We focused our attention on the retrograde labelling of the perivascular areas of the basal prosencephalon and brainstem. We also studied the co-location of NADPH-diaphorase (NADPH-d) and HRP in the nitrergic areas of the basal prosencephalon and brainstem in eight of the above cases, in which we performed injections of HRP into MD and A.

The animals were always anaesthetized before surgery and perfusion.

Results

Afferent projections to MD and A. An overall view

Regarding retrograde cortical labelling, A injections labelled the medial surface of the cerebral cortex, and mainly the cingular cortex. MD injections labelled the cingular cortex but also more rostral areas, and mainly the prefrontal cortex and less so the motor, premotor and somatosensory cortices. The occipital cortex does not project either to MD or A. In both cases the retrograde cell labelling was always observed in the deeper layers of the cerebral cortex.

The retrograde labelling in the basal prosencephalon was observed specially after injections in the intermediate band of MD and of the anteroventral and anteromedial nuclei of the A complex. The lateral hypothalamic area was labelled mainly after injections in the intermediate and lateral bands of MD (Figure 2A), as well as in the

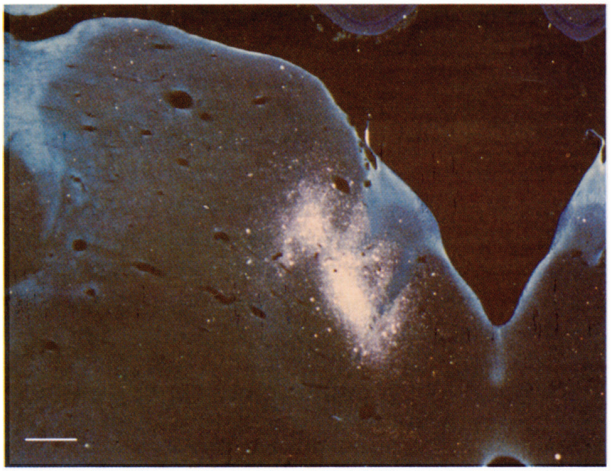

Fig. 1. Photomicrograph of HRP-WGA injection in MD (polarized light illumination). Calibration bar = 1 mm.

