Synthetic Cannabinoid Receptor Agonist 'JWH-018' does not mimic Serotonin

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ABSTRACT

Synthetic cannabinoid receptor agonists (SCRAs) are popular recreational drugs. It has been suggested that indole-containing SCRAs may have activity at several serotonergic targets, owing to their structural similarity to the neurotransmitter serotonin (5-HT). This similarity may be responsible for features of toxicity similar to serotonin syndrome observed in cases of SCRA intoxication. To determine whether indole-SCRAs have activity at serotonergic targets we investigated the effect of JWH-018, an indole-containing SCRA, with a non-indole-containing SCRA (CP55,940) and an endocannabinoid (anandamide) on 5-HT neuronal functions in rat brain slices; 5-HT neuronal activity was examined using *in vitro* extracellular single-unit electrophysiology in the dorsal raphe nucleus and 5-HT presynaptic uptake was examined using radiolabeled 5-HT(^{3}[H] 5-HT) in the hippocampus. 5-HT (50µM) inhibited the 5-HT neuronal firing rate but JWH-018 (50µM) had no effect on the basal or NMDA (30µM) augmented firing rate. In contrast, anandamide (10uM) increased the NMDA-augmented firing rate of 5-HT neurons, but not the basal rate. The selective serotonin reuptake inhibitor fluoxetine (0.001 – 10 µM) inhibited 5-HT reuptake but JWH-018 (0.001 – 10 µM) had no effect on serotonin uptake. Our data suggest that indole-SCRAs and non-indole SCRAs do not directly interact with serotonergic targets in either the dorsal raphe nucleus or hippocampus. Further mechanistic studies are needed to determine if SCRAs affect serotonergic neurotransmission through the modulation of afferents to 5-HT neurons.

Keywords

Synthetic Cannabinoids, Serotonin, JWH-018, Dorsal Raphe Nucleus, Hippocampus.

تصنيع مستقبلات ناهض القنب JWH-018 لا ينافس السيروتونين

الخلاصة

تعد ناهضات مستقبلات اشباه القنب الصناعية (SCRA) من العقاقير الترويحية الرائجة. يدعو التشابه الهيكلي بين SCRA الحاوية على حلقة الاندول والناقل العصبي سيروتونين للاعتقاد بان هذه المركبات قد تمتلك فعالية في عدة مواقع ذات نشاط سيروتونيني. و قد يعزى لهذا التشابه التسبب باعراض سمية مشابهة لمتلازمة زيادة السيروتونين. ولتحديد اذا ما كان مركبات ال SCRA الحاوية لحلقة الاندول تتفاعل مع مستقبلات السيروتونين , فقد قمنا باختبار مادة JWH-018وهو نوع من أنواع الـ SCRA الذي يحتوي على الاندول في تركيبه الهيكلي ومقارنته مع SCRA غير محتوي على اندول (CP55,940) ومركب اناناميد (وهو من اشباه القنب الداخلية) ودراسة تأثيرهم على الوظائف العصبية السيروتونينية باستخدام شرائح من ادمغة الجرذان. تم فحص النشاط العصبي السيروتونيني باستخدام تقنية الفيزيولوجيا الكهربائية للوحدة المفردة خارج الخلية في نواة الرفاء الظهرية بينما تم فحص التأثير على إعادة الامتصاص ما قبل التشابكية للسيروتونين , باستخدام السيروتونين الموسوم اشعاعيا على منطقة الهيبوكامبس في ادمغة الجرذان. تسبب السيروتونين بتركيز 50µM بتثبيط معدل التحفيز للخلايا العصبية بينما لم يكن لمركب ال JWH-018 بتركيز 50µMأي تأثير على كل من معدلي الاطلاق للخلايا الأساسي او المعزز ب 30µM من مادة ال NMDA. في المقابل زادت مادة الاناناميد بتركيز 10µMمن معدل الاطلاق لخلايا السيروتونين العصبية المعززة ب 10µM من مادة ال NMDA ولكن ليس للمعدل الأساسي. منع

مثبط امتصاص السيروتونين الانتقائي فلوكستين بتركيز (10-0.001 µM) أعادة امتصاص السيروتونين ولكن JWH-018بتركيز (10-0.001 µM) لم يكن له أي تأثير على إعادة امتصاص السيروتونين. تشير نتائجنا الى ان مركبات SCRAالحاوية وغير الحاوية على الاندول لا تتفاعل بشكل مباشر مع مستقبلات السيروتونين سواء في نواة الرفاء الظهرية او الهيبوكامبس. قد تكون هناك حاجة لمزيد من الدراسات الميكانيكية لتحديد ان كانت الـ SCRAs تؤثر على النقل العصبي السيروتونيني عبر تعديل التغذية الواردة ما قبل الخلايا العصبية السيروتونينية.

