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Distribution of Cannabinoid Receptors in the Central and Peripheral Nervous System

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Abstract CB_1 cannabinoid receptors appear to mediate most, if not all of the psychoactive effects of delta-9-tetrahydrocannabinol and related compounds. This G

protein-coupled receptor has a characteristic distribution in the nervous system: It is particularly enriched in cortex, hippocampus, amygdala, basal ganglia outflow tracts, and cerebellum—a distribution that corresponds to the most prominent behavioral effects of cannabis. In addition, this distribution helps to predict neurological and psychological maladies for which manipulation of the endocannabinoid system might be beneficial. CB_1 receptors are primarily expressed on neurons, where most of the receptors are found on axons and synaptic terminals, emphasizing the important role of this receptor in modulating neurotransmission at specific synapses. While our knowledge of CB_1 localization in the nervous system has advanced tremendously over the past 15 years, there is still more to learn. Particularly pressing is the need for (1) detailed anatomical studies of brain regions important in the therapeutic actions of drugs that modify the endocannabinoid system and (2) the determination of the localization of the enzymes that synthesize, degrade, and transport the endocannabinoids.

Keywords Immunocytochemistry · In situ hybridization · Autoradiography · Cholecystokinin · Synapse

1 Introduction

1.1 Background

The CB_1 cannabinoid receptor is the major mediator of the psychoactive effects of cannabis and its derivatives. In addition, this G protein-coupled receptor transduces many of the effects of the endogenous cannabinoids. Understanding the distribution of CB_1 receptors has proved helpful to both predict and understand the effects of cannabinoids. For example, the high CB_1 receptor levels found in cortex, basal ganglia, and cerebellum coincide with the prominent effects cannabinoids have on functions subserved by these brain regions. By comparison, the low levels present in the medullary nuclei responsible for regulating respiration are consistent with the modest effects cannabinoids have on respiratory drive. Furthermore, the strong presynaptic localization of the receptor found in ultrastructural studies underscores its major role as a modulator of neurotransmitter release.

The distribution of cannabinoid receptors has been extensively mapped by quantitative autoradiography, in situ hybridization, and immunocytochemistry. Each of these techniques has its strengths and weaknesses. Properly calibrated, quantitative autoradiography provides the best measure of absolute receptor density. Nonetheless, its spatial resolution is limited and specificity depends on the ligand used. In situ hybridization identifies the cells synthesizing CB_1 mRNA. However, mRNA levels and protein levels may not necessarily correlate. Immunocytochemistry provides outstanding spatial resolution; however, fixation artifacts and unanticipated antibody crossreactivity must be assiduously avoided. For the most

part, the results obtained from these three approaches have provided complementary and logically consistent results. In addition to these anatomical approaches, it is possible to obtain a measure of CB_1 receptor function by guanosine triphosphate (GTP)γS binding, giving spatial resolution similar to quantitative autoradiography. Finally, the results of experiments using regionally or neuron specific CB_1 knockout mice can give additional insight into receptor localization.

1.2 Autoradiography

Miles Herkenham performed the first CB_1 receptor distribution studies using autoradiography with the tritiated CB_1 agonist, CP55,940. Examples of his results from human brain are shown in Fig. 1. A striking feature of the autoradiographic studies was the extraordinarily high levels of CB_1 receptors found in substantia nigra, globus pallidus, hippocampus, cerebellum, and cortex. The levels of CB_1 receptors found in these brain regions in the rat approached 6 pmole/mg (Herkenham et al. 1991). To give a sense of the magnitude of CB_1 receptor expression, CB_1 receptors are tenfold denser than D_2 receptors in the basal ganglia and have a density similar to cortical ionotropic glutamate receptors. The specificity of these results was verified by Virginia Seybold and her colleagues, who performed a systematic autoradiographic study of rat brain using tritiated WIN55,212-2, a structurally distinct CB_1 agonist (Jansen et al. 1992). These thorough studies in rodents have been complemented by autoradiographic studies in human brain (Glass et al. 1997; Mato et al. 2003). The results of the human and rodent studies are qualitatively similar once the evolutionary changes associated with the development of the human brain are considered.

1.3 In Situ Hybridization

Cloning the CB_1 receptor (Matsuda et al. 1990) made it possible to identify CB_1 synthesizing cells by in situ hybridization (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993). Correlating the results of the autoradiographic and in situ hybridization studies reveals several common themes of the CB_1 system. The first was that in some brain regions, particularly forebrain (for example, cortex, amygdala, and hippocampus), CB_1 receptors are expressed at very high levels in a very restricted set of neurons. These neurons then project widely, resulting in a dense network of CB_1-positive axons. The second was that CB_1 receptors were primarily found on axons and terminals. For example, high levels of CB_1 are present in the striatonigral pathway and substantia nigra, yet nigral neurons express no CB_1 mRNA. These findings strongly suggest CB_1 receptors are synthesized in the striatal projection neurons (medium spiny neurons—which contain moderate levels of CB_1 mRNA) and are trafficked to their axons. The axonal and terminal localization of CB_1 receptors, coupled with the observation that CB_1 receptors inhibit presynaptic calcium channels, implied that a major function of CB_1 receptors would be to

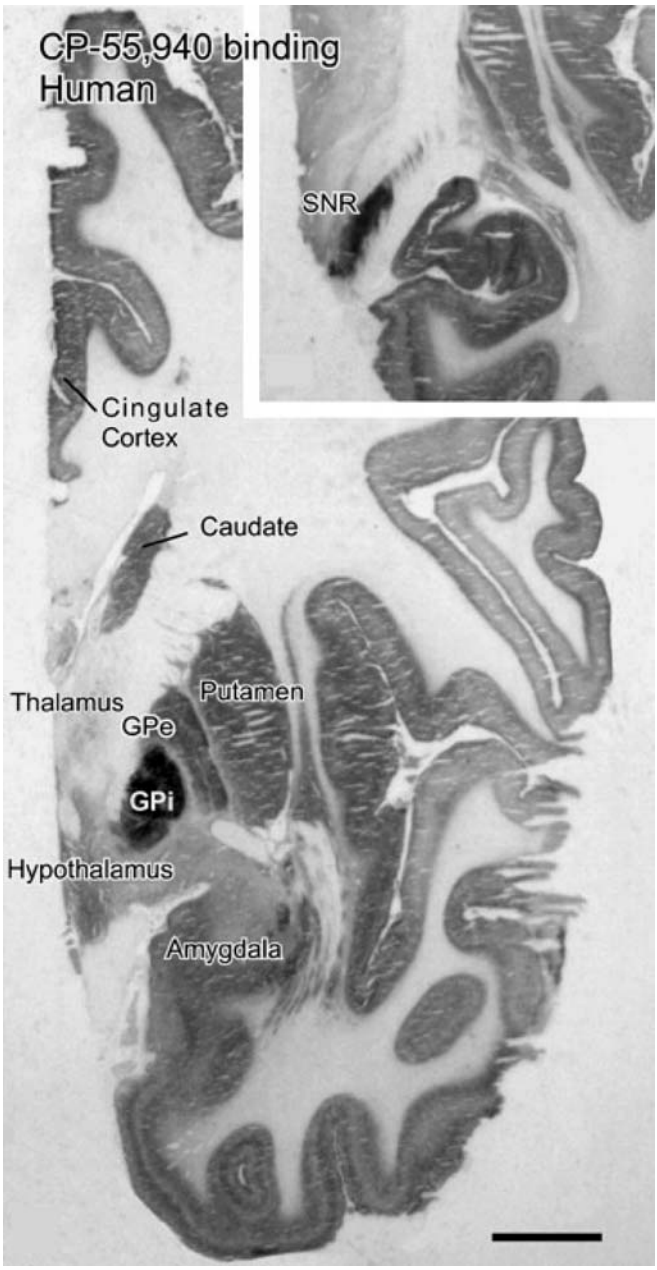

Fig. 1. CB_1 expression in human brain. CB_1 receptors were detected by quantitative autoradiography using tritiated CP55,940. Strikingly high levels are found in the substantia nigra pars reticulata (*SNR*) and the internal segment of the globus pallidus (*GPi*). Moderate levels are present in the caudate, putamen, the external segment of the globus pallidus (*GPe*), amygdala, and cortex. Lesser levels are present in hypothalamus, and very low expression is apparent in most areas of the thalamus. The laminar nature of CB_1 expression is apparent in the most rostral parts of the cortex. *Scale bar* = 1 cm. (Original figure provided by Miles Herkenham)

