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Salvinorin A analogs and other kappa opioid receptor compounds as treatments for cocaine abuse

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Abstract

Acute activation of κ opioid receptors produces anti-addictive effects by regulating dopamine levels in the brain. Unfortunately, classic κ opioid agonists have undesired side effects such as sedation, aversion and depression which restrict their clinical use. Salvinorin A (Sal A), a novel κ opioid receptor agonist extracted from the plant *Salvia divinorum*, has been identified as a potential therapy for drug abuse and addiction. Here, we review the preclinical effects of Sal A in comparison with traditional κ opioid agonists and several new analogues. Sal A retains the anti-addictive properties of traditional κ opioid receptors agonists with several improvements including reduced side effects. However, the rapid metabolism of Sal A makes it undesirable for clinical development. In an effort to improve the pharmacokinetics and tolerability of this compound, κ opioid receptor agonists based on the structure of Sal A have been synthesized. While work in this field is still in progress, several analogues with improved pharmacokinetic profiles have been shown to have anti-addiction effects. While in its infancy, it is clear that these compounds hold promise for the future development of anti-addiction therapeutics.

Keywords

Kappa opioid receptor; Salvinorin A; addiction; drug abuse; *Salvia divinorum*

Introduction to Salvinorin A

Salvinorin A (Sal A) is the active compound isolated from *Salvia divinorum*, a member of the Lamiaceae (mint) family (Ortega, Blount, & Manchand, 1982) classified in 1962 by Epling and Jativa (Epling & Jativa-M, 1962). Traditionally *Salvia divinorum* has been used as a medicine of the Mazatec Indians of Oaxaca Mexico. It has hallucinogenic effects in humans (Siebert, 1994) and is reported to be the most potent naturally occurring hallucinogen. Unlike other known hallucinogens, it does not bind or activate serotonergic pathways (5HT_{2A}), and is a potent and selective κ -opioid receptor (KOPr) agonist (Roth, Baner, Westkaemper, Siebert, Rice, Steinberg et al., 2002). In humans, Sal A does not

produce the same effects in resting electroencephalogram when compared to other hallucinogens such as mescaline or ketamine suggesting that it has different psychomimetic actions (Ranganathan, Schnakenberg, Skosnik, Cohen, Pittman, Sewell et al., 2012). Another unique property of Sal A is that it was the first identified KOPr agonist with a non-nitrogenous structure.

Sal A was found to be a full agonist at the KOPr (Roth et al., 2002) and has similar efficacy to 2-(3,4-dichlorophenyl)-N-methyl-N-[(2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide (U50,488), N-methyl-2-phenyl-N-[(5R,7S,8S)-7-pyrrolidin-1-yl-1-oxaspiro[4.5]decan-8-yl]acetamide (U69,593) and the endogenous KOPr peptide dynorphin A in GTP- γ S assays (Chavkin, Sud, Jin, Stewart, Zjawiony, Siebert et al., 2004; Prevatt-Smith, Lovell, Simpson, Day, Douglas, Bosch et al., 2011).

The novel properties of Sal A has led many researchers to re-evaluate the KOPr system for potential therapies known to be modulated by kappa mediated pathways including anti-addiction effects, often in comparison with the endogenous KOPr ligands and traditional acrylacetamide KOPr agonists (Morani, Kivell, Prisinzano, & Schenk, 2009; Shippenberg, Zapata, & Chefer, 2007; Wang, Sun, Tao, Chi, & Liu, 2010) (See Wee & Koob, 2010) for recent review)). Sal A reduces the adverse actions of morphine such as tolerance, reward, learning and memory (reviewed in Wang et al., 2010), and can be used to treat pain (for review see: McCurdy, Sufka, Smith, Warnick, & Nieto, 2006), particularly when KOPr agonists are peripherally restricted (reviewed in Kivell & Prisinzano, 2010). Sal A has also been investigated as a non-addictive analgesic (Groer, Tidgewell, Moyer, Harding, Rothman, Prisinzano et al., 2007; McCurdy et al., 2006), and neuroprotective agent (Su, Riley, Kiessling, Armstead, & Liu, 2011; Wang, Ma, Riley, Armstead, & Liu, 2012). While Sal A has been found to have many actions similar to traditional kappa opioid agonists there are many differences in its actions. Sal A has been shown to induce analgesia (McCurdy et al., 2006), has both aversive (behavioural conditional place aversion models) (Zhang, Butelman, Schlussman, Ho, & Kreek, 2005) and rewarding effects (Braidia, Limonta, Capurro, Fadda, Rubino, Mascia et al., 2008) as well as pro-depressive (Carlezon, Beguin, DiNieri, Baumann, Richards, Todtenkopf et al., 2006; Morani, Schenk, Prisinzano, & Kivell, 2012) and anti-depressive effects (Braidia, Limonta, Pegorini, Zani, Guerini-Rocco, Gori et al., 2007; Hanes, 2001). While many of these contradicting effects can be explained by use of different doses and acute versus chronic administration, a clearer understanding of these effects and their underlying mechanisms are needed. Recent developments in the understanding of 'functional selectivity' or 'biased agonism' whereby multiple agonists acting on the same receptor are able to have different effects has led to greater interest into the effects of KOPr agonists and potential signalling pathways relating to various behavioural effects. There is renewed hope that KOPr agonists possessing desirable anti-addiction effects without unwanted side effects may be identified.

To this end, many of the studies conducted to determine the biological and cellular effects of Sal A have been done in comparison to classic KOPr agonists such as U50,488 or, U69,593, enadoline or dynorphin A. These compounds have all been investigated for their ability to modulate addiction related behaviours and are briefly outlined here followed by comparisons with the effects of Sal A.

Kappa Opioid Receptors and the Endogenous Opioid System

KOPr is a pertussis toxin sensitive G-protein coupled receptor that exerts its effects in the brain and intestines (Avidorreiss, Zippel, Levy, Saya, Ezra, Barg et al., 1995). There are 3 known pharmacological variants of KOPr: KOPr1, KOPr2, and KOPr3 but the only subtype that has been cloned to date is KOPr1 (Heyliger, Jackson, Rice, & Rothman, 1999; Horan, Decosta, Rice, Haaseth, Hruby, & Porreca, 1993; Yasuda, Raynor, Kong, Breder, Takeda, Reisine et al., 1993). KOPr is enriched in brain circuitry involved in the control of motivation and mood, and is found in various neocortical areas, including the olfactory bulb, amygdala, basal ganglia, external globus pallidus, hippocampus, thalamus, hypothalamus, ventral tegmental area (VTA) and locus coeruleus (Simonin, Gaveriaux-Ruff, Befort, Matthes, Lannes, Micheletti et al., 1995).

