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Review

Endocannabinoid system and stress and anxiety responses

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Abstract

Cannabinoid agonists induce complex and often contradictory effects on anxiety in humans and experimental animals. The data from animal tests provide evidence of dose-dependent bidirectional modulation of anxiety by the cannabinoid system and the importance of environmental context. The mechanisms mediating the effects of cannabinoids on anxiety-related responses appear to involve CB_1 and non-CB_1 cannabinoid receptors. In addition, the CRH, $GABA_A$, cholecystokinin, opioid and serotonergic systems have also been implicated. Brain regions such as the amygdala, hippocampus and cortex, directly involved in the regulation of emotional behavior, contain high densities of CB_1 receptors. Mutant mice lacking CB_1 receptors show anxiogenic-like and depressive-like phenotypes in several tests, as well as profound alterations in their adrenocortical activity. Pharmacological blockade of CB_1 receptors induces anxiety in rats, and inhibition of anandamide metabolism produces anxiolytic-like effects. Thus, the endocannabinoid system appears to play a pivotal role in the regulation of emotional states and may constitute a novel pharmacological target for anti-anxiety therapy.

Keywords: Cannabinoids; Anxiety; Bimodal effects; Adrenocortical activity; $GABA_A$; Cholecystokinin; Opioid and Serotonergic systems

Contents

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1. Introduction

Anxiety can be regarded as a "normal" emotion and an adaptive component of the acute stress response under circumstances that threaten the integrity of the individual. However, if anxiety is disproportional in intensity or chronicity, or is not associated with any actual risk, it constitutes a maladaptive response or even a psychiatric disorder. A diversity of mechanisms appears to be involved in the regulation of anxious states, which may contribute to an appropriate emotional response to aversive events. In addition to the GABAergic, serotonergic and noradrenergic systems, many other neurotransmitters and modulators have been implicated. Accordingly, though benzodiazepines and agents acting on serotonergic system are currently the main drugs employed in the management of anxiety disorders, there is considerable scope for the development of alternative therapies (Sandford et al., 2000; Millan, 2003). In this context, there is an increasing interest in the endocannabinoid system as part of the complex circuitry that regulates anxiety. In reviewing the involvement of the endocannabinoid system in the control of anxiety-related responses, the present article focuses on the following points: effects of cannabinoid receptor agonists on anxiety-related responses in humans and experimental animals and the possible mechanisms underlying these effects, results obtained from both pharmacological and genetic strategies that highlight the role of the endogenous cannabinoid system in the regulation of anxiety, interactions between cannabis and other drugs of abuse in the control of anxious states, and the endocannabinoid system as a new pharmacological target for treating anxiety-related disorders.

2. Effects of cannabinoids on anxiety-related responses

The main feature of the recreational use of cannabis is that it produces a euphoriant effect. This "high" can be accompanied by decreased anxiety and increased sociability. However, cannabis can also produce dysphoric reactions, feelings of anxiety, panic, paranoia and psychosis (Hollister, 1986; Hall et al., 1994; Hall and Solowij, 1998; Ashton, 2001; Patton et al., 2002; Tournier et al., 2003; Dannon et al., 2004). It is possible that the reasons for this lie in bidirectional effects of cannabinoids on anxiety, with low doses having anxiolytic, and high doses anxiogenic effects, as well as in the previous history of the individual and the environmental context. The data from animal tests provide further evidence for the complexity of the scenario. Low doses of the cannabinoid receptor agonists, nabilone (Onaivi et al., 1990), CP 55,940 (Genn et al., 2003; Marco et al., 2004a) and Δ^9-tetrahydrocannabinol (THC) (Berrendero and Maldonado, 2002) induced anxiolytic-like effects in the elevated plus-maze and light–dark crossing tests. A low dose of CP 55,940 blocks successive negative contrast on post-shift day 2, an action that has been interpreted as reducing anxiety, and one that is similar to that of benzodiazepines (Genn et al., 2004b). However, in this test situation a high dose was without effect. In contrast, high doses of the cannabinoid agonist HU-210 produced anxiogenic-like responses in the defensive withdrawal test (Rodríguez de Fonseca et al., 1996) and enhanced emotional responding to tactile stimulation (Giuliani et al., 2000), whereas mid-high doses of CP 55,940 had anxiogenic-like effects in the plus-maze (Arévalo et al., 2001; Genn et al., 2003; Marín et al., 2003; Marco et al., 2004a) and in the social interaction test (Genn et al., 2004a) (Table 1). In this latter study, we found no evidence for an anxiolytic effect in the social interaction test following low doses of CP 55,940, and thus, in this situation, there did not seem to be bidirectional effects. It is perhaps not surprising that the effects in this test differ from those seen in the elevated plus-maze. There is evidence from factor analysis studies that the two tests measure different states of anxiety (File, 1992) and considerable evidence that they are mediated by different neurobiological pathways (File et al., 1996; González et al., 1996, 1998; Cheeta et al., 2000). This raises the interesting possibility that the cannabinoid system may be differently involved in different states of anxiety. A recent report indicates that exposure to chronic stress enhances the anxiety-like responsiveness to cannabinoids in rats (Hill and Gorzalka, 2004) (Table 1). A phenomenon that is also observed in humans.