inhibit neurotransmitter release. The third theme was that in a few brain regions (for example, anterior olfactory nucleus, caudate nucleus and cerebellum) CB_1 receptors are uniformly expressed at moderate levels on a single class of neurons.

1.4 Immunocytochemistry

Elucidation of the primary sequence of the CB_1 receptor allowed for production of numerous CB_1 receptor antibodies. There have been two thorough immunocytochemical mapping studies in rodent brain (Tsou et al. 1998a; Egertová and Elphick 2000) and one in spinal cord (Farquhar-Smith et al. 2000). These generally support the results from the autoradiographic studies, with some differences in relative intensity of staining. These variations may be due to differences in antibody access to specific epitopes, variable post-translational modification of an epitope (e.g., phosphorylation), or fixation conditions. There is little evidence for alternative splicing in the coding region of rodent CB_1 receptors (Matsuda 1997; Lutz 2002), despite the report of alternatively splicing of the human CB_1 receptor (Shire et al. 1995; Matsuda 1997; Lutz 2002); so alternative splicing is less likely to explain the reported differences.

The immunocytochemical studies have led to additional insights into cannabinoid action. The first is that rigorous electron microscopic studies in the hippocampus demonstrated that in this region CB_1 is undetectable on somatic cell membranes and dendrites, yet is very highly expressed in axon terminals and preterminal segments (Hajos et al. 2000; Katona et al. 2000, 2001). An example of this is shown in Fig. 2, with the labeling of four consecutive ultrathin sections of a cortical axodendritic synapse. The second is that double-label immunostaining experiments demonstrated that in forebrain there is a striking correlation between cholecystokinin (CCK) and CB_1 receptor expression (Katona et al. 1999, 2001; Tsou et al. 1999). These findings have been confirmed and extended with double-label in situ hybridization studies (Marsicano and Lutz 1999). Thus, the cells expressing the highest levels of CB_1 receptors in forebrain are γ-aminobutyric acid (GABA)ergic, CCK-positive interneurons. Although inhibition of GABA release is measured in the in vitro electrophysiological studies, activation of CB_1 receptors in vivo will attenuate both inhibitory transmission (generally fast, mediated by GABA A receptors) (Wilson et al. 2001) as well as the slow, excitatory actions mediated by CCK receptors (Beinfeld and Connolly 2001). Thus, the localization of CB_1 receptors on CCK-containing neurons suggests that CB_1 receptors are well positioned to modulate complex network behaviors (Freund 2003).

Once antibodies to the anandamide-degrading enzyme, namely fatty acid amide hydrolase (FAAH), became available, it was apparent that in many regions FAAH and CB_1 expression is reciprocal in nature (Egertová et al. 1998, 2003; Tsou et al. 1998b). For example, FAAH, but not CB_1 is highly expressed in the somata and proximal dendrites of hippocampal pyramidal cells and cerebellar Purkinje neurons. These neurons are, in turn, densely innervated by CB_1-positive fibers. Thus, it has been proposed that anandamide, despite its possible presynaptic site

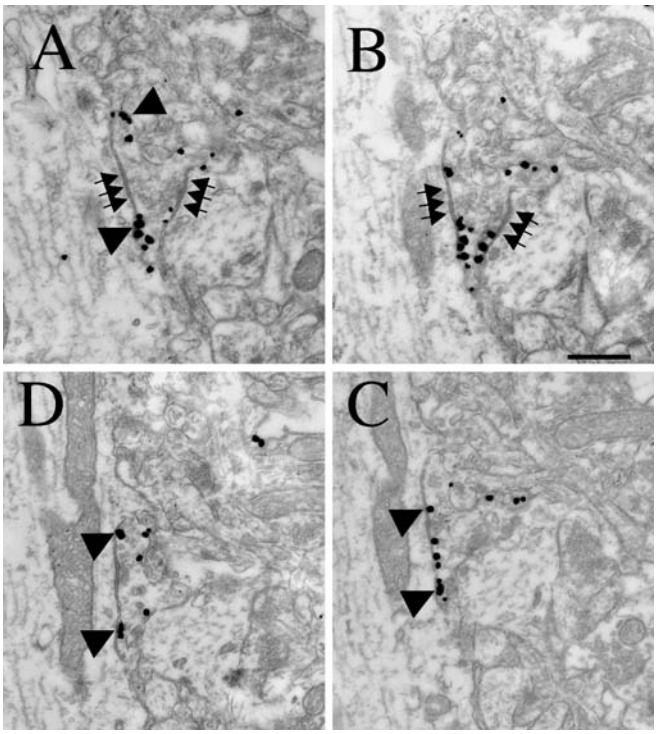

Fig. 2A–C. CB_1 expression in serial sections of a γ-aminobutyric acidergic (GABAergic) terminal synapsing onto an apical dendrite in cortex. CB_1 receptors (*arrowheads*) were detected with an antibody directed against the C terminus of rat CB_1 using pre-embedding immunogold with silver enhancement. The boutons are forming symmetric synapses (*arrows*), characteristic of GABAergic axon terminals, onto the apical dendrite of a cortical pyramidal cell. *Scale bar* = 0.5 µm. (Original photomicrograph provided by Tamas Freund and Agnes Bodor)

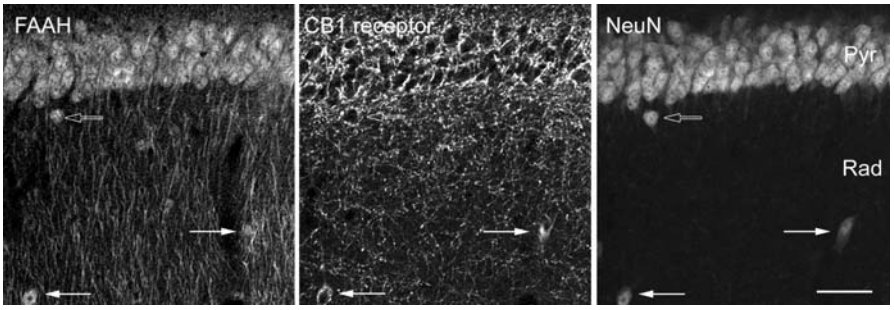

Fig. 3A–C. Reciprocal expression of CB_1 and FAAH in mouse hippocampus. FAAH was detected using an antibody raised against the last 200 residues of FAAH, CB_1 receptors were detected by an antibody directed against its C terminus, and neuronal nuclear antigen (NeuN) was detected using a mouse monoclonal antibody from Chemicon. FAAH is expressed uniformly by pyramidal neurons (*Pyr*) including the apical dendrites. FAAH is also expressed in interneurons (*open and filled arrows*). CB_1 receptors are present in axons investing the pyramidal cell layer and also some interneuron cell bodies (*filled arrows*), but not in others (*open arrow*). Staining of neurons by NeuN identifies neuronal nuclei in the field. *Scale bar* = 18 µm. (Figure provided by Tibor Harkany)

