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Antinociceptive effects of tetrahydrocannabinol side chain analogs: dependence upon route of administration

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Abstract

The role of flexibility of the alkyl side chain in the tetrahydrocannabinols to cannabinoid activity has been delineated in previous studies with side chain analogs of Δ^8-tetrahydrocannabinol with double or triple bonds. This study investigated the site of antinociceptive action for these analogs through analysis of structure–activity relationships following different routes of administration. In analogs without terminal substitutions, potency was greater following intrathecal (i.t.) injection than with intracerebroventricular (i.c.v.). Further, optimal structural features differed for each route of administration. Absolute position of the double or triple bond best predicted i.t. potency. In contrast, i.c.v. potency was best predicted by the size of the alkyl substituent beyond the point of unsaturation. Terminal substitutions tended to increase i.c.v. potency while decreasing or not affecting i.t. These results suggest that receptor mechanisms for cannabinoid antinociceptive effects differ in brain and spinal cord, although potential pharmacokinetic differences in rate of local distribution cannot be eliminated.

Keywords: Antinociception; Cannabinoid; Intrahecal; Intraventricular; Structure–activity relationship; Tetrahydrocannabinol

1. Introduction

Psychoactive cannabinoids from each of the major cannabinoid classes (tetrahydrocannabinols, bicyclic cannabinoids, aminoalkylindoles, and anandamides) bind to cannabinoid CB1 receptors in the brain and produce a similar profile of pharmacological effects in mice, including hypoactivity, hypothermia, antinociception and catalepsy (Martin et al., 1991; Smith et al., 1994). Although tetrahydrocannabinols and bicyclic cannabinoids (e.g., Δ^9-tetrahydrocannabinol and CP 55,940 [(−)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol], respectively) are equipotent (across tests) and equally efficacious in producing these four pharmacological effects, differences in potencies and/or magnitudes of maximal effect in tests with aminoalkylindoles and anandamides have been observed. For example, the potencies of aminoalkylindoles for suppression of locomotor activity are greater than their potencies in producing the other three cannabimimetic effects in mice (Compton et al., 1992). In addition, whereas anandamide is more efficacious than Δ^9-tetrahydrocannabinol in producing catalepsy, it is only a partial agonist for reducing body temperature and in other pharmacological assays (Mackie et al., 1993; Smith et al., 1994). Collectively, these results suggest that the interaction of the different classes of cannabinoid agonists with the cannabinoid CB1 receptor may not be identical, although the possibility of multiple cannabinoid receptors cannot be eliminated.

A primary goal of empirical study of structure–activity relationships among cannabinoids has been to clarify the nature of these agonist interactions with cannabinoid CB1 receptors. Such studies have revealed that manipulation of the length of and terminal substitution in the alkyl side chain in the tetrahydrocannabinols and bicyclic cannabinoids (or its equivalent in aminoalkylindoles and anandamides) affects pharmacological activity and receptor recognition (Melvin et al., 1993; Razdan, 1986; Ryan et

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al., 1997; Seltzman et al., 1997; Wiley et al., 1998). In previous studies (Martin, B.R. et al., 1999; Ryan et al., 1995), we examined the importance of the flexibility of this side chain in Δ^8-tetrahydrocannabinol analogs through systematic incorporation of either double or triple bonds along the side chain as well as addition of terminal functional groups that are known to influence other classes of ligands (Charalambous et al., 1991; Martin, B.R. et al., 1993). One of the findings of this study (Martin, B.R. et al., 1999) was that, unlike the parent compound, some of these rigid side chain analogs showed antinociceptive selectivity. Since any pharmacokinetic differences among the analogs that might have been produced by structural manipulation should affect all centrally mediated pharmacological effects equally, this result suggests potential differences in receptor interaction or activation. In order to further investigate the level at which these pharmacodynamic differences might be occurring, we measured the antinociceptive effect of these rigid side chain analogs following intrathecal (i.t.) and intracerebroventricular (i.c.v.) administration.

