Acta Neuropathol (1991) 82: 225–228

Acta Neuropathologica

Short original communication

Neuronal autophagy in experimental scrapie

J. W. Boellaard[1], M. Kao[2], W. Schlote[3], and H. Diringer[4]

[1] Institut für Hirnforschung der Universität, Calwerstraße 3, W-7400 Tübingen, Federal Republic of Germany
[2] The Wistar Institute of Biology and Anatomy, 36th Street at Spruce, Philadelphia, PA 19104/4268, USA
[3] Neurologisches Institut der Universität, Deutschordenstraße 46, W-6000 Frankfurt/M 71, Federal Republic of Germany
[4] Robert Koch Institut des Bundesgesundheitsamtes, Nordufer 20, W-1000 Berlin 65, Federal Republic of Germany

Received March 29, 1991/Accepted May 6, 1991

Summary. In this study we report the formation of giant autophagic vacuoles (AV) in neurons in experimental scrapie in hamsters. Autophagy is an important step in the cellular turnover of proteins and organelles. It is known to occur in neurons under physiological as under pathological conditions. Giant AV, however, are seen very rarely only in pathological states. In our model AV are much more numerous after intracerebral (i.c.) transmission of the scrapie agent than after the transmission via the intraperitoneal route which points to a correlation between the intensity of the process and the period of incubation. As the appearance of the AV in our model is correlated chronologically with that of scrapie-associated fibrils, at least after i.c. transmission, the process may be related to a disturbance of cellular protein metabolism and, thus, to the processing of prion protein.

Key words: Scrapie – Prion – Neuronal autophagy – Lysosomes – Lipofuscin

Cellular autophagy is well known as a way of cellular protein turnover in neurons [4, 14] and other cells [8]. It has also been discussed as a way for the cell to get rid of damaged cytoplasmic constituents [10, 14]. The morphological manifestation of autophagic activity is the autophagic vacuole (AV) [6]. Boellaard et al. [2] reported giant AV in experimental Creutzfeldt-Jakob disease (CJD) in mice [2]. We wondered whether this process could also be found in experimental scrapie in rodents. Such studies in the cerebral cortex of mice and hamsters have been performed only recently [13]. We extended our study to the thalamus, the brain stem and the cerebellar cortex. The results have been preliminarily reported elsewhere [3].

Offprint requests to: J.W. Boellaard (address see above)

Material and methods

Fifteen outbred Syrian hamsters, 7–10 weeks old were inoculated intracerebrally (i.c.) with 50 µl of a 1% scrapie brain homogenate containing about 10^5–10^7 LD_{50} infective units of the 236K strain of scrapie [11]. The animals were housed singly and allowed to live 33–90 days post inoculation (p.i.). Two animals were inoculated intraperitoneally (i.p.) to avoid cerebral damage and killed only 90 days p.i. in view of the increased incubation period. Ten control animals were sham inoculated i.c. with normal hamster brain suspension and killed 33–68 days p.i. Two untreated animals were used as controls for the i.p. test (Table 1). Animals were killed by supravital perfusion of a picric acid-paraformaldehyde-glutaraldehyde fixative [19]. The perfusion was performed through a cannula inserted into the left ventricle of the heart [16]. Brain specimens were obtained from the following locations: cerebral cortex right and left, thalamus right and left, midbrain, and cerebellar cortex. Postfixation was performed in osmium tetroxide and block staining with uranyl acetate, dehydration in graded series of ethanol and embedding in Epon. Semithin sections were cut and stained with toluidine blue. Ultrathin sections were stained with lead acetate and examined in a Zeiss EM9 or EM10 electron microscope.

Results

Light microscopically the semithin sections revealed no signs of neuronal damage. In general no vacuolation was seen in the layers 2–7 of the cerebral cortex nor in the thalamus. Only the test animals killed after 73 and 90 days p.i. showed diffuse vacuolation in the cerebral cortex. Electron microscopically the four test animals killed 33 and 40 days p.i. showed no conspicuous alterations; the small AV seen in neuronal somata were also present in the controls. From 54 days p.i. large membrane-bound vacuoles (Fig. 1b) and numerous

