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© Med Sci Monit, 2005; 11(5): MS47-53
PMID: 15874900

www.MedSciMonit.com
Mini-Symposium

Received: 2005.03.14
Accepted: 2005.03.16
Published: 2005.05.05

Pain, immunity, opiate and opioid compounds and health

George B. Stefano[1], Gregory L. Fricchione[2], Yannick Goumon[3], Tobias Esch[4]

[1] Neuroscience Research Institute, State University of New York, College at Old Westbury, Old Westbury, NY, U.S.A.
[2] Department of Psychiatry, Division of Psychiatry and Medicine, Massachusetts General Hospital, Boston, MA, U.S.A.
[3] Inserm, 575 "Physiopathology of the Nervous System", Strasbourg, France
[4] Charité – University Medicine Berlin, Institute for General Practice and Family Medicine, Berlin, Germany

Source of support: This work was supported in part, by the following grants: MH47392 and DA09010

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Summary

We surmise that opioid peptides, i.e., methionine enkephalin, first arose during evolution as modulators of cellular immune function given their immune actions and the presence of enkelytin, a potent antibacterial peptide, and its precursor proenkephalin in animals 500 million years divergent in evolution. Pain probably emerged from this perspective because of its association with proinflammatory events. Endogenous morphine appears to exert positive effects on homeostasis by limiting the degree of excitation. Supporting this view is the fact that the mu3 opiate receptor subtype, which is opioid peptide insensitive and morphine selective, is coupled to constitutive nitric oxide release, which also has this down regulating action in neural, immune, vascular and gastrointestinal tissues. Thus, morphine down regulates immune processes in addiction, an action/function that it appears to normally perform when the situation calls for this action and by so doing in this natural setting, sustains life.

key words: **morphine • pain • amygdala • nitric oxide • μ3 opiate receptor • opioid peptides • methionine enkephalin • enkelytin**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/16165
Word count: 3568
Tables: 1
Figures: 3
References: 51

Author's address: Dr. G.B. Stefano, Neuroscience Research Institute, State University of New York, College at Old Westbury, Old Westbury, NY 11568-0210, U.S.A., e-mail: gstefano@sunynri.org

BACKGROUND

Over hundreds of millions of years living organisms evolved numerous and diverse strategies to protect themselves against threats, i.e., microbes, harsh environments and the trauma associated with rapidly changing circumstances. These environmental challenges are usually referred to as perturbations if weak and 'stress' if stronger, and protective mechanisms to overcome stressful situations include the so called "stress response" [1,2]. Those successful strategies gave such endowed organisms the ability to pass on these processes, including those that could be considered health promoting. Thus, long living organisms today retain many successful strategies for survival, enabling them to live a relatively long life in reasonably good health. Probably the most advantageous coping strategy that evolved recently is cognition [3], enabling man to effect this process directly, speeding up the evolvement of even more protective strategies. As with any process, too much "thinking" can lead to inactivity and by so doing becomes detrimental [4,5]. Taken together, we surmise that our physiological processes contain health-promoting information, which we have only begun to understand. In this review, we examine only opioid and opiate chemical messengers for their health promoting actions in diverse organisms. This is important since opiate alkaloids are primarily identified for their negative role in addiction and related activities.

OPIOID PEPTIDES

Opioid pentapeptides, i.e., methionine enkephalin, stimulate cytokine release and immunocyte chemotaxis and induce conformational changes in immunocytes indicative of activation [6]. These activities are phylogenetically ancient since they also occur in invertebrates, animals that are 500 million years divergent in evolution from mammals [6,7]. Invertebrates contain a mammalian-like proenkephalin molecule [7], which contains the potent antibacterial peptide enkelytin [7] that exhibits a 98% sequence identity with mammalian enkelytin [7]. The presence of enkelytin strengthens the association of opioid peptides with immune-related activities.

Since these chemical messengers are present in both invertebrates and man, in the past we demonstrated that opioid peptides stimulate immunocyte chemotaxis and phagocytosis as well as the secretion of cytokines, and the simultaneously liberated enkelytin would attack bacteria immediately (Figures 1,2) [8]. This process would allow time for the immunocyte-stimulating capabilities (i.e., recruitment) of the opioid peptides to manifest themselves. Interestingly, this same scenario may occur in neural tissues, i.e., via microglia. In neural tissues, proenkephalin processing may also lead to enkelytin 'liberation', suggesting that neurons may exhibit bactericidal innate immune functions. We surmise these mechanisms have evolved to supplement immune actions by covering the latency period before total or partial immune activation occurs. The same phenomenon, e.g., simultaneous increase in opioid peptides and enkelytin, is found in human plasma in patients undergoing coronary artery bypass grafting [9]. Taken together, it can be argued that the co-processing and liberation of enkelytin and [Met]enkephalin represents a unified neuroimmune protective response to an immediate threat to the organism, regardless of what form the stimulation takes. Such a unified response might thus represent an important survival strategy since it promotes health [8].