Though most of the literature about acute effects of cannabinoids comes from adult animals, there are also some results obtained from juvenile (Romero et al., 2002) and infant (Borcel et al., 2004) rats. The data clearly showed that cannabinoids are able to induce at least three of their typical responses, i.e. antinociception (this effect was slight), reduction of motor activity and stimulation of adrenocortical activity, in immature rats. Cannabinoids may also affect the emotional state of immature animals. Thus, in rats of 12 days of age, CP 55,940 caused a dose-dependent anxiolytic-like reduction of the separation-induced ultrasonic vocalizations that was completely reversed by the CB_1 receptor selective antagonist SR 141716A (rimonabant) (McGregor et al., 1996a). In addition, neonatal (McGregor et al., 1996a) and juvenile (Romero et al., 2002) rats treated with high doses of CP 55,940 have been also reported to emit audible vocalizations when picked up by the experimenter, which might be interpreted as an aversive or anxiogenic-like response (Table 1).

The anxiogenic effects of abused drugs may constitute salient stimuli to which drug-associated negative symptoms are classically conditioned. For example, the phenomenon of test-specific conditioned anxiety to an anxiogenic dose of nicotine has been demonstrated in both the social interaction and the elevated plus-maze tests (File et al., 2002a; Tucci et al., 2002). Other drugs of abuse, such as cocaine (DeVries and Pert, 1998), produce a similar pattern of conditioned anxiety and this phenomenon has been closely linked to cocaine's abuse potential (Goeders, 1997, 2002). In a recent

Table 1
Effects of cannabinoids and FAAH inhibitors on anxiety related responses

Agonist	Specie	Doses	Apparatus	Effects	References
THC	Rats (SD)	1–10 mg/kg i.p.	PM	+	Onaivi et al., 1990
	Mice (ICR)	10–20 mg/kg i.p.		+	
	Mice (CD-1)	0.3 mg/kg i.p.	Light–dark box	–	Valjent et al., 2002
		5 mg/kg i.p.		+	
	Mice (CD-1)	0.3 mg/kg i.p.	Light–dark box	–	Berrendero and Maldonado, 2002
CP 55,940	Rats (W)	10–100 μg/kg i.p.	CCP	Place avoidance	McGregor et al., 1996b
	Rats (LE) 12PN	100–1000 μg/kg i.p.	UV test	Inhibition of vocalizations	McGregor et al., 1996a
		1000 μg/kg i.p.	Observation	Audible vocalizations	
	Rats (W)	75–125 μg/kg i.p.	PM	+	Arévalo et al., 2001
	Rats (W) 40 PN	0.6 mg/kg s.c.	Observation	Audible vocalizations	Romero et al., 2002
	Rats (W)	75 μg/kg i.p.	PM	+	Marín et al., 2003
	Rats (HL)	2.5–5 μg/kg i.p.	PM	–	Genn et al., 2003
		40 μg/kg i.p.		+	
	Rats (W)	1 μg/kg i.p.	PM	–	Marco et al., 2004a
		50 μg/kg i.p.		+	
	Rats (HL)	2.5–10 μg/kg i.p.	SI–HU	0	Genn et al., 2004a
		2.5–10 μg/kg i.p.	SI–HF	0	
		40 μg/kg i.p.		+	
		10–20 μg/kg i.p.	SI–LF	0	
		40 μg/kg i.p.		+	
HU210	Rats (W)	Novelty 4 μg/kg. i.p.	D–W test	–	Rodríguez de Fonseca et al., 1996
		Habituated 4–100 μg/kg i.p.		+	
	Rats (W)	25 μg/kg i.p. Observation: Enhanced responsiveness to tactile stimulation	X-maze	+	Giuliani et al., 2000
	Rats (LE)	Unstressed 10 μg/kg i.p.	PM	–	Hill and Gorzalka, 2004
		Unstressed 50 μg/kg i.p.		+	
		Stressed 10 and 50 μg/kg i.p.		+	
Nabilone	Mice (ICR)	10–100 μg/kg i.p.	PM	–	Onaivi et al., 1990
Cannabidiol (CBD)	Mice (ICR)	1–10 mg/kg i.p.	PM	–	Onaivi et al., 1990
	Rats (W)	2.5–10 mg/kg i.p.	PM	–	Guimarães et al., 1990
		20 mg/kg i.p.		0	
	Rats (W)	5 mg/kg i.p.	PM	–	Guimarães et al., 1994
CBD derivatives	Rats (W)	HU-219: 0.03–1 mg/kg i.p.	PM	–	Guimarães et al., 1994
		HU-252: 1 mg/kg i.p.		–	
		HU-261: 1 mg/kg i.p.		–	
FAAH inhibitors	Rats (W)	URB532: 1–10 mg/kg i.p.	Zero-maze	–	Kathuria et al., 2003
		URB587: 0.1 mg/kg i.p.	UV test	–	

Effects: + = anxiogenic effect; 0 = no effect; – = anxiolytic effect. Specie: Rats: HL = Hooded Lister; W = Wistar; SD = Sprague–Dawley; LE = Long–Evans. Apparatus: PM = plus-maze; CCP = conditioned place preference; UV test = ultrasonic vocalization test; SI = social interaction test, HU = high light unfamiliar, HF = high light familiar, LF = low light familiar; D–W test = defensive–withdrawal test.

