# Intranuclear rodlets in retrochiasmatic area neurons of the hypothalamus of the rat

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*Summary.* Microfilamentous spindle-shaped nuclear rodlets are described in neurons of the retrochiasmatic area in the rat hypothalamus. The authors consider these structures as normal and dynamic nuclear inclusions of these neurons, although their significance remains unknown.

Nuclear rodlets (NR) are rare and unusual inclusions consisting of microfilaments and/or microtubules present mainly in the nucleus of some neurons. They were initially studied by Ramón and Cajal[1] by optical microscopy. Since then they have been reported in a wide variety of species and loci[2–7], both by light and electron microscopy. Recently, Seite et al.[8,9] described 3 types of NR in peripheral sympathetic neurons: a) microfilamentous spindle-shaped inclusions, b) microfilamentous-microtubular spindle-shaped inclusions and c) crystalloids composed by layers of tubular or fibrillar lattices.

It has been suggested a relation of NR with pathological processes[10–12], ageing[13], sexual behaviour[7] and the level of neuronal activity[6,9,14]. However, neither the distribution nor the functional significance of NR has been clarified so far. In the present work we report an ultrastructural study of NR in the retrochiasmatic area of rat hypothalamus. To our knowledge, this is the first

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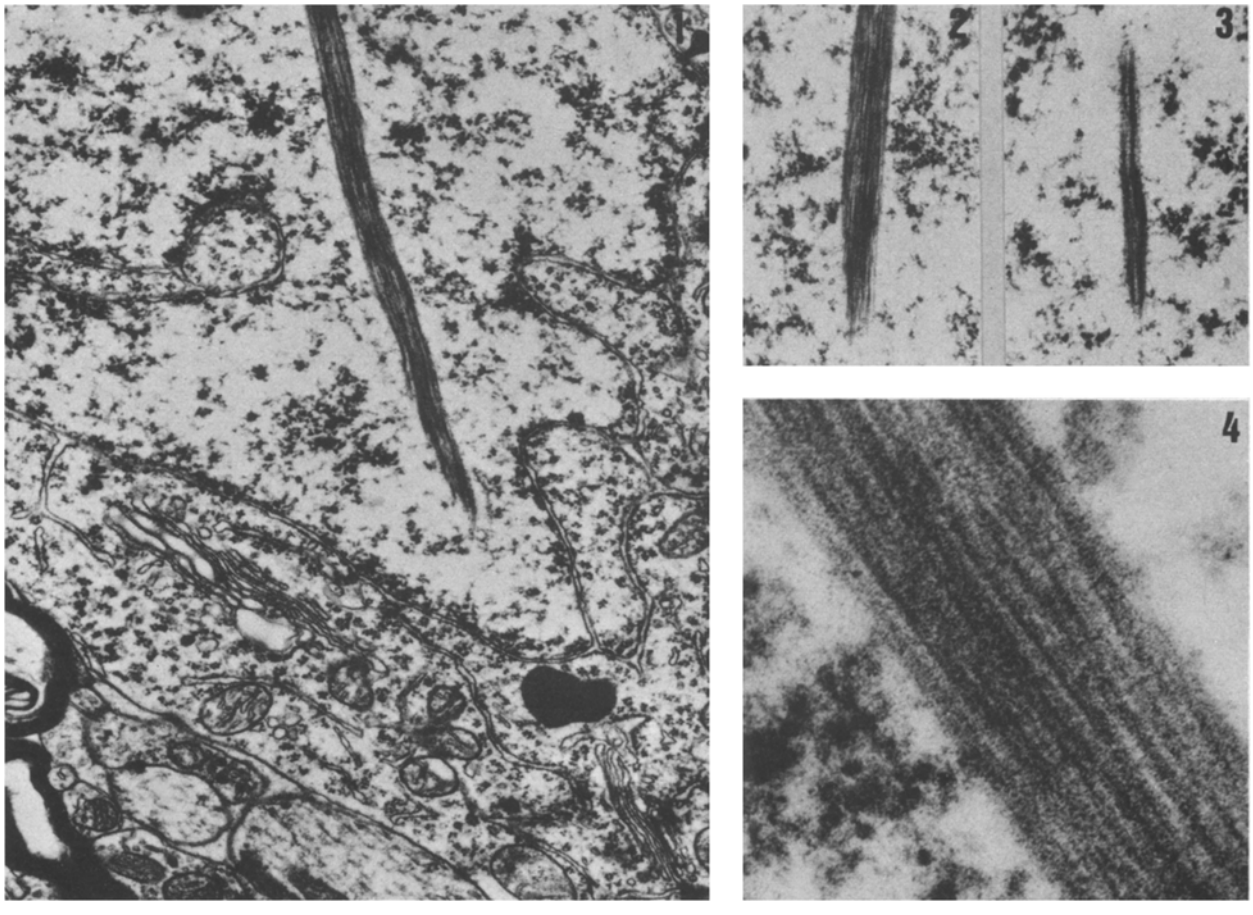


Fig. 1–4. Microfilamentous spindle-shaped nuclear rodlets. Longitudinal section. *1* ×19,200, *2* ×14,200, *3* ×16,200 and *4* ×120,000.

observation of this type of intranuclear inclusions in the neurons of the retrochiasmatic area of any mammalian, and also the first report of NR in the hypothalamus of the rat.

