SHORT COMMUNICATION

Synaptology of the proximal segment of pyramidal cell basal
dendrites

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Abstract

Pyramidal neurons are covered with dendritic spines, the main postsynaptic targets of excitatory (asymmetrical) synapses. However, the proximal portion of both the apical and basal dendrites is devoid of spines, suggesting a lack of excitatory inputs to this region. In the present study we used electron microscopy to analyse the proximal region of the basal dendrites of supra- and infragranular pyramidal cells to determine if this is the case. The proximal region of 80 basal dendrites sampled from the rat hindlimb representation in the primary somatosensory cortex was studied by electron microscopy. A total of 317 synapses were found within this region of the dendrites, all of which were of the symmetrical type. These results suggest that glutamate receptors, although present in the cytoplasm, are not involved in synaptic junctions in the proximal portion of the dendrites. These data further support the idea that inhibitory terminals exclusively innervate the proximal region of basal dendrites.

Introduction

Typically, pyramidal neurons possess one apical dendrite and several basal dendrites that radiate from the base of the cell body. These dendrites are covered with spines that constitute the main postsynaptic targets for asymmetrical (excitatory) synapses in the cerebral cortex (reviewed in White, 1989; DeFelipe & Fariñas, 1992).

Excitatory synapses are also present on dendritic shafts of neocortical pyramidal cells, although infrequently (Hersch & White, 1981, 1982; White & Hersch, 1981, 1982), but they have not been described on the proximal dendrites, cell bodies (Feldman, 1984; Peters & Harriman, 1990; Fariñas & DeFelipe, 1991a; White et al., 1994) or axon initial segments (Sloper & Powell, 1979; Fairén & Valverde, 1980; Somogyi et al., 1982; Fariñas & DeFelipe, 1991b). Similar observations have been made in hippocampal CA1 pyramidal cells (Megías et al., 2001; Papp et al., 2001). Symmetrical synapses, which are believed to be inhibitory (Ribak, 1978), often terminate on the cell body and axon initial segments of pyramidal cells, as well as on their dendritic shafts (reviewed in White, 1989; DeFelipe & Fariñas, 1992). Asymmetrical synapses to neocortical pyramidal cells are provided by afferent inputs and cortical spiny neurons (e.g. pyramidal and spiny stellate cells), whereas most symmetrical synapses originate from nonsynaptic, nonpyramidal cells (also known as aspyrin interneurons) (Colonnière, 1968; Peters et al., 1968; Jones & Powell, 1969; Peters & Kaiserman-Abramof, 1970; for reviews see Feldman, 1984; White, 1989; DeFelipe & Fariñas, 1992).

Considerable data provided by light microscopy have shown that the proximal portions of pyramidal cell basal dendrites (approximately 10–15 μm from the soma) are devoid of dendritic spines (for a review see Elston & DeFelipe, 2002) but, as yet, there are insufficient data to determine whether asymmetrical synapses are formed directly with the shafts of proximal dendrites. This information is needed to better understand the wiring characteristics of pyramidal cells. In the present study we used conventional electron microscopy to examine the fine structure of the proximal regions of basal dendrites belonging to supra- and infragranular pyramidal cells to study the ultrastructural features of proximal dendritic cytoplasm. We confirmed that the aspiny proximal portion of the basal dendrites is devoid of asymmetrical synapses. In addition, we found that the distribution of synapses along the proximal portions of the basal dendrites was different for supra- and infragranular pyramidal cells as compared with infragranular cells.

Materials and methods

All experiments were performed according to the European Communities Council Directive (86/609/EEC) for the use and care of experimental animals and the experimental procedures approved by the Animal Welfare Committee of the Cajal Institute.

Intracellular injection

Three 5-month-old Wistar rats of either sex (two females and one male) were perfused through the heart, under sodium pentobarbital anaesthesia, with physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was then removed and postfixed for 24 h at 4 °C. Coronal sections (200 μm) were cut through
the hindlimb representation in the primary somatosensory area with the aid of a vibratome. Cell injection methodology has been described in detail elsewhere (Elston et al., 1997).

Electron microscopy

Five female Wistar rats (5 months old) were used for electron microscopic studies. The animals were overdosed with a lethal i.p. injection of Nembutal and decapitated. The brains were removed immediately and immersed in cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 36 h at 4 °C. The blocks containing the hindlimb representation (between bregma −0.26 mm and bregma −2.12 mm; Paxinos & Watson, 1977) were sectioned at 50 μm in the coronal plane with a vibratome. Sections were postfixed in a solution containing 2% glutaraldehyde in phosphate buffer for 1 h and then osmicated in 1% osmium tetroxide, dehydrated and flat-embedded in Araldite resin. Plastic-embedded sections were studied by a correlative light and electron microscopic method (DeFelipe & Fairén, 1993). Semithin sections containing pyramidal cells showing, in addition, basal dendrites were resectioned into serial ultrathin sections with a silver–grey interference colour method. The ultrathin sections were collected on formvar-coated, single-slot grids, stained with uranyl acetate and lead citrate and examined with a JEOL 1200 EX electron microscope. Preparations were photographed (×10000) and printed at a final magnification of ×30000. Morphometric examinations of the dendrites were performed using a digitizing tablet (Summasketch III; Summagraphics, Seymour, USA). Statistical analyses were made with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Estimations of the lengths of basal dendrites that were devoid of spines were made on intracellularly injected cells from both supragranular (layers II and III) and infragranular (layer V) layers. A total of 36 basal dendrites from nine pyramidal cells in layers II and III and seven from layer V were examined at the light microscopic level. In all cases, no spines were observed on the proximal 10 μm of the basal dendrites (Fig. 1). A total of 80 basal dendrites were analysed from 76 pyramidal cells (43 supra- and 33 infragranular cells) by correlative electron microscopy (Fig. 2). In all cases, dendrites were examined for a length of 10–20 μm in continuity with the soma. Thirty-two of these dendrites were examined in two to three ultrathin sections.

