MINIREVIEW—SPECIAL ISSUE IN MEMORY OF AVRAM GOLDSTEIN

Dynorphin—Still an Extraordinarily Potent Opioid Peptide

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ABSTRACT

This issue of *Molecular Pharmacology* is dedicated to Dr. Avram Goldstein, the journal's founding editor and one of the leaders in the development of modern pharmacology. This article focuses on his contributions to the discovery of the dynorphins and evidence that members of this family of opioid peptides are endogenous agonists for the kappa opioid receptor. In his original publication describing the purification and sequencing of dynorphin A, Avram described this peptide as "extraordinarily potent" ("dyn" from the Greek, *dynamis* = power and "orphin" for endogenous morphine peptide). The name originally referred to its high affinity and great potency in the bioassay that was used to follow its activity during purification, but the name has come to have a second meaning: studies of its physiologic function in brain continue to provide powerful insights to the

molecular mechanisms controlling mood disorders and drug addiction. During the 30 years since its discovery, we have learned that the dynorphin peptides are released in brain during stress exposure. After they are released, they activate kappa opioid receptors distributed throughout the brain and spinal cord, where they trigger cellular responses resulting in different stress responses: analgesia, dysphoria-like behaviors, anxiety-like responses, and increased addiction behaviors in experimental animals. Avram predicted that a detailed molecular analysis of opiate drug actions would someday lead to better treatments for drug addiction, and he would be gratified to know that subsequent studies enabled by his discovery of the dynorphins resulted in insights that hold great promise for new treatments for addiction and depressive disorders.

Introduction

It is appropriate that this issue of *Molecular Pharmacology* is dedicated to Dr. Avram Goldstein, who died June 1, 2012, one month before his 93rd birthday. Avram was the journal's founding editor in 1965, and he was a major force in the development of modern molecular pharmacology through his textbook, *Principles of Drug Action*, written with Lewis Aronow and Sumner Kalman, and first published in 1968 (Goldstein et al., 1968). In his long career as a professor of pharmacology at Stanford University, he made numerous contributions to neuroscience, but perhaps he is best known for his leadership in the study of drug addiction, where he applied biochemical principles to the understanding of opiate and nicotine drug actions, sociological approaches to an understanding of the impacts of drug addiction on our communities, and treatment research designed to identify more effective addiction therapies. In this brief review, I focus on his studies in their

historical context and describe some of their continuing implications and ramifications.

Discovery

The 1970s and 1980s were a golden era of neuropeptide discovery that dramatically changed our conceptions of neurotransmitter structure and function. The presence of endogenous morphine-like substances in brain was first reported by Terenius and Walstrom (1974), using a radioligand binding method first suggested by Avram and colleagues (Goldstein et al., 1971). Subsequently, John Hughes and Hans Kosterlitz succeeded in identifying the first endogenous opioid peptides: methionine and leucine enkephalin (Hughes et al., 1975). Also in 1975, Avram and colleagues detected peptide-like opioid activity in bovine and porcine pituitary extracts (Cox et al., 1975; Teschemacher et al., 1975). Shortly afterward, C.-H. Li and David Chung reported the sequence of a 31 amino acid polypeptide, β -endorphin isolated from pituitary extracts having homology with methionine enkephalin (Li and Chung, 1976) and demonstrated its opioid receptor activity with the help of Brian Cox and Avram Goldstein (Cox et al., 1976). The highly basic fraction of the pituitary extracts (originally

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ABBREVIATIONS: β -CNA, β -chlornaltrexamine; DADLE, [D-Ala², D-Leu⁵]-enkephalin; ERK, extracellular signal-regulated kinase; GPI, guinea pig ileum; JDTC, ((3R)-7-hydroxy-N-[(2S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-yl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide); Ke, equilibrium dissociation constant derived from Schild analysis; MAPK, mitogen-activated protein kinase; MVD, mouse vas deferens; norBNI, norbinaltorphimine (17,17'-(dicyclopropylmethyl)-6,6',7,7'-6,6'-imino-7,7'-bimorphinan-3,4',14,14'-tetrol).

