Prevention of Opioid Addiction

Stephanie A Ihezie and Nachum Dafny*

Department of Neurobiology and Anatomy, McGovern Medical School, The University of Texas Health Science Center at Houston, USA

ABSTRACT

Opioid addiction is classified as a Substance Use Disorder (SUD), a complex and chronic health condition with physical, social, and psychological consequences. While there is no cure for it, we present a novel approach towards preventing a hallmark feature of addiction— the opiate withdrawal syndrome. Opioids exert numerous effects, acutely and chronically, on the nervous system with physical dependence, tolerance, and withdrawal being the most adverse chronic features. The degree of opioid dependence can be quantified by the frequency and/or intensity of the behavioral expression of withdrawal seen after abrupt termination of opioid consumption or after treatment with an opioid antagonist such as naloxone. Although the Central Nervous System (CNS) is the primary area of opioid impact, the involvement of the immune system in modifying CNS phenomena was suggested nearly two centuries ago and proved by several groups within the last few decades. Through a series of studies with immunomodulators alpha interferon, cyclosporine A, and cortisol, preclinical experiments show that administration of these agents prior to chronic morphine exposure prevents the expression of opiate withdrawal, a hallmark feature of addiction. This review provides updates on current developments in the management of the opioid epidemic and an overview of studies on preventative immunomodulation prior to repetitive opioid administration as a means of addressing one of the underlying symptomatology driving the epidemic.

INTRODUCTION

The rise of the opioid epidemic

Morphine is a legally prescribed opioid analgesic, and as the first alkaloid isolated from opium by German pharmacist Friedrich Sertürner in the early 1800s, it is the standard by which other opioids are tested [1,2]. The current opioid epidemic began its precipitous incline in the 1990s when opioids were widely prescribed for post-surgical and chronic pain [1]. The rationale for this boom is that opioids were known to be potent but falsely projected as not having any serious adverse effects. It was during this decade that the incidence of first-time opioid analgesic abuse rose from 628,000 individuals in 1990 to 2.4 million in 2001 [3]. One of the more recent attempts to curb this trend was when the FDA encouraged the reformulation of OxyContin in order to make it more difficult to abuse, but this has been associated with a subsequent rise in heroin use [4,5].

About 20–30% of patients who are prescribed opioids for chronic pain misuse them, and about 80% of heroin users first misuse prescription opioids [6,7]. More than 210 million opiate prescriptions were filled in 2010 with close to 12 million people admitting to abusing these drugs by taking them for non-medical reasons. Results from a 2014 National Survey on Drug Use and Health revealed that about 4.3 million Americans aged 12 or older had used opioid for non-medical purposes the month prior to the survey interview [8]. Other factors highlighting the gravity of the opioid epidemic are overdose-related emergency room visits and deaths. In 2015, an estimated 20,101 deaths occurred due to prescription painkillers and 12,990 deaths due to heroin use specifically, but even more people—591,000—were reported to...
Side effects and long-term effects of opioid use

Opioid addiction can arise from both the abuse of illicit opiates such as heroin and the misuse of prescription pain relief medications such as morphine, hydrocodone, oxycodone, and codeine, and misuse can result in life-threatening health problems. During the acute period of use, opioids elicit a sense of well-being that can become addictive. However, increased doses or inadequate systemic clearance can induce respiratory depression, constipation, urinary retention, drowsiness, nausea, vomiting, and hypothermia or more fatally cardiac arrest and death [2,18]. Long-term use can also elicit opioid-induced hyperalgesia, a phenomenon most often seen with higher doses of parenteral morphine and hydromorphone, in which there is increased sensitivity to pain despite increased opioid dosing and diffuse extension of the pain and allodynia [19,20].

Current management of opioid addiction

Presently, no treatment regimen prevents the development of opiate dependence. Management of opioid abuse is mostly reactive rather than proactive. Therapy is limited to treating overdose with naloxone. However, reversing an overdose does not stop relapse. When discussing opioid abuse and addiction, it is important to consider that there are non-medical and medical uses of opioids. Non-medical uses refer to consuming opioids when they are not prescribed for medical use, whereas medical uses refer to prescription pain relievers. It is this latter group of prescription opiates that contribute more to the opioid crisis, but strategies have focused on preventing non-medical use at the expense of preventing and treating opioid addiction in both medical and non-medical users [21].