of action, is synthesized and degraded in the postsynaptic neuron. An example of this reciprocal localization in the CA1 region of mouse hippocampus is shown in Fig. 3. The situation for monoacylglycerol (MAG) lipase, the major 2-arachidonoyl glycerol-degrading enzyme, is still being clarified. However, a recent paper suggests that MAG lipase, in contrast to FAAH is predominately localized presynaptically (Gulyas et al. 2004). As the majority of CB_1 receptors a presynaptic, location of MAG lipase near these receptors would mean the endogenous cannabinoid 2-AG would be metabolized at its likely site of action, rather than having to diffuse back across the synapse. Thorough studies on the anatomical distribution of the endocannabinoid-synthesizing enzymes, diacylglycerol lipase (Bisogno et al. 2003) and the *N*-acyl phosphatidylethanolamine-preferring phospholipase D (Okamoto et al. 2004), remain to be done.

1.5 Functional Studies

Functional studies have provided another dimension in cannabinoid receptor localization. The most pertinent studies for this chapter are GTPγS studies and results inferred from studies with CB_1 knockout mice. The chapter by Lindsey et al. (this volume) will consider advances in positron emission tomography (PET), single-photon emission computed tomography (SPECT) and 2-deoxy-glucose imaging of CB_1 receptors and their activation. GTPγS studies provide a measure of regional CB_1 receptor activation of G proteins with a spatial resolution similar to other autoradiographic studies. Informative results from these studies include the observation that CB_1 receptors are relatively inefficient activators of G protein (for example, sevenfold less efficient than μ- or δ-opioid receptors) and that activation of G proteins by CB_1 receptors desensitizes strongly with chronic tetrahydrocannabinol (THC) treatment (Sim et al. 1996a,b). As mentioned below, the region-specific CB_1 knockout mice experiments support the contention that some CB_1 receptors may be expressed on hippocampal pyramidal neurons.

2 CB_1 Expression in Specific CNS Regions

2.1 Olfactory Areas

The highest levels of CB_1 receptors in olfactory bulb are in the inner granule cell layer, followed by the inner plexiform layer. The external plexiform layer, the mitral cell (glomerular) layer, and the accessory olfactory bulb have few CB_1 receptors (Herkenham et al. 1991; Tsou et al. 1998a; Egertová and Elphick 2000). The anterior olfactory nucleus and anterior commissure, which connects the olfactory bulbs, both contain high levels of CB_1 receptor. In contrast to neighboring regions, CB_1 receptors are expressed uniformly by most neurons in the anterior olfactory

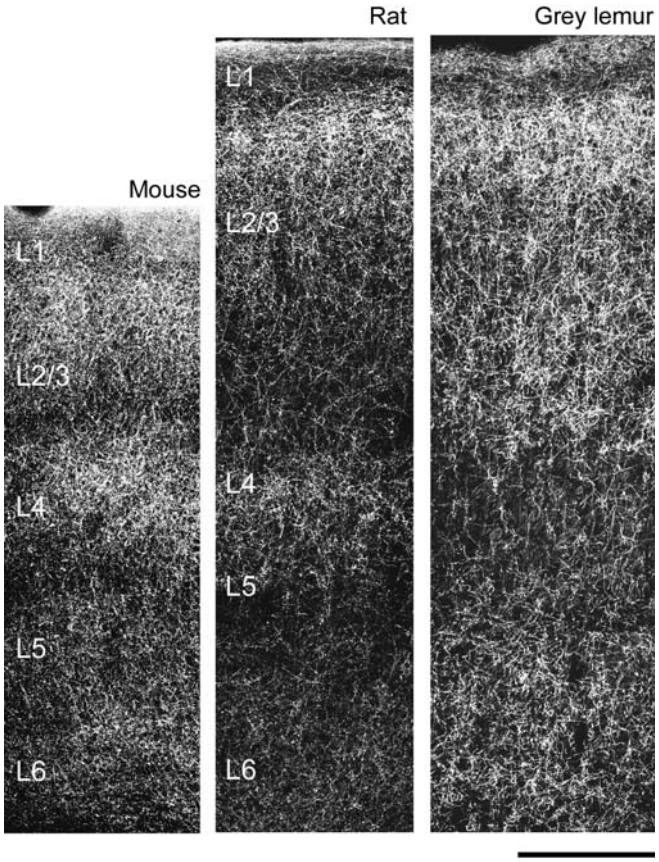

Fig. 4. Laminar CB_1 expression in cortex of three mammals. CB_1 receptors were detected with an antibody directed against the C terminus of rat CB_1. Particularly high levels of CB_1 are found in lamina layers II, upper III (*L2/3*), IV (*L4*), and VI (*L6*). *Scale bar* = 250 μm. (Figure provided by Tibor Harkany)

nucleus (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Tsou et al. 1998a; Marsicano and Lutz 1999; Egertová and Elphick 2000). CB_1 receptors are also found in the supporting cells of the olfactory epithelium as well as axon bundles of the lamina propria (M. Caillol, personal communication).

2.2 Neocortex

CB_1 receptors are densely expressed in all regions of the cortex (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Glass et al. 1997; Tsou et al. 1998a; Egertová and Elphick 2000). The variation in CB_1 expression across cortical regions has been examined most extensively in human brain using receptor autoradiography. Here there is variation between regions, with higher

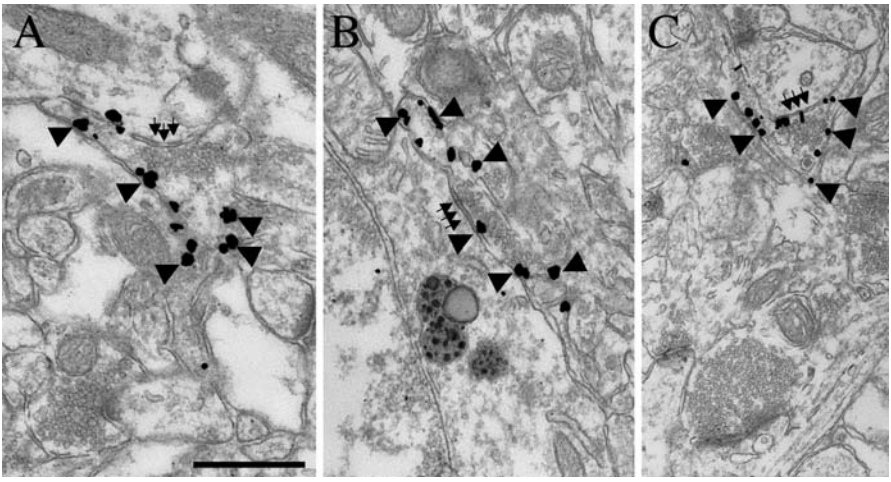

Fig. 5A–C. CB_1 expression on GABAergic terminals in rat somatosensory cortex. CB_1 receptors (*arrowheads*) were detected with an antibody directed against the C terminus of rat CB_1 using pre-embedding immunogold with silver enhancement. The boutons are forming symmetric synapses (*arrows*), characteristic of cortical GABAergic axon terminals. CB_1-positive terminals form synapses with pyramidal cell bodies (**A**), main apical dendrites (**B**), and fine-caliber dendrite branches (**C**). *Scale bar* = 0.5 µm. (Original photomicrograph provided by Tamas Freund and Agnes Bodor)