الكلمات المفتاحية: تصنيع القنب، السيروتونين، JWH- 018، نواة الرفاة الظهرية ، الحصين.

INTRODUCTION

Synthetic cannabinoid receptor agonists (SCRAs) are novel psychoactive substances that have become popular as recreational drugs. Recently, increasing numbers of SCRA users are presenting to emergency departments with severe acute intoxication (Waugh, Najafi, and Hawkins 2016). Like Δ9-tetrahydrocannabinol (Δ9-THC), the major psychoactive component of cannabis, SCRAs activate cannabinoid 1 receptors (CB_1Rs). SCRA intoxication has been associated with clinical features consistent with serotonin (5-HT) toxicity (Louh and Freeman 2014; Papanti D. 2015). Serotonin toxicity is seen in overdoses of drugs that inhibit uptake, attenuate metabolism or promote the release of 5-HT or act as agonists at 5-HT receptors (Boyer and Shannon 2005).

CB_1R modulation of 5-HT neurotransmission is complex and there are conflicting reports of inhibitory, excitatory, and no effect at various 5-HT targets (Fisar 2012). Conflicting indirect effects on serotonergic neurotransmission have also been observed: the activation of inhibitory CB_1Rs on GABAergic interneurons reduces their inhibitory influence on dorsal raphe nuclei (DRN) 5-HT neurons, increasing 5-HT release (Tao and Ma 2012; Geddes et al. 2016; Mendiguren and Pineda 2009), whilst activation of CB_1Rs on 5-HT neuronal terminals inhibits 5-HT release (Nakazi et al. 2000). Many recreational SCRAs contain an indole moiety, which is found in serotonin and other substances that activate serotonin receptors or act as substrates for the serotonin reuptake transporter (see Figure 1; Papanti 2015). It has been suggested that indole-containing SCRAs, in addition to theCB_1R effects discussed above, could mimic serotonin and precipitate serotonin toxicity (Papanti 2015). In this study, we evaluated the effects of JWH-018, an indole-containing SCRA, on serotonin neuronal functions to determine if it acts as a mimic of serotonin or drugs targeting the serotonergic system. Rat brain slices containing serotonin neuronal cell bodies in the dorsal raphe nucleus, and presynaptic terminals in the hippocampus were used. This allowed us to evaluate several serotonergic responses, across multiple brain areas within locally intact neural architecture.

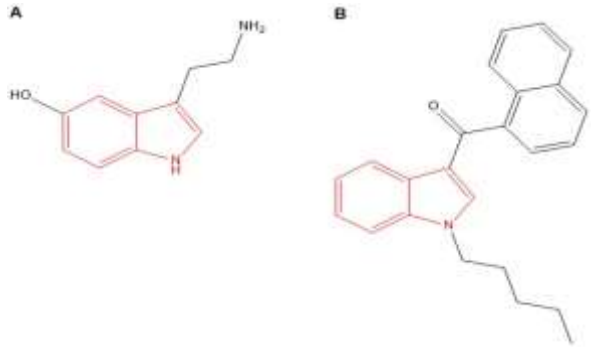

Figure 1. A. Serotonin (5-HT). **B.** JWH-018. The indole core of each molecule is highlighted in red.

MATERIALS AND METHODS

Animals

Experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 and the European Union Directive (2010/63/EC). Adult male Lister Hooded rats (Charles River, UK; *n* = 18) housed as previously described (Judge, Ingram, and Gartside 2004) overdosed with isoflurane and the brain was rapidly removed. Coronal slices were cut in ice-cold oxygenated sucrose slush using a vibrating microtome.

Drugs

The drugs N-arachidonoylethanolamine (anandamide), naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) and 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP55, 940) were purchased from Tocris Bioscience (UK); NMDA, phenylephrine and serotonin were purchased from Sigma-Aldrich (UK).

