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Review Article

Pharmacologically-induced stress: a cross-species probe for translational research in drug addiction and relapse

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Abstract: Stress plays a major role in the process of drug addiction and various stressors are known to increase measures of craving in drug dependent human laboratory subjects. Animal models of stress-induced reinstatement of drug-seeking have also been developed in order to determine the neuropharmacological and neurobiological features of stress-induced relapse. Here, we review experimental approaches that use various pharmacological agents to induce a stress response and subsequent craving or drug-seeking for drugs of abuse. The advantages of such an approach are that the exact same stressor can be used in different species, pharmacological stress activation works on identifiable pathways, and stress levels can be varied via dose dependent manipulations. To date, successful use of such probes in both humans and experimental animals have been achieved with noradrenergic compounds and corticotrophin-releasing hormone (CRH). Other possible approaches, such as neuroactive peptides related to central stress responses (e.g., vasopressin and substance P) and inverse benzodiazepine agonists show some promise, and we discuss recent experiments using these compounds. Future development and application of pharmacological stressors across species will be useful in assessing stress-induced craving and relapse in both human drug addiction and animal models of relapse. Through this translational approach, novel treatment interventions for addiction may be designed and tested.

Keywords: Addiction, corticotropin-releasing hormone, craving, reinstatement, relapse, stress, yohimbine

Introduction

Theories of addiction postulate that stress plays an important role in determining an individual's vulnerability and motivation to abuse addictive substances [1,2]. Generally, these models suggest that acute and chronic stress states can drive the process of drug use in order to modulate tension and distress. Over time, dependence can develop, and withdrawal phenomenon, conditioned cues, as well as stress relief and mood enhancement may motivate maladaptive drug taking [3]. Clinical studies have supported an association between adverse life events, chronic stress, and substance use disorders that would be consistent with these models [4,5], although clear causal relationships have not been established.

The definition of stressors varies widely from dramatic events (e.g., divorce, automobile accidents, crime) to the chronic irritants of daily life. Drug or alcohol consumption has been associated with subjective reports of stress, lack of support, and avoidance coping [6-8]. Drug use as a coping response to stress has also been associated with the development of dependence and compulsive use [9,10]. For example, as compared to non-alcoholics, alcoholics increased alcohol intake in response to stressful situations, or reported more stress reduction after alcohol ingestion [11]. Furthermore, painful emotional states and interpersonal stress are primary determinants of relapse in crack cocaine users [12], and in cigarette smokers, smoking increased after exposure to high anxiety as compared to low anxiety situations [13]. These findings indicate that, across substances, stress exposure enhances drug-seeking and drug use.

A number of studies have shown that internal mood states related to stress and negative emotion can elicit craving in a laboratory set-

ting, even in the absence of drug associated cues [14-16]. In cocaine-dependent individuals, personalized imagery of stress situations, as well as exposure to drug-related cues, produced significant increases in cocaine craving, heart rate, cortisol, and subjective anxiety [16]. In studies conducted in subjects with comorbid PTSD and cocaine dependence, exposure to trauma-related images produced as much physiological responsivity and craving as exposure to cocaine-related cues [17]. Furthermore, several studies have found stress/mood-elicited craving predicted relapse to substance use [15,18]. Such studies indicate that stress not only elicits craving, but may also independently predict relapse, supporting the targeting of stress-activated pathways for anti-relapse medication development [19].

Animal models of addiction and relapse

Non-human primates and rodents will reliably self-administer most drugs abused by humans [20,21], and intravenous drug self-administration is well established as a model of addiction with good predictive and construct validity. Compared to other models of addiction (e.g., conditioned place preference), self-administration more closely models the abuse of drugs by humans based on both the route of drug administration (intravenous, rather than intraperitoneal or subcutaneous) and the response-contingent mode of administration (i.e., not given by the experimenter). Drug self-administration studies are typically divided into four temporally distinct phases: acquisition, maintenance, extinction/withdrawal, and reinstatement. The acquisition phase is defined as the period of time required to attain a stable rate of drug self-administration. This phase is followed by a maintenance phase of a duration that ranges from days to weeks. In relapse models, subjects enter an extinction phase, during which response behavior is extinguished by removing the primary drug reinforcer. Extinction is carried out to achieve an operationally defined criterion that can involve within-session parameters on a single test trial, or multiple daily sessions where no drug reinforcement is available. The reinstatement phase occurs when the persistence of drug-seeking behavior is measured by responding on an operandum (usually a lever) where the drug was previously available. Alternatively, subjects may be tested after a period of withdrawal in the absence of explicit extinction trials (abstinence) when returned to the previous environment where drug was available [22]. The reinstatement paradigm is well suited as an animal model for the study of relapse after prolonged drug use and discontinuation [23,24]. Three general procedures have been used for reinstatement of extinguished responding for drugs of abuse: conditioned-cues previously paired with drug taking, acute drug "priming" injections, and external stressors. For detailed descriptions of conditioned-cue and drug-primed reinstatement, the reader is referred to several comprehensive reviews [25-29].

