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Opiate and Cocaine Addiction: From Bench to Clinic and Back to the Bench

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Summary

This review primarily focuses on our recent findings in bidirectional translational research on opiate and cocaine addictions. First, we present neurobiological and molecular studies on endogenous opioid systems (e.g., proopiomelanocortin, mu opioid receptor, dynorphin, and kappa opioid receptor), brain stress responsive systems (e.g., orexin, arginine vasopressin, V1b receptor, and corticotropin-releasing factor), hypothalamic-pituitary-adrenal axis and neurotransmitters (especially dopamine), in response to both chronic cocaine or opiate exposure and to drug withdrawal, using several newly developed animal models and molecular approaches. The second aspect is human molecular genetic association investigations including hypothesis-driven studies and genome-wide array studies, to define particular systems involved in vulnerability to develop specific addictions, and response to pharmacotherapy.

Introduction

In the field of drug addiction, like many others, for years there has been a focus of “bench-to-clinic,” that is translational, research. In our field, we have emphasized that there remains a continuing need to make careful observations in the clinic, as well as in real-world settings, and then to model findings of pattern, mode, and frequency, as well as specific types and routes of administration of drugs of abuse, for designing studies for research at “the bench” [1]. There, animal models, and molecular and cellular constructs are used, as appropriate, employing a variety of techniques of modern science, ranging from analytical and medicinal chemistry, and the newer techniques of molecular and cell biology and imaging, at the molecular up through the whole animal research, along with integrated neurobiological and behavioral studies. Our Laboratory of the Biology of Addictive Diseases focuses on what we began to refer to, in the late-1980s, as “bidirectional translational research.” It is enormously exciting to unravel the molecular and chemical mechanisms of the brain that underlie learning, memory, and behaviors. However, in our field, it is also very important, as promptly as possible, to translate those findings into the clinical setting for improvement of prevention and treatment of addictions. In this effort, an additional field is proving to be extremely helpful, that is, human molecular genetics.

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In this brief concept paper, we will focus on two types of addiction: opiate addiction (to heroin, as well as to prescription opiates illicitly used) and cocaine addiction. We will present findings on the role of the endogenous opioid system, including the mu opioid receptor (MOP-r) system, which is involved in reward, the hypothalamic-pituitary-adrenal (HPA) stress-responsive system, and the countermodulatory kappa opioid receptor (KOP-r) system, as well as related classical neurotransmitter systems and other neuropeptides, in the pathophysiology of the addictive diseases. We also will cover some highlights of topics from recent work of human molecular genetics of these addictions, including hypothesis-driven studies and genome-wide array studies.

Although we have very effective treatments, when properly used, for opiate addiction, i.e., methadone maintenance, first developed by our laboratory in 1964 [2-3], and also buprenorphine maintenance, our laboratory and numerous others are still struggling to find a compound or several different compounds which may be demonstrated to be significantly effective in treatment of cocaine addiction, with willingness of clinical scientists and regulatory agencies to accept any specific medication documented to be helpful, on a long-term basis, to as low as 20% of those persons suffering from chronic cocaine addiction.

I. MOP-r, the HPA axis, and other stress responsive systems

Methadone (a selective MOP-r agonist, long-acting in humans) is widely used in treatment of short-acting opiate (primarily heroin) addiction [2,3]. Methadone maintenance can decrease cocaine use in heroin-cocaine codependent patients [4,5]. Consistent with our clinical and laboratory studies, other investigators have observed up-regulation of MOP-r binding in cocaine-dependent individuals, which is associated with cocaine craving [6]. In rats, after acute or chronic cocaine there is an increase in gene expression and MOP-r density in mesocorticolimbic regions [7,8]. However, we have not found any increase in proopiomelanocortin (POMC) gene expression or one of its products, beta-endorphin, which is the longest of the endogenous opioid peptides, that normally acts at MOP-r.

In collaboration with Leri and colleagues, we investigated the effect of methadone on cocaine conditioned place preference (CPP) in rats, a rodent model for cocaine wanting/liking behaviors. Since methadone has a short half-life in rodents (about 60-80 min) [3], methadone was delivered through osmotic pumps [9] to mimic steady-state methadone (SSM) maintenance in humans. We found that 1) rats with SSM prior to cocaine conditioning did not develop and/or express cocaine CPP; 2) rats with SSM after cocaine conditioning expressed neither spontaneous nor cocaine-evoked CPP; and 3) increased MOP-r mRNA levels in nucleus accumbens (NAc) core after cocaine conditioning was dose-dependently prevented by SSM. Together, our results suggest that SSM blocks cocaine wanting and drug-precipitated cocaine "liking," possibly by preventing MOP-r alterations in NAc core and thus avoiding a relative endorphin deficiency [10•].

