Fibromyalgia and the endocannabinoid system

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Chapter Eleven

11

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CHAPTER CONTENTS

Introduction

Fibromyalgia has been characterized as an 'endocannabinoid (eCB) deficiency syndrome', along with other refractory maladies such as irritable bowel syndrome, migraine, premenstrual syndrome and other pain-processing disorders (Russo 2004). This syndrome may arise from diminished receptor expression, inadequate ligand biosynthesis or gain-of-function mutations in ligand-catabolizing enzymes. Helping you to understand these basic science concepts (and applying them) will be the goal of this chapter. Our trawl through basic science begins with definitions:

- Ligands are natural or synthetic compounds that bind to receptors.
- Ligands may activate receptors ('agonists') or deactivate receptors ('inverse agonists').
- Endogenous ligands (ligands produced by our own bodies) are synthesized by anabolic enzymes.
- To serve in self-regulatory roles, ligands must be broken down by catabolic enzymes.

Clinicians with a biomechanical or structural orientation may better understand the chemical concepts underlying eCB research by realizing that *chemistry is structure* (Ingber 1998). For example, the pharmacological principle of structure–activity relationships (SAR) is analogous to the anatomical concept of structure–function relationships.

On a molecular level, the eCB system resembles the better-known endorphin system. The endorphin system was indirectly discovered in 1801, when morphine was isolated from opium. The mechanism of action of morphine remained a mystery until the μ-opioid receptor was discovered by Candice Pert in 1973. That discovery begged the question: Why do humans have a receptor for an opium plant compound? Shortly thereafter, scientists discovered the enkephalins and endorphins, endogenous compounds that are mimicked by the plant compound (reviewed by Pert 1997).

According to Dr Andrew Taylor Still, the founder of osteopathic medicine, 'Man should study and use the drugs compounded in his own body' (Still 1897). Still hypothesized that osteopathic manipulative treatment (OMT) stimulated the production of endogenous compounds that promoted homeostasis and healing. Soon after enkephalins and endorphins were discovered, researchers at schools of osteopathy, chiropractic, physical therapy, massage therapy and acupuncture carried out endorphin research. The initial wave of enthusiasm dampened after studies produced conflicting evidence. In fact, endorphins and enkephalins may not be modulated by OMT, chiropractic manipulation, massage therapy, acupuncture or even 'runner's high' (reviewed in Dietrick & McDaniel

2004, Harbach et al 2007, McPartland et al 2005, Schultz et al 2000). In the past few years, research has swung from endorphins to the eCB system. In 1992, the year eCBs were discovered, an internet query using the PubMed search engine (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) with the term 'endorphin' returned 596 hits, and 'endocannabinoid' returned only two hits. In a search limited to 2008, 'endorphin' returned 141 hits whereas 'endocannabinoid' returned 565 hits.

The eCB system embodies our holistic concept that the body possesses self-regulatory mechanisms that are self-healing in nature. The eCB system has emerged as an important regulator of mind–body structure and function. This self-regulatory capacity can be rephrased as the maintenance of homeostasis. The eCB system's capacity to maintain homeostasis will be cited many times in this chapter. As Dr Still emphasized: 'To find health should be the object of the doctor. Anyone can find disease' (Still 1897).

Cannabis and cannabinoid receptors

Discovery of the μ-opioid receptor in 1973 launched a search for cannabinoid receptors, which are named after the C*annabis* plant, the source of cannabis (marijuana, hashish). Cannabis has long been recognized for its anti-inflammatory, analgesic (pain-relieving) and muscle relaxant qualities. Over 4000 years ago the Chinese physician Shen Nung recommended cannabis for rheumatic pains (cited in Mechoulam 1986). Nearly a century ago, Sir William Osler considered cannabis the 'most satisfactory remedy' for migraine headache (Osler & McCrae 1915). At that time, cannabis was dispensed as an orally administered fluid extract, sold by all the leading pharmaceutical companies. Unfortunately, orally administered cannabis is erratically absorbed by the gut. This factor, coupled with variable product potency, unreliable sources of supply and poor storage stability, led to fluid extracts falling out of favour (McPartland 2008a). The decline in popularity was hastened by new synthetic medicines, such as aspirin. Concern with 'reefer madness' led to cannabis prohibition, despite vigorous opposition to prohibition by the American Medical Association that continues today (Fishbein 1937, Okie 2005).

The primary psychoactive ingredient in cannabis, Δ^9-tetrahydrocannabinol (THC), was discovered in 1964. Over 70 C_{21} terpenophenols unique to cannabis, collectively called the cannabinoids, have been identified by Raphael Mechoulam, Roger Pertwee and others (reviewed in Mechoulam 1986, Pertwee 2005). Synthetic THC (dronabinol, Marinol) was approved as a schedule II drug in 1986, and moved to schedule III in 1999. Its medical indications include nausea and vomiting associated with cancer chemotherapy, and appetite loss and weight loss in people with acquired immunodeficiency syndrome (AIDS). Nabilone (Cesamet), a synthetic analogue of THC, was also approved by the US Food and Drug Administration (FDA), with the same indications.

The search for a cannabinoid receptor was stymied by THC's poor performance as a molecular probe (THC is lipophilic and sticks to everything), so receptor discovery awaited development of a synthetic, water-soluble THC analogue. This was accomplished in 1988 when researchers showed that [^{3}H]CP55,940 bound specifically to a cannabinoid receptor located in neuron cell membranes. Two years later the gene for the receptor was cloned (reviewed in Howlett et al 2002). The gene translates into a chain of 472 amino acids that weave back and forth across the cell membrane seven times – the structure of a G-protein coupled receptor (GPCR). GPCRs are named for their G-proteins (short for guanine-nucleotide-binding proteins), which function as intracellular 'molecular switches'. Each GPCR possesses a unique binding pocket with affinity for specific ligands, like a lock-and-key mechanism. A short list of GPCRs includes opioid receptors, dopamine receptors, some serotonin receptors, some GABA receptors and beta-adrenergic receptors.