*Methods.* The ultrastructure of the NR of the hypothalamic retrochiasmatic area was studied in normal adult Wistar rats of both sexes. The rats were fixed by perfusion with 3% glutaraldehyde and the hypothalamic blocks were post-fixed in 2% osmium tetroxide. Both fixatives were maintained at pH 7.4 with 0.12 M phosphate buffer. The hypothalamic blocks were dehydrated in acetone and embedded in Durcupan (Fluka). Ultrathin sections were stained with 1% aqueous uranyl acetate and then lead citrate, and examined in a Philips EM-201 electron microscope.

*Results.* The neurons of the retrochiasmatic area display an irregular nucleus with extensive infoldings of the nuclear membrane. The electron-lucid cytoplasm contains mitochondria, free polyribosomes and isolated cisternae of granular endoplasmic reticulum (figure 1).

In some neurons of this area, both in male and female rats, microfilamentous spindle-shaped rodlets are observed. The rodlets have an indeterminate length and they are 0.1–0.3 µm width (figures 1–3). These structures are composed of a bundle of numerous closely packed microfilaments oriented along its log axis (figures 1 and 4). These microfilaments, of about 60–70 Å in diameter, are generally straight. They are arranged in parallel with a centre-to-centre spacing of 140–150 Å (figure 4).

The position of the rodlets within the nucleus is variable, but they are never related to any other nuclear features, such as the nucleolus or the nuclear envelope. They are not surrounded by a narrow-zone free of chromatin granules, and frequently contacts between the rodlets and chromatin granules can be observed (figures 1 and 4).

*Discussion.* This study shows that NR are present in neurons of the retrochiasmatic area of the hypothalamus of the healthy adults rats. Although in some instances its presence in the central nervous system has been related to pathological processes[10–12], they must be considered as normal nuclear inclusions in the neurons of this area, both in male and female rats. Since NR are widely distributed in various types of neurons, it has been proposed that they should be considered as a normal cellular organelle[6,9,14]. Our report of these structures in an area where they have not been previously observed support this suggestion.

The structure and shape of NR observed in this area correspond to the microfilamentous spindle-shaped inclusions described in other neuronal locations[2–7]. Other types of NR such as microfilamentous-microtubular spindle-shaped or crystalloids[8] have not been found.

The origin of NR has been studied in detail in embryonic material by Masurovsky et al.[5]. In our study, in the adult rat, we found NR of different thickness, suggesting that they are dynamic structures of the neuron formed by progressive aggregation of protein subunits. This observation is in agreement with the results of Seite et al.[14,15]. According to these authors, it could be suggested that their formation is related with the level of neuronal activity. The irregular distribution of these structures we have observed within the neurons of this retrochiasmatic area supports this hypothesis.

Seite et al.[9] have demonstrated recently that the cyclic AMP induces the formation of NR in the neurons of the sympathetic ganglion. They suggest that this effect is a consequence of the increase of the neuronal activity mediated by the modulating effect of the cyclic AMP on the synaptic transmission. The facts that cyclic AMP plays a prominent role in the catecholaminergic transmission[16], and that this kind of transmission is specially important in the retrochiasmatic area we have studied[17], may suggest a relation between the presence of NR and catecholaminergic neurons. The occurrence of NR in other hypothalamic areas rich in catecholaminergic neurons, like arcuate nucleus[7], supports the above hypothesis.

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## The effect of tolbutamide on early embryos of *Xenopus laevis*

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*Summary.* The effect of tolbutamide on the early development of Xenopus laevis has been studied. The results suggest that continuous exposure to $3 \times 10^{-4}$ M tolbutamide causes abnormal development.

Tolbutamide, N′-4-methylbenzenesulphonyl-N″-butylurea, is a therapeutic agent useful for the control of diabetic hyperglycemia. Although its side effects in adults are minimal, there is evidence that such hypoglycemic drugs are teratogenic in mammals[2,3]. However, it is not clear whether its teratogenic effects in mammals are due to tolbutamide itself or to the metabolic state of the pregnant female induced by tolbutamide. As part of a series of experiments designed to assess whether sea-urchin embryos could be used as a test system for pharmacological agents, Hagström and Lönning[4] studied the effects of tolbutamide on the gametes and early embryos of Paracentrotus lividus. Although tolbutamide did not affect fertilization, it did have rather specific effects on early development. Embryos exposed to tolbutamide showed inhibition of the formation of the endoderm, a greater number of yolk platelets than controls, and a change in the ultrastructural appearance of the yolk platelets[4]. Any agent which effects specifically one embryonic tissue might prove a powerful tool for the analysis of early differentiation, and it is important

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