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CB1 receptor localization in rat spinal cord and roots, dorsal root ganglion, and peripheral nerve

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ABSTRACT

AIM: The localization of CB1 receptors in the spinal cord, spinal roots, dorsal root ganglion (DRG), and peripheral nerve of the rat was determined. **METHODS**: We studied the distribution of CB1 cannabinoid receptors by immunohistochemistry using an antibody raised against the N-terminal of the receptor. **RESULTS**: The spinal cord showed numerous transverse fibers labelled for CB1 receptors throughout and concentrated in the dorsal horn. Lightly-stained cells were observed throughout the spinal cord gray matter. The DRG also showed cells and fibers labelled for CB1 receptors. Labelled fibers were observed in both dorsal and ventral roots as well as in peripheral nerves. **CONCLUSION**: The presence of CB1 receptors in the DRG, the dorsal root, and the dorsal horn is in accordance with the analgesic effects of cannabinoids. The presence of labelled cells and fibers in the ventral horn and ventral root provides a substrate for cannabinoid-induced muscle relaxant and antispastic effects.

INTRODUCTION

Cannabinoids produce antinociceptive effects by actions in the spinal cord and at peripheral sites, besides their actions in the brain[1-8] and they produce muscle relaxation by a hypothesized action at the neuromuscular junction and/or the spinal cord[9,10]. Previous work has revealed the distribution of CB1 binding, CB1 mRNA, and CB1 immunoreactivity in the brain, and we previously reported the presence of CB1-like immunoreactivity in the spinal cord[11-17]. Autoradiographic studies revealed the binding of cannabinoid receptors in the spinal cord and CB1 receptor mRNA in the DRG[11,16]. However, there are no detailed descriptions of the distribution of immunoreactive CB1 receptors in the spinal cord[13], its roots, DRG, or peripheral nerves. This study examined the localization of CB1 receptor-like immunoreactivity in these areas providing information about its distribution at the cellular level.

MATERIALS AND METHODS

Subjects Ten male Sprague-Dawley rats (Charles River Laboratories), approximately 250 - 300 g served as subjects. They were housed in single cages on a 12-h light/dark cycle with food and water *ad lib*.

Immunohistochemistry Rats were deeply anesthetized with sodium pentobarbital (75 mg/kg, ip). The cardiovascular system was flushed transaortically with heparinized 0.9 % saline, followed by perfusion with Zamboni's fixative. Brains were removed, postfixed in the same fixative overnight and cryoprotected by immersing the tissues in 20 % sucrose potassium phosphate-buffer saline (KPBS) 50 mmol·L^{-1}. The brains were cut on a Reichert cryostat (30 μm). Adjacent sections were collected for Nissl staining with Neutral Red or Cresyl Violet to facilitate the identification of the location of immunoreactivity in the immunohistochemical study.

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Floating slices were washed in KPBS 50 mmol/L, incubated with the affinity purified CB1 receptor antibodies (1 : 2000 dilution in KPBS 50 mmol/L, 0.4 % Triton, 1 % bovine serum albumin) at 4 ℃ for 48 h. The sections were washed with KPBS 50 mmol/L, incubated with biotinylated goat antirabbit antibodies (1 : 200) at room temperature for 1 h followed by avidin-biotin complex (Vector Elite, Burlingame, CA). Visible reaction product was produced by treating the sections with 0.04 % diaminobenzidine (DAB), 2.5 % nickel sulfate, and 0.01 % H_2O_2, dissolved in sodium acetate 0.1 mol/L. To terminate the reaction, the sections were washed twice with 0.9 % NaCl. The sections were mounted on gelatin-coated slides. After drying in open air, the sections were treated with graded alcohols, xylene, and coverslipped with Permount. Controls for immunohistochemistry in slices included the preabsorption and co-incubation of the antibodies with the immunizing protein and incubation with anti-GST antibodies. The observations and photography of the slides were performed using a Nikon Labophot-2 microscope.

