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Review

σ Receptors: potential medications development target for anti-cocaine agents

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Abstract

The ability of cocaine to interact with σ receptors suggests a viable target for medications development. Recently, numerous novel compounds and antisense oligodeoxynucleotides targeting σ receptors have been synthesized and shown to prevent the behavioral toxicity and psychomotor stimulant effects of cocaine in animals. Protective doses of σ receptor antagonists have also been shown to prevent changes in gene expression that are induced by cocaine. Together, the studies provide insight and promising future directions for the development of potential medications for the treatment of cocaine addiction and overdose.

Keywords: Antisense; Cocaine; Conditioned place preference; Convulsion; Gene expression; Locomotor activity; Microarray; Sensitization; σ Receptor

1. Introduction

Cocaine abuse is a major public health problem and there are no effective treatments for it (Carroll et al., 1999). Of the many strategies that can be adopted to develop an effective anti-cocaine agent, the one that has been most avidly pursued is the development of antagonists that compete with cocaine for its target proteins.

Since cocaine is generally thought to act as a dopamine reuptake inhibitor to produce its reinforcing effects (Kuhar et al., 1991), much of the drug development effort in recent years has been aimed at making a compound that can interfere with cocaine's access to dopamine transporters, while allowing the reuptake of dopamine into the pre-synaptic nerve terminal. Unfortunately, of the hundreds of compounds that have been developed and tested, the vast majority of them exhibit neurochemical and behavioral profiles similar to cocaine (Carroll et al., 1999). Although several of these compounds have been reported to attenuate responding to cocaine in pre-clinical studies (Nader et al., 1997; Villemagne et al., 1999), thus far, no medication for the treatment of cocaine addiction has emerged from these efforts (Carroll et al., 1999; Newman, 2000). These disappointments, together with the fact that mice lacking dopamine transporters still respond to the rewarding properties of cocaine (Rocha et al., 1998; Sora et al., 1998), suggest the need to explore other viable targets for the development of anti-cocaine agents.

In addition to blocking the reuptake of dopamine, cocaine also inhibits the reuptake of serotonin and norepinephrine and binds to a number of neurotransmitter receptors (Ritz and George, 1993). Of the myriad of sites with which cocaine interacts, the monoamine transporters, muscarinic receptors, and σ receptors are thought to be most relevant in mediating the psychological and physiological properties of the drug because the affinity of cocaine for these proteins fall within a concentration range that can be achieved in vivo (Ritz and George, 1993). Earlier investigations on serotonergic, noradrenergic, and muscarinic mechanisms have succeeded in confirming a role for these systems in the actions of cocaine, but suggest that they may be less optimal drug development targets because of limited efficacy and/or unfavorable side effect profiles. Therefore, current studies have targeted the most recently discovered of these sites, the σ receptor.

This review begins with an overview on σ receptors. Then, the ability of σ receptor antagonism to attenuate

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cocaine-induced behaviors is summarized, with an emphasis on the contribution of different σ receptor subtypes to the protective actions. Next, the ability of a σ receptor antagonist to prevent cocaine-induced changes in gene expression is presented. Finally, the potential mechanisms involved in these anti-cocaine effects are briefly described, along with future directions for research. Together, the data suggest that σ receptors are logical and promising medications development targets for the treatment of cocaine abuse.

2. σ Receptors

2.1. Historical perspective

σ Receptors were first postulated by Martin et al. (1976) based on the actions of SKF 10,047 (*N*-allylnormetazocine) and related benzomorphans. The name "σ" originated from the first letter "S" in SKF 10,047, which was thought to be the prototypic ligand for these binding sites. Unfortunately, SKF 10,047 is now recognized as a non-selective ligand, which contributed to the turbulent early history surrounding σ receptors. The seminal studies of Martin et al. involved racemic SKF 10,047, which is a mixture of (+) and (−) forms of the drug. As shown in Fig. 1, this drug produces actions through at least three distinct sites. The (−)-isomer of SKF 10,047 produces actions that are reversible by naloxone and these interactions are attributable primarily to the kappa type of opiate receptor. The (+)-isomer of SKF 10,047, on the other hand, interacts with at least two sites: (a) phencyclidine binding sites within the ionophore of the *N*-methyl-D-aspartate (NMDA) receptor, and it is due to this interaction that the term σ-phencyclidine (PCP) was prevalent in the early 1980s; and (b) another site which today retains the designation of σ. It is this third, non-opiate, non-NMDA σ site that is the focus of the review.

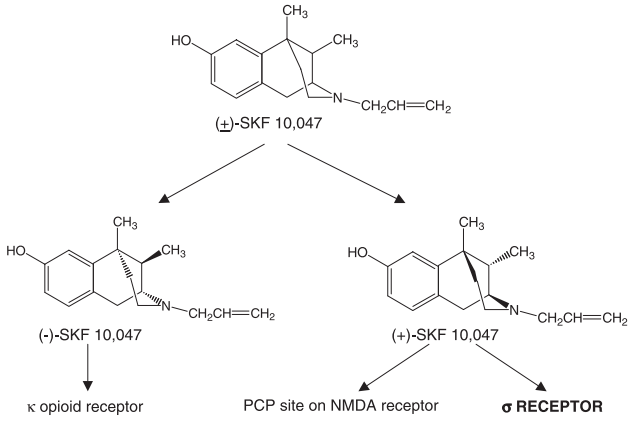

Fig. 1. SKF 10,047 binding sites. The (−)-isomer of SKF 10,047 interacts with kappa opiate receptors in a naloxone-sensitive manner. The (+)-isomer for SKF 10,047 interacts with sites within the ionophore of NMDA receptors, and also with other binding sites that today retain the designation of σ receptors. σ Receptors are thus distinct from both opiate and NMDA receptors.