supplied by J.D. Fisher, Armour Pharmaceuticals, as byproducts of commercial corticotropin production, also contained a different, trypsin-sensitive opioid activity that gave a slower onset of action and reversed more slowly than previously described opioid peptides (Cox et al., 1975). The latter opioid peptide was ultimately purified, sequenced, and named dynorphin-A (a 17 amino acid polypeptide having homology with leucine enkephalin) (Goldstein et al., 1979, 1981). In parallel, Hisayuki Matsuo and colleagues were isolating a different group of opioid peptides from porcine hypothalamus (Kangawa and Matsuo, 1979) and determined the complete sequence of α -neo-endorphin shortly thereafter (Kangawa et al., 1981). The sequence of a precursor polypeptide containing dynorphin-A, followed by a second related sequence, dynorphin B, was next described (Fischli et al., 1982). The same 13 amino acid, dynorphin B sequence, was also isolated from bovine pituitary and named "rimorphin" (Kilpatrick et al., 1982) (Table 1). In addition, two C-terminally extended forms of dynorphin B (big-dynorphin and leumorphin) were described (Fischli et al., 1982; Kilpatrick et al., 1982). During this time, powerful new methods of neuropeptide detection, purification, and sequence identification were being developed that greatly enhanced the rate of discovery of many other neuropeptides in brain and fundamentally changed our conception of neuro-signaling.

An intense effort by many groups at the time was devoted to identifying other members of this family of opioid peptides, discovering the biosynthetic pathways involved, and describing the structural relationships between these neuropeptides. This effort culminated in the cloning and sequencing of the cDNAs for corticotropin- β -lipotropin (the β -endorphin precursor) (Nakanishi et al., 1978), preproenkephalin (the precursor of methionine and leucine enkephalins) (Noda et al., 1982), and preprodynorphin (the precursor for dynorphin A[1-17], dynorphin-A[1-8], dynorphin B[1-13], α -neo-endorphin, β -neo-endorphin, big-dynorphin, and leumorphin) (Kakidani et al., 1982) (Table 1). Since that time, the neurophysiological actions of these opioid peptides have been the subject of considerable research effort: enkephalins (6143 citations), endorphins (9042 citations), and dynorphins (2395 citations), with no evidence of slowing (Schwarzer, 2009).

The rapid pace of the initial descriptions of these peptides (less than 8 years from an initial suggestion of their existence in 1974 to the full characterization of their primary structures, biosynthesis, and brain distributions in 1982) led to great

optimism that their roles in nociception, opiate addiction, and mental health would soon follow. In his Nathan B. Eddy Award lecture (1981), Avram speculated that opiate addiction is associated with a disturbed regulation of an endogenous opioid, but concluded that promising studies of the functions of the endogenous opioids, including dynorphin, were only just beginning.

Receptors

Perhaps with the benefit of hindsight and after the cloning of the three opioid receptor genes during 1992–1993, the conceptual struggles to define the nature of the opiate receptor and distinguish multiple forms of the receptor during the preceding decades may seem overblown. But contemporaneous with the struggle to define the structures and properties of the endogenous opioid peptides, an intense effort was devoted to the characterization of the receptors mediating their actions. In reality, this struggle continues to this day, because the roles of receptor splice variants and heterooligomeric forms of the receptors are still not resolved (Rozenfeld and Devi 2011; Barrie et al., 2012). In addition, new concepts of functional selectivity and ligand-directed signaling (Urban et al., 2007; Bruchas and Chavkin, 2010) cloud any simple definition of opioid receptor types. The original conception of a monomeric seven-transmembrane protein, free-floating in the membrane to interact with heterotrimeric G-proteins, has been replaced by a more nuanced, multicomponent, macromolecular complex combining the receptor, accessory proteins, and signaling effectors in which tissue-specific composition may influence the ligand binding properties and functional consequences of opioid receptor activation. Nevertheless, Avram Goldstein made important contributions to the characterization of opioid receptors during the 1970s and 1980s (described below).