GPCRs are tensegrity structures that span the cell membrane. A ligand that loads the receptor's *extracellular* surface will distort the shape of its *transmembrane* weave of amino acids, thereby altering the *intracellular* side of the receptor and its interface with the G-protein. Cannabinoid receptors associate with several subtypes of G-proteins, such as Gi, Gs and Go subtypes. The 'i' and 's' abbreviate 'inhibitor' and 'stimulator', which describes the oppositional effects these G-proteins have on their targets. Importantly, research has shown that different cannabinoid agonists preferentially activate different G-proteins subtypes. This 'agonist trafficking' of G-proteins lysergically alters

the classic key-in-lock metaphor; instead, different keys (different cannabinoid agonists) fit the same lock (the CB receptor), but open the door into three different rooms (Gi, Go or Gs). This may explain why different strains of cannabis produce different psychoactive effects. For example, afghani strains seem to preferentially activate Gi and cause an inhibitory, stony, narcotic-like effect, whereas plants from Thailand seem to preferentially activate Gs and cause a speedy, buzzy high.

A second cannabinoid receptor was discovered in 1993, so the receptors were renamed CB_1 and CB_2. The two receptors express slightly different structures, and therefore express slightly different functions: CB_1 principally functions in the nervous system, whereas CB_2 is primarily associated with cells governing immune function, such as white blood cells. Taken together, CB_1 and CB_2 span the field of psychoneuroimmunology and represent a microcosm of mind–body medicine.

CB_1 is the most common GPCR in the human brain. Its uneven distribution in the brain reflects the well-known effects of cannabis upon humans and other animals. Highest densities of CB_1 are found in the hippocampus (affecting short-term memory) and parts of the basal ganglia – for example, substantia nigra, globus pallidus and striatum (caudate and putamen). CB_1 in these nuclei coordinate motor function and movement, as does CB_1 in the cerebellum. High densities in the cerebral cortex, amygdala and dorsal horn of the spinal cord affect cognition, mood and emotion, and pain perception. The brainstem nuclei that govern 'cardiorespiratory drive' express very few CB_1 receptors, which probably accounts for the lack of lethal effects from cannabis overdose (reviewed in Howlett et al 2002).

The genes for CB_1 (termed *CNR1*) and CB_2 (*CNR2*) are paralogues (genes separated by a gene duplication event), and their orthologues (genes separated by a speciation event) have been identified in all vertebrate species. A cannabinoid receptor gene tree within a species tree is illustrated in Figure 11.1. The species tree was constructed from 8 species whose entire genomes have been sequenced, specifically chosen to obtain a balanced species divergence within the evolutionary 'tree of life'. The human, mouse and puffer fish genomes all express *CNR1* and *CNR2* genes, whereas the sea squirt genome expressed only one gene, which we called the ancestral *CBR* gene (McPartland et al 2006). No cannabinoid genes were found in

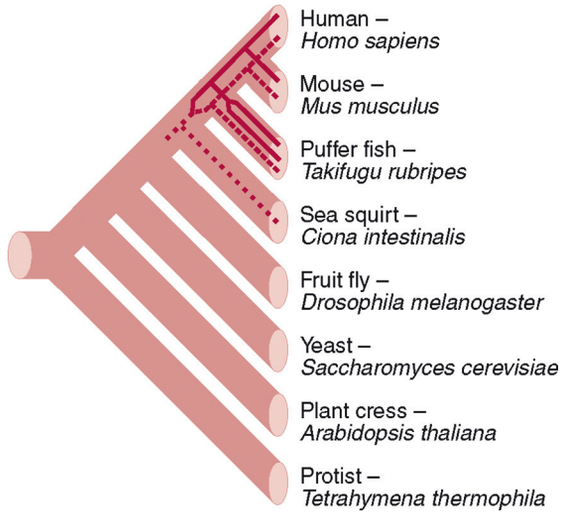

Figure 11.1 • The cannabinoid receptor gene tree within a species tree. The species tree is represented by thin tubular lines, with the gene tree represented by thicker lines, either solid (representing ancestral CBR gene orthologues and CB_1 gene orthologues after the gene duplication event) or dashed lines (representing CB_2 gene orthologues). (Reproduced with permission from McPartland 2008a.)

the 'lower' organisms with deeper evolutionary roots. These findings suggest that the gene duplication event that gave rise to *CNR1* and *CNR2* occurred in the ancestor of vertebrates. The ancestral *CBR* gene that preceded the duplication event may have evolved in the ancestor of sea squirts, about *600 million years ago* (McPartland et al 2007). Several lines of evidence suggest the solitary ancestral *CBR* receptor probably functioned more like CB_1 than CB_2 (McPartland & Glass 2003, Matias et al 2005). Duplicated genes often show asymmetric rates of evolution, with one paralogue retaining its ancestral function and preserving its gene sequence, while the other paralogue undergoes neofunctionalization accompanied by a burst of sequence evolution. Consistent with this, the cannabinoid gene duplication event gave rise to one paralogue that continued its CB_1-like function, whilst the second paralogue diverged to take up new functions. *CNR1* has become stabilized, whilst *CNR2* reflects neofunctionalization and its mutation rate is four-fold greater than *CNR1* (McPartland et al 2007).

Mutant CB_1 (–/–) knockout mice have been created that lack CB_1 receptors. Surprisingly, the transgenic CB_1 (–/–) mice survive gestation, but they suffer increased morbidity and premature

mortality compared to wild-type mice (Zimmer et al 1999). Young CB_1 (–/–) mice perform as well as wild-type mice, or often better, in a number of learning and memory paradigms. But mature CB_1 (–/–) mice perform much worse, suggesting that age-related cognitive decline is accelerated in the absence of CB_1 (Bilkei-Gorzo et al 2005). CB_1 (–/–) mice show greater aggression, epilepsy, age-related neuron loss, anxiogenic-like behaviour, depressive-like behaviour, anhedonia and fear of newness (Martin et al 2002). Their very survival speaks volumes regarding the resiliency of life, and probably depends on the recruitment of vestigial receptor systems.

Endocannabinoids and their enzymes

Humans did not evolve cannabinoid receptors for a *Cannabis* compound. The cannabinoid receptor definitely evolved long before *Cannabis*, which is not more than 34 million years old (McPartland & Guy 2004). The best-known endogenous cannabinoid, anandamide (AEA), was discovered by Mechoulam in 1992 (28 years after he discovered THC), followed by 2-arachidonoylglycerol (2-AG). AEA and 2-AG are metabolites of arachidonic acid. Their structures do not resemble the THC structure, but all three compounds nonetheless fit the CB_1 and CB_2 binding pockets. Thus the effects of THC, AEA and 2-AG substantially overlap, because they activate the same receptors (reviewed in Mechoulam et al 1998).

