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SR-141716A-induced stimulation of locomotor activity A structure–activity relationship study

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Abstract

The central cannabinoid receptor (CB_1) antagonist, SR-141716A, has been used extensively to ascertain that cannabinoids interact with the CB_1 receptor. SR-141716A has been shown to produce effects opposite of cannabinoids when administered alone. It has been theorized that SR-141716A may act as an inverse agonist at the CB_1 receptor or by disinhibiting an endogenous cannabinoid tone. In an effort to ascertain the exact interaction between SR-141716A and the CB_1 receptor, we have conducted a structure–activity relationship study to compare CB_1 receptor affinity of SR-141716A analogs with their ability to produce an increase in locomotor activity. SR-141716A produced a significant increase in locomotor activity in mice within the first hour of administration. Twenty SR-141716A analogs from five different chemical series were also tested. Our data implicate particular regions of the SR-141716A molecule that may be involved in stimulation and depression of locomotor activity. When the K_I of the analogs was plotted against the percent stimulation that each analog produced, it is evident that there is no correlation between the ability of the analogs to stimulate locomotor activity and their affinity for the CB_1 receptor. [^{35}S]GTPγS binding data indicate that SR-141716A and five of the analogs are inverse agonists. However, none of the analogs demonstrating inverse agonism produce stimulation of locomotor activity. It is therefore concluded that the SR-141716A-induced stimulation in locomotor activity is not the result of inverse agonist activity at the CB_1 receptor or by disinhibition of an endogenous tone.

Keywords: SR-141716A; Locomotor activity; Stimulation; Behavior; Cannabinoid receptor

1. Introduction

SR-141716A is a potent antagonist to the central cannabinoid receptor (CB_1) (Rinaldi-Carmona et al., 1994) that has proven to be very useful in elucidating the effects of various compounds on the cannabinoid system. SR-141716A binds with high affinity to the central cannabinoid receptor, designated CB_1, and with much lower affinity for the peripheral cannabinoid receptor, designated CB_2 (Rinaldi-Carmona et al., 1994). This selective antagonism is important in determining if a particular drug response is mediated through the central cannabinoid system. However, despite its extensive use, a number of questions remain as to how SR-141716A produces its antagonism. Many studies have demonstrated that the compound can induce pharmacological effects by itself that are opposite of those produced by cannabinoid receptor agonists. Compton et al. (1996) demonstrated that SR-141716A alone produced an increase in locomotor activity in mice. SR-141716A has also been shown to produce hyperalgesia in mice (Richardson et al., 1997), increased arousal in rats (Santucci et al., 1996) and improvement in short-term memory in both rats and mice (Terranova et al., 1995). Such findings indicate that SR-141716A may act as an inverse agonist. It has been postulated that the CB_1 receptor exists in two conformational states, one in which the receptor is precoupled to secondary effector systems and one in which the receptor is uncoupled (Milligan et al., 1995). It is possible that SR-141716A may preferentially bind to the uncoupled state, thereby shifting the equilibrium of the CB_1 receptor pool to

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the uncoupled, inactivated state. It has also been theorized that SR-141716A may disinhibit an endogenous cannabinoid tone produced by such compounds as anandamide or 2-arachidonylglycerol. In either case, the administration of SR-141716A alone would lead to a pharmacological action opposite of a cannabinoid agonist. However, it is also possible that, despite its high affinity for the CB_1 receptor, SR-141716A is acting at different receptors or through non-receptor-mediated means.

SR-141716A-induced stimulation of locomotor activity is often cited as an example of inverse agonism. However, there is no direct evidence indicating that this behavior is the result of inverse agonism or is even mediated through the CB_1 receptor. We have chosen to explore the structure–activity relationship of various SR-141716A analogs in an effort to determine if SR-141716A-induced stimulation in mice is CB_1 receptor mediated and, if so, which structural features of SR-141716A are responsible for the stimulation. Such information should establish whether SR-141716A is acting as an inverse agonist at the CB_1 receptor or as an agonist at another receptor to produce locomotor stimulation.

2. Materials and methods

2.1. Subjects

Male ICR mice (Harlan Laboratories, Indianapolis, IN) weighing between 24 and 30 g were used in all experiments. Mice were maintained on a 14:10-h light/dark cycle with food and water available ad lib. SR-141716A was obtained from the National Institute on Drug Abuse and the SR-141716A analogs were synthesized by the authors.

2.2. Drug preparation and administration

All drugs were dissolved in a 1:1:18 solution of ethanol, emulphor and saline, respectively. The mice were injected intraperitoneally with either vehicle or drug and immediately placed in individual photocell activity chambers. Spontaneous activity was monitored for 2 h in a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH) as measured by the number of interruptions of 16 photocell beams per chamber. The activity count was accumulated in 10-min bins.