Electrophysiology Studies

Slices (300 µM) containing the DRN (bregma -7.6mm to -8.3mm) were perfused with oxygenated artificial cerebrospinal fluid (aCSF) containing the α_1-adrenoceptor agonist phenylephrine (10 µM) to simulate *in vivo* firing (Gartside et al. 2007; Judge, Ingram, and Gartside 2004). The extracellular activity was recorded from neurons displaying the electrophysiological characteristics of 5-HT neurons (Judge, Ingram, and Gartside 2004; Gartside et al. 2007). Neurons inhibited by 5-HT were classified as 5-HT neurons (*n* = 52; 63.2 ± 3.7% inhibitory response). In some experiments N-methyl-D-aspartate (NMDA) was applied for 2 minute periods to activate CB_1R expressing GABAergic inputs to the 5-HT neurons (Tao and Ma 2012; Mendiguren and Pineda 2009) which are normally silent in the DRN brain slices (Judge, Ingram, and Gartside 2004; Gartside et al. 2007). Responses to drug applications were expressed as a percentage of baseline firing rate, except excitatory NMDA responses which expressed as extra spikes. Drug (or vehicle) responses during NMDA augmented firing were then calculated as a percentage of the control NMDA response. Outliers (> mean ± 2SD) were removed from the analysis (*n* = 3). Within neuron, comparisons were made using paired t-tests or Wilcoxon signed ranks tests. Data are expressed as mean ± SEM.

Uptake Studies

Left and right dorsal hippocampi were dissected from coronal slices (400 µM; bregma -3 to -4.5 mm, 7-8 per rat), weighed (5.6 ± 0.2 mg, *n* = 151), and incubated in oxygenated aCSF (34 °C) for 30 mins. Slices were then incubated for 30 mins with drugs (or vehicle) and ^{3}[H]5-HT (8.6 nM, 26.2 kBq, PerkinElmer). ^{3}[H] was measured in incubation media and slices (incubated overnight in perchloric acid) in a scintillation counter. 5-HT uptake (Tissue (DPM/mg) / Media (DPM/ml)) for each hippocampi was calculated and non-specific uptake (ice control) subtracted (0.38 ± 0.06 T:M; *n* = 18). Average control uptake was 15.1 ± 1.3 T:M (*n* = 24). Outliers (> mean ± 2SD) were removed from the analysis (*n* = 2). ANOVAs were used to make comparisons between drugs. Data are expressed as mean ± SEM.

RESULTS

JWH-018 does not change 5-HT neuronal firing rate in the dorsal raphe nucleus.

The basal firing rate of the 5-HT neurons was 1.8 ± 0.1 Hz (*n* = 52). 5-HT inhibited the basal firing rate, whereas JWH-018 and the CB_1R agonist anandamide had no effect (Figure 2A-B). To determine if JWH-018 affects 5-HT neuronal firing when GABAergic inputs are activated, NMDA (30 µM) which is known to activate GABAergic terminals (Judge, Ingram, and

Gartside 2004) was applied. NMDA alone briefly increased the firing rate of 5-HT neurons by 143 ± 13 extra spikes (n = 38); repeated application of NMDA alone did not affect the NMDA-augmented firing rate (p = 0.14; n = 13). JWH-018 (3 min before and 2 min during NMDA application) and a non-indole-containing SCRA, CP55, 940, did not affect NMDA-augmented firing activity (Figure 2C-F). In contrast, the application of the endogenous cannabinoid, anandamide (3 min before and 2 min during NMDA application) increased the NMDA-augmented firing rate (Figure 2F). Neither the JWH-018 / CP55, 940 vehicle, DMSO (0.25%; 5 min; p = 0.92; Z -0.1; n = 16) nor the anandamide vehicle, ethanol (0.07%; 5 min; p = 0.74; Z -0.3; n = 7) affected NMDA-augmented firing rate.