In reference to the stress-induced reinstatement model of relapse, stress can be operationally defined as "forced exposure to events or conditions that are normally avoided by the non-human lab subject" [30]. In animal models of stress-induced relapse, studies have usually employed intermittent footshock to induce reinstatement of drug-seeking behavior [31-33] and to study the neural substrates of stress-induced reinstatement [34,35]. The footshock reinstatement paradigm provides a useful animal model for the study of stress activation of craving states as evidenced from clinical studies that use acute stressors [33]. Footshock stress has been shown to reinstate responding of the previously drug-paired response [31,36], although it is not consistently found [37,38] and certain environmental parameters of the footshock stress exposure dictate the degree of reinstatement that can be produced [23]. It also appears to be selective for reinstatement of drug-seeking, as footshock does not reinstate lever pressing for food [39], nor does it appear to be related to any immediate pain produced by footshock, since footshock given outside the context of the drug-taking environment does not reinstate drug-seeking [23]. Other than footshock stress, several other external stressors have been tested that do not produce reinstatement of drug-seeking in rodent models, including restraint stress and predator odor stress [23,40], even though they elicit similar behavioral and physiological responses aside from reinstatement [41-43].

Pharmacological stress and relapse

In developing translational approaches to the study of relapse with drugs of abuse, it would be advantageous to use stressors that have a high

degree of homology across species. In this regard, external stressors used in clinical laboratory studies of drug craving are often unique to humans, such as script-guided imagery of stressful situations [44] or the Trier Social Stress Test [45]. Physical stressors akin to those used in animal models have been applied in human studies, such as cold pressor stress [46], but such methods are limited due to minimal effect size and potential ethical issues. Furthermore, the subjective comparison of such physical stressors in laboratory animals relative to humans is problematic.

An experimental approach with clear translational potential is the administration of pharmacological agents known to induce stress states in both humans and animal models. The approach of pharmacologically activated stress has been well demonstrated in clinical laboratory research in psychiatry, particularly for acute symptom evocation in syndromes such as post-traumatic stress disorder [47] and phobias [48]. The use of a pharmacological stressor has some obvious limitations, particularly in that this approach does not fully model the impact of a typical external stressor, such as social stress, or the constellation of stress factors that individuals experience in daily life. However, from an experimental perspective, pharmacologically-induced stress offers the clear advantage of acting via similar neurobiological mechanisms in both humans and laboratory animals. Furthermore, the use of pharmacological stressors provides a means to "titrate" stress activation in a dose dependent manner generally unattainable with external stressors. Here, we review studies to date that have utilized pharmacological agents to induce stress states and subsequent craving and drug-seeking in both animal models and human drug addicts.

Corticotropin releasing hormone (CRH)

The most widely tested approach to directly stimulating stress-induced relapse to drug-seeking has been to affect the hypothalamic-pituitary-adrenal (HPA) axis, as the HPA axis is central to mediating stress responses. Corticotropin releasing hormone (CRH) (also referred to as corticotropin releasing factor – CRF) is released in the hypothalamus, and leads to stimulated release of glucocorticoids from the adrenal cortex to the bloodstream [49]. In addition, CRH receptors (CRH1 and CRH2) are broadly localized within the brain and play a key role in mediating stress-related responses [50]. While glucocorticoids have been implicated in the early stages of drug addiction [51,52], their role in craving and relapse is not as definitive.

In animal models of reinstatement to drug-seeking, surgically or pharmacologically attenuating the effects of glucocorticoids fails to affect relapse behavior [53], and human studies demonstrate that glucocorticoid levels do not predict the probability of relapse [44], although these levels are predictive of the amount taken during a relapse event. However, CRH itself does seem to play a significant role in stress-induced drug-seeking at the time of reinstatement [53], but in a manner independent of its classic role in the HPA axis [54]. Multiple extra-hypothalamic brain regions involved in motivation and stress response utilize CRH as a neurotransmitter, and CRH activity in these brain areas strongly influences both stress responses and drug-seeking [3]. Two of these regions, the bed nucleus of the stria terminalis (BNST) and the ventral tegmental area (VTA), highly express CRH receptors and play key roles in stress-induced relapse [35,55]. CRH infusion into either of these brain regions will reinstate drug-seeking [56,57], while CRH receptor antagonists infused into these areas blocks stress-induced reinstatement [57,58].

