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Some Effects of CB1 Antagonists with Inverse Agonist and Neutral Biochemical Properties,^{a,b}

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Abstract

The CB1 inverse agonist/antagonist SR141716A recently has been introduced for the management of obesity (rimonabant; Acomplia) and appears to have beneficial effects. However, its utility may be hampered in some individuals by adverse effects including nausea or emesis or by mood depression. The recent development of biochemically ‘neutral’ antagonists such as AM4113 (Sink et al. 2007) has allowed an initial evaluation of the proposition that the adverse effects of SR141716A are associated with its inverse agonist activity. Thus far, data comparing SR141716A and AM4113 across several species indicate that both drugs produce dose-related direct effects on operant behavior within the same range of doses that serve to antagonize the behavioral and hypothermic effects of a CB1 agonist. However, initial observations suggest that AM4113 may not produce preclinical indications of nausea or emesis. Further studies with AM4113 and other novel CB1 antagonists differing in efficacy should amplify our understanding of the relationship between the pharmacological activity of CB1 antagonists and their behavioral effects.

Keywords

SR141716A; rimonabant; AM4113; AM4054; inverse agonism; neutral antagonism; behavior; hypothermia

A rapid succession of events in the first half of the 1990s including the discovery of the cannabinoid-1 (CB1) receptor and the isolation and synthesis of its endogenous ligands anandamide and 2-AG, energized the explosion of scientific interest in cannabinoid pharmacology and the development of novel ligands, including those that produced Δ^9 THC-like effects and those could counter, i.e., antagonize, the effects of Δ^9 THC and other CB1 agonists at the CB1 receptor. It is interesting that, notwithstanding the acknowledged medicinal value of cannabis products with CB1 agonist actions, the first major therapeutic agent to emerge from these research efforts has been the CB1 antagonist/inverse agonist SR141716A (rimonabant; [1]). Perhaps it is not surprising that its initial therapeutic targets have been based

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on actions that are directly opposite to those of cannabis products and synthetic CB1 agonists, for example, the enhancement of appetite and food consumption.

SR141716A

Although SR141716A can be classified as a CB1 antagonist, its inverse agonist actions are well documented. Thus, its biochemical or behavioral effects generally are opposite in direction to effects produced by Δ^9 THC or other CB-1 agonists and can be antagonized by prior treatment with CB-1 agonists [2]. Biochemically, SR141716A can inhibit mitogen-activated protein kinase activity, adenylyl cyclase activity, and GTP γ S binding in selected brain regions [3,4]. Behaviorally, relatively low doses of SR141716A (0.1 mg/kg i.v. or 1–3 mg/kg by other injection routes) increase nociceptive responsivity, decrease food intake and body weight, disrupt operant behavior, and produce observable behavioral responses that suggest its effects may be noxious [5,6,7,8,9]. For example, SR141716A has been shown to enhance the taste aversion produced by lithium chloride, often considered an indicator of noxious effects. Notably, such findings of enhanced taste aversion are consistent with the production of conditioned gaping, an indicator of nausea and food-related malaise, that has been reported for other CB1 inverse agonists (see below; [10,11])

Clinically, SR141716A has been developed for weight reduction and as a pharmacological aid for smoking cessation. Confirming its value for the treatment of weight reduction, SR141716A appears to produce relatively large and sustained reductions in measurements of obesity in man [12,13]. Although clearly a CB1-related effect, the precise mechanism by which SR141716A reduces eating and weight gain remains unknown, but may involve actions on metabolic processes as well as appetite [14]. From the perspective of drug development and the potential clinical applications of CB1 ligands, it is important to realize that although such effects clearly are desirable in treating obesity, decreases in eating behavior may be disadvantageous for other proposed clinical uses of SR141716A or other CB1 antagonists, e.g. as a treatment for smoking cessation or to combat cannabis addiction and dependence.