Pain

The concept of pain [10] has proved a difficult issue to approach. Nevertheless, there is a wealth of literature documenting the ability of opioid peptides and opiate alkaloids in ameliorating the sensation of pain [11,12]. In this regard, why, then, is an antibacterial peptide found within proenkephalin containing methionine enkephalin, a naturally occurring analgesic inducing molecule, and why has this association endured for at least 500 million years? The close association of enkelytin and opioid peptides, i.e., methionine enkephalin, probably reflects the fact that both types of molecules have evolved to fight the presence of microbes. Bacteria and viruses are persistent factors in the environment and are a threat to any organism, regardless of the evolutionary time period. Thus, in order to survive and reproduce, organisms had to evolve processes to combat this immediate non-cognitive threat. The association of enkelytin and opioid peptides probably represents such a successful strategy [8].

Pain and immune function

Once the association between enkelytin and opioid peptides was established in evolutionary terms, organisms needed an early detection/surveillance system by which they could continuously monitor microbial penetration and growth. What better alerting process than one that signaled attention by creating a noxious sensation and, maybe later in evolution, pain? However, it would be counterproductive if this sensory experience could "cripple" an organism into inactivity. The following scenario might be envisaged: Under serious situations commanding attention, the sensation of pain must subside momentarily (i.e., analgesia) to allow for an appropriate response to the stimulus. While the organism is orienting itself to the stimulus, the simultaneous release of enkelytin, analgesic and immunocyte-stimulating opioid peptides combats the pathogenic challenge of a bacterial presence [8]. Once this is over, the pain-provoking process can emerge once more, possibly even at a stronger level, resulting in behaviors designed to alleviate the condition.

Indeed, if pain evolved to fit this function, it evolved in association with immune processes. Furthermore, these opioid mediated activities were probably enhanced during evolution as the central nervous system (CNS) became closed off from the circulatory system by the blood-brain barrier, ultimately isolating the ganglionic neural activities that also required immune surveillance. However, regardless of the barrier, various immune cells, i.e., those responsive to opioid peptides, were always allowed access to the isolated tissues, some taking up residence in the CNS as microglia. The reason for the evolving relationship between opioid neural and immune processes now appears quite simple, that is, analgesic priority-setting activities associated with an anti-infectious/anti-inflammatory process. This combination would provide a high degree of survival benefit to any organism, since it would ensure appropriate behavior to meet not only these noncognitive challenges, but also cognitive ones [8].

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G     65    CKDLLQVSKQELPQEGASSLRESGKQDESHLLSKKYGGFMKRYGGFMKKVDELYPVEPEE 124
M     24    CK-IFQYRLQKCPSLKASSLRESGKQDESHLLSKKYGGFMKRYGGFMKKVG-----EPE-  76

G     125   EANGGEILTKRYGGFMKKDAEDGDALANSSDLLKELLGTGDDRDRENHHQEGGDSDEGVS 184
M     77    EILTKR-----YGGFMKKD-EAAQAANSSDLLKELLGTGDDRDRENHHQEGGDSDEGVS 130

G     185   KRYGGFMRGLKRSPQVEDEAKELQKRYGGFMRRVGRPEWWMDYQKRYGGFLKRFAEFLPS 244
M     131   KRYGGFMRGLKRSP---KSRSSEQ---------V--------VQKRYGGFLKRFAEFLPS 170