2.3. Data analysis

The data from the first six bins were pooled and analyzed by repeated-measures ANOVA. The data from the entire 12 bins were also analyzed. A compound was determined to significantly alter locomotor activity if the ANOVA resulted in a P level <.05. Additionally, the total activity was determined by adding the individual photocell beam interruptions over the 2-h period together and analyzed by Student's t test with significance defined as a P level <.05.

2.4. Receptor binding

[^{3}H]CP-55,940 (K_D=690 pM) binding to whole brain P_2 membranes was conducted as described earlier (Compton et al., 1993). Displacement curves were generated by incubating drugs with 1-nM [^{3}H]CP-55,940. Assays were performed in triplicate and results represent the combined data from three individual experiments. Equilibrium Binding Data Analysis (EBDA) (Biosoft, Milltown, NJ) software was used to determine the K_I values from the displacement data.

2.5. Guanosine-5′-O-(3-[^{35}S]thio)-triphosphate binding

The methods used for measuring agonist-stimulated [^{35}S]GTPγS binding were modified from those developed by Sim et al. (1995). Ten micrograms of rat cerebellar membranes were incubated with 30-μM GDP, 0.5-nM [^{35}S]GTPγS and either increasing concentrations of the SR-141716A analog or ethanol control in glass tubes. The total assay volume was 0.5 ml. The tubes were incubated at 30 °C for 1 h. The reaction was stopped by rapid filtration under vacuum through Whatman GF/B glass-fiber filters followed by three washes with 2-ml ice-cold wash buffer (50-mM Tris–HCl, 5-mM $MgCl_2$, pH 7.4). Filters were placed in 7-ml plastic scintillation vials (RPI, Mount Prospect, IL), which were then filled with 5-ml BudgetSolve scintillation fluid. Dpm were determined by liquid scintillation spectrophotometry. Nonspecific binding was determined using 10-μM unlabelled GTPγS and basal binding was determined in the absence of drug. Inhibition is defined as the percentage decrease below basal levels. Percent change from basal binding is determined as:

$$\%\ \text{change} = [\text{dpm (agonist)} - \text{dpm (no agonist)}] / \text{dpm (no agonist)} \times 100.$$

An analog was determined to be an inverse agonist if it demonstrated a dose-dependent inhibition of [^{35}S]GTPγS binding.

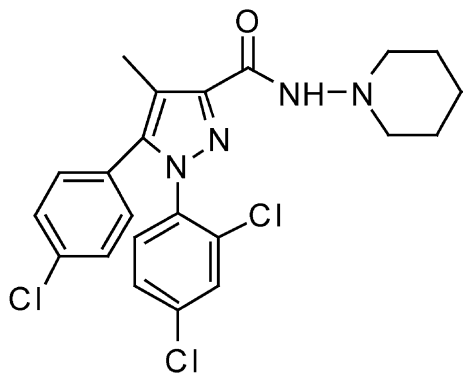


Fig. 1. Structure of SR-141716A.

3. Results

3.1. Effect of SR-141716A on locomotor activity

The structure of SR-141716A is depicted in Fig. 1. The motor stimulation produced by SR-141716A at a dose of 30 mg/kg is depicted in Fig. 2A. SR-141716A-treated mice produced an average of 8055±491 beam interruptions within the first hour in the chamber versus 5675±1024 interruptions in vehicle-treated mice (Table 5). Repeated-measures ANOVA demonstrated that this was a significant increase in locomotor activity. The 10 and 60 mg/kg doses were tested several times and occasionally produced a significant increase in locomotor activity (data not shown). When stimulation was produced by these doses, it was comparable to that seen with the 30 mg/kg doses. For example, 8205±861 counts were produced by mice treated with 10 mg/kg of SR-141716A compared to 5447±860 counts produced by vehicle-treated mice. In other experiments, this dose did not produce any significant increase in counts. However, the 30 mg/kg dose consistently produced a significant increase in locomotor activity. Therefore, all subsequent analogs were tested at a dose of 30 mg/kg.