AEA and 2-AG are not stored in vesicles like classic neurotransmitters. Instead they are synthesized 'on demand' from precursor phospholipids within the lipid cell membrane, and released when needed. The biosynthesis of AEA may follow several pathways and involve several enzymes. Its precursor phospholipid (abbreviated NAPE) can be cleaved by a NAPE-selective phospholipase D enzyme (NAPE-PLD) to directly release AEA. NAPE also serves as a substrate for the abhydrolase domain-containing protein (ABHD4) enzyme, giving rise to phosphoglycerol-AEA which is cleaved by a phosphodiesterase into AEA. NAPE can be hydrolysed via a PLC pathway into phospho-AEA before being further hydrolysed to AEA by the protein tyrosine phosphatase, non-receptor type 22 enzyme (PTPN22). Lastly, a pathway involving a secretory phospholipase 2 enzyme followed by a lysophospholipase D enzyme may convert NAPE to AEA (reviewed by McPartland et al 2007). For AEA to work in a homeostatic fashion, it must be catabolized (broken down) after it activates a cannabinoid receptor. The best-known catabolic enzymes of AEA are fatty acid amide hydrolase (FAAH), fatty acid amide hydrolase 2 (FAAH2) and *N*-acylethanolamine acid amidase (NAAA).

The 'life-cycle' of 2-AG appears to be more straightforward. 2-AG is biosynthesized by two diacylglycerol lipases, DAGLα and DAGLβ, and 2-AG is catabolized by monoacylglycerol lipase (MAGL) and by cyclooxygenase 2 (COX-2, prostaglandin-endoperoxide synthase). Recently, an FAAH-blocking agent was described, which prolonged AEA activity in the synapse, analogous to a serotonin uptake inhibitor. Pharmacologists are racing to find inhibitors of the other eCB enzymes as well (Pertwee 2005).

The eCBs, their enzymes and the cannabinoid receptors collectively regulate many aspects of homeostasis, including embryological development (and adult neurogenesis via the same mechanisms), neuroprotection and neural plasticity, autonomic function with links to immunity and inflammation, apoptosis and carcinogenesis, hunger and feeding, biological oscillators and pacemakers cells, and perhaps most importantly for the purposes of this book, nociception, pain and emotional memory (suffering).

Nociception and pain

The effects of eCBs upon nociception and pain will focus on four areas: 1) the peripheral terminals of nociceptors; 2) the dorsal horn in the spinal cord; 3) the descending pain inhibitory pathway; and 4) supratentorial sites.

Pain begins in peripheral tissues as nociception, transmitted by A-δ and C-fibre afferent sensory neurons (nociceptors). *Polymodal* nociceptors contain receptors for mechanical pressure, thermal stimuli and many chemicals: potassium ions, protons and free O_2 radicals (by-products of muscle metabolism and tissue injury), histamine (released from mast cells), serotonin (released from platelets after exposure to platelet-activating factor released from mast cells) and bradykinin (cleaved from serum proteins). All of these chemical 'activators' bind to receptors in the nociceptor and initiate an action

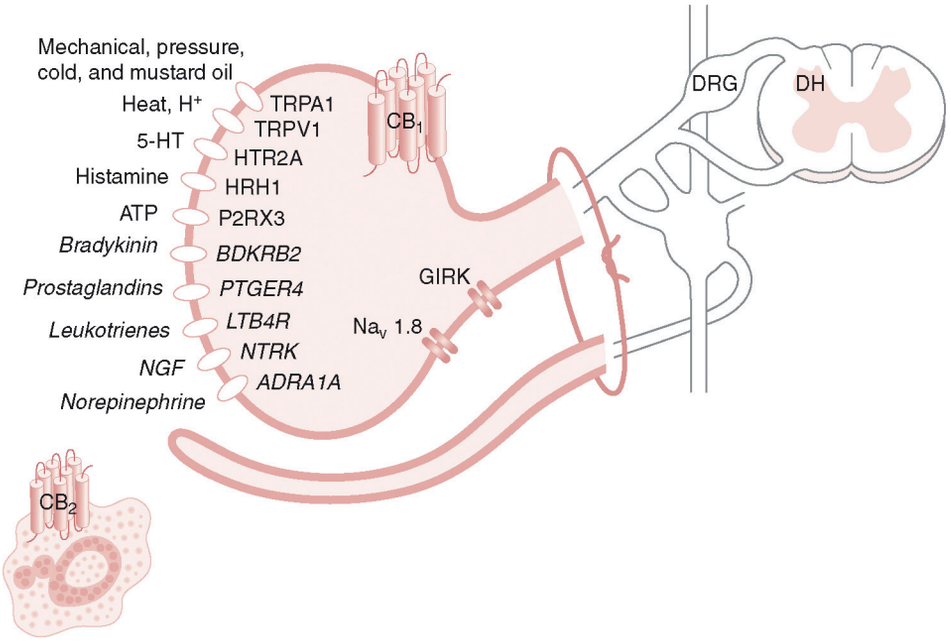

Figure 11.2 • Schematic illustration of a polymodal C-fibre nociceptor, with an enlarged view of its distal terminal, its cell body in the dorsal root ganglion (DRG) and central terminal in the dorsal horn (DH). A suture loop separates the enlarged view from the rest of the nociceptor. A sympathetic postganglionic neuron and a lymphocyte expressing CB_2 are illustrated below the distal terminal. Within the distal terminal are five receptors for activators (regular font) and five receptors for sensitizers (italic font), named by their gene symbols. Also embedded in the distal terminal are two ion channels (GIRK and Na_v 1.8) and CB_1. (Reproduced with permission from McPartland 2008a.)

potential (Fig. 11.2). 'Sensitizers' are also released from damaged tissue (e.g. prostaglandins and leukotrienes), neighbouring autonomic nerves (noradrenaline/norepinephrine) and from the nociceptor itself (substance P and calcitonin gene-related peptide). Sensitizers decrease the activation threshold of a neuron, so that the nociceptor fires with less activation (Fig. 11.2).