The existence of a specific opiate receptor had already been postulated by Beckett and Casy (1954) on the basis of their analysis of the stereochemistry of morphine-type analogs and the structural properties of opiate antagonists. Differences in the modes of drug-receptor interactions had also been noted by Phil Portoghese, who postulated the existence of opiate receptor dualism (Portoghese, 1965). These multiple opioid receptor concepts were refined by Bill Martin and colleagues, who proposed the existence of distinct mu, sigma, and kappa receptors, based on differences in the physiologic responses

TABLE 1
Three families of endogenous opioid peptides and their primary sequences

Proenkephalin-derived opioids
Methionine-enkephalin (YGGFM)
Leucine-enkephalin (YGGFL)
Several additional C-terminally extended forms have been described, but physiologic significance is not established
Proopiomelanocortin-derived opioids
β -Endorphin (31 amino acid sequence beginning with YGGFM)
Several C-terminally truncated forms have been described, but physiologic significance is not established
Prodynorphin-derived opioids
Dynorphin A(1-17) (YGGFLRRIRPKLWDNQ)
Dynorphin A(1-8) (YGGFLRRI)
Dynorphin B (YGGFLRRQFKVVT) (a.k.a. rimorphin)
α -Neo-endorphin (YGGFLRKYPK)
β -Neo-endorphin (YGGFLRKYP)
Big dynorphin (YGGFLRRIRPKLWDNQKRYGGFLRRQFKVVT)
Leumorphin (YGGFLRRQFKVVTQSQQDPNPAYYGGFLFNV)

(e.g., miosis and bradycardia) and cross-tolerance between classes of opiate drugs (Martin et al., 1976). Strong evidence for multiple opioid receptors was also provided by the studies by Hans Kosterlitz and colleagues, who distinguished differences between mu-, delta-, and kappa-type opioid actions in the *in vitro* smooth muscle contraction bioassays (Lord et al., 1977). *In vitro* bioassays had advantages over behavioral pharmacological measures, because pharmacokinetic and metabolic differences between drugs are less likely to confound the biologic response. In the Lord et al. (1977) study, potencies in radioligand displacement in guinea pig brain membrane binding assays were compared with agonist potencies in the guinea pig ileum (GPI) and mouse vas deferens (MVD) bioassays. The sensitivities to naloxone antagonism of the different agonists were particularly revealing: mu-type agonists were equally sensitive to naloxone in GPI and MVD (naloxone $K_e = \sim 2$ nM), whereas naloxone was 10-fold less potent in blocking the effects of the enkephalins in MVD than in GPI (naloxone K_e : ~ 22 nM vs. ~ 2 nM). The authors interpreted these data as suggesting that enkephalins activated mu receptors in GPI and delta receptors in MVD. Of interest, they also noted that opiates in the class of drugs Martin called kappa were less sensitive to naloxone than were mu-type opiates in both MVD and GPI (naloxone $K_e = \sim 9$ – 15 nM).

These *in vitro* bioassay results provided strong support for the concept of physically distinct types of opioid receptors, but alternative interpretations were still plausible. Specifically, a competing concept at the time was that the opioid receptor was a single binding protein that adopted different conformations depending on interactions with auxiliary proteins. Evidence supporting the concept of interconverting receptor states was provided by Candace Pert's group, who suggested that the opioid receptor could adopt different conformations based on allosteric interactions (Bowen et al., 1981). This latter idea is not fundamentally different from how we currently view the effects of receptor dimerization on ligand potencies (Jordan and Devi, 1999), by which opioid drug potencies and efficacies depend on which receptors (e.g., kappa:delta, kappa:kappa, or kappa:mu) are physically associated.