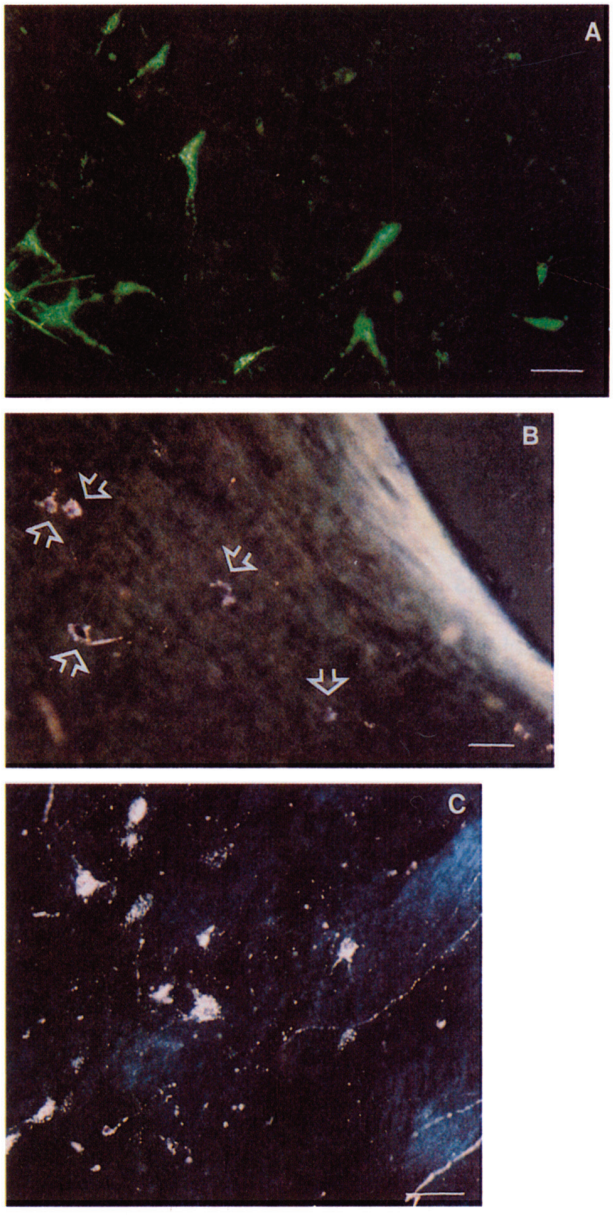

Fig. 2. Photomicrographs under polarized light illumination showing the retrograde cell labelling in different subcortical prosencephalic structures after HRP injections in MD (**A**: labelling in the lateral hypothalamic area), in the paraventricular nucleus of the thalamus (**B**: labelling in the suprachiasmatic nucleus), in the anteroventral nucleus of the A complex (**C**: labelling in the dorsal and rostral portion of the reticular thalamic nucleus). Calibration bars = 50 μm.

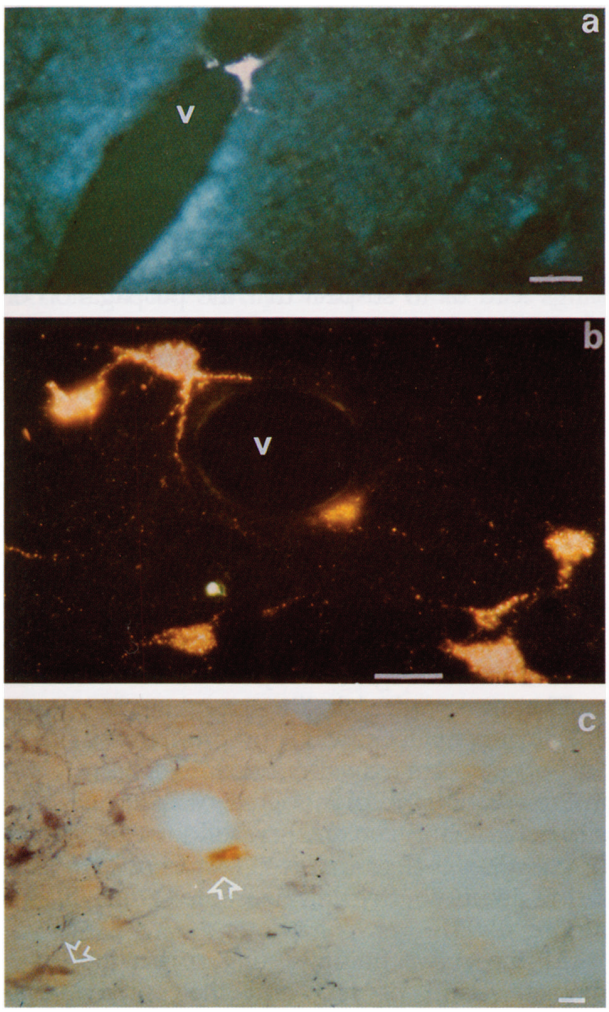

Fig. 3. Photomicrographs under polarized light illumination showing retrograde labelled neurons around vessels after HRP injections in the intermediate band of MD (**a**, **b**) and a massive HRP injection in MD (**c**). v = vessels. Calibration bars = 50 µm.
a): HRP-labelled neuron in the basal prosencephalon.
b): HRP-labelled neurons around a vessel in the coeruleus area.
c): NADPH-d neurons and neurons simultaneously colocating HRP and NADPH-d in the coeruleus area (white arrows).

