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## A major glucuronidated metabolite of JWH-018 is a neutral antagonist at CB1 receptors

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### Abstract

Recently, hydroxylated metabolites of JWH-018, a synthetic cannabinoid found in many K2/Spice preparations, have been shown to retain affinity and activity for cannabinoid type 1 receptors (CB1Rs). The activity of glucuronidated metabolites of JWH-018 is not known; hence this study investigated the affinity and activity of a major metabolite, JWH-018-N-(5-hydroxypentyl)  $\beta$ -D-glucuronide (018-gluc), for CB1Rs. The 018-gluc binds CB1Rs ( $K_i = 922$  nM), has no effect on G-protein activity, but antagonizes JWH-018 activity at CB1Rs. The data suggests that hydroxylation by cytochrome P450s and subsequent glucuronidation by UDP-glucuronosyltransferases produces a metabolite, 018-gluc, which possesses antagonistic activity at CB1Rs.

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On March 2, 2011 the U.S. Drug Enforcement Administration (DEA) temporarily classified five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, CP-47,497, and cannabicyclohexanol) as Schedule I substances,<sup>1</sup> but synthetic cannabinoid abuse is still increasing with more than 6,000 calls to Poison Control Centers in 2011,<sup>2</sup> more than double the previous year. In addition, one in nine high school seniors admitted to using a synthetic cannabinoid in the past year.<sup>3</sup> Little is known about the pharmacokinetics, metabolism, and toxicology of the synthetic cannabinoids other than their ability to induce psychoactive effects by activating one of the most abundant G-protein coupled receptors in the central nervous system, the cannabinoid type 1 receptor (CB1R).<sup>4, 5</sup>

Several published studies have investigated the metabolism and biological activity of various metabolites of the common synthetic cannabinoids JWH-018 and JWH-073.<sup>6-9</sup> A number of hydroxylated metabolites of JWH-018 retain significant *in vitro* and *in vivo* activity.<sup>6</sup> Since glucuronides of JWH-018 have been identified *in vitro* using recombinant UDP-glucuronosyltransferases (UGTs)<sup>7</sup> and almost all of the JWH-018 metabolites are

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excreted in the form of glucuronides in human urine,<sup>8</sup> studies investigating the biological activity of the glucuronide conjugates of JWH-018 are essential. This study investigated the activity and affinity of a major glucuronidated metabolite of JWH-018, a common constituent in K2/Spice, for the CB1R.

CB1R affinity was determined in [<sup>3</sup>H]CP-55,940 competition binding assays employing mouse brain homogenates. Consistent with previous reports<sup>6</sup> JWH-018 and  $\Delta^9$ -tetrahydrocannabinol (THC) bind to CB1Rs with high affinities,  $0.56 \pm 0.22$  nM and  $8.0 \pm 2.7$  nM, respectively (Figure 1A). Importantly, a major glucuronidated JWH-018 metabolite JWH-018-N-(5-hydroxypentyl)  $\beta$ -D-glucuronide (018-gluc),<sup>7</sup> which is excreted in human urine, binds to CB1Rs ( $K_i = 922$  nM, Figure 1A). In contrast, a major glucuronidated THC metabolite (+)-11-Nor- $\Delta^9$ -THC-9-carboxylic acid  $\beta$ -D-glucuronide (THC-gluc) does not bind CB1Rs ( $K_i > 10,000$  nM). [<sup>35</sup>S]GTP $\gamma$ S binding using mouse brain homogenates examined the functional activity of these compounds at CB1Rs (Figure 1B). These studies revealed that JWH-018 and THC parent compounds exhibit agonistic activity at CB1Rs, as expected, maximally activating G-proteins ( $0.115 \pm 0.016$  pmol/mg and  $0.085 \pm 0.004$  pmol/mg, respectively) when using 100 nM for JWH-018 and 1  $\mu$ M for THC. In contrast, 10  $\mu$ M of neither 018-gluc nor THC-gluc produced significant stimulation of G-protein activity ( $0.012 \pm 0.014$  pmol/mg and  $0.007 \pm 0.018$  pmol/mg, respectively). Together, these data suggest 018-gluc lacks intrinsic activity, but retains receptor binding affinity. Thus, 018-gluc may function as a neutral antagonist at CB1Rs.

