Giant granular filamentous bodies in the cytoplasm of arcuate nucleus neurons of castrated rats

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Summary. We have performed an ultrastructural and quantitative study of granular filamentous bodies (GFBs) present in the cytoplasm of some arcuate nucleus neurons of rats of both sexes castrated at one month of age and sacrificed one or three months later, as well as untreated and sham-operated animals of the same ages. GFBs appear as round or ovoid cytoplasmic inclusions of granular-filamentous texture and generally lack a limiting membrane; their sizes vary from 0.7 to 2.8 μm (average 1.7 μm). GFBs are present more frequently in the perikarya, but they also occur in dendrites. In rats of both sexes castrated at one month of age and sacrificed three months later a noticeable finding was the presence of some giant GFBs, whose major axis could reach up to 6 μm. The nomenclature, the origin and the possible significance and function of GFBs are discussed.

Key words: Granular filamentous bodies, Arcuate nucleus, Castrat ion, Rat

Introduction

Granular filamentous bodies (GFBs) are round or ovoid cytoplasmic inclusions, of 1-2 μm in diameter, appearing as accumulations of electron-dense material generally devoid of a surrounding membrane. They have also been termed nematosomes, nucleolus-like bodies, chromatoid bodies or cytoplasmic filamentous bodies (see discussion). GFBs occur in the cytoplasm of certain neurons of the central nervous system (Kawabata, 1965; Shimizu and Ishii, 1965; Gambetti et al., 1968; Brawer, 1971; Le Beux, 1971, 1972; Le Beux et al., 1971; Sano and Sotokawa, 1971; Kishi, 1972; Anzil et al., 1973; Santolaya, 1973; Hindelang-Gertner et al., 1974; Millhouse, 1978; Van Houten and Brawer, 1978; Groves and Wilson, 1980; Lafarga et al., 1980; Dellmann, 1982; Katoh and Shimizu, 1982; Ledesma-Jimeno et al., 1982; Van Den Pol, 1982; Jennes et al., 1985; Kiss, 1985; Leranth et al., 1985, 1991; Amat et al., 1987; Menéndez Peláez and Alvarez-Uría, 1987; Peláez et al., 1991); in cells of the Harderian gland (López et al., 1990); and in other cell types (see Takeuchi and Takeuchi, 1982).

In a previous work (Peláez et al., 1991) we performed an ultrastructural and quantitative study of GFBs -termed in that paper cytoplasmic filamentous bodies- present in hypothalamic arcuate nucleus (ARCN) neurons of male rats treated with a single dose of monosodium glutamate (4 mg/g b.w.) at four days of age and sacrificed 7, 30 and 120 days after administration of the neurotoxic substance. The results suggested a possible relationship between the number of GFBs and neuronal activity.

In this work we study the GFBs present in ARCN neurons in rats of both sexes castrated at the age of one month and sacrificed at one or three months after surgery and in sham-operated and untreated animals of the same ages, with the aim of reporting on some features of GFBs of castrated rats and clarifying the confusion generated in the literature owing to the use of different nomenclatures by several authors.

Materials and methods

Seventy-two Sprague-Dawley rats of both sexes were used. From weaning the animals were housed under standard stabiling conditions, caging them by litters and sex, temperature 20±2 °C, relative humidity 50±5% and free access to food and water.

The animals were divided into groups as follows: 1) 24 rats (12 male, 12 female) were castrated at one month of age (castrated rats, CR); 6 animals of each sex were sacrificed one month after castration (CR1+1) and the other rats three months after surgery (CR1+3). 2) 24 rats (12 male, 12 female) were sham-castrated at one month of age (sham-castrated rats, SC); 6 animals of each sex were sacrificed one month after surgery (SC1+1) and the other rats three months after sham-intervention (SC1+3). 3) We also used 12 untreated rats (UR; 6 male, 6 female) sacrificed at two months of age (UR1+1) and 12 untreated rats (6 male, 6 female) sacrificed at four months of age (UR1+3). Castration was performed via
the posterior and scrotal routes for females and males, respectively, and verified after the sacrifice of the animals. Sham-castrated and untreated female rats were sacrificed in proestrus.

Under intraperitoneally-administered sodium thiopental anaesthesia (45 mg/kg b.w.) all the animals were perfused through the left ventricle with a fixative solution of 5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4, at 4 °C following a wash of the vascular tree with a 150 mM sodium chloride solution. After perfusion, the hypothalamus was removed. Blocks containing the median eminence-arcuate nucleus region were kept in the fixative solution over 12 h and then post-fixed in 1% osmium tetroxide in the same solution of 5% glutaraldehyde in 0.1M phosphate buffer for 90 min at 4 °C. They were then dehydrated in acetone, contrasted with 2% uranyl acetate and embedded in Durcupan (Fluka). Ultrathin sections were taken on an LKB Ultratome III 8800 ultramicrotome and studied with a Philips EM-201 electron microscope.

