

# State of the art

## *Opioids, dopamine, stress, and the addictions*

Mary Jeanne Kreek, MD



*The articulated goals of Dialogues in Clinical Neuroscience are to serve as "an interface between clinical neuropsychiatry and the neurosciences by providing state-of-the-art information and original insights into relevant clinical, biological, and therapeutic aspects." My laboratory, the Laboratory of the Biology of Addictive Diseases at The Rockefeller University, has for years been focused on "bidirectional translational research," that is, learning by careful observations and study in patient populations with the disorders under study, in this case primarily specific addictive diseases, and then using that knowledge to create improved animal models or other laboratory-based research paradigms, while, at the same time, taking research findings made at the bench into the clinic as promptly as that is appropriate and feasible. In this invited review, therefore, the focus will be on perspectives of our Laboratory of the Biology of Addictive Diseases and related National Institutes of Health/National Institute on Drug Abuse research Center, including laboratory-based molecular neurobiological research, research using several animal models designed to mimic human patterns of drug abuse and addiction, as well as basic clinical research, intertwined with treatment-related research.*

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Unnumerable reviews on addictive disorders have been written by many groups, including our own, over the past decade.<sup>1-17</sup> We have contributed over 20 reviews, commentaries, perspectives, or viewpoints in the last 5 years. In 2004, my laboratory published a review article on "Evolving perspectives in neurobiological research in the addictions."<sup>18</sup> Therefore, for this state-of-the-art review with conceptual insights, focus will be placed on research conducted in our Laboratory and Center over the last 5 years. For further information and for some relevant citations of other research groups, one can consult some reviews which we have prepared on basic molecular neurobiology, with a focus on cocaine and other stimulant addictions, opiate addiction, and alcoholism.<sup>1-6</sup> We have published other reviews and perspectives on research related to stress responsivity, and also genetics related to stress responsivity, and with emphasis on the role of stress responsivity.<sup>5,7,8,11</sup> Further, and relatively exhaustive, reviews on human molecular genetics related to the addictions may be found in yet other recent publications from our laboratory.<sup>9,10</sup> Finally, reviews of the history of treatment research in our own laboratory, as well as overviews of recent contributions of our group and others, have been published within the last 5 years.<sup>12-17</sup>

**Keywords:** *opioids; dopamine; stress; opiate addiction; cocaine addiction; polymorphisms*

**Author affiliations:** Patrick E. and Beatrice M. Haggerty Professor, Head of the Laboratory of the Biology of Addictive Diseases, The Rockefeller University, Senior Physician, The Rockefeller University Hospital, New York, NY, USA

**Address for correspondence:** Mary Jeanne Kreek, MD, Laboratory of the Biology of Addictive Diseases, The Rockefeller University, Senior Physician, The Rockefeller University Hospital, 1230 York Avenue – Box 171 New York, NY 10021  
(e-mail: [kreek@rockefeller.edu](mailto:kreek@rockefeller.edu))

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## Selected abbreviations and acronyms

<b>ACTH</b>	<i>adrenocorticotropin hormone</i>
<b>CRF</b>	<i>corticotropin-releasing factor</i>
<b>HPA</b>	<i>hypothalamic-pituitary-adrenal</i>
<b>nor-BNI</b>	<i>nor-binaltorphimine</i>
<b>POMC</b>	<i>proopiomelanocortin</i>

This review will be presented in three sections: (i) laboratory-based molecular neurobiological and neurochemical studies related to cocaine and opiate addiction and potential new approaches to treatment thereof; (ii) role of stress responsivity in the acquisition and persistence of specific addictive diseases, and the impact of chronic exposure to drugs of abuse and withdrawal therefrom on components of the stress-responsive system, along with identification of potential new targets for therapeutic intervention; and (iii) basic clinical research related to specific addictive diseases, with emphasis on stress responsivity: all research focused on treatment improvement.

## Laboratory-based molecular neurobiological and neurochemical studies related to cocaine and opiate addiction, and potential new approaches to treatment thereof

Over the past several years, we have developed several animal models for acute, subacute, and chronic exposure to specific drugs of abuse, with emphasis on cocaine, morphine and heroin, and alcohol. One of these models, which we have developed, validated, and used extensively in our studies, is “binge”-pattern cocaine administration mimicking the most common pattern of human abuse. In studies from our laboratory in which animals were allowed to self-administer cocaine with presentation of high, as well as moderate, and the usual low doses of cocaine, and with extended access (10 hours) provided, we found that animals will escalate their use of cocaine.<sup>19</sup> In fact, by 5 days of extended access to high doses of cocaine rats will self-administer more than twice the dose which we had usually used in our chronic “binge-pattern” cocaine administration (15 mg/kg x3, that is 45 mg/kg/day). We have extended this “binge-pattern,” using both the steady-dose and escalating-dose “binge-pattern” administration of cocaine.<sup>19</sup> We have been able to study various behavioral factors, as well as impact on gene expression, comparing these two models. One of the most important early findings from our labo-

ratory (and others) on gene expression has been the finding of significant increased preprodynorphin gene expression in the striatum of rodents after acute, subacute, and chronic cocaine administration (eg, refs 20,21). This is especially important since we and others have shown that dynorphin peptides, which are the natural endogenous opioid ligands of the kappa-opioid receptors, serve to modulate dopaminergic tone and countermodulate cocaine-induced dopaminergic surges. In a recent study, we examined the effects of steady-dose versus escalating-dose binge-pattern cocaine administration upon striatal preprodynorphin messenger ribonucleic acid (mRNA) levels, and also on behavioral stereotypy.<sup>22</sup> We found that both steady-dose and escalating-dose binge cocaine administration resulted in increased preprodynorphin mRNA levels in the caudate-putamen, but not in the nucleus accumbens. These are similar to all our earlier studies of the impact of acute, subacute, and chronic cocaine administrations. In this study, there were no significant differences in preprodynorphin mRNA levels when escalating doses (up to 30 mg/kg x 3, or a total of 90 mg/day) were administered during the last five days of 14-day chronic dosing, compared with a total of 45 mg/day, the steady dose “binge pattern.”<sup>22</sup> These data showed that the enhancement of gene expression of dynorphin response to cocaine has probably reached its maximum level at a dose of 45 mg/kg/day of cocaine, and may or may not be dose-dependent at lower doses. Further, in this study it was found that cocaine significantly affected body weight in both paradigms, and that both resulted in expression of behavioral stereotypy. However, of note, one component of stereotypy, that is, intense rapid head movements, was found to be both dose- and time-dependent, with more profound effects in the escalating-dose model.<sup>22</sup>