RESULTS

Numerous transversely cut fibers that appeared as dots were found throughout the white and grey matter of the spinal cord (Fig 1A, 2A) and were especially numerous in the dorsal horn surrounding small spheric immunonegative areas (Fig 1B). Fibers extending from the white matter into the grey matter and reaching both sides of the dorsal and ventral horns were observed underneath the central canal (Fig 1C). Fig 1D showed a detail of the central canal surrounded by transverse fibers labelled for the CB1 receptor. Cells with a very light sheet of immunoreactivity were observed throughout the grey matter of the spinal cord, and it was difficult in most cases to determine whether the cell was labelled or surrounded by immunoreactivity present on afferents to the cell. Very lightly labelled neurons and their processes were observed in the ventral horn (Fig 1F, G).

The amount of immunoreactivity for CB1 was much higher in the DRG than that in the spinal cord (Fig 2A). Virtually all cells were labelled with varying intensities for the CB1 receptor antibody in the DRG (Fig 2C, E). In some pairs of apposed DRG cells, demarcated strips of CB1-like immunoreactivity were observed on both sides of a parallel immunonegative strip that appeared to represent the glial sheath separating the cells (Fig 2E, inset).

Both dorsal and ventral roots exhibited labelled fibers (Fig 1E, 2A, C) as did the peripheral nerve (Fig 2F). Fig 2B and D were adjacent sections to A and C respectively, but Nissl-stained with Cresyl Violet.

DISCUSSION

The results of this study are in general agreement with the previous studies of cannabinoid receptor markers[11-17] and provide a more detailed examination of the distribution of CB1 receptors because of the greater cellular resolution of immunohistochemistry. The main difference between this study and a previous study of the distribution of drug binding and CB1 receptor mRNA[16] is the observation that virtually all cells in the DRG are labeled, whereas the previous study found that only a small percentage of small neurons were labeled[16]. The larger number of small cells labeled by immunohistochemistry might have been due to differences in sensitivity of the two methods. The presence of CB1-like immunoreactivity in the dorsal root and the proximal nerve suggestes transport of CB1 receptors to both the distal and central terminals of primary afferent neurons. The finding of substantial levels of CB1 receptor immunoreactivity in the smaller DRG cells and the widespread labelling of the dorsal root implies that the ability of cannabinoids to suppress C-fiber mediated spinal responses may be mediated by direct presynaptic actions on primary afferent neurons[7]. The presence of labelled fibers distal to the DRG suggests that it is a source of cannabinoid receptors to the periphery, in accord with previous studies suggesting distal transport of cannabinoid receptors[17] and peripherally-mediated analgesic action of cannabinoids[8].

The present study further indicates that CB1 receptors are located on DRG cell bodies. This is almost certainly the case since there are few, if any, synapses in the DRG obviating the possibility that the labelling is due to presynaptic receptors on any afferents to these cells. These receptors may play a role in cannabinoid analgesia, though their role in normal