Activators and sensitizers cause *peripheral sensitization* – a phenomenon known to people with fibromyalgia as *hyperalgesia* (abnormally increased sensation of pain) and *allodynia* (pain from normally non-painful stimuli). Peripheral sensitization elicits a homeostatic response by the eCB system: CB_1 activation causes a decrease in the release of activators and sensitizers around the site of tissue injury, and CB_1 opens K^+ channels in the nociceptor cell membrane, so the nerve becomes hyperpolarized and less likely to fire. CB_2 signalling decreases the release of activators and sensitizers by neighbouring mast cells and macrophages (reviewed by Walker & Hohmann 2005). Functioning of the eCB system at the peripheral terminal of the nociceptor provides the 'first line of defence against pain' (Agarwal et al 2007).

In the dorsal horn (the gateway to the central nervous system or CNS) the nociceptor synapses with a sensory neuron that ascends to the brain. Normally a nociceptor action potential arrives at the dorsal horn, causes a release of glutamate and substance P into the *synaptic cleft*, and these neurotransmitters bind to their respective receptors in the *postsynaptic* cell. Activation of the postsynaptic cell initiates another action potential, which ascends to the brain (Fig. 11.3). Abnormal persistent activation of a nociceptor causes excessive glutamate release in the dorsal horn synapse. This maladaptively upregulates glutamate receptors in the postsynaptic cell and causes an influx of Ca^{2+} in the postsynaptic cell, which leads to central sensitization, 'wind-up' or 'dorsal horn memory'. The sensitized dorsal horn becomes a 'neurologic lens'. It consolidates other nociceptive signals that converge upon the same segment of the spinal cord, including nociceptive signals from other somatic dysfunctions and visceral dysfunctions. As a result, postsynaptic

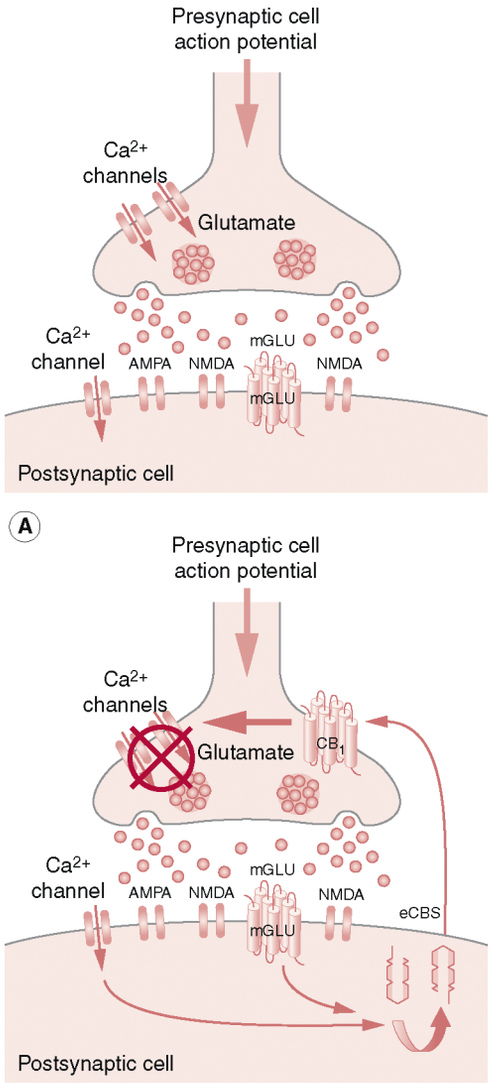

Figure 11.3 • The eCB system dampens excessive nociception at the dorsal horn. **A** Persistent firing of a C-fibre nociceptor opens voltage-gated calcium channels (VgCCs) in the presynaptic axon terminal. Calcium influx causes presynaptic vesicles of glutamate to release into the synaptic cleft. Excessive activation and upregulation of glutamate receptors in the postsynaptic cell causes the opening of calcium channels. **B** Open calcium channels in the postsynaptic cell stimulate DAGLα enzymes to synthesize 2-AG, which is released into the synapse and activates CB_1 in the presynaptic cell. The G-proteins from activated CB_1 close GCCs, thereby halting release of presynaptic glutamate vesicles. (Reproduced with permission from McPartland 2008a.)

spinal neurons have *decreased* activation thresholds, and *increased* response magnitudes. They fire with increased frequency or fire spontaneously (Simons et al 1999).

Again, the eCB system may come to the rescue: Ca^{2+} influx into the postsynaptic cell causes DAGLα enzymes located in that cell to synthesize 2-AG. The 2-AG moves *retrograde* across the synapse (opposite the direction of glutamate) to CB_1 located on the presynaptic neuron (see Fig. 11.3). Activated CB_1 closes presynaptic Ca^{2+} channels, which halts glutamate vesicle release. This newly discovered *retrograde transmission* is called 'depolarization-induced suppression of excitation' (DSE; Mátyás et al 2007). The eCB system induces 'dorsal horn memory loss' and short circuits central sensitization (Morisset & Urban 2001), requiring neuroscientists to rewrite textbooks that describe the synapse as a 'one-way street'. Retrograde signalling enables the postsynaptic cell to control its own incoming synaptic traffic. The eCB system also employs the same negative feedback mechanism to temporarily halt the synaptic release of gamma-aminobutyric acid (GABA). GABA is an inhibitory neurotransmitter, so this mechanism is termed depolarization-induced suppression of inhibition (DSI, Straiker & Mackie 2006). DSI is a ubiquitous phenomenon and modulates neurotransmission in the hippocampus, cerebellum, basal ganglia, cerebral cortex and amygdala.

The 'descending pain inhibitory pathway' is activated by the perception of pain in the brain. The pathway descends from the cerebral cortex and thalamus through the periaqueductal grey (PAG) and periventricular grey (PVG) in the midbrain, to the nucleus raphe magnus (NRM) in the rostroventral medulla, and down to the dorsal horn of the spinal cord. Endorphins, eCBs, serotonin, noradrenaline (norepinephrine) and adenosine play important roles in the pathway. CB_1 and eCBs are found in high concentration in the PAG, PVG, NRM and dorsal horn, where they suppress GABA-releasing interneurons that inhibit neurons in the descending pathway (reviewed by Walker & Hohmann 2005). The coordinated release of eCBs in this pathway mediates 'stress-induced analgesia' (Suplita et al 2007), the well-known phenomenon in which people are less responsive to pain following an environmental stressor (i.e. soldiers wounded in battle or athletes injured in sports events may not feel pain during the battle or game). The eCB and endorphin systems co-localize within the pathway circuitry. This is how THC and eCBs work synergistically with morphine, and

provide a 'morphine-sparing effect' (Walker & Hohmann 2005). A rodent study also showed that activated CB_2 receptors stimulate the release of beta-endorphins (Ibrahim et al 2005).