To determine if 018-gluc is an antagonist at CB1Rs, [<sup>35</sup>S]GTP $\gamma$ S binding studies were conducted to examine if 018-gluc antagonizes G-protein activation produced by the agonist JWH-018. In Figure 2A, a 10 nM concentration of JWH-018 produced stimulation of CB1R G-protein activation ( $0.137 \pm 0.029$  pmol/mg protein) in mouse brains homogenates. An established CB1R antagonist, O-2050,<sup>10</sup> attenuated JWH-018-induced G-protein stimulation by 82.5% ( $0.024 \pm 0.022$  pmol/mg). Similarly, 10  $\mu$ M of 018-gluc significantly antagonized JWH-018-induced G-protein stimulation by 56.3% ( $0.060 \pm 0.018$  pmol/mg,  $P < 0.05$ ). Determination of the antagonist dissociation constant ( $K_b$ ) for 018-gluc provided an additional quantitative measurement of antagonistic affinity of 018-gluc for CB1R by measuring the effect on G-protein activation produced by co-incubation with 10  $\mu$ M of 018-gluc in the presence of increasing concentrations of JWH-018 (Figure 2B). A single 10  $\mu$ M concentration of 018-gluc significantly shifted the JWH-018 curve 3.9-fold to the right ( $ED_{50} = 9.4 \pm 1.6$  nM to  $37.0 \pm 7.2$  nM,  $P < 0.01$ ) where the antagonist dissociation constant ( $K_b$ ) of 018-gluc for CB1Rs is 3406 nM. Co-incubation with 018-gluc did not significantly alter the maximal level of G-protein activation ( $E_{max}$ ) produced by JWH-018. Hence, data indicates 018-gluc, a major glucuronidated metabolite of JWH-018, may act as a neutral, competitive antagonist at CB1Rs *in vitro*.

As previously reported, hydroxylation of JWH-018 can produce several mono-hydroxylated metabolites that retain significant agonistic activity at CB1Rs in both *in vitro* and *in vivo* models.<sup>6</sup> Glucuronidation by human UGTs of JWH-018 results in the formation of 018-gluc,<sup>7</sup> a metabolite that retains affinity and activity as a neutral antagonist at CB1Rs. The preserved affinity of the 018-gluc for CB1Rs, unlike the major glucuronide of THC may be due to the metabolic placement of the glucuronide groups on the cannabinoid structure. For example, unlike the more rigid placement of the  $\beta$ -D glucuronide on the THC structure, the glucuronide of 018-gluc is positioned on the long alkyl side chain that may allow the structure to remain flexible and retain the ability to bind the receptor (Figure 3).

While this potential mechanism requires further study, other reports support this notion by demonstrating the biological activity of JWH-018 and THC derived compounds depends on the positioning of new functional groups to the parent structure.<sup>11, 12</sup> Notably, previous

studies have established that the potency of THC-derivatives is influenced by long, alkyl side chains<sup>12</sup> that may allow the structure to remain flexible and easily interact with CB1Rs.