Currently, SR141716A (rimonabant)—in conjunction with exercise and diet—has been approved in Europe for the management of obesity, but not yet as an aid for smoking cessation. Despite some delays in receiving approval in the United States, the therapeutic potential of rimonabant has generated considerable excitement. Complementing its reported success in reducing body weight and fat mass, rimonabant also has been in trials for the prevention of diabetes, the treatment of dyslipidaemia, the prevention of atherosclerosis, and the prevention of coronary heart disease. However, in the midst of such evaluations of the potential range of rimonabant's clinical applications, it must be remembered that this CB1 antagonist also can produce untoward effects. In this regard, subjects receiving rimonabant in clinical trials have reported adverse events (e.g., dizziness, diarrhea, nausea, vomiting) and discontinued treatment more often than those given placebo [13,15]. Presumably, the untoward effects of rimonabant in man parallel some of its direct effects in preclinical studies, e.g., on measures of taste aversion in rats (see above), and are mediated by comparable pharmacological actions at the CB1 receptor. In addition to effects on feeding-related endpoints, rimonabant also has been reported to increase reports of depression and anxiety in man. Although these latter effects remain to be firmly verified, it is noteworthy that the European Medicines Agency is recommending that rimonabant not be used in patients with major depression or in patients who would be concurrently taking antidepressant drugs [1]. In conjunction, the several adverse or potentially adverse effects of rimonabant suggest that the identification of CB1 antagonists with a reduced side-effect profile is a therapeutically relevant goal in further CB1 drug discovery and development.

Inverse vs. neutral antagonism

Among different approaches to the development of novel CB1 antagonists, the strategy of eliminating inverse agonist activity, that is, of developing 'neutral' antagonists is especially appealing. Inverse agonist activity generally is attributed to binding to sites coupled to ion channels or constitutively active G-proteins in a manner that results in effects qualitatively opposite to those of receptor agonists. For example, the β -carboline β -CCE or the benzodiazepine sarmazenil have, respectively, full and partial inverse agonist activity at benzodiazepine recognition sites on the GABA_A complex, and induce physiological and behavioral effects opposite to those associated with classical benzodiazepines such as diazepam or midazolam [16,17,18]. In contrast to inverse agonists, a 'neutral' antagonist can be envisioned as a drug that binds to a receptor or recognition site without triggering changes in cellular signaling. Continuing the above example, flumazenil is well established as a relatively 'neutral' antagonist at benzodiazepine recognition sites, although there is increasing recognition that its characterization may vary in a context-dependent manner (e.g., [19]).

It is important to recognize that the biochemical and behavioral effects of a ligand can vary with different levels of constitutive activity within the cellular milieu. This recently has been nicely illustrated in studies with opioid antagonists. For example, the mu-opioid receptor antagonist naltrexone is a relatively 'neutral', i.e., silent, antagonist under many circumstances but has pronounced effects in a direction opposite to those of mu opioid agonists in morphine-treated tissue or subjects. Presumably, this results from 'inverse' agonist actions that emerge as constitutive activity in the morphine-treated condition changes. Interestingly, recent reports have suggested that other mu opioid antagonists, e.g. 6 β -naltrexol and -naloxol, and 6 β -naltrexamine, retain neutral antagonist activity, even in morphine-treated tissue [20].

The preceding discussion is of relevance to the clinical utility of CB1 antagonists with inverse agonist activity, including SR141716A or other currently available antagonists such as AM251, CP-272871, or Ave1625 [21,22,9,3,23]. As described above for SR141716A, antagonist/inverse agonists that have been thoroughly investigated in laboratory and/or clinical studies appear to produce direct physiological and behavioral effects that may limit their therapeutic application. While these may be inescapable attributes of antagonism at the CB1 receptor, it also is conceivable that some or all of such untoward effects may be functional consequences of their inverse agonist activity, i.e., a suppression of basal signaling levels. This is a potentially attractive concept; yet, there currently is very little information to either support or challenge this proposition. Previous studies of allosteric regulation of the CB1 receptor showed that allosteric regulators could be used to manipulate the affinity of CB1 agonists but not of SR141716A, suggesting that it functioned as a neutral antagonist in such binding assays [24]. It also has been reported that the magnitude of inverse agonist action that is produced by antagonist concentrations of SR141716A on forskolin-stimulated accumulation of cAMP is moderate, raising the possibility that SR141716A may serve as a partial inverse agonist in this assay. However, such discussion of the contribution of inverse agonist actions and their behavioral consequences are purely speculative in the absence of data documenting the effects of more efficacious CB1 inverse agonists and of CB1 neutral antagonists. Thus, the behavioral significance of inverse agonist activity at the CB1 receptor in biochemical studies remains uncertain.