'Supratentorial sites' (a cranial osteopathic term for parts of the brain lying above the tentorium cerebelli) control the acquisition and storage of aversive memories. Painful memories, fear and anxiety are factors that turn *chronic pain* into *chronic suffering*. The eCB system facilitates the extinction of aversive memories ('active forgetting') through selective inhibitory effects on local inhibitory networks in the limbic system (especially the amygdala), and also squelches hippocampus-dependent fear conditioning. Thus the eCB system may benefit hospice patients and people unable to extinguish painful memory (e.g. post-traumatic stress disorder; Lafenetre et al 2007). Fear of pain and fear of movement are significant concerns for people with fibromyalgia, and elevated fear correlates with greater pain severity, depressed mood and disability (Turk et al 2004).

Around the edges of fibromyalgia

CB_1 receptors in nociceptors are synthesized in the dorsal root ganglion and carried by axoplasmic flow to insertion sites in the distal terminal of the nerve (see Fig. 11.2). In a rodent study of the sciatic nerve (Hohmann & Herkenham 1999), it was shown that a mechanical barrier (a loop of suture) that restricted axoplasmic flow actually prevented CB_1 receptors from reaching the distal terminal. The ligation loop in Figure 11.2 represents myofascial dysfunctions seen in people with fibromyalgia – thoracic outlet restriction, piriformis syndrome, carpal tunnel syndrome, etc. Thus myofascial dysfunction may diabolically exacerbate eCB system dysfunction in a positive-feedback loop.

The pathophysiology of fibromyalgia involves the autonomic nervous system, stress hormones, the immune system, the sleep cycle, tender points and trigger points, and connective tissue in general. The eCB system affects autonomic outflow through the peripheral and central nervous systems. Sympathetic nerve terminals contain CB_1, and the activation of these receptors inhibits noradrenaline (norepinephrine) release and dampens sympathetically mediated pain (Pacher et al 2006). The sympathetic nervous system drives the hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–locus coeruleus–noradrenaline (norepinephrine) (HLN) axis. Psychological stress activates the HPA axis and results in corticosteroid release, whereas stress-induced activation of the HLN axis results in noradrenaline (norepinephrine) release. These stress responses are opposed by the eCB system (Pacher et al 2006).

Activation of the HPA axis hinders the immune response. Cannabinoids oppose the HPA, and therefore act as immuno*modulators*, and not simply immuno*suppressors* as characterized in the 1970s (Ashton 2007). Cannabinoids do indeed suppress production of Th1 (T-helper 1, cellular immunity) cytokines such as interleukin (IL)-2 and interferon gamma (INFγ), as well as tumour necrosis factor alpha (TNFα). On the other hand, cannabinoids *increase* the secretion of Th2 (T-helper 2, humoral immunity) cytokines such as IL-4, IL-5 and IL-10. The alkylamide ligands produced by *Echinacea* potently stimulate CB_2, and stimulate phagocytosis by white blood cells (Ashton et al 2008). Lack of psychoactivity by *Echinacea* can be attributed to its CB_2-specific selectivity and the relative lack of CB_2 in the brain.

The eCB system alters every biological oscillator or pacemaker cell investigated to date, including cells that govern the sleep cycle (reviewed in McPartland 2008a). The pineal gland produces melatonin as well as 2-AG in a circadian rhythm driven by the suprachiasmatic nucleus, regulated in part by CB_2. The eCB may also be involved in sleep induction (Mechoulam et al 1997), an important consideration in people with fibromyalgia.

The major diagnostic criterion of fibromyalgia – the presence of tender points – may be a symptom of eCB deficiency. During the menstrual cycle, AEA decreases during the luteal phase (*c.* day 21) and rises during the follicular phase (*c.* day 10) due to the progesterone-induced upregulation of FAAH (enzyme that breaks down AEA) in the luteal phase. In a study of healthy women with normal menstrual cycles, the decrease in AEA corresponded with hypersensitivity to algometer-induced pressure pain during the luteal phase. Several subjects 'changed' fibromyalgia diagnosis during the course of a menstrual cycle, fulfilling the tender point criterion (tenderness $\leq$4 kg at $\geq$11 points) during the AEA-deficient luteal phase or menstrual phase, but never during the AEA-rich follicular phase (Dunnett et al 2007).

The differences between myofascial tender points and myofascial trigger points (MTrPs) are

described elsewhere in this book. The eCB system modulates both. Travell & Simons implicated dysfunction at the motor endplate (i.e. the neuromuscular junction) as the cause of MTrPs. At the motor endplate, an α-motor neuron terminates upon its target muscle. The nerve terminates in multiple swellings (termed presynaptic boutons) that contain acetylcholine (ACh) vesicles, with voltage-sensitive calcium channels (VsCCs, specifically *P/Q-type* VsCCs) clustered nearby (Fig. 11.4). Normally an α-motor neuron action potential arrives at the bouton and opens VsCCs, leading to an influx of Ca^{2+} into the bouton, which causes a release of ACh. ACh diffuses across the synaptic cleft and binds to nicotinic ACh receptors (nAChs) embedded in the cell membrane of the postsynaptic muscle fibre (see Fig. 11.4). This mechanism is analogous to the dorsal horn (see Fig. 11.3), but uses ACh instead of glutamate. Activation of postsynaptic nAChs depolarizes the postsynaptic cell, forming a miniature endplate potential (MEPP). A sufficient number of MEPPs activate postsynaptic VsCCs (specifically *L-type* VsCCs), which subsequently trigger the ryanodine receptor. Activation of the ryanodine receptor releases Ca^{2+} from the sarcoplasmic reticulum into the cytoplasm of the muscle cell. This triggers the interaction between actin and myosin, and the muscle contracts (Simons et al 1999).