It was in this historical context that the initial characterizations of dynorphin-A(13) actions in the GPI assay were performed (Goldstein et al., 1979). Although the dynorphin-A(1-13) fragment was extraordinarily potent in the guinea pig ileum bioassay (>700 times more potent than leucine enkephalin), its effects were one-thirteenth as sensitive to naloxone antagonism in this assay. The lower sensitivity to naloxone was shared by ethylketocyclazocine, a drug related to ketocyclazocine, which is the prototype used by Bill Martin and colleagues (Martin et al., 1976) to propose the existence of mu, kappa, and sigma type opioid receptors. In the MVD assay, dynorphin A(1-13) was 3-times more potent than leucine enkephalin, and the effects of both peptides were substantially less sensitive to naloxone antagonism. Strong conclusions about the relationship between the dynorphin receptor and other possible receptor forms were not made in the 1979 article: "If this tissue contains more than one type of opiate receptor, it follows that the [Leu]enkephalin receptor is different from the dynorphin receptor with respect to affinity both for these peptide ligands and for naloxone" (Goldstein et al., 1979, p. 6669). This conservative stance was presumably because the two principal competing conceptions ("physically distinct opioid

receptor types" and "interconverting conformations of a single opioid receptor") had not been convincingly resolved.

There next followed a period of intense examination of the properties of the dynorphin receptor. Wuster and Schulz (1980c) suggested that dynorphin receptor was the kappa receptor on the basis of further comparison of potassium sensitivities between dynorphin-A(1-13) and kappa opioid effects in the MVD assay. Others drew attention in print to the resemblance between the dynorphin receptor and the kappa-type receptor (Huidobro-Toro et al., 1981; Oka et al., 1982; Yoshimura et al., 1982), but these studies reiterated the previously established resemblance between dynorphin-A(1-13) and kappa-type opioids in their sensitivity to antagonists in bioassay without resolving the fundamental relationship. Efforts to resolve this question using radioligand binding assay were also published. Remi et al. (Remi et al., 1981) found that dynorphin-A(1-17) and dynorphin-A(1-13) did not have different affinities for mu, delta, and kappa binding sites in rat striatum labeled with [3 H]-dihydromorphine, [3 H]-[D-Ala², D-Leu⁵]encephalin, and [3 H]-ethylketocyclazocine, respectively. Pfeiffer and Herz (1982) developed a computer curve-fitting technique to resolve the radioligand binding sites and concluded that dynorphin-A(1-13) had highest affinity for the [3 H]-ethylketocyclazocine binding site; Corbett et al. (1982) did a similar analysis to show that dynorphin A(1-8) and dynorphin A(1-9) also had their highest binding affinity for the [3 H]ethylketocyclazocine site. Radioligand binding is a powerful tool, but has intrinsic limits; specifically, the conformation of the opioid receptor binding site (and, thus, its ligand selectivity) is strongly affected by assay temperature and concentrations of sodium ions, GTP, and buffer molarity (Simon and Groth, 1975; Werling et al., 1984), none of which are within the physiologic range in the radioligand competition assays typically used.

A more persuasive strategy was developed by Wuster and colleagues (Wuster et al., 1980a; Wuster et al., 1981) who used a selective tolerance approach to distinguish opioid receptors in the MVD. Mice were implanted with osmotic minipumps that delivered either the stable analog [D-Ala², D-Leu⁵]enkephalin (DADLE) or the potent and selective mu agonist sufentanil for 6 days before isolating the vas deferens tissue for organ bath bioassays. Pretreatment with DADLE selectively reduced the potency of delta-type agonists without affecting dynorphin or mu-type agonists, and pretreatment with sufentanil selectively reduced the potencies of mu-type agonists without affecting delta-type or dynorphins. In retrospect, of surprise, the simultaneous infusion of DADLE and sufentanil strongly shifted the potency of ketocyclazocine in MVD without affecting the potency of dynorphin A(1-13) (Wuster et al., 1980a,b). Pretreatment with the more selective kappa opioid ethylketocyclazocine shifted the potency of α -neoendorphin but had only a weak effect on dynorphin-A(1-13) potency in MVD (Wuster et al., 1981; Schulz et al., 1982). These studies provided important evidence that the receptors mediating the effects of mu, delta, and kappa opioids in MVD could be distinguished by selective-tolerance methods and that dynorphin-A(1-13) and α -neoendorphin likely acted through the kappa opioid receptor. However, issues of incomplete cross-tolerance between dynorphin and ethylketocyclazocine and uncertainty about the molecular mechanisms of tolerance remained to be resolved.