AM4113

The recent development of the novel CB1 'neutral' antagonist AM 4113 (Sink et al. 2007) is an important step in directly exploring the above questions regarding the behavioral relevance of differences in efficacy among CB1 antagonists. In side-by-side comparisons, AM4113 appears to be approximately 10-fold more potent than SR141716A (<1nM vs. 10 nM), and has

comparable 10:1 selectivity for binding CB1 rather than CB2 receptors. Data from studies of forskolin-stimulated cAMP production reveal a dose-related increase in cAMP production in the presence of SR141716A but not AM4113. Thus, SR141716A-induced increases begin at a concentration of approximately 300 nM and plateau at about 140% of control at concentrations ranging up to 10 μ M. In contrast, concentrations up to 10 μ M AM4113 have no effect on cAMP production. These data provide strong evidence that the effects of AM4113 on receptor-mediated cAMP accumulation differ qualitatively from those of both CB1 agonists which decrease cAMP accumulation and CB1 inverse agonists which, like SR141716A, enhance forskolin-stimulated cAMP accumulation. This profile of action is consistent with that of a neutral antagonist, at least with regard to the effects of CB1 ligands on adenylate cyclase-mediated signalling processes.

Initial reports of the behavioral effects of AM4113 have revealed both similarities and differences between its effects and those of antagonists with inverse agonist properties such as SR141716A or AM251. For example, Sink et al. (2007) show that doses of AM4113 that block motoric and antinociceptive effects of the CB1 full agonist AM411 also directly suppress food-maintained operant behavior and reduce food intake across a range of palatability. These effects are comparable to those of AM251 or SR141716A [9], which suggests that such effects are not exclusively associated with inverse agonist activity at the CB1 receptor. On the other hand, doses of AM 4113, which reduced food-related behavior, did not induce the previously-discussed behavior of 'conditioned gaping' in rats (Sink et al. 2007). These data contrast dramatically with the effects of behaviorally active doses of inverse agonists including SR141716A and AM251. These drugs either enhance the effects of lithium chloride in a taste aversion assay or produce 'conditioned gaping' in rats ([11], Sink et al. 2007). To the extent that taste aversion or 'conditioned gaping' are markers for nausea or malaise in rats, these findings raise the possibility that nausea, among the most common adverse events in man reported with rimonabant [13,15] may be less likely with neutral antagonists such as AM4113. Preliminary observations by Chambers et al. [25] indicating that AM4113—unlike SR141716A—does not induce vomiting in ferrets, further support this possibility. Certainly, further studies comparing rimonabant with a neutral antagonist like AM4113, either in nonhuman primates or directly in man, are needed to corroborate these initial experiments. Nevertheless these initial observations with AM4113 are very encouraging, and additional findings consistent with those already reported in rats and ferrets would confirm a significant advance in our understanding of the relationship between the nauseating and/or emetic effects of CB1 antagonists and their neutral or inverse agonist activity.

Other studies to further compare the direct and antagonist actions of SR141716A with the novel CB1 neutral antagonist AM 4113 are ongoing in rats and monkeys. First, the anti-hypothermic effects of both drugs are being evaluated in rats using colonic probes in six-hr experimental sessions. In these subjects, i.p. administration of the CB1 full agonist AM 4054 produces a time-dependent and dose-dependent decrease in colonic temperature. For example, 0.1 mg/kg AM4054 decreases colonic temperature by approximately 1.2 degrees after 60 minutes and approximately 3 degrees after 3 hrs. Pretreatment with s.c. SR141716A serves to antagonize the CB1-mediated hypothermia in a dose-dependent manner, with a near-complete blockade of the effect following 1.0 mg/kg of SR 141716A. Moreover, administration of 1.0 mg/kg of SR141716A, when given at the time of peak effect of AM4054, appears to fully reverse the ongoing hypothermia produced by the CB1 agonist. Comparison studies indicate that the novel neutral antagonist AM 4113 (1.0 mg/kg, i.p.) also fully antagonizes the hypothermic effects of AM4054, as evident both in the blockade of effects by pretreatment and the reversal of ongoing hypothermic effects. Of interest, neither the antagonist dose of SR141716A or of AM4113 produce appreciable increases in colonic temperature when administered alone. These data suggest that the two types of CB1 antagonists, neutral and inverse agonist, modify CB1-

mediated hypothermia in a qualitatively comparable manner and that there is little evidence of inverse agonist activity of SR141716A using hyperthermia as an endpoint.