levels found in cingulate gyrus, frontal cortex, and secondary somatosensory and motor cortex. Lesser levels are found in primary somatosensory and motor cortex (Glass et al. 1997). The laminar nature of CB_1 expression within the neocortex is striking. The relative levels of expression between regions vary (Glass et al. 1997). However, as an example, in rat somatosensory cortex, CB_1 levels are relatively higher in layers II, upper III, IV, and VI. In contrast, CB_1 receptor expression is relatively less in deeper layer III and layer V (Freund et al. 2003). Layer I appears almost devoid of CB_1 receptors. Examples of CB_1 immunoreactivity in mouse, rat, and mouse lemur cortex are shown in Fig. 4. While the general laminar pattern between species is preserved, the amount of CB_1 expression appears to increase, particularly in layers III and V in the primate. Ultrastructural studies reveal that in cortex, CB_1-positive terminals synapse onto pyramidal cell bodies, apical dendrites, and smaller caliber branches (Fig. 5).

In neocortex, almost all neurons expressing CB_1 at high or moderate levels are likely to be inhibitory due to the tight correlation between GAD65 and CB_1 mRNA expression (Marsicano and Lutz 1999). However, there appear to be CB_1-mediated actions on glutamatergic transmission in cortex (Sjostrom et al. 2003). The localization and nature of these cannabinoid receptors remain to be identified. As in most other forebrain areas, the majority of strongly CB_1-positive axons in the cortex appear to arise from CCK-expressing interneurons (Marsicano and Lutz 1999). However, among cortical neurons, those expressing lower levels of CB_1 receptors represent a more heterogeneous population, with 20% of the CB_1-positive cells not expressing detectable levels of CCK mRNA (Marsicano and Lutz

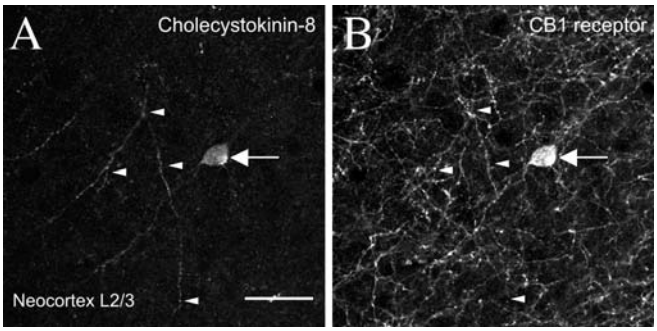

Fig. 6A, B. Co-localization of CCK with CB_1 in neocortex. **A** Expression of CCK in a cortical interneuron (*arrow*) and CCK-positive processes (*arrowheads*). **B** CB_1 is widely expressed in cortical axons. CCK-positive processes are often CB_1 positive as well (*arrowheads*). *Scale bar* = 25 µm. (Figure provided by Tibor Harkany)

1999). An example of this for layer II/III cortex is shown in Fig. 6. Here, a strongly CB_1-expressing neuron co-localizes with CCK immunoreactivity, and most CCK-containing fibers also are immunopositive for CB_1. However, there are also many CB_1-positive fibers that do not appear to contain CCK.

2.3 Hippocampal Formation

2.3.1 Hippocampus

The hippocampus expresses high levels of cannabinoid receptors. Because of the cognitive effects of cannabinoids, this brain region has received much attention as a site of action of endogenous and exogenous cannabinoids. The first autoradiographic studies found very high levels of CB_1 receptors in all subfields of the hippocampus as well as the dentate gyrus (Herkenham et al. 1991; Jansen et al. 1992). In situ hybridization studies revealed that most of this CB_1 receptor expression arose from a restricted subset of interneurons (Matsuda et al. 1990, 1993; Mailleux and Vanderhaeghen 1992). Immunocytochemical studies identified a dense plexus of CB_1-containing axon terminals surrounding the pyramidal cell layer (perisomatic labeling), consistent with CB_1 receptor expression on basket cell axons (Tsou et al. 1998a, 1999; Katona et al. 1999; Egertová and Elphick 2000). This is illustrated in Figs. 3 and 7.

Basket cells can be conveniently separated into two groups distinguished by CCK or parvalbumin expression (Freund and Buzsaki 1996; Freund 2003). Double-label immunocytochemistry has shown that high levels of CB_1 receptor expression are restricted to the CCK-expressing interneurons (Katona et al. 1999; Tsou et al. 1999). Given that the CCK-expressing interneurons may be involved in the more subjective (emotional and motivational) aspects of information processing, it is likely that endocannabinoids are involved in the normal function of these circuits, and exogenous cannabinoids may serve to disrupt them in some fashion. This

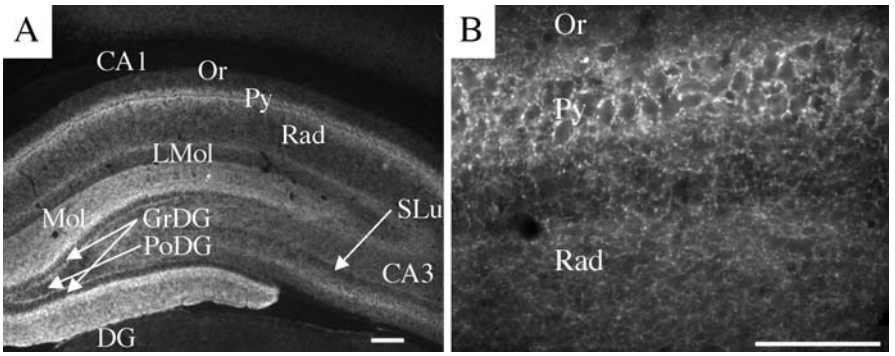

Fig. 7A, B. CB_1 expression in rat hippocampal formation. **A** CB_1 cannabinoid receptors were detected with an antibody raised against the C terminus of rat CB_1. Receptor levels are particularly high in the pyramidal cell layer (*Py*), the molecular layer (*Mol*) of the dentate gyrus (*DG*), and at the base of the granule cell layer (*GrDG*) of the dentate gyrus. Lesser levels are found in the stratum oriens (*Or*), stratum radiatum (*Rad*), stratum lucidum (*SLu*), and the polymorphic layer of the dentate gyrus (*PoDG*). *CA1*, field CA1 of the hippocampus; *CA3*, field CA3 of the hippocampus. **B** CB_1-positive fibers surround the somata of pyramidal cells (*Py*) in CA1. Numerous varicosities, corresponding to terminals, are apparent. CB_1 receptors are also seen on axon fibers, although at lower levels, in stratum oriens (*Or*) and stratum radiatum (*Rad*). For both images, *scale bar* = 100 µm. (Original photomicroph provided by Marja Van Sickle and Keith Sharkey)

pattern of selective interneuron and axonal CB_1 receptor expression is preserved at all stages of postnatal development in the rat (Morozov and Freund 2003).