Contemporaneously, Avram felt that the interconverting states and physically distinct forms alternative conceptions needed to be directly addressed before conclusions about the nature of the dynorphin receptor could be made. For this purpose, we adopted a selective-receptor protection strategy using the nitrogen mustard analog of naltrexone, β -chlornaltrexamine (β -CNA), that had recently been developed by Portoghese et al. (1978). β -CNA is a site-directed alkylating agent that covalently binds to the opioid receptor(s) and nonselectively inhibits the effects of all classes of opioid agonists. By pretreating with either the stable enkephalin analog, DADLE or dynorphin-A(1-13) before β -CNA treatment, we were able to selectively protect from inactivation either the dynorphin receptors or the enkephalin receptors present in the GPI (Chavkin and Goldstein, 1981a). Because the receptors protected by dynorphin remained dynorphin-selective for hours after washing out the β -CNA and did not interconvert, we could conclude that receptors were physically distinct (Fig. 1). We then showed that selective protection with dynorphin A(1-13) equally protected the receptors in GPI mediating the effects of dynorphin and ethylketocyclazocine without protecting leucine-enkephalin or normorphine's potency (Chavkin et al., 1982). The selective protection studies also revealed that ethylketocyclazocine preferred the kappa receptor, but was not as selective as dynorphin-A(1-13). The lack of strong receptor specificity of ethylketocyclazocine helped explain some of the ambiguous results obtained in the selective tolerance studies cited above. Selective protection had been previously used by Robson and Kosterlitz (1979) and Smith and Simon (1980) to resolve delta and mu binding sites in brain homogenate binding assays. Our 1981 study extended those findings by distinguishing mu and dynorphin receptors in a functional opioid receptor bioassay, and the 1982 study strongly supported the concept that the

dynorphin A was an endogenous ligand for a physically distinct, non-interconverting kappa opioid receptor.

Of interest, low doses of β -CNA produced a parallel shift in the agonist dose-response curves in the GPI assay, suggesting for the first time that spare opioid receptors could control opioid sensitivity in tissue (Chavkin and Goldstein, 1981a). Differences in dynorphin potency in the GPI and MVD were subsequently shown to be a consequence of differences in tissue expression of kappa opioid receptors (Cox and Chavkin, 1983). In addition, the presence of spare opioid receptors suggested that morphine tolerance could be a consequence of the loss of spare receptors (Chavkin and Goldstein, 1982), and this concept was further established in subsequent studies (Porreca and Burks, 1983; Chavkin and Goldstein, 1984).

As described in the initial article on dynorphin (Goldstein et al., 1979), dynorphin-A(1-13) had both high potency and low sensitivity to naloxone. Its high potency is a consequence of its high receptor binding affinity, intrinsic efficacy, and spare receptor fraction. Its effects in the GPI and MVD are fully blocked by naloxone, which is the principal definition of an opioid receptor-mediated response, but the ~ 10 -fold lower sensitivity to naloxone is consistent with its kappa opioid receptor selectivity. Structural analysis of the dynorphin sequence revealed that the strongly basic residues in its C-terminal domain (arginine-7, lysine-11, and lysine-13) were cumulatively responsible for kappa selectivity in dynorphin-A (Chavkin and Goldstein, 1981b). Dynorphin-B and α -neo-endorphin also have strongly basic residues in comparable positions (dynorphin B: arginine-7, lysine-10; α -neo: arginine-7, lysine-10) and also show strong kappa receptor selectivity (James et al., 1984). In contrast, natural fragments of prodynorphin include dynorphin A(1-8) and β -neo-endorphin, which lack the C-terminal lysines and have lower kappa receptor potencies and selectivities (Chavkin and Goldstein, 1981b; James et al., 1984) (Table 1).