Since the present results indicate that 018-gluc can antagonize the activity of the parent compound at CB1Rs *in vitro*, 018-gluc may attenuate the pharmacological properties of JWH-018 *in vivo* if sufficient concentrations are formed at the site of action. However, extrapolating these *in vitro* findings is difficult because the human pharmacokinetic properties and metabolism of JWH-018 is unknown. If metabolism of JWH-018 leads to antagonism of the psychoactive effects of JWH-018 in humans, escalated use of the parent compound is expected for achieving similar levels of intoxication. Further, if the half-life of 018-gluc is shown to be longer than JWH-018, chronic use of K2 or Spice products containing JWH-018 would be expected to potentially perpetuate abuse and may lead to increased levels of tolerance and dependence in relation to cannabis abuse. For example, a recent drug discrimination study conducted using monkeys where JWH-018 possessed the same discriminatory stimulus as THC, but the duration of psychoactive effects by THC were twice as long as JWH-018.<sup>13</sup> The shorter duration of action of JWH-018 relative to THC may be due to the antagonism of JWH-018 by 018-gluc whereas THC-gluc lacks the ability to attenuate the effects of THC.

In summary, this study is the first to demonstrate that a major glucuronidated metabolite of JWH-018, a synthetic cannabinoid often found in K2 or Spice, acts as a neutral antagonist at CB1Rs *in vitro*. Future studies are required to determine if 018-gluc antagonizes the actions of JWH-018 *in vivo*.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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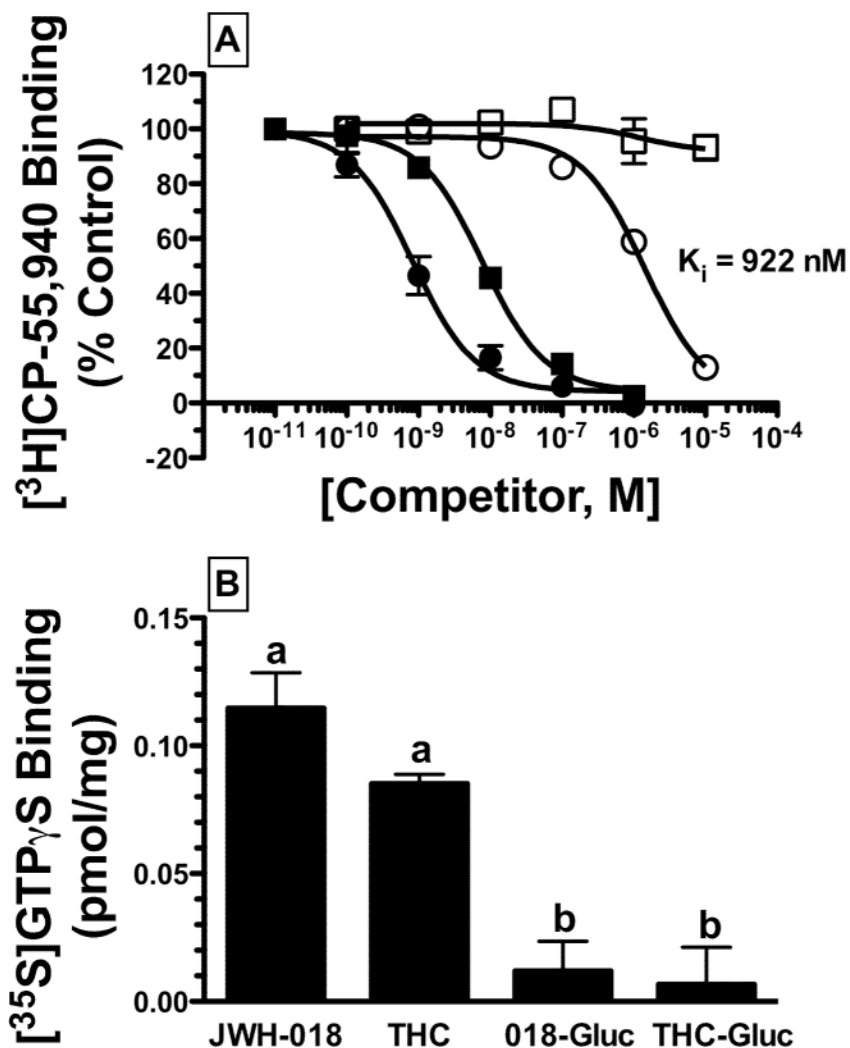
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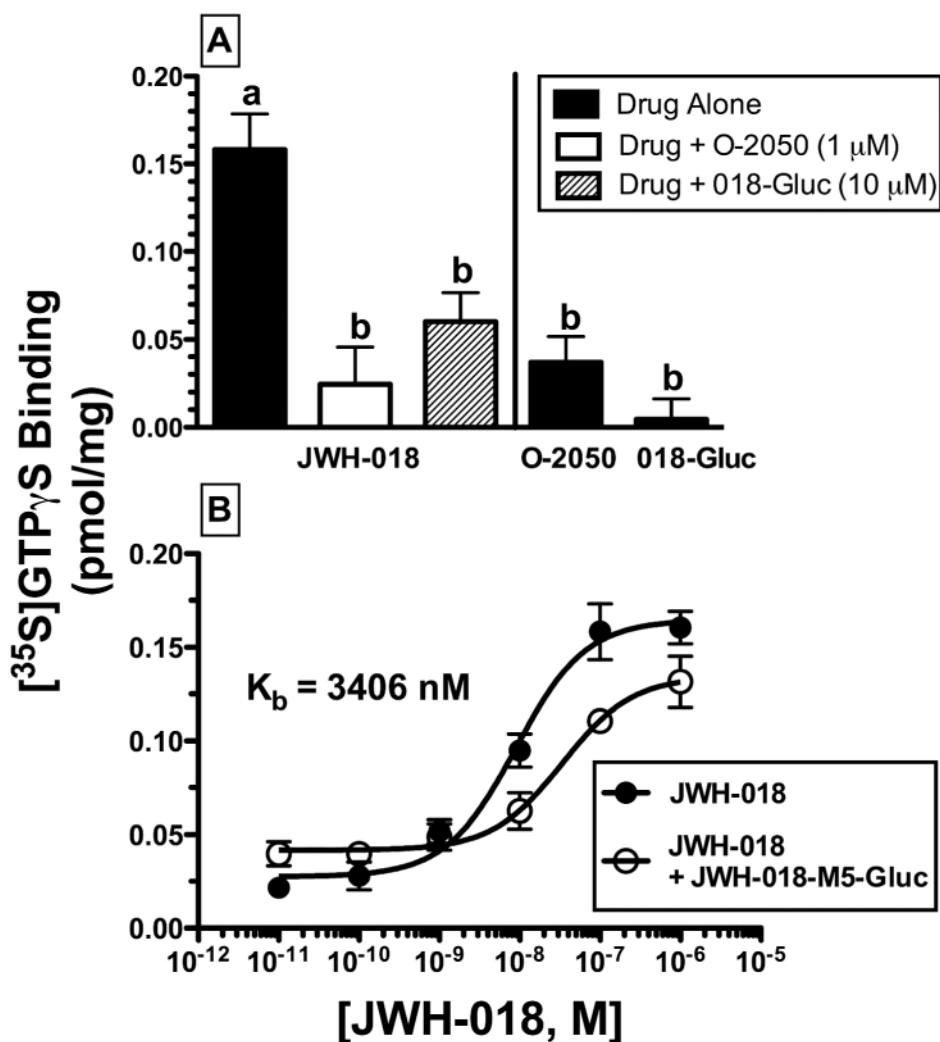
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## Abbreviations