Tight functional separation of GABAergic input onto CA1 pyramidal cells has also been demonstrated in an elegant electrophysiological study where only large, fast GABAergic inhibitory postsynaptic currents (IPSCs) mediated by inhibitory terminals expressing N-type [(Cav1.2); but not P-type (Cav1.1)] calcium channels were subject to depolarization-induced suppression of inhibition (Wilson et al. 2001). These electrophysiological results are satisfyingly consistent with the anatomical localization of the CB_1 receptor on perisomatic GABAergic terminals.

The expression of CB_1 receptors on principal cells of the hippocampus is a source of some controversy (as reviewed by Freund et al. 2003). On one hand, careful electron microscopic immunocytochemical studies with specific and sensitive CB_1 receptor antibodies have consistently failed to find CB_1 receptor expression in pyramidal cells (Katona et al. 1999, 2000; Hajos et al. 2000; Chen et al. 2003). On the other hand, in situ hybridization studies consistently show low levels of CB_1 mRNA in the stratum pyramidale (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Marsicano and Lutz 1999). Complicating interpretation of these studies are the observations that several drugs acting at CB_1 receptors (for example, WIN55,212-2 and SR141716) also inhibit glutamate release from pyramidal neurons in a CB_1 receptor-independent fashion [that is, they inhibit release in CB_1 knockout mice (Hajos et al. 2001; Hajos and Freund 2002)]. The electrophysiological and in situ data could conceivably be reconciled by crossreactivity of the in situ probes with a receptor closely related to the CB_1 receptor. However, this does not seem to be the case, as targeted deletion of CB_1 receptors from hippocampal pyramidal neurons (sparing CB_1 receptors in the interneurons) eliminates

cannabinoid-mediated protection in a kainate neurotoxicity model (Marsicano et al. 2003). Although this issue is not yet resolved, a parsimonious explanation of experimental results thus far is that hippocampal pyramidal neurons may express CB_1 receptors, albeit at far lower levels than the CCK-containing basket cells.

2.3.2 Dentate Gyrus

As in the hippocampus, CB_1 receptors in dentate gyrus are primarily found in CCK-containing basket cells—parvalbumin-positive basket cells and the granule cells do not express CB_1 (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993; Katona et al. 1999; Marsicano and Lutz 1999; Tsou et al. 1999). This results in high levels of CB_1 receptors in the inner third of the molecular layer and at the base of the granule cell layer in the dentate gyrus (Fig. 7). While it has not been studied anatomically, functional studies suggest the glutamatergic terminals of the perforant path may express CB_1 receptors (Kirby et al. 1995).

2.4 Amygdala

CB_1 receptor distribution in the amygdala is markedly heterogeneous (Katona et al. 2001; McDonald and Mascagni 2001). High levels are found in the basolateral complex (comprising the lateral, basal, and accessory basal nucleus), nucleus of the lateral olfactory tract, the periamygdaloid cortex, and amygdalohippocampal areas. In contrast, CB_1 receptors are sparsely expressed in the medial, central, and intercalated nuclei (Fig. 1). As in other regions of the forebrain, CB_1 receptors are primarily expressed on large, GABAergic, CCK-containing axon terminals (Katona et al. 2001; McDonald and Mascagni 2001). Activation of these CB_1 receptors by cannabinoids decreases GABA release from these terminals, which may disinhibit the basolateral glutamatergic pyramidal cells (Katona et al. 2001). As in other forebrain regions, there is also a relatively high concordance between CB_1 and serotonin-3 (5-HT3) receptor expression in amygdala (Morales et al. 2004). Compelling evidence suggests that endocannabinoids play a role in modulating fear conditioning at the level of the amygdala (Marsicano et al. 2002), and amygdaloid CB_1 receptors may play a role in the panic states occasionally seen following consumption of prodigious quantities of cannabis.

2.5 Subcortical CB_1 Receptors

2.5.1 Basal Forebrain

Moderate levels of CB_1 receptors are present in the basal forebrain. Autoradiographic studies found CB_1 in the medial and lateral septum and the intermediate

nucleus of the lateral septum (Herkenham et al. 1991). CB_1 mRNA is present at moderate levels in many cells of the medial septum and the nucleus of the diagonal band (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993). A recent immunocytochemical study in mouse revealed that the tenia tecta, ventral pallidum, and substantia innominata all contained a dense network of CB_1-positive fibers. In contrast, a fine meshwork of CB_1 receptor-containing fibers was present in the medial septum, diagonal bands, and nucleus basalis (Harkany et al. 2003). No CB_1 immunoreactivity was detected in basal forebrain cholinergic cells; instead these cells contained high levels of FAAH (Harkany et al. 2003). These results are in contrast to a report in monkey, which found CB_1 expression in cholinergic forebrain neurons (Lu et al. 1999). This discrepancy may be due to a difference between species or methodologies.

2.5.2 Basal Ganglia

The subcortical structures with the highest level of CB_1 receptor expression are the basal ganglia. In fact, the highest levels of CB_1 receptors in the brain detected in autoradiography studies were found in the substantia nigra (Herkenham et al. 1991). In situ hybridization studies demonstrated that many striatal medium spiny neurons express CB_1 receptors (Matsuda et al. 1993; Julian et al. 2003). In contrast, adult pallidal and nigral neurons contain little or no CB_1 mRNA (Matsuda et al. 1993; Julian et al. 2003). Rather, CB_1 receptors in the globus pallidus and substantia nigra are localized to the axons traversing or terminating in these structures (Tsou et al. 1998a; Egertová and Elphick 2000). Thus, the high levels of pallidal and nigral CB_1 receptor binding and protein observed in autoradiographic and immunocytochemical studies mostly arise from GABAergic neurons projecting from the caudate putamen. Figure 8 illustrates the intense immunostaining of CB_1 receptors that begins at the border between the caudate putamen and globus pallidus. It is possible that dopaminergic neurons may transiently express CB_1 receptors during development, as CB_1 co-localizes with tyrosine hydroxylase in cultured mesencephalic neurons (Hernandez et al. 2000).

Both autoradiographic and immunocytochemical studies show a gradient of CB_1 expression in the rodent caudate putamen with the highest levels found dorsolaterally (Tsou et al. 1998a; Egertová and Elphick 2000). Both the matrix and patch structures of the caudate putamen contain CB_1 receptors, where they partially overlap with μ-opioid receptors (Rodriguez et al. 2001). CB_1 receptors are present on both the striatonigral (prodynorphin or preprotachykinin A positive) and striatopallidal (proenkephalin positive) projection pathways (Hohmann and Herkenham 2000). Thus, CB_1 receptors are positioned to modulate both the direct and indirect striatal output pathways.