G     245   DEEGESYSKEVPEMEKRYGGFMRF
M     171   EEEGESYSKEVPEMEKRYGGFMRF
```

Figure 1. Comparison of invertebrate and mammalian proenkephalin. Common amino acids are shown in red for comparison with the mammalian material, opioid peptides in blue and the enkelytin (green) sequence, including [Met]enkephalin-Arg-Phe (blue) is underlined, G – Guinea pig, M – *Mytilus edulis*, dashed lines represent spaces in the proenkephalin molecules as determined by a best fit model.

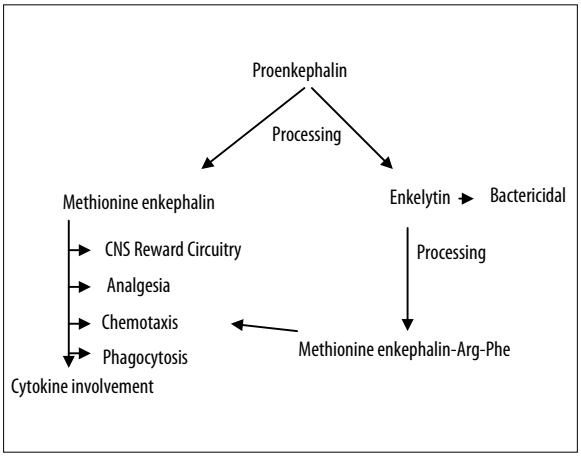

Figure 2. Complementary immune actions of [Met]enkephalin and enkelytin. Bacterial products (e.g., lipopolysaccharide) or tissue trauma (e.g., a cut) can induce the simultaneous release of [Met]enkephalin and enkelytin from immune cells. [Met]enkephalin induces immunocyte chemotaxis and the release of other signaling molecules (i.e., cytokines), whereas enkelytin exerts an antibacterial action. Within minutes enkelytin too is processed to yield [Met]enkephalin-Arg-Phe that further augments the immunocyte response.

Furthermore, given the presence of the same signaling molecules in central nervous system reward circuits, i.e., opioid and opiate molecules [11,12], one can also surmise that here too, processing of proenkephalin leads to an enkelytin presence. Thus, feeling good, indicating opioid peptide possessing, may also create a healthy internal environment and may form the basis of a novel protective survival strategy. Given the belief and trust components of feeling good, involving these signal molecules, this may be the basis of promoting health as can be demonstrated in placebo experiments as well as complementary and alternate medical therapies [11,13–15].

Endogenous Opiate Alkaloids: Morphine

In the past we have noted the many reports documenting the presence of morphine and its metabolites and precursors in animals, including invertebrates, strongly suggesting that this chemical messenger can be made by animals [16–18]. Recently, two reports have emerged noting that the addition of opiate alkaloid precursors results in enhanced morphine levels in cancer cell lines and incubating normal healthy ganglia, demonstrating the synthesis of morphine [19–21]. These studies are complemented by the demonstration of a mu opiate receptor subtype, mu3, that is opiate alkaloid selective and opioid peptides insensitive present on immune, vascular and neural tissues [22].

Functional roles of endogenous morphine

In this discussion of the possible activities of endogenous opiates we are guided by information collected in numerous studies on the pharmacological responses to the administration of exogenous morphine and related drugs. One feature that appears to be characteristic of exogenous opiate compounds, exemplified by their known antinociceptic effects, is that they lower thresholds under a variety of physiological and pathological conditions. It is, therefore, reasonable to speculate that endogenous opiates may act in a similar capacity, wherever a situation calls for it.

The presence of opiate alkaloids in the circulation and of special opiate receptors on immunocytes, demonstrated in vertebrates as well as invertebrates, enables these compounds to participate directly in auto- and immunoregulatory activities [6,16]. Indeed, some reports have noted excitatory effects of morphine, however, in part, we have demonstrated that this may occur from a rebound from inhibition – its just that the observation time points in those studies did not pick up the initial inhibitory effects [23–27]. These direct activities may be judged to be largely of an inhibitory nature. In addition, circulating opiates may contribute to the total sum of directives mediated by signal molecules

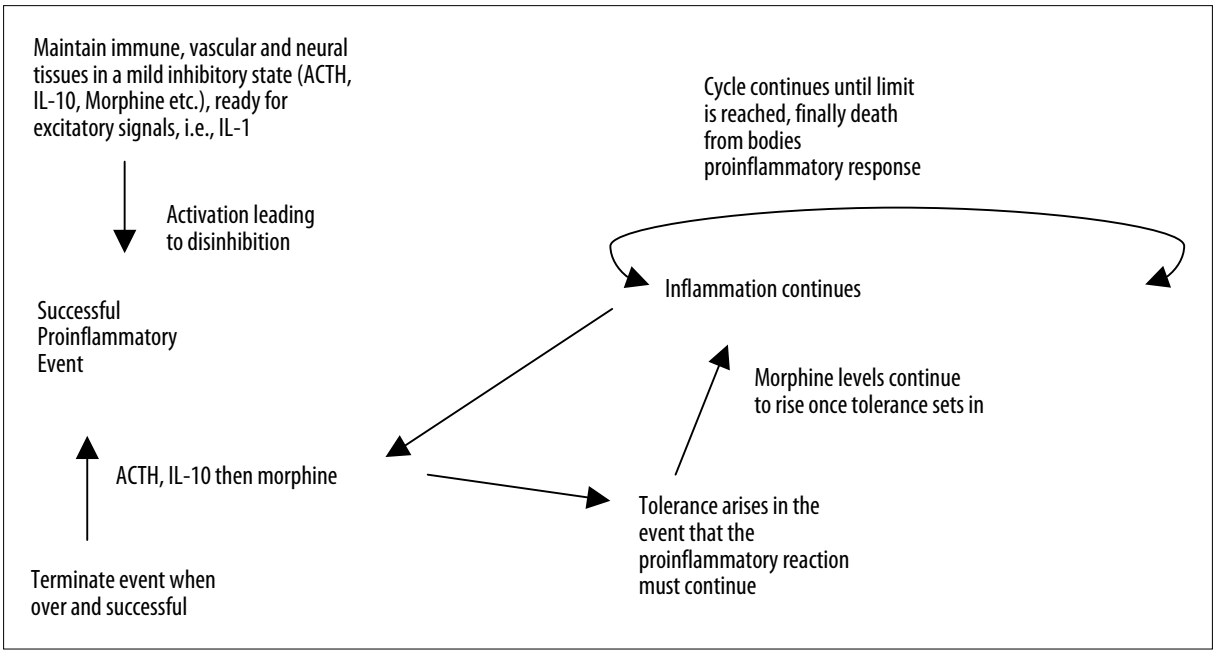

Figure 3. Morphine's natural function to limit excitation. For example, in an immune event, "tonal/basal" morphine levels initially remain low, mildly inhibiting the cellular components. Once a sufficient stimulus is induced/present, the system becomes disinhibited, leading to activation. After this process continues, the body's processes (i.e., brain and adrenal gland) attempt to restore normal function via down regulating signal molecule production, i.e., morphine, which exhibits a latency period before its increased concentration can be detected. However, if excitation continues to rise, the augmented morphine levels go unfelt, i.e., tolerance, allowing the process to continue. We surmise the body repeats this cycle, when more and more activity is called for until the down regulating capabilities are lost, suggesting the individual may succumb to the bodies widespread activation, which when left unchecked becomes toxic, i.e., sepsis.