Extending our much earlier studies in the rat, the effects of the natural kappa-opioid receptor agonist, dynorphin A(1-17), on both basal striatal dopamine levels and on cocaine-induced increases in striatal dopamine levels, as well as on cocaine-induced conditioned place preference, was studied in C57BL/6J mice.<sup>23</sup> In earlier studies conducted in the rat, we had shown that dynorphin applied directly into the striatum causes a dose-dependent reduction in dopaminergic levels. In this recent study, dynorphin, at four different doses, was infused into the caudate-putamen, and dopamine levels were quantitatively measured, using high-performance liquid chromatography, in the extracellular fluid obtained during in vivo

microdialysis in that brain region.<sup>23</sup> Also, the effect of a relatively high dose of dynorphin A on increases in dopamine levels caused by 15 mg/kg of cocaine was measured using *in vivo* microdialysis. In related studies, the effect of this dose of dynorphin A on cocaine-induced conditioned place preference was studied.<sup>23</sup> We found that dynorphin significantly decreased basal dopamine levels in a dose-dependent manner and by more than 60% at the highest dose. Further, this effect was blocked by preinjection with a selective kappa-opioid receptor antagonist, nor-binaltorphimine (nor-BNI).<sup>23</sup> Further, it was found that the highest dose of dynorphin studied (4.4 nanomolar) resulted in a complete block of the cocaine-induced increases in dopamine levels, and also attenuated locomotor activity induced by 15 mg/kg of cocaine, and blocked the formation of cocaine-induced conditioned place preference.<sup>23</sup> These findings suggest that a dynorphin agonist might be helpful in managing cocaine and other stimulant dependency by preventing cocaine or other stimulant-induced dopamine surges. However, on the other hand, any significant lowering of basal dopaminergic tone could lead to dysphoria, and thus more craving for a drug of abuse such as cocaine. Therefore, it has made our laboratory suggest that a potentially effective kappa-opioid receptor-directed compound for management of cocaine addiction would probably be a kappa partial agonist, that is, with modest agonist activity, but also antagonist activity, which should render stable basal dopaminergic tones, yet significantly attenuate cocaine- or other stimulant-induced dopamine surges, as well as "liking of" cocaine.

In related studies, Zhang et al studied a related potent synthetic kappa-agonist, R-84760, on cocaine-induced increases in striatal dopamine levels in cocaine-induced conditioned place preference in C57BL/6J mice.<sup>24</sup> R-84760 is a novel nonpeptidic potent synthetic selective kappa-opioid receptor agonist that has been studied to a limited extent in humans for other indications. It was found that, similarly to dynorphin itself, this compound would effect a dose-dependent reduction in dopaminergic tone, as measured during *in vivo* microdialysis in the striatum.<sup>24</sup> Also, it was shown that, like dynorphin, a low dose (0.1 mg/kg) of R-84760 would block cocaine-induced increases in the dopamine levels. Also, it was found that similarly low doses of R-84760 would completely prevent the development of cocaine-induced conditioned place preference and would attenuate locomotor activity in the conditioning chamber.<sup>24</sup> Further, it was

documented that these effects of R-84760 on lowering dopaminergic tone and cocaine-induced surges were completely blocked by a selective kappa-antagonist, nor-BNI.<sup>23</sup> Thus, these effects were documented to be mediated exclusively by the kappa-opioid receptor.

In different studies, we further explored the impact of extended-access (10 hours) versus short-access (3 hours) and also high- versus low-dose cocaine impact on self-administration, cocaine-induced reinstatement, and on brain mRNA levels.<sup>25</sup> It was again found that the escalation of cocaine self-administration under long-access conditions was greater than under short-access, and was dose-dependent. Further, we showed that such long-access, with animals who were allowed self-administration for 10 hours at high doses, resulted in an increased susceptibility to drug-induced relapse.<sup>25</sup> There were also differences in neurobiological indices, specifically levels of gene expression in those animals who were allowed to have long access and high doses, compared with short access. There were significant increases in proenkephalin gene expression in the caudate-putamen following long-access and high-dose self-administration.<sup>25</sup> Further, it was found that dopamine D2 receptor mRNA levels in the caudate-putamen and nucleus accumbens were significantly correlated with cocaine reinstatement.<sup>25</sup> However, there was no significant correlation between neuropeptide mRNA levels and cocaine-induced reinstatement.<sup>25</sup> Body weight progressively declined in the long-access self-administering rats.<sup>25</sup> In parallel to these findings, food consumption was also significantly reduced in each group during self-administration, but the reduction in food intake was much greater in the long-access rats.<sup>25</sup> During the 10-day extinction period, food consumption was significantly greater in the long-access, high-dose rats compared with both the short-access and the low-dose rats, and, in fact, food consumption during extinction in the high-dose group was significantly greater than pre-self-administration baseline levels.<sup>25</sup> These findings are similar to observations made by our group in human cocaine addicts in a controlled research setting. They have negative implications for some groups of people, where the desire for thinness, or the desire for attaining the self-image of thinness, may contribute to continued cocaine (or other stimulant) self-administration. The many findings from these long-access, high-dose cocaine self-administration rodent studies, both our more recent ones, as well as our earlier ones, along with the studies from other groups, particularly those of Koob and of Miczek,

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suggest that the findings may not only be relevant potentially for the human situation, but provide new insights for further study both in laboratory-based and human research paradigms.<sup>5,7,25</sup>

We have conducted studies in collaboration with the Laboratory of Dr Paul Greengard in which we have studied the impact of change of a single amino acid in an important signal transduction protein on (i) both dopaminergic responses to binge-pattern cocaine; as well as (ii) acquisition; and then (iii) persistence and amounts of self-administration of cocaine.<sup>26</sup> These studies were conducted in four separate lines of mutant mice, each with a mutation code for alanine introduced into the gene for the protein DARPP-32 at four sites of phosphorylation.<sup>26</sup> The four sites of phosphorylation chosen were: (i) the protein kinase A site, threonine 34A; (ii) the cyclin-dependent kinase-3 site, threonine 75A; (iii) the kinase CK2 site, serine 97A; and finally, (iv) the kinase CK1 site, serine 130A. In each case, animals were bred so that both the mutant strain, as compared with the wild-type strain, could be studied, with the single amino acid change introduced into one of these four sites of critical phosphorylation involved in different pathways of the dopamine D1 receptor signal transduction through the DARPP-32 cascade pathway.<sup>26</sup> Acquisition of self-administration required significantly more time in the threonine 34A<sup>-/-</sup> mice. However, once self-administration was established, both threonine 34A and the serine 130A DARPP-32 mutant mice administered significantly more cocaine than did their wild-type controls.<sup>26</sup> This became especially apparent after training each of these strains on a high dose of cocaine (1 mg/kg) and then starting the self-administration studies for each strain using an even higher dose of cocaine per injection (2 mg/kg), but then progressing downward in concentration to 1.0, .05, and .01 mg/kg per injection. As the dose was reduced below 1.0 mg/kg per injection, both the threonine 34A and the serine 130A mice significantly increased lever pressing to obtain more cocaine than did their matched wild-type controls.<sup>26</sup> Such an increase during reduction of cocaine concentration was not seen in either the threonine 75A or the serine 97A mice.<sup>26</sup> This suggests that although somewhat slower to acquire self-administration, both the threonine 34 site and the serine 130 site of DARPP-32 phosphorylation are important for the persistence of, and though not studied, possibly also to relapse to, cocaine self-administration. Further, and in support of these findings, studies using microdialysis in the freely-moving mice

could be carried out in three of the four strains (the fourth strain was not available in adequate numbers for study.) When this was performed, it was found that the same two strains that administered more cocaine, that is, the threonine 34A and the serine 130A, experienced a much lower rise in extracellular fluid dopamine after each of three binge cocaine injections than did the control mixed wild-type animals.<sup>26</sup> Further, this did not happen in the threonine 75A; these animals had a much higher level of dopamine achieved after each dose of binge cocaine, and these were animals that showed no difference between the single amino acid change mutant strain and the wild-type strain. These findings suggest that a single amino acid change of a critical phosphorylation site may alter the behaviors of self-administration; they also give further support to the concepts of many groups, that a lower dopaminergic tone either at rest, or achieved after any normal (for instance, a liked or desired food) or abnormal (for instance, cocaine) self-administration, may result in a lesser increase in dopamine tone. Thus, such animals (or possibly people) could be expected to seek more activation of this pleasure-related dopaminergic system, and thus have a greater vulnerability to developing an addiction.