Results

In the ARCN neurons of untreated, sham-castrated and castrated rats of both sexes, the GFBs appeared as round or ovoid cytoplasmic inclusions which consisted of granular and fibrillar material. They occurred in the perikarya of the neurons (Figs. 1, 2 4-10) and occasionally in dendrites (Fig. 3). Generally, the GFBs lacked a limiting membrane, although on rare occasions GFBs partially surrounded by cisternal rings near to the cellular nucleus were seen (Fig. 5). The cisternae did not form complete rings; rather, they consisted on an inner membrane and an outer membrane separated by a pale halo.

The ultrastructural aspect of these cytoplasmic inclusions was variable. Certain GFBs were dense and homogeneous (Figs. 1, 3 8-10), but there were also GFBs containing clear spaces, appearing as uni- or multi-locular cavitations containing a flocculent matrix, ribosome-like granules as other organelles such as clear or granular vesicles (Fig. 4). An unusual finding was the GFB of figure 2 which showed two electron-dense zones, one central and another peripheral, separated by a pale intermediate zone. We also found cytoplasmic inclusions with an ultrastructural aspect that was intermediate between the homogeneous GFBs and those displaying cavitations (Figs. 5-7).

The presence of electron-dense granules lacking a membrane at the periphery of some GFBs (Fig. 1, arrows) or attached to them was observed (Figs. 6, 7, arrows). Next to certain GFBs dense-core vesicles were also found (Figs. 2, 6 arrowheads).

The major axis of the GFBs of arcuate neurons of our animals (untreated, sham-castrated and castrated rats) usually ranged from 0.7 to 2.8 μm (average 1.7). However, in rats of both sexes castrated at one month of age and sacrificed three months after castration, we observed some very large GFBs (Figs. 8-10). These «giant» GFBs were very homogeneous. The major and minor axes of the GFB of figure 8 measured 5 and 4 μm, respectively, and those of the GFB of Figure 10 measured 6 and 3.75 μm.

Sometimes, the GFBs were present in the vicinity of axodendritic (Fig. 3) or axosomatic synapses (Figs. 5, 8, 9), although no morphological relationship between the GFB and the postsynaptic web was observed.

Discussion

We have used the term granular filamentous bodies (GFBs) - a name previously employed by King et al. (1974) - to refer to inclusions present in the cytoplasm of some ARCN neurons of castrated, sham-castrated and untreated rats. These inclusions consist of filamentous and granular elements and generally lack a limiting
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membrane.

References in the literature concerning cytoplasmic inclusions that are ultrastructurally identical to those reported in the present work are very numerous. However, the use of different terminologies by the different authors has given rise to not a little confusion. Additionally, the origin, the significance and the function of these cytoplasmic inclusions remains to be elucidated. In this discussion we shall analyze successively the terminology used to designate the GFBs, the hypotheses that have been put forward to account for their origin and nature, and their possible functions.

A) The confusing nomenclature of the GFBs

Cytoplasmic inclusions ultrastructurally identical to GFBs have been called nematosomes. However, it should be pointed out that the term nematosomes was first used by Grillo (1970), with the meaning of thread-like body, to refer to a cytoplasmic inclusion present in sympathetic neurons of adult rats, whose ultrastructural aspect differs considerably from that of the GFBs. In fact, this author drew attention to the difference between the structures described by her (nematosomes) and «...the granulofilamentous inclusions described by some investigators as nuclear or nucleolar-like in appearance...». Later, structures identical or very similar to those reported by Grillo have been observed in rat sympathetic post-ganglionic neurons (Heym and Addicks, 1982), in human and mouse placenta (Ockleford et al., 1987) and in external horizontal cells of white perch retina (De Juan et al., 1991). The confusion arises because, despite the warning of Grillo, some authors have used the term nematosome to refer to cytoplasmic inclusions similar to the GFBs that differ ultrastructurally from Grillo’s inclusions (Hindelang-Gertner et al., 1974; Tasso and Rua, 1978; Gash et al., 1980; Hervás and Lafarga, 1980; Hervás et al., 1980; Lafarga et al., 1980; Walsh et al., 1982; Jennes et al., 1985; Leranth et al., 1985, 1991). In addition Le Beux (1972) uses the term nematosome to denote both inclusions similar to the GFBs and other structures that are in no way ultrastructurally similar to these or to the inclusions reported by Grillo. It is surprising that in an earlier work Le Beux et al. (1971) state: «Although the organelle that we described in a previous paper is structurally different from that reported by Grillo (1970), this does not eliminate a possible identity in the function of these two cytoplasmic inclusions. Organelles of this type will be therefore more conveniently designated throughout this paper by the descriptive term “nematosome”». Despite the fact that we cannot establish whether the nematosomes of Grillo and the GFBs are of the same nature, it is evident that the ultrastructural aspects of both are very different. We therefore believe that the term nematosome should be avoided when referring to cytoplasmic inclusions similar to those described in the present work (i.e., GFBs). Additionally, it should be stressed that, as far as we are aware, inclusions resembling those reported by Grillo have not been described in hypothalamic neurons.