3.2. Effects of O-compounds on locomotor activity

3.2.1. Fused ring series

The first series of compounds were formed by the fusion of the central pyrazole group of SR-141716A with its 5-(4-chlorophenyl) to form a central indazole ring (Table 1). The resulting 6-chlorine in the indazole ring was alternately retained, removed or substituted. In two cases, O-1412 and O-1343, the 1-(2,4-dichlorophenyl) ring in SR-141716A was substituted with a methyl group or a bulky alkyl group. Of the six compounds in this series, only two produced a significant increase in locomotor activity, O-1247 and O-1248 (Fig. 2B and C). O-1247 administration resulted in 9236±1437 average beam interruptions as opposed to 4898±975 average interruptions in vehicle-treated animals. O-1248 treatment resulted in an average of 7872±878 beam interruptions versus 3817±352 average interruptions of vehicle-treated animals. The presence of the indazole along with either a chloro or a nitro moiety at the 6-position resulted in significant stimulation. Replacing the 6-chlorine in the indazole ring with a bromine or hydroxyl group attenuated the ability of the compound to produce stimulation. Removal of the 6-chlorine and replacement of the 1-(2,4-dichlorophenyl) ring substituent in SR-141716A with either a methyl or a bulky alkyl group also resulted in loss of stimulation. These data indicate that the addition of structural rigidity in the pyrazole ring of SR-141716A does not inhibit the ability of the compound to stimulate motor behavior.

3.2.2. Substituted 2,4-dichlorophenyl series

The compounds in this series were generated by substitution of the 1-(2,4-dichlorophenyl) ring in SR-141716A with a phenyl ring possessing a bulky alkyl group in the

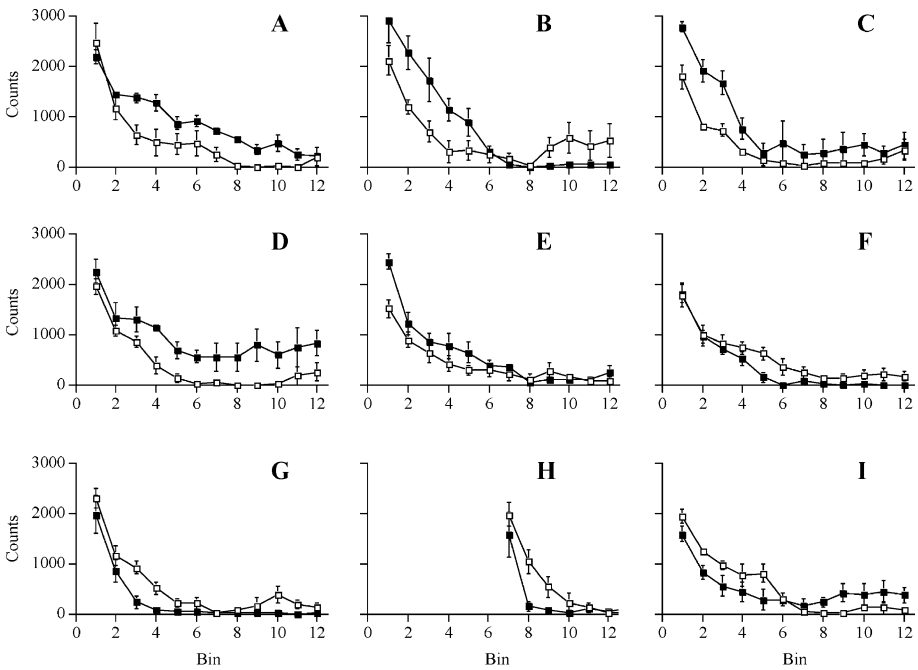
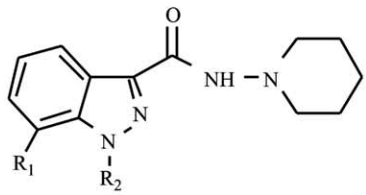
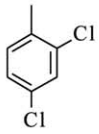
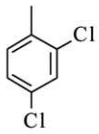
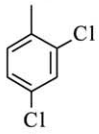
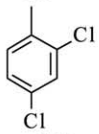
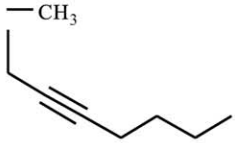


Fig. 2. SR-141716A analogs with the ability to alter locomotor activity. Open squares represent the vehicle-treated mice, while closed squares represent the drug-treated mice. Each point represents the average±S.E. of total activity per 10-min bin for at least six mice. Data from the first 6 bins and total 12 bins were analyzed by repeated-measures ANOVA. SR-141716A (A), O-1247 (B), O-1248 (C), O-1300 (D) and O-1691 (E) significantly increased locomotor activity. O-1270 (F), O-1803 (G), O-1269 (H) and O-1710 (I) significantly decreased locomotor activity. Mice given O-1269 (H) were not placed in chamber until 1 h after intraperitoneal injection.

Table 1
Summary of structures from the fused ring series



Analog	R_1	R_2	K_I	Counts (first hour)	
				Vehicle	Drug
O-1247	$-NO_2$		3533±170	4898±975	9236±1437[a]
O-1248	—Cl		475±6	3817±352	7872±878[b]
O-1654	—Br		881±44	5178±411	6418±571
O-1655	—OH		592±27	5235±1154	7377±823
O-1412	—H	$-CH_3$	>10,000	5154±1511	4240±976
O-1343	—H		1487±99	6115±657	5436±845

Mice were injected intraperitoneally with 30 mg/kg and monitored for spontaneous activity for 2 h. The results of the first hour of observation are presented here as mean counts±S.E.