Neurotransmitter Properties of Dynorphin

The cloning of the prodynorphin cDNA by Kakidani et al. (1982) revealed that the prodynorphin sequence contained three opioid domains with the leucine enkephalin pentapeptide sequence followed by three different, highly basic C-terminal extensions (Table 1). Working with Lakshmi Devi, Avram began a study of the processing enzymes responsible for generating the active peptides from the precursor (Devi and Goldstein, 1984, 1986), and subsequent studies identified the thiol protease present in brain responsible for cleaving the single and paired basic sites as prohormone convertase PC2 (Berman et al., 2000). The range of prodynorphin opioids derived from the precursor is fairly broad, extending from large forms (big-dynorphin and leumorphin) intermediate-sized, kappa-selective forms (dynorphin A, dynorphin B, and α -neoendorphin), to shorter forms that do not distinguish between the opioid receptor types [dynorphin A(1-8) to leucine enkephalin]. The ability of prodynorphin to act as a precursor for the latter two peptides was convincingly established (Weber et al., 1982; Whitnall et al., 1983; Zamir et al., 1984); however, the functional implications of having a precursor able to generate peptide products with differing degrees of kappa receptor selectivity are not yet clear. Furthermore,

Fig. 1. The two principal competing conceptions describing opioid receptors during the 1960–1970. In the upper panel, a single opioid receptor structure could adopt differently shaped binding sites depending on the actions of allosteric modulators and distinguished by different opioid ligands. In the second conception, multiple types of opioid receptor proteins exist in cells, and opioids differ in their affinities and selectivities for the different physical forms. A selective protection strategy is outlined in the lower panel. β -CNA can nonselectively bind and inactivate both OpR1 and OpR2 in the absence of a reversible protective ligand. After excess β -CNA and the reversible ligand are washed away, the protected population of receptors remain available for activation. If the receptor forms are freely interconverting, no selective protection will be evident.

preprodynorphin is also the precursor for peptides that do not activate opioid receptors (Walker et al., 1982; Lai et al., 2006).

Since 1921, when Otto Loewi first characterized cholinergic transmission in the heart (Loewi, 1921), the criteria necessary to establish that a candidate is a neurotransmitter includes (1) presence in presynaptic terminals, (2) release after physiologic stimulation, (3) postsynaptic actions that can be blocked by selective antagonist and mimicked by selective agonist, and (4) metabolic mechanisms of its elimination. The specific distribution of the dynorphin peptides in brain and peripheral tissues at the cellular and ultrastructural levels has been well-defined using highly selective antibodies (Akil et al., 1984). The pro-dynorphin-derived opioids can be released from rat brain tissue in a calcium-dependent manner and positively identified by HPLC-C18 resolution, followed by specific radioimmunoassay (Chavkin et al., 1983). These released dynorphins were found to selectively bind to kappa opioid receptors after release (Wagner et al., 1991). Focal stimulation of dynorphin-containing pathways caused presynaptic inhibition of excitatory synaptic transmission in hippocampus that could be selectively blocked by kappa receptor antagonism (Wagner et al., 1993; Weisskopf et al., 1993; Drake et al., 1994; Terman et al., 1994). These studies are summarized in more complete reviews (Castillo et al., 1996; Simmons and Chavkin, 1996) and diagrammed in Fig. 2. The final criterion of specific metabolic clearance is more difficult for peptide transmitters, because their actions are typically terminated by nonspecific peptidases in the extracellular matrix, rather than by reuptake or selective degradation mechanisms.