Basing their ideas on the ultrastructural resemblance of the GFBs to the nucleolus and assuming a nuclear origin for such inclusions, some authors have employed the term nucleolar-like bodies (Shimizu and Ishi, 1965; Kishi, 1972; Anzil et al., 1973; Santolaya, 1973; Hindelang-Gertner et al., 1974; Ford and Milks, 1978; Tasso and Rua, 1978; Gash et al., 1980; Groves and Wilson, 1980; Katoh and Shimizu, 1982; Menéndez Peláez and Alvarez Uría, 1987). Moreover, other authors have used the term nucleolar-like body to refer to structures identical to the nematosomes of Grillo (Heym and Addicks, 1982; De Juan et al., 1991). The terminological confusion is completed when several authors indiscriminately use the terms nucleus-like body and nematosome to designate inclusions identical to the GFBs (Hindelang-Gertner et al., 1974; Ford and Milks, 1978; Tasso and Rua, 1978; Hervás and Lafarga, 1980; Hervás et al., 1980; Lafarga et al., 1980; Van Den Pol, 1982). In our opinion, which agrees with that of Van Houten and Brawer (1978), the term nucleus-like body is not appropriate because it assumes a nuclear origin for the GFBs, a hypothesis posed some time ago (see below) but which for the time being remains to be demonstrated.

Occasionally, the term chromatoid bodies has been used to designate the GFBs (Millhouse, 1978; Ledesma-Jimeno et al., 1982; Kiss, 1985).

The term cytoplasmic filamentous bodies has also been used to refer to the GFBs (Van Houten and Brawer, 1978; Walsh and Brawer, 1979; Walsh et al., 1982; Peláez et al., 1991). We believe that this term might be valid, although the ultrastructural aspect of these inclusions, in which the granular component is outstanding, together with the fact that most authors describe that they are formed of granular and fibrillar material, suggests to us that the term granular filamentous bodies (GFB) would be more appropriate.

It should be noted that the problem posed by the different denominations used is not, in the case of the GFBs, a merely «academic» or «terminological»
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question. A brief review of the literature on cytoplasmic inclusions reveals that the inappropriate use of equivocal terms such as nematosomes or nucleolus-like bodies has led many authors to lump together inclusions that, ultrastructurally, differ widely from one another, such as the true nematosomes of Grillo, the «reticular nematosomes» of Le Beux or the GFBs.

B) Hypothesis on the origin and nature of the GFBs

Both nuclear and cytoplasmic origins have been proposed for the GFBs. In this sense, it has been suggested that the GFBs might arise from a process of nucleolar extrusion (Shimizu and Ishii, 1965; Sano and Sotokawa, 1971). An aspect in favour of this hypothesis is the observation that some of these structures appear surrounded by a double membrane and are located in the vicinity of the cell nucleus (Sano and Sotokawa, 1971; Kishi, 1972; Van Houten and Brawer, 1978; see also our Fig. 5). In our material the GFBs surrounded by a double membrane were scarce. Van Houten and Brawer (1978), in ventromedial hypothalamic nucleus of male rats, observed some GFBs only partially surrounded by cisternal rings and others completely enveloped. These authors state: «Thus, there would seem to be a range of intermediate morphologic associations between completely encapsulated CFBs and those entirely free in the cytoplasm. This range of possible intermediates is consistent with the nuclear extrusion hypothesis». According to this suggestion, the GFBs surrounded by membrane would correspond to inclusions recently extruded from the nucleus.

In order to clarify the origin of GFBs several authors have carried out cytochemical studies, although these results are not fully conclusive. Kishi (1972) affirms that GFBs are proteinaceous in nature, while for other authors these inclusions consist of ribonucleoproteins (Hindelang-Gertner et al., 1974; Santolaya, 1973; Takeuchi and Takeuchi, 1982; Takeuchi and Sonta, 1983). These latter observations are consistent with a nuclear origin for GFBs.