study (Genn et al., 2004a) we investigated whether anxiety could be conditioned to an anxiogenic dose of CP 55,940 in the social interaction test. CP 55,940 at a dose of 40 μg/kg significantly decreased the time spent in social interaction, indicating an anxiogenic effect. In rats tested undrugged 24 h after testing with 40 μg/kg there was a significant anxiogenic effect, indicating conditioned anxiety. Further experiments will be needed to determine the mechanisms mediating the conditioned anxiogenic effect of CP 55,940, but corticotropin releasing hormone (CRH) may be a candidate since it has been shown to mediate conditioned anxiety to both nicotine and cocaine (DeVries and Pert, 1998; Tucci et al., 2003). In the same study mentioned above, we also found that a group of rats injected with 40 μg/kg immediately after the social interaction test showed an unexpected significant anxiolytic effect when tested undrugged 24 h later (Genn et al., 2004a). This finding was somewhat similar to those of Valjent and Maldonado (2000). They found a conditioned place aversion with 5 mg/kg THC and no effect with 1 mg/kg using a standard protocol. However, if mice received a priming dose of THC in their home cage, 24 h before starting the place preference conditioning procedure, they showed a place preference with 1 mg/kg THC and no effect of 5 mg/kg THC. Although the paradigms are very different, there are important analogies between the two experiments. Valjent and Maldonado (2000) avoided place aversion (5 mg/kg THC) and revealed place preference (1 mg/kg THC) by preventing

the association of the aversive reaction of the animals to the first exposure to the drug, with the apparatus. In our case, an anxiolytic effect of a relatively high dose of CP 55,940 was found 24 h after its administration, if the drug was administered after the first exposure to the apparatus, thus avoiding the association of the short-term anxiogenic effect of the drug with the apparatus, i.e. with the context. Therefore, it is not surprising that low doses of CP 55,940 have been associated with both conditioned place preference (20 μg/kg) (Braida et al., 2001) and conditioned place aversion (10 μg/kg) (McGregor et al., 1996b), as the timing between injection and pairing plays a large part in the expression of these affective differences (Lepore et al., 1995). Other data further highlight the importance of environmental factors in relation to the effects of cannabinoids on anxiety (see below).

3. Mechanisms underlying the effects of cannabinoids on anxiety-related responses

3.1. Biphasic effects of cannabinoids: proposed hypotheses

As indicated in the previous section, the effects of cannabinoid agonists on anxiety are biphasic, with low doses being anxiolytic and high doses anxiogenic. It is worth noting that, in addition to anxiety, there are other behavioral responses, such as motor activity and exploration (McGregor et al., 1996b; Chaperon and Thiebot, 1999; Marín et al., 2003; Genn et al., 2004a; Marco et al., 2004a) that are affected by cannabinoid agonists in a biphasic manner. In general, low doses are stimulatory whereas high doses are inhibitory. One possible explanation for these biphasic effects of cannabinoids in different neurobiological responses is that distinct receptors with a differential sensitivity to cannabinoids are implicated in the inhibitory/anxiogenic and the stimulatory/anxiolytic effects of these compounds. In this respect, it has been shown that two distinct cannabinoid sensitive presynaptic receptors regulate network activity in the hippocampus. Activation of CB_1 receptors (selectively expressed in a subset of GABAergic inhibitory interneurons), reduces GABA release from presynaptic terminals, thereby increasing the excitability of principal cells (i.e. pyramidal and dentate granule cells). Novel, non-CB_1 cannabinoid sensitive receptors are present on the hippocampal excitatory axon terminals which suppress glutamate release. The two receptors have different pharmacological features. WIN 55,212-2 is an order of magnitude less potent in reducing glutamatergic transmission than in inhibiting GABAergic postsynaptic currents, and the novel receptor binds vanilloid receptor ligands (Hàjos and Freund, 2002). Cannabinoids also modulate neuronal firing in the rat basolateral amygdala by both CB_1 and non-CB_1 receptors and the vanilloid antagonist capsazepine acted as an antagonist at this non-CB_1 receptors (Pistis et al., 2004). Given the important role of the hippocampus and the basolateral amygdala in the regulation of anxiety, it is conceivable that a novel cannabinoid–vanilloid sensitive non-CB_1 receptor is involved in the effects of cannabinoid agonists on anxiety. In support of this hypothesis, it has recently been suggested that there is a role for a novel cannabinoid receptor in the mediation of anxiety (Haller et al., 2002), since both wild type and CB_1 knockout mice show changes in anxiety in the plus-maze in response to the functional CB_1 receptor antagonist rimonabant. This antagonist binds to the putative novel cannabinoid-sensitive receptor (Haller et al., 2002), which constitutes a candidate for the mediation of preserved effects of cannabinoids in CB_1 knockout mice (Jarai et al., 1999; Zimmer et al., 1999; Di Marzo et al., 2000; Breivogel et al., 2001; Hàjos et al., 2001). This novel receptor may differ from CB_1 receptors in several pharmacological features, including the degree of sensitivity to cannabinoid agonists. Other possible explanation for the bimodal effects of cannabinoids is the implication of distinct neuroanatomical CB_1 receptors with a differential sensitivity to cannabinoids. Thus, the administration of WIN 55,212-2 resulted in a biphasic, dose-dependent effect on hippocampal acetylcholine (ACh): a low and a high dose of the compound induced a transient stimulation and a prolonged inhibition of hippocampal ACh efflux, respectively. These amphidromic responses appeared to involve the same structural entities (Gi-coupled CB_1 receptors) but different neuroanatomical sites. High-dose inhibitory effects were mediated locally in hippocampus whereas low-dose excitatory effects were mediated in septum. Moreover, the stimulatory and the inhibitory effect of the cannabinoid agonist involved dopamine D1 and D2 receptors activation, respectively (Tzavara et al., 2003). A third hypothesis which might account for the biphasic effects of cannabinoids is the possible differential implication of Gs and Gi proteins in the stimulatory and inhibitory effects, respectively (Sulcova et al., 1998). It would be interesting to test this hypothesis in vivo, in relation to anxiety-related effects.