Using small interfering RNAs (siRNA) targeting MOP-r, specifically in the ventral tegmental area/sub-substantia nigra compacta, we recently found that mice with reduced expression of MOP-r showed attenuated heroin-enhanced locomotor activity and CPP expression [11•]. Our results demonstrate the utility of siRNA in integrated neurobiological studies of specific genes expressed in specific brain regions, an approach which may have potential therapeutic value in the future.

Studies from our laboratory and others have examined the effects of withdrawal from chronic opioid administration on MOP-r gene expression in animal brain. Administration of opiates using pellets, used in some studies, differs from heroin addicts who use an intermittent pattern of self-administration to achieve rewarding effects and expose to withdrawal in between-dose intervals [3]. Therefore, an administration paradigm of chronic intermittent escalating-dose

heroin or morphine was developed in our laboratory. Using this paradigm, we found that acute morphine withdrawal (but not chronic exposure) resulted in increased MOP-r mRNA levels in NAc core, lateral hypothalamus (LHypo) and caudate-putamen [12], again further supporting the need for chronic MOP-r agonist or partial agonist treatment of opiate addiction.

Recent evidence suggests an important role for orexins/hypocretins in modulation of drug reward and addiction-like behaviors in rodents [13]. Preprodynorphin (pDYN) expresses in all the orexin neurons in LHypo. While acute cocaine withdrawal increased both LHypo orexin and pDYN mRNA levels [14], morphine withdrawal only increased orexin levels [12], suggesting that the orexin/dynorphin system is differentially involved in cocaine and opiate withdrawal (see section II). The orexin system may be a target for therapeutic interventions.

Recent human studies have demonstrated that severe or unavoidable psychological stressors elevate cocaine and alcohol craving and HPA activity, whereas modest activation of the HPA axis, for instance by MOP-r antagonist administration, may reduce craving, and the HPA responses in part predict subsequent drug relapse [15•,16]. In rats, chronic cocaine and its withdrawal dynamically alter HPA activity and expression levels of stress responsive genes (Table 1) [17]. Stress-related anxiety and depression are major psychiatric consequences of chronic drug abuse, especially during withdrawal [18•]. Cocaine withdrawal increases arginine vasopressin (AVP) mRNA levels in rat medial amygdala [19]. Amygdalar AVP mRNA increases are further found during early heroin withdrawal and also after foot shock in the rat withdrawn from heroin for 2 weeks [20•]. There are three subtypes of AVP receptors: V1a, V1b, and V2. While V2 is located in the kidney and mediates the antidiuretic action, V1a and V1b are expressed in extended amygdala. Selective V1b antagonists dose-dependently attenuated foot-shock-induced reinstatement of heroin-seeking behavior, and blunted the HPA activation by stress [20•]. Our data suggest that the stress-responsive AVP/V1b system may provide new therapeutic targets for prevention of drug relapse.

II. KOP-r/pDYN system

The KOP-r system, and its endogenous peptide agonists (dynorphins), have been implicated in the acute and chronic modulation of neurobiological and behavioral effects of drugs of abuse, including opiates and psychostimulants, and alcohol. This modulation is largely thought to be due to the prominent localization of this neuropeptide system in the mesocorticolimbic and nigrostriatal dopaminergic pathways, major direct and downstream targets of drugs of abuse. Acutely administered drugs of abuse (e.g., opiates, alcohol and psychostimulants) cause elevations in extracellular fluid dopamine in the striatum (including NAc), and dopamine surges are thought to partially underlie initial rewarding effects [21]. KOP-r agonists (synthetic or endogenous) tend to have the opposite effect, as a counter-regulatory mechanism. Also, the KOP-r/pDYN system is reoccurently up-regulated in response to both acute and chronic drugs of abuse, possibly priming a state of heightened vulnerability to relapse [7].