In addition to medium spiny neurons, anatomical and functional studies identified CB_1 receptors on the terminals of the corticostriatal pathway (Gerdeman and Lovinger 2001; Huang et al. 2001; Rodriguez et al. 2001) and GABAergic aspiny interneurons (Hohmann and Herkenham 2000). In contrast, CB_1 receptors

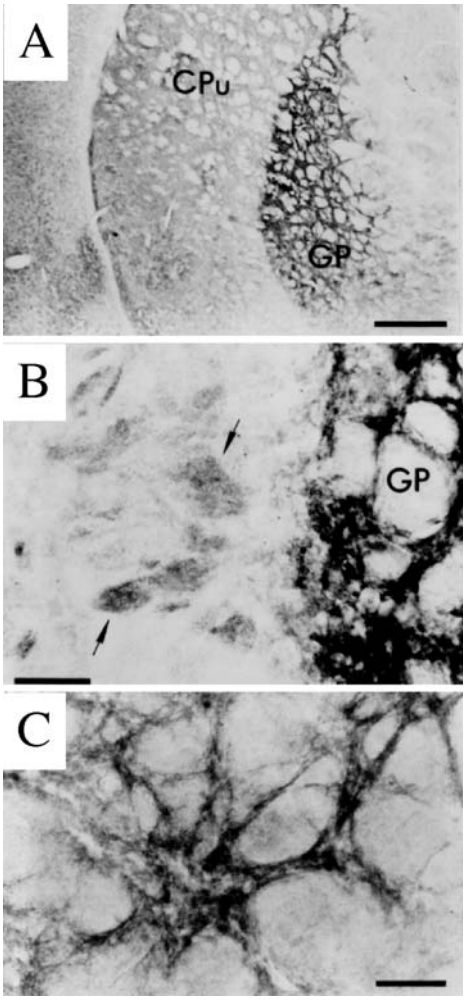

Fig. 8A–C. CB_1 expression in basal ganglia detected by an antibody raised against the amino terminus of rat CB_1. **A** Low-power view showing moderate levels of CB_1 in caudate putamen (*CPu*) and very high levels in the globus pallidus (*GP*). The sharp demarcation between the two structures is evident. **B** Boundary of CPu and GP. Two moderately stained fiber bundles are indicated by the *arrows*. **C** High-power view of globus pallidus with fine, strongly immunoreactive, non-varicose processes corresponding to medium spiny neuron axons. *Scale bars* = 500 µm (**A**); 50 µm (**B** and **C**). (Modified from a photomicrograph provided by Kang Tsou)

do not appear to be expressed in the large aspiny cholinergic interneurons or somatostatin-containing interneurons (Hohmann and Herkenham 2000). CB_1 receptors are also present on the neurons in the subthalamic nucleus (Matsuda et al. 1993). Taken together, the presence of CB_1 receptors on diverse neuronal populations in the basal ganglia can account for the complex effects of cannabinoids on motor behaviors (Sanudo-Pena et al. 1999b; Romero et al. 2002).

2.5.3
Nucleus Accumbens

CB_1 receptors are also expressed at low to moderate levels in the nucleus accumbens. Here CB_1 receptors are found in a pattern reminiscent of the striatum. CB_1 receptors are expressed on terminals of the glutamatergic prefrontal cortex accumbens pathways (Robbe et al. 2001). They are also present on the accumbens medium spiny neurons. They appear to be absent from the dopaminergic terminals projecting to the accumbens from the ventral tegmentum. Consequently, cannabinoid stimulation of dopamine release in nucleus accumbens (Tanda et al. 1997) appears to be an indirect effect, perhaps mediated by inhibition of GABA release (Szabo et al. 1999, 2002).

2.5.4
Thalamus

Expression of CB_1 receptors in the thalamus is low (Herkenham et al. 1991; Jansen et al. 1992; Matsuda et al. 1993; Glass et al. 1997; Tsou et al. 1998a; Egertová and Elphick 2000). Regions of the thalamus with some CB_1 expression include the (lateral) habenular nucleus, the anterior dorsal thalamic nucleus, and the reticular thalamic nucleus (Herkenham et al. 1991; Mailleux and Vanderhaeghen 1992; Tsou et al. 1998a).

2.5.5
Hypothalamus

Given the marked effects of CB_1 receptor agonists on body temperature and antagonists on consumptive behavior, it is not surprising that CB_1 receptors are present in the hypothalamus. Low to moderate levels of CB_1 immunoreactivity are found in the paraventricular nucleus, ventral medial hypothalamic nucleus, infundibular stem, and lateral hypothalamic area (Tsou et al. 1998a). There are in situ data suggesting CB_1 receptors in the hypothalamus are primarily present on glutamatergic neurons (Marsicano and Lutz 1999). Intriguingly, although the levels of CB_1 receptors in hypothalamus are fairly low, functional studies with GTPγS suggests these CB_1 receptors are more strongly coupled to G proteins than are most CB_1 receptors (Breivogel et al. 1997). A careful and detailed anatomical study of CB_1 expression in hypothalamus is needed because of the likely involvement of this region in the anti-appetitive actions of CB_1 antagonists.

2.6
Midbrain

2.6.1
Substantia Nigra

A striking feature of CB_1 receptor expression is the high number of CB_1 receptors found in the substantia nigra (Fig. 9A and 9B). As mentioned above, these receptors

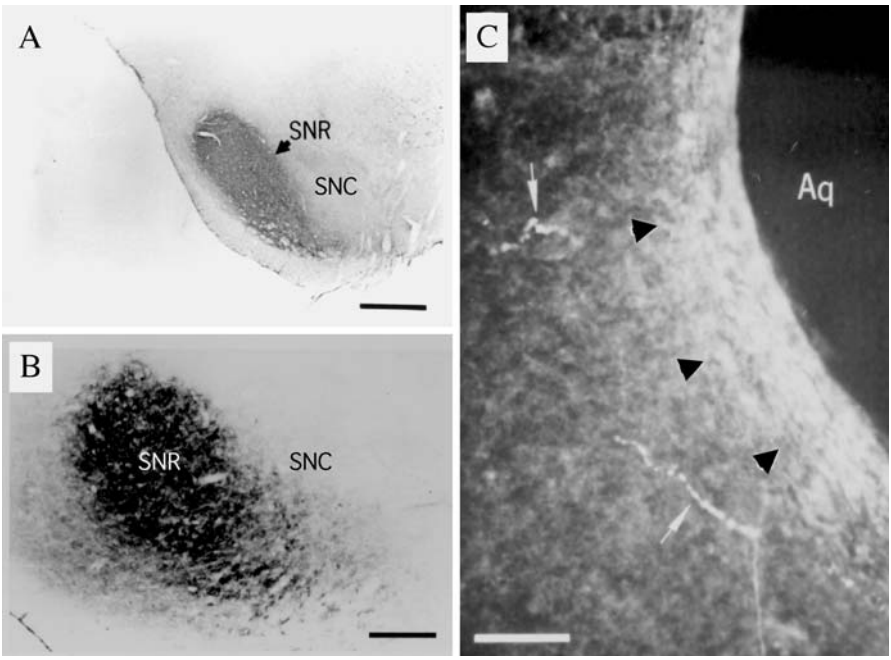

Fig. 9A–C. CB_1 receptor expression in midbrain structures detected by an antibody against the amino terminus of rat CB_1. **A** CB_1 immunostaining is very strong in substantia nigra pars reticulata (*SNR*) but virtually absent in substantia nigra pars compacta (*SNC*). **B** Higher magnification view of SNR. When the plane of the section is perpendicular to the striatonigral pathway, immunoreactivity is apparent as puncta, from the high levels of axonal CB_1 expression. **C** In caudal periaqueductal gray, CB_1-positive fibers (*arrows*) and intensely labeled neuropil (*arrowheads*) are apparent. *Aq*, lumen of the aqueduct. *Scale bars* = 500 μm (**A**), 50 μm (**B**), and 20 μm (**C**). (Modified from a photomicrograph provided by Kang Tsou)

appear to be restricted to the GABAergic axons of the putamen medium spiny neurons—the nigral dopaminergic neurons appear to be devoid of CB_1 receptors (Matsuda et al. 1993; Julian et al. 2003). Anatomical and functional evidence also suggests that the excitatory glutamatergic projection from the subthalamic nucleus to the substantia nigra contains CB_1 receptors (Mailleux and Vanderhaeghen 1992; Sanudo-Pena and Walker 1997; Sanudo-Pena et al. 1999b).