3.2. Cannabinoids, adrenocortical activity and the corticotropin-releasing hormone (CRH) system

There is substantial evidence indicating that cannabinoid agonists induce a CB_1 cannabinoid receptor-mediated activation of the hypothalamus–pituitary–adrenal (HPA) axis in rodents (Weidenfeld et al., 1994; Wenger et al., 1997; Martín-Calderón et al., 1998; Manzanares et al., 1999a; Romero et al., 2002; Marín et al., 2003). The stimulatory effects of cannabimimetics on pituitary–adrenal responses appear to be centrally mediated, probably through an increased release of corticotropin-releasing hormone (CRH), and with the contribution of extrahypothalamic inputs from stress-responsive limbic nuclei (Martín-Calderón et al., 1998; Wenger et al., 1997). The effect appears to be dose-dependent since we have found that 75 μg/kg of CP 55,940 (Marín et al., 2003), but not lower doses of 50 or

1 μg/kg of the compound (Marco et al., 2004b), increased serum corticosterone levels in rats. Given the key role of corticotrophin-releasing hormone (CRH) and the hypothalamic–pituitary–adrenal axis (HPA) in the regulation of stress and anxiety-related responses (De Kloet, 2003; Muller et al., 2003), there may be a functional relationship between the effects of cannabinoids on anxiety and HPA axis. In fact, it has been proposed that the stimulating effect of cannabinoids on the HPA axis might contribute to unpleasant side effects (Chaperon and Thiebot, 1999). It has been shown that a CRH receptor antagonist attenuated the anxiogenic-like effects of the cannabinoid agonist HU-210 on defensive withdrawal behavior, indicating that the CRH system may participate in cannabinoid-induced anxiety (Rodríguez de Fonseca et al., 1996). High doses of CP 55,940 (75 μg/kg) induced both, anxiogenic-like effects in the plus-maze and stimulation of adrenocortical activity (Marín et al., 2003). However a dose of 50 μg/kg induced an anxiogenic-like effect in the same test, without increasing corticosterone concentrations (Marco et al., 2004b). Thus, at certain doses, the effects of cannabinoids on anxiety can be dissociated from their effects on adrenocortical activity.

An electrophysiological study by Di et al. (2003) has revealed a mechanism of rapid glucocorticoid feedback inhibition of hypothalamic hormone secretion via endocannabinoid release in the paraventricular nucleus. By this mechanism, endocannabinoids may be involved in the modulation of a number of peptidergic systems, including CRH. More recently, it has been confirmed that, in vivo, endocannabinoid signaling negatively modulates HPA axis function in a context-dependent manner (Patel et al., 2004).

3.3. $GABA_A$ system and cholecystokinin

The CB_1 receptors are expressed in high density in structures such as the amygdala, hippocampus, anterior cingulate cortex and prefrontal cortex (Herkenham et al., 1990, 1991; Glass et al., 1997; Katona et al., 2001; Hàjos and Freund, 2002; Tzavara et al., 2003; Pistis et al., 2004) which are key regions in the regulation of anxiety. With respect to cellular localization, CB_1 cannabinoid receptors can be found on the axon terminals of a subset of GABAergic inhibitory interneurons containing the neuropeptide cholecystokinin (CCK) (Katona et al., 1999; 2001; Marsicano and Lutz, 1999; Tsou et al., 1999). The CCK system, in particular CCK_B receptors, appears to play a role in the modulation of anxiety (Rotzinger and Vaccarino, 2003) and cannabinoid agonists have been shown to affect the release of CCK (Beinfeld and Connolly, 2001). Therefore, it is possible that interaction between endogenous cannabinoids and CCK are implicated in the control of anxious states.

The benzodiazepine/$GABA_A$ receptor may be involved in both the anxiogenic and the anxiolytic reactions to cannabinoid agonists. Flumazenil has been shown to antagonize both the anxiogenic-like effect of THC and the anxiolytic-like actions of nabilone and cannabidiol in the plus-maze (Onaivi et al., 1990).