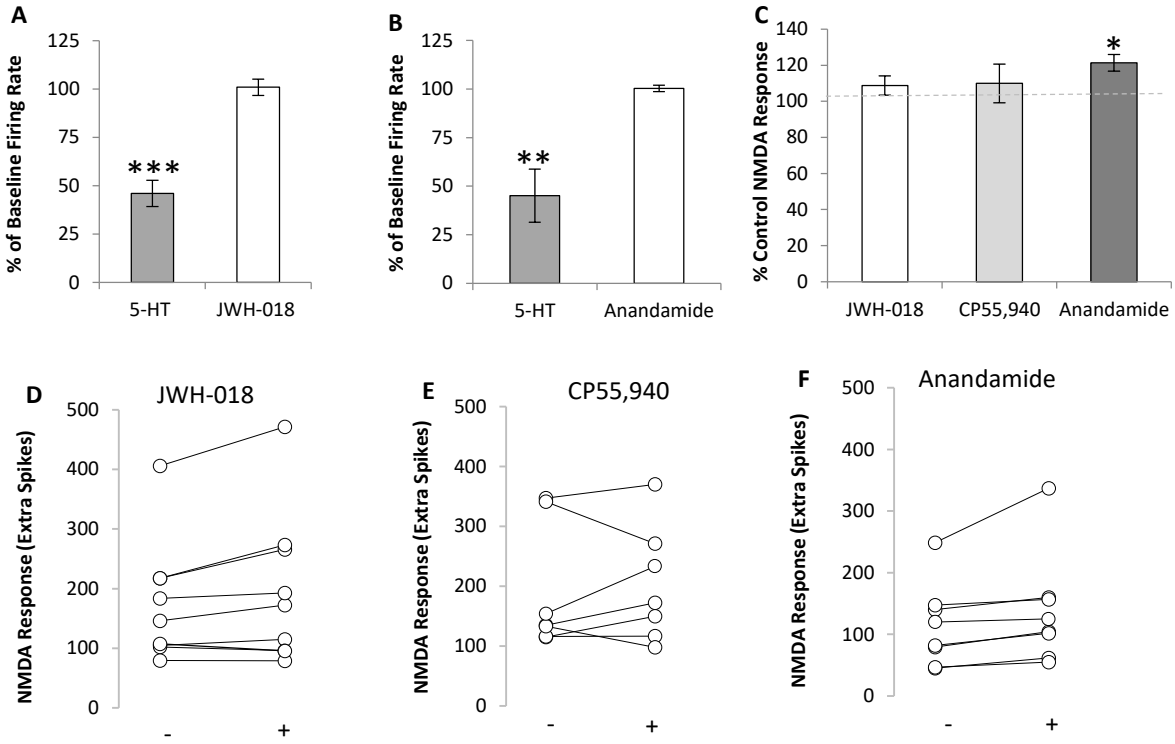

Figure 2. JWH-018 does not change the 5-HT neuronal firing rate in the dorsal raphe nucleus. A, B. 5-HT (50 µM; 2 min) inhibited the firing activity of 5-HT neurons in the DRN (**A**, n = 10, $p < 0.001$; **B**, n = 5, $p < 0.01$), but JWH-018 (**A**, 50 µM; 2 min) and anandamide (**B**, 10 µM; 2 min) had no effect on the firing rate of the same neurons. **C.** Average 5-HT firing activity. **D, E, F.** Individual firing activity in the absence (-) and presence (+) of agonists. NMDA-augmented firing activity (expressed as extra spikes) was not significantly affected by JWH-018 (**D**, 50 µM, 5 min; n = 9; p = 0.09; Z -1.7) or CP55,940 (**E**, 50 µM; 5 min; n = 7; p = 0.09; Z -1.7) but was increased by anandamide (**F,** 10 µM; 5 min; n = 8; $p < 0.05$; Z -2.5). Data shown are mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

JWH-018 does not inhibit 5-HT uptake at presynaptic terminals in the hippocampus.

The selective serotonin reuptake inhibitor fluoxetine (0.1 – 100 µM) dose-dependently inhibited ^{3}[H] 5-HT uptake into 5-HT neuronal presynaptic terminals (Figure 3A), whereas JWH-018 (0.001 – 10 µM) did not. To determine if the lack of effect of JWH-018 on 5-HT uptake was due to counteracting mechanisms of action, the effect of JWH-018 on 5-HT uptake was examined in the presence of the monoamine oxidase inhibitor pargyline and the CB_1R antagonist rimonabant. Pargyline increased control 5-HT uptake by 34 % (15.1 ± 1.3 [n = 24] vs 20.3 ± 2.0 [n = 10]). Neither JWH-018 nor CP55, 940 affected 5-HT uptake in the absence or presence of rimonabant (Figure 3B).