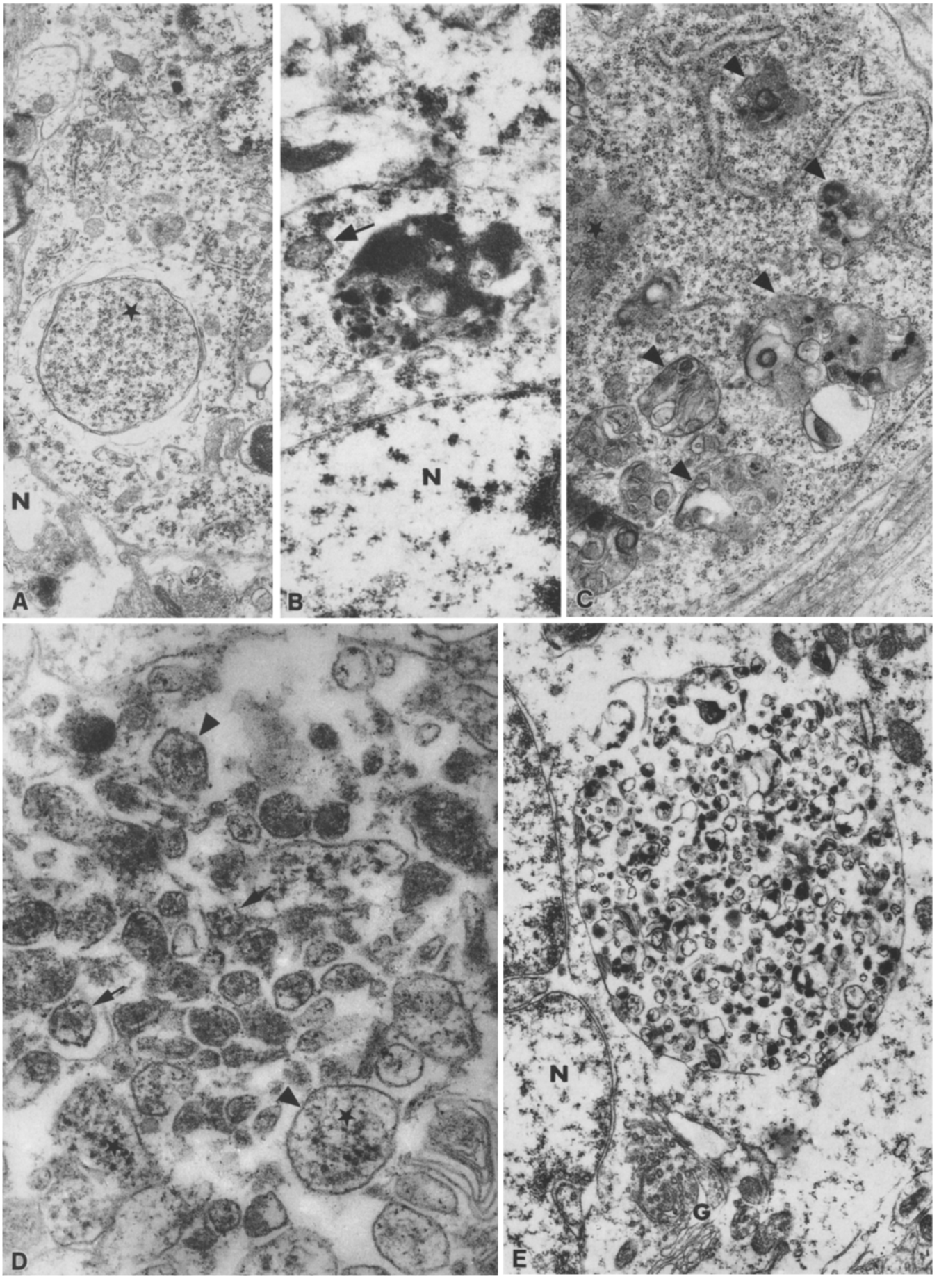
N
A
N
B
C
D
N
G
E

Table 1. Summary of experimental animals and presence of autophagic vacuoles (AV)

Test animals inoculated: i.c.	Day p.i.[a]	AV	Controls inoculated: i.c.	Day p.i.[a]	AV
1	33	–	1	33	–
2	33	–	2	33	–
3	40	–	3	40	–
4	40	–	4	40	–
5	54	×	5	54	–
6	54	×	6	54	–
7	61	×	7	61	–
8	61	×			
9	66	×			
10	66	×			
11	68	×	8	68	–
12	73	×	9	68	–
13	73	×	10	68	–
14	90	×			
15	90	×			
i.p.			i.p.		
16	90	(×)	11	120	–
17	90	(×)	12	120	–