On admitting the nuclear extrusion hypothesis for the GFBs, it becomes necessary to entertain the idea of the existence of a later aggregation process inside the cytoplasm. Indeed, it is very difficult to imagine the nuclear extrusion of structures measuring up to 6 μm in diameter, such as some of those observed by us in this work.

Regardless of what the origin (nuclear or cytoplasmic) of the GFBs might be, certain ultrastructural images are in accordance with a process of cytoplasmic aggregation. Thus, close to some GFBs, we have seen dense-core vesicles and small granules; the latter lack a membrane and are in contact with the GFBs while the vesicles are slightly removed from them (Figs. 1, 2, 6 and 7). Similar images have been reported by Ford and Milks (1978) in ARCN neurons of male rats following adrenalectomy. These images could suggest that the GFBs would be formed, at least partially in the cytoplasm through the aggregation of small granules of unknown origin.

C) The possible function(s) of the GFBs

Despite the large number of publications concerning the GFBs, their significance and function still remain unknown. In our opinion, an interesting observation is the presence of GFBs in embryonic cells (Takeuchi and Takeuchi, 1982) and during the first days of post-natal life (Walsh and Brawer, 1979; Lafarga et al., 1980; Dellmann, 1982; Menéndez Peláez and Alvarez-Uría, 1987; López et al., 1990; Peláez et al., 1991). We feel that this would allow one to discard the idea that GFBs would be age-related degenerative structures.

It has been proposed that GFBs may represent a reserve of ribonucleoprotein or preribosomal material in germinial cells (Weakley, 1971; Azvedo, 1984), in embryonic cells (Takeuchi and Takeuchi, 1982) and in neurons (Shimizu and Ishii, 1965; Katoh and Shimizu, 1982, Menéndez-Peláez and Alvarez-Uría, 1987). It has also been suggested that GFBs might be accumulations of material synthesized by neurons and that they would later be used and/or released (Anzil et al., 1973). It has even been speculated «that the cells containing such bodies might synthesize some proteinaceous substances, which serve as unknown neurotransmitters;...» (Katoh and Shimizu, 1982).

GFBs have been considered as a sign of cellular hyperactivity (Norström and Hansson, 1973; Hindelang-Gertner et al., 1974; Tasso and Rua, 1978; Menéndez Peláez and Alvarez-Uría, 1987; López et al., 1990). In male rats treated with a single dose of monosodium glutamate (4 mg/kg b.w.) at four days of age and sacrificed 7 days after administration of neurotoxic substance we have observed an increase in the number of GFBs present in arcuate neurons coinciding with ultrastructural features indicative of cellular hyperactivity (Peláez et al., 1991). In addition, an increase in the number of GFBs after treatment with high doses of estradiol valerate has been reported in animals previously ovariectomized, both in the rat (Leranth et al., 1985) and in the monkey (Leranth et al., 1991).

According to the latter authors, GFBs are always present in glutamic acid decarboxylase-containing neurons, while for other authors these inclusions consist of ribonucleoproteins (Hindelang-Gertner et al., 1974; Santolaya, 1973; Takeuchi and Takeuchi, 1982; Takeuchi and Sonta, 1983). These latter observations are consistent with a nuclear origin for GFBs.

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Giant granular filamentous bodies (GFBs) (up to 6 µm in diameter) in ARCN neurons of rats of both sexes that had been castrated at one month of age and sacrificed three months later. Because the major axis of the GFBs in the arcuate neurons of the sham-castrated and untreated rats of our material ranged from 0.7 to 2.8 µm (average 1.7 µm) and the size of these inclusions referred to in the literature (see Table 1), it is undoubted that a GFB whose major axis measures 5 to 6 µm is a «giant» GFB. To our knowledge, GFBs as large as we observed in this work have never been described previously in the literature. We believe that the genesis of these giant GFBs may be due to an increase in the cellular activity of some ARCN neurons following castration. The exact nature of these neurons should be elucidated in further studies.

Finally, it has been suggested that GFBs may be involved in the formation of the subsynaptic web (Le Beux et al., 1971; Le Beux, 1972). In our study the involved in the formation of the subsynaptic web (Le Beux et al., 1971; Le Beux, 1972; Millhouse, 1978; Brawer J.R. (1971). The role of the arcuate nucleus in the brainpituitary-gonad axis. J. Comp. Neurol. 143, 411-436.


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