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Research Articles

Central terminals of capsaicin-sensitive primary afferent make synaptic contacts with neuronal soma in the mouse substantia gelatinosa

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Abstract. Degeneration of primary afferent central terminals (C-terminals) that contact neuronal soma in the substantia gelatinosa of the spinal dorsal horn was examined by electron microscopy 2 h after s.c. injection of capsaicin into newborn and adult mice. The C-terminals were small, dark, sinuous or slender terminals with clear synaptic vesicles in the early postnatal period. They are thought to develop into scalloped CI-terminals, surrounded by dendrites and a few axonal endings, forming synaptic glomeruli. The same type of nonglomerular terminals making presynaptic contacts with neuronal soma showed degeneration in both the newborn and adult animals, and were identified as capsaicin-sensitive CI-terminals. This finding suggests that capsaicin-sensitive C-fibers have a modulatory role on their own nociceptive input besides functioning in nociceptive transmission in the substantia gelatinosa.

Key words. Primary afferent central terminal; substantia gelatinosa; capsaicin; degeneration; neuronal soma; synapses.

There are some reported ultrastructural studies of degeneration of primary afferent central terminals (C-terminals) in the superficial dorsal horn of newborn rats[1–5] and mice[6,7], and in adult rats[8], after administration of capsaicin. Results have indicated that capsaicin causes selective damage to one type of glomerular C-terminals in the early postnatal period[3–5]: the small, dark, round, sinuous or slender terminals filled with clear synaptic vesicles and surrounded by many dendrites, which later develop into the electron-dense terminals with a scalloped contour known as CI-terminals[9]. The same type of terminals making contact with neuronal soma were seen in the substantia gelatinosa of newborn and adult mice[6,10]. Identification of morphological features of the capsaicin-sensitive CI-terminals and their anatomical relationship to surrounding dendrites, boutons and soma, are of fundamental importance for understanding nociceptive transmission in the superficial dorsal horn. We therefore examined whether capsaicin-sensitive CI-terminals make contact with neuronal soma in the substantia gelatinosa.

Materials and methods

DDY mice of 2 neonatal (day 2 after birth) and adult mice were given a s.c. injection of 50 mg/kg capsaicin (Nacalai Tesq., Kyoto). Two neonatal and adult control DDY mice were given a s.c. injection of the vehicle only (10% alcohol and 10% Tween-80 in physiological saline). Two hours later, two newborn animals from each group were anesthetized with ether and immersed in a solution of 1.2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The lumbar spinal cord was flushed with the fixative by pipette under a binocular microscope. Similarly, two adult mice from each group were perfused through the left cardiac ventricle first with saline, then with the same fixative, 2 h after capsaicin or vehicle injection. The lumbar spinal cord was dissected out. After midsaggital sectioning, the spinal cord was cut into segments. The tissue blocks were immersed in fresh fixative of the same composition for 2 h at 4 °C. The specimens were then washed with phosphate buffer, and postfixed in 1% O_sO_4 for 2 h at 4 °C. After dehydration in an ethanol series, the tissues were embedded in Epon. For detection of well preserved contours of the dorsal horn, semi-thin sections stained with toluidine blue were examined by light microscopy. Ultrathin sections were cut transversely, stained with uranyl ácetate and lead nitrate, and examined in a Hitachi H-800 electron microscope. The substantia gelatinosa was identified on the basis of the presence of clusters of small roundish neurons with scanty cytoplasm.