We have conducted studies in which morphine was self-administered by animals and was available 18 hours/session/day.<sup>27</sup> In these studies, animals were allowed to select a more concentrated or less concentrated morphine solution and once stable choice was established, the concentrations were increased. The animals allowed such a choice both escalated their morphine use to a much greater extent than did steady-dose animals. After 14 days the animals were self-administering extremely large amounts of morphine in the extended-access and escalating high-dose model.<sup>27</sup> These studies showed that the average daily morphine self-administration increased from 22.5 mg on day 1 up to 66.4 mg by day 14.<sup>27</sup>

In addition to our neurobiological studies of drug addiction by more traditional methods, such as gene expression, we have been collaborating with Dr Virginia Pickel's laboratory in the use of immunogold electron microscopy (EM) to study drug-induced receptor trafficking. In these studies we have been exploring the effects of chronic intermittent self-administration of escalating doses of morphine on ionotropic glutamate receptor subunit trafficking in postsynaptic (ie, dendritic) sites in neurons, a process that is emerging as a critical cellular substrate of neural plasticity. Because immunogold

EM can be used to localize receptors near intracellular organelles, as well as presumably functional areas of the plasma membrane, this approach provides a more functional view than many of the more conventional methods of measuring receptor levels. We have been using immunogold EM to study glutamate receptor localization in neurons in portions of limbic-autonomic brain areas, namely the reciprocally connected nucleus tractus solitarius (NTS) and central (CeA) and basolateral (BLA) nuclei of the amygdala, a brain circuit that may play a critical role in homeostatic adaptations associated with repetitive drug use.<sup>28,29</sup> We have reported that the N-methyl-D-aspartate (NMDA)-NR1 receptor subunit is decreased on the dendritic plasma membrane of NTS neurons in animals self-administering morphine, compared with control animals not exposed to morphine.<sup>28</sup> Further, morphine self-administering rats showed region-dependent changes in the subcellular location of the AMPA-GluR1 receptor subunit in the amygdala. Specifically, there was an increase in AMPA-GluR1 labeling on the dendritic plasma membrane of BLA neurons and a concomitant decrease in dendritic AMPA-GluR1 in CeA neurons from animals self-administering morphine compared with control animals.<sup>29</sup> These findings suggest that chronic opiate self-administration is associated with a redistribution of postsynaptic plasma membrane glutamate receptor subunits that play an important role in neural plasticity in brain circuitry regulating homeostatic processes. These adaptations may be an important neural substrate for alterations in drug reward, autonomic function, and behavioral processes, each of which may be associated with the acquisition and persistence of an addiction.<sup>28,29</sup>

In four separate earlier studies from our laboratory we have shown that chronic (14 days) binge-pattern cocaine administration increases mu-opioid receptor mRNA levels and also increases density of mu-opioid receptors in specific brain regions where there are abundant dopaminergic terminals from neurons located in the ventral tegmental area.<sup>30-33</sup> In recent studies, Bailey and our group have shown that early withdrawal from chronic binge cocaine administration results in a recurrence of an increase in mu-opioid receptor mRNA levels in the rat frontal cortex, but only in this region.<sup>34</sup> In further studies, Bailey found that there is a persistent upregulation of mu-opioid receptors following long-term withdrawal from escalating-dose binge-pattern cocaine.<sup>35</sup> In these

studies, animals were treated with our new modified paradigm of escalating-dose binge cocaine over 14 days, which also results in an increase of mu-opioid receptor density, but with no increase in endogenous endorphin levels.<sup>35</sup> Following 14 days of withdrawal, there was still a highly significant increase in mu-opioid receptor density, and primarily in specific brain regions, again where there are dopaminergic terminals from the ventral tegmental area neurons and in fields in close proximity to both mu-opioid receptor mRNA levels in the neurons producing mu-opioid receptors and presenting them on the cell surface.<sup>35</sup>

In a further set of studies, Bailey explored changes in the kappa-opioid receptors following 14-day withdrawal from escalating-dose binge-pattern cocaine.<sup>36</sup> Here, very different findings were made. Whereas in multiple studies from our laboratory we have found both increases in gene expression of dynorphin, and increases in kappa-opioid receptor densities, and a correlated increase in kappa-opioid receptor mRNA levels, with kappa, unlike mu-opioid receptors, which are found to be persistently increased in density following 14 days of withdrawal from binge-pattern escalating-dose cocaine, in this study there was lowering of kappa-opioid receptors in two specific brain regions in animals in long-term withdrawal from cocaine. These areas included the basolateral amygdala and septum. Such a decrease in density was not found in other regions, but also with no persistence of increase in density. These selective brain regions of decrease in kappa-opioid receptor might contribute, in part, to the biological substrate for the development of dysphoria, which is usually observed in drug-free former cocaine-dependent individuals.<sup>36</sup>

### **Role of stress responsivity in the acquisition and persistence of specific addictive diseases, and the impact of chronic exposure to drugs of abuse and withdrawal therefrom on components of the stress-responsive system, along with identification of potential new targets for therapeutic intervention**

In our recent studies, we have also further explored the relative role of dopamine D1 and dopamine D2 receptors in various specific neurobiological changes, or neural plasticity, resulting from chronic exposure to cocaine. Since it has been well established that dopamine plays a major role

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in the rewarding properties of cocaine, and since it has been established for a long time that one of cocaine's primary sites of action is the presynaptic reuptake transporter for dopamine, where cocaine, by blocking reuptake, effects a flooding of perisynaptic space with dopamine, we have tried to dissect out the relative role of dopamine D1-like versus dopamine D2-like receptors in some of the resultant changes, both in behaviors, but also in gene expression and neuropeptide levels. During the last 5 years, we have completed further studies of the effects of selective dopamine D1-like and also dopamine D2-like receptor antagonists during acute binge-pattern cocaine administration on corticotropin-releasing factor (CRF) mRNA levels and pro-opiomelanocortin (POMC) mRNA levels in the hypothalamus. In earlier studies, we have found that both dopamine D1-like and also dopamine D2-like antagonists attenuate the chronic binge-pattern cocaine-induced increase in adrenocorticotropin hormone (ACTH) and corticosterone levels.<sup>37</sup> Further, we have shown that an attenuation of cocaine-induced changes in stress hormone levels similarly occurs in animals with complete deletion of the DARPP-32 protein, which is involved directly in dopamine D1 receptor signal transduction.<sup>38</sup> In our recent studies, we again found that both dopamine D1-like and dopamine D2-like antagonists attenuated the elevation of corticosterone levels by acute, as well as in our earlier studies of chronic, binge-pattern cocaine.<sup>39</sup> The previously identified acute binge cocaine-induced increases in hypothalamic CRF mRNA levels were not found in rats pretreated either with a dopamine D1-like or D2-like antagonist. Further, we found that neither the dopamine D1-like or dopamine D2-like receptor antagonists alone, in the absence of cocaine, altered mRNA levels of CRF in the hypothalamus. Thus, these results further support our earlier concept, that both dopamine D1 receptors and dopamine D2 receptors mediate acute as well as chronic cocaine's stimulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis.<sup>39</sup> Since neurobiological evidence has suggested that there are functional interactions between the dopaminergic and opioid systems regulating preproenkephalin and prodynorphin expression in the striatum, and since there is increasing evidence there may be direct connections between the dopaminergic system in the striatum and the stress-responsive components of the hypothalamus, we also raised the question of whether dopamine D1-like or dopamine D2-like antagonists could play a role in regulation of POMC mRNA levels in the hypothalamus.<sup>39</sup> In this

part of these studies, it was found that dopamine D2-like receptor blockade increased the POMC mRNA levels in the hypothalamus, a site with a different function than POMC mRNA levels in the anterior pituitary.<sup>39</sup> These findings suggest that activation of the dopamine D2 receptor may play a tonic inhibitory tone on hypothalamic POMC gene expression. However, neither dopamine D2 blockade nor acute binge cocaine altered POMC mRNA levels in the amygdala, the anterior pituitary, or the neurointermediate level of the pituitary. Also, dopamine D1 receptor blockade had no impact on hypothalamic POMC expression. Thus, these results both suggest a possible specific role for dopamine D2 in at least acute cocaine effects on hypothalamic POMC gene expression.<sup>39</sup>