3.4. Opioids

There is evidence indicating the existence of functional interactions between the cannabinoid and the opioid systems in the modulation of analgesic responses (Manzanares et al., 1999b; Welch and Eads, 1999; Valverde et al., 2000), in addiction-related processes (Manzanares et al., 1999b; Valverde et al., 2001; Zimmer et al., 2001; Ghozland et al., 2002), and in the induction of antidepressant-like effects (Valverde et al., 2001) in rodents. Cannabinoid receptor agonists enhance the release of endogenous opioids, which might account for these functional interactions (Pugh et al., 1997; Manzanares et al., 1999b; Houser et al., 2000; Valverde et al., 2001). More recently, Berrendero and Maldonado (2002) studied the possible involvement of the opioid system in the anxiolytic-like effects of cannabinoids in mice. They showed that the μ-opioid receptor antagonist β-funaltrexamine and the δ-opioid receptor antagonist naltrindole, but not the κ-opioid receptor antagonist nor-binaltorphimine abolished THC anxiolytic-like effects in the light-dark box. Thus, μ- and δ-opioid receptors appear to be involved in the anxiolytic-like effect of Δ^9-tetrahydrocannabinol (THC). Data obtained from various anxiety tests as well as from place preference/aversion paradigms suggest that the activation of the κ-receptor system induces anxiogenic/proaversive effects (De Rosset and Holtzman, 1985; Nobre et al., 2000; Sante et al., 2000), and the cannabinoid agonist CP 55,940 enhances the release of dynorphins (endogenous opioid ligands acting at κ-receptors) (Pugh et al., 1997; Houser et al., 2000). On the basis of these data we expected that the anxiogenic-like effect of CP 55,940 could be mediated by the κ-opioid receptor. In accordance with our hypothesis we demonstrated that, in rats, the anxiogenic-like effect of CP 55,940 in the plus-maze was antagonized by the κ-opioid receptor antagonist nor-binaltorphimine, but not by either a μ- (cyprodime) or a δ- (naltrindole) receptor antagonist (Marín et al., 2003). The κ-opioid system appears to be involved in other effects of cannabinoid receptor agonists. CP 55,940 induces the release of dynorphins (Pugh et al., 1997; Houser et al., 2000) which through activation of κ-opioid receptors participate in the antinociceptive effect of the cannabinoid receptor agonist. Other data obtained from dynorphin-deficient mice (Zimmer et al., 2001) and κ-deficient mice (Ghozland et al., 2002) have revealed that the absence of either dynorphin or κ-receptors suppressed the negative motivational effects of THC in place conditioning paradigms. Moreover, nor-binaltorphimine also blocked the establishment of THC-induced conditioned place aversion (Zimmer et al., 2001). Concordant with these studies, our results indicated that an activation of the dynorphin-κ receptor system likely participates in the anxiogenic-like effect of CP 55,940. Our results also indicated that other

behavioral effects of CP 55,940 such as decreased motor activity and exploration and the stimulating effect of the drug on adrenocortical activity were independent of the opioid receptors (Marín et al., 2003). Thus, our results supported the view that the effects of cannabinoids on motor activity, exploration, anxiety and adrenocortical activity are mediated by distinct mechanisms. Moreover, taken as a whole, the results by Berrendero and Maldonado (2002) and Marín et al. (2003) show that the anxiogenic-like and the anxiolytic-like effects of cannabinoids appear to be mediated by distinct mechanisms.

3.5. Serotonergic system

There is abundant evidence from both, pharmacological (Griebel, 1995; Griebel et al., 1997, 2000) and genetic (Holmes, 2001) studies for a role of the serotonergic system in the regulation of anxiety. The nature of this physiological role of serotonin (5-HT) is complex and different mechanisms, mediated by different receptor subtypes, appear to be involved in the genesis of anxiety (Griebel, 1995). The 5-HT_{1A} receptors located in serotonergic pathways projecting from midbrain raphe nuclei to limbic areas have received special attention. Studies of the mechanisms underlying the anxiolytic properties of 5-HT_{1A} receptor agonists tend to favor a presynaptic action, at least in some models, although an involvement of postsynaptic mechanisms cannot be ruled out (File et al., 1996; Barnes and Sharp, 1999; Sakaue et al., 2003). Although there is relatively scarce information about interactions between the serotonergic and endocannabinoid systems, some data support certain relationships. There are data indicating that cannabinoid receptor agonists inhibit 5-HT_3 receptor mediated currents and other results suggest that endocannabinoids may act by 5-HT_2 receptor blockade (Fride and Shohami, 2002). It has been shown also that the somatodendritic 5-HT_{1A} receptors are implicated in the hypothermic effect of cannabinoids (Malone and Taylor, 2001). On the basis of the above evidence, we hypothesized that 5-HT_{1A} receptors might be involved in the anxiety-related responses induced by the cannabinoids. In order to address this hypothesis, we studied the possible interaction between CP 55,940 and the 5-HT_{1A} receptor antagonist WAY 100635 (WAY) in the modulation of plus-maze and holeboard activity in rats. Our data indicated that WAY antagonized the reducing effect of a mid-high dose of the cannabinoid agonist on exploratory activity and attenuated its anxiogenic effect. However, the 5-HT_{1A} antagonist did not antagonize either the anxiolytic or the increasing effect on exploration of a low dose of the cannabinoid agonist. The reduction of directed exploration in the holeboard and the anxiogenic effect of CP 55,940 were not reversed by the selective CB_1 receptor antagonist rimonabant (Arévalo et al., 2001; Romero et al., 2002). However, the reducing effect of a high dose of CP 55,940 on exploratory activity (likely related to the increased anxiety) is mediated via 5-HT_{1A} receptors. The data also indicated that there may be an endogenous serotonergic tone, mediated by 5-HT_{1A} receptors, which participates in the regulation of grooming behavior, and that this latter effect can be modulated by CP 55,940 (Marco et al., 2004a). Since grooming behavior is a parameter indicative of an increased stress level, the interaction between the serotonergic and the cannabinoid system in the modulation of this behavior deserves further investigation.