2.2. Anatomical distribution

σ Receptors are widely distributed in the body. They are found in many peripheral organs such as the heart, lung, liver, kidney, intestines, and reproductive organs (Kawamura et al., 1999, 2000; Wolfe et al., 1989), where their physiological roles are still poorly characterized. σ Receptors are also concentrated in the central nervous system (Aanonsen and Seybold, 1989; Bouchard and Quirion, 1997; Graybiel et al., 1989; Gundlach et al., 1986; Jansen et al., 1991; Mash and Zabetian, 1992; McLean and Weber, 1988; Walker et al., 1992; Weissman et al., 1988), where their actions have been best studied. In the brain, the highest concentrations of σ receptors are found in brainstem motor regions, with significant densities also in limbic structures (Bouchard and Quirion, 1997; Walker et al., 1990).

While there is still much to be learned about the actions of σ receptors in various organ systems, it must be acknowledged that the presence of these receptors in the brain and heart, two important target organs for the actions of cocaine, are consistent with a role for these receptors in the addictive and toxic actions of cocaine. Furthermore, antagonist compounds that prevent cocaine from accessing these targets would provide a logical strategy by which anti-cocaine effects can be achieved.

2.3. σ Receptor subtypes

Biochemical and pharmacological studies indicate the existence of multiple σ receptor subtypes. This review will focus on the two established subtypes, σ_1 and σ_2, with some of their salient characteristics summarized in Table 1. The characterization of other putative subtypes is still in its infancy and no consensus has been reached regarding them. With regards to σ_1 and σ_2 receptors, these two subtypes can be distinguished by their drug selectivity patterns and molecular sizes. The σ_1 subtype is thought to be a 25–29-kDa protein (Hellewell and Bowen, 1990; Hellewell et al., 1994; Kavanaugh et al., 1988; Mei and Pasternak, 2001; Moebius et al., 1993a, 1996; Wilke et al., 1999), whereas the σ_2 receptor is thought to be slightly smaller at 18–22 kDa, and perhaps existing as a heterodimer (Hellewell and Bowen, 1990; Hellewell et al., 1994; Moebius et al., 1993a,b). Although the (+)-isomer of benzomorphans and morphinans interacts with the two subtypes with similar affinities, the relative interactions of the (−)-isomers of these compounds can be discriminating. At the σ_1 subtype, the (+)-isomer has a better affinity than the (−)-isomer, whereas at the σ_2 subtype, the (−)-isomer has a better affinity than the (+)-isomer (Quirion et al., 1992). Cocaine itself interacts with both σ_1 and σ_2 receptors, but has about a 10-fold better affinity for the σ_1, as compared to the σ_2,

Table 1
Characteristics of σ_1 and σ_2 receptors

	σ_1	σ_2	
Physical characteristics			
Size	25–29 kDa	18–22 kDa	
Sequence (GenBank accession number)	AF004927	not determined	
Relative proportions in tissues			
Brain	60%	40%	
Heart	90%	10%	
Putative agonists (K_i in nM)			
DTG	74 ± 15	61 ± 13	Bowen et al., 1993
(+)-Pentazocine	7 ± 1	1361 ± 134	Bowen et al., 1993
Putative antagonists (K_i in nM)			
BD1063	9 ± 1	449 ± 11	Matsumoto et al., 1995
BD1047	0.9 ± 0.1	47 ± 0.6	Matsumoto et al., 1995
LR132	2 ± 0.1	701 ± 375	Matsumoto et al., 2001b
NE-100[a]	2 ± 0.3	85 ± 33	Chaki et al., 1994
(±)-SM 21	>1000	67 ± 8	Mach et al., 1999

[a] IC_{50} in nM.

subtype (Matsumoto et al., 2002b). Moreover, while both subtypes are present in the rodent brain (Bouchard and Quirion, 1997), almost all of the σ receptors in the heart are of the σ_1 subtype (Novakova et al., 1995).

Of the σ receptor subtypes, only the σ_1 receptor has thus far been cloned. It has been isolated with high homology in mouse brain, rat brain, guinea pig liver, and human placenta (Hanner et al., 1996; Kekuda et al., 1996; Seth et al., 1997, 1998). It is located on human chromosome 9, band p13 (Prasad et al., 1998), and it is distinct from any known mammalian protein. The σ_1 receptor gene consists of four exons and three introns (Seth et al., 1997). The translation start site is coded for in exon 1 and the termination codon is in exon 4. The receptor is thought to function as a single putative transmembrane domain, with the protein coding sequence located in exons 2 and 3 and portions of exons 1 and 4, suggesting that all four exons contribute to the coding of the functional receptor protein (Prasad et al., 1998). Moreover, the ligand binding domain and its obligatory anionic amino acid residues (D126, E172) appear to be in or near the region coded for by exon 3 (Seth et al., 2001). The σ_1 receptor gene lacks a TATA box, which is the classical promoter element for the transcription start site (Seth et al., 1997). However, it contains CCAATC and GC boxes immediately upstream of the transcription start site that are recognition sites for SP1 (Prasad et al., 1998; Seth et al., 1997). This is significant because SP1 is a transcription factor that is thought to have an important role in the expression of TATA-less genes. In addition to σP1 binding sites, the promoter region contains putative binding sites for activator protein (AP)-1, AP-2, GATA-1, a variety of cytokine responsive factors, and consensus sequences for steroid, aryl hydrocarbon, and retinoic acid receptors (Prasad et al., 1998; Seth et al., 1997).