[a] Day post inoculation (p.i.) on which the animal was killed
i.c.: Intracerebrally; i.p.: intraperitoneally; –: absent; ×: present

smaller ones (Fig. 1c) were the most striking finding in the perikarya of some neurons. They were solitary, about 1.7 μm in size, or smaller and multiple about 0.3 μm in size. These vacuoles contained ribosomes (Fig. 1a), debris (Fig. 1c) and lipofuscin granules (Fig. 1b), thus displaying the typical morphology of AV. The surrounding membrane was usually single, sometimes double (Fig. 1a) and seldom multiple. In places, we found collections of small membrane-bounded spherical profiles containing osmiophilic bodies (Fig. 1d). These were occasionally situated in giant membrane-bounded vacuoles (Fig. 1e). It must be emphasized that only a few neurons harbored giant AV and that these cells showed no signs of damage. Their Golgi fields were well developed. There were no conspicuous differences between the frequency of vacuoles in either cerebral hemisphere. Neurons with AV were most numerous in the thalamus, sparsely scattered in the basilar portion of the pons and extremely rare in the cerebellar cortex, where they were present in a few Golgi cells of the granular cell layer. They were never found in Purkinje cells (Table 1). AV of several sizes could be found in neuronal processes in the neuropil. In the two i.p.-injected animals, which had a significantly longer incubation period than i.c.-injected animals, AV were very rare. In all cases the number and appearance of primary lysosomes were not remarkable. The number and size of lipofuscin granules had only increased and coarsened in some neuronal perkarya. No signs of increased phagocytosis could be found in astrocytes. Spongiform changes of the neuropil were patchy and very rare in most of the animals. In the test animals at 73 and 90 days, p.i., however, spongiosis was seen in the cerebral cortex and in the thalamus but less in the midbrain. There was no obvious correlation between spongiform changes and the presence or frequency of AV. Neuronal perikarya were sometimes depleted of organelles but there were no vacuoles.

Fig. 1. A Early autophagic vacuole (AV, *asterisk)* filled with ribosomes and surrounded by a double membrane. *N:* Neuropil; 73 days post inoculation (p.i.); × 12,600. **B** Late AV filled with debris. *Arrow:* Lysosome; *N:* nucleus; 90 days p.i.; × 25,000. **C** Neuronal perikaryon filled with multiple smaller AV (*arrowheads*). *Asterisk:* Flat section of the nuclear envelope with nuclear pores; 54 days p.i.; × 20,000. **D** Multiple spheroid bodies loosely distributed in the neuronal cytoplasm. They are surrounded by a single unit membrane (*arrowheads*). Some of them are filled with ribosomes (*asterisk*) others with osmiophilic granules (*arrows*); 66 days p.i.; × 50,000. **E** Giant vacuole filled with numerous spherical bodies containing osmiophilic dots. *N:* Nucleus; *G:* Golgi fields; 61 days p.i.; × 20,000

Discussion

Neuronal autophagy has been reported under physiological conditions [4, 9] and increased in some experimental models, involving: colchicine [21]; vinblastine [20]; trauma [1]; and X-irradiation [14]. Increased neuronal autophagy with giant AV has been reported in experimental CJD in mice [2]. Recently similar results have been seen in experimental scrapie in rodents [13] and in hamsters [3]. In this latter model, previous clinical and biochemical studies [5] permit reliable relationship to morphological aspects. According to these latter authors, brain tissue in this model is infective from the 20th day p.i., although scrapie-associated fibrils (SAF) do not appear earlier than 41 days and clinical signs are not observed before 65 days p.i. As the giant AV develop between 40 and 54 days p.i. (Table 1), their origin may be correlated with that of the SAF. Their size is much larger than that seen in our controls and generally described in literature as about twice that of secondary lysosomes [7]. These „normal" AV are thought to mark the turnover of proteins and other plasma constituents in the normal and in the stress state [8, 15]. The giant AV may indicate focal cytoplasmic degradation [10] and isolation of injured cytoplasmic areas in damaged cells [13].

Apart from giant AV we found collections of small spherical bodies in neuronal perikarya of test animals. They differ from those described hitherto but, as they sometimes contain ribosomes, we regard them as AV similar to those seen in injured neurons with chromatolysis [9].

In our experiments the giant AV and their obvious chronological correlation with SAF raise the question of whether they may be related to the processing of prion protein (PrP). This view is supported by the fact that almost no giant AV are to be found in the cerebellum, since it is known that the production of PrP in the cerebellum is very low [12]. Therefore, we assume a link between autophagy and an altered cellular protein metabolism after infection with the scrapie agent.