Dynorphin is a posttranslational product of the PDYN gene. Prodynorphin is cleaved into several types of dynorphin by proprotein convertase 2 including dynorphin A, dynorphin B, and big dynorphin (Marinova, Vukojevic, Surcheva, Yakovleva, Cebers, Pasikova et al., 2005). Dynorphins are widely distributed throughout the central nervous system (Watson, Khachaturian, Coy, Taylor, & Akil, 1982) with high levels found in the substantia nigra (SN), hypothalamus, caudate nucleus, pallidus and putamen (Gramsch, Holtt, Pasi, Mehraein, & Herz, 1982). Lower amounts of dynorphin can also be found in the amygdala, hippocampus, periaqueductal gray, colliculi, pons, medulla and the area postrema (Gramsch et al., 1982). Dynorphin A (1–17), the most active form of dynorphin, preferentially activates KOPr although it does have some affinity for μ -opioid receptor and δ -opioid receptor (Chavkin, James, & Goldstein, 1982; Merg, Filliol, Usynin, Bazov, Bark, Hurd et al., 2006).

Activation of the endogenous KOPr system leads to several negative behavioural effects including stress and aversion (Wee & Koob, 2010), depression (Knoll & Carlezon Jr, 2010), anxiety (Van't Veer & Carlezon Jr, 2013), hypothermia (Spencer, Hruby, & Burks, 1988), increased submissive behaviour (Shippenberg et al., 2007), sedation (Dykstra, Gmerek, Winger, & Woods, 1987), and modulation of drug-seeking behaviours (Bruchas, Land, & Chavkin, 2010; Butelman, Yuferov, & Kreek, 2012; Liu-Chen, 2004; reviewed in Wee and Koob, 2010). It is generally accepted that stimulation of the KOPr/dynorphin system antagonises the hedonic or rewarding effects of drugs of abuse (Nestler, 2001; Shippenberg et al., 2007). It has also been suggested that these effects are via punishment or aversive-like effects, which directly oppose the actions of the μ -opioid system. Accordingly, the KOPr system is upregulated by acute and chronic exposure to drugs of abuse such as cocaine and morphine (Tjon, Voorn, Vanderschuren, DeVries, Michiels, Jonker et al., 1997; Unterwald, Rubenfeld, & Kreek, 1994; Wang, Zhou, Spangler, Ho, Han, & Kreek, 1999).

Stimulation of the dopaminergic system with cocaine has also been shown to increase dynorphin levels (Daunais, Roberts, & McGinty, 1993; Spangler, Unterwald, & Kreek, 1993). Further support for the role of KOPr in depressive-like behaviours and anhedonia following KOPr activation are that these behavioural effects are blocked by prior administration of KOPr antagonists (Chartoff, Sawyer, Rachlin, Potter, Pliakas, & Carlezon, 2012; Shirayama, Ishida, Iwata, Hazama, Kawahara, & Duman, 2004; Zhang, Shi, Woods,

Watson, & Ko, 2007). The dysphoric and aversive effects of KOPr activation have been shown to require intact mesoaccumbal dopamine (DA) neurotransmission and studies also indicate that decreased DA neurotransmission underlies these dysphoric effects (Dichiara & Imperato, 1988; Shippenberg, Balskubik, & Herz, 1993; Wee & Koob, 2010). KOPr activation also decreases 5-HT release in freely moving rats (Tao & Auerbach, 2002, 2005) and mediates stress-induced anxiety like behaviours in mice (Bruchas, Land, Lemos, & Chavkin, 2009). Recently, a review of positron emission tomography studies highlighted the importance of the KOPr/dynorphin system in regulation of DA transmission contributing to the hypodopaminergic state observed in cocaine addiction (Trifilieff & Martinez, 2013).

Kappa Opioid Regulation of Dopamine Systems

KOPr's are located in tyrosine hydroxylase positive neurons that are found in locations directly opposed to dopamine transporters (DAT) (Svingos, Chavkin, Colago, & Pickel, 2001). DAT is a Na^+/Cl^- symporter protein from the SLC6 gene family that is expressed in neurons located in the VTA, SN, dorsal striatum (dStr) and nucleus accumbens (NAcb), areas crucial to the brain reward pathway (Boja & Kuhar, 1989; Scheffel, Pogun, Stathis, Boja, & Kuhar, 1991). Dopaminergic neurons that innervate the NAcb were found to contain DAT protein on their axons, dendrites and cell bodies (Nirenberg, Chan, Pohorille, Vaughan, Uhl, Kuhar et al., 1997). DAT functions as a major regulator of DA levels in the synapse and acts by reuptaking DA into presynaptic terminals, where it is either recycled for further use or degraded by monoamine oxidases or catechol- O-methyltransferase (Guo, Zhou, Sun, Wu, Haile, Kosten et al., 2007). Administration of synthetic KOPr agonists have been shown to regulate extracellular DA concentrations by both decreasing DA release and increasing DAT activity in the NAcb core and shell (Thompson, Zapata, Justice, Vaughan, Sharpe, & Shippenberg, 2000).

The cell surface expression of DAT is regulated by DA, with acute exposure leading to increased DAT expression and chronic exposure leading to a decrease in DAT cell surface expression (Chi & Reith, 2003; Furman, Chen, Guptaroy, Zhang, Holz, & Gnegy, 2009; Gulley, Doolen, & Zahniser, 2002; Saunders, Ferrer, Shi, Chen, Merrill, Lamb et al., 2000). Thompson et al. (2000) found that administration of KOPr agonists increased DAT activity, effectively reducing the concentration of extracellular DA. It has been suggested that this is one of the possible mechanisms by which KOPr agonists exert their effects. These KOPr effects on DA uptake and release functionally oppose the actions of cocaine and other drugs of abuse that reduce DA uptake (Collins, D'Addario, & Izenwasser, 2001; Thompson et al., 2000). Activation of mesoaccumbal DA neurotransmission and a decrease in dopamine D_1 receptor activation has been shown to underlie the aversive effects of KOPr agonists (Shippenberg, Bals-Kubik, Huber, & Herz, 1991; Shippenberg et al., 1993; Shippenberg & Herz, 1987, 1988). Differential regulation of DA levels by multiple signalling pathways and mechanism, such as DA release and reuptake in various brain regions is another possible mechanism by which Sal A and novel KOPr agonists may regulate addiction, stress, depression, sedation and aversion.

Effects of Drugs of Abuse on the Kappa Opioid System

Both acute and chronic administration of drugs of abuse upregulates the kappa opioid system. Spangler et al. (1997) found that preprodynorphin mRNA in the caudate putamen of rats was increased after acute administration of cocaine. This was shortly followed by preproenkephalin mRNA and KOPr mRNA after the second day of 'binge' cocaine (Spangler, Zhou, Maggos, Schlussman, Ho, & Kreek, 1997). Initial cocaine exposure in rhesus monkeys self-administering cocaine also activated the PDYN mRNA expression in the caudate putamen (Fagergren, Smith, Daunais, Nader, Porrino, & Hurd, 2003).