To further our studies on the relative role of the D1-like and D2-like (and also D3-like, which are D2-like) dopamine receptors in the setting of drug abuse, and, specifically the impact of binge-pattern cocaine administration, we have conducted studies using D1-/- or D3-/- selective dopamine receptor gene deletion mice.<sup>40</sup> In these studies, we examined mu-opioid receptor gene expression in response to binge-pattern cocaine. We found that, at basal state, there was a significant increase in mu-opioid receptor mRNA levels in the frontal cortex of both the D1-/- and D3-/- dopamine receptor gene deletion mice, as compared with each of their wild-type controls.<sup>40</sup> However, there were no differences in basal levels of mu-opioid gene expression in the nucleus accumbens or in the caudate-putamen in these gene deletion mice. Strikingly, and in an opposite direction from some of our earlier findings in wild-type rat models, acute binge cocaine 15 mg/kg x 3 doses resulted in the restoration of frontal cortex mu-opioid receptor mRNA levels in the gene deletion mice to the levels of those in wild-type mice.<sup>40</sup> Further, in the nucleus accumbens core, after acute binge cocaine, there was an actual decrease in mu-opioid receptor levels in the D1-/- mutant mice, whereas in that brain region there was an increase in mu-opioid receptor gene expression in D3-/- mice.<sup>40</sup> The opposite pertained in the caudate-putamen, with an increase in mu-opioid receptor levels after binge cocaine in the caudate-putamen of the D1-/- mice and a decrease in the dopamine D3-/- mice.<sup>40</sup> In addition, a decrease in basal orexin mRNA levels was found in the lateral hypothalamus of the D3-/- mice, which did not change with cocaine.<sup>40</sup> These findings suggested that both D1 and D3 receptors are involved in mu-opioid receptor gene regulation in the frontal cortex, and also that D1 and D3

receptors may play opposite roles in the effects of cocaine on mu-opioid receptor gene expression in two striatal areas, the caudate-putamen and the nucleus accumbens core. In the control wild-type mice for the D1 receptor gene deletion, binge-pattern cocaine, as expected, increased mu-opioid receptor gene expression. However, in the wild-type controls for the dopamine D3 receptor knockout mice, there was a very modest, but not significant, increase in mu-opioid gene expression after binge cocaine, which was unexpected.<sup>40</sup> These findings were made both in the caudate-putamen and in the nucleus accumbens core, suggesting that in the actual breeding of the wild-type animals, the controls for the D3 knockout groups may have been substantially different from the wild-type mice which were the controls to the D1 knockout mice. Of particular note was the finding of increased basal levels of mu-opioid gene expression in both the D1 and D3 knockout mice, though only in the frontal cortex. These curious findings need to be studied further in D1 and D3 gene deletion mice, and also in different strains of wild-type mice.<sup>40</sup>

Most of the other studies of the impact of drug-induced stress on many different parameters, with emphasis on documentations of specific changes or evidence of neuroplasticity, have been conducted in rat models. In one sequence of studies, we examined the effects of acute morphine administration; chronic intermittent escalating-dose morphine (from 7.5 mg/kg/day on day 1 up to 120 mg/kg/day on day 10); and spontaneous 12-hour withdrawal from chronic morphine administration, using the escalating dose 10-day paradigm.<sup>41</sup> There were no changes in mu-opioid receptor mRNA levels in the lateral hypothalamus, the nucleus accumbens core, the caudate-putamen, or the amygdala following acute single injection of morphine, nor after chronic 10-day intermittent escalating-dose morphine.<sup>41</sup> However, after 12 hours of withdrawal from 10-day chronic morphine administration, several indices documenting stress response in the HPA axis were found, including increased POMC mRNA levels in the anterior pituitary, coupled with increases in ACTH levels, and also increased mu-opioid receptor mRNA levels in the lateral hypothalamus, the nucleus accumbens core, and the caudate-putamen. The changes in mu-opioid receptor gene expression suggest both a rebound from the abrupt withdrawal from large doses of the exogenous opioid morphine, as well as changes integral to the HPA stress-responsive axis, as well as in the hypothalamus.<sup>41</sup>

Several studies from other laboratories have demonstrated a role of lateral hypothalamic orexin (hypocretin) activation in drug-related positive reward, as well as in withdrawal effects; therefore gene expression of this peptide was also studied. It has been established by others that around half of the lateral hypothalamic orexin neurons concomitantly express mu-opioid receptors. In parallel to the increase in mu-opioid receptor gene expression found in the lateral hypothalamus in acute morphine withdrawal, similarly the levels of orexin mRNA in the lateral hypothalamus were also found to be increased.<sup>41</sup> No changes were found in the lateral hypothalamic levels of preprodynorphin mRNA, a gene which is known to be usually coexpressed with orexin in that hypothalamic region. These findings suggest that many different responses to the stress of morphine withdrawal occur, or, alternatively, changes which occur in the setting of withdrawal may drive the HPA axis activation and stress of withdrawal, just as we have found to be the case in our clinical studies.<sup>42,43</sup> Further, they suggest that in the lateral hypothalamus activation of orexin gene expression occurs in parallel to mu-opioid receptor gene expression. These findings suggest a novel target for managing opiate withdrawal.<sup>41</sup>

In a subsequent series of studies, a similar but somewhat different opioid administration paradigm was used. In these studies, heroin, the most common human opiate of abuse, was used,<sup>44</sup> coupled with a chronic, intermittent escalating-dose administration paradigm and conducted with doses of heroin ranging on day 1 from 7.5 mg/kg up to 60 mg/kg by day 10 (it should be noted that in this intermittent morphine escalating-dose paradigm, the starting dose was the same for heroin and morphine (7.5 mg/kg), but after 10 days, the escalation was up to 120 mg/kg when morphine was used, and 60 mg/kg when heroin was used.<sup>44</sup> One group of animals was then studied at the end of chronic escalating heroin administration; other animals were studied during early 12-hour withdrawal from such chronic heroin exposure; and a third group was studied after late 10 days of withdrawal from chronic heroin exposure.<sup>44</sup> In this study, it was found that arginine vasopressin mRNA levels were significantly increased during early spontaneous withdrawal, and, of several brain regions examined, only in the amygdala.<sup>44</sup> Further, separate studies showed that arginine vasopressin mRNA levels were increased not only in early spontaneous withdrawal from heroin in the amygdala, but also following foot-shock in rats withdrawn from

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heroin self-administration.<sup>44</sup> Such findings were not made in the self-administration control, heroin-naïve rats. This increase in arginine vasopressin mRNA levels was no longer observed following 10 days of withdrawal from chronic heroin. As in earlier studies, POMC mRNA levels in the anterior pituitary were found to be increased, both 30 min after chronic heroin administration, which probably is a sign of very early withdrawal, as well as at 12 hours of withdrawal from heroin. POMC mRNA levels had returned to normal after 10 days of withdrawal. Similarly, ACTH levels were increased in early withdrawal, coupled with a significant increase in plasma corticosterone, after 12 hours of withdrawal. Although the levels of both ACTH and corticosterone at the end of the chronic heroin administration, and thus 30 min, after the last dose, were somewhat greater than those in the saline-treated controls, these changes were not significant.<sup>44</sup>