Thus it has been hypothesized that ligands acting at KOP-r may be potential pharmacotherapeutic agents for specific stages in the treatment of addictive diseases [1]. Acutely, high efficacy KOP-r agonists have been shown to block the rewarding and psychostimulant effects of cocaine [22]. However, high efficacy KOP-r agonists are known to have psychotomimetic effects in humans [23], and it is unknown whether doses of these compounds that do not produce such undesirable effects would be chronically active in the blockade of cocaine-induced reward. The shortcomings potentially associated with the use of high efficacy KOP-r agonists may suggest that a KOP-r partial agonist may effectively modulate hypo- or hyper-activity in the KOP-r/pDYN system, in the absence of severe psychotomimetic effects. There are currently no well studied selective KOP-r partial agonists, however several clinically used compounds have partial KOP-r agonist effects in addition to

their actions at other sites (typically MOP-r sites; e.g., nalbuphine, butorphanol, pentazocine). Notably, nalmefene (a clinically available MOP-r antagonist) has also been shown to be a KOP-r partial agonist in humans, and does not produce psychotomimetic effects, consistent with its low efficacy at KOP-r [24]. Thus nalmefene, or a congener with reduced secondary affinity at MOP-r, may represent a valuable pharmacotherapeutic approach in the management of psychostimulant addiction.

Consistent with an alternative hypothesis that activation of the KOP-r/pDYN system may enhance vulnerability to relapse, a selective KOP-r antagonist, JDTic, was able to block shock-induced (a stressor), but not cocaine-induced, reinstatement of cocaine self-administration in rodents [25]. Similar to other KOP-r antagonists, JDTic also produced antidepressant-like effects [25]. Since depressed mood states and stress may be important vulnerability factors in relapse to cocaine addiction, administration of a selective KOP-r antagonist may be a potential pharmacotherapeutic approach. Of note JDTic, like most other known selective KOP-r antagonists, is of very long duration *in vivo* (at least several days). The molecular underpinnings, and pharmacotherapeutic consequences, of these long-lasting effects of KOP-r antagonists are potential areas for investigation.

III. Human molecular genetic studies

1. Genome-wide case-control association studies

Three pooled DNA studies of polysubstance abuse were reported by the Uhl group. The first study [26] scanned 1,494 SNPs, in European Americans (EA) and African Americans (AA), provided 41 candidate chromosomal regions including one spanning the genes encoding brain-derived neurotrophic factor (BDNF) and alcohol dehydrogenase 3. A 10K scan [27] identified 38 risk variants including genes implicated in “cell adhesion” and alcohol dehydrogenase gene clusters. The third 639K study [28] identified 89 potential risk genes including genes implicated in cell adhesion.

A recent 10K scan from our laboratory [29•] was conducted in an EA population of former heroin addicts in methadone treatment. The most significant associations were obtained with two intergenic variants (rs965972 and rs1986513), and the intronic SNP rs1714984 (myocardin gene). An association was also shown for a genotype pattern (rs1714984, rs965972 and rs1867898). Evidence for association was also detected for SNPs located upstream/downstream (11-75 kb) of the hypothesis-driven genes encoding MOP-r, metabotropic receptors mGluR6 and mGluR8, nuclear receptor NR4A2 as well as cryptochrome 1 (photolyase-like).

2. Hypothesis-driven gene association studies

Hypothesis-driven gene association studies have reported association of heroin addiction with variants in various genes (Table 2). These genes are encoding the MOP-r, KOP-r, delta-opioid receptor (DOP-r), dopamine receptors D2 and D4, serotonin receptor 1B, serotonin transporter, gamma-aminobutyric acid (GABA) receptor gamma 2, catechol-*O*-methyltransferase (COMT, involved in metabolism of estrogen and dopamine), period circadian protein 3, proenkephalin, POMC, tryptophan hydroxylase 2, BDNF and melanocortin receptor type 2 (ACTH receptor). In a recent hypothesis-driven multi-gene study [30••], we scanned 1350 variants in 130 candidate genes in an EA population of 412 former severe heroin addicts in methadone treatment, and 184 healthy controls. The nine variants that showed the most significant associations were in non-coding regions of the genes encoding the MOP-r, KOP-r and DOP-r, serotonin receptor 3B, galanin (involved in regulation of food intake, behavioral and neurochemical effects of opiates and high stress response), and casein kinase 1epsilon

(participates in important signaling pathways including the phosphorylation of the circadian rhythm protein, period).