Pharmacological management of opioid addiction

In the mid-1960s, a methadone maintenance treatment was initiated to manage heroin dependence long-term [22]. Methadone acts as an agonist at the opioid receptor and, along with partial agonist buprenorphine, works to control cravings [21]. Alternatively, naltrexone is an antagonist that blocks the effects of opioids. At first glance it may appear as the best pharmacological option out of the three, but relapse occurs very often. Therefore, not every patient is suited for naltrexone. For acute cases of opioid overdose, antagonist naloxone is a solution to life-threatening respiratory depression. It can reverse opioid overdose within minutes and has proven to be an effective strategy for rescue therapy.

Long treatment periods are required for most addiction therapies including Suboxone, Zubsolv, Probuphine, Sublocade, Bunavail, naltrexone, methadone, buprenorphine, CAM2038, and lofexidine (an alpha-2 adrenergic agonist that minimizes the symptoms of naloxone-induced withdrawal) [23,24]. Buprenorphine implants are relatively new, but initial findings show promise for therapeutic effectiveness and safety [25,26].
However, because most of the previously mentioned drugs belong to the opioid family there is concern that the above treatments are simply substituting one opioid for another which may prolong a type of dependence. Many of these drugs are for opiate withdrawal management, so they can lead to stronger cravings and relapse. Furthermore, there is little evidence for patient willingness to comply with these long-term treatments, and patients treated with these drugs may be at increased risk for subsequent overdose. Thus, an out of the box non-opioid regimen to prevent the induction of opiate withdrawal syndrome, a key contributor to the opioid epidemic, is needed. Additional novel treatments are under development including vaccines, trans-cranial current stimulations, and methods for improving treatment delivery [23].

Non-pharmacological management of opioid addiction

In addition to the above drugs, counseling, behavioral therapies, and social/spiritual support for long periods of time are essential. Psychosocial approaches, policies, and regulations are established methods of non-pharmacologic management. Residential treatment and rehabilitation, mutual-help programs such as Narcotics Anonymous, and 12-Step regimens are valuable options that can be used alone or in conjunction with medication [21].

Neuroimmune effects of opioids

Wybran showed that morphine does not only impact neuronal function but also affects the immune system, a finding that spearheaded investigations revealing that many neuropeptides, hormones, and neurotransmitters can alter components of the immune response [27,28]. In their study, they employed both endogenous opioids and exogenous opioids and found the former led to an increase in active T-cell rosettes and the latter led to a decrease in active T-rosettes [27]. Immune cells, such as T-cells, secrete endogenous opioid peptides which bind opioid receptors to alleviate pain and reduce inflammation, but exogenous opioids like fentanyl and morphine impair the function of T-cells, macrophages, and Natural Killer (NK) cells and circumvent them in order to bind opioid receptors [29]. Additionally, Kohno noted the role of neuroinflammation in the genesis, maintenance and treatment of SUD, and concluded that the pathologic activity induced by drugs of abuse contributes to the immune response [30]. Other studies have confirmed these results that endogenous opioids activate the immune system, and exogenous opioids suppress it [2,31]. Yet the level of immunosuppression by exogenous opioids is dependent on the type of opiate.

The endogenous opioid system is composed of widely scattered neurons that produce endorphins, enkephalins, and dynorphins which all function both as neurotransmitters and/or neuromodulators at the mu (μ), delta (δ), and kappa (κ) opioid receptors [32]. Exogenous opioids include morphine, buprenorphine, fentanyl, and several others. Morphine takes a dual approach to immunosuppression in that it binds directly to μ-Opioid Receptors (MORs) on macrophages, monocytes, NK lymphocytes, B-cells, and T-cells and indirectly to MORs in the CNS. Downstream pathways of the Hypothalamic-Pituitary-Adrenal (HPA) and sympathetic nervous system are activated and release glucocorticoids and noradrenaline, respectively, which both act on leukocytes to negatively modify immune function [33]. Buprenorphine is a partial MOR agonist and κ-Opioid Receptor (KOR) antagonist [34,35]. Fentanyl is a synthetic opioid that is 30–50 times more potent than heroin and 80–100 times stronger than morphine. These different exogenous opioids have varying levels of immunomodulation whether stimulatory, suppressive, or both [22,33]. For example, in rodent studies, buprenorphine did not affect splenic NK-lymphocyte, T-cell, or macrophage function but morphine significantly suppressed immunity [33,36]. In another rodent study on the development of tumors and surgery-induced immunosuppression underlying metastasis, tramadol increased NK Lymphocyte (NKL) activity while morphine suppressed it [37]. Fentanyl also decreases NK lymphocyte cytotoxicity thereby increasing the risk of tumor metastasis [38]. These findings were consistent with Sacerdote results from a study of surgery-induced immunosuppression in rats: buprenorphine returned NKL activity to preoperative baseline, morphine partially returned NKL activity, and fentanyl failed to return NKL activity [33]. In a mouse model of long-term opioid administration, after 7 days of infusion of fentanyl, there was reduced lymphoproliferation, interleukin-2, and interferon-gamma, but after 7 days of buprenorphine, there were no immune alterations [39]. Therefore, putting all these findings together it can be posited that tramadol is immunostimulatory, buprenorphine is immune-protective, and fentanyl and morphine are immunosuppressive.