Detailed studies of the neurotransmitter properties of the endogenous dynorphin system have documented its broad distribution in brain, consistent with the broad range of

pharmacological effects of dynorphin and kappa-selective drug actions. Prodynorphin-derived opioids are contained in large dense core vesicles and principally released in a calcium-dependent manner as dynorphin B, dynorphin A(1–8), dynorphin A(1–17), and α -neoendorphin forms. They can be released from nerve terminals to cause presynaptic auto-inhibition (e.g., mossy fiber terminals in the CA3 region of the hippocampus or hilar collaterals in the dentate gyrus). They can also be released from dendrites to cause retrograde inhibition of excitatory afferents (e.g., in the molecular layer of the dentate gyrus). Biophysical measurements of dynorphin diffusion rate in the extracellular space, and the kinetics of action support an estimate of the dynorphin synapse dimensions as being approximately 50–100 μ m from sites of release to sites of action (Drake et al., 1994). At the sites of action, they principally activate kappa opioid receptors selectively (no other source of endogenous kappa opioid has been identified). Kappa receptor activation is acutely inhibitory through the activation of potassium channels (Kir3 and Kv), inhibition of voltage-gated calcium channel opening, or direct inhibition of synaptic vesicle exocytosis through a $G\beta\gamma$ mechanism. In addition, sustained kappa receptor activation also results in stimulation of mitogen-activated protein kinase (MAPK) pathways (ERK1/2, p38 MAPK, and c-Jun kinase) (Bruchas and Chavkin, 2010), and the activation of p38 α MAPK in serotonergic neurons has been shown to be necessary for the dysphoric/aversive effects of stress-induced dynorphin release (Bruchas et al., 2011).

Dynorphins and Addiction

The preceding summary of the molecular physiology of the dynorphins supports the conclusion that dynorphins are endogenous neurotransmitters that function through kappa opioid receptors in brain; these insights would have gratified Avram, but he was particularly interested in how dynorphins might help explain drug addiction risk. Several clues to this question have emerged in the past 25 years. Herz and colleagues clearly established that kappa opioid agonists are profoundly dysphoric when administered to humans (Pfeiffer et al., 1986) and aversive when given to rodents (Shippenberg et al., 2001). Dynorphins are released during exposure of rats or mice to stressful behavioral experiences. Rodents subjected to repeated forced swim show norbinaltorphimine (norBNI)-sensitive increases in immobility (a depression-like behavior) (Mague et al., 2003; McLaughlin et al., 2003), and this effect is blocked by prodynorphin or kappa receptor gene knockout (McLaughlin et al., 2003; McLaughlin et al., 2006). Stress-induced dysphoria or anxiety is known to increase the risk of drug abuse in people (de Kloet et al., 2005) and to reinstate extinguished drug seeking in rodents (see Shaham et al., 2000). Repeated stress exposure produces dynorphin-dependent dysphoria (Bruchas and Chavkin, 2010; Bruchas et al., 2010; Knoll and Carlezon, 2010). Of importance, stress-induced release of dynorphin increases the rewarding effects of cocaine and nicotine in a conditioned place preference model (McLaughlin et al., 2003, 2006; Schindler et al., 2010, 2012; Smith et al., 2012), and stress-induced release of dynorphin reinstates cocaine self-administration and drug seeking (Beardsley et al., 2005; Redila and Chavkin, 2008). Walker and Koob (2008) also found that kappa antagonism reduces ethanol consumption in dependent rats.