Several studies reported association of cocaine addiction (with or without alcohol comorbidity) with genetic variants (Table 2). These include the genes encoding DOP-r, pDYN, COMT, dopamine transporter 1, dopamine receptor D2, cannabinoid receptor 1, tachykinin receptor 3 (involved in activation of phospholipase C by binding of substance P, associated with the regulation of anxiety, depression and stress), Homer1 (a dendritic protein that has a role in signal transduction and regulates group 1 mGluR function), and nicotinic cholinergic receptor subunits alpha 3 and 5. Also, associations were reported for mixed addictions (cocaine and/or heroin) with variants in the genes encoding the MOP-r, multiple *ADH* genes, proenkephalin, POMC, cholinergic muscarinic 2 receptor, cannabinoid receptor 1 and dopamine receptor D2 (Table 2).

3. P-glycoprotein gene (*ABCB1*, *MDR1*) variants and methadone dose requirements

The inter-individual variability in methadone dose effectiveness may be determined in part by genetic factors. Since methadone is a substrate of P-glycoprotein, we assessed the association between *ABCB1* SNPs and methadone dose requirements in 98 methadone-maintained heroin addicts from Israel [31•]. A significant difference in genotype frequencies was found between the “higher” (>150 mg/day) and “lower” (≤150 mg/day) dose groups for the synonymous SNP 1236C>T (rs1128503) and for a 3-locus genotype pattern TT-TT-TT (rs1045642, rs2032582 and rs1128503).

4 A functional *PDYN* haplotype is associated with cocaine/alcohol dependence

In a recent study [32•] we tested for association of *PDYN* polymorphisms with vulnerability to develop cocaine and/or cocaine/alcohol dependence, in EA and AA subjects. An association was found with three linked 3' UTR SNPs, independently and as a haplotype, in EA. We showed differential allelic expression for SNP rs910079 in caudate and nucleus accumbens of human postmortem brains. There were also lower levels of total *PDYN* expression of postmortem brains in subjects having the risk haplotype.

Each of these genes, enumerated above, may turn out to be interesting targets for further studies in animal modeling and might, in the future, yield targets for specific compounds for challenge studies in humans and possibly therapeutic agents [33].

IV. Conclusion

The bidirectional translational approach continues to yield both discovery of new potential targets, as well as confirm or refute hypothesis-driven sites of action, downstream events, and the various dynamic changes which occur during the development and persistence of, as well as relapse to and addiction to a specific drug of abuse. This type of bench-to-bedside and back again is undoubtedly critical for future advances in our field.

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Table 1

Effects of chronic “binge” pattern cocaine administration (3×15 mg/kg/day i.p. at hourly intervals) across 14 days (A) and across 10 days of withdrawal from 14 days of chronic “binge” cocaine (B) on plasma ACTH (pg/ml) and corticosterone (ng/ml) levels and on POMC, CRF, and CRF-R1 mRNA levels (pg/ μ g total RNA) in the hypothalamus (Hypo), anterior pituitary (AP), frontal cortex (FCx) and amygdala (Amy) of the rat. Note: $\uparrow$, significant increase vs. Control; $\downarrow$, significant decrease vs. Control; ^a, 14-day cocaine significantly lower than 3-day cocaine. Data published earlier in graphic form [17,34].