[a] Significantly different from the control group in the first hour ($P<.05$).

[b] Significantly different from the control group in both the first hour and the total 2 h ($P<.05$).

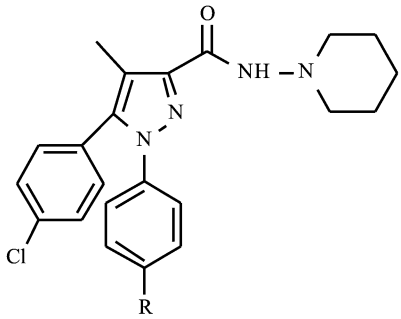
4-position. The results of the four compounds in this series are summarized in Table 2. The presence of the bulky alkyl group attenuated the stimulation. However, when the dichlorophenyl ring was replaced with a phenyl ring, with no bulky alkyl group in the 4-position (O-1300), stimulation was restored (Fig. 2D). O-1300 administration resulted in an average of 7384±652 beam interruptions as opposed to 4553±330 interruptions in the vehicle-treated mice. This indicates that the chlorines are not essential to the ability of SR-141716A to stimulate. Furthermore, the addition of a sterically hindering bulky alkyl group on the phenyl ring prevents stimulation, indicating that the compound requires flexibility in this region to stimulate motor function.

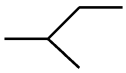
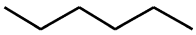
3.2.3. Substituted 3-carboxamide series

This series of SR-141716A analogs involves the substitution at the 3-carboxamide side chain (Table 3). Two analogs, O-1269 and O-1270, are the result of a substitution of the *N*-piperidine moiety with a bulky alkyl group (Fig. 2H and F). In both cases, administration of these compounds resulted in significant depression in locomotor activity. O-1270 administration produced an average of 4251±291 beam interruptions, while vehicle administration resulted in an average of 5446±888 beam interruptions. It should be noted that mice from the O-1269 experiments did not produce a significant change in locomotor activity when placed in the chambers immediately after injection. However, it was observed that the mice were noticeably depressed in locomotor activity well after the 2 h of measurement. When the mice were placed in the chambers 1 h after the injection and 12 10-min bins were measured, the resulting data indicated a significant decrease in locomotor activity (Fig. 2H). O-1269-treated mice produced an average of 2000±589 beam interruptions within the first hour of being placed in the chambers, while vehicle-treated animals produced an average of 3934±699 beam interruptions.

O-1271 and O-1272 involved the replacement of both the *N*-piperidine moiety and the substitution of a ketone for the

Table 2
Summary of structures from the substituted 2,4-dichlorophenyl series

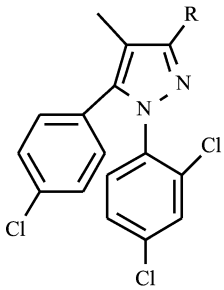


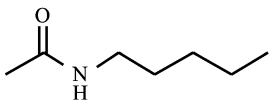
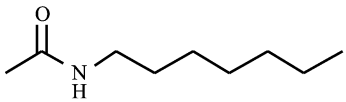
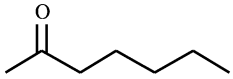
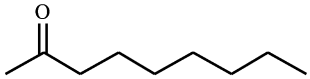
Analog	R	K_I	Counts (first hour)	
			Vehicle	Drug
O-1253		47±2	5846±469	5062±922
O-1254		226±4	5384±876	4657±504
O-1255		433±103	4691±337	5160±940
O-1300	—H	150±20	4553±330	7384±652[a]

Mice were injected intraperitoneally with 30 mg/kg and monitored for spontaneous activity for 2 h. The results of the first hour of observation are presented here as mean counts±S.E.

[a] Significantly different from the control group in both the first hour and the total 2 h ($P<.05$).

Table 3
Summary of structures from the substituted 3-carboxamide series



Analog	R	K_I	Counts (first hour)	
			Vehicle	Drug
O-1269[a]		32±5	3934±699	2000±589[b]
O-1270		48±12	5446±888	4251±291[c]
O-1271		82±10	4903±552	5888±956
O-1272		221±36	3835±958	5212±673

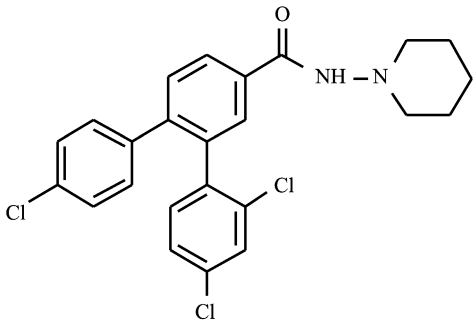
Mice were injected intraperitoneally with 30 mg/kg and monitored for spontaneous activity for 2 h. The results of the first hour of observation are presented here as mean counts±S.E.