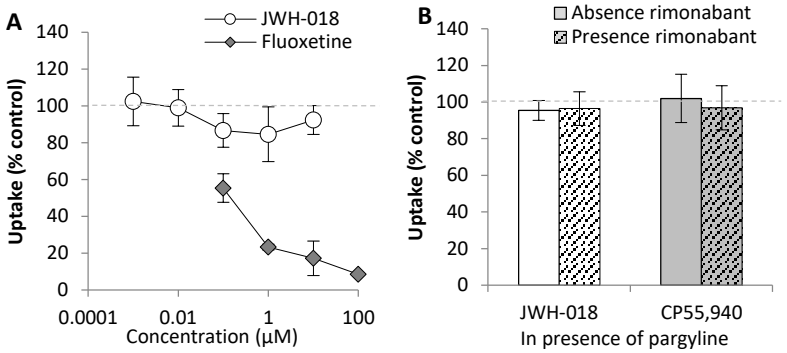

Figure 3. **JWH-018 does not inhibit 5-HT re-uptake in the hippocampus. A**. Fluoxetine inhibited ^{3}[H] 5-HT uptake into rat hippocampal terminals ($F_{4,11} = 49.4$; $p > 0.001$; $n = 3$ slices per concentration), whereas JWH-018 did not ($F_{5,63} = 1.5$; $p = 0.2$; $n = 6$ to 16 slices per group). **B**. In the presence of pargyline (10 µM) neither JWH-018 (10 µM; $F_{2,12} = 0.08$; $p = 0.92$; $n = 4$ slices per concentration) nor CP55,940 (10 µM; $F_{2,22} = 0.04$; $p = 0.96$; $n = 6$ to 8 slices per group) affected 5-HT uptake with or without rimonabant (1 µM). Data shown are mean ± SEM.

DISCUSSION

Little information is known about the effects of SCRA on serotoninergic systems in the brain. The understanding of the impact of cannabinoids on serotonin could help to understand the interaction between the two systems.

In the DRN, 5-HT neuronal firing decreases in response to 5-HT, 5-HT_{1A} agonists, and 5-HT reuptake inhibitors (Egashira et al. 2002) and monoamine oxidase inhibitor agents (Evans et al. 2008). In the hippocampus, 5-HT reuptake decreases in hippocampal pre-synaptic terminals in response to 5-HT and 5-HT reuptake inhibitors and increases with monoamine oxidase inhibitors (Azmitia and Marovitz 1980; Blackburn, French, and Merrills 1967; Ross and Renyi 1969). Our data suggest that JWH-018 does not activate 5-HT_{1A} receptors, nor does it act as a substrate or block the hippocampal 5-HT reuptake transporter or inhibit monoamine oxidase. The lack of effect at neuronal cell bodies or presynaptic terminals indicates that serotonergic neurons are unlikely to be the primary site of action of SCRAs for the observed features of serotonin toxicity. A study demonstrated that 5F-ADB, an indazole analogue of JWH-018, did not have effects on midbrain serotonergic neuron firing rate (Asaoka et al. 2016) consistent with our data. Velenovská et al. (2007) investigated the effect of cannabinoids on 5-HT function on platelets from chronic cannabis smokers (Velenovská and Fišar, 2007). They reported that high concentrations of cannabinoids (Δ9 THC, anandamide and WIN 55,212-2) can inhibit 5-HT transporter activity acutely. This inhibition is non- competitive, which indicates that cannabinoids indirectly inhibited 5-HT transporter activity through the changes on membrane lipids. Although studying 5-HT uptake using platelets models has been shown to be a successful method (Stahl and Meltzer, 1978, Lesch et al., 1993), it does not represent the complexity of the brain where there is an integration and interaction between different receptors, neurotransmitters and /or transporters. In addition, Velenovská noted that high concentrations of tested cannabinoids are required to induce 5-HT uptake inhibition. In contrast, this study tested 5-HT uptake in rat brain hippocampus, where most of the serotonergic features are expressed.

The possible serotonergic effects of cannabinoids may be mediated through modulation of projections to serotonin neurons from other brain areas (Geddes et al. 2016), however, our evidence would suggest GABAergic inputs are not responsible. Further mechanistic studies should be conducted to evaluate the effects of SCRAs

on projections to serotonergic neurons from other brain areas.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Asaoka, Nozomi, Hiroyuki Kawai, Naoya Nishitani, Haruko Kinoshita, Norihiro Shibui, Kazuki Nagayasu, Hisashi Shirakawa, and Shuji Kaneko. 2016. 'A new designer drug 5F-ADB activates midbrain dopaminergic neurons but not serotonergic neurons', *The Journal of toxicological sciences*, 41: 813-16.

Azmitia, E. C., and W. F. Marovitz. 1980. 'In vitro hippocampal uptake of tritiated serotonin (3H-5HT): A morphological, biochemical, and pharmacological approach to specificity', *Journal of Histochemistry & Cytochemistry*, 28: 636-44.