2.4. Signal transduction

σ Receptors have also been linked to the modulation or production of intracellular second messengers, such as cGMP (Mamiya et al., 2000; Rao et al., 1991), inositol phosphates (Novakova et al., 1998; Yamamoto et al., 1995b), protein kinases (Derbez et al., 2002; Morin-Surun et al., 1999; Nuwayhid and Werling, 2003), and calcium (Brent et al., 1997; Hayashi et al., 2000; Vilner and Bowen, 2000). Although the interaction of σ receptors with recognized signal transduction systems is a feature expected of physiologically functioning receptors, it should be emphasized that these proteins produce their actions through a mechanism that is distinct from classical ionotropic and metabotropic receptors. Such a unique mechanism is consistent with the lack of homology of σ receptors with other established neurotransmitter receptors.

Current thinking in this area is that σ receptors, particularly the σ_1 subtype, modulate intracellular signaling cascades via a mechanism involving translocation to and from various cellular compartments (Morin-Surun et al., 1999). In addition to translocating during signaling, σ_1 receptors associate with a number of other proteins, including ankyrin B, heat shock protein 70 (hsp70), heat shock conjugate protein (hsc 70), and glucose-regulated protein (GRP78/BiP) (Hayashi and Su, 2001; Su and Hayashi, 2001; Yamamoto et al., 2002). Therefore, σ_1 receptors appear to function more like growth factor receptors or receptor tyrosine kinases, than classical neurotransmitter receptors. In contrast to σ_1 receptors that readily translocate, σ_2 receptors appear to be lipid raft proteins that affect calcium signaling via sphingolipid products (Crawford et al., 2002; Gebreselassie and Bowen, 2002). Although additional studies are needed to fully define the mechanisms involved in σ receptor signaling, it is clear that these receptors may transduce the actions of cocaine by interacting with a number of potential signaling molecules and cascades.

2.5. Endogenous ligands

Although not fully characterized and understood, there is evidence for endogenous ligands for σ receptors. Many neuroactive steroids have been reported to interact with σ receptors (Su et al., 1988). The best characterized of these include progesterone which appears to act as a σ receptor agonist, and dehydroepianderosterone (DHEA) which appears to act as a σ receptor antagonist (Monnet et al., 1995; Urani et al., 2001). In addition to neurosteroids, several laboratories have independently isolated and reported brain extracts that are capable of binding to σ receptors (Contreras et al., 1987; Su et al., 1986; Zhang et al., 1998). Since these extracts have yet to be purified, the specific identities of these compounds are still unknown.

Their functional relevance is nevertheless suggested by physiological studies demonstrating the release of endogenous σ receptor binding substances from specific brain pathways under conditions associated with neurotransmitter release (Neumaier and Chavkin, 1989; Connor and Chavkin, 1991).

Therefore, although σ receptors have had a rather turbulent history, the advances of recent years underscores the physiologically relevance of these receptors. Moreover, these receptors appear to be viable targets for the development of anti-cocaine drugs.

2.6. Interaction of σ receptor subtypes with cocaine

The ability of cocaine to bind to σ receptors was first reported in Sharkey et al. (1988). However, the physiological relevance of this interaction remained controversial for many years. The reason for the controversy stemmed from the fact that cocaine has micromolar affinity for σ receptors, a finding that has been confirmed by several other laboratories (Matsumoto et al., 2001a, 2002b; Ritz and George, 1993; Ramamoorthy et al., 1995). Despite the relatively low affinity of cocaine for σ receptors, cocaine has been shown to achieve micromolar concentrations in the body (Mittleman and Wetli, 1984; Spiehler and Reed, 1985), suggesting that the interactions demonstrated in the receptor binding studies are physiologically relevant.

However, early attempts to validate the physiological relevance of this interaction by using pharmacological antagonists at σ receptors to attenuate the actions of cocaine were somewhat equivocal. Early generations of σ receptor antagonists, such as rimcazole, BMY-14802 (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine butanol monohydrochloride) and NPC16377 (6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methylflavone) produced interesting initial insights, but generally did not possess high affinity and selectivity for σ receptors (Bristow et al., 1991; Ferris et al., 1986; Karbon et al., 1993). Therefore, novel compounds and sequence-specific antisense oligos that have been developed since then have proved invaluable in demonstrating the importance of the interaction between cocaine and σ receptors, and in establishing the viability of targeting these receptors for the development of anti-cocaine agents.