Chronic cocaine administration upregulates KOPr expression in the NAc shell, caudate putamen, claustrum and endopiriform nucleus (Collins, Kunko, Ladenheim, Cadet, Carroll, & Izenwasser, 2002). This increase is also accompanied by an elevation in preprodynorphin mRNA levels (Collins et al., 2001; Collins et al., 2002; Tzaferis & McGinty, 2001). The kappa opioid system remains upregulated for several days following cocaine use, which has been linked to dysphoric effects during withdrawal (Hurd & Herkenham, 1993; Kreek, 1996; Sivam, 1989; Smiley, Johnson, Bush, Gibb, & Hanson, 1990). Repeated cocaine also alters mesoaccumbal, mesocortical (Gehrke, Chefer, & Shippenberg, 2008) and nigrostriatal DA neurotransmission and results in decreased basal DA neurotransmission during cocaine withdrawal in mice (Zhang, Schlussman, Rabkin, Butelman, Ho, & Kreek, 2013).

Effects of Kappa Opioid Receptor Agonists on Drug Addiction

KOPr agonists have little abuse potential and are not self-administered (Tang & Collins, 1985). The ability of KOPr agonists to have profound effects on motivational reward and emotional states have led to studies manipulating this system with the aim of identifying the way KOPr agonists exert anti-addiction effects. In the same manner, the ability of KOPr antagonists to modulate depressive and anxiety related behaviours, particularly relating to stress induced relapse, are also studied.

There are three major stages of the addiction cycle, the initial bingeing stage, followed by withdrawal (and the negative effects associated with withdrawal) and finally the preoccupation or anticipation stage that leads to further drug taking (Koob & Le Moal, 2008). These hypotheses of the drug addiction cycle incorporate reinforcement of the drugs of abuse as well as neuroadaptations in the reward and stress systems within the brain (Koob, 2008). KOPr agonists are hypothesised to play a role in combating addiction at the binge or intoxication phase by attenuating the rewarding effects of drugs of abuse. The mesolimbic DA system, containing DA projections from the VTA to the NAc is modulated by KOPr agonists including endogenous dynorphin which functions to decrease the rewarding effects of drugs of abuse, for recent reviews see: (Butelman et al., 2012; Picetti, Schlussman, Zhou, Ray, Ducat, Yuferov et al., 2013; Shippenberg, 2009; Wee & Koob, 2010). On the other hand, KOPr antagonists are of potential therapeutic use later in the addiction cycle as they have been shown to modulate stress pathways in addiction. KOPr antagonists hold therapeutic promise for their ability to reduce stress-induced relapse (Chavkin, 2011; Picetti et al., 2013). The *in vivo* effects of KOPr antagonists are variable with long-lasting increases in alcohol self-administration seen in rats exposed to a single

injection of nor-BNI. These results also suggest that the effects of KOPr agonists are due to direct modulation of the reward circuitry, rather than by increasing the aversive effects of KOPr agonists (Mitchell, Liang, & Fields, 2005). Other studies show that nor-BNI decreases ethanol self-administration in ethanol dependent rats (Walker, Zorrilla, & Koob 2010). Nor-BNI alone did not alter cocaine self-administration in rhesus monkeys but did prevent KOPr agonist induced decreases in cocaine self-administration in rhesus monkeys (Glick, Maisonneuve, Raucci, & Archer, 1995) and rats (Negus, Mello, Portoghese, & Lin, 1997) and in an extended cocaine access model in rats attenuated cocaine intake (Wee, Orio, Ghirmai, Cashman & Koob; 2009). Studies utilising the KOPr antagonist JDTC have also been shown to attenuate alcohol self-administration in the rat (Schank, Goldstein, Rowe, King, Marusich et al., 2012). Recently, attenuation of stress-induced drug-seeking by KOPr antagonists has been shown to hold promise for potential therapeutics (Beardsley, Pollard, Howard, and Carroll, 2010; Butelman et al., 2012), for recent review of pre-clinical and clinical effects of KOPr antagonists see (Carroll & Carlezon, 2013). Here we will focus on KOPr agonists and their potential role in the development of pharmacotherapies to reduce the rewarding effects of drugs of abuse, particularly cocaine.

Activation of the kappa opioid system opposes the actions of the μ -opioid system. It is generally accepted, based on the large body of evidence in animal models, that stimulation of the kappa system antagonises the rewarding effects of drugs of abuse (Mello & Negus, 2000; Shippenberg, Chefer, Zapata, & Heidbreder, 2001) by modulating DA levels in the central nervous system (Dichiara & Imperato, 1988; Jackisch, Hotz, & Hertting, 1993; Margolis, Hjelmstad, Bonci, & Fields, 2003; Spanagel, Herz, & Shippenberg, 1992; Suzuki, Kishimoto, Ozaki, & Narita, 2001; Werling, Frattali, Portoghese, Takemori, & Cox, 1988). It has also been suggested that these effects are via punishment or aversive-like effects. In particular, KOPr activation modulates DA uptake in the NAc via DAT in the rat (Thompson et al., 2000) and directly inhibits DA neurons in the midbrain in whole-cell patch-clamp recordings in rat brain slices (Margolis et al., 2003). Repeated treatment with the traditional KOPr agonist U69,593 also alters dopamine D₂ receptor mRNA levels (Perreault, Graham, Scattolon, Wang, Szechtman, & Foster, 2007) and function in the rat (Acri, Thompson, & Shippenberg, 2001). Recently, Mori et al. (2012) showed that in discriminative stimulus tests in the rat, the dopamine D₂ receptor agonist sulpiride generalised to the effects of U50,488 (3mg/kg), an effect that was not seen with the dopamine D₁ antagonist SCH23390. This suggests that postsynaptic dopamine D₂ receptors are critical for the induction of the discriminative stimulus effects induced by U50,488. This was also suggested to be due to suppression of the Akt pathway (Mori, Yoshizawa, Ueno, Nishiwaki, Shimizu, Shibasaki et al., 2013). Given the recently identified physical and functional interaction between dopamine D₂ receptors and DAT (Bolan, Kivell, Jalingam, Oz, Jayanthi, Han et al., 2007) and regulation of DAT by KOPr agonists there is further need for investigations into the role KOPr agonists have on these proteins regulating DA levels.