Blackburn, K. J., P. C. French, and R. J. Merrills. 1967. '5-Hydroxytryptamine uptake by rat brain in vitro', *Life sciences*, 6: 1653-63.

Boyer, Edward W., and Michael Shannon. 2005. 'The serotonin syndrome', *New England Journal of Medicine*, 352: 1112-20.

Egashira, N., K. Mishima, S. Katsurabayashi, T. Yoshitake, Y. Matsumoto, J. Ishida, M. Yamaguchi, K. Iwasaki, and M. Fujiwara. 2002. 'Involvement of 5-hydroxytryptamine neuronal system in Delta(9)-tetrahydrocannabinol-induced impairment of spatial memory', *Eur J Pharmacol*, 445: 221-9.

Evans, A. K., N. Reinders, K. A. Ashford, I. N. Christie, J. B. Wakerley, and C. A. Lowry. 2008. 'Evidence for serotonin synthesis-dependent regulation of in vitro neuronal firing rates in the midbrain raphe complex', *Eur J Pharmacol*, 590: 136-49.

Fisar, Z. 2012. 'Cannabinoids and monoamine neurotransmission with focus on monoamine oxidase', *Prog Neuropsychopharmacol Biol Psychiatry*, 38: 68-77.

Gartside, S. E., A. J. Cole, A. P. Williams, R. McQuade, and S. J. Judge. 2007. 'AMPA and NMDA receptor regulation of firing activity in 5-HT neurons of the dorsal and median raphe nuclei', *Eur J Neurosci*, 25: 3001-8.

Geddes, S. D., S. Assadzada, D. Lemelin, A. Sokolovski, R. Bergeron, S. Haj-Dahmane, and J. C. Beique. 2016. 'Target-specific modulation of the descending prefrontal cortex inputs to the dorsal raphe nucleus by cannabinoids', *Proc Natl Acad Sci U S A*, 113: 5429-34.

Judge, S. J., C. D. Ingram, and S. E. Gartside. 2004. 'GABA receptor modulation of 5-HT neuronal firing: characterization and effect of moderate in vivo variations in glucocorticoid levels', *Neurochem Int*, 45: 1057-65.

Lesch, K. P., WOLOZIN, B. L., MURPHY, D. L. & RIEDERER, P. 1993. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. Journal of neurochemistry, 60, 2319-2322.

Louh, I. K., and W. D. Freeman. 2014. 'A 'spicy' encephalopathy: synthetic cannabinoids as cause of encephalopathy and seizure', *Crit Care*, 18: 553.

Mendiguren, A., and J. Pineda. 2009. 'Effect of the CB(1) receptor antagonists rimonabant and AM251 on the firing rate of dorsal raphe nucleus neurons in rat brain slices', *Br J Pharmacol*, 158: 1579-87.

Nakazi, M., U. Bauer, T. Nickel, M. Kathmann, and E. Schlicker. 2000. 'Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors', *Naunyn-Schmiedeberg's archives of pharmacology*, 361: 19-24.

Papanti D., Orsolini L., Bonavigo T., Sandri F., Pascolo-Fabrici E., Schifano F. 2015.

"Synthetic Cannabinoids and the Serotonin Syndrome: An Unforseen Association" In *IV International Congress Dual Disorders. Addictions and other mental disorders*, edited by IV International Congress Dual Disorders. Addictions and other mental disorders, 117. Barcelona: IV International Congress Dual Disorders. Addictions and other mental disorders.

Ross, S. B., and A. L. Renyi. 1969. 'Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue', *European journal of pharmacology*, 7: 270-77.

Tao, R., and Z. Ma. 2012. 'Neural Circuit in the Dorsal Raphe Nucleus Responsible for Cannabinoid-Mediated Increases in 5-HT Efflux in the Nucleus Accumbens of the Rat Brain', *ISRN Pharmacol*, 2012: 276902.

Stahl, S. M. & MELTZER, H. Y. 1978. A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with central serotonergic neurons. Journal of Pharmacology and Experimental Therapeutics, 205, 118-132.

Velenovska, M. & Fisar, Z. 2007. PRECLINICAL STUDY: Effect of cannabinoids on platelet serotonin uptake. Addiction biology, 12, 158-166.

Waugh, J., J. Najafi, and L. Hawkins. 2016. 'Epidemiology and clinical features of toxicity following recreational use of synthetic cannabinoid receptor agonists. A report from the United Kingdom National Poisons Information Service', *Clinical Toxicology*, 54: 543-43.