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BRAIN RESEARCH

Research Report

Supravital microwave experiments support that the formation of "dark" neurons is propelled by phase transition in an intracellular gel system

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ABSTRACT

Background: Based on circumstantial evidences, we (Gallyas, F., Farkas, O., Mázló, M., 2004. Gel-to-gel phase transition may occur in mammalian cells: Mechanism of formation of "dark" (compacted) neurons. Biol. Cell 96, 313–324.) proposed that the formation of "dark" neurons (striking compaction of visibly normal ultrastructural elements accompanied with large-scale fluid excretion), which occur in many neurological diseases such as ischemia, proceeds with a non-enzymatic mechanism. Objective: To support this proposition, the present paper deals with the results of supravital experiments using microwave irradiation. Method: After transcardial glutaraldehyde fixation followed by decapitation, a pin was stuck into rat brains just before and just after they were warmed up to 80 °C and maintained at this temperature for various periods of time by controlled microwave irradiation. Results: Independently of the duration of irradiation, the pre-irradiation pin sticking produced numerous "dark" neurons in an approximating 500-μm-wide zone around its track whereas the post-irradiation pin sticking did the same only when the irradiation was shorter than 55 s. The excreted fluid was present in neighbouring astrocytic processes but not in the extracellular space. Conclusions: The formation of "dark" neurons is completed in less than 55 s under the circumstances of the experiment. As neurons are poor in readily consumable chemical energy in the absence of blood circulation, this rapid and massive fluid excretion cannot be explained by any enzyme-mediated membrane-related pump mechanism. An osmotic mechanism can also be discounted. In contrast, it is in conformity with the above mentioned non-enzymatic (physicochemical) phenomenon, the phase transition of a supra-cytoskeletal gel network storing free energy in the form of non-covalent interactions.

1. Introduction

Many diseases including ischemia, hypoglycemia and status epilepticus (Graeber et al., 2002), and experimental conditions such as various kinds of head injury (Gallyas and Zoltay, 1992; Csordás et al., 2003) can cause a common type of morphological damage to neurons. Such neurons, which are usually referred to as "dark", display the following unique characteristics (reviewed by Gallyas, 2007): (i) The "dark" neurons are strikingly shrunken (as a result of dramatic compaction of their ultrastructural elements) and (ii) hyperbasophilic (as a result of acquisition of excess negative charges). (iii) The "dark" neurons are non-systematically scattered among normal-looking neurons even of the same phenotype,

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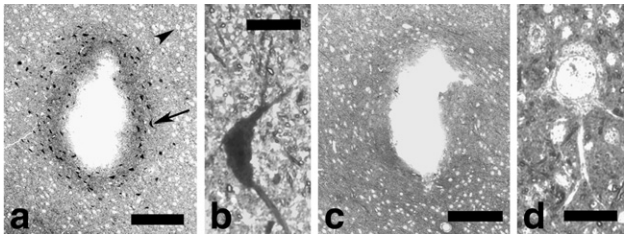

Fig. 1 – "Dark" (a and b) and normal-looking (a, c and d) neurons in a rat brain that was pin-stuck before (a, b and d) and after (c) microwave irradiation. In panels a and c, the normal neurons appear as small round patches lighter than the background. In panel a, an arrow points to the "dark" neuron enlarged in panel b, and an arrowhead to the normal-looking neuron enlarged in panel d. As seen in panel d, the somata and dendrites of normal-looking neurons are lighter than the background. Scale bars: a and c — 400 μm, b and d — 40 μm.

chemo-type and function (despite of the ubiquitous presence of the circumstances initiating their formation). (iv) The fluid excreted from the "dark" neurons can be found in neighbouring astrocytic processes (but not in the surrounding extracellular space). (v) The above characteristics are uniformly observable throughout the soma-dendrite domains of the "dark" neurons (all-or-nothing phenomenon), and (vi) are independent of the circumstances that initiated their formation. (vii) The in-vivo formation of the "dark" neurons in experimental animals can be initiated not only by diseases such as those mentioned above (chemical noxae), but also by momentary mechanical or electric injuries (physical noxae). (viii) Physical noxae can initiate the formation of "dark" neurons post mortem too, even under circumstances that are extremely unfavourable for enzyme-mediated processes. Because of these peculiarities, the mechanism of formation of "dark" neurons has been a century-old enigma in neuropathology.