reaching the central nervous system from various sources, including the immune system.

Another, i.e., indirect, pathway for the suppression of immune processes by morphine can be postulated**,** namely that via the hypothalamic-hypophysial-adrenal axis (Figure 3). In this case, the final outcome, immunosuppression, must be visualized to be initiated by a stimulatory signal from morphine, furnished by the brain, to CRH-producing neurons of the hypothalamus, the first way station in this axis. This concept is supported by the immunocytochemical demonstration of a morphine-like compound in the rat hypothalamus as well as morphine's action in releasing CRF, which would then enhance plasma ACTH levels followed by that of glucocorticoid [28–30].

The direct and indirect down regulating activities attributed to endogenous morphine should be considered in the context with those of other chemical mediators known to act in the same capacity. One of these inhibitory molecules is the cytokine interleukin-10 (IL-10), which is released by macrophages to counteract excessive immunostimulation caused by other cytokines which, under certain conditions of activation, are produced and released by the same cells (see [31,32]). Another inhibitory signal molecule produced by immunocytes is ACTH [33], which, like IL-10, can be considered to participate in autoimmunoregulatory activities and can be stimulated by morphine [30].

The question arises in which manner and under which circumstances the immunosuppressive activity of endogenous morphine is called into action that can be interpreted as positive for the organism. It is reasonable to speculate that the need for an additional control system may arise under conditions making unusual demands.

There seems to be general agreement on the fact that serious or life threatening challenges create a state of alertness, brought about by the instant release of stimulatory messenger molecules (opioid peptides and others), during which all available energies are directed toward meeting the emergency [1,34]. What should be considered to be equally important is that these stimulatory signals need to be stopped as soon as they are no longer required, so as to prepare the organism for a subsequent challenge. Endogenous morphine would seem to be an appropriate candidate to meet this demand.

For example, during major surgical interventions, the immunosuppressive effect of ACTH and IL-10 produced by immunocytes may not suffice to lower the hyperstimulation of granulocytes and macrophages attributable to their release of IL-1 and TNF due to this trauma. It seems reasonable to suggest that, under these circumstances, morphine may be called upon to down regulate the process so as to restore the normal level of activity [34]. The validity of this proposal is supported by tests carried out with blood samples taken from patients during cardiopulmonary bypass operations. In preparations exposed to morphine, signs of cellular activity were less pronounced than in untreated samples, and plasma morphine levels in surgical patients were high, following a 24 hour latency period [35,36].

The results of another experiment, indicative of the down regulating capacity of endogenous morphine under conditions

of stress, deserve attention, since it was first reported in an invertebrate. The design was to follow the sequence of activities generated by subjecting the mollusc Mytilus edulis to stressful interventions (electrical shock, prevention of valve movements [37]). The immediate response was activation of the animal's defense system, as judged by conformational changes in its immunocytes, and interpreted to be brought about by the release of endogenous opioid peptides and additional molecules. Twenty-four hours later, when the state of alertness had subsided, the return of the immunoactive hemocytes to a more "inactive" conformation was found to concur with a temporary but significant rise in the opiate content of nervous tissue and hemolymph [35,38]. The conformation of the immunocytes observed at this point in time resembled that of unstressed animals exposed to exogenous morphine.