Autophagy of hepatocytes in vitro [17] and in situ [15] is dependent on the amino acid content of the medium and may lead to "autophagic suicide" in the case of continuous amino acid deprivation [18]. Mortimore and Schworer [15] suggested that autophagy may serve as a mechanism for the cell to degrade normal components for energy needs. Likewise, AV in experimental prion diseases may be caused by a disturbance of cellular protein metabolism during the production and/or processing of prion protein.

In addition, there seems to exist a correlation between the intensity of the autophagic process (or the cellular process leading to it) and the route of infection. As in experimental CJD [2], the incubation period in this scrapie model is much longer after i.p. transmission than after the i.c. route of infection and neuronal AV are dramatically increased in the latter. Whatever the cause of autophagy in this model of experimental scrapie infection, the observation of unusual giant AV indicates a need to study the protein metabolism of neuronal cells and the role of neuronal processing of abnormal (prion) proteins after infection with the scrapie agent.

References

1. Barron KD, Means ED, Larsen, E (1973) Ultrastructure of retrograde degeneration in thalamus of rat I. Neuronal somata and dendrites. J Neuropathol Exp Neurol 32:218–244
2. Boellaard JW, Schlote W, Tateishi J (1989) Neuronal autophagy in experimental Creutzfeldt-Jakob disease. Acta Neuropathol 78:410–418
3. Boellaard JW, Kao M, Schlote W, Diringer H (1991) Neuronal autophagy in experimental scrapie. Clin Neuropathol 10:98
4. Brunk U, Ericsson ILE (1972) Electron microscopic studies on rat brain neurons. Localisation of acid phosphatase and mode of formation of lipofuscin bodies. J Ultrastruct Res 38:1–15
5. Czub M, Braig HR, Diringer H (1988) Replication of scrapie agent in hamsters infected intracerebrally confirms the pathogenesis of an amyloid-inducing virosis. J Gen Virol 69:1753–1756
6. de Duve C, Wattiaux R (1966) Functions of lysosomes. Annu Rev Physiol 28:435–492
7. Holtzman E (1969) Lysosomes in the physiology and pathology of neurons. In: Dingle JT, Fell HB (eds) Lysosomes in biology and pathology. North Holland, Amsterdam London, pp 192–216
8. Holtzman E (1989) Lysosomes. Plenum Press, New York, pp 243–318
9. Holtzman E, Novikoff AB, Villaverde H (1967) Lysosomes and GERL in normal and chromatolytic neurons of the rat ganglion nodosum. J Cell Biol 33:419–435
10. Hruban Z, Spargo B, Swift H, Wissler RW, Kleinfeld RG (1963) Focal cytoplasmic degradation. Am J Pathol 42:657–677
11. Kimberlin RH, Walker CA (1977) Characteristics of a short incubation model of scrapie in the golden hamster. J Gen Virol 34:294–304
12. Kretschmar HA, Prusiner SB, Stowring LE, DeArmond SJ (1986) Scrapie prion proteins are synthesized in neurons. Am J Pathol 122:1–5
13. Liberski PP, Yanagihara R, Gibbs CJ, Jr, Gajdusek DC (1991) Neuronal autophagic vacuoles in experimental Creutzfeld-Jakob disease and scrapie. Acta Neuropathol (in press)
14. Masurovsky EB, Bunge MB, Bunge RP (1967) Cytological studies of organotypic cultures of rat dorsal root ganglia following X-irradiation. I. Changes in neurons and satellite cells. J Cell Biol 32:467–496
15. Mortimore GE, Schworer CM (1977) Induction of autophagy by amino acid deprivation in perfused rat liver. Nature 270:174–176
16. Palay SL, McGee-Russell SM, Gordon S, Grillo MA (1962) Fixation of neural tissue for electron microscopy by perfusion with solutions of osmium tetroxide. J Cell Biol 12:385–410
17. Schwarze PE, Seglen PO (1980) Protein metabolism and survival of rat hepatocytes in early culture. Exp Cell Res 130:185–190
18. Seglen PO (1987) Regulation of autophagic protein degradation in isolated liver cells. In: Glaumann H, Ballard FJ (eds) Lysosomes: their role in protein breakdown. Acad Press, London New York, pp 371–414
19. Somogyi P, Takagi H (1982) A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neuroscience 7:1779–1783
20. Terry RD, Wisniewski H, Johnson AB (1970) Studies on the formation of autophagic vacuoles in neurons treated with spindle inhibitors (colchicine and vinblastine). J Neuropathol Exp Neurol 29:142–143
21. Wisniewski H, Terry RD (1967) Experimental colchicine encephalopathy. I. Induction of neurofibrillary degeneration. Lab Invest 17:577–587