3. Antagonism of σ receptors attenuates cocaine-induced behaviors

3.1. Comparison of antagonism using σ receptor antagonists and antisense oligos

Functional antagonism of σ receptors can be achieved using either pharmacological antagonists or antisense oligodeoxynucleotides. Pharmacological antagonists act by interfering with the access of cocaine to σ receptors (Fig. 2A). Antisense oligos, on the other hand, deplete the number of σ receptors that are available for cocaine binding by interfering with the synthesis of new receptor proteins (Fig. 2B). The end result in both cases is a reduction in the number of σ receptors that are accessible to cocaine. Both of these strategies have been utilized to functionally antagonize σ receptors and the results from these studies are summarized in the sections that follow. Of the many behavioral endpoints that can be monitored, the studies to date have focused on cocaine-induced convulsions, lethality, locomotor activity, and conditioned place preference.

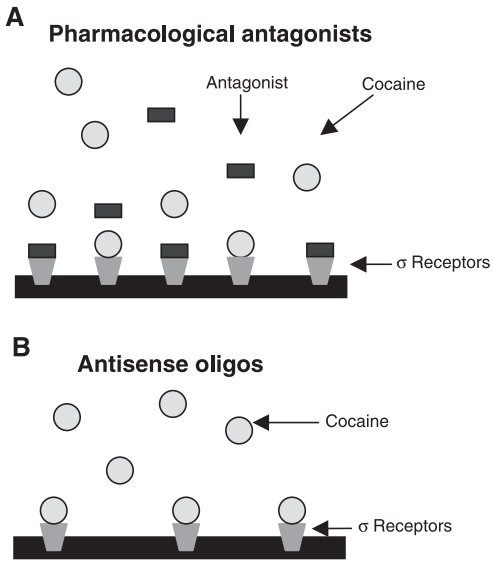

Fig. 2. Antagonism of σ receptors using pharmacological antagonists vs. antisense oligonucleotides. (A) Pharmacological antagonists compete with cocaine for access to its receptors. (B) Antisense oligonucleotides inhibit the synthesis of new receptor proteins thereby depleting the number of receptors available for cocaine binding.

3.2. Cocaine-induced convulsions

Convulsions represent a measure of behavioral toxicity with clinical significance because they are a recognized complication of cocaine intoxication in humans (Pascual-Leone et al., 1990). The mechanisms underlying cocaine-induced convulsions have yet to be fully characterized (Lason, 2001), and it is a form of convulsions that can be resistant to antiepileptic drugs in current clinical use (Witkin and Katz, 1992).

Of the behavioral endpoints that are available to test for medications development potential, cocaine-induced convulsions are a useful screening tool for assessing σ-active compounds because of the relative ease with which these acute studies can be conducted. In addition, since antagonists that attenuate cocaine-induced convulsions also tend to attenuate cocaine-induced locomotor activity, but not vice versa, it provides a way of identifying the most promising compounds to treat both the toxic and stimulant actions of cocaine. The studies that have been conducted to date demonstrate that antagonism of σ receptors using either

pharmacological antagonists or antisense oligos attenuates cocaine-induced convulsions.

3.2.1. Pharmacological antagonists attenuate cocaine-induced convulsions

Historic σ receptor antagonists, such as BMY-14802 and haloperidol, have been reported to significantly attenuate cocaine-induced convulsions in mice (Matsumoto et al., 2001c; Ushijima et al., 1998). In addition to these historic antagonists, numerous novel compounds with antagonistic actions at σ receptors have been reported in recent years to have anti-cocaine properties.

The largest characterized series are the ethylenediamines, comprised of BD1008 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine) and its analogs. Compounds from this synthetic series that significantly attenuate the convulsive effects of cocaine include: BD1008, BD1018 (3*S*-1-[2-(3,4-dichlorophenyl)ethyl]-1,4-diazabicyclo-[4.3.0]nonane), BD1047 (*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine), BD1060 (*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl) ethylamine), BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine), BD1067 (*N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-ethyl-2-(1-pyrrolidinyl)ethylamine), LR132 (1*R*,2*S*-(+)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine), LR172 (*N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine), LR176 ((*R*)-(+)-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-1-methyl-2-(1-pyrrolidinyl)ethylamine), YZ-011 (*N*-[2-(*m*-methoxyphenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-016 (*N*-[2-(*p*-methoxyphenyl) ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-018 (*N*-[2-(*o*-methoxyphenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-027 (*N*-[2-(*m*-nitrophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-028 (*N*-[2-(*o*-nitrophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-029 (*N*-[2-(*p*-nitrophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-030 (*N*-[2-(*p*-aminophenyl) ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine), YZ-032 (*N*-[2-(*o*-aminophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl) ethylamine), YZ-033 (*N*-[2-(*m*-aminophenyl)ethyl]-*N*-methyl-2-(1-pyrrolodinyl)ethylamine) (Matsumoto et al., 2001b,c, 2002b; McCracken et al., 1999a). Notable compounds from this series include BD1063 and BD1047, which have high affinity and selectivity for σ receptors (Matsumoto et al., 1995) and exhibit antagonistic effects through these receptors in other physiological systems (Freeman and Young, 2001; Garrone et al., 2000; Hamabe et al., 2000; Matsumoto et al., 1995; Meyer et al., 2002; Monnet et al., 1995, 1996; Rawls et al., 2002; Tran et al., 1998; Ueda et al., 2001; Urani et al., 2001; Vilner and Bowen, 2000).