In much earlier basic clinical research studies, performed in a stress-minimized research unit, documented that plasma levels of ACTH and cortisol became elevated before any signs and symptoms of opioid abstinence were observed or reported following very-low-dose opioid antagonist administration in opioid-dependent persons, suggesting that HPA axis activation drives, in part, the stress of opioid withdrawal, rather than reflecting a response to that stress.<sup>42,43</sup>

In separate, but related, studies, a model of heroin self-administration was used. The dose of heroin administration was 0.05 mg/kg per infusion, and 7 daily short-access (3-hour) sessions were used.<sup>44</sup> Since vasopressin mRNA elevations had been observed in animal models of heroin withdrawal, these studies were designed to look at the effects of a vasopressin receptor (V1B receptor) antagonist, SSR149415, in that setting. Administration of this compound was before the first extinction, or drug withdrawal, session. The vasopressin receptor antagonist dose-dependently attenuated foot-shock-induced reinstatement and blocked heroin-induced reinstatement.<sup>44</sup> This antagonist also blunted HPA axis activation by foot-shock.<sup>44</sup> All these data suggest that arginine vasopressin activation may occur during withdrawal from opiates, and suggest that this peptide also may contribute to relapse or reinstatement. Further, it is shown that, in the stress of withdrawal, when foot-shock is added, there is a significant increase in gene expression, and thus probably in the arginine vasopressin peptide. Most important, the data suggest that a vasopressin antagonist might attenuate either stress (in these experiments, withdrawal-induced

and foot-shock-induced), and also drug-induced reinstatement and relapse to opiate self-administration or use. Further studies in rodent models are needed. The arginine vasopressin receptor may become a novel target for therapeutics.<sup>44</sup>

In other separate studies, possible alterations of arginine vasopressin mRNA levels in the amygdala were studied in animals undergoing acute withdrawal from cocaine.<sup>45</sup> In these studies, our model of steady-dose binge-pattern (15 mg/kg every hour x 3 hours with no cocaine for 22 hours) administration for 14 days was used, followed by acute withdrawal (3 hours), subacute withdrawal (24 hours), and long-term withdrawal (10 days).<sup>45</sup> It was found that, although there were no changes in arginine vasopressin mRNA levels in the amygdala immediately following 14 days of cocaine administration, there were increases in arginine vasopressin mRNA levels in acute withdrawal (3 hours) from cocaine. Further, it was found that the selective opioid receptor antagonist naloxone blocked this increase.<sup>45</sup> As found in previously reported studies from our laboratory, chronic cocaine did not result in increased mu-opioid mRNA levels in the amygdala, nor did acute withdrawal from cocaine in these studies. At 24 hours of withdrawal, significant increases in arginine vasopressin mRNA levels in the amygdala were still observed. However, these levels had returned to normal after 10 days of withdrawal.<sup>45</sup> As found in our previous studies, adaptation or tolerance to the cocaine effects on the HPA axis activation also was observed during chronic binge cocaine.<sup>45</sup> However there were still modestly elevated levels of ACTH during acute withdrawal. As expected, naloxone produced modest elevations in ACTH levels in cocaine-naïve rats; naloxone did not have such an effect in the acute or subacute cocaine-withdrawn animals. There were no changes in arginine vasopressin, or POMC, or mu-opioid receptor mRNA levels in the hypothalamus following chronic cocaine administration, and acute withdrawal from cocaine.<sup>45</sup> These findings suggested that opioid receptors may mediate the increase in arginine vasopressin in the amygdala during acute cocaine withdrawal, and suggest a potential role for arginine vasopressin in the amygdala in some of the adverse effects of withdrawal from cocaine as well as in withdrawal from opiates.<sup>45</sup>

A recent set of laboratory-based studies in rats affirm, and further suggest a mechanism, for observations which we have made in two separate clinical studies, around 7 years apart, and in two parts of the world.<sup>46-48</sup> We have deter-

mined that steady-state methadone may attenuate or eliminate the liking of cocaine, and may do so by a mu-opioid receptor-mediated mechanism<sup>49,50</sup> In several earlier studies, as discussed above, we have shown that chronic binge-pattern cocaine administration results in an increase in mu-opioid receptor density in multiple, but not all, brain regions, and specifically in regions where there are abundant dopaminergic terminals from dopamine neurons in the ventral tegmental area and substantia nigra compecta.<sup>31-33</sup> Further, we have shown that acute and subacute, but not chronic, cocaine administration results in an increase in mu-opioid receptor mRNA levels.<sup>30</sup> In these recent studies, different paradigms were used.<sup>41</sup> In one set of studies, rats were implanted with either saline- or methadone-filled osmotic minipumps and then conditioned with 1, 5, or 20 mg/kg cocaine intraperitoneally. Animals with the 20 mg/kg/day or 55 mg/kg/day methadone-filled osmotic pumps did not express cocaine-induced place preference.<sup>46</sup> However, methadone pumps at two doses (30 and 55 mg/kg/day) did not alter intravenous self-administration of cocaine using a continuous schedule of reinforcement with different doses of cocaine (0.1, 0.5, and 2.0 mg/kg/infusion) studied. Mu-opioid receptor mRNA levels were measured in animals treated with cocaine as part of conditioning for place preference. As in earlier studies, it was shown that this subacute cocaine administration resulted in increased mu-opioid receptor mRNA levels in the nucleus accumbens core and in the frontal cortex 10 days after cocaine conditioning.<sup>46</sup> However, this increase in mu-opioid receptor mRNA levels was attenuated or eliminated by the steady-dose infusion of methadone. Earlier studies have shown that the dose of 55 mg/kg/day subcutaneously by pump in the rat results in a plasma level similar to that in patients seen in methadone maintenance.<sup>49</sup> These studies showed that, although high doses of methadone delivered by pump did not alter the direct reinforcing effects of cocaine as seen in self-administration, those doses of methadone did block both spontaneous and cocaine-induced “seeking” or “liking” 10 days after cocaine conditioning. Further, we have suggested that this may be through the mechanism of methadone attenuating or preventing the relative endorphin deficiency resulting from the increased mu-opioid receptor density preceded by increased mu-opioid receptor gene expression, but with no concomitant increase in the endogenous opioids that bind to the mu receptor, that is, no increase in beta-endorphin or in the enkephalin peptides.<sup>46</sup>

These studies also build upon the early and also much more recent findings that, despite the fact that up to 70% of all persons in the middle Atlantic states, as well as currently in Tel Aviv, Israel, have concomitant dependence upon cocaine, when presenting for treatment for long-standing dependence on heroin, after 1 year or more of methadone treatment, as expected, the numbers using heroin dropped precipitously, to less than 20% of patients using heroin at any time (as contrasted to heroin use by all patients 3 to 6 times a day prior to entry). This was accompanied by the more surprising findings that during steady-dose methadone maintenance treatment, the percentage of persons dependent on cocaine drops down to less than 20%, and those using any cocaine to less than 30%.<sup>47,48</sup> Although these beneficial results of methadone maintenance on managing cocaine addiction were always attributed to the counseling and other psychosocial benefits derived from a good methadone maintenance program, we have, over the last decade, hypothesized that a pharmacological mechanism also is in place, a hypothesis based on our findings that binge cocaine increases acutely mu-opioid receptor gene expression and on a chronic basis, mu-opioid receptor density, and further, that a relative endorphin deficiency thus develops in humans, since there is no concomitant increase of beta-endorphin or enkephalins, as may be directly documented by stress-responsive metyrapone testing.<sup>50</sup> These findings suggest that possibly an opioid agonist such as methadone, or possibly a partial agonist, such as buprenorphine, might be able to be effectively used to treat very severe, long-term, cocaine-dependent persons who have not responded to any other available current treatment. Since there are no effective targeted pharmacotherapies for cocaine addiction, the potential target of the mu-opioid receptor, with now a neurobiological basis for such treatment, might be warranted.