Chronic cocaine exposure results in adaptations in the kappa opioid system (Butelman et al., 2012; Gehrke et al., 2008); while KOPr activating drugs prevent alterations in brain function that occur as a consequence of repeated drug use (Chefer, Czyzyk, Bolan, Moron, Pinar, & Shippenberg, 2005; Chefer, Moron, Hope, Rea, & Shippenberg, 2000; El Daly, Chefer, Sandill, & Shippenberg, 2000). These studies reinforce the function of the kappa opioid

system as a part of a negative feedback loop that buffers the increases in DA levels in response to cocaine and functions to maintain a steady state (Chefer et al., 2005; Spanagel et al., 1992). These studies strongly support the potential of KOPr agonists as anti-addictive pharmacotherapies. Unfortunately, classic selective and potent KOPr agonists such as U50,488 and U69,593 produce undesired side effects such as aversion, depression, dysphoria, emesis and sedation (Prisinzano, Tidgewell, & Harding, 2005; Shippenberg et al., 2007; Todtenkopf, Marcus, Portoghese, & Carlezon, 2004; Wee & Koob, 2010). Clinically, activation of the kappa opioid system has been associated with adverse effects such as confusion, hallucinations and visual distortions (Johnson, MacLean, Reissig, Prisinzano, & Griffiths, 2011; Pfeiffer, Brantl, Herz, & Emrich, 1986; Walsh, Geter-Douglas, Strain, & Bigelow, 2001). These side effects have restricted the use of classic KOPr agonists as anti-addictive agents (Walsh, Strain, Abreu, & Bigelow, 2001). Several strategies are being employed to develop KOPr based pharmacotherapies with varying binding and selectivity for kappa and other opioid receptors.

Previous studies have shown that dynorphin A (1) blocks cocaine-induced increases in striatal dopamine levels; (2) blocks cocaine-induced place preference; and (3) attenuates cocaine induced locomotor activity (Zhang, Butelman, Schlussman, Ho, & Kreek, 2004). However, the therapeutic potential of KOPr agonist selective peptides is relatively unexplored compared to small molecules. This is likely to continue given the difficulties in overcoming pharmacokinetic problems and rapid metabolism.

The structurally unique properties of Sal A have identified this novel KOPr activating compound as a lead for the development of new anti-addiction pharmacotherapies. There are studies that show differences between Sal A and traditional KOPr agonists in terms of structural binding, activation, behavioural as well as cellular effects. In the following sections, we will discuss both the behavioural and cellular effects of Sal A and its analogs.

Animal Studies with Salvinorin A

Sal A has been shown to decrease the effects of cocaine in preclinical models. Studies have shown that Sal A modulates cocaine-seeking behaviour and attenuates behavioural sensitisation by modulating DA levels within the reward pathways. Recently Sal A (0.3mg/kg) was shown to attenuate cocaine-prime-induced reinstatement, similar to U69,593 (0.3 mg/kg), U50,488 (30 mg/kg) and spiradoline (1 mg/kg) (Morani et al., 2009). Sal A also attenuated the expression of cocaine-induced behavioural sensitization in rats (Morani et al., 2012) comparable to U69,593 (Heidbreder, Babovicvuksanovic, Shoaib, & Shippenberg, 1995). The decrease in cocaine self-administration with traditional KOPr agonist U69,593 was accompanied by a decrease in food intake in rhesus monkeys (Mello & Negus, 1998; Negus, et al., 1997) and decreases in self-administration were only apparent when rats were trained with a cue (Schenk, Partridge, & Shippenberg, 1999). These data suggest that traditional KOPr agonists may decrease the general responding of lab animals rather than specifically targeting the rewarding effects of cocaine. In contrast to these effects, Sal A did not attenuate responses for a natural reward in rats (10% sucrose solution) at the dose (0.3 mg/kg) (intraperitoneal, ip) that attenuated cocaine prime-induced reinstatement (Morani et al., 2009). Morani et al. (2012) also showed that 0.3 mg/kg of Sal A had no effect on

spontaneous locomotion, cocaine induced stereotypy, cocaine induced hyperactivity, or conditioned taste aversion, suggesting that the Sal A dose sufficient to produce attenuation of drug-seeking in rats does not cause sedation or aversion. However, decreased swimming and increased immobility times were observed in the forced swim test (FST) indicating that pro-depressive effects are still seen at this dose. Together, this data distinguishes, in part, the behavioural effects of Sal A from traditional KOPr agonists.

Studies to compare the effects of Sal A with traditional KOPr agonists in their ability to modulate mesocortical limbic DA signalling have also been conducted recently. Gehrke et al. (2008) utilised quantitative microdialysis techniques to show that acute, locally administered (200 nM), but not repeated systemic Sal A (5 days 3.2 mg/kg) decreased DA levels in the dorsal striatum (dStr) in a KOPr dependent manner in the mouse. This is similar to traditional KOPr agonists, which were shown to decrease DA release in rat striatal synaptosomes (Ronken, Mulder, & Schoffelmeer, 1993) and in mice following intra-NAcb perfusion of U69,593 (Chefer et al., 2005). DA levels in the NAcb have an important role in modulating the rewarding effects of drugs of abuse, food, and sex (Nestler & Carlezon, 2006; Wise, 1998), and decreased levels of DA within the NAcb have been previously shown to lead to anhedonia in the rat (Weiss, Markou, Lorang, & Koob, 1992).

Regulation of DA levels in the NAcb may be the mechanism underlying the behavioural effects of Sal A. Further evidence to support this is a recent study by Ebner et al. (2010) who showed that Sal A (2 mg/kg) decreased phasic DA release in the rat NAcb 5–135 min post injection without increasing DA reuptake, with max effects seen at 15 min. A lower dose of Sal A (0.25 mg/kg) did not alter DA release in the NAcb (Ebner, Roitman, Potter, Rachlin, & Chartoff, 2010), which is in contrast to the study in mice by Gehrke et al. (2008). However, species differences and the route of Sal A administration (local versus systemic administration) are likely explanations for the difference seen in these studies. The timing of these effects on decreased DA release in the NAcb are consistent with the rapid pharmacokinetic effects of Sal A (Butelman, Prisinzano, Deng, Rus, & Kreek, 2009; Ranganathan et al., 2012; Schmidt, Schmidt, Butelman, Harding, Tidgewell, Murry et al., 2005; Teksin, Lee, Nemieboka, Othman, Upreti, Hassan et al., 2009).

Acute U69,593 administration increases DA uptake in the NAcb in addition to decreasing DA release (Thompson et al., 2000), an effect that is not seen with Sal A in the dStr (Gehrke et al., 2008) or NAcb (Ebner et al., 2010). Further studies are needed to confirm whether acute Sal A modulates DA uptake in the NAcb following local administration. However, results to date indicate significant differences in DA uptake between Sal A and traditional KOPr agonists U50,488 and U69,593.