As indicated above, cannabinoids are potent activators of the HPA axis. In turn, serotonin, possibly through the activation of 5-HT_{1A} receptors (Vicentic et al., 1998), also exerts a stimulatory influence on the HPA axis function (Fuller, 1992). Doses of the cannabinoid agonist CP 55,940 that induce anxiogenic-like effects and increase serum corticosterone concentrations in the rat (Marín et al., 2003), also modify brain levels of serotonin (Arévalo et al., 2001). Thus, it seems likely that the serotonergic system is involved in the effects of cannabinoids on adrenocortical activity. We have recently shown that, in animals pre-exposed to the plus-maze, the combined treatment of CP 55,940 (at a dose that did not modify corticosterone levels) and the 5-HT_{1A} antagonist WAY 100635 (which when administered alone induced a modest decrease in corticosterone concentration) induced a significant increase of corticosterone responses. These results may indicate that there is a functional relationship between cannabinoid CB_1 and 5-HT_{1A} receptors in the modulation of adrenocortical activity (Marco et al., 2004b). Since in this study the animals were subjected to behavioral testing before they were sacrificed, it is likely that under this state of stress both, the HPA axis and the 5-HT_{1A} receptors which participate in its control, were activated. We are currently investigating the possible interactions between the cannabinoid and the serotonergic systems in the control of baseline corticosterone levels.

4. Role of the endocannabinoid system in the regulation of anxiety

The development of knockout mice deficient in CB_1 receptors has provided an excellent tool to evaluate the physiological roles of the endocannabinoid system, and in particular its possible implication in the regulation of anxiety (Table 2). The CB_1 knockout mice showed an increase in the aggressive response measured in the resident–intruder test and an anxiogenic-like response in the light–dark box. Even more, the mutant mice showed a higher sensitivity to exhibit depressive-like responses in the chronic unpredictable mild stress procedure, which suggests an increased susceptibility to develop an anhedonic state (Martín et al., 2002). These results clearly indicate that endogenous cannabinoids, through the activation of CB_1 receptors are implicated in the control of emotional behavior. The CB_1 knockout mice also showed an anxiogenic-like behavior in the plus-maze test and in the social

Table 2
Behavioral features of CB_1 knockout mice in relation to anxiety

Reference	Genetic background	Behavioral paradigm	Phenotype
Marsicano et al., 2002	C57 mice	Plus-maze (PM)	Normal anxiety levels
Martín et al., 2002	CD-1 mice	Light–dark box	Increased anxiety
		Resident–intruder test	Increased aggressive responses
Haller et al., 2002	CD-1 mice	Plus-maze (PM)	Anxiogenic-like phenotype
Maccarrone et al., 2002	CD-1 mice	Light–dark box	
	Young=1-month-old		Mild anxiety-related behavior
	Old=4-month-old		Normal anxiety levels
Urigüen et al., 2004	CD-1 mice	Light–dark box	High anxiety-like behavior
		Plus-maze (PM)	Hypersensitivity to stress
		Social interaction test	Impaired actions of anxiolytic drugs
Haller et al., 2004	CD-1 mice	PM low light (0.5 lx)	No differences WT vs. KO
		PM high light (200 lx)	Increased anxiety
		Social interaction test (novel cage/unfamiliar)	Increased anxiety
		Resident–intruder test (home cage)	Aggressive behavior

interaction test (Haller et al., 2002; Urigüen et al., 2004), pronounced alterations in the HPA axis (reduced basal corticosterone secretion and POMC gene expression in the anterior pituitary gland and hypersensitivity to restraint stress) and impaired action of anxiolytic drugs such as bromazepam and buspirone (Urigüen et al., 2004). This latter result suggests that functional integrity of cannabinoid CB_1 receptors is necessary to achieve a complete efficacy of anxiolytic drugs, which may have consequences in the treatment of mood-related disorders, including those derived from cannabinoid abuse. Furthermore, the impaired action of buspirone (which exerts its anxiolytic action by acting as a partial agonist at the $5\text{-}HT_{1A}$ receptor) in mutant mice further supports the possible interaction between the cannabinoid and the serotonergic systems in the modulation of anxiety (Marco et al., 2004a). In contrast to some of the results indicated above, Marsicano et al. (2002) did not find an anxiogenic-like response in the plus-maze in their CB_1 knockout mice. These apparent discrepancies might be explained, at least partially, by differences in environmental factors. Recently, it has been shown that CB_1 knockout mice only show an anxiogenic-like phenotype under conditions of high stress: light in the plus-maze and unfamiliar environment in the social interaction test (Haller et al., 2004). On the other hand, the CB_1-deficient mice from Marsicano et al. (2000) showed strongly impaired short-term and long-term extinction in auditory fear-conditioning test, with unaffected memory acquisition and consolidation. Thus, the endogenous cannabinoid system may have a central function in extinction of aversive memories and could represent a useful pharmacological treatment of pathologies associated with inappropriate retention of aversive memories, such as post-traumatic stress disorder (Marsicano et al., 2002).