[a] Mice were injected 1 h prior to being placed in chambers.

[b] Significantly different from the control group in the first hour ($P<.05$).

[c] Significantly different from the control group for the entire 2 h ($P<.05$).

Table 4
Summary of substituted phenyl



Analog	K_I	Counts (first hour)	
		Vehicle	Drug
O-1803	113±20	5328±634	3257±624[a]

Mice were injected intraperitoneally with 30 mg/kg and monitored for spontaneous activity for 2 h. The results of the first hour of observation are presented here as mean counts±S.E.

[a] Significantly different from the control group in both the first hour and the total 2 h ($P<.05$).

carboxamide series. This substitution resulted in the loss of ability to stimulate or depress activity, indicating that the carboxamide unit is necessary for either stimulation or depression.

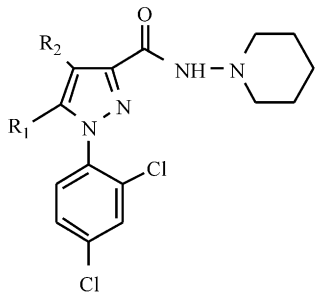
3.2.4. Substituted phenyl

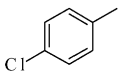
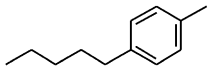
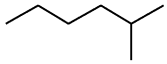
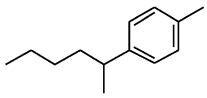
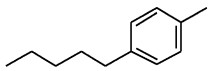
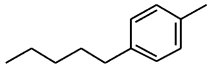
In order to determine whether the pyrazole backbone was essential for the stimulatory activity, O-1803 was prepared in which a phenyl served as the central core of the molecule. Results are summarized in Table 4 and depicted in Fig. 2G. O-1803 produced a significant decrease in locomotor activity with drug-treated mice producing an average of 3257±624 beam interruptions versus 5328±634 produced by vehicle-treated mice.

3.2.5. Substituted 5-(4-chlorophenyl) series

This series of compounds arises from substitutions at the 4- and 5-positions in the pyrazole. Results are summarized in Table 5. Of the five compounds tested, four involve the substitution of the chlorine in the phenyl with a bulky alkyl

Table 5
Summary of structures from the substituted 5-(4-chlorophenyl) series



Analog	R_1	R_2	K_I	Counts (first hour)	
				Vehicle	Drug
SR-141716A		—CH_3	7±2	5675±1024	8055±491[a]
O-1302		—CH_3	2.14±0.08	5621±647	6258±913
O-1559		—CH_3	233±3	3804±851	5901±845
O-1690		—CH_3	2.62±0.13	4899±750	5629±617
O-1691		—Br	1.55±0.22	4142±636	6426±822[b]
O-1710		—H	26.9±0.86	5507±590	3054±184[b]

Mice were injected intraperitoneally with 30 mg/kg and monitored for spontaneous activity for 2 h. The results of the first hour of observation are presented here as mean counts±S.E.

[a] Significantly different from the control group in both the first hour and the total 2 h ($P<.06$).

[b] Significantly different from the control group in the first hour ($P<.05$).

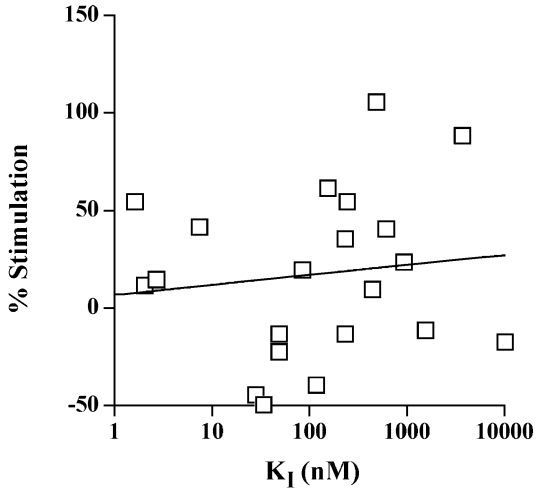


Fig. 3. Lack of correlation between locomotor stimulation and affinity for the CB_1 receptor. Percent stimulation was determined by dividing the average of total counts from 2 h in the activity chamber of the analog-treated groups by the average of the total counts of the vehicle-treated groups. A negative percent stimulation indicates an analog that resulted in depression of locomotor activity. Each point represents either SR-141716A or 1 of the 20 analogs. Curve fit analysis results in a correlation coefficient of .015.