Another synthetic series yielding effective anti-cocaine compounds is comprised of the historic "σ receptor antagonist" rimcazole and several of its analogs. Although rimcazole has historically been referred to as σ receptor antagonist, it has relatively weak affinity for σ receptors (micromolar range) and exhibits a significantly higher affinity for dopamine transporters (Ferris et al., 1986; Husbands et al., 1997, 1999). Rimcazole itself is not particularly effective in mitigating the convulsive effects of cocaine (Matsumoto et al., 2001a; Skuza, 1999), but analogs that exhibit protective actions include: SH1/57 (9-[3-[*cis*-3,5-dimethyl-4-(2-isothiocyanotoethyl)-1-piperazinyl]propyl]carbazole), SH2/21 (3-[*cis*-3,5-dimethyl-1-piperazinyl)propyl]diphenylamine), SH3/24 (3-(*cis*-3,5-dimethyl-4-[3-phenylpropyl]-1-piperazinyl)-propyl]diphenylamine) (Matsumoto et al., 2001a). It is further noteworthy that the ability of these compounds to prevent cocaine-induced convulsions is correlated with the affinities of these ligands for σ receptors rather than dopamine transporters (Matsumoto et al., 2001a).

Other individual agents possessing putative antagonistic actions at σ receptors and significantly attenuating cocaine-induced convulsions in rodents include: AC927 (*N*-phenethylpiperidine oxalate) (Gilmore et al., 2002), AC928 (1,4-dibenzylpiperazine oxalate) and its analogs (Foster et al., 2003), EMD 57445 ((*S*)-(−)-[4-hydroxy-4-(3,4-benzodioxol-5-yl)-piperidin-1-ylmethyl]-3-(4-methoxyphenyl)-oxazolidin-2-one) (Skuza, 1999), NPC 16377 (6-[6-(4-hydroxypiperidinyl)hexyloxy]-3-methylflavone) (Witkin et al., 1993), (+)-SM-21 (3α-tropanyl-2-(4-chlorophenoxy)butyrate) (Matsumoto and Mack, 2001), YZ-069 (*N*-phenylpropyl-*N*′-(3,4-dichlorophenethyl)piperazine) and its analogs (Matsumoto et al., 2003).

Almost all of the σ receptor antagonists identified to date possess significant affinity for both σ_1 and σ_2 receptors. However, anti-cocaine agents that possess a >10-fold preference for a particular σ receptor subtype include LR132 (σ_1) and (+)-SM-21 (σ_2), suggesting that both subtypes can be targeted to attenuate the convulsive effects of cocaine. This is further supported by data from the rimcazole series in which the ability of the compounds to attenuate cocaine-induced convulsions was significantly correlated with their binding affinities for both σ_1 and σ_2 receptors (Matsumoto et al., 2001a).

3.2.2. Antisense oligonucleotides attenuate cocaine-induced convulsions

Since pharmacological antagonists at σ receptors produce anti-cocaine actions, antisense oligos targeting these receptors are expected to elicit a similar functional outcome. Two different antisense oligos targeting σ_1 receptors indeed significantly attenuate cocaine-induced convulsions in mice, under conditions where there is about a 40% reduction in the number of σ receptors in the brain (Matsumoto et al., 2001b, 2002b). In contrast, administration of control oligos, comprised of sense or mismatch sequences, does not alter the responsiveness of the animals to cocaine-induced convulsions (Matsumoto et al., 2001b, 2002b). The ability of antisense oligos and pharmacological antagonists that target σ_1 receptors to each reduce the convulsive effects of cocaine suggests that interfering with cocaine's access to these receptors is sufficient to prevent this behavioral toxic effect.

3.2.3. σ Receptor agonists exacerbate cocaine-induced convulsions

In contrast to the behavioral protective effects produced by the antagonists and antisense oligos, σ receptor agonists exacerbate the toxic effects of cocaine and/or shift the dose–response curve for cocaine-induced convulsions to the left. Pre-treatment of mice with the well-established σ receptor agonist di-*o*-tolylguanidine (DTG), or the novel compounds BD1031 (3*R*-1-[2-(3,4-dichlorophenyl)ethyl]-1,4-diazabicyclo[4.3.0]nonane) and BD1052 (*N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-allyl-2-(1-pyrrolidinyl)ethylamine), shift the dose–response curve for cocaine-induced convulsions to the left (Matsumoto et al., 2001b,c, 2002b; McCracken et al., 1999a). In addition, DTG worsens the toxicity of cocaine, with some mice dying in response to doses that are not normally lethal (Matsumoto et al., 2002b; McCracken et al., 1999a). Although most of the agonists tested have mixed interactions at both σ_1 and σ_2 receptors, the apparent significance of σ_1 receptors is again suggested by the ability of the novel σ_1 receptor agonist SA4503 to prolong and enhance the convulsive effects of cocaine (Skuza, 1999).

3.2.4. Summary of cocaine-induced convulsions and σ receptors

Together, the studies indicate that antagonism of σ receptors attenuates the convulsive effects of cocaine. σ Receptor agonists, in contrast, appear to exacerbate the toxic effects of cocaine. Moreover, antagonism of σ_1 receptors alone is sufficient to produce a protective effect, although antagonism of the σ_2 subtype also appears effective. The stronger influence of the σ_1 subtype in producing a protective effect is consistent with the significant affinity of cocaine for σ_1 receptors at concentrations that are achievable in vivo, and the weaker affinity of cocaine for σ_2 receptors.