Sal A (100 nM) also inhibited 5-HT and DA release and induced noradrenaline (NA) overflow in mouse striatal and prefrontal cortex synaptosomes, effects that were not observed in the presence of pertussis toxin or norbinaltorphimine (nor-BNI). This is in contrast to U69,593 which had no effect on NA levels. These effects were not mediated by μ -opioid receptors but the δ -opioid receptor antagonist naltrindole did have an effect (Grilli, Neri, Zappettini, Massa, Bisio, Romussi et al., 2009). Again this is another example highlighting different effects of Sal A compared to traditional KOPr agonists.

When a compound fully substitutes for another in the discriminative stimulus test it suggests that these compounds are similar in their actions (Peet & Baker, 2011). Sal A has been found to reliably, and fully substitute for U69,593 and U50,488 in drug discriminating studies in rodents following Sal A (1 mg/kg) (ip) (Baker, Panos, Killinger, Peet, Bell, Haliw et al., 2009) and also in primates (0.001–0.032 mg/kg) (subcutaneous, sc) (Butelman, Harris, & Kreek, 2004; Butelman, Rus, Prinszano, & Kreek, 2010). Butelman et al. (2004) showed a reversal of the discriminative stimulus effects of Sal A (0.001–0.032 mg/kg) (sc) by the KOPr antagonist quadazocine but not another KOPr antagonist 5'-guanidinonaltrindole (GNTI). This may be due to different binding site for Sal A compared to traditional KOPr agonists or differences in selectivity, onset of action or pharmacokinetic properties. These effects were also not blocked by the 5-HT₂ antagonist ketanserin (0.1 mg/kg) (Butelman et al., 2010). The onset of the Sal A effects started at 5–15 min and dissipated by 120 min. In addition to these studies, Sal A was shown to have effects on the neuroendocrine system by increasing prolactin levels in rhesus monkeys (Butelman, Mandau, Tidgewell, Prinszano, Yuferov, & Kreek, 2007). Discriminative stimulus effects of Sal A were not substituted for by serotonergic hallucinogens, ketamine or cannabinoids (Butelman et al., 2007; Killinger, Peet, & Baker, 2010; Walentiny, Vann, Warner, King, Seltzman, Navarro et al., 2010). These effects combined further supports the KOPr selectivity of Sal A *in vivo*, and also highlights differences between Sal A and traditional KOPr agonists.

The behavioural effects of Sal A differ according to the duration of exposure (acute versus chronic) or the administered dose (low versus high). Sal A does not modulate cocaine induced locomotion in mice (Gehrke et al., 2008) or rats (Morani et al., 2009) in contrast to U69,593 which shows attenuation of cocaine-induced locomotor activity (Collins et al., 2001; Heidbreder, Schenk, Partridge, & Shippenberg, 1998). However, acute Sal A at 2 mg/kg has also been shown to attenuate cocaine induced locomotor activity in the rat while repeated Sal A potentiated cocaine induced locomotor activity (Chartoff, Potter, Damez-Werno, Cohen, & Carlezon, 2008). Repeated Sal A administration at a high dose (3.2 mg/kg) showed a different effect to U69,593, with increased cocaine-evoked DA levels in the dStr without decreasing cocaine-induced hyperactivity in the rat (Gehrke et al., 2008). A high acute dose of Sal A (2 mg/kg) lowered the breakpoint in progressive ratio responding to sucrose, suggesting a decrease in the reinforcing effects of sucrose. This effect was seen between 20 and 40 min post injection. A lower dose of Sal A (0.25 mg/kg) had no effect on progressive ratio responding. This data showing that low dose Sal A does not alter the rewarding properties of sucrose (Ebner et al., 2010) supports results from previous studies which showed no differences in sucrose responding based on a fixed ratio schedule following 0.3 mg/kg or 1 mg/kg Sal A over a 60 min time period (Morani et al., 2009). Previous studies comparing progressive and fixed ratio self-administration schedules suggest that the two paradigms typically produce similar effects in reinforcement (Winger & Woods, 1985). Another study in mice showed that Sal A (1.0 mg/kg and 3.2 mg/kg, (ip) caused conditioned place aversion and decreased locomotor activity. There was no change in DA levels in the NAcB, but significantly decreased DA levels in the caudate putamen (Zhang et al., 2005).

Given this data, it is likely that high doses Sal A (2 mg/kg) have predominantly aversive effects including the ability to decrease DA release, reward and motivation. It also attenuates the effects of cocaine hyperactivity when administered acutely, but potentiates the same effects when chronically administered. On the other hand, low doses of Sal A (< 0.25 mg/kg) (ip) consistently display less aversive effects (Morani et al., 2009; Morani et al., 2012) and is within the range with known anti-cocaine effects. Given this, Sal A possesses desirable anti-addiction effects with fewer side effects compared to traditional KOPr agonists. Although the pharmacokinetic properties of Sal A are not desirable as an anti-cocaine pharmacotherapy (discussed below), its novel structure may aid in the development of new compounds with better pharmacokinetics and side effect profiles.

Effects of Salvinorin A on Depression

Salvinorin A has been shown to have both pro- (Morani et al., 2012) and anti-depressant effects *in vivo* (Braidia, Capurro, Zani, Rubino, Vigano, Parolaro et al., 2009; Braidia et al., 2008; Braidia et al., 2007; Harden, Smith, Niehoff, McCurdy, & Taylor, 2012). In contrast, the effects traditional KOPr agonists such as U69,593 tend to be consistently pro-depressive and aversive (Van't Veer & Carlezon Jr, 2013). Known KOPr mediated effects on depression and stress related behaviours, particularly in the ability of KOPr agonists to potentiate stress induced relapse has been a major limiting factor in the development of KOPr agonist based anti-addiction pharmacotherapies. In this regard, the effects of Sal A are somewhat different to traditional KOPrs. Major differences in these effects are reviewed below.

Anti-Depressive Effects of Salvinorin A

In zebra fish, swimming behaviour and conditioned place preference tests (CPP) have shown that low doses of Sal A have antidepressant effects (Braidia et al., 2007). It is generally accepted that the effects of Sal A on depression are not due to the effects of reduced locomotion or in a reduced ability to perform tasks, particularly at the lower Sal A doses (<0.5 mg/kg) where no changes in locomotor activity are reported in rats (Carlezon et al., 2006; Chartoff et al., 2008; Morani et al., 2009). The anti-depressant-like effects of Sal A, have also been reported with low doses of Sal A (10 µg/kg) in rats and mice (0.001 and 10 µg/kg). At these doses, Sal A decreased immobility and increased swimming times in the FST in a KOPr and cannabinoid receptor type 1 (CB₁) dependent manner as the effects were blocked by respective antagonists. Low dose Sal A (0.1–40 µg/kg) also showed cocaine CPP and intracerebroventricular self-administration, suggestive of rewarding effects (Braidia et al., 2009). The low doses of Sal A used in this study can potentially account for the variation in effects seen, with low dose Sal A being considered anti-depressive (Braidia et al., 2009; Braidia et al., 2008; Braidia et al., 2007) or neutral and higher doses pro-depressive (Carlezon et al., 2006; Morani et al., 2012; Potter, Damez-Werno, Carlezon, Cohen, & Chartoff, 2011). DA levels in the NAc shell were also increased with administration of low dose Sal A (40 µg/kg), indicative of reward. Sal A (0.1–160 µg/kg) has also been shown to have anxiolytic effects 20 min post injection (ip) in rats. These effects were shown to be nor-BNI reversible at the lowest 0.1 µg/kg dose, indicating selective KOPr mediated effects. However, effects were also reversed by the CB₁ antagonist AM251 indicating a role of CB₁ in these effects