group. One compound, O-1559, involved the substitution of the phenyl ring entirely with a bulky alkyl group. All of the substitutions attenuated the ability of the compounds to increase locomotor activity. However, stimulation returned when one of the analogs, O-1302, was further modified such that the 4-methyl group was replaced with a bromine, resulting in the analog O-1691 (Fig. 2E). O-1691-treated mice produced an average of 6426±822 beam interruptions, while the vehicle-treated group produced an average of 4142±636. When the methyl was removed altogether, as in analog O-1710, depression occurred (Fig. 2I). An average of 3054±184 beam interruptions was produced in O-1710-treated mice versus 5507±590 in vehicle-treated mice. This indicates that the presence of a sterically hindering group on the 5-phenyl side chain does not hinder the ability of SR-141716A to stimulate locomotor activity. Furthermore, it may imply that the 4-methyl group is an important area for determining the ability of the SR-141716A analogs to produce motor stimulation or depression.

3.3. Relationship between analog affinity and ability to stimulate locomotor activity

The correlation between the pharmacological potency of the analogs and their affinity for the CB_1 receptor was very low. When the percent stimulation of the analogs are plotted against their affinities, a correlation coefficient of .015 results (Fig. 3). This lack of correlation is typified by analogs such as O-1302, which possesses very high affinity for the CB receptor (K_I=2.14±0.08), but failed to stimulate motor activity. O-1247 has very weak affinity for the CB_1 receptor (K_I=3530±170) yet possesses the ability to induce stimulation in locomotor activity. It is possible that including all analogs in the analysis hides a correlation between potency and affinity. However, when analyzing the correlation between stimulation and affinity for only those compounds that significantly stimulate or depress locomotor activity, a low correlation coefficient of .123 results (data not shown). This further indicates that the ability of these compounds to affect locomotor activity does not correlate well with their ability to bind to the CB_1 receptor.

3.4. [^{35}S]GTPγS binding

The [^{35}S]GTPγS binding data indicate that SR-141716A is an inverse agonist. SR-141716A produced a dose-dependent decrease in basal [^{35}S]GTPγS binding. The maximal level of inhibition occurred at the 10-μM dose of SR-141716A and reached a level of 45±6.3%. Of the 20 analogs tested, only five exhibited a dose-dependent decrease in basal [^{35}S]GTPγS binding. The concentration–effect curve for SR-141716A compares well with previously published

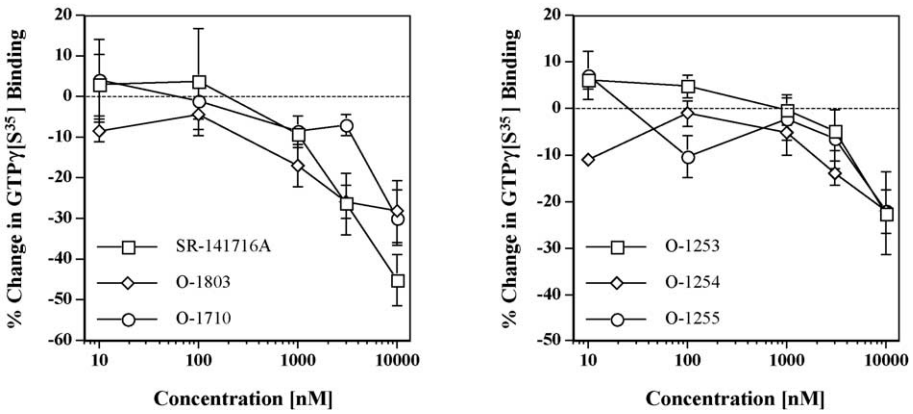


Fig. 4. Dose–effect curves of inverse agonists. SR-141716A and each of the 20 analogs were tested for their ability to inhibit [^{35}S]GTPγS binding. The percent change from basal binding was determined for increasing concentration of drug. Of the 20 SR-141716A analogs, only five compounds demonstrated inverse agonist properties including O-1803, O-1710, O-1253, O-1254 and O-1255.

results (Sim-Selley et al., 2001) and is presented in Fig. 4 along with the other five inverse agonist curves. Of the six inverse agonists, only SR-141716A, O-1803 and O-1710 were determined to significantly affect locomotor activity. SR-141716A significantly increased locomotor activity, while O-1803 and O-1710 significantly decreased locomotor activity. The remaining three inverse agonists, O-1253, O-1254 and O-1255, did not significantly affect locomotor activity. None of the five analogs that were capable of producing inverse agonism were able to stimulate locomotor activity. Analyses were conducted to determine if a relationship exists between [^{35}S]GTPγS binding and locomotor activity (data not shown). There is no apparent relationship even when examining only those compounds that stimulate or depress locomotor activity as well as those compounds that have high or low affinity for the CB_1 receptor.