2. Materials and methods

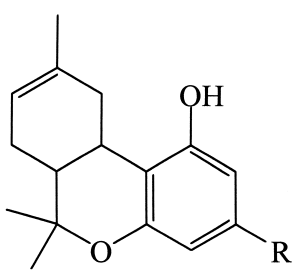
2.1. Subjects

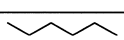
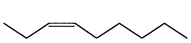
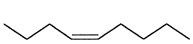
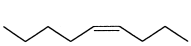
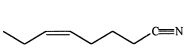
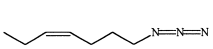
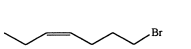
ICR male mice (Harlan Laboratories, Indianapolis, IN) weighing 25 to 30 g were used in all experiments. Mice were maintained on a 14:10-h light/dark cycle with free access to food and water. Separate mice were used for testing each drug dose with each route of administration.

2.2. Drugs

Δ^9-Tetrahydrocannabinol (National Institute on Drug Abuse, Rockville, MD) was mixed in dimethylsulfoxide. Δ^8-tetrahydrocannabinol side chain analogs were synthe-

Table 1
Antinociceptive effects of side-chain analogs with double bonds after i.t. or i.c.v. administration[a]



#	Chemical Name	R	KD[b] (nM)	TF[a] (i.t.)	TF (icv)
	Δ^9-THC		41±1.7	29 (24-36) 91[100]	32 (26-39) 100[100]
O-1318	*cis*-3-(2-Octenyl)-Δ^8-THC		3.19±0.92	6 (1-26) 71[50]	27 (14-52) 79[75]
O-1319	3-(3-Octenyl)-Δ^8-THC		3.36±0.91	3 (2-5) 98[50]	22 (15-33) 90[75]
O-1083	*cis*-3-(4-Octenyl)-Δ^8-THC		11±3.2	26 (19-35) 100[100]	— 52[200]
O-1237	*Cis*-3-(6-Cyano-2-hexenyl)-Δ^8-THC		1.25±0.51	11 (7-18) 90[50]	28 (19-39) 92[100]
O-1238	*Cis*-3-(6-Azido-2-hexenyl)-Δ^8-THC		3.32±0.59	2.4 (1.2-4.7) 85[10]	5.6 (2.9-11) 85[30]
O-1236	*Cis*-3-(6-Bromo-2-hexenyl)-Δ^8-THC		1.66±0.66	11 (7-17) 90[5]	19 (12-30) 100[100]

[a] The results are presented as ED50 (95% confidence limits in parenthesis) and expressed as ug/mouse for both routes of administration. Below the ED50 is the maximal effect and the dose in brackets at which it occurred.
[b] Values from Martin, B.R. et al. (1999).

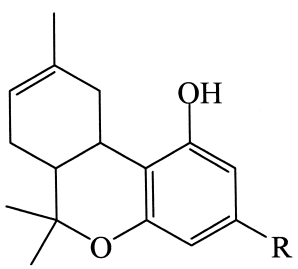
sized in our labs (Organix, Woburn, MA) and were also mixed in dimethylsulfoxide.

2.3. Procedure

Antinociception was defined as the latency to remove the tail from a heat stimulus (Dewey et al., 1970). Control values were determined for tail-flick latency (in s) immediately preceding injection. Tail-flick latency was measured again at 10-min post-injection. Maximum latency of 10 s was used, at which time the mouse's tail was removed from the heat source by the experimenter. Antinociception was expressed as percent of maximum possible effect {%MPE = [(test-control latency)/(10 − control)] × 100}. Control latencies typically ranged from 1.5 to 4.0 s. Different mice ($n = 6–8$ per dose) were tested at each dose of each compound.

Mice were injected either i.t or i.c.v.. I.t. injections were administered to unanesthesized mice between the L5 and L6 area of the spinal cord with a 30-gauge, 0.5-in. needle. For i.c.v. injections, an incision was made to expose bregma. Injections were given with a 26-gauge needle to a site 2-mm lateral and 2-mm caudal to bregma at a depth of 2 mm. Each mouse received an i.t. or i.c.v. injection volume of 5 μl of a given concentration, regardless of weight. For i.c.v. injection, mice were prepared under light ether anesthesia, as required by the Institutional Animal Care and Use Committee. Anesthesia (either ether or pentobarbital) has been used for i.c.v. injections in past studies to which we intend to compare our results (e.g., Martin, W.J. et al., 1999; Welch et al., 1995). The influence of ether or pentobarbital anesthesia on cannabinoid antinociceptive potency has not been systematically investigated, although it is standard procedure to use it in this type of study.