Various hypotheses have been offered to explain the mechanisms underlying opioid withdrawal and tolerance. Most withdrawal behaviors have been accepted as primarily CNS-mediated phenomena, but further investigations have shown that opioid dependence has an immune system involvement. As early as the 1980s the question arose of using immunomodulatory agents to test for a possible link between the immune system and the expression of opiate withdrawal [17,41,42]. It was found that immune ablated rats treated with chronic morphine did not exhibit withdrawal behavior following naloxone injection [43]. Thereby demonstrating that an intact immune system is essential to the expression of opiate withdrawal. Soon after that, studies revealed that opioids change the percentage of T-lymphocytes forming active rosettes and their reactivity to mitogenic stimulation [44]. Opioids also affect cytotoxicity of natural killer cells, decrease the capability of cells to produce α-interferon, and decrease the levels of endogenous circulating α-interferon [45]. An alternative hypothesis is that the function of T-cells and natural killer cells is suppressed by stress which is
mediated by endogenous opioids [46]. When associated with immunosuppression, opioid use can enhance tumor progression and increase susceptibility to infection [33]. Indeed, clinical studies have demonstrated that opioid abusers are more susceptible to opportunistic infections including pneumonia and HIV which can develop from lowered levels of α-IFN, retrovirus restriction factors TRIM22 and TRIM5α, and APOBEC3G [45,47,48].

Glial cells and opiate addiction

Traditionally, glial cells were considered passive accessories to neurons. However, it was demonstrated that these cells actively participate in synaptogenesis, neuronal excitability, and neurotransmission [49]. Additionally, the glial system exhibits robust synaptic plasticity via changes in its morphology and physiology in response to opioid exposure within key brain sites contributing to addiction [50–52]. Synaptic plasticity is a hallmark of neurons and involves changes in synaptic strength which are believed to be the basis of learning and memory.

Recent studies have increased understanding of the interactions between the CNS and immune system that likely play causal roles in the pathophysiology of multiple psychiatric illnesses [14]. Microglia in the CNS regulate both addiction and analgesia, and due to their general role in modulating inflammation they, along with astrocytes, are a source of cytokine up regulation in the brain after opioid exposure [14,50,53,54]. Additionally, since opioid receptors have been identified outside of the CNS, it has been shown that opioids modify both the innate and acquired immune responses at several levels [55]. Gial cells express receptors for most neurotransmitters and release neuroactive substances. As a result, glial cells have been shown to modulate synaptic plasticity in many ways from changes in synaptic coverage to release of chemokines and cytokines. Moreover, the glial cells are part of the immune system. Gia cells are nomadic immune cells of the brain and active participants in the generation of innate immune response [56]. The Toll–Like Receptor 4 (TLR4) is a potential site for opioid-induced glial activation [57]. By binding the same binding site as bacterial lipopolysaccharide on the TLR4–Myeloid Differentiation Factor 2 (TLR4–MD2) complex, opioids can stimulate signaling downstream to TLR4. It has been suggested that opioid induced immune signaling does not occur through classical opioid receptors because the opioid receptor active metabolite of morphine, M6G, cannot bind and activate TLR4 whereas the metabolite without opioid receptor activity, M3G, can. Morphine modifies gene expression profiling in glial cells and promotes the release of factors such as IFN-γ, IL1-β, IL-6, IL-10, CCL4, and CCL17. Through the release of pro-inflammatory cytokines and chemokines, glial cells contribute to opioid reward, and through regulation of synaptic transmission and plasticity contribute to opioid-elicited addictive behaviors [57].