While brain site selective CRH delivery can discern the localized action of CRH in mediating stress-induced reinstatement, such an approach cannot be easily applied in human studies. Interestingly, while peripheral cocaine dependent humans exhibit exaggerated subjective and heart rate responses to systemic administration of CRH (compared to non-cocaine using controls), the HPA axis response does not differ between cocaine-dependent and control groups, supporting a non-hypothalamic CRH mechanism in the increased stress responsivity of cocaine addicts [59]. A recent clinical study showed that response to CRH, as measured by elevated subjective craving and stress, was a significant predictor of later cocaine use [45]. Such findings are congruent with animal model data showing reinstatement of cocaine-seeking after intra-cerebroventricular (ICV) CRH [60], an effect enhanced in animals with more extensive histories of cocaine intake [61]. Some receptor specificity has been demonstrated in the reinstating effects of CRH, as ICV CRH1, but not

CRH2 receptor antagonists successfully blocked footshock-induced stress [38,53,62]. Surprisingly, when these compounds are infused directly into the VTA, this trend is reversed, as CRH2 antagonists effectively blocked footshock-induced reinstatement, whereas CRH1 antagonists were ineffective [63]. While these results introduce some questions as to the exact role played by CRH receptor subtypes in relapse, future studies directed at activation of CRH receptor subtypes may be a valuable approach for stress-induced reinstatement paradigms in both animal and human laboratory studies.

Norepinephrine

Like CRH, central norepinephrine (NE) release is involved in stress responses [64]. Evidence of a role for NE in stress-induced reinstatement of drug-seeking has been demonstrated in that β1 and β2 NE receptor antagonists infused into the BNST or central amygdala blocked footshock-induced reinstatement of cocaine-seeking [65]. Furthermore, systemic administration of the α-2 NE autoreceptor agonists, clonidine and guanabenz, also attenuated footshock-induced reinstatement of cocaine-seeking [66].

Several pharmacological agents can be used to activate NE function when given systemically. Of particular note is yohimbine, which increases synaptic NE by antagonizing the α-2 autoreceptor, elicits an increase in subjective anxiety, and activates autonomic stress measures, such as increased blood pressure [67,68]. Yohimbine has been extensively used to elicit stress and anxiety responses in clinical laboratory studies [67,69] and to produce stress states in rodent models [70,71].

When administered to abstinent opiate addicts, yohimbine increased subjective anxiety and drug craving [72], thus lending support for the use of yohimbine as a pharmacological probe to activate stress responses and subsequent craving and drug-seeking. In the first demonstration of yohimbine as a means of stress-induced reinstatement in rodents, Shepard and colleagues found that yohimbine dose-dependently reinstated extinguished methamphetamine-seeking in rats [73]. Subsequently, yohimbine has been shown to reliably reinstate drug-seeking in rats after a history of chronic self-administration of cocaine [74,75], heroin [76], alcohol [77], and palatable food [78]. Furthermore, yohimbine potently enhances the reinstating effects of previously drug-paired cues in reinstatement models [74,76]. Finally, both yohimbine and another α-2 autoreceptor antagonist, RS-79948, reinstated cocaine-seeking in monkeys [79].

While yohimbine is a useful pharmacological tool to induce stress in different species, the neuropharmacology of yohimbine in stress responses is complex, as seen by a recent study in which ICV NE induced reinstatement was blocked by pretreatment with the CRH receptor antagonist, D:-Phe CRF(12-41); however, CRH-induced reinstatement was not attenuated by pretreatment with the α2- noradrenergic receptor agonist, clonidine [80]. Furthermore, pretreatment with D:-Phe or clonidine did not block yohimbine-induced reinstatement. Thus, while activating NE systems induces stress and contributes to relapse, these effects may involve a complex interaction with upstream and downstream CRH regulation. Future studies are clearly warranted that may employ more selective NE agents, as well as dual approaches to target NE and CRH.

Other stress-activating neuropeptides

While initial studies have focused on CRH and NE in stress-induced relapse, other important neuropeptides play a role in stress-mediated responses and offer potential targets of study for stress-induced relapse. These peptides share close associations with brain systems involved in both motivation and stress responses, and thus would be predicted to show some level of involvement in craving and relapse behavior. While minimal work has been done to date with these systems in either human or animal models of addiction, they represent potential future targets for pharmacologically-induced relapse. For an in-depth description of additional neuropeptides that potentially play a role in stress induced drug-seeking, the reader is referred to the recent review by Shalev [54].

A neuropeptide that has recently received much attention in the field of addiction is orexin (or hypocretin), which is expressed in two different forms, A and B. Orexins interact with a variety of brain systems involved in motivation, stress responses, and arousal. Accordingly, orexins have been proposed to play a key role in addiction related behaviors [81]. For example, cen-

tral administration of orexins induces stress and reinstates drug-seeking, while the orexin A receptor antagonist SB-334867 blocks footshock-induced reinstatement [82]. While there are no systemic orexin receptor agonists available, future exploration of orexin-mediated stress activation of drug-seeking is a promising area.