3.3. Cocaine-induced lethality

3.3.1. Pre-treatment with σ receptor antagonists attenuates cocaine-induced lethality

Since death is the ultimate toxic endpoint, it is noteworthy that σ receptor antagonists can mitigate the lethal effects of cocaine. Pre-treatment of mice with the following σ receptor antagonists have been reported to attenuate cocaine-induced lethality: BD1008, BD1018, BD1047, BD1060, BD1063, BD1067, BMY-14802, haloperidol, LR132, LR172, LR176, reduced haloperidol, YZ-011, YZ-027, YZ-032 (Matsumoto et al., 2001b,c, 2002b; McCracken et al., 1999a). Although the pre-treatment studies demonstrate that interfering with the access of cocaine to σ receptors reduces its toxic effects, to be of clinical value, the antagonists must be effective when administered after an overdose.

3.3.2. Post-treatment with σ receptor antagonists attenuates cocaine-induced lethality

Therefore, it is noteworthy that post-treatment of mice with the following σ receptor antagonists significantly attenuates cocaine-induced lethality: LR132, YZ-011 (Matsumoto et al., 2001b, 2002b). Following a normally lethal overdose of cocaine, mice exhibit a series of behaviors that begin with hyperactivity, followed by convulsions, respiratory depression, and ultimately death. In the post-treatment studies, administration of the antagonists was delayed until the mice began convulsing, leaving only a few minutes to rescue them from death. The ability of select σ receptor antagonists to prevent death under these rigorous, and clinically relevant, conditions suggests that additional studies are warranted to further explore their medications development potential.

3.4. Cocaine-induced locomotor activity

In addition to its toxic effects that are operational in overdose situations, cocaine also possesses psychomotor stimulant effects that contribute to its addiction potential. Therefore, it is noteworthy that σ receptor antagonists and antisense oligos have been reported to attenuate the acute locomotor stimulatory actions of cocaine. In addition, pharmacological antagonists can attenuate the locomotor sensitization that develops after subchronic exposure to cocaine.

3.4.1. Effects of σ ligands on the acute locomotor effects of cocaine

The ability of compounds to attenuate the acute locomotor effects of cocaine is often used as an initial screening tool to identify agents that have the ability to block the psychomotor stimulant properties of cocaine. The following σ ligands have been reported to attenuate the locomotor stimulatory effects of cocaine in rodents: BD1008, BD1018, BD1047, BD1063, BMY-14802, EMD 57445, LR132, LR172, NPC 16377, rimcazole, S14905 (isobutyl-*N*-(1-indan-2yl-piperid-4-yl)*N*-methyl carbamate furamate), (+)-SM-21, SR 31742A (*cis*-3-(hexahydroazepin-1-yl)1-(3-chloro-4-cyclohexylphenyl)propene-1), YZ-011, YZ-027, YZ-032 (Hascoet et al., 1995; Maj et al., 1996; Matsumoto and Mack, 2001; Matsumoto et al., 2001b, 2002b; McCracken et al., 1999a,b; Menkel et al., 1991; Skuza, 1999; Witkin et al., 1993). The ability of these compounds to attenuate the locomotor stimulatory effects of cocaine involves, at least in part, σ receptors because administration of either of two antisense oligos that knock down the levels of brain σ_1 receptors in mice produces a similar effect (Matsumoto et al., 2002b). In contrast to reducing the locomotor stimulatory effects of cocaine through antagonism of σ receptors, the σ receptor agonist DTG has been reported to enhance the locomotor stimulatory effects of cocaine in rats (Skuza, 1999). Therefore, similar to the pattern observed with the behavioral toxic endpoints, antagonism of σ receptors with either pharmacological antagonists or antisense oligos attenuates the locomotor stimulatory actions of cocaine, whereas agonists at these receptors enhance the actions of cocaine.

3.4.2. Effects of σ ligands on cocaine-induced locomotor sensitization

Repeated administration of cocaine to animals can result in behavioral sensitization or reverse tolerance, in which the animals develop an enhanced response to a given dose of cocaine over time. Although the ramifications of this phenomenon to the clinical situation are unclear, it serves as a useful, measurable index of nervous system plasticity that results upon repeated exposure to cocaine. In this regard, it is significant that a number of σ receptor ligands significantly attenuate the development of cocaine-induced locomotor sensitization: BMY-14802, NPC 16377, rimcazole, SR 31742A (Ujike et al., 1996; Witkin et al., 1993). These data suggest that σ receptor antagonists have the ability to prevent neural adaptations that occur upon repeated exposure to cocaine in addition to blocking its acute psychomotor effects.

3.5. Cocaine-induced conditioned place preference

The conditioned place preference paradigm is an ingenious experimental method to quantify the rewarding properties of drugs (Tzschentke, 1998). It is based on the fact that when given a choice, animals will return to and spend more time in an environment in which they previously experienced a drug that was rewarding. Conversely, animals will avoid environments in which they previously experienced an aversive drug. Thus, the extent to which a novel compound may prevent the rewarding effects of cocaine can be quantified as a reduction in the preference (or time spent) in the environment in which they had previously experienced cocaine.