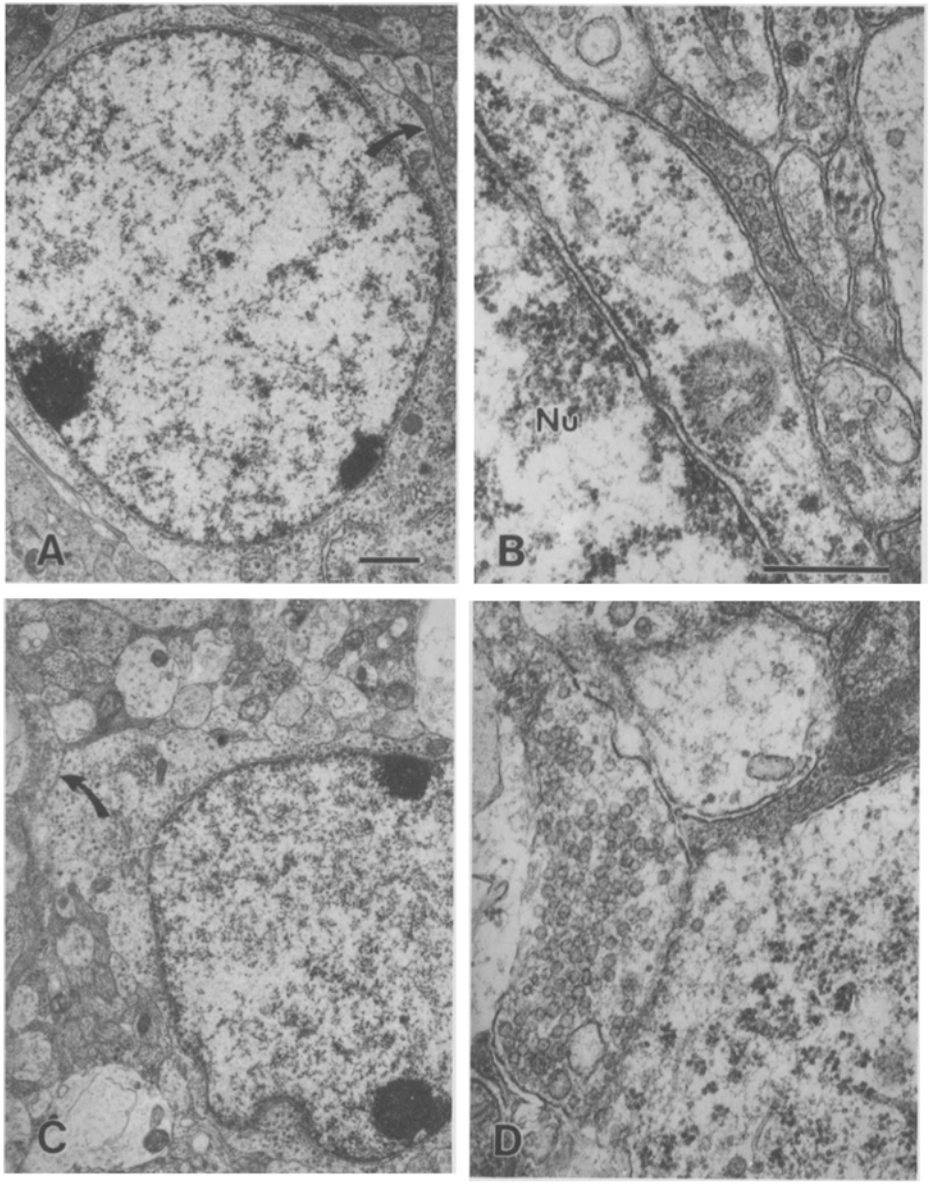

Figure 1.