The CB1 antagonist effects of AM4113 also are being studied in monkeys, using both behavioral and hypothermia endpoints. In behavioral studies, subjects are seated in customized chairs and press a lever for milk delivery under a 30-response fixed-ratio schedule. Under this schedule, every 30th leverpress triggers the delivery of 0.3 ml of milk into a receptacle that is accessible to the monkey. In these subjects, the effects of SR141716A and AM4113 are being studied by determining the direct effects of each drug on leverpressing response rates and, also, how pretreatment with each drug modifies the response rate-decreasing effects of 1.0 mg/kg of the CB1 agonist AM411. Thus far, results show that both SR141716A and AM4113 can have direct effects on reinforced behavior. Both drugs decrease response rates in a dose-related manner, with <3-fold difference in potency. Direct effects on response rates generally are not evident 24-hr following administration with either drug. Both SR141716A and AM4113 also appear to comparably antagonize the marked rate-reducing effects of AM411. However, the onset of action appeared to be somewhat quicker for 1.0 mg/kg AM4113 (full effects within 30 min) than for 3.0 mg/kg SR141716A (full effects within 4 hrs). Both drugs also appear to retain their antagonist effectiveness for at least 24 hrs following administration, i.e., at a time when direct effects of either the inverse agonist or neutral antagonist were no longer observed.

In hypothermia studies in monkeys, colonic temperature is measured while subjects are seated in customized chairs within a sound-attenuated chamber that is maintained at a constant temperature of 23°C. After acclimatization, the effects of the novel CB1 full agonist AM4054 were studied alone and following treatment with either the inverse agonist/antagonist SR141716A or the neutral antagonist AM4113. As in rats, the CB1 agonist AM4054 produces dose-related reductions in colonic temperature; the dose that markedly decreased behavior (0.03 mg/kg) reduces temperature by approximately 1° C. The onset of these effects of AM4054 generally is evident within 60 min and continues to grow over the next several hours. Prior treatment with either SR141716A (3.0 mg/kg) or AM4113 (1.0 mg/kg) appear to greatly attenuate these hypothermic effects of AM4054 and, as in rats, these doses of the CB1 antagonists do not themselves increase colonic temperature.

Overall, the results of ongoing studies further extend the range of conditions under which the pharmacological actions of the neutral antagonist AM4113 and the inverse agonist SR141716A are similar and, importantly, also extend these comparisons to a primate species. The two types of CB1 antagonists produce comparable CB1 antagonist actions in monkeys and both drugs have direct behavioral effects, which appear to dissipate faster than their antagonist activity. It remains to be determined whether, as in rats and ferrets, AM4113 can be distinguished from SR141716 or other CB1 inverse agonists using measures of nausea/emesis in a primate species.

Conclusion

Remarkable advances in CB pharmacology within the past two decades have yielded many new insights into the biological significance of endocannabinoid systems and, as well, a first therapeutic candidate, the antagonist/inverse agonist SR141716A (rimonabant; [1]). At this time, rimonabant has been approved for the management of obesity in some countries and, by current standards, appears to have good therapeutic efficacy (e.g., [26]). Like many useful therapeutics, however, it also produces some adverse effects that may compromise its clinical applications. One possibility is that the adverse effects of rimonabant are associated wholly or in part with its inverse agonist activity. This remains speculative in the absence of studies with antagonists that differ widely in the range of their inverse agonist efficacy and in the absence of studies with neutral antagonists. However, the recent development of AM4113, a neutral antagonist, has permitted some initial investigation of this proposition. The available data

suggest that, like other currently studied antagonists, AM4113, when administered alone, can produce effects on schedule-controlled (i.e., motivated) behavior in rats and monkeys. However, unlike rimonabant or other currently studied antagonists, AM4113 appears not to produce effects in rats or ferrets that are thought to predict nauseating or emetic effects in man. Whether or not such differences in the behavioral profile of AM4113 ultimately can be ascribed to its neutral antagonist actions, these are encouraging findings for the further development of clinically useful CB1 antagonists.

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