Table 2
Antinociceptive effects of side-chain analogs with triple bonds after i.t. or i.c.v. administration[a]



#	Chemical Name	R	KD[b] (nM)	TF (i.t.)	TF (icv)
O-964	3-(1-Heptynyl)-Δ[8]-THC		36±0.8	43 (22-84) 77[100]	127 (92-174) 74[200]
O-615	3-(2-Hexynyl)-Δ[8]-THC		11±1.0	14 (8-23) 87[30]	— 58[200]
O-584	3-(2-Octynyl)-Δ[8]-THC		4.9±2.0	17 (9-29) 78[60]	52 (21-129) 93[200]
O-630	3-(2-Nonynyl)-Δ[8]-THC		3.7±1.3	24 (14-40) 84[100]	65 (40-104) 77[150]
O-1004	3-(3-Butynyl)-Δ[8]-THC		367±23	46 (21-101) 82[200]	99 (65-149) 86[300]
O-1020	3-(3-Octynyl)-Δ[8]-THC		9.0±1.3	50 (28-88) 80[200]	55 (30-103) 77[200]
O-1052	3-(4-Octynyl)-Δ[8]-THC		19±1.3	202 (108-377) 68[400]	— 42[400]

[a] See footnote for Table 1.
[b] Values from Martin, B.R. et al. (1999).

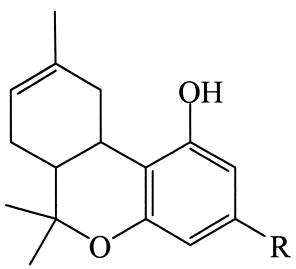
2.4. Data analysis

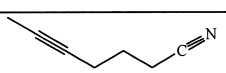
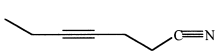
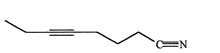
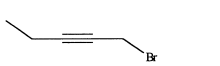
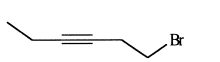
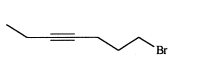
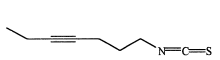
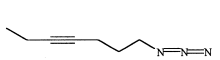
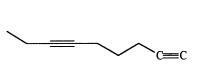
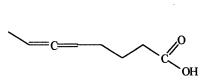
ED_{50}s were calculated separately for each drug using least-squares linear regression on the linear part of the dose–effect curve, plotted against $\log_{10}$ transformation of the dose. In addition, Pearson product-moment correlation coefficients (with associated significance tests) were calculated between binding affinity (expressed as log K_i) and in vivo potency (expressed as log ED_{50}) for each route of administration for all active cannabinoids.

3. Results

The first series of compounds contained either an octenyl side chain with the double bond located at different positions or a 2-hexenyl side chain with various substitutions on the terminal carbon (Table 1). These double-bond compounds in general had high receptor affinity and were effective i.t. and i.c.v. For example, the 2-octenyl derivative was more potent than Δ^9-tetrahydrocannabinol after i.t. (~6-fold) injection but both were approximately

Table 3
Antinociceptive effects of side-chain analogs with triple bonds and terminal substitution after i.t. or i.c.v. administration[a]