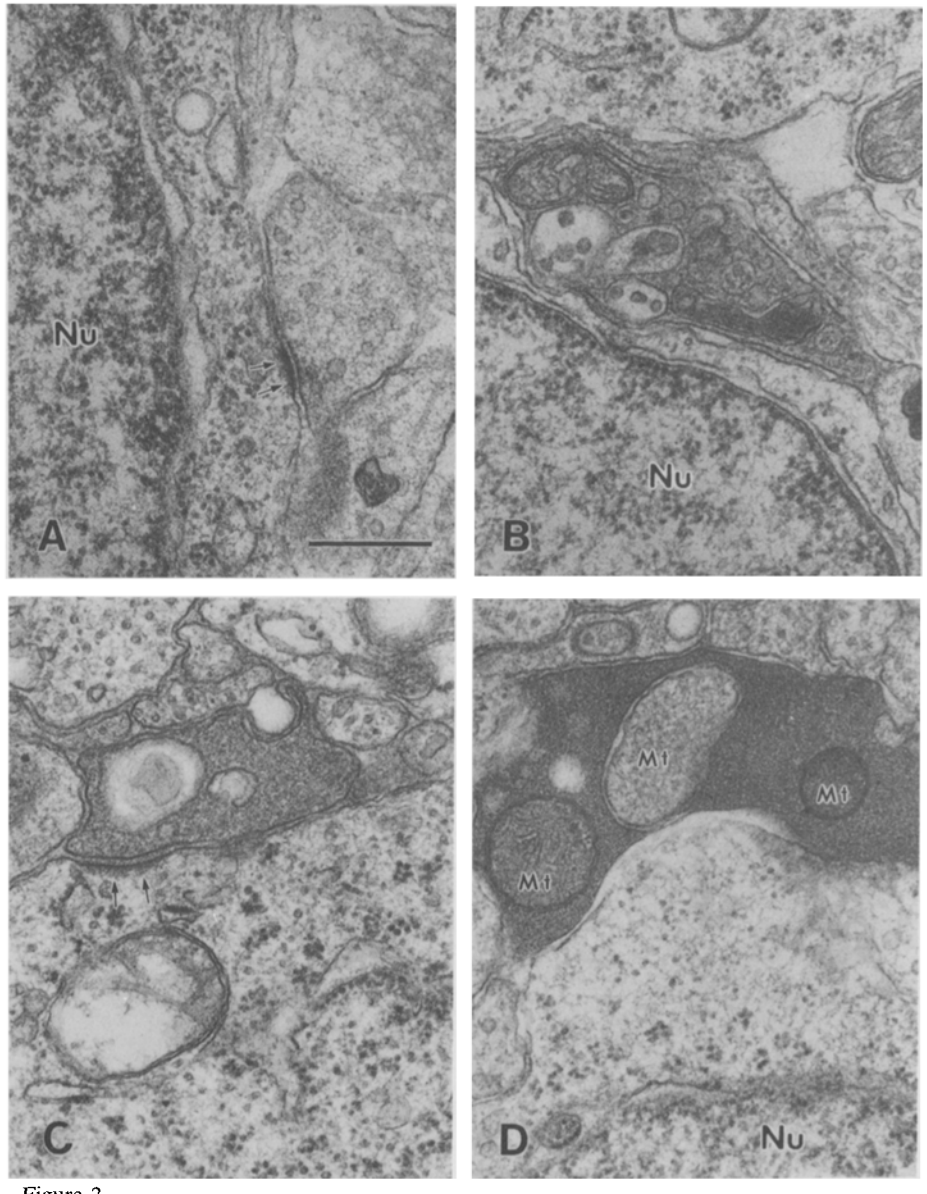

Figure 2.

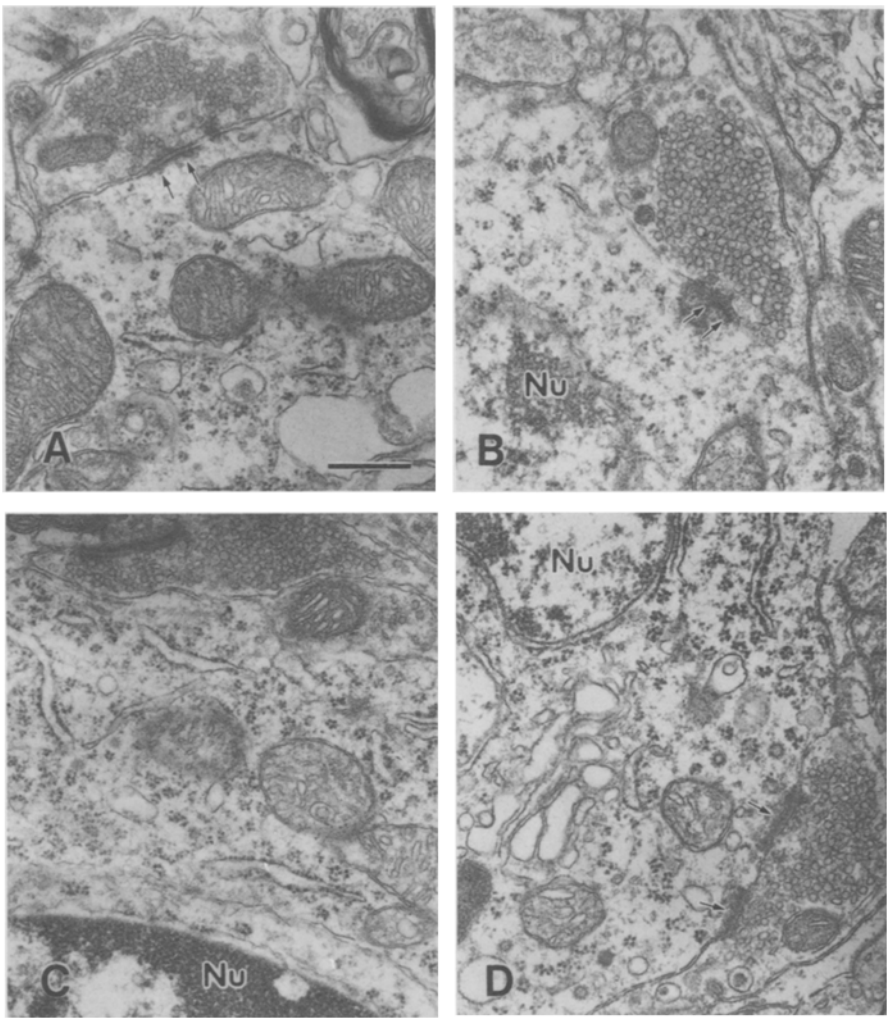

Figure 3. Synaptic contacts of nonglomerular CI-terminals with neuronal soma in the substantia gelatinosa of adult mice 2 h after vehicle injection (control). *A*, *B*, *D*, many CI-terminals, showing scalloped contours with closely packed clear synaptic vesicles, make presynaptic contacts with interneuronal soma. *C* (uppermost), a clear synaptic structure is not visible, probably because the section was cut outside the synaptic structure. Arrows indicate postsynaptic site. Nu – nucleus. Bar represents 0.5 μm.