4. Discussion

The results presented in this study support previous findings that SR-141716A administration can produce stimulation of locomotor activity (Compton et al., 1996). The Compton study determined that SR-141716A produced an increase in locomotor activity with an ED_{50} of approximately 4.7±1.5 mg/kg when administered intravenously. The doses utilized in this study were much higher as the SR-141716A was administered intraperitoneally. However, this alone cannot account for the extremely high doses needed to produce stimulation of locomotor activity. While a traditional dose–response curve was not conducted, it was determined that the administration of 10 or 60 mg/kg SR-141716A produced stimulation inconsistently while 30 mg/kg SR-141716A produced stimulation very consistently. Presumably, the lower dose of 10 mg/kg is at or near the ED_{50} level for stimulation produced by SR-141716A administered intraperitoneally, which would explain why significant stimulation was not always observed. The higher dose of 60 mg/kg may produce inconsistent stimulation as a result of toxic effects or an increased activity at noncannabinoid receptor sites.

It is sometimes difficult to analyze locomotor activity as external factors can often complicate the results. In an effort to minimize the variability, each experiment was conducted at least twice. Each experiment was also conducted at the same time each day to minimize influences of the diurnal cycle. Finally, each drug-treated group was run concomitantly with a vehicle-treated group in an effort to ensure that both groups were exposed to the same conditions in the activity chambers.

The primary focus of this paper was to determine which structural elements of SR-141716A are necessary to produce the stimulation in locomotor activity that occurs with the administration of high doses of SR-141716A. While we have in fact defined some of the potentially important regions of the SR-141716A compound that may contribute to this effect, it is notable that we have determined not which structural elements are necessary for stimulation but rather which elements are not required. For example, the fused ring series of compounds introduced a large degree of structural rigidity within the central region of the compound. Although the majority of analogs from this series did not produce stimulation, two were capable with very minor changes in the first position of the indazole ring, indicating that introduction of structural rigidity in the central ring does not inhibit stimulation. The chlorines in the 2,4-dichlorophenyl side chain are not necessary for stimulation; however, the addition of a moiety that sterically hinders the compound in this region prevents stimulation. The piperidine moiety from the 3-carboxamide side chain may be required to induce stimulation, as all compounds tested that did not possess this moiety would not stimulate. However, the substitution of an alkyl group for the piperidine moiety resulted in depression of locomotor activity, indicating that this region is a critical determinant for distinguishing between motor stimulation and depression. Depression or stimulation of activity did not occur when the 3-carboxamide was replaced with ketones that were structurally similar to the substituted carboxamides in the depression-inducing analogs. It is thus possible that replacement of the piperidine with bulky alkyl groups or the combination of the bulky alkyl groups with the carboxamide is needed to produce the depression. The compound from the fourth series, O-1803, demonstrates that the substitution of a benzene ring for the pyrazole leads to depression in locomotor activity. It is difficult to draw conclusions from data with a single compound, but it would appear that the pyrazole or a similar nitrogen-containing backbone is necessary for motor stimulation.

Finally, the fifth series demonstrates that the addition of a sterically hindering group in the 4-position of the phenyl in the 5-substituted pyrazole does not prevent either stimulation or depression in locomotor activity. Furthermore, it implicates the methyl in the 4-position of the pyrazole as potentially essential in determining whether the analog stimulates or depresses. In total, these data provide some intriguing clues as to the structural elements that may lie at the root of SR-141716A-induced stimulation of locomotor activity. It also demonstrates the structural diversity that can exist within the various analogs that do affect locomotor activity.

This study also sought to determine if SR-141716A-induced stimulation of locomotor activity is mediated through the CB_1 receptor. The ability of SR-141716A to stimulate locomotor activity is often cited as an example of a behavioral consequence of inverse agonism (Compton et al., 1996). However, despite the biochemical evidence that SR-141716A is indeed an inverse agonist, there are no data that establish inverse agonism as the mechanism of action for SR-141716A-induced stimulation. In this study, it was determined that many of the SR-141716A analogs that produced significant stimulation or depression in this study had very low affinity for the CB_1 receptor. When the affinities of the various compounds for the CB_1 receptor

are compared to their ability to induce changes in locomotor activity, it is apparent that there is no correlation. Furthermore, SR-141716A is the only inverse agonist tested in this study that is capable of stimulating locomotor activity. All other analogs that were determined to be inverse agonists, as measured by inhibition of [^{35}S]GTPγS S binding, were not capable of producing stimulation of locomotor activity. These data indicate that SR-141716A-induced stimulation in locomotor activity does not appear to result from activity at the cannabinoid receptor or from inverse agonism. While this is somewhat surprising, it is not altogether unfounded. This could explain the inability of SR-141716A to block the pharmacological actions of anandamide in mice (Adams et al., 1998). The lack of correlation between the affinity of the analogs for the CB_1 receptor and the ability to stimulate motor activity suggests that these compounds may have a different site of action. Furthermore, the amount of motor depression induced by some of the SR-141716A analogs does not correlate with their affinities for the CB_1 receptor, further implicating that even these traditionally cannabinoid effects may not be mediated through the CB_1 receptor.