(Braida et al., 2009). However, a more recent study has suggested that this reversal is explained by the actions of AM251 at the KOPr not CB₁ receptors (Walentiny et al., 2010). Recently, additional studies have also suggested a biphasic effect of Sal A on reward (Potter et al., 2011). Utilizing an intracranial self-stimulation (ICSS) model to measure reward sensitivity, Potter et al. (2011) showed that repeated high dose Sal A (2 mg/kg/day) resulted in increased baseline thresholds indicating that Sal A decreased the reward potentiating effects of a cocaine challenge. However, when the same doses used in rats and mice by Braida et al. (2009) were used in this study, no effects on ICSS baseline thresholds were seen in rats (Potter et al., 2011). A recent study by Harden et al. (2012) assessed the effects of Sal A on chronic mild stress in rats. In this study, Sal A (1 mg/kg) reversed anhedonia. The authors chose this model as it is believed to be a superior model to the FST model of depression because it does not apply the stressor at the same time as performing the test. In this same study, Sal A had no effect on sucrose intake in the absence of chronic mild stress suggesting that Sal A does not alter the hedonic effects of sucrose. This study supports the study conducted by Morani et al. (2009) where Sal A (0.3 mg/kg and 1 mg/kg) also did not alter sucrose responding.

Pro-Depressive Effects of Salvinorin A

Activation of KOPr's by both traditional KOPr agonists and Sal A has been shown to produce pro-depressive effects in laboratory animals. In the FST, Carlezon et al. (2006) showed that Sal A decreased swimming and increased immobility times following systemic administration at doses of 0.25–2 mg/kg (ip). Sal A also dose dependently elevated ICSS thresholds, with significant increases seen at doses ranging from 0.5–2 mg/kg (ip) in the rat. Elevated ICSS thresholds have been shown to be representative of depressive effects in humans (Carlezon et al., 2006). No attenuation of locomotor activity was observed within these doses, consistent with other findings for Sal A, and in contrast to traditional agonists such as U69,593 which show sedative effects at similar doses. Decreased DA levels were also seen in the NAcB, at the dose where Sal A shows pro-depressive effects in the FST and ICSS models. No changes in 5-HT concentrations were observed, suggesting a specific KOPr mediated effect. This correlation suggests that decreased DA levels within the NAcB may contribute to these depressive effects.

It is clear from these studies that there are no consistent effects for Sal A in its ability to modulate both pro and anti-depressive behaviours. However, it is clear that the properties of Sal A differ from that of traditional KOPr agonists. Further studies are needed to evaluate these differences at both the behavioural and cellular levels to determine the mechanisms underlying these actions. Based on the studies listed above, there is evidence to suggest that Sal A has the potential to yield compounds that have anti-addiction properties without inducing depression.

Signalling Pathways Regulated by Salvinorin A

Some recent studies have investigated the ability of Sal A to regulate known KOPr signalling pathways and compared these with traditional KOPr agonists. The transcription factor cAMP response element-binding protein (CREB) is known to regulate dynorphin

levels (Carlezon, Thome, Olson, Lane-Ladd, Brodtkin, Hiroi et al., 1998). Phosphorylated CREB levels in the rat have been shown to increase in the NAcB in response to stress (Pliakas, Carlson, Neve, Konradi, Nestler, & Carlezon, 2001), an effect that is behaviourally correlated to an increase in immobility in the FST, a preclinical measure of depression (Porsolt, 1979). The stress related effects of corticotrophin-releasing factor have recently been shown to be mediated by KOPr's (Land, Bruchas, Schattauer, Giardino, Aita, Messinger et al., 2009). Elevations in CREB have also been shown to reduce the rewarding effects of cocaine (a measure of anhedonia). Endogenously, in the NAcB, elevated CREB-mediated dynorphin levels leads to decreased DA and pro-depressive behaviours. In the presence of cocaine, the endogenous KOPr system decreases DA levels in the NAcB, opposing the actions of cocaine (and other stimulants). This is also the same mechanism hypothesised to be responsible for pro-depressive effects and other undesirable effects associated with drug withdrawal. Dynorphin mediated activation of CREB is postulated to be the cause of relapse, where drug taking activities are resumed in order to decrease the adverse effects of withdrawal. For a recent review on the role of CREB and dynorphin see (Muschamp & Carlezon, 2013). The role Sal A in modulating CREB levels remains to be determined. One study has shown that acute Sal A (2 mg/kg) does not change CREB protein levels in the NAcB or caudate putamen but repeated Sal A increases CREB activation in the NAcB of the rat (Potter et al., 2011).

CREB is activated by extracellular signal-regulated kinases (ERK1/2), and both KOPr and DAT proteins have been shown to signal via ERK1/2 pathways (Potter et al., 2011; Rothman, Dersch, Carroll, & Ananthan, 2002; Yoshizawa, Narita, Saeki, Narita, Isotani, Horiuchi et al., 2011). Activation of ERK1/2 leads to an increase in DAT function and cell surface expression (Bolan, Kivell, Jalgam, Oz, Jayanthi, Han et al., 2007; Moron, Zakharova, Ferrer, Merrill, Hope, Lafer et al., 2003), and ERK1/2 inhibitors (but not P38 MAPK inhibitors), cause a concentration and time dependent decrease in DA uptake, an effect shown to be due to decreased DAT cell-surface expression (Moron et al., 2003). ERK 1/2 activation is known to have both β arrestin dependent and β arrestin independent phases (McLennan, Kiss, Miyatake, Belcheva, Chambers, Pozek et al., 2008). The early ERK1/2 activation phase (5–15 min) is via activation of G $\beta\gamma$ subunits that are phosphoinositide-3-kinase, Ca²⁺, and Protein Kinase- ζ dependent (Belcheva, Clark, Haas, Serna, Hahn, Kiss et al., 2005), whereas late phase ERK1/2 activation (2 hrs) requires recruitment of β arrestin (McLennan et al., 2008). Both acute and repeated Sal A increased ERK1/2 phosphorylation in the NAcB. Acute Sal A increased ERK1/2 phosphorylation rapidly at 15 min, while repeated Sal A induced a delayed increase in ERK1/2 phosphorylation (Potter et al., 2011).