Evidence for an endogenous anxiolytic cannabinoid tone also comes from the intrinsic effects of the CB_1 receptor antagonist rimonabant. This drug has anxiogenic effects in the defensive withdrawal and elevated plus-maze tests in adult rats (Navarro et al., 1997; Arévalo et al., 2001) (Table 3). As indicated in a previous section, CP 55,940 reduced ultrasonic vocalization in rat pups separated from their mother, indicating an anxiolytic effect. The CB_1 receptor antagonist rimonabant not only reversed this effect, but also enhanced pup ultrasonic vocalizations when administered alone (McGregor et al., 1996a). These results further support the view that there is an endogenous regulation of emotional states by cannabinoid systems, which could be present since early developmental stages. As for CB_1 knockout animals, certain results obtained in mice with

Table 3
Effects of rimonabant on anxiety-related responses

Specie	Apparatus	Dose	Effect	Reference
Rats (LE) 12 PN day	Ultrasonic Vocalization test	20 mg/kg i.p.	Anxiogenic-like	McGregor et al., 1996a
Rats (W)	Defensive–withdrawal test	3 mg/kg i.p.	Anxiogenic-like	Navarro et al., 1997
	Plus-maze			
Rats (W)	Plus-maze	3 mg/kg i.p.	Anxiogenic-like	Arévalo et al., 2001
CD-1 mice (WT and KO CB_1 mice)	Plus-maze	3 mg/kg i.p.	Anxiolytic-like	Haller et al., 2002
Swiss–Webster mice	Plus-maze trial 1	1 mg/kg i.p.	No effects	Rodgers et al., 2003
	Plus-maze trial 2		Anxiolytic-like	

rimonabant appear to be contradictory since this compound was shown to be anxiolytic in the plus-maze (Haller et al., 2002). These data may reflect species differences, however it is likely that the environmental context and baseline anxiety may account for at least some of the different results. The context dependency is indirectly supported by the "one-trial sensitization" phenomenon described by Rodgers et al. (2003) in the plus-maze. In these experiments rimonabant had no behavioral effects in maze-naive animals, but induced an anxiolytic-like effect in the second trial of the test. Interestingly, benzodiazepines have been shown to lose efficacy in maze-experienced animals ("one-trial tolerance") (File et al., 1990). These results further suggest that cannabinoids and benzodiazepines may modulate distinct types of anxiety.

As indicated in other parts of this issue, the enzyme fatty acid amide hydrolase (FAAH) catalyzes the hydrolysis of the endogenous cannabinoid anandamide. Pharmacological blockade of this enzyme by URB597 and URB532 produces anxiolytic-like effects in the elevated zero-maze in adult rats and in the isolation-induced ultrasonic emission test in rat pups (Table 1). These effects were accompanied by augmented brain levels of anandamide and were prevented by CB_1 receptor blockade. Moreover, the anxiolytic actions of the FAAH inhibitor URB597 were not accompanied by other actions which are signs of cannabinoid intoxication in rodents such as catalepsy or hypothermia. These results indicate that anandamide participates in the modulation of emotional states and point to FAAH inhibition as an innovative approach to anti-anxiety therapy (Kathuria et al., 2003).

The distribution of CB_1 receptors in rat brain is also consistent with an involvement of this system in the regulation of emotional reactivity, with high levels of CB_1 expression in structures such as the amygdala, hippocampus, anterior cingulate cortex and prefrontal cortex (Herkenham et al., 1990, 1991; Glass et al., 1997; Katona et al., 2001; Hàjos and Freund, 2002; Tzavara et al., 2003; Pistis et al., 2004). The CB_1 agonist CP 55,940 increased Fos immunoreactivity in brain structures known to be involved in anxiety and fear-related responses such as the central nucleus of the amygdala, the periaqueductal grey and the paraventricular nucleus of the hypothalamus (Arnold et al., 2001).

On the basis of the above mentioned results, a model has been proposed to explain the possible mechanism by which the system anandamide-central cannabinoid receptor(s) may participate in the control of anxious states. Endocannabinoid substances could be generated in the amygdala during anxiety and might regulate emotional states by influencing amygdala outputs (Gaetani et al., 2003). This view is supported also by the fact that anandamide content in the mouse basolateral amygdala rises when the animal is conditioned to expect a foot shock after hearing a tone (Marsicano et al., 2002). Thus, the endocannabinoid system, and anandamide in particular, might be activated in response to anxiogenic situations and this activation could be part of a negative feedback system that limits anxiety (Gaetani et al., 2003).