Results

In the earlier postnatal periods glomerular CI-terminals were small, dark, round, sinuous or slender terminals with closely packed clear synaptic vesicles, and were centrally located and surrounded by several dendrites and a few axonal endings. They developed into dark, scalloped CI-terminals with densely packed clear synaptic vesicles.

Besides the glomerular CI-terminals, nonglomerular CI-type terminals made contact with neuronal soma in the substantia gelatinosa of newborn and adult mice (figs 1 and 3). The number of nonglomerular CI-terminals were smaller than glomerular type, but it is not difficult to find the former. In some cases, a few nonglomerular CI-terminals made contact on a neuronal somata. Frequently, specialized (clear) synaptic structures were visible in the adult mice (figs 3 and 4). We examined the degenerated CI-terminals for the presence of a rounded aggregation of part of the axoplasm

Figure 1. Synaptic contacts of nonglomerular CI-terminals with neuronal soma in the substantia gelatinosa of neonatal mice 2 h after vehicle treatment (control). An intact dark, sinuous CI-terminal with clear synaptic vesicles (arrow, *A*, enlarged in figure *B*). An intact CI-terminal with closely packed clear synaptic vesicles (arrow, *C*, enlarged in figure *D*). Nu – nucleus. Bar represents 1.0 μm in *A* and *C*, and 0.5 μm in *B* and *D*.

Figure 2. Some examples of synaptic contacts of degenerating nonglomerular CI-terminals on neuronal soma in the substantia gelatinosa of neonatal mice 2 h after capsaicin treatment. Note the CI-terminals showing various degrees of degeneration. *A*, a small roundish degenerating CI-terminal making presynaptic contact with neuronal somata; *B*, a degenerating CI-terminal showing dark axoplasm with degraded synaptic vesicles and mitochondria; *C*, *D*, severely degenerated homogeneous more highly electron-dense CI-terminals with degenerated mitochondria and many vesicles. Degeneration is progressive from *A* to *D*. Arrows indicate postsynaptic site. Mt – mitochondria, Nu – nucleus. Bar represents 0.5 μm.