P38 α Mitogen-Activated Protein Kinase (MAPK) activation by KOPr agonists in the dorsal raphe has been shown to be required for KOPr mediated dysphoria (Land et al., 2009) and behavioural stress effects in the mouse (Bruchas, Schindler, Shankar, Messinger, Miyatake, Land et al., 2011). This is also believed to require β -arrestin recruitment (Bruchas, Land, Aita, Xu, Barot, Li et al., 2007). In theory, should this hold true, then KOPr agonists that do not activate P38 α MAPK or β -arrestin may be devoid of dysphoric effects (Muschamp, Van't Veer, & Carlezon, 2011). Previous studies have shown that MOM Sal B fails to recruit β arrestin (Beguín, Potuzak, Xu, Liu-Chen, Streicher, Groer et al., 2012), but Morani et al. (paper submitted for review) still report pro-depressive effects in the rat with MOM Sal B

(0.3 mg/kg) (ip) in the FST with the same dose that displayed anti-addiction effects. The differences seen between assays may be due in part to the pharmacokinetic properties of MOM Sal B. However, there is little information on the signalling pathways activated by Sal A and even less is known about the signalling pathways of novel Sal A analogs. Correlation between cellular, behavioural, and pharmacokinetic effects is likely to lead to a better understanding of the complex signalling mechanisms responsible for both the desired anti-addiction effects and unwanted side effects.

Pharmacokinetics of Salvinorin A

Sal A is well known to have a short half-life and rapid onset of action (Butelman et al., 2009; Hooker, Xu, Schiffer, Shea, Carter, & Fowler, 2008; Ranganathan et al., 2012; Teksin et al., 2009). Behavioural studies in humans consistently report very short-lasting psychoactive effects with onset of seconds to minutes (MacLean, Johnson, Reissig, Prisinzano, & Griffiths, 2013). Sal A concentration in plasma peaks at 10 minutes and returns to baseline within 30 min (Ranganathan et al., 2012). In primates, Sal A had peak effects on facial relaxation and ptosis at between 5 and 15 min following sc injection and 1–2 min following intravenous administration of Sal A (0.001–0.032 mg/kg) (Butelman et al., 2010). Teksin et al. (2009) showed that following a 10 mg/kg dose of Sal A the $T_{\max}$ was 10–15 min in both plasma and brain with a half-life of 36 min in the brain. This study also showed that Sal A increased the activity of permeability glycoprotein ATPase activity suggesting it is a substrate for this protein (Teksin et al., 2009). These studies correlate well with behavioural data in animals showing that Sal A pre-treatment of longer than 20 min often failed to have behavioural effects. Characterisation of new Sal A analogs with longer acting effects has the potential to lead to new more effective KOPr activating compounds with improved pharmacokinetics to Sal A.

Salvinorin A Analogs

One of the most important factors to consider in the development of Sal A analogs is an improved pharmacokinetic profile. With this in mind several groups have utilised the neoclerodane diterpene structure of Sal A to develop novel compounds with varying opioid binding properties (Lovell, Vasiljevik, Araya, Lozama, Prevatt-Smith, Day et al., 2012; Munro, Rizzacasa, Roth, Toth, & Yan, 2005; Prevatt-Smith et al., 2011; Prisinzano, 2009; Prisinzano & Rothman, 2008; Tidgewell, Harding, Schmidt, Holden, Murry, & Prisinzano, 2004; Valdes, Chang, Visger, & Koreeda, 2001; Vortherms & Roth, 2006) (reviewed in Grundmann, Phipps, Zadezensky, and Butterweck (2007)).

Ethoxymethyl ether Salvinorin B (EOM Sal B) and methoxymethyl ether Salvinorin B (MOM Sal B) are two potent and selective analogs with C-2 substitutions (Figure 1). Both MOM Sal B and EOM Sal B have been shown to have a longer half-life *in vivo* compared to Sal A (Baker et al., 2009; Wang, Chen, Xu, Lee, Ma, Rawls et al., 2008). Hooker et al. (2009) compared EOM Sal B to the parent compound, Sal A and showed that EOM Sal B had increased metabolic stability and decreased plasma protein affinity are likely mechanisms for its increased duration of effects (Hooker, Patel, Kothari, & Schiffer, 2009).

In vitro studies indicate that MOM Sal B is five- and seven-fold more potent at KOPr in GTP- γ S assays than U50,488 and Sal A, respectively (Wang et al., 2008). EOM Sal B and MOM Sal B have increased binding to KOPr compared to Sal A (Munro, Duncan, Xu, Wang, Liu-Chen, Carlezon et al., 2008) and MOM Sal B has been shown to be longer acting *in vivo* than Sal A in hotplate and hypothermic assays in the rat (Wang 2009). In a study by Hooker (2009) EOM Sal B gave 3 fold higher levels in the brain 65 min following ip. injection, indicating slower metabolism. A recent study by Peet and Baker (2011) compared MOM Sal B and EOM Sal B for their ability to produce discriminative stimulus effects in rats trained to discriminate Sal A. The discrimination assays are a method to investigate similarities between known drugs of abuse and novel compounds with respect to their interoceptive stimulus properties. Male Sprague-Dawley rats were trained to discriminate Sal A (2 mg/kg) from vehicle (75% DMSO, 25% water) prior to stimulus generalization testing with EOM Sal B and MOM Sal B. Time course tests showed that both EOM Sal B and MOM Sal B had greater potency and substituted fully for U50,488 and Sal A (Peet and Baker, 2011). It is also worth noting that, although EOM Sal B (ED_{50} 0.65 nM) is 10 times more potent (ED_{50} in GTP- γ S assays) than MOM Sal B (ED_{50} 6 nM) *in vitro* (Munro et al., 2008), the study by Peet and Baker (2011) did not show this magnitude of effect *in vivo*. Therefore further characterization of effects *in vivo* is necessary to identify the bioavailability and pharmacokinetic effects of these Sal A analogs. To our knowledge this is the first study showing that the synthetic derivatives of Sal A produce similar discriminative stimulus effects. Differences between Sal A, EOM Sal B and MOM Sal B were also noted, in that, unlike Sal A, they also partially substituted for ketamine and lysergic acid diethylamide (LSD). It remains to be determined if these compounds have other differences *in vivo* including anti-addiction effects, effects on mood, locomotion, and aversion.