The cytoplasm of the proximal portion of basal dendrites displayed a large number of microtubules parallel to the surface of the dendrite (Fig. 2). Mitochondria and other organelles, such as multivesicular

Fig. 1. Photomicrographs of a supragranular pyramidal cell from the hindlimb representation of the primary somatosensory cortex of the rat. (A) This neuron was injected with Lucifer Yellow, processed with DAB and stained subsequently for Nissl substance. Note the complete absence of dendritic spines on proximal dendritic segments. (B) Higher magnification of (A) to better illustrate the lack of spines on the proximal segment of a basal dendrite. Scale bar (in B), 15 μm (A); 5 μm (B).
bodies, endoplasmic reticulum and polyribosomes, were spread throughout the cytoplasm but also formed clusters near the surface (Figs 2 and 3). Polyribosomal rosettes were frequently found close to postsynaptic densities but also at nonsynaptic sites (Fig. 3).

Synapses with a prominent postsynaptic density (40–50 nm in thickness) were identified as asymmetrical, whereas those with a thinner postsynaptic density (roughly 20 nm in thickness) (Gray, 1959; Colonnicier, 1968, 1981; Peters, 1987; White, 1989; Peters et al., 1991; Peters & Palay, 1996) were identified as symmetrical. As we did not reconstruct the basal dendrites in serial sections, the data below are rough approximations for the purpose of comparing synaptic densities on dendrites from different layers. A total of 317 synaptic contacts were found on the dendrites, all of which were symmetrical (Fig. 3). The density of synapses was higher for dendrites of cells in supra- rather than infragranular layers. We measured the dendritic diameters in order to ascertain whether these differences were related to differences in dendritic size. We found that the diameters were similar in supra- and infragranular cells (mean ± SD, 1.25 ± 0.41 and 1.47 ± 0.55 μm, respectively). Therefore, the differences observed were not related to dendritic diameter. A repeated measures analysis of variance over the first 10 μm of the basal dendrites revealed a significant difference in synaptic density between supra- and infragranular cells ($P = 0.017$). Posthoc analysis revealed the difference to be significant over the first 5 μm. The number of synaptic terminals in this 5 μm (mean ± SEM) was 2.22 ± 0.18 and 1.62 ± 0.16 in supra- and infragranular layers, respectively. Furthermore, the greatest differences in synapse density between supra- and infragranular pyramidal cells were found at 2 and 3 μm from the soma. Interestingly, less than 20% of the dendritic surface was covered with axon terminals (19 and 16% for cells in supra- and infragranular layers, respectively).

Discussion

In agreement with previous findings in various cortical layers of different species (for a review see Elston & DeFelipe, 2002), we found that the proximal portions of pyramidal cell basal dendrites in rat hindlimb representation in the somatosensory cortex lack dendritic spines. In this and in other ways, the proximal regions of pyramidal cell basal dendrites bear strong similarities to their parent cell bodies, both are observed to be essentially spine-free and, with rare exceptions (Liu
et al., 1991), are postsynaptic nearly exclusively at symmetrical synaptic junctions (e.g. Boothe et al., 1979; Feldman, 1984; Diao & So, 1991).

As reported previously (Bodian, 1972; Peters et al., 1991), the proximal portions of the basal dendrites of pyramidal cells contain numerous clusters of polyribosomes, suggesting that the machinery for protein synthesis and synapse formation is present within the dendrites (reviewed in Steward & Schuman, 2001; Villanueva & Steward, 2001). Polyribosomal rosettes are found in nonsynaptic regions (present results) and also beneath both symmetrical and asymmetrical synaptic sites on the dendrites (Steward, 1983; reviewed in Steward & Schuman, 2001) near the postsynaptic densities. However, asymmetrical synapses were not observed on the proximal dendritic segments examined in the present study.

One possible explanation for the absence of asymmetrical synapses from proximal dendrites is that the cytoskeleton in this region of pyramidal cells is not permissive to localization/anchoring of glutamatergic receptors, thus preventing the formation/stabilization of excitatory synapses (for a review see Craig & Boudin, 2001). Such nonpermissiveness may be attributed to a number of factors (see Elston & DeFelipe, 2002 for a review). For example, the cytoskeletal scaffold may vary along the length of the dendrite, resulting in regional specialization in synapse distribution. Such specialization has been demonstrated for polysialized acid–neural cell adhesion molecule and serotonin receptors on the axon initial segment of pyramidal cells (Azmitia et al., 1996; DeFelipe et al., 2001; Arellano et al., 2002) and for specific glutamate receptor subunits on dendrites (Stowell & Craig, 1999).

Further studies are necessary to identify the mechanisms that contribute to regional specificity in the anchoring of receptor complexes and synapse formation. Only then will we begin to understand the specifics of address selection of inputs to cells in determining specificity in cortical circuits.

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References