Indeed, acute and chronic morphine and psycho stimulant use activate specific components of the innate immune system [56]. Given the known involvement of the immune system, immunotherapy is now being considered in the management of addiction [56]. It has been suggested that the effects of drugs of abuse on the immune cells in the brain can be summarized into three steps: 1) opioids act on glia cells to generate and release proinflammatory cytokines, 2) these cytokines induce activation of quiescent astrocytes and microglia which in turn enhance the inflammatory response, and 3) the significant pathways that are initiated by these cytokines activate the immune cells and alter function [53]. Opioids activate CNS microglia to release various factors, which in turn contribute to opioid tolerance, dependence and alleviation of the withdrawal symptoms. Thus, Evans and Cahill suggest that repeated opioid consumption induces adaptive changes that modify neuronal circuitry, alter transcription, and spur dendritic spine changes, all of which create an altered “normality” pushing the individual into a new allostatic state or “drug dependent” state [52].

Immunomodulators attenuate behavioral opiate withdrawal

Further evidence for immune system implication in the expression of opioid withdrawal come from studies showing that its obliteration significantly reduced, if not eliminated, withdrawal behaviors [58–61]. Key immunomodulators that have been shown to diminish the opioid effect include α-interferon (α-IFN), cortisol, and cyclosporine A though there are several others under current investigation as well such as minocycline and ibudilast, but for the purposes of this review we will focus on the former three α-interferon (α-IFN) (Figure 1).

As a biological response modifier, α-IFN is often used as immunotherapy to regulate functions of the immune cells. It is part of an endogenous family of proteins found in vertebrates. α-IFN possess non-specific, potent immunomodulatory activity and is the most rapidly produced defense against foreign macromolecules [62,63]. α-IFN stimulates cells to produce other proteins, and it enhances the proliferation of human B cells and activates NK cells. It activates dendritic cells, initiating immune responses, and induces the expression of inducible-protein 10 (IB-10), a chemokine that promotes a TH1 inflammatory response [64].

In addition to immunologic properties, α-IFN poses both neurologic and endocrine activity and elicits corticosteroid secretion [65,66]. It has been argued that the immune system is the source for α-IFN, ACTH, and endorphins, but experimental studies divide this association with descriptions of α-IFN linking the endocrine and immune systems or linking the immune system and the CNS [68–73].

Blalock and Smith suggest that the action of α-IFN on
neurons operates through opiate receptors [68]. α-IFN was responsible for modulating opiate mediated phenomenon by direct action on the brain and participated in pain, temperature, and food intake [71,74,75]. Inflammatory changes have also been implicated in modifying dopamine signaling and decreasing dopamine synthesis by affecting important cofactors and possibly dopamine transporter function [14]. For example, patients receiving infusions of pro-inflammatory cytokine α-IFN had altered activity of the basal ganglia and reduced dopamine [76]. Neurons operate through opiate receptors [68]. α-IFN was responsible for modulating opiate mediated phenomenon by direct action on the brain and participated in pain, temperature, and food intake [71,74,75]. Inflammatory changes have also been implicated in modifying dopamine signaling and decreasing dopamine synthesis by affecting important cofactors and possibly dopamine transporter function [14]. For example, patients receiving infusions of pro-inflammatory cytokine α-IFN had altered activity of the basal ganglia and reduced dopamine [76].

Since opioid addiction stems from increased dopamine neurotransmission, applying α-IFN before drug administration could lower the effects of opiates arising from activated dopaminergic pathways. Therefore, α-IFN was utilized in our lab’s studies to determine if it can prevent the opiate withdrawal syndrome. It was found that α-IFN treatment prior to chronic morphine exposure attenuated withdrawal in morphine dependent rats [77-81] (Figure 1). Because opioid use decreases circulating levels of α-IFN, it is likely that application of α-IFN before repetitive opioid administration stimulated the immune response leading to changes that reduced dopamine signaling thereby diminishing subsequent opioid effects [45,47,48,82-89].