There are some other possible mechanisms of action to explain SR-141716A-induced stimulation of locomotor activity. Recent evidence indicates the presence of a non-CB_1 and non-CB_2 cannabinoid receptor binding site in the brain of CB_1 knockout mice (Breivogel et al., 2001). It has also been demonstrated that CB_1 receptors are located presynaptically on hippocampal interneurons and that these receptors are responsible for a cannabinoid-induced inhibition of GABA release (Katona et al., 1999). Another recent study has demonstrated that a non-CB_1 cannabinoid-sensitive receptor exists in the hippocampus of CB_1 knockout mice (Hajos et al., 2001). This novel cannabinoid receptor appears to account for the cannabinoid-induced inhibition of glutamate release only and not GABA in hippocampal slices. These data indicate that a novel cannabinoid receptor exists in the brain that preferentially regulates glutamate release. An interesting possibility is that SR-141716A may selectively or preferentially block an endogenous tone to one of the cannabinoid receptors in the brain. This would lead to an imbalance between the production of GABA and glutamate. Such an imbalance between these inhibitory and excitatory neurotransmitters may lead to the stimulation of locomotor activity that is observed in vivo. The possibility also exists that SR-141716A can act as an agonist at this non-CB_1 cannabinoid receptor. Such an activity could explain the behavioral effects of SR-141716A when administered alone. However, while there is substantial evidence supporting its existence, this non-CB_1 receptor has yet to be thoroughly characterized. A full understanding of how SR-141716A affects this putative receptor cannot be obtained until more is known about the localization, structure and pharmacology of such a receptor.

Another possible mechanism that may explain SR-141716A-induced stimulation of locomotor activity is that the high doses of SR-141716A utilized in the present study may be acting on a combination of cannabinoid receptors or other neuroreceptors. This would also explain why the structural requirements for SR-141716A-induced stimulation in locomotor activity are difficult to define, particularly if two different receptors contribute to the effect. Furthermore, it may explain why some analogs depress and others stimulate. The affinity of these analogs for a possible second receptor is unknown and it is therefore possible that pharmacologically active analogs with low affinity for the CB_1 receptor could have high affinity for this unknown receptor. Other analogs may have high affinity for both receptors. It is also possible that affinities of the SR-141716A analogs for the CB_1 receptor as reported here are not relevant. If an analog is an inverse agonist, it will preferentially bind to the uncoupled receptor. However, the K_I values were determined by the displacement of [^{3}H]CP-55,940. It is therefore possible that the analogs may display a slightly different affinity for an unoccupied receptor. A dose of 3 mg/kg of SR-141716A completely antagonizes cannabinoid action at the CB_1 receptor. However, the doses for SR-141716A-induced stimulation of locomotor activity range at least between 10 and 60 mg/kg. Such high concentrations of the drug could lead to binding and activation of a receptor that would normally have low affinity for SR-141716A. Furthermore, while the dose of SR-141716A used in these studies seems extremely high, it is impossible to compare the doses needed for CB_1 mediated effects, such as antagonism, with those that are non-CB_1 mediated. Finally, there is some evidence that agonist-selective G protein signaling occurs with CB_1 and CB_2 receptors (Glass and Northup, 1999). It is therefore possible that the SR-141716A analogs activate different subsets of G-proteins, thereby leading to different behavioral consequences.

In summary, our results strongly suggest that SR-141716A-induced stimulation of locomotor activity is not mediated through the CB_1 receptor alone and is not the result of inverse agonism alone. This is apparent in that analogs of SR-141716A that have very little affinity for the CB_1 receptor can stimulate locomotor activity. Also, the analogs that produce inverse agonism do not stimulate locomotor activity. It is therefore apparent that inverse agonism at the CB_1 receptor cannot account for SR-141716A-induced stimulation of locomotor activity. There are numerous reports of agonist activity from SR-141716A administration. While it appears that stimulation of locomotor activity is not mediated through inverse agonism, other SR-141716A-induced effects, such as stimulation of appetite and improvement in short-term memory, may be the result of inverse agonism, although such hypotheses should be tested in the future. Finally, while this study presents some possible structural clues as to which regions of the SR-141716A compound are necessary for stimulation, the lack of a clear mechanism through which stimulation occurs prevents a complete analysis of the SAR data.

Acknowledgements

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