2.6.2 Ventral Tegmentum

CB_1 expression and function in the ventral tegmental area (VTA) is of interest because of the euphoric and reinforcing properties of cannabinoids—evident in carefully conducted studies. There is no evidence for CB_1 receptor expression on the tegmental dopamine neurons (Herkenham et al. 1991). Emerging functional evidence (detailed immunocytochemical studies remain to be done) suggests that CB_1 receptors are present on intrinsic GABAergic terminals, GABAergic terminals

present on accumbens neurons projecting to VTA, and glutamatergic terminals (Szabo et al. 2002; Riegal et al. 2003; Melis et al. 2004). These findings suggest that CB_1 receptor activation may play a role in the reinforcing effects of cannabinoids and, more provocatively, that disorders in endocannabinoid-mediated synaptic plasticity may be important in a broader range of addictive disorders.

2.6.3 Periaqueductal Gray

Moderate levels of CB_1 receptor are also found in several other regions of the midbrain. One of these is the periaqueductal gray (PAG) (Fig. 9C). Here CB_1 receptors are found on the terminals of GABAergic neurons. In contrast to opiate receptors on GABAergic aqueductal neurons, CB_1 receptors are preferentially localized in the dorsal portion of the PAG (Tsou et al. 1998a). Autoradiographic studies indicate that CB_1 receptors are also found at moderate levels in the reticular formation and raphe nucleus (Glass et al. 1997).

2.7 Brainstem

Expression of CB_1 receptors in brainstem is relatively low. In contrast to the opioid receptors, few cannabinoid receptors are found in the medullary respiratory control centers (Herkenham et al. 1991; Glass et al. 1997). This likely underlies

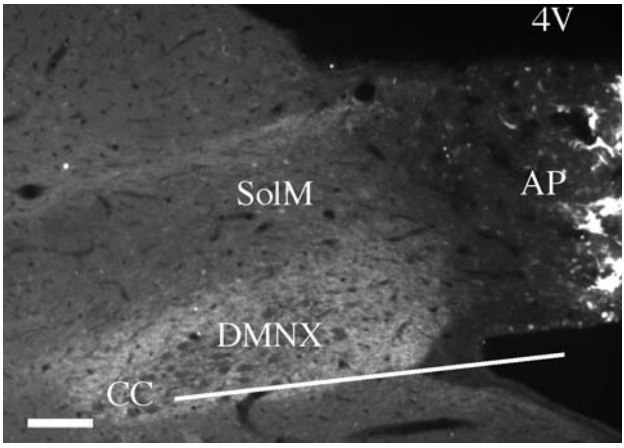

Fig. 10. CB_1 expression in emetic centers. CB_1 is prominently expressed in the ferret area postrema (*AP*), dorsal vagal complex (*DMNX*), and associated regions involved in emesis as detected with a C-terminal CB_1 receptor antibody. Particularly strong immunostaining is present in a restricted group of cells in the area postrema as well as diffusely through the dorsal motor nucleus of the vagus (notice the lack of staining of cell bodies in DMNX), and the medial nucleus of the solitary tract (*SolM*). *4V*, fourth ventricle; *CC*, central canal. *Scale bar* = 100 μm. (Original photomicrograph provided by Marja Van Sickle and Keith Sharkey)

the low lethality of high doses of cannabinoids. One exception to the low levels of cannabinoid receptor in the brainstem is the medullary nuclei associated with emesis (Van Sickle et al. 2001). Here, as illustrated in Fig. 10, relatively high levels of CB_1 receptor are found in the dorsal motor nucleus of the vagus and the medial subnucleus of the nucleus of the solitary tract. Moderate levels are present in the subnucleus gelatinosus of the solitary tract (Fig. 10). Occasional, very strongly stained cells are evident in the area postrema (Fig. 10). In most cases, CB_1 receptors appear to be localized to terminal structures. Interestingly, FAAH immunoreactivity was restricted to the cell bodies invested by the CB_1-positive fibers (Van Sickle et al. 2001), continuing the theme of complementary expression of CB_1 receptors and FAAH. Compelling evidence suggests that a major portion of the antiemetic actions of cannabinoids is a consequence of CB_1 receptor activation in these nuclei (Van Sickle et al. 2001, 2003).

2.8 Cerebellum

CB_1 receptor expression in the cerebellum follows a striking and very predictable pattern. Autoradiographic and immunocytochemical studies show very strong labeling of the molecular layer (Fig. 11A), while in situ hybridization studies show robust expression in the granule cell layer (Matsuda et al. 1990; Herkenham et al. 1991; Glass et al. 1997; Tsou et al. 1998a; Egertová and Elphick 2000). Combining these results with functional studies suggests CB_1 receptors are expressed in climbing fibers and parallel fibers, as well as the basket cells, particularly at the basket cell–Purkinje cell synapse (Fig. 11A, B). In contrast, there is little evidence that Purkinje neurons express CB_1 receptors (Matsuda et al. 1990). Thus, both ma-

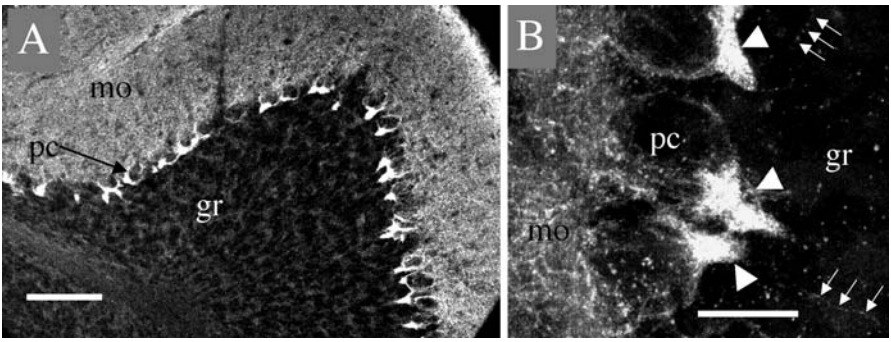

Fig. 11. CB_1 is highly expressed in the molecular layer and on the basket cell–Purkinje neuron synapse of the mouse cerebellum. **A** Using an antibody directed against the C terminus of the CB_1 receptor, strikingly high levels of CB_1 receptors are apparent at basket cell synapses onto the Purkinje neurons (*pc*) as well as diffusely high levels in the molecular layer (*mo*), corresponding to the parallel fiber–Purkinje neuron synapse. **B** Higher magnification view showing intense labeling of basket cell synapses (*arrowheads*), labeled fibers in the granule cell layer (*gr*) (*arrows*), and diffuse labeling in the molecular layer. *Scale bars* = 150 µm (**A**), and 15 µm (**B**). (Modified from a photomicrograph provided by Jane Lauckner)

jor glutamatergic inputs and at least some of the GABAergic input onto Purkinje neurons are subject to modulation by cannabinoids. These anatomical observations are supported by several elegant electrophysiological studies demonstrating a role for endogenous cannabinoid inhibition of glutamatergic and GABAergic neurotransmission onto Purkinje neurons (Kreitzer and Regehr 2001; Maejima et al. 2001; Diana et al. 2002; Kreitzer et al. 2002; Brenowitz and Regehr 2003). While most of the actions of exogenous and endogenous cannabinoids can be interpreted as effects on presynaptic CB_1 receptors, there is also solid evidence for somatic expression of CB_1 receptors. This comes from experiments by the Regehr lab showing that the release of endocannabinoids from Purkinje neurons can slow the firing rate of basket cells, consistent with an activation of somatic potassium channels (Kreitzer et al. 2002).