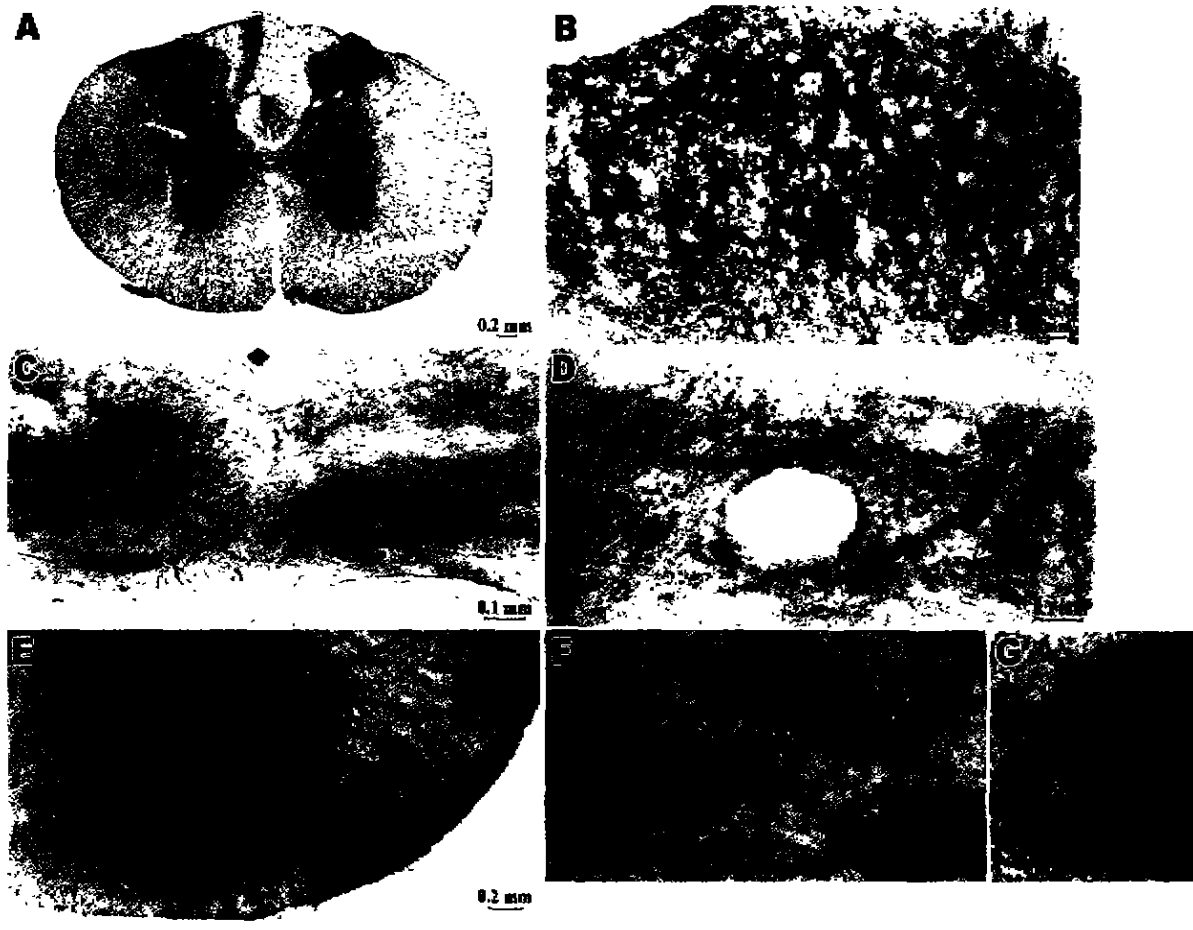

Fig 1. A: Immunohistochemistry of rat spinal cord for CB1 receptors. B: Enlargement of dorsal horn grey (left), white matter (arrow pointing dorsally). Note the numerous transversely cut immunoreactive fibers. C: Enlargement of the central canal area. Note the immunoreactive fibers entering the grey matter from the white matter underneath the central canal (denoted by ◆). D: Immunoreactivity surrounding the central canal in lamina X. E: Enlargement of the ventral horn. Note the immunoreactive motoneurons axons converging towards the ventral root (arrow). F: Lightly stained cells for the CB1 receptor in the ventral horn. G: Higher magnification of the cell on the right of F (arrow).

physiology is unknown. The presence of CB1 receptors on DRG somata, together with the presence of ion channel-types known to be modulated by cannabinoids[19-21], and the high levels of the endocannabinoid 2-arachidonylglycerol in the DRG[22], may reflect a novel form of intercellular communication in the DRG.

Previous work indicated that cannabinoids produce muscle relaxation in quadraplegic patients and patients with multiple sclerosis[9,10], highly suggestive of direct motor actions of cannabinoids on either the spinal cord, the neuromuscular junction or both. The presence of labelled cells in the ventral horn and labelled fibers exiting the ventral root provides a potential substrate for