Fig. 2. A granule cell from the dentate gyrus of the hippocampal formation. These neurons contain both excitatory amino acids and prodynorphin-derived opioid peptides. When activated by excitatory synaptic input in the perforant path from the entorhinal cortex, granule cells release glutamate to excite hilar interneurons and CA3 pyramidal cells. Kappa receptor agonists reduce granule cell excitation by inhibiting glutamate release from the perforant path fibers, reduce hilar neuron activation by inhibiting glutamate release from mossy fiber collateral fibers, and reduce CA3 pyramidal cell activation by presynaptic inhibition of the mossy fibers. High-frequency activation of the granule cells causes dynorphin release at each of these sites in the hippocampus, which also reduces excitation at these synapses. Biophysical studies of dynorphin transmission in the hippocampus have helped to define the special dimensions of this neuropeptide synapse in brain.

These results suggest that dynorphins acting through kappa opioid receptors encode the dysphoric, aversive, and anxiogenic effects of stress in ways that increase the risk of drug abuse and addiction. The abstinent state during drug withdrawal is also profoundly dysphoric, and dynorphin activation of kappa opioid receptors may contribute to the intense craving that results in drug-seeking behaviors in humans and animal models of drug addiction. A role for the endogenous dynorphins in mediating the dysphoric effects of drug withdrawal has been demonstrated after cessation of morphine (Carroll et al., 2005), tetrahydrocannabinol (Zimmer et al., 2001), nicotine (McCarthy et al., 2010), cocaine (Chartoff et al., 2012), and ethanol (Walker and Koob, 2008; Schank et al., 2012; Valdez and Harshberger, 2012). Animals lacking a functional kappa opioid system through either genetic deletion or receptor antagonism seem to be stress-resilient and less likely to seek drugs. Kappa antagonists do not affect addictive drug consumption in the absence of stress and do not affect cue-induced drug reinstatement; thus, they are unlikely to be broadly effective as anti-addiction medications. These results in preclinical animal models suggest that kappa receptor antagonists might be therapeutically effective in treating the anxiety and depressed affective states during withdrawal. These antagonists could become an important adjunctive treatment of addiction, but whether kappa antagonists reduce craving (and, thus, reduce drug seeking) or reduce the negative consequences of withdrawal (and, thus, promote drug use) for humans remains to be established. However, the concept that enhancing stress resilience in vulnerable individuals could protect them from addiction seems to be plausible, and the therapeutic potential of kappa opioid receptor antagonists in promoting stress resilience seems to be worth further study (Chavkin, 2011).

Highly selective kappa receptor antagonists have been developed; Portoghese and colleagues initially introduced norBNI (Portoghese et al., 1987), and Carroll and colleagues subsequently developed JDTC (Carroll et al., 2004). Both norBNI and JDTC have antidepressant-like and anxiolytic-like properties in rodent models of stress behaviors (Bruchas and Chavkin, 2010; Knoll and Carlezon, 2010), and these actions may be therapeutically useful. However, both norBNI and JDTC have very long durations of action (>3 weeks in rodents after a single dose), and this long-duration of effect is likely to be a consequence of kappa receptor inactivation through a c-Jun N-terminal kinase 1-dependent mechanism (Melief et al., 2011). Short-acting, selective kappa antagonists that do not activate c-Jun kinase have more recently been developed by scientists working at AstraZeneca (Peters et al., 2011), Pfizer (Grimwood et al., 2011), and Eli Lilly (Mitch et al., 2011), and further development of these and related compounds is ongoing. Developing safe and drug-like compounds is an expensive and difficult process, but hopefully, clinical trials will soon reveal whether antagonism of endogenous dynorphin tone in humans can be an effective treatment of depression, anxiety, or addiction disorders.

Conclusions

Substantial progress in the molecular, physiologic, and behavioral characterizations of the endogenous dynorphin/kappa opioid system and its relationship to brain function has been made in the past 30 years. This brief review focused on the initial

contributions of Avram's laboratory, then expanded on the specific topics that he was interested in addressing. In many ways, the recent history of this field validates his original belief that a molecular understanding of opiate drug action would lead to important therapeutic advances. New treatments are not yet in hand, but they seem to be nearly in grasp. Dynorphin has been an extraordinarily potent peptide, and this would have greatly pleased Avram.

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