2.9 Spinal Cord

Because of the efficacy of intrathecal cannabinoids in various pain models, it is not surprising that moderate levels of CB_1 receptor are found in the regions of the spinal cord associated with analgesia. In particular, the superficial layers of the dorsal horn, the dorsolateral funiculus, and lamina X all have moderate levels of CB_1 receptor (Farquhar-Smith et al. 2000). Cannabinoids inhibit glutamate release from afferents in lamina I of the dorsal horn in a CB_1 receptor-dependent fashion (Jennings et al. 2001; Morisset and Urban 2001). Providing anatomical support for these functional studies, CB_1 receptors are found in the dorsal horn in a characteristic twin band corresponding to lamina I and the inner portion of lamina II (Farquhar-Smith et al. 2000).

The source of CB_1 receptors in the dorsal horn remains controversial. One immunocytochemical study found little decrease in CB_1 receptor immunoreactivity following dorsal rhizotomy or hemisection of the spinal cord, suggesting CB_1 receptors are primarily expressed on interneurons (Farquhar-Smith et al. 2000). In contrast, another study using autoradiography to quantify CB_1 expression found a 50% decrease in CB_1 expression following dorsal rhizotomy, suggesting that approximately 50% of CB_1 receptors are found on primary afferents while the balance are on interneurons and descending pathways (Hohmann et al. 1999). Additional evidence supporting functionally significant levels of CB_1 expression on primary afferents includes the findings that CB_1 receptor activation inhibits glutamate release in lamina I (Jennings et al. 2001; Morisset and Urban 2001), only low levels of CB_1 mRNA are present in spinal cord (Mailleux and Vanderhaeghen 1992), and CB_1 receptor mRNA and protein are both expressed in dorsal root ganglia cells (Hohmann et al. 1999; Hohmann and Herkenham 1999b; Bridges et al. 2003). Despite the presence of CB_1 receptors on some C fibers, many more are present on large, myelinated fibers (Abeta and Adelta) (Hohmann and Herkenham 1998, 1999b; Bridges et al. 2003; Price et al. 2003). In balance, it is likely that the analgesic effects of CB_1 receptor activation in the spinal cord are due to interplay between cannabinoid actions on primary afferents, interneurons, and descending pathways.

Emerging evidence suggests that CB_2 agonists are analgesic in a number of neuropathic and inflammatory pain models (Ibrahim et al. 2003; Nackley et al. 2003; Hohmann et al. 2004). There is little evidence for CB_2 expression in normal spinal cord (for example, Buckley et al. 1998). However, CB_2 expression is induced in the spinal cord, likely in microglial cells, following nerve injury and the development of a neuropathic state (Zhang et al. 2003). Precise localization of these receptors using immunocytochemistry remains to be performed. Intriguingly, CB_2 receptor expression was not increased in an inflammatory pain model, despite the efficacy of CB_2 agonists as analgesics in this model. This suggests that CB_2 receptors are selectively upregulated only after specific forms of nerve injury. It also implies that peripherical CB_2 receptors mediate some of the effects of CB_2 agonists, at least some inflammatory pain states.

While expression of CB_1 in the dorsal horn is well established, its expression in spinal cord areas associated with movement is less certain. However, some immunocytochemical evidence suggests CB_1 receptors are found in the ventral horn (Tsou et al. 1998a; Sanudo-Pena et al. 1999a). Interestingly, FAAH is also found in the cell bodies of ventral horn neurons (Tsou et al. 1998b). The localization of CB_1 receptors and FAAH in neuronal circuits associated with movement may underlie the antispastic effects of cannabinoids.

3
Peripheral Nervous System

3.1
Peripheral Nerves

There is strong evidence for CB_1 receptor expression in the periphery. For example, ligation of the sciatic nerve leads to accumulation of CB_1 receptors proximal to the ligation (Hohmann and Herkenham 1999a) and peripherally administered, but systemically inactive, doses of CB_1 agonists can be analgesic (Calignano et al. 1998). To date, no studies have been published examining CB_1 receptors in the periphery beyond major nerves (e.g., sciatic). The development of sufficiently sensitive techniques to study CB_1 and CB_2 expression in the periphery is needed to thoroughly understand the peripheral actions of these compounds. Cannabinoids also regulate autonomic nervous system function. Examples include cannabinoid inhibition of neurotransmitter release in ileum (Roth 1978; Pertwee et al. 1992; Croci et al. 1998) and vas deferens (Nicolau et al. 1978; Pertwee et al. 1992).

3.2
Enteric Nervous System

CB_1 receptors are richly distributed throughout the enteric nervous system; their function has been the focus of reviews (Pertwee 2001; Pinto et al. 2002). Cannabis and its psychoactive extracts inhibit intestinal motility (Shook and Burks 1989; Izzo

et al. 1999). Detailed anatomical studies have found high levels of CB_1 receptor in specific populations of nerves innervating the gut (Kulkarni-Narla and Brown 2000; Coutts et al. 2002; MacNaughton et al. 2004). Studies of guinea pig ileum suggest that CB_1 receptors are localized, in part to the cholinergic myenteric motor neurons (Coutts et al. 2002). Activation of these presynaptic CB_1 receptors inhibits acetylcholine release, decreasing longitudinal muscle contractions. Intestinal motility mediated by non-adrenergic, non-cholinergic (NANC) neurotransmission is also decreased by CB_1 agonists (Izzo et al. 1998); likewise, CB_1 receptors are also found on some NANC neurons (MacNaughton et al. 2004). Activation of CB_1 receptors also decreases fluid secretion in the stomach and intestine. Consistent with this, CB_1 receptors are present in both cholinergic and non-cholinergic sensorimotor submucosal neurons (Tyler et al. 2000; Adami et al. 2002; MacNaughton et al. 2004). CB_1 receptors are also present on some vagal afferents, where their expression is decreased by food intake and CCK (Burdyga et al. 2004).

3.3 Pelvic Viscera

Several studies suggest CB_1 receptor activation has effects on bladder, vas deferens, and uterine function, in both normal and pathophysiological states (Nicolau et al. 1978; Pertwee et al. 1992; Pertwee and Fernando 1996; Dmitrieva and Berkley 2002; Farquhar-Smith et al. 2002). While CB_1 receptors are expressed on tyrosine hydroxylase (noradrenaline)-positive pelvic neurons (Pan et al. 1998), detailed studies on CB_1 receptor distribution to these organs remains to be performed.

4 Summary

The pattern of CB_1 expression in the brain generally correlates with its function both at the macroscopic and microscopic levels. High levels of cannabinoid receptors are found in brain regions implicated in the behavioral effects of cannabinoids, particularly cortex, hippocampus, amygdala, basal ganglia, cerebellum, and the emetic centers of the brainstem. Conversely, low levels are found in other regions, such as the thalamus, pons, and the remainder of the brainstem. Correspondingly, these areas have generally not been implicated in playing a major role in the actions of cannabis or cannabinoids. Undoubtedly, the future will bring further refinement in the localization of CB_1 receptors as well as the badly needed details on where endocannabinoid synthesizing and degrading enzymes are found. Together, this information will aid in our understanding of the role of CB_1 receptors in the function of the CNS, both in normal physiology as well as in pathological states.

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