#	Chemical Name	R	KD[b] (nM)	TF (i.t.)	TF (icv)
O-1068	3-(5-Cyano-1-pentynyl)-Δ^8-THC		104±12	— 48[400]	— 34[200]
O-1106	3-(5-Cyano-2-pentynyl)-Δ^8-THC		31±8.3	5 (3-9) 90[50]	32 (22-48) 90[100]
O-823	3-(6-Cyano-2-hexynyl)-Δ^8-THC		0.77±0.05	15 (11-19) 97[50]	1.5 (0.6-3.5) 100[30]
O-1187	3-(4-Bromo-2-butynyl)-Δ^8-THC		143±31	275 (231-328) 80[400]	— 30[400]
O-1105	3-(5-Bromo-2-pentynyl)-Δ^8-THC		25±4.2	11 (6-18) 86[60]	17 (11-26) 85[60]
O-806	3-(6-Bromo-2-hexynyl)-Δ^8-THC		1.2±0.1	15 (9-24) 83[60]	2.1 (1.0-4.5) 93[30]
O-1176	3-(6-Isothiocyano-2-hexynyl)-Δ^8-THC		11.5±2.3	— 38[200]	— 26[200]
O-1184	3-(6-Azido-2-hexynyl)-Δ^8-THC		2.14±0.44	2 (1-4) 100[50]	4 (2-9) 89[50]
O-965	3-(2,7-Octadiynyl)-Δ^8-THC		4.7±0.4	71 (54-92) 80[200]	153 (129-181) 93[300]
O-1174	3-(6-Carboxy-1,2-hexadienyl)-Δ^8-THC		3170±105	107 (77-149) 78 [200]	141 (100-198) 97 [400]

[a] See footnote for Table 1.
[b] Values from Martin, B.R. et al. (1999).

equipotent after i.c.v. administration. The primary exception was the 4-octenyl analog (O-1083) that was moderately potent after i.t. injection and very weak after i.c.v. administration It produced only 52% MPE at an i.c.v. dose of 200 μg/mouse (approximately 6 mg/kg). It is worth noting that the C-terminal substitution of either a cyano (O-1237) or a bromo (O-1236) had relatively little impact on i.t. and i.c.v. potency. Interestingly, azido substitution (O-1238) enhanced i.c.v. potency in the double-bond series.

As for incorporating an acetylene in the side chain, both chain length and location of unsaturation were examined (Table 2). Analogs with a 1-heptynyl (O-964), 2-octynyl (O-584), 2-nonynyl (O-630), and 3-octynyl (O-1020), were antinociceptive by both routes of administration. However, some of these derivatives failed to produce maximal effects even at relatively high doses. Partial agonist effects were more obvious with compounds such as O-615 (2-hexynyl) and O-1052 (4-octynyl) which failed to achieve 100% effect by both routes, but particularly by the i.c.v. route. On the other hand, the 3-butynyl analog had very low receptor affinity and failed to produce antinociception at i.v. doses up to 30 mg/kg (Martin, B.R. et al., 1999); yet, it was capable of some antinociceptive activity by both i.t. and i.c.v. routes. As with the previous side chain double-bond series, the antinociceptive doses following i.t. and i.c.v. administration were comparable to their i.v. doses for most of these aceylenic derivatives, when estimated on a mg/kg basis. The most prominent exception was O-1004 (3-butynyl), as mentioned previously.

The final series of compounds contained side chains of varying length, an acetylene at different positions, and substituent substitutions at the terminus (Table 3). The 6-cyano-1-pentynyl analog (O-1068) had low receptor affinity and was only a partial agonist following i.v. administration (Martin, B.R. et al., 1999). As would be predicted, it produced little antinociceptive effect after either i.t. or i.c.v. injection. In contrast, moving the acetylene from C1–C2 to C2–C3 (O-1106) led to potent, efficacious antinociception by both i.t. and i.c.v. routes. Extending the side chain length by one carbon to form 6-cyano-2-hexynyl (O-823) provided a profile similar to that of O-1106. This high affinity analog was highly potent and efficacious after i.c.v. administration. It was about 10 times less potent after i.t., as compared to i.c.v., but still fully efficacious. Three bromo derivatives in the 2-ynl series were prepared with varying side chain length. The butynyl analog (O-1187) exhibited little effect by either route of administration which was consistent with its low receptor affinity. However, increasing the chain length by 1 carbon atom (O-1105) increased receptor affinity and potencies i.t. and i.c.v. to a considerable degree. Increase in carbon side chain length by one additional carbon (O-806) further increased receptor affinity and produced an approximate eightfold increase in potency over that of O-1105 by the i.c.v. route with little change in potency by i.t. injection.