5. Interaction between cannabinoids and other drugs of abuse in relation to anxiety

Functional interactions between endogenous systems mediating the effects of drugs of abuse are of special interest in the context of polydrug abuse. Thus, it is conceivable that human addicts use mixtures of drugs either to augment the sensation of pleasure or to reduce the withdrawal and other aversive effects of a given substance. For instance, the drug most commonly taken with MDMA in human users is cannabis. More than 90% of ecstasy users take cannabis regularly (Siliquini et al., 2001; Topp et al., 1999). Cannabis is frequently used to mitigate the MDMA "comedown" (Winstock et al., 2001), but is also commonly used before and during dance parties at which MDMA is taken (Boys et al., 1997). In a recent paper it has been shown that co-administered cannabinoids may attenuate the 5-HT depletion and some of the anxiety-related behaviors that are observed weeks after MDMA administration in rats (Morley et al., 2004).

The endocannabinoid transmission appears to be a component of the brain reward system and to play a role in dependence/withdrawal to different habit-forming drugs (Arnone et al., 1997; Comings et al., 1997; Ledent et al., 1999; Manzanares et al., 1999b; Mascia et al., 1999; Hungund and Basavarajappa, 2000; Yamamoto and Takada, 2000). It has been shown that chronic exposure to nicotine, ethanol or cocaine induce diverse region-specific changes in endocannabinoid contents (arachidonoyethanolamide, AEA and 2-arachidonoyl-glycerol, 2-AG) (González et al., 2002). Since, as discussed above, there may be a tonic activity of the endocannabinoid system in the regulation of anxiety, it is likely that changes in the levels of endocannabinoids may mediate the anxiety-related symptoms of the withdrawal syndrome corresponding to different drugs of abuse.

The simultaneous use of nicotine and cannabis is very frequent, particularly among adolescents. In addition to the already reviewed effects of cannabinoids on anxiety, nicotine has also been shown to induce diverse sex-dependent effects on anxiety in both, adolescent humans (File et al., 2001, 2002b) and experimental animals (Cheeta et al., 2001). Thus, the interaction between these drugs in relation to anxious/aversive states is of special interest. Mice co-treated with nicotine and THC showed attenuation of THC tolerance and an enhancement in the somatic expression of antagonist-precipitated THC withdrawal (Valjent et al., 2002). The rewarding effect of nicotine appeared to be absent in CB_1 knockout mice, whereas no change in the severity of nicotine withdrawal was observed in these mice (Castañé et al., 2002). Administration of

rimonabant decreased nicotine self-administration in rats (Cohen et al., 2002), which supports a possible utility of the CB_1 receptor antagonist as an aid for smoking cessation.

The possible functional interaction between cannabis and other drugs of abuse in the modulation of anxiety in young animals of both genders deserves further investigation.

6. The endocannabinoid system as a potential pharmacological target for the treatment of anxiety disorders

Marijuana and its derivatives have been used with medicinal purposes for many centuries and during the last years there has been a renewed interest in their possible therapeutic uses. A number of animal studies and clinical trials indicate that cannabinoids may have clinical application in emesis, loss of appetite and nausea associated with AIDS and cancer chemotherapy, neurodegeneration and brain trauma, tumors, spasticity associated with multiple sclerosis, and neuropathic pain (Galve-Roperh et al., 2000; Williamson and Evans, 2000; Porter and Felder, 2001; Croxford, 2003; Massi et al., 2004). The experimental results presented in this review suggest that beyond these applications, cannabinoids and related compounds may also have a therapeutic potential in anxiety-related disorders. The endogenous cannabinoid system could represent a therapeutic target for the treatment of diseases associated with inappropriate retention of aversive memories such as post-traumatic stress disorders (Marsicano et al., 2002; Haller et al., 2004). Perhaps more importantly, it is conceivable that potential patients treated with cannabinoids or related compounds for multiple sclerosis or chronic neuropathic pain might benefit also from the anxiolytic effects of the drug (Robson, 2001). Inhibitors of either metabolism or transport of endocannabinoids could be a more physiological and safer (in terms of side effects) approach than exogenous agonists. Another promising compound is cannabidiol, a major non-psychotropic constituent of Cannabis (Howlett et al., 2002). This compound has been shown to reduce anxiety in experimental animals and humans and to reduces anxiety induced by THC (Zuardi et al., 1982; Guimarães et al., 1990, 1994; Williamson and Evans, 2000; Crippa et al., 2004). The use of such nonpsychoactive compound may allow dissociation of unwanted psychoactive effects from potential therapeutic effects (Croxford, 2003).

7. Concluding remarks

During the last few years, the increasing interest in the relationships between cannabinoids and anxiety has led to a number of interesting data derived from animal studies. These results may contribute to understand the underlying mechanisms of complex effects of cannabinoids in humans and certain associations between cannabis abuse and mental disorders.

The use of transgenic mice lacking CB_1 receptors and inhibitors of endocannabinoids metabolism has allowed to suggest the existence of an intrinsic endocannabinoid tone which contributes to the regulation of emotion and anxiety. Activation of endocannabinoid signaling during stress may attenuate anxiety and CRH release. Thus, the endocannabinoid system might constitute an interesting pharmacological target for the development of anti-anxiety drugs.

The effects of combined treatments with cannabinoids and other drugs of abuse on anxiety-related responses may provide new insights for a better understanding of the polydrug-abuse phenomenon.

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