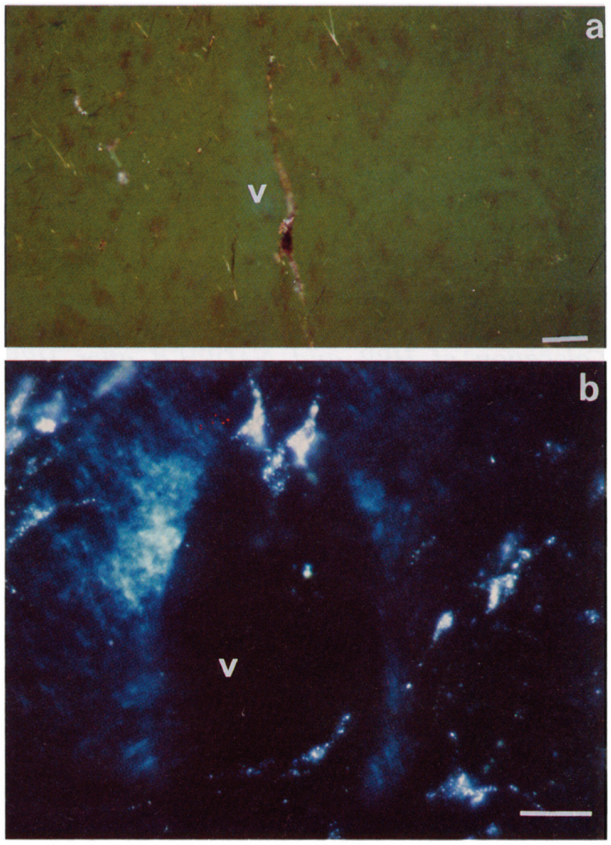

Fig. 4. Photomicrographs under polarized light illumination showing a retrograde-labelled neuron close to vessels in the basal prosencephalon after an HRP injection in the anteroventral nucleus of the A complex (**a**, **b**). v = vessels. Calibration bars = 50 µm.

anteroventral and anteromedial nuclei of the A complex. The suprachiasmatic nucleus was particularly well labelled after tracer injections in the intermediate band of MD, although in a scant amount. It is important to mention that after reviewing the rest of animals undergoing thalamic injections we observed some retrogradely-labelled neurons in the suprachiasmatic nucleus in the case of injections made in the paraventricular nucleus of the thalamus (Figure 2B). The reticular thalamic nucleus was abundantly labelled after injections in the three bands of MD and sparingly labelled after injections in the anteroventral nucleus of the A complex (Figure 2C) (in this case, mainly after injections of fast blue).

The retrograde cell labelling of the brainstem was more prominent after tracer injections in MD than in A, with a topography in agreement with our previous descriptions (Velayos and Reinoso-Suárez, 1982). Afferent projections to A (and especially to the anteroventral nucleus) could come from the parabrachial areas.

Retrograde labelling of perivascular areas of the basal prosencephalon and brainstem after tracer injections in the anterior, medial and intralaminar nuclei of the thalamus and in the habenula

Most of the retrogradely-labelled neurons in perivascular location corresponded to the injections of the tracers in MD, and especially of its intermediate band. We found these neurons in the brainstem (Figure 3B) (mainly in the locus coeruleus and the dorsal tegmental nucleus of Gudden) and, in lower amounts, in the basal prosencephalon (Figure 3A) (especially in the diagonal band of Broca and the neighbouring preoptic area).

Tracer injections in the intralaminar nuclei resulted in scarce perivascular neuron labelling in the basal prosencephalon and the brainstem.

Perivascular cells labelled after injections in the habenula were small and few and were located in the basal prosencephalon. Injections in the midline thalamic nuclei resulted in a scarce perivascular retrograde labelling.

The number of labelled perivascular neurons in the brainstem was low after injections in A; regarding the basal prosencephalon, although they were not abundant, they were observed after anteroventral nucleus injections (Figure 4).

Colocation of HRP and NADPH-d after HRP injections in MD and A.

We studied NADPH-d co-location in neuronal cell bodies in relation to retrograde HRP labelling after MD injections of HRP. We observed that the basal prosencephalon presented this pattern of cells (mainly in the diagonal band of Broca); in the brainstem, the most abundant co-location was situated in the dorsal tegmental nucleus of Gudden and the coeruleus complex (Figure 3C). An important number of this type of cells was observed near the vessels. The co-location was mainly observed after injections in the intermediate band of MD.

We also studied the NADPH-d co-location in neuronal bodies in relation to retrograde labelling after HRP injections in the anteroventral nucleus of the A complex. The number of these cells was lower than in the MD cases, and the cells were mainly situated in the dorsal tegmental nucleus and coeruleus complex; some of these cells were situated near the vessels.

Discussion

The anatomopathological findings of the cases of FFI studied until now show that after a time the cerebral cortex is affected in its deep layers (Manetto et al., 1992; Parchi et al., 1995). In our experiments, we observed the retrograde labelling of these layers after tracer injections in MD and A.