Based on the experiments that proved item (viii) of the above characteristics, we proposed a non-enzymatic (physicochemical) mechanism of the formation of "dark" neurons (Gallyas et al., 2004, Kellermayer et al., 2006; Kovács et al., 2007) that can explain their other enigmatic characteristics too (see the review of Gallyas, 2007). A further argument in favour of the non-enzymatic nature of the formation of "dark" neurons (and thereby in favour of the physicochemical mechanism at issue) would be an experimental proof that the process of their formation is completed in seconds, rather than in minutes or hours, as assumed so far in neuropathology.

It is known that, at room temperature, transcardial perfusion of experimental animals with a glutaraldehyde fixative allows the ultrastructural compaction in neurons to take place completely even when initiated by a mechanical noxa several hours after the start of fixation, but not later (Cammermeyer, 1960; Gallyas et al., 2004). However, it was reported (Loberg and Torvik, 1993) that warming the rat brain up to 70 °C by means of microwave irradiation after transcardial perfusion with an aldehyde fixative hinders the production of "dark" neurons by mechanical injuries caused during post-irradiation removal of the brain from the skull, while does not disturb the morphological features of those formed in vivo around cryogenic lesions 1 h earlier. This report gave us the idea of the present study, in which a pin was stuck, through predrilled holes in the skulls, into brains of glutaraldehyde-fixed rats about 2.5 s before and 2.5 s after temperature-controlled microwave treatments lasting 25, 55, 115 or 235 s. The brain temperature reached 80 °C in about 10 s and was kept constant until the end of irradiation.

2. Results

In the case of the 55-second microwave treatment, numerous neurons (Figs. 1a, b) were strikingly shrunken and hyperbasophilic (homogenously and intensely stained with toluidine blue, the positively charged molecules of which bind to negative tissue sites) in an approximating 500-μm-wide zone around the track of the pre-irradiation pin sticking in each rat. These were randomly scattered among normal-looking neurons. Outside this zone every neuron was normal-looking (Figs. 1a, d). The somata (Figs. 2c, d) and dendrites (Figs. 3c, d) of the shrunken and hyperbasophilic neurons displayed increased electron-density and striking compaction of ultrastructural elements, as compared with the normal-looking

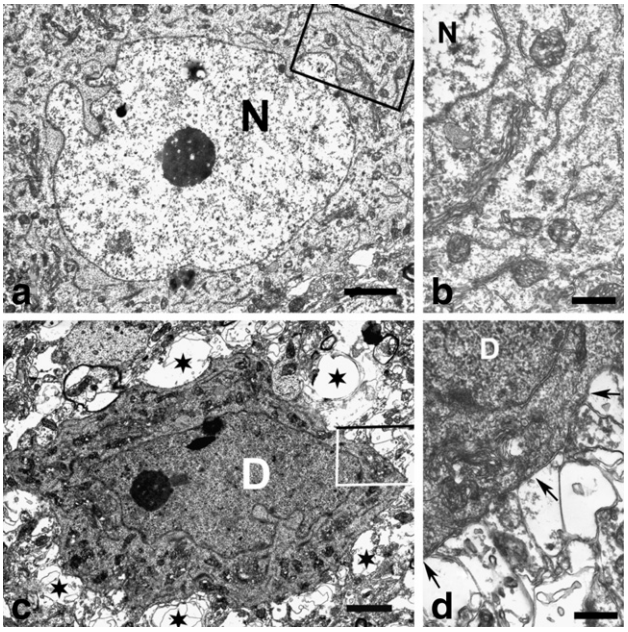

Fig. 2 – Electron-microscopic appearance of normal-looking (a and b) and compacted (c and d) neuronal cell bodies in a rat that was pin-stuck after transcardial perfusion with a glutaraldehyde fixative and just before microwave irradiation. Panels b and d are enlargements of the areas boxed in panels a and c, respectively. In panels a and b, N denotes the nucleus of a normal-looking neuron. In panels c and d, D denotes the nucleus of a "dark" (compacted) neuron. In panel c, asterisks denote strikingly swollen astrocytic processes. In panel d, arrows in strikingly swollen astrocytic processes point to the non-dilated extracellular space between them and the "dark" neuron. Scale bars: a and c — 2 μm, b and d — 500 nm.