Corticosteroids are nonspecific immunosuppressors because they affect macrophages and monocytes as well as T-cells and B-cells, and hence cell-mediated and humoral-mediated immune processes [90]. Aside from inducing a lymphopenia by redistributing lymphocytes to other lymphoid compartments [91-97], glucocorticoids suppress type 2 cytokines such as IL-2 and IL-4 which are responsible for memory T cell differentiation as well as helper T cell differentiation and B-cell isotype switching to IgE [98]. Cortisol was also able to reduce the naloxone-prefectuated withdrawal syndrome [99] (Figure 2). Cortisol is produced in the adrenal glands and is responsible for regulating a wide range of processes including immunomodulation and stress response. Almost every cell contains receptors for cortisol, so it is not just an anti-inflammatory agent but also controls salt and water balance, influences blood pressure, controls glycemic levels and thus regulates metabolism, and influences cognitive function [100-102]. Activation of the HPA axis arises from IL-1 stimulating hypothalamic Corticotrophin Releasing Hormone (CRH) thereby activating Adrenocorticotrophic Hormone (ACTH) in the pituitary which then activates the adrenal cortex to release glucocorticoids, and increased plasma concentrations of glucocorticoids depress the immune system [103-105].

In rodents, acute opioid administration increases ACTH and glucocorticoids levels which in turn leads to immunosuppression, but chronic opioids either decrease or have no effect on these hormone levels. Cortisol is anti-inflammatory acutely and pro-inflammatory in the long-term [14,106]. In humans, exogenous glucocorticoids are given to decrease inflammation and pain but long-term can cause immunosuppression. Although both α-IFN and cortisol decrease the occurrence of withdrawal behaviors when given prior to repetitive morphine exposure, they have opposing functions, cortisol is anti-inflammatory and α-IFN is pro-inflammatory. However, cortisol also directly operates as part of the HPA with a feedback mechanism. As a result, it is likely that administration of cortisol before repetitive morphine provided negative feedback to the HPA axis preventing further release of glucocorticoids and, subsequently, immune suppression.
Cyclosporine A (CsA) is a cyclic, undeca-polypeptide extracted from soil fungus and used in organ and tissue transplantation to prevent graft rejection [107,108]. CsA utility in transplants comes from its immunosuppressive properties with preferential action against helper T-lymphocytes through the arrest of the cell in the G0 or G1-phase. [107,109,110].

Although cyclosporine A is primarily used as an immunosuppressant, it also affects the CNS. In one of our previous studies we directly measured the cyclosporine levels in rat brain tissue 1 hour following intraperitoneal injection. Significant levels of the drug were observed in comparison to the control group. This indicates that the drug does reach the brain area where it conceivably may have a direct effect on the CNS [41].

Interaction of immunomodulators and opioids

Either ablating the immune system—which would only be indicated in humans in very rare cases—or stimulating it before repeated opioid administration resulted in attenuated opioid withdrawal. Two possible explanations for this finding is that 1) since opioids negatively affect the immune response, if there is no immune system for the drug to impact, the effects are negligible, or 2) stimulation of the immune system before repetitive drug administration can counter the immunosuppressive effects of opioids so that the physiological effects of withdrawal are diminished. Moreover, it has been reported that the interplay between the CNS and immune system is active during the immune response, a response that is altered by drugs of abuse [14]. The reciprocal interaction between the CNS and the immune system has gained traction because of the demonstration of a putative pathway between these two systems. This interaction occurs essentially at two levels: 1) cell–to–cell contact and 2) release of soluble mediators that bind to cell surface receptors [91,92]. Thus, immunomodulators such as...
as α-IFN, cyclosporine, and cortisol possess both immune and neural actions, permitting their modulation of the behavioral expression of opioid addiction. These treatments carry potential therapeutic implications for substance use disorders.