The anatomopathological findings also show a constant and important affectation of the cingular cortex (Parchi et al., 1995), and this cortex projects to MD and A (Velayos et al., 1993; Velayos and Cruz, 1994). The least affected cortex is the occipital one (Parchi et al., 1995; Cortelli et al., 1997); in our experiments we hardly found any retrogradely-labelled neuron in this cortical area (Velayos and Reinoso-Suárez, 1985; Velayos et al., 1993; Velayos and Cruz, 1994; Velayos, 1997). In addition to this, the thalamic lateral geniculate body is not affected in FFI. All these anatomical findings lead us suspect that the propagation of the infective proteins from MD and A to the cerebral cortex must mainly occur through a retrograde pathway, the pathway of corticothalamic connections.

Projections of the basal prosencephalon mainly course towards the intermediate band of MD and anteroventral and anteromedial nuclei of the A complex. Heckers et al. (1992) have stressed the importance in humans of the projections from the basal prosencephalon to the medial band of MD. Barriga et al. (1997) have observed that the basal prosencephalon sends more projections to those nuclei than it receives; these findings lead us to suspect that the propagation of modified prion proteins could occur mainly in a retrograde way from MD and A to the basal prosencephalon. McGinty et al. (1994) have underlined the importance of the basal prosencephalon in the regulation of sleep-wakefulness cycle and as a possibly affected structure in FFI.

The rest of the subcortical structures send more projections to MD and A than in the opposite direction (Divac and Passingham, 1980); this also suggests a more prominent propagation of prion proteins to the brainstem in a retrograde than in an anterograde way.

The retrograde labelling seen in the suprachiasmatic nucleus was not prominent, but it was observed mainly after tracer injections in the intermediate band of MD. As is well known, the suprachiasmatic nucleus is related to the control of circadian cycles, and in FFI an important alteration in these rhythms is seen (Edgar, 1994; Moore, 1994).

All the above findings suggest that the intermediate band of MD and the anteroventral nucleus of the A complex (which are primarily affected in FFI) are structures with a different connectivity from the rest of portions or bands of MD and A and the rest of thalamic nuclei studied.

In our study, most of the retrogradely labelled neurons in a perivascular location were observed after HRP injections in the intermediate band of MD, in both the basal prosencephalon and brainstem (Figure 3). This has been partly described in an earlier report (Velayos et al., 1998). It is worth noting that the intermediate band of MD is a primarily affected structure in FFI, and in this illness important modifications occur in vegetative and visceral functionality; both the brainstem and prosencephalic areas where we found such labelled neurons are related to this control (Sakaguchi et al., 1979; Szymusiak et al., 1991; Sinnamon, 1992; Alam and Mallick, 1994; Chen and Herbert, 1995).

It should be noted that the HRP injections in the rest of the nuclei studied showed scanty labelling close to the vessels and that in A, the anteroventral nucleus (a primarily affected structure in FFI) is the one which receives more projections from perivascular neurons, mainly from the basal prosencephalon (Figure 4).

Studying the co-location of HRP and NADPH-d after MD and A injections of HRP, we obser-

ved this kind of neurons situated in the basal prosencephalon and the brainstem, and mainly after the HRP injections in the intermediate band of MD. At least some of the perivascular neurons that project to this MD band could be nitrergic. Nitric oxide (NO) is important in the regulation of vascular flow (Do et al., 1996) and it could be speculated that in FFI an alteration would occur in nitrergic areas because of a possible propagation of the infective proteins to those zones. It is well known that excess of NO can be harmful (Dawson and Dawson, 1995; Benzing and Mufson, 1995); however the present data do not indicate either an increase or a decrease in NO in the basal prosencephalon and/or the brainstem. In Alzheimer's disease an increase in NADPH-d neurons in the substantia innominata has been observed (Benzing and Mufson, 1995), and in scrapie-infected mice and PrP knockout mice an abnormal localization and activity of neuronal nitric oxide synthase have been found (Keshet et al., 1999). On the other hand, cholinergic/nitrergic mesopontine neurons connect profusely with the thalamus in the rat (Williams et al., 1997), suggesting that they may play a role in EEG desincronization. The question therefore arises as to whether a decrease will occur in NADPH-d-positive neurons in the brainstem of FFI patients.

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