In other studies, conducted by a collaboration with our colleagues at the Karolinska Institute in Stockholm, Sweden, yet another potential target for future therapeutic use, a nociceptin/orphanin FQ receptor agonist (Ro64-6198) was found to reduce alcohol self-administration, and, further, and importantly, to prevent relapse to alcohol drinking in a rat model.<sup>51</sup> Other orphanin-nociceptin (ORL-1) receptor agonists may be found to have effectiveness in treatment of alcoholism and possibly other specific addictive diseases, which involve interactions between the dopaminergic system and different components of the opioid and opioid-like system.<sup>51</sup>

# State of the art

## Basic clinical research related to specific addictive diseases, with emphasis on stress responsivity: all research focused on treatment improvement

Corticotropin-releasing factor (CRF), synthesized and released in the hypothalamus, passes through the portal blood system to the anterior pituitary, where it effects processing and release of the single gene product of the POMC gene (reviewed in ref 7). This large peptide is then further processed to yield many biologically active and important neuropeptides, including the major stress-responsive and glucocorticoid-regulated peptide, ACTH, as well as the longest (31 amino acids) of the endogenous opioids, and a primary ligand of the endogenous mu-opioid receptor, beta-endorphin. ACTH and beta-endorphin are released in equimolar amounts from the anterior pituitary sites in humans (who, unlike rodents, do not possess an intermediate lobe in the pituitary except transiently during pregnancy.) ACTH and beta-endorphin pass into the general circulation. ACTH impacts directly upon the adrenal cortex to bring about the processing and release of the major glucocorticoid in humans, cortisol, in addition to altering and enhancing the biotransformation and release of several other steroid hormones. Beta-endorphin may act at many peripheral sites. There is some evidence that there may be retrograde passage of these two neuropeptides back into the hypothalamic region, which in human and nonhuman primates, but not in rodents, lies partially outside the brain barrier. Glucocorticoids have been documented for a very long time to negatively regulate the HPA axis in a negative-feedback mode, with cortisol being the primary glucocorticoid in humans, non-human primates and guinea pigs, and corticosterone, the primary glucocorticoid having this effect in rats and mice. Thus, cortisol acts at both the hypothalamic sites of CRF production and at the anterior pituitary sites of POMC processing and release, to transiently attenuate or inhibit the release of these hormones. A 24-hour circadian rhythm is thus achieved, with the lowest levels of CRF, ACTH, beta-endorphin and thus cortisol in the late afternoon and early evening in humans, and with levels rising again in the early morning hours, the opposite times pertain in rodents, with highest hormone levels at night, at the beginning of the activity period.

Based on early findings of Volavka, our group and a few others years ago began to study the possible role of the

endogenous opioid system, in particular, the mu-opioid receptor system, in also modulating the HPA axis. In several studies we have shown that the HPA axis is inhibited by the mu-opioid receptor system (reviewed in refs 5,7,8). In one study from our group, we looked at high and very high doses of two different selective mu-opioid receptor antagonists, both of which can be administered intravenously in humans, naloxone and nalmefene.<sup>52</sup> Studies using nonhuman primate membranes and, more recently, studies using cloned human genes in proper molecular-cellular constructs, have shown that, in contrast to rodents, naloxone binds almost exclusively to the mu-opioid receptor and acts as an antagonist.<sup>52</sup> Nalmefene, on the other hand, binds to both mu- and kappa-opioid receptors. Very recently, in collaboration with the group of Bidlack, we have shown that the kappa opioid receptor effect of nalmefene is that of a partial agonist (that is, with some agonist and some antagonist properties), whereas the mu component is pure mu-opioid receptor antagonist.<sup>53</sup> Since we have studied both of these compounds in several earlier clinical research studies, we elected to use high and very high doses of each, to be sure that the ceiling of the effective doses in humans was exceeded. We found, as we and others had shown before, that naloxone activates the HPA axis by disinhibition and causes significant increases in both ACTH and cortisol. Of great interest in this study, however, was the finding that nalmefene causes a significantly greater activation of the HPA axis, with higher resultant peripheral levels of ACTH and cortisol.<sup>46</sup> Our more recent studies, in which we found that the kappa component of nalmefene is a partial agonist, suggest that whereas the mu antagonists act at mu-opioid receptors of the hypothalamic and anterior pituitary sites, and through the mechanism of disinhibition bring about the increased release of CRF and ACTH and beta-endorphin, the kappa partial agonist component of nalmefene may act directly to enhance release of CRF and/or of the POMC peptides, ACTH and beta-endorphin, thus directly activating the HPA stress-responsive axis, which has been suggested by several workers in preclinical studies.<sup>52,53</sup> This possibility has not, however, been well studied with any of the very few selective kappa agonists which have ever been introduced to human use, and only a few additional studies of these kappa agonists or partial agonists have been conducted in nonhuman primates.

In earlier studies, it has been shown that activation of the HPA axis, with increased levels of plasma ACTH and

cortisol, occurs after administration of alcohol or cocaine, and many groups have made similar findings in animal models. Further, we have shown that tolerance develops to this HPA activation effect of both cocaine and alcohol. In other studies, we have suggested that activation of the HPA axis is sought by the rat or mouse, and by the human. In human studies conducted, in collaboration, by O'Malley at Yale in a clinical research setting, naltrexone, a selective mu-opioid antagonist with some kappa antagonist activity, was administered for 1 week to alcoholics and compared with placebo administered for one week to a similar group.<sup>54</sup> Then a laboratory session was conducted in which limited alcohol self-administration was permitted for up to 2 hours. We found, just as in the numerous field trials, that alcoholics receiving naltrexone drank significantly fewer drinks.<sup>54</sup> Because of the naltrexone disinhibition of the hypothalamic-pituitary sites of the HPA axis, there was a significant increase in levels of ACTH and cortisol in alcoholics treated with naltrexone after consumption of fewer than two drinks, whereas the much larger amounts of alcohol consumed by the alcoholics receiving placebo resulted in no significant activation of this axis.<sup>54</sup> Further, on responding to specific questionnaires, the alcoholics receiving naltrexone, and who had consumed only a small amount of alcohol, but had experienced modest activation of the HPA axis, felt no further "craving," or desire to drink alcohol, and this decrease in craving was correlated to the increase of serum cortisol levels. The opposite pertained in those alcoholics receiving a placebo, who had consumed more alcohol, but had no activation of the HPA axis, and no increase in cortisol, a significant urge to drink alcohol persisted.<sup>54</sup>

Many of our earlier studies have shown that short-acting opiates, opposite from the effects of cocaine and alcohol in the HPA axis, profoundly attenuate or suppress the HPA axis, resulting in lowered levels of ACTH and cortisol after opiate administration. However, after tolerance and physical dependence have developed, in the setting of withdrawal from opiates, profound activation of the HPA axis occurs with increases in levels of ACTH and cortisol. The neuroendocrine changes of opiate withdrawal look very similar to the normal response to a specific mu opioid receptor antagonist, such as naltrexone, when given to a healthy volunteer. Therefore, it is not surprising, as we had predicted, that most opiate addicts will not willingly accept chronic daily naltrexone or other opioid antagonist treatment once experienced, whereas alco-

holics would accept such treatment, and might be directly benefited. Giving an opioid antagonist to any opiate-dependent person is contraindicated, because profound activation of the stress-responsive axis will occur and creates a very adverse and noxious experience. In many of our earlier studies, we have shown that during chronic methadone maintenance treatment, which provides steady perfusion with a synthetic ligand of the mu-opioid receptor, complete normalization of the HPA axis occurs, including normalization of basal levels of hormones, as well as responsiveness in various functional tests.