The σ receptor antagonists BD1047 and NE-100 (*N*,*N*-dipropyl-2-[4-methoxy-3-(2-phenylenoxy)-phenyl]-ethylamine monohydrochloride) have been reported to attenuate cocaine-induced conditioned place preference, suggesting the ability of these compounds to reduce the rewarding properties of cocaine (Romieu et al., 2000). The involvement of σ receptors in these effects is confirmed by the ability of an antisense oligo against σ_1 receptors to also attenuate cocaine-induced conditioned place preference (Romieu et al., 2000). Under conditions where the compounds reduced the conditioned place preference produced by cocaine, they produced no rewarding or aversive properties on their own (Romieu et al., 2000). Furthermore, the antagonists were effective in reducing both the development and expression of cocaine-induced conditioned place preference (Romieu et al., 2002). Thus, σ receptor antagonists appear capable of preventing alterations that occur in response to repeated administration of cocaine and to additionally reverse the functional consequences of these changes once they have occurred.

4. Antagonism of σ receptors attenuates cocaine-induced changes in gene expression

The ability of σ receptor antagonists to prevent the toxic and psychomotor effects of cocaine is now well documented. However, the mechanisms associated with the behavioral protective actions of σ receptor antagonists have yet to be fully characterized. In an effort to begin identifying candidate protection genes, a recent study combined behavioral pharmacological studies with microarray analysis and reverse transcriptase-polymerase chain reaction (RT-PCR) confirmations (Liu et al., 2002).

4.1. Experimental design

Since these studies have only been published thus far in abstract form, the design of the study is briefly outlined here. Mice were assigned to different experimental groups: naive, saline + cocaine, BD1063 (a prototypic σ receptor antagonist) + cocaine, BD1063 + saline. The dose of cocaine used (10 mg/kg, i.p.) was one that produced robust locomotor stimulatory effects in previous studies (Matsumoto et al., 2001b, 2002b; McCracken et al., 1999a,b); the dose of BD1063 used (30 mg/kg, i.p.) was one that produced significant attenuation of cocaine-induced locomotor activity in earlier studies (Matsumoto et al., 2001b; McCracken et al., 1999b). Following the drug treatments, the locomotor activity of the animals was quantified, and the brains harvested at a time point at which cocaine produces its peak locomotor stimulatory effects (20 min). The expression of 1176 genes in the brains of these animals was then profiled using cDNA microarrays. Significant changes in gene expression were defined as >5-fold changes that were statistically significant by analyses of variance and post hoc tests, and correlated with the locomotor behavioral changes. Those changes deemed significant based on the microarray data were then confirmed using RT-PCR.

4.2. Cocaine-induced changes in gene expression

The brains of mice exposed to cocaine had a significant up-regulation of 20 genes, and down-regulation of 16 genes. Of these genes, the microarray analysis and subsequent RT-PCR confirmation revealed that the σ receptor antagonist BD1063 prevented cocaine-induced changes in three genes: fos-related antigen 2 (fra-2), G-protein coupled receptor 27 (GPCR 27), and ataxia telangiectasia murine homolog (ATM) (Fig. 3).

Of the three genes identified, changes in fra-2, a member of the fos family of transcription factors is particularly noteworthy. Cocaine is known to stimulate the expression of other fos-related transcription factors (cf. Herdegen and Leah, 1998), but this is one of the first reports showing that cocaine can induce the expression of fra-2. Earlier studies have shown that the expression of fra-2 is stimulated by second messengers such as cAMP and calcium (Rutberg et al., 1997; Rezzonico et al., 1995), suggesting that BD1063 may produce anti-cocaine actions, at least in part, by blocking the activation of these second messengers (Vilner and Bowen, 2000). Similar to other members of the fos family, Fra-2 binds to AP-1 sites as heterodimeric complexes with

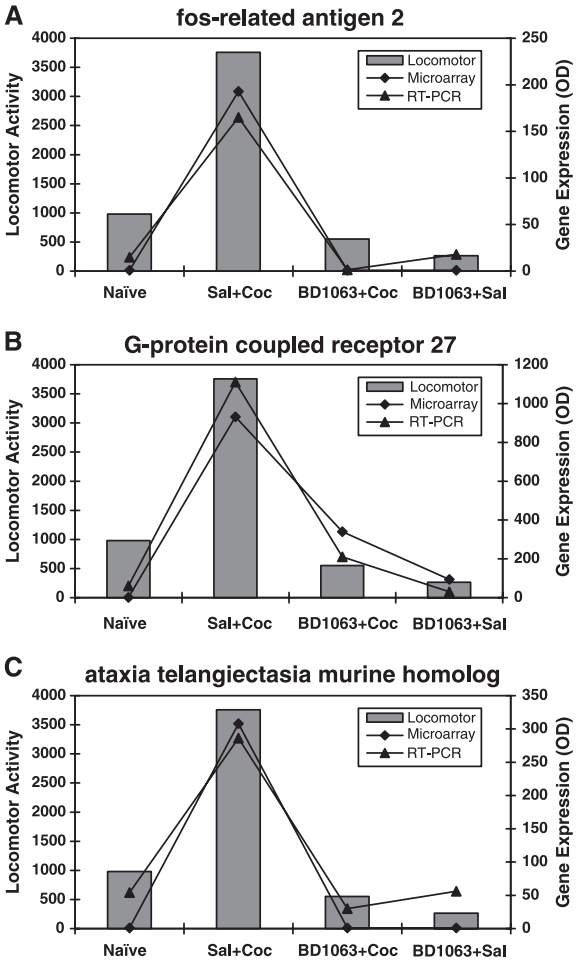

Fig. 3. Association between changes in gene expression and locomotor behavior for fos-related antigen 2 (A), G-protein coupled receptor 27 (B), and ataxia telangiectasia murine homolog (C). The average locomotor activity score for each of the experimental groups is represented in each panel on the left *y*-axis. The average gene expression (in optical density units) for each of the experimental groups is represented in each panel on the right *y*-axis. The data from the microarray studies are connected by circles. The data from the RT-PCR studies are connected by triangles and have been multiplied by the following factors to fall into the same range as the microarray units: X3 in panel A, X30 in panel B, and X2 in panel C. Sal = saline, Coc = cocaine.