The 2-hexynyl analogs O-1176, O-1184 and O-965 can be considered isothiocyanate, azido and acetylyne substitutions at C6 for purposes of comparison to the 6-bromo-2-hexynyl analog (O-806). While both isothiocynate and azido analogs exhibited high receptor affinity and potency (i.v.; Martin, B.R. et al., 1999), their potencies were markedly different by the i.t. and i.c.v. routes. The azido analog (O-1184) was very potent at both sites, whereas the isothiocyanate (O-1176) produced little response at a dose of 200 μg/mouse after i.t. and i.c.v. injections. O-965 represents a substitution of an acetylene for the isothiocyano in O-1176 or the axido in O-1184. The resulting analog was 30–40 times less potent than the azido analog at both sites despite having similar receptor affinities. O-965 and O-1184 had similar potencies i.v. (Martin, B.R. et al., 1999). The last compound that was evaluated contained a 1,2-hexadienyl with a 6-carboxyl group (O-1174). This analog has almost no receptor affinity and showed only partial efficacy after i.t. injection. Although fully efficacious i.c.v., O-1174 was weakly potent via this route.

Correlations between binding affinities and potencies for all active analogs were highest (and significant; $P < 0.05$) following i.c.v. administration, but were low (and

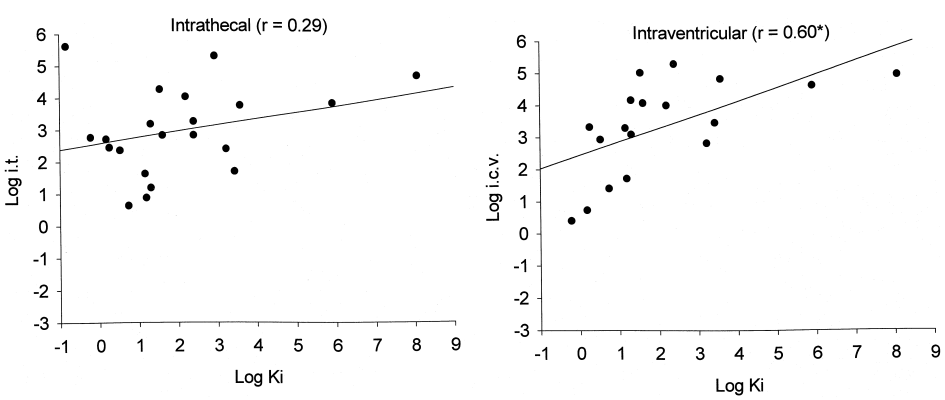


Fig. 1. Scatterplots and regression lines of log K_i plotted against log ED_{50} for antinociception for both routes of administration (i.t. and i.c.v.) of a series of side chain analogs of Δ^8-tetrahydrocannabinol. (* indicates that the correlation coefficient was significantly different from 0.)

nonsignificant) after i.t. administration (Fig. 1). The correlations between binding affinity and antinociceptive potency were 0.29 and 0.60 following i.t. and i.c.v. injection, respectively.

4. Discussion

Results of previous studies provide evidence for both spinal and supraspinal sites of cannabinoid-induced antinociception (Martin, W.J. et al., 1993, 1999; Hohmann et al., 1998; Smith and Martin, 1992). Δ^9-Tetrahydrocannabinol, CP 55,940, WIN 55,212-2 {*R*-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol (1,2,3-*de*]-1,4-benzoxazin-6-yl)(1-naphthalenyl)methanone monomethanesulfonate}, and other classical cannabinoids showed antinociceptive effects when administered either i.t. or i.c.v. (Martin, W.J. et al., 1993; Welch et al., 1995). Further, antinociceptive potencies via both routes of administration were approximately equal for Δ^9-tetrahydrocannabinol (present study). Mechanisms for this effect at each location, however, may differ. Welch et al. (1998) found that the cannabinoid CB1 receptor antagonist SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride] completely and dose-dependently blocked the antinociceptive effects of i.c.v. administered tricyclic and bicyclic cannabinoids when it was also injected i.c.v., but i.t. administered SR141716A only partially blocked the antinociceptive effects of the same cannabinoids administered i.t. In addition, SR141716A (i.t.) did not completely block the antinociceptive effects of anandamide (i.t.). One possible explanation for these results is that sub-types of central cannabinoid receptors exist and that these receptor isoforms may be differentially distributed in the brain and spinal cord. Except for cloning of a cannabinoid CB_{1A} receptor (a splice variant of the cannabinoid CB1 receptor) (Shire et al., 1995), however, putative central cannabinoid receptor sub-types have not been identified. Alternatively, different intracellular messenger systems or differential effects of the same intracellular messenger may be involved in spinal and supraspinal mediation of cannabinoid-induced antinociception. Lending support to this hypothesis is the finding that modulators of the two intracellular messengers, Ca^{2+} and cAMP, affected antinociception induced by tricyclic and bicyclic cannabinoids differently depending upon whether the cannabinoid was administered i.t. or i.c.v. (Welch et al., 1995). In addition, cannabinoid enhancement of morphine-induced antinociception was associated with differential modulation of these intracellular messengers in brain versus spinal cord synaptosomes (Pugh et al., 1994).