To dissect further the relative contribution of the glucocorticoid system contrasted to the mu-opioid receptor endogenous ligands, that is, beta-endorphins and enkephalins, we have conducted further studies using metyrapone. In humans, metyrapone blocks the final step of cortisol synthesis, that is, 11- $\beta$ -hydroxylation. In the single oral dose test using metyrapone, the synthesis of cortisol is blocked for about 8 hours, and then returns to normal. Therefore, one can measure the levels of ACTH (which also reflect the equimolar release and levels of beta-endorphin) following metyrapone administration which are elevated because with cortisol synthesis blocked, and the normal negative feedback is transiently cut off. In healthy human beings, with normal endogenous opioid systems, the mu-opioid receptor system responds to bring a check, or brake, to the increased release and levels of ACTH (and beta-endorphin). However, we had shown in several earlier studies that in medication-free, drug-free former heroin addicts, there is no such mu-opioid receptor-mediated brake, and thus hyper-responsivity to metyrapone testing is observed (reviewed in refs 5,7). Further, we had reported that in abstinent cocaine addicts a similar hyper-responsivity to metyrapone testing exists.<sup>50</sup> This hyper-responsivity, therefore, suggests a relative endorphin deficiency, which our laboratory-based studies also support.<sup>30-33,50</sup> As discussed above, we have found that chronic binge cocaine administration causes an increase in gene expression in the mu-opioid receptor, as well as an increase in density in mu-opioid receptors, in specific brain regions with abundant dopaminergic terminals, and, further, in recent studies, we have found that this increase in mu-opioid receptor density persists for a protracted period of time after last cocaine exposure.<sup>30-32,35</sup> However, we have also shown that there is no increase of the endogenous opioids that bind at the mu receptor. Thus a relative endorphin deficiency develops (or possibly was present a priori on a genetic or environmentally-induced basis).

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Subsequently, Frost and colleagues, using positron emission tomography (PET) showed similarly the mu-opioid receptor density being increased in recently-abstinent cocaine addicts, and further more recently have shown that this increase persists for protracted periods of time into successful cocaine abstinence.<sup>55,56</sup> Thus, a relative endorphin deficiency has been documented both in humans as well as in rodent models, in humans directly shown by testing of the stress-responsive system. In several studies, we have found that metyrapone responsiveness is abnormal in opiate addicts, but becomes normalized in methadone maintenance patients (reviewed in refs 5,7). We also have shown that abnormal hyper-responsivity occurs in cocaine addicts.<sup>57</sup> In a more recent study, we again documented the normalization during methadone maintenance treatment.<sup>50</sup> We also conducted studies in a subgroup of methadone maintenance patients who continued during 6-month treatment or more to meet the criteria of cocaine dependence.<sup>50</sup> This group was maintained on moderate doses of methadone (60 to 90 mg/day). As discussed above, an early clinical study from our laboratory, a very recent clinical study from our laboratory, and a recent laboratory-based study have all suggested that increasing the dose of methadone may decrease cocaine addiction in maintenance patients with dual-dependency, and further, in the rodent model, that the addition of steady-state methadone may prevent alterations in mu-opioid receptor gene expression and attenuate or prevent conditioned place preference to cocaine.<sup>46-48</sup>

In another set of studies reported in the last decade we have re-explored the glucocorticoid negative feedback both in methadone-maintained former heroin addicts, as well as those with ongoing cocaine dependence.<sup>57</sup> In all our earlier studies, we found, surprisingly, that all of the methadone-maintained patients had normal suppression to dexamethasone and, in this study, we also used two lower doses than the usual suppression dose, that is, 0.5 and .125 mg and found that all subjects suppressed completely (as reviewed in refs 5,7,57). All the cocaine-dependent methadone-maintained patients also suppressed completely. Although not significant, the glucocorticoid feedback effects in the cocaine-dependent, methadone-maintained patients, and also in the otherwise well-stabilized methadone-maintained patients appeared to be greater than the normal volunteers in the late afternoon, suggesting that there may be a modestly altered, or enhanced, negative feedback by glucocorti-

coids, in at least some subjects.<sup>57</sup> This, in turn, may contribute to the observed attenuation of both basal and cocaine-induced responsivity of the HPA axis in humans and in rodents in other studies from our laboratory and others.

In another study, we examined the effect of corticotropin-releasing factor in methadone-maintained versus control subjects. In this study, we found differences between long-term well-stabilized methadone-maintained subjects as compared with normal control subjects.<sup>58</sup> In this study, two doses of CRF were used; one lower than the usual dose (0.5 µg/kg) and one dose higher (2.0 mg/kg) than usually used in the neuroendocrine diagnostic procedure (100 µg, irrespective of weight).<sup>58</sup> There was no difference in hormonal measurements between the two groups following placebo administration, nor during low-dose hCRF administration. However, following high-dose CRF administration, the methadone-maintained patients displayed a significantly greater increase in plasma ACTH levels than did the normal volunteers.<sup>58</sup> This suggested that in long-term methadone-maintained patients some abnormalities in HPA axis responsivity may pertain, in this case, a greater sensitivity of the anterior pituitary to CRF stimulation. In turn, these findings suggest that the basal and peak levels of CRF may be slightly reduced in stable methadone maintenance patients, possibly related to the increased sensitivity to negative feedback by glucocorticoids, as discussed above, or due to the steady but high and exogenous opioid tone in patients in treatment with the long-acting mu agonists.<sup>57,58</sup> Further studies to explore this altered sensitivity in other persons with specific addictive diseases, not in treatment, as well as in treatment, are in progress.

In another series of studies, we have been able to pursue in humans findings which we and others had made in rodents, that is, that dynorphin, the natural endogenous opioid ligand of the kappa-opioid receptor, may directly act to alter (lower) dopaminergic tone. We have been able to access dynorphin A(1-13), a natural-sequenced dynorphin four residues shorter than the natural dynorphin A(1-17) for research use under an investigator-initiated investigational new drug application (IND) approved by the US Food and Drug Administration. Building upon the established biological fact that, in humans, prolactin release is almost exclusively under dopaminergic tone, and thus, that a lowering of dopamine in the tuberoinfundibular dopaminergic region results in

a rise in prolactin levels, we conducted studies first in healthy volunteers using two different doses of intravenously-administered dynorphin A(1-13) (120 µg/kg and 500 µg/kg). Since in humans some of the hypothalamus lies outside the blood-brain barrier, we assumed that the peptide dynorphin would be able to act on this tuberoinfundibular dopaminergic system. When we conducted these studies in a stress-minimized environment of our Rockefeller Hospital clinical research center, we found that peripheral administration of dynorphin A(1-13) gave a prompt dose-dependent increase in serum prolactin levels, which then returned to normal within 120 minutes.<sup>59</sup> This duration of action was much longer than we predicted, based on our *in vitro* biotransformation studies in which we established the probable half-life of dynorphin A(1-13) in human blood.<sup>60</sup> Of interest, with respect to the possible effect of dynorphin on the HPA axis, we found no increment in ACTH or CRF following peripheral dynorphin administration.<sup>59</sup> To document whether the dynorphin effect was modulated by the endogenous opioid system, we conducted studies using the lower dose of dynorphin A(1-13) following pretreatment with either naloxone or nalmefene, both selective mu-opioid receptor antagonists, and one (nalmefene) with partial kappa-opioid agonist activity.<sup>59</sup> We found pretreatment with either of these compounds attenuated the rise in serum prolactin.<sup>59</sup> In these initial studies, a further very provocative (but not completely unexpected given the physiological differences) observation was made, an unusual finding in our human studies of addictive diseases, specifically that female subjects had modestly higher basal levels of prolactin than males, but when given dynorphin, gave a significantly exaggerated response, with higher levels of prolactin achieved after dynorphin administration and thus the reduction of dopamine in the tuberoinfundibular region.<sup>59</sup> Thus, a clear gender difference was observed. Our subsequent studies of dynorphin effects now must be done always considering males and females separately.