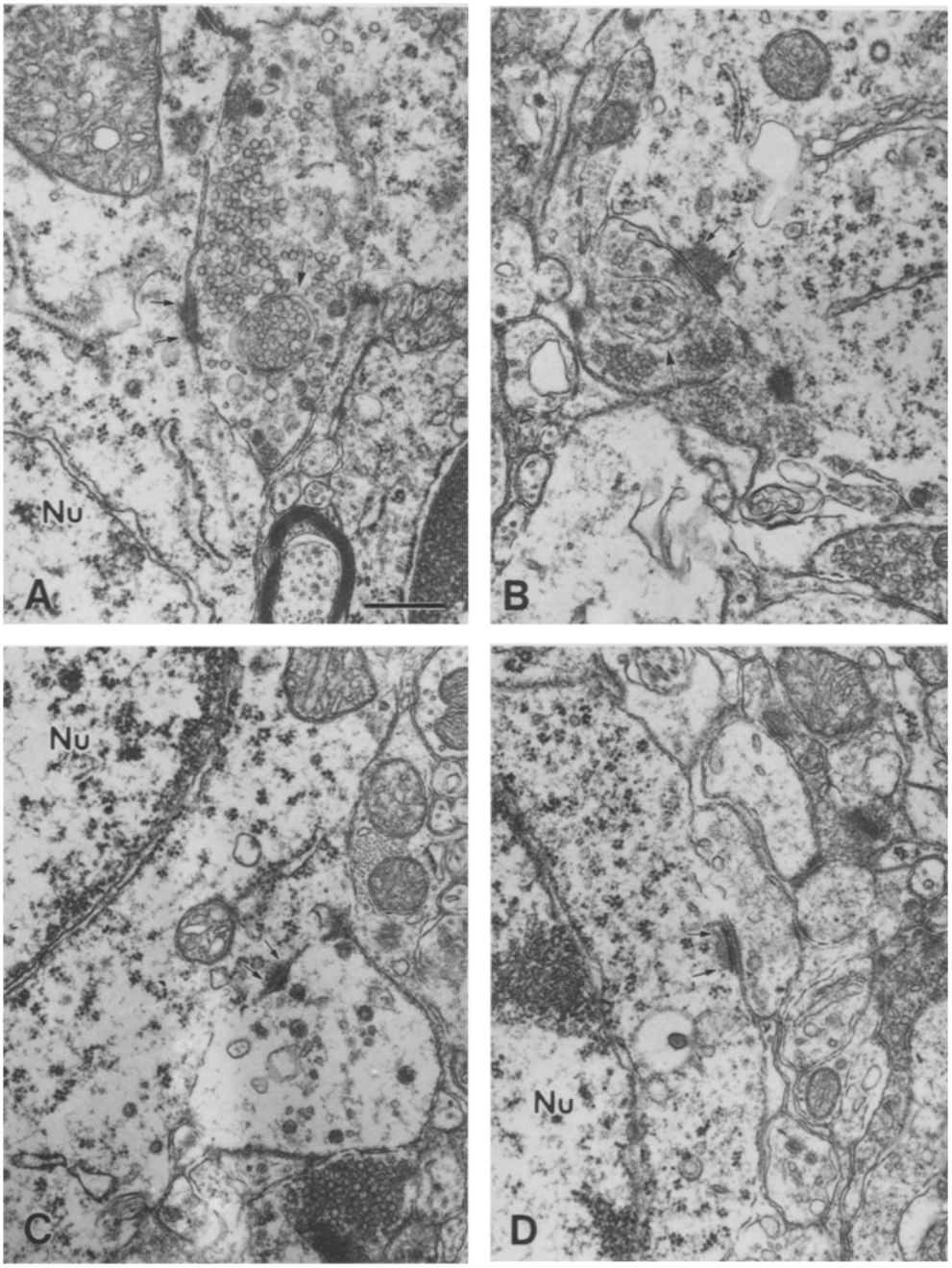

Figure 4. Some examples of synaptic contacts of degenerating nonglomerular CI-terminals on neuronal soma in the substantia gelatinosa of adult mice 2 h after capsaicin treatment. *A*, *B*, early degenerating CI-terminals, showing rounded aggregations of part of the axoplasm (large arrows). *C*, *D*, degenerating CI-terminals showing degradation of synaptic vesicles and homogeneous electron-lucent axoplasm. Note that all the degenerating CI-terminals make presynaptic contacts with the interneuronal soma. Arrows indicate postsynaptic site. Nu – nucleus. Bar represents 0.5 μm.

(cytolysosome) and disrupted synaptic vesicles, showed homogeneous high electron-dense axoplasm with numerous vacuoles and clearly degenerated mitochondria, or electron-lucent axoplasm devoid of axoplasmic organelles[7]. Judged by these criteria, nonglomerular CI-type terminals making contact with neuronal soma in the substantia gelatinosa of both newborn and adult mice showed degeneration (figs 2 and 4) similar to that of glomular CI-terminals. No such degenerative figures were seen in controls (figs 1 and 3).

Discussion

Capsaicin is known to affect selectively CI-terminals of smaller primary afferent neurons[3–5,8]. The present study showed that the degenerating nonglomerular CI-terminals affected by capsaicin make contact with neuronal soma in the substantia gelatinosa, which consists of tightly packed, small, round or fusiform cells[11]. In the adult animals particularly, nonglomerular CI-terminals formed presynaptic contacts on the interneuronal soma.

The unclear synaptic structure of nonglomerular CI-terminals in the newborn animals may indicate an immature formation of synapses in the neonatal period. Synapses of capsaicin-sensitive CI-terminals on neuronal soma have not been reported previously. Quantitative analysis of the number of nonglomerular CI-terminals was not performed in this study, but there were quite a few nonglomerular CI-terminals forming synapses on interneuronal soma. As nonglomerular CI-terminals synapsing on neuronal soma are seen in both newborn and adult mice, such synaptic contact is maintained throughout life. Although Willis and Coggeshall[12] claimed that the gelatinosa is not a closed system (certain cells in lamina II project to the thalamus, brain stem and lamina I), most neurons in the dorsal horn are interneurons[13]. Recently, we showed that in the mouse, CI-type terminals make presynaptic contacts with GABAergic dendrites[10] and soma[14] in the substantia gelatinosa. Thus, the anatomical relationship between CI-terminals and interneurons suggests the regulation of pain transmission by C-fibers themselves in the substantia gelatinosa.

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