Delineation of structure–activity relationships represents one method by which receptor interactions may be characterized. Although numerous previous studies have reported results of structure–activity relationship investigations with systematically administered cannabinoids, examination of cannabinoid structure–activity relationships following i.c.v. or i.t. administration is limited or nonexistent. In the present study, we manipulated route of administration and examined the effect on antinociception produced by a series of side chain analogs of Δ^8-tetrahydrocannabinol with double or triple bonds. Several patterns emerged from this analysis. First, all of the analogs without terminal substitutions (first part of Tables 1 and 2) were active following i.t. administration and potencies were consistently greater than after i.c.v. injection, with the exception that O-1020 was equipotent i.t. and i.c.v. This selectivity has also been observed with anandamide which is active following i.t. administration, but not after i.c.v. injection (Smith et al., 1994), suggesting that this phenomenon is not unique to rigid side chain analogs of the tetrahydrocannabinols. Second, the structural features which predicted antinociceptive potency in this series of non-substituted rigid side chain analogs differed between i.t. and i.c.v. routes of administration. The primary structural feature which predicted i.t. antinociceptive potency was absolute position of the double or triple bond, with the C2 position being optimal regardless of length of the entire side chain (within the range of six to nine carbons). Interestingly, the three triple-bond analogs with greatest i.t. antinociceptive potency (O-615, O-584, and O-630) also showed the highest antinociceptive selectivity for producing cannabimimetic effects in mice i.v. (Martin, B.R. et al., 1999), suggesting that the position of the rigid bond may also affect selectivity of cannabimimetic effects. In contrast, the primary structural factor, which predicted i.c.v. antinociceptive potency in the nonsubstituted analogs, was the size of the alkyl substituent beyond the point of unsaturation. Positioning of the double or triple bond such that the terminal alkyl substituent was short (two carbons in acetylenic series and three carbons in the ethylenic series) greatly attenuated i.c.v. antinociceptive activity (compared to i.t. potency). Although removing the terminal akyl group (O-1004) increased potency slightly, non-substituted acetylenic analogs with longer terminal side chains (three to five carbons) showed the greatest antinociceptive potency i.c.v. Consistent with these results, terminal side chain length affected binding affinity of a series of methylated pentyl side chain analogs of Δ^8-tetrahydrocannabinol. Compounds with methylation at positions farther from the terminal carbon showed greater affinity for cannabinoid CB1 receptors than did analogs with methylation nearer the terminus even though the length of the entire side chain was identical for all of the analogs (Huffman et al., 1997). A secondary factor that predicted i.c.v. antinociceptive potency in the present study was flexibility at the proximal end of the double bond side chain. Double bonds at more proximal positions within the side chain decreased potency whereas those at more distal positions increased potency, as long as the terminal alkyl substituent was maintained at four to five carbons. In

addition, the more flexible non-substituted ethylenic analogs were more potent i.c.v. than were the corresponding acetylenic analogs. The fact that structural features which predicted antinociceptive potency following i.t. and i.c.v. injection were not identical lends support to the hypothesis that spinal and supraspinal cannabinoid binding sites and/or intracellular mechanisms may differ, albeit a relatively small number of non-substituted cannabinoid side chain analogs were evaluated and the role of pharmacokinetic differences in rates of local distribution cannot be eliminated.