In a second set of studies, we have addressed the question of whether or not the dynorphin responsivity, with respect to lowering dopaminergic tone, will occur similarly in healthy long-term well-stabilized methadone-maintained subjects.<sup>61</sup> Two doses of dynorphin again were used for study in both a new group of healthy volunteer subjects and in a group of long-term stable methadone-maintained patients.<sup>61</sup> Again, in the healthy volunteer subjects, a dose-dependent rise in serum prolactin was

observed after dynorphin administration.<sup>61</sup> Similarly, in the methadone-maintained patients (receiving 80 to 120 mg/day of methadone), a dose-dependent rise in prolactin occurs.<sup>61</sup> Because years ago (published in 1978 by our group), we had shown that methadone itself, acting as a mu opioid receptor agonist, acts to lower dopaminergic tone, causes increase in serum prolactin, which occurs at time of peak plasma levels of methadone (that is, around 2 to 4 hours after oral methadone dose), in the dynorphin studies, we withheld the methadone dose until 60 minutes after the dynorphin was given.<sup>62</sup> In these subjects, as in our much earlier studies, we showed a second and separate brisk rise in prolactin levels, beginning at 2 hours after methadone administration and remaining elevated at 5 hours after methadone administration. Again, in the methadone-maintained patients, as in both groups of healthy volunteer subjects, there was a dose-dependent dynorphin-induced rise in prolactin levels which returned to basal levels by 90 to 120 minutes. Thus, in this study, we were able to observe both the dynorphin- and methadone-induced lowering of tuberoinfundibular dopaminergic tone, resulting in both rises in serum prolactin levels.<sup>61,62</sup>

In yet another series of studies, we had observed that when given to healthy volunteers nalmefene caused a small but modest rise in serum prolactin levels.<sup>53</sup> Therefore, we entered into a collaboration with Bidlack, and in that collaboration addressed directly the issue of whether the kappa opioid receptor activity of nalmefene is antagonist, or possibly, as we hypothesized, partial agonist. It was found clearly that nalmefene possesses kappa-opioid receptor partial agonist activity in *in vitro* studies using appropriate molecular cellular constructs.<sup>53</sup> It was reconfirmed that the mu opioid receptor action of nalmefene is only that of antagonism; the kappa opioid receptor action is both agonism (partial agonist) and antagonism.<sup>53</sup> Further, we were able to show that nalmefene effects a modest elevation of prolactin levels, suggesting a modest lowering of dopaminergic tone. This suggests, however, that nalmefene or other mu-opioid receptor antagonists, which have kappa-partial agonism (probably also true for naltrexone) may have augmented benefit for management of alcoholism, and possibly even for treatment for stimulant, such as cocaine, dependency, since a modest lowering of dopaminergic tone could be helpful in decreasing or attenuating the "reward" effect, whereas the inhibition of the mu-opioid receptor regulation of the stress-responsive HPA axis could provide

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modest activation of this axis, which we have directly documented to be sought by alcoholics, and in our animal modeling suggests is also sought by the cocaine self-administering animals.<sup>19,54</sup> In these basic clinical research studies, we have again found an extremely important role of the mu-opioid receptor system, as well as identifying a previously not-appreciated role of the kappa-opioid receptor system in modulation of the human stress-responsive HPA axis.

Our genetics work, including our work in physiogenetics, has not been discussed herein, but has been reviewed elsewhere, as discussed above.<sup>5,8-11</sup>

All these findings have taught us that physiogenetics may

occur, that is, difference in our response to our own proteins, peptides, neurotransmitters, or steroids, based on a polymorphism of a receptor or some polymorphism of the ligand or the pathway producing the ligand. Further, such studies, in the future, may give us increasing insights into targets for therapeutics, as well as providing a basis for effective primary prevention of specific addictive diseases. □

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## **Opioides, dopamina, estrés y las adicciones**

*Los objetivos expresados por Dialogues in Clinical Neuroscience son servir como interfase entre la neuropsiquiatría clínica y las neurociencias al entregar información actualizada y conocimientos originales de aspectos clínicos, biológicos y terapéuticos relevantes. Mi laboratorio, el Laboratorio de la Biología de las Enfermedades Adictivas de la Rockefeller University, se ha orientado por años a la "investigación translacional bidireccional", es decir, el aprendizaje mediante cuidadosas observaciones y análisis de poblaciones de pacientes con la enfermedad en estudio, en este caso, enfermedades adictivas primariamente específicas, y luego utilizar ese conocimiento para crear modelos animales mejorados u otros paradigmas de investigación basados en el laboratorio; y al mismo tiempo, trasladar los resultados de estudios realizadas en el mesón del laboratorio a la clínica tan pronto como sea apropiado y posible. En esta revisión por invitación, el foco de atención serán las perspectivas de nuestro Laboratorio de la Biología de las Enfermedades Adictivas, del Instituto Nacional de Salud y del Centro de Investigación del Abuso de Drogas del Instituto Nacional, incluyendo investigación neurobiológica molecular basada en el laboratorio, investigación con algunos modelos animales diseñados para simular patrones humanos de abuso y adicción a drogas, y también investigación clínica básica entrelazada con investigación relacionada con el tratamiento.*

## **Opioides, dopamine, stress et addictions**

*L'objectif des Dialogues in Clinical Neuroscience est de servir « d'interface entre la neuropsychiatrie clinique et les neurosciences en délivrant une information à la pointe des connaissances et des points de vue originaux sur des aspects cliniques, biologiques et thérapeutiques pertinents ». Le laboratoire de Biology of Addictive Diseases que je dirige à l'université Rockefeller, se concentre depuis des années sur « la recherche translationnelle bidirectionnelle », composée de recueil d'informations par l'observation soignée et l'étude de populations de patients atteints de troubles donnés, dans le cas de notre laboratoire plus spécifiquement de maladies addictives, et de l'utilisation ultérieure de ces connaissances pour créer un modèle animal amélioré ou d'autres modèles de recherche en laboratoire. Parallèlement, il s'agit d'exporter les résultats de recherche obtenus in vitro aussi vite que cela est opportun et faisable, vers la clinique. Dans cette revue, nous avons donc mis l'accent sur les perspectives du laboratoire de Biology of Addictive Diseases and related National Institutes of Health/National Institute on Drug Abuse research Center ; elles incluent la recherche neurobiologique moléculaire de laboratoire qui utilise différents modèles animaux afin d'imiter les modèles humains d'abus de drogues et d'addiction, aussi bien que la recherche clinique de base, étroitement liée à la recherche thérapeutique.*

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