In an attempt to address these important issues (Morani et al., manuscript submitted for review), we tested the effects of MOM Sal B on cocaine-seeking using the cocaine prime-induced reinstatement paradigm in rats. We also investigated the side effects of MOM Sal B such as modulation of motor function (spontaneous locomotion and cocaine induced hyperactivity), reward reinforcement (sucrose reinforcement), aversion (conditioned taste aversion) and depression (forced swim test). These results were consistent with the effects previously reported for Sal A (Morani et al., 2009; Morani et al., 2012). This study showed that MOM Sal B (0.3 mg/kg) attenuated cocaine-primed induced reinstatement in a similar way to Sal A (0.3 mg/kg), with no change in activity in either cocaine-induced hyperactivity or spontaneous open field activity tests. However MOM Sal B, unlike Sal A attenuated sucrose reinforcement indicating that its anti-addiction effects may be non-specific, by altering natural reward pathways. Increased immobility and decreased swimming times in the forced swim test were also observed. This indicates that MOM Sal B has an improved side-effect profile compared to traditional KOPr agonists, however; pro-depressive effects and effects on natural reward remain. MOM Sal B has a much higher potency in GTP- γ S assays at KOPr receptors ($ED_{50} = 6 \pm 1$) compared to Sal A ($ED_{50} = 40 \text{ nM} \pm 10$) and this may be responsible for the increased side-effects seen in this study.

Recently, a novel Sal A analog lacking a hydrolysable ester at C-2, a modification that is likely to yield longer acting compound was synthesized (Wang et al., 2008). β -

Tetrahydropyran Sal B (Figure 1), was also shown to have anti-addiction effects using the pre-clinical self-administration model of cocaine prime-induced relapse (Prevatt-Smith et al., 2011). β -Tetrahydropyran Sal B attenuated cocaine prime-induced drug seeking behaviour at a dose of 1 mg/kg in a similar way to Sal A (0.3 mg/kg). The increased dose required to see these effects may be explained by differences in absorption, distribution, metabolism and excretion as β -Tetrahydropyran Sal B has similar potency in GTP- γ S assays ($ED_{50} = 60 \text{ nM} \pm 6$), to Sal A ($ED_{50} = 40 \text{ nM} \pm 10$).

These findings form the proof of principal that Sal A analogs have anti-addiction effects. It remains to be determined if these compounds also have improved side effect profiles. It is clear from these behavioural results that highly potent selective KOPr agonists such as MOM Sal B, while having reduced side effects compared to traditional KOPr's still exhibit undesirable pro-depressive effects which are likely to limit their therapeutic utility. However, valuable information gained will aid the development of further compounds and develop a selection criterion for additional compounds to test preclinically.

Partial agonists, or functional agonists that are able to activate signalling pathways differentially may hold the key to the discovery analogs with anti-addiction effects without unwanted side effects. No derivatives of Sal A have been identified as KOPr partial agonists or antagonists to date. It is possible, by studying the signalling activity of Sal A and novel analogs, particularly with their abilities to recruit β arrestin and induce phosphorylation of kinases including ERK(1/2), and P38, that compounds with the desired effects may be identified. In order to do this, further studies on KOPr signalling pathways is needed.

Conclusions

Recent studies have investigated the preclinical effects of Sal A on behaviours such as reward, aversion and depression. However, many of these studies show conflicting results. It has become clear that the choice of behavioural model, dose of Sal A administered and duration of Sal A administration is responsible for the majority of the differences seen. While high doses of Sal A causes side effects typical of traditional KOPr agonists, lower doses of Sal A do not present these effects. It is therefore exciting to note that the anti-addiction/anti-reward effects of Sal A are present at levels below the threshold of many known side effects such as sedation, aversion and attenuation of natural reward. Although Sal A has been found to display pro-depressive effects, the reduced side effects profile of Sal A compared to classic KOPr agonists makes Sal A a compound of high therapeutic promise in treating drug abuse.

The potential of Sal A as a pharmacotherapy can be further determined by characterisation of the cellular signalling pathways responsible for each of these behavioural effects seen, and whether it is, in fact, possible to separate these undesirable side-effects from the anti-addiction effects. It has been hypothesised that partial KOPr agonists, or 'functional agonists' that are able to activate signalling pathways differentially may hold the key to development of anti-addictive compounds without the side effects. Recently, p38 activation, β arrestin recruitment and late activation of ERK have been identified as potential candidates responsible for undesirable side effects. Sal A analogs have recently been synthesised in an

effort to improve the behavioural effects and pharmacokinetic properties of Sal A. None of these derivatives have been identified as KOPr partial agonists or antagonists to date. However, as there is currently limited information on the behavioural effects and cellular signalling pathways of these compounds, the potential of these analogs as an anti-addiction therapy remain to be seen. Further studies in this field are warranted as Sal A and its analogs hold definite promise as treatments for drug abuse.

ABBREVIATIONS

5-HT	Serotonin
CB₁	Cannabinoid receptor type 1
CPP	Conditioned place preference
CREB	cAMP response element-binding protein
DA	Dopamine
DAT	Dopamine transporter
DMSO	Dimethyl sulfoxide
dStr	Dorsal striatum
ED₅₀	Effective dose for 50% of maximal response
ERK1/2	Extracellular signal-regulated kinases
FST	Forced swim test
GTP-γS	[³⁵ S] guanosine gamma thio-phosphate
ip	Intraperitoneal
KOPr	κ-opioid receptor
MAPK	Mitogen-activated protein kinase
NA	Noradrenaline
Nacb	Nucleus accumbens
nor-BNI	Norbinaltorphimine
Sal A	Salvinorin A
sc	Subcutaneous
SN	Substantia nigra
U50,488	2-(3,4-dichlorophenyl)-N-methyl-N-[(2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide
U69,593	N-methyl-2-phenyl-N-[(5R,7S,8S)-7-pyrrolidin-1-yl-1-oxaspiro[4.5]decan-8-yl]acetamide
VTA	Ventral tegmental area

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Salvinorin A

ED₅₀ (nM) = 40 ± 1
 KOPr Ki ± SD (nM) = 7.4 ± 0.7

β-Tetrahydropyran Sal B

ED₅₀ (nM) = 60 ± 6
 KOPr Ki ± SD (nM) = 6.21 ± 0.40

EOM Sal B

ED₅₀ (nM) = 0.65 ± 0.17
 KOPr Ki ± SD (nM) = 3.13 ± 0.40

MOM Sal B

ED₅₀ (nM) = 6 ± 1
 KOPr Ki ± SD (nM) = 1.9 ± 0.2

Figure 1. Kappa opioid receptor agonists

Opioid receptor binding ([³H] U69,593) in CHO cells expressing human KOP receptors (Ki ± SD nM). ED₅₀ = effective dose for 50% of maximal response in [³⁵S] GTP-γS binding.

Data for EOM SalB, MOM SalB and β-Tetrahydropyran Sal B taken from Prevatt-Smith et al. (2011) and data for Sal A taken from Lozama et al. (2011) (Lozama, Cunningham, Caspers, Douglas, Dersch, Rothman et al., 2011).