The neuroactive peptide substance P and its primary receptor neurokinin 1 (NK1) are closely associated with pain responses, as well as more general stress responses. Substance P interacts with the mesolimbic dopamine system through actions in the VTA, BNST, and nucleus accumbens [83]. Given these connections, it is not surprising that substance P may play a role in addictive behaviors, including stress-induced relapse. Application of footshock results in an excitatory effect of substance P in the VTA, which enhances dopamine release from these cells [84]. In line with these effects, infusion of substance P receptor agonists into the VTA reinstates cocaine-seeking, while substance P receptor antagonists block footshock stress-induced relapse when infused into the VTA [85,86]. Since substance P and NK1 receptors are co-localized with central CRH and CRH receptors, substance P likely interacts with CRH systems in mediating stress activated relapse [83].

Arginine vasopressin (AVP) is a neuroactive peptide that activates multiple stress systems, including the HPA axis and elements of the extended amygdala, and modulates central reward systems, including mesolimbic dopamine [87,88]. An initial connection of AVP to drug-seeking was revealed by the observation that markers of AVP are increased in the amygdala during both cocaine [89]and heroin withdrawal [90]. Furthermore, a connection between AVP and relapse is suggested by the observed increases in AVP levels in the extended amygdala during footshock-induced reinstatement of heroin-seeking, as well as the blockade of footshock-induced reinstatement by a centrally acting AVP/V1b receptor antagonist [90]. New drugs that target central AVP receptors may thus serve as useful probes in stress-induced relapse.

Benzodiazepine receptors

The inhibitory neurotransmitter γ-amino butyric acid (GABA) has been extensively explored in the mediation of stress and anxiety. Benzodiazepines (BZs) exhibit agonist action at the BZ component of $GABA_A$ receptors, and BZs are commonly used to interrupt and diminish stress responses in both humans and animal models [91]. BZs can decrease the anxiety associated with acute administration of psychostimulants [92], and attenuate the withdrawal symptoms of psychostimulants, opiates, and alcohol [93]. These withdrawal symptoms activate stress systems and are a primary instigator of relapse [94,95]. Therefore, relieving these symptoms may decrease the probability of a relapse event. Accordingly, systemic BZ administration blocks the reinstating effects of drug-paired cues [96]. BZ effects on cue-induced craving may be due to the attenuation of the stressful component of cues, as cues can evoke stress in abstinent human drug addicts [97].

If BZs act to dampen stress-induced relapse, inverse receptor agonists at the BZ binding site may serve as potential pharmacological probes to increase stress and drug craving. In contrast with the behavioral and physiological effects of BZs, the BZ partial inverse agonist, FG 7142, increases subjective and behavioral measures of stress and anxiety, as well as activation of the HPA axis in both humans and rodents [98,99]. While it would be expected that FG 7142 should enhance drug-seeking, we have obtained preliminary data indicating that FG 7142 does not reinstate cocaine-seeking or affect the reinstating qualities of conditioned cues [100]. Interestingly, FG 7142 also fails to elicit anxiety in cocaine-trained rats as seen by performance on an elevated plus maze, a standard indicator of anxiety-like behavior. These results indicate the possibility that $GABA_A$ receptors are modified in the addicted state, such that a diminished stress response occurs with acute BZ inverse agonism. Supporting this idea, rats with a history of repeated cocaine administration exhibited diminished levels of BZ receptors in the nucleus accumbens and prefrontal cortex [101].

Conclusions

Stress is a critical mediator of the process of drug addiction, particularly during periods of withdrawal and abstinence, when stressors can elicit drug craving and renewed drug use. While not extensively utilized in the drug addiction field, pharmacological elicitation of stress states

offers a potentially powerful approach to studying the fundamental mechanisms of craving and stress-induced relapse and developing successful addiction treatments. Drugs such as yohimbine can be reliably employed in parallel studies of stress activation of drug craving in humans and drug-seeking in animal models, thus facilitating translational investigation in ways that are superior to other forms of experimentally-induced stress. Pharmacological probes allow for improved parameters of activation of stress responses and may better elucidate some of the underlying neuronal mechanisms that mediate stress-driven relapse. Finally, pharmacological probes for stress-induced relapse offer an excellent experimental tool for measuring *in vivo* brain function (e.g., fMRI or PET) in both humans and experimental animals in addiction research. As detailed in this review, several neurotransmitter and neuropeptide systems offer potential avenues of exploration for developing appropriate pharmacological agents. As newer drugs and better routes of administration become available, it will be critical to apply such agents in the translational study of addiction and relapse.

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