CONCLUSION

Using immunomodulators to prevent opioid adverse effects

Opioid abuse can lead to addiction. Our previous studies on the neurophysiological mechanisms underlying drug dependence have established that repetitive administration of morphine and other drugs of abuse modulate the behavioral and neuronal Baseline (BL) activities. These changes correlate closely with the development of tolerance and withdrawal. In other words, when the drug is used repeatedly and then withdrawn, it results in a biochemical chain of events that produces changes in the neuronal BL firing patterns of involved brain regions, and it is these electrophysiological changes that are involved with causing the subsequent behavioral expressions of withdrawal [95,96,115-118]. Although addiction is primarily a CNS process, it is linked to the immune system. Several studies recounted above show that if we can modulate the immune system before repetitive morphine are able to significantly reduce the severity of opiate withdrawal behavior precipitated by naloxone injection [119-124]. Preventing opiate withdrawal syndrome will decrease relapse and toxicity and potentially prolong lives. These immunomodulators are safe and effective therapies that have long been on the market and used for other disorders as well [125-129]. They hold promise in combating the on-going opioid epidemic. Given the discussions in this review of the underlying basis of opioid withdrawal and proposed alternative approach of using non-opioid immunomodulators to prevent it, it may be beneficial to utilize preventative immunomodulation in the management of opioid abuse in an effort to overcome the current opioid epidemic.

ACKNOWLEDGMENT

Supported in part by NIH grant RO1 DA 00803.

References


61. Dougherty PM, Drath DB, Dafny N. Evidence of an immune system to brain
68. Dafny N, Rigor BM. Characterization of unit activity recorded from septum, thalamus,
67. Blalock JE, Smith EM. Human leukocyte interferon: Structural and biological
52. Evans CJ, Cahill CM. Neurobiology of opioid dependence in creating addiction
49. Watkins LR, Hutchinson MR, Johnston IN, Maier SF. Glia: novel counter-regulators
73. Reyes-Vázquez C, Mendoza-Fernandez V, Herrera-Ruiz M, Dafny N. Interferon
modulates glauconicsensitive neurons in the hypothalamus. Exp Brain Res. 1997
48. Dafny N. Interferon alters the regulation and control of the action potential in
29. Dafny N, Rigor BM, Pellis NR. The immune system and opiate withdrawal. Int J
69. Dafny N, Reyes-Vazquez C. Three different types of alpha-interferons alter naloxone-
68. Dafny N, Lincoln J. The Role of Interferons on the Central Nervous System in Health
59. Dafny N, Vázquez-Cruz, Cruz, Gómez, Dafny N. Interferon induces abstinence in morphine addicted rats. Immunopharmacology. 1985 
70. Dafny N, Reyes-Vázquez C, Prieto-Gómez B, Dafny N. Effects of chronic interferon- 

101. Eisenstein TK, Rahim RT, Feng P, Thingalaya NK, Meissler JJ. Effects of opioid
99. Law R, Clow A. Stress, the cortisol awakening response and cognitive function. Int
94. Kharas N, Reyes-Vazquez C, Dafny N. Locus coeruleus neuronal activity correlates
93. Kharas N, Whitt H, Reyes-Vazquez C, Dafny N. Methylphenidate modulates dorsal
82 in
24520393; PMCID: PMC3919782.
129. Sarpatwari A, Avorn J, Kesselheim AS. Using a drug-safety tool to prevent
128. Qiao JT, Dafny N. Dorsal raphe stimulation modulates nociceptive responses
125. National Academies of Sciences, Engineering, and M., Health and Medicine Division,
124. Frolov A, Reyes-Vasquez C, Dafny N. Behavioral and neuronal recording of the
123. Dong WQ, Wilson OB, Skolnick MH, Dafny N. Hypothalamic, dorsal raphe and external
122. Owens T, Khorooshi R, Wlodarczyk A, Asgari N. Interferons in the central nervous
120. Shreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506.
118. Tang B, Dafny N. Dorsal raphe neuronal activities are modulated by methylphenidate.
116. Tang B, Dafny N. Behavioral and dorsal raphe neuronal activity following acute and
115. Venkataraman SS, Claussen CM, Kharas N, Dafny N. The prefrontal cortex and
114. Venkataraman SS, Joseph M, Dafny N. Concomitant behavioral and prefrontal
cortex neuronal responses following acute and chronic methylphenidate exposure
113. Venkataraman SS, Claussen CM, Kharas N, Dafny N. The prefrontal cortex and the
caudate nucleus respond conjointly to methylphenidate (Ritalin). Concomitant behavioral
112. Venkataraman SS, Claussen CM, Kharas N, Dafny N. Dorsal raphe neuronal activity
following acute and chronic morphine withdrawal. Int J Immunopharmacol. 1987 Sep