MTrPs may be evoked by the abnormal depolarization of motor endplates, caused by presynaptic, synaptic and postsynaptic mechanisms (McPartland & Simons 2007). Presynaptic dysfunction may arise from excessive release of ACh, synaptic dysfunction may be a defect of acetylcholinesterase (the enzyme responsible for ACh breakdown) and postsynaptic dysfunction may be caused by an upregulation of nAChs. McPartland & Simons hypothesized that CB_1 in motor endplates dampens ACh release, and perhaps plays a role in preventing or treating MTrPs. This hypothesis has been supported by two new animal studies showing that CB_1 activation in motor endplates dampens ACh release (Newman et al 2007, Sánchez-Pastor et al 2007).

Fascia and connective tissues are also modulated by the eCB system. A recent study revealed that fibroblasts, myofibroblasts, chondrocytes and synoviocytes expressed CB_1, CB_2, and eCB ligand-metabolizing enzymes (McPartland 2008b). Fibroblast CB_1 levels became upregulated after exposure to inflammatory cytokines, and after mechanical stretching of fibroblasts. Within the cell membrane, CB_1 is localized to scaffolding microdomains known as 'lipid rafts' (Rimmerman et al 2007). Lipid rafts in fibroblast cells anchor *integrins*, which are transmembrane receptors that link ligands in the extracellular matrix (such as collagen and fibronectin) to the intracellular cytoskeleton. Integrin receptors transmit signal via intracellular enzymes (e.g. FAK, Rac and Rho), and these in turn regulate the actin–microtubule–cytoskeleton system. The integrin-centred cluster of signalling proteins is known as a 'focal adhesion' and it regulates fibroblast growth, remodelling and migration. It is easy to speculate that focal adhesions are modulated by a mechanism that is CB_1-dependent (Aguado et al 2007).

Inflammatory degradation of connective tissues may be dampened by CB_1. Fibroblast-like synovial cells exposed to inflammatory TNFα secrete metalloproteinase enzymes, which facilitate articular cartilage destruction (Johnson et al 2007). Johnson and colleagues experimentally decreased metalloproteinase secretion by treating fibroblast cells with a synthetic cannabinoid, ajulemic acid (AjA). Related research has shown that articular cartilage destruction (*and* nitric oxide-induced proteoglycan degradation *and* collagen breakdown) are all decreased by AEA (Mbvundula et al 2005).

Enhancing the eCB system

Obviously, enhancing the eCB system would benefit people with fibromyalgia. Clinicians who use bodywork (i.e. osteopathy, chiropractic, physical therapy, massage therapy, Rolfing, etc.) induce psychological changes in their patients, such as anxiolysis, easement of suffering, an increased sense of well-being and even euphoria. These supratentorial effects have been dubbed 'cannabimimetic' because they are evoked by the administration of cannabis. Perhaps bodywork evokes cannabimimetic effects by augmenting the production of eCBs in our patients. We conducted a randomized, blinded, controlled clinical trial (McPartland et al 2005) that measured serum AEA levels twice in subjects, pre- and post-osteopathic manipulative treatment (OMT). The OMT intervention consisted of myofascial release, muscle energy techniques and thrust techniques. Subjects receiving OMT experienced cannabimimetic effects such as 'high, happy, lightheaded and hungry' (measured with a visual analogue-type questionnaire). The increase in