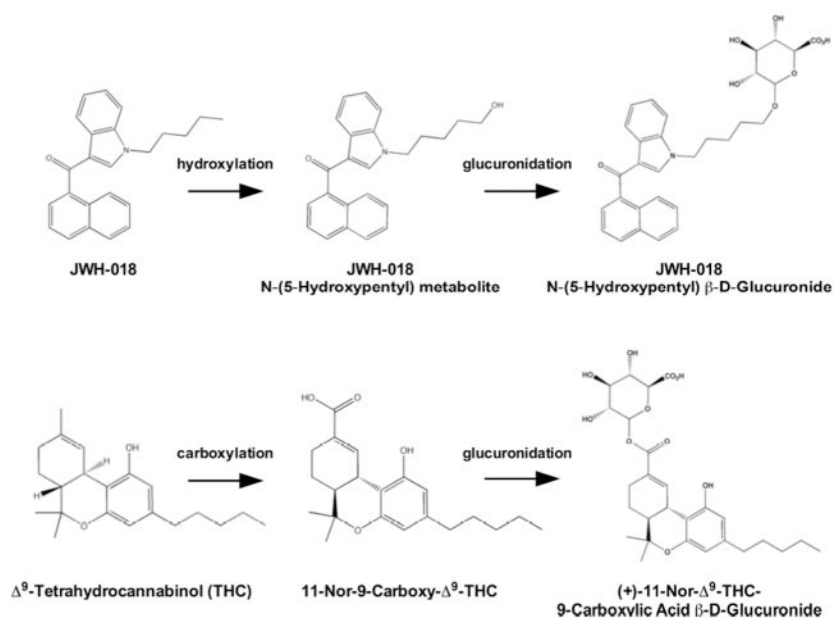
<b>018-gluc</b>	JWH-018-N-(5-hydroxypentyl) $\beta$ -D-glucuronide
<b>THC-gluc</b>	(+)-11-Nor- $\Delta^9$ -THC-9-carboxylic acid $\beta$ -D-glucuronide
<b>CB1Rs</b>	cannabinoid type 1 receptors
<b>(UGTs)</b>	UDP-glucuronosyltransferases
<b>THC</b>	$\Delta^9$ -tetrahydrocannabinol



**Figure 1.** Receptor binding and intrinsic activity of cannabinoids. JWH-018 (●) and THC (■) bind CB1Rs with nanomolar affinity (Panel A,  $n = 3$ ). 018-gluc (○) binds to CB1Rs with nanomolar affinity ( $K_i = 922 \pm 104 \text{ nM}$ ,  $n = 3$ ) whereas THC-gluc (□) does not bind CB1Rs ( $K_i > 10,000 \text{ nM}$ ,  $n = 3$ ). Panel B is the G-protein activity of JWH-018 ( $10^{-7} \text{ M}$ ,  $n = 4$ ), THC ( $10^{-6} \text{ M}$ ,  $n = 5$ ), 018-gluc ( $10^{-5} \text{ M}$ ,  $n = 3$ ) and THC-gluc ( $10^{-5} \text{ M}$ ,  $n = 3$ ) where different letters signify statistical differences between groups ( $P < 0.05$  by a one-way ANOVA with Newman-Keuls Multiple Comparison *post-hoc* test).



**Figure 2.** Antagonistic activity of 018-gluc. As shown in Panel A, JWH-018 ( $10^{-8}$  M,  $n = 6$ ) is significantly antagonized by an established CB1R antagonist O-2050 ( $10^{-6}$  M,  $n = 3$ ) and 018-gluc ( $10^{-5}$  M,  $n = 6$ ). O-2050 and 018-gluc have no significant intrinsic activity at CB1Rs. Different letters indicate statistical differences between groups ( $P < 0.05$  by a one-way ANOVA with Newman-Keuls Multiple Comparison *post-hoc* test). In Panel B, a dose-response curve of JWH-018 (●,  $ED_{50} = 9.4 \pm 1.6$  nM,  $n = 3$ ) is significantly shifted to the right during co-incubation of  $10^{-5}$  M 018-gluc (○,  $ED_{50} = 37.0 \pm 7.2$  nM,  $n = 3$ ,  $P < 0.01$  by Student's *t*-test). The  $K_b$  for 018-gluc determined by this method is 3406 nM.



**Figure 3.** Hydroxylation or carboxylation of JWH-018 and THC with subsequent glucuronidation produces the  $\beta$ -D glucuronide derivatives.