other proteins (Herdegen and Leah, 1998; Smith et al., 2001), but the target genes for Fra-2 remain largely unknown. Since σ receptors are localized intracellularly, in addition to their presence in the cell membrane, and the 5′-flanking region of the σ receptor gene contains putative binding sites for AP-1 (Seth et al., 1997), it is possible that they serve as targets for Fra-2. If so, the interaction between Fra-2 and σ receptors would have implications not only for the immediate response to cocaine, but also for longer lasting consequences.

There are no published reports concerning the function of GPCR 27, making it difficult to hypothesize a role for this gene in the behavioral protective effects of BD1063 against cocaine. However, it is noteworthy that in a parallel study in which BD1063 was used to attenuate the locomotor stimulatory effects of methamphetamine, the only significant gene candidate common to this study was GPCR 27 (Matsumoto et al., 2002a). Therefore, it is hypothesized that GPCR 27 may be involved in locomotor function and/or the actions of psychostimulant drugs.

The function of ATM is likewise still being defined. ATM is known to encode a serine protein kinase, with both nuclear and cytoplasmic functions. At the nuclear level, ATM regulates DNA repair in response to double strand breaks (Khanna et al., 2001; Rotman and Shiloh, 1998). It is also capable of phosphorylating various targets at different cell cycle checkpoints (Khanna et al., 2001; Rotman and Shiloh, 1998). The role of ATM in the cytoplasm of neuronal cells has yet to be characterized, but it is thought to participate in sensing and initiating signal transduction pathways. Therefore, antagonism of σ receptors may prevent the activation of ATM-sensitive signal transduction pathways that mediate the actions of cocaine.

The studies indicate that the σ receptor antagonist BD1063 has the ability to prevent alterations in the expression of a number of genes that are induced by cocaine. Some of these changes have implications for the immediate response to cocaine, while others have longer lasting consequences. It therefore appears that some of the protective actions of σ receptor antagonists involve transcriptional mechanisms.

5. Hypothesized mechanism of anti-cocaine actions

The ability of σ receptor antagonists to attenuate a number of cocaine-induced behaviors is thought to result from their ability to intervene in the actions of cocaine at many different levels: (1) direct interference at the receptors, which are localized in key organ systems that are involved in cocaine's actions, (2) modulation of downstream neurotransmitter systems that are involved in the actions of cocaine, and/or (3) alterations in gene expression that are associated with the long-term consequences of cocaine. By competing with cocaine for binding to σ receptors, which are localized in key organ systems such as the brain and heart, σ receptors antagonists can act at one of the initiating points of cocaine's actions. In addition, σ receptors are known to modulate neurochemical systems that have traditionally been linked to the psychomotor and toxic effects of cocaine. For example, dopamine is an important neurotransmitter system that is involved in the psychomotor stimulatory actions of cocaine. The interaction between σ and dopamine systems is well documented, including the ability of σ receptor agonists to cause dopamine synthesis and release (Bastianetto et al., 1995; Booth and Baldessarini, 1991; Gonzalez-Alvear and Werling, 1994; Iyengar et al., 1990; Patrick et al., 1993; Weiser et al., 1995). Although less studied, σ ligands also modulate the effects of serotonin and norepinephrine, two additional neurotransmitter systems that are affected by cocaine (Campbell et al., 1989; Massamiri and Duckles, 1991). In the case of a cocaine

overdose, an important downstream event appears to be excessive activation of NMDA receptors (Brackett et al., 2000), and σ ligands have the ability to modulate NMDA-mediated responses (Iyengar et al., 1990; Monnet et al., 1992; Yamamoto et al., 1995a). Moreover, recent studies demonstrate that σ receptor antagonists prevent cocaine-induced changes in gene expression (Liu et al., 2002), suggesting additional behavioral protective mechanisms occurring via genomic processes. Thus, σ receptor antagonists have the ability to counteract the actions of cocaine at many different levels. These complementary mechanisms are thought to contribute to the ability of σ receptor antagonists to combat an array of cocaine-induced behaviors. Additional studies are needed to further characterize the interaction between σ receptors and these processes.

6. Conclusions

In conclusion, the data indicate that σ receptors are viable targets for the development of anti-cocaine agents. σ Receptors are localized in key organ systems such as the brain and heart, which mediate the psychostimulant and toxic effects of cocaine. Cocaine interacts with these receptors at concentrations that can be achieved in vivo, further supporting the physiological relevance of these sites. Pharmacological studies demonstrate that antagonism of σ receptors, particularly the σ_1 subtype, mitigates a number of cocaine-induced behaviors in mice, including locomotor activity, conditioned place preference, convulsions, and lethality. The contribution and viability of targeting σ_2 receptors to attenuate the actions of cocaine may also be feasible, but additional studies are needed to validate this. Together, the data indicate that antagonism of σ receptors is a promising strategy for the development of medications to treat cocaine addiction and overdose.

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