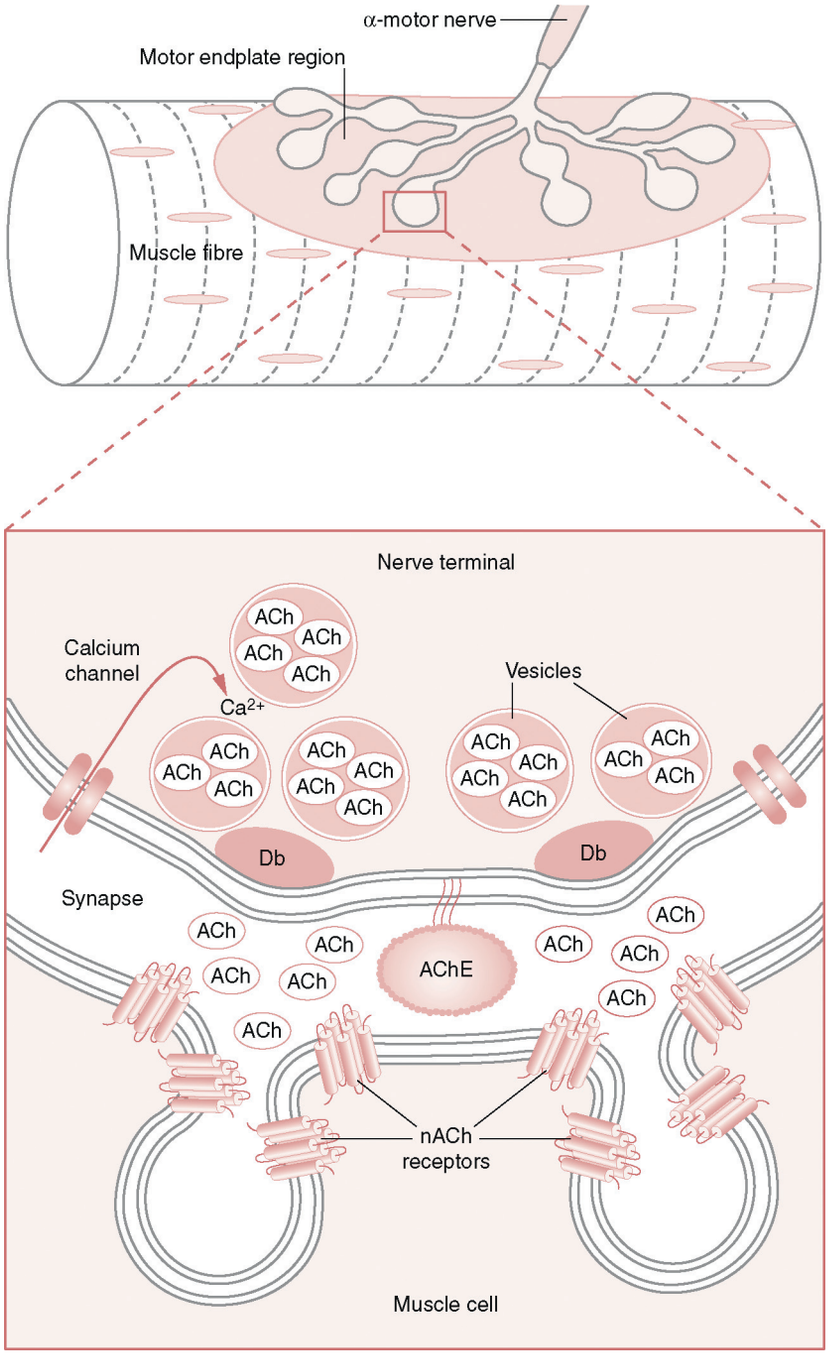

Figure 11.4 • The motor endplate – proposed site of trigger point dysfunction. Top: The junction between the α-motor neuron and the muscle fibre. Bottom: Presynaptic boutons are separated from the postsynaptic muscle cell by the synaptic cleft. Within each bouton are many vesicles containing ACh, clustered around dense bars (Db). Also clustered around the Db are calcium channels. The Db is the site of ACh release into the synaptic cleft. Across the synaptic cleft from the Db, the postsynaptic muscle cell membrane forms junctional folds that are lined with nicotinic ACh receptors (nACh). ACh released into the synaptic cleft activates nACh receptors, then is inactivated by the acetylcholinesterase enzyme (AChE). (Reproduced with permission from McPartland & Simons 2007.)

cannabimimetic effects correlated with an elevation in post-OMT serum AEA levels (more than double pre-OMT levels). Neither cannabimimetic effects nor changes in AEA levels occurred in control subjects. A second smaller clinical trial that investigated the effects of OMT upon eCB levels reported little change in AEA levels, but showed significant post-OMT augmentation of *N*-palmitoylethanolamine (PEA), a short-chain analogue of AEA (Degenhardt et al 2007). The lack of change in AEA levels may have been from differences in measuring AEA, but may have been due to the fact that the OMT intervention in the Degenhardt study did not include myofascial release. Myofascial release imparts strong and prolonged mechanical shearing forces upon the skin. Previous researchers have hypothesized that eCB release in the skin may be the source of 'runner's high' – the cannabimimetic emotions evoked by feet repetitively 'pounding the pavement' (Dietrick & McDaniel 2004).

As noted in the previous section describing thoracic outlet restriction and piriformis syndrome, myofascial dysfunction may recursively loop into eCB system dysfunction. Myofascial barriers can restrict axoplasmic flow and prevent CB_1 from reaching peripheral sites. Thus bodywork that reduces or eliminates myofascial barriers will restore axoplasmic flow and facilitate CB_1 transport to peripheral sites of action.

The cellular mechanisms underlying OMT have been modelled by in vitro stretching of fibroblasts in a Flexercell apparatus (Dodd et al 2006). Unfortunately these researchers did not investigate the effects of stretching upon eCB ligands or CB_1 activation. We reported a doubling of CB_1 *expression* in fibroblasts following stretching in a Flexercell apparatus, but expression is not the same as activation (McPartland 2008b). Speculatively, stretching may activate CB_1 in the *absence* of an eCB agonist. Recall that an agonist works by reshaping its receptor into an 'active conformation', thereby activating a G-protein. Similar reshaping occurs when hydrostatic pressure stretches the angiotensin 1 receptor into an active conformation (Zou et al 2004). This makes sense, because the angiotensin 1 receptor occurs in smooth muscle cells in blood vessel walls and modulates blood pressure. Receptor activation of G-protein in the absence of ligand is termed 'constitutive activity'. Constitutive activity may arise spontaneously in CB_1, but the causes of CB_1 constitutive activity remain unknown (reviewed in Howlett et al 2002).

We speculated a hydrostatic mechanism might stretch CB_1 into an active conformation during a cranial osteopathic 'CV-4' treatment (McPartland 2008b). CV-4 (compression of the fourth cerebral ventricle) transiently increases hydrostatic pressure in the cerebral ventricular system (Adams et al 1992), and cells that line the cerebral ventricles are enriched with CB_1 (Curtis et al 2006). However, cells lining the cerebral ventricles also express enzymes for AEA (Ashton et al 2004), so we have also speculated that the CV-4 technique may trigger a release of eCBs (McPartland & Skinner 2005). Activation of the eCB system may explain many CV-4 effects, such as relaxation and drowsiness, decreased sleep latency and decreased sympathetic nerve activity (Cutler et al 2005). This is not a particularly original thought – Pert (2000) previously hypothesized that energy therapists heal patients by inducing a vibrational tone that shifts neuroreceptors into active conformations, or the vibrational tone triggers release of endorphins that active the neuroreceptors. Oschman (2000) described crystalline materials within biological structures that generate piezoelectric fields when compressed or stretched. Examples of crystalline materials applicable to fibromyalgia include the phospholipids that surround CB_1 within cell membranes, and collagen in the ECM that surrounds fibroblasts.

The eCB system modulates at least three cranial phenomena: the motility of neurons and glial cells (Harkany et al 2007), the rate and amplitude of CSF production (Mancall et al 1985), and restraint of suture ossification (Ofek et al 2006). Lastly, OMT may stimulate nitric oxide and improve cardiovascular circulation, perhaps via eCB release (Stefano & Salamon 2006). Improved cardiovascular circulation is one mechanism by which OMT improves health: 'the rule of the artery is supreme' (Still 1897).

Other approaches

Clinicians wield other tools for augmenting eCB activity in people with fibromyalgia. These include lifestyle modifications (exercise, stress reduction, dietary supplements, drug and alcohol restraint) and pharmaceutical approaches (acetaminophen, NSAIDs, antidepressants, exogenous cannabinoids).

Fibroblasts react to acupuncture needle rotation, a response modulated by Rho and Rac signalling (Langevin et al 2006). Recall that Rho and Rac

signalling are part of 'focal adhesions' and the eCB system works through Rho and Rac (e.g. Berghuis et al 2007, He & Song 2007). Indeed, acupuncture may work through the eCB system (Li et al 2007), rather than the endorphin system as previously assumed (Harbach et al 2007). Similarly, the eCB system rather than the endorphin system may be responsible for 'runner's high' – exercising on a treadmill or stationary bicycle raises serum AEA levels (Dietrich & McDaniel 2004, Sparling et al 2003). Encouraging patients to exercise is a key part of *stress reduction*. Chronic stress downregulates CB_1 expression (Hill et al 2005), so any form of stress reduction may enhance the eCB system.