A.				
	Saline Control	1-day Cocaine	3-day Cocaine	14-day Cocaine
ACTH	17 $\pm$ 4	$\uparrow$ 60 $\pm$ 10	$\uparrow$ 145 $\pm$ 27	50 $\pm$ 20 ^a
Corticosterone	10 $\pm$ 2	$\uparrow$ 55 $\pm$ 7	$\uparrow$ 90 $\pm$ 16	$\uparrow$ 47 $\pm$ 11 ^a
CRF in Hypo	0.39 $\pm$ 0.03	$\uparrow$ 1.10 $\pm$ 0.20	0.34 $\pm$ 0.03	$\downarrow$ 0.26 $\pm$ 0.02
POMC in Hypo	32.3 $\pm$ 3.5	$\downarrow$ 26.0 $\pm$ 2.0	29.0 $\pm$ 1.2	33.0 $\pm$ 1.5
CRF-R1 in AP	0.79 $\pm$ 0.03	0.74 $\pm$ 0.05	0.85 $\pm$ 0.04	$\uparrow$ 0.96 $\pm$ 0.05
POMC in AP	260 $\pm$ 15	270 $\pm$ 12	310 $\pm$ 22	$\uparrow$ 350 $\pm$ 25
CRF in FCx	0.21 $\pm$ 0.01	0.22 $\pm$ 0.02	$\uparrow$ 0.29 $\pm$ 0.02	0.24 $\pm$ 0.02
CRF in Amy	0.057 $\pm$ 0.003	$\uparrow$ 0.119 $\pm$ 0.026	0.063 $\pm$ 0.003	0.063 $\pm$ 0.002
B.				
	Acute (1-2 day) Withdrawal Control	Acute (1-2 day) Cocaine Withdrawal	Chronic (10-day) Withdrawal Control	Chronic (10-day) Cocaine Withdrawal
ACTH	50 $\pm$ 6	$\uparrow$ 138 $\pm$ 39	33 $\pm$ 13	62 $\pm$ 19
Corticosterone	4.9 $\pm$ 0.7	$\uparrow$ 14.0 $\pm$ 3.9	7.0 $\pm$ 1.7	6.2 $\pm$ 1.3
CRF in Hypo	0.33 $\pm$ 0.06	0.34 $\pm$ 0.04	0.29 $\pm$ 0.02	0.27 $\pm$ 0.02
POMC in Hypo	33 $\pm$ 4.0	42 $\pm$ 5.0	31 $\pm$ 2.0	30 $\pm$ 3.0
CRF-R1 in AP	0.85 $\pm$ 0.08	1.13 $\pm$ 0.25	0.59 $\pm$ 0.07	0.70 $\pm$ 0.10
POMC in AP	213 $\pm$ 18	307 $\pm$ 62	182 $\pm$ 15	183 $\pm$ 7
CRF in FCx	0.22 $\pm$ 0.01	0.25 $\pm$ 0.02	0.22 $\pm$ 0.01	0.20 $\pm$ 0.05
CRF in Amy	0.055 $\pm$ 0.003	$\uparrow$ 0.069 $\pm$ 0.004	0.051 $\pm$ 0.008	0.043 $\pm$ 0.002

Table 2

Genes associated with heroin and/or cocaine addiction by hypothesis-driven single or multiple gene association studies

Gene	Protein	Drug	Selected References ¹
<i>OPRM1</i>	mu opioid receptor	h, dd	30,35,36,37
<i>OPRK1</i>	kappa opioid receptor	h	30,38,39
<i>OPRD1</i>	delta opioid receptor	h, c	30,40
<i>PDYN</i>	prodynorphin	c	32,41,42,43
<i>PENK</i>	proenkephalin	h, dd	44
<i>POMC</i>	proopiomelanocortin	h, dd	44
<i>HOMER1</i>	homer homolog 1	c	45
<i>TACR3</i>	tachykinin receptor 3	c	46
<i>MC2R</i>	melanocortin receptor type 2 (ACTH receptor)	h	47
<i>DRD2</i>	dopamine receptors D2	h, c, dd	48
<i>DRD4</i>	dopamine receptors D4	h, c	49
<i>SLC6A3</i>	dopamine transporter 1 (DAT1)	c	50
<i>COMT</i>	catechol-O-methyltransferase	h, c	51
<i>HTR1B</i>	serotonin receptor 1B	h	52
<i>HTR3B</i>	serotonin receptor 3B	h	30
<i>SLC6A4</i>	serotonin transporter (SERT)	h	53
<i>TPH2</i>	tryptophan hydroxylase 2	h	54
<i>GABRG2</i>	GABA _A receptor gamma 2	h	55
<i>CNR1</i>	cannabinoid receptor 1	c, dd	56,57,58
<i>CHRM2</i>	cholinergic muscarinic 2 receptor	dd	59
<i>BDNF</i>	brain-derived neurotrophic factor	h, c	60
<i>PER3</i>	period circadian protein 3	h	61
<i>CSNK1E</i>	casein kinase 1 epsilon	h	30
<i>GAL</i>	galanin	h	30
<i>ADH</i>	alcohol dehydrogenase gene cluster (multiple genes)	dd	62

h: heroin, c: cocaine, dd: drug dependence (cocaine and/or heroin).

¹ For additional references (prior to 2005) see [33].