The influence of chain length and position of unsaturation was further delineated in the acetylenic and ethylenic analogs with terminal substitutions. With the exception of an inactive analog (O-1068), the rigid part of the side chain was placed at the C2–C3 position in all of these substituted analogs. Ethylenic and acetylenic analogs with double or triple bonds in the C2–C3 position of pentyl and hexyl side chains and with terminal substitutions of cyano, bromo, azido, octadiynyl or carboxy groups were fully efficacious i.t. and i.c.v., but many of the acetylenic analogs were less efficacious i.v. (Martin, B.R. et al., 1999). With a shorter side chain and terminal bromo substitution (O-1187), i.c.v. activity was lost and i.t. potency was drastically decreased, suggesting again that length of the terminal side chain is an important factor in the i.c.v. potency of these rigid side chain analogs. Perhaps the most interesting pattern observed with these substituted rigid side chain analogs, however, was the differential effects of some of the substitutions on i.c.v. versus i.t. potency. Previous research found that terminal substitution of bromo or cyano increased systemic potency of Δ^8-tetrahydrocannabinol or Δ^8-tetrahydrocannabinol-dimethylheptyl side chain analogs without double or triple bonds (Charalambous et al., 1991; Martin, B.R. et al., 1993; Singer et al., 1998; Wiley et al., 1996). Similarly, cyano and bromo substitutions, as well as octadiynyl and carboxy substitutions, increased i.c.v. antinociceptive potency; however, each of these four substitutions decreased (O-965 and O-1174) or did not affect (O-823 and O-806) i.t. potency. Indeed, i.c.v. potency exceeded that of i.t. potency by 7- to 10-fold for the cyano and bromo substituted 2-hexynyl analogs (O-823 and O-806), an occurrence that was not encountered with any of the non-substituted acetylenic analogs nor with the corresponding ethylenic compounds.

The correlations between binding affinities and potencies for the ethylenic and acetylenic analogs tested in this study was moderate following i.c.v. administration ($r = 0.60$), but was notably lower after i.t. administration ($r = 0.29$). A possible explanation of this difference is that in vivo pharmacological effects might occur predominantly at supraspinal sites following i.c.v. administration, whereas spinal sites are involved following i.t. administration. Lichtman and Martin (1991) showed that spinal transection between the sixth and seventh thoracic vertebrae attenuated, but did not completely eliminate cannabinoid-induced antinociception following systemic administration. All binding assays for these cannabinoids were performed in brain tissue. The low correlation between binding and i.t. antinociceptive potency would be expected if there were differences between cannabinoid receptors in the brain and spinal cord. Although correlations between binding and i.c.v. antinociceptive potency for all of the active analogs in this acetylenic and ethylenic series were lower than those for i.v. administered tricyclic and bicyclic cannabinoids (Compton et al., 1993), they were comparable to that obtained for the i.v. antinociceptive potency of indole- and pyrrole-derived cannabinoids (Wiley et al., 1998). The reduced correlations between affinity and antinociceptive potency within the group of compounds based on an aminoalkylindole structure and within the present series of rigid side chain analogs of Δ^8-tetrahydrocannabinol is not surprising, given that these two groups of cannabinoids are more structurally diverse than are tricyclic and bicyclic cannabinoids without double or triple bonds in the side chain. However, it should be pointed out that fewer compounds were included in the correlation for the ethylenic and acetylenic side chain analogs than for more traditional cannabinoids (Compton et al., 1993).

In summary, although not conclusive, the results of the present study support a role for differential spinal and supraspinal mechanisms in the antinociceptive effects of cannabinoids. Since there is little evidence for multiple cannabinoid receptor sub-types in the central nervous system, it is reasonable to speculate that different transduction pathways may be involved in mediation of antinociceptive effects in the brain and spinal cord. This hypothesis has received support from research showing that cannabinoids interact with more than one intracellular messenger (e.g., cAMP and Ca^{2+}) and that their effects on intracellular events are dependent upon route of administration. Alternatively, as yet unidentified, cannabinoid receptor variants may be expressed in the spinal cord but not in the brain. Additional structure–activity relationship studies are needed to further delineate the different structural requirements of cannabimimetic activity spinally and supraspinally.

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