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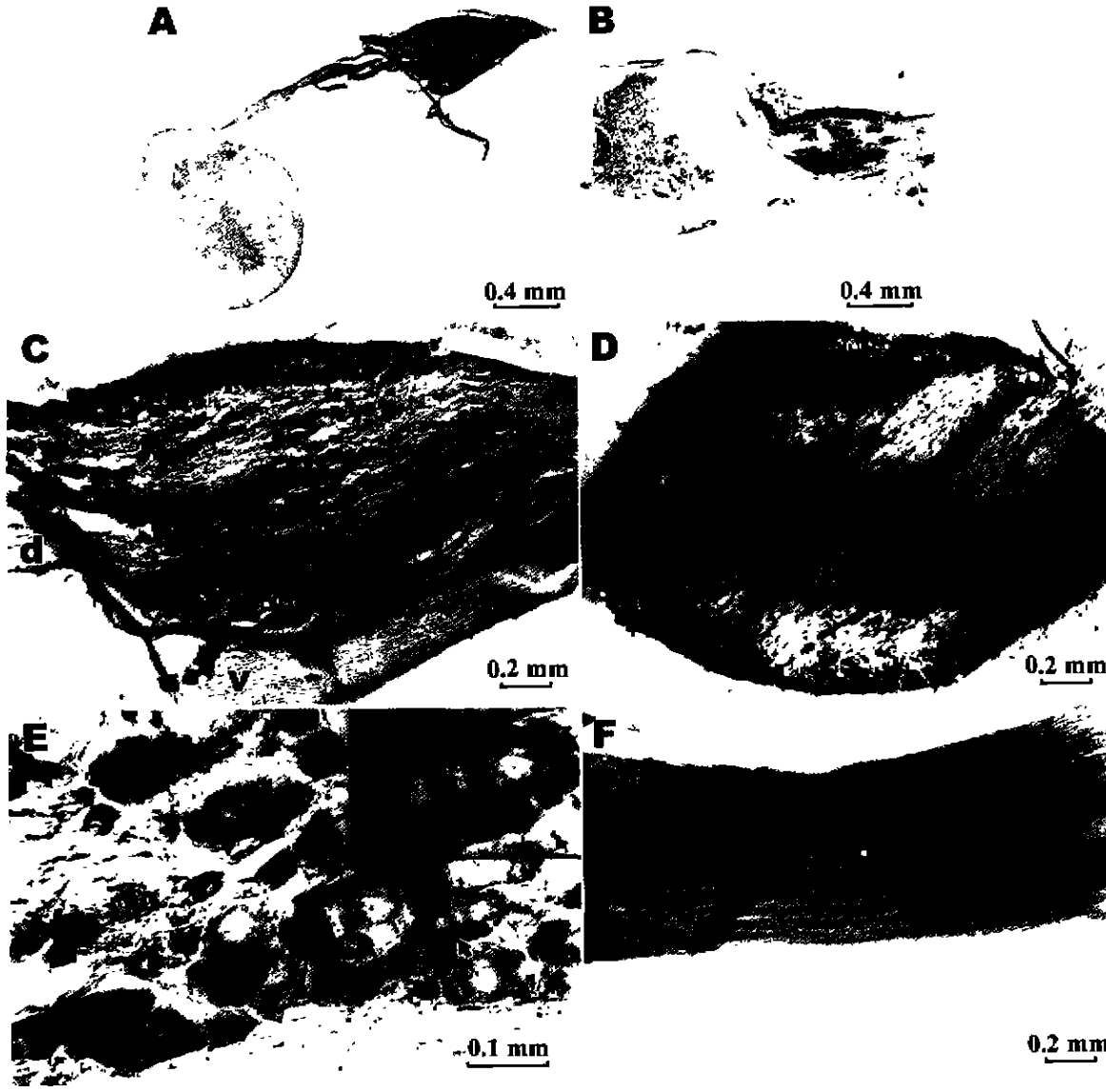

Fig 2. A: Immunohistochemistry of rat spinal cord and dorsal root ganglion (DRG) for CB1 receptors. Note the markedly higher level of labelling in DRG compared with spinal cord. B: Cresyl violet of adjacent section of spinal cord and DRG. C: Higher magnification of DRG immunolabelled for CB1 receptors. Virtually all cells appeared labelled to some degree. Note the immunoreactivity in the dorsal (d) and ventral (v) roots. D: Higher magnification of DRG stained with cresyl violet. E: Higher magnification of labelled cells for CB1 receptors in the DRG. Different cell types exhibit markedly different degrees of immunostaining. Arrow and inset: note the parallel strips of label on the apposing cells separated by the immunonegative sheet of glia. F: Enlargement of peripheral nerve to show the immunostaining of its fibers for CB1 receptors.

these muscle relaxant actions of cannabinoids, consistent with their effects at the neuromuscular junction[23].

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大麻酚类受体-1在脊髓、背角、背根神经节和周围神经的分布

关键词 大麻酚类; 大麻酚类受体; 脊神经节; 脊神经根; 周围神经; 中枢性肌松弛药; 镇痛

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