Studies have shown that acute ethanol ingestion decreased AEA and 2-AG in most brain regions (Gonzales et al 2002) and chronic ethanol downregulated CB_1 expression (Ortiz et al 2004). Dietary inclusion of fish oils containing DHA (docosahexaenoate) and other omega-3 polyunsaturated fatty acids increased AEA and 2-AG levels in the brain (Berger et al 2001, Watanabe et al 2003). Oral administration of *Lactobacillus* upregulated CB_2 in intestinal epithelial cells and relieved symptoms of irritable bowel syndrome (Rousseaux et al 2007).

Pharmaceuticals may augment the eCB system. Acetaminophen (paracetamol) is converted into *N*-arachidonoylphenolamine by the liver, a compound that activates CB_1 (Hogestatt et al 2005). Ibuprofen and other non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2, an enzyme that breaks down 2-AG, so NSAIDs may prolong 2-AG activity. NSAIDs also inhibit FAAH and therefore enhance AEA activity (Fowler 2004). In animal models, the coadministration of NSAIDs with eCBs acted synergistically (Guindon et al 2006). This may explain why NSAIDs sometimes cause sedation and other unexpected psychotropic effects in our patients. Dexamethasone potently upregulated CB_1 in rodents (Wang et al 2007) and may account for the strange 'steroid-induced euphoria' seen in patients on high doses of these drugs. Diazepam and eCBs produce synergistic anxiolytic effects in mice, leading researchers to propose that enhancement of eCB function increases the effectiveness of diazepam (Naderi et al 2008). The tricyclic antidepressant desipramine increased CB_1 levels in the brain (Hill et al 2006), whilst fluoxetine decreased CB_1 expression (Oliva et al 2005). This may be why people with fibromyalgia often respond to low doses of tricyclic antidepressants, whereas SSRIs yield mixed results.

What about drugs that directly activate CB_1 and CB_2? Adelmidrol, a synthetic analogue of PEA, has been topically applied to improve lateral epicondylitis (Sioutis et al 2004). At least three clinical studies are currently testing THC or nabilone (a synthetic THC analogue) for the treatment of fibromyalgia (see http://www.clinicaltrials.gov). Two other studies have been completed. Subjects given nabilone reported significant improvements in visual analogue scales (VAS) for pain and anxiety, and in the Fibromyalgia Impact Questionnaire (a functional improvement scale) (Skrabek et al 2008). The drug was well tolerated, although the nabilone group experienced more side-effects than the placebo group. A previous pilot study of synthetic THC (Schley et al 2006) reported improvement in some fibromyalgia patients, but over half the subjects withdrew due to adverse side-effects.

Adverse side-effects are the primary reason why many patients prefer cannabis to synthetic THC (McPartland & Pruitt 1999). Cannabis is more than THC. Other ingredients provide additional benefits, as well as mitigate the side-effects of THC. Cannabidiol (CBD), for example, reduces symptoms of dysphoria and anxiety provoked by THC, while contributing its own anxiolytic, antipsychotic, analgesic, anticarcinogenic, antioxidant and neuroprotective effects (Russo & Guy 2006). Sativex, a botanical extract standardized to contain a 50:50 mix of THC and CBD, has been approved for Phase III trials in the USA. The product is sprayed under the tongue, where it is absorbed into the bloodstream. Sativex is licensed in Europe and Canada for multiple sclerosis, neuropathic pain and cancer-related pain. Extension studies have shown that Sativex retains efficacy for at least 4 years, without drug tolerance or dose escalation, and with no evidence of dependency or abuse (Russo 2007).

Myofascial pain is a common reason why patients self-medicate with cannabis (Ware et al 2005). Cannabis is mostly illegal, although it is being reinstated as a controlled pharmaceutical drug by an increasing number of countries (in Europe, Canada and elsewhere, and in a dozen states in the USA). Suboptimal routes of administration continue to hamper its use. The oral administration of cannabis shares drawbacks with THC or nabilone capsules – they all suffer from erratic gut bioavailability and poor dose titration. THC taken by mouth is converted to an 11-hydroxy-THC metabolite with two to four times more psychoactivity, which is why people get whacked by 'pot brownies' (McPartland & Pruitt 1997).

Smoking cannabis is a health hazard due to polyaromatic hydrocarbons (PAHs) formed during combustion. Vaporization of cannabis provides an alternative to smoking, recently described and illustrated in the *New England Journal of Medicine* (Okie 2005). THC vaporizes at a temperature *below* the ignition point of combustible plant material, so few PAHs appear in the vapour.

THC and CBD may widen their own therapeutic windows by increasing AEA levels, and THC surprisingly upregulated CB_1 expression when administered acutely (reviewed in McPartland & Guy 2004). Low doses of THC (subtherapeutic doses) markedly potentiate pain relief imparted by endogenous cannabinoids (Suplita et al 2007). Chronic high doses of THC, however, cause downregulation and desensitization of cannabinoid receptors (they involute from the cell surface), resulting in the development of drug tolerance (Breivogel et al 2003). Tolerance develops at varying rates and magnitudes in different brain regions – for example, it occurs faster and greater in the hippocampus compared to the basal ganglia (Breivogel et al 2003). This may explain why frequent users of cannabis develop tolerance to its effects upon memory loss but not to its euphoric effects, which are believed to be mediated by the hippocampus and basal ganglia, respectively (D'Souza et al 2008).

Although controversial, the eCB system has been associated with psychotic disorders. Individuals with schizophrenia have elevated levels of AEA in their cerebrospinal fluid, but the elevated levels are negatively correlated with psychotic symptoms (Giuffrida et al 2004). This suggests that abnormal activation of postsynaptic D_2 receptors triggers release of AEA and retrograde signalling via CB_1, thus homeostatically attenuating dopamine release. High doses of THC may therefore provoke psychiatric illness in susceptible individuals, by downregulating and desensitizing CB_1 receptors and diminishing retrograde signalling (Giuffrida et al 2004). On the other hand, CBD shows promise as an antipsychotic agent (Zuardi et al 2006).

Conclusions

The eCB system is a key regulator of psychoneuroimmunological function. As fibromyalgia may represent an eCB deficiency syndrome, enhancing eCB function provides a new approach for treating fibromyalgia. The strategies described in this chapter should be added to existing regimens, because fibromyalgia is a complex condition that requires a multifactorial approach to management.

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