The insights gained from the study of the various traumatic situations cited suggest that in the heirarchy of available down regulating mechanisms, morphine operates as a strong backup system. The observation that this secondary system goes into effect after a latency period during which endogenous opiate levels rise, is in line with the fact that the μ_3 opiate receptor has an affinity constant in the range of 10^{-8} M [22,39].

Evidently, the availability of a network of effective immunostimulatory agents has great survival value for vertebrates and invertebrates alike. It is, therefore, understandable that the development of its elements, including those operating in immunoregulation, can be traced far back on the evolutionary scale. The need for the operation of more than one immunosuppressive mechanism is as obvious as that for the availability of effective immunostimulatory agents. It is our belief that it is one of morphine's important tasks to meet this vital demand.

In addition to those discussed here, endogenous morphine and related opiates may be presumed to engage in a variety of other activities, for example some operating within the confines of the nervous system [18].

TOLERANCE

With the above discussion in mind we will now consider the phenomenon of tolerance with regard to endogenous opiate alkaloid compounds, morphine, morphine-6-glucuronide, etc. since they too are endogenous to various animal tissues, including invertebrates [40–43]. With continued exposure to the same dose of an opiate, various physiological systems exhibit a decrease in their response. This phenomenon is referred to as tolerance. As with the study of opiates, our historic interest in this phenomenon is focused around anti-nociception. However, given that morphine is a naturally occurring signal substance, we must ask another question. Since tolerance occurs, what would its "normal" function be? We have examined the need for 'turn-on' molecules as well as the need for 'turn-off' molecules in various systems. Now we must consider what turns off or down regulates this 'off' system, i.e., morphine.

We believe the answer, in part, to this question is in the phenomenon of tolerance. Once the down regulatory process has been initiated and the level of these molecules (i.e., morphine) rise to competitively overcome the influences of the initial stimulatory molecules, the inhibitory molecule's level, i.e., morphine (as also noted by the K_d for morphine on the mu3 receptor, approx. 15–50 nM), would be hard to overcome, as would be its continued presence due to its relatively long half-life as well as its conversion to morphine-6-glucuronide, extending its actions longer in time [44]. Thus, stimulatory signal molecules could not activate the system during this down regulation unless their concentrations rose well above those levels in the initial event. Indeed, at this moment, the activities generated by an additional phase of excitatory molecule release would upset the now instituted down regulation, given its competitive and reversible signal molecule nature. This is especially true of morphine [44]. However, the only mechanism that can effectively diminish the inhibitory actions over a relatively short period would be one in which the very same effector cell system progressively becomes desensitized to the presence of morphine. In this way, the down regulating influence would be terminated regardless of the concentration of morphine present during a single event. Thus, the effector cells become tolerant. Interestingly, tolerance would only set in once down regulation had been achieved**,** because desensitization relies on higher or extended morphine concentrations – a dose that would have already exerted its down regulating properties before. Thus, the 'brake' would be administered on a 'need' basis along with the dynamic capability of progressive adjustments in this process if required. Moreover, tolerance, once achieved, also would allow for further stimulation of the system if it was required, since morphine's presence would not be "sensed".

In summary, tolerance represents a dynamic mechanism that can be used to augment various regulatory processes whether they are involved in excitation or inhibition. Simply stated, tolerance is a process that allows for the termination of morphine's action while it is still present in the environment. It is still present in the environment because it is a general, yet specific, mechanism operating only at concentrations above basal levels, concentrations that would terminate excitatory processes. However, tolerance ensures that immuno-inhibition, for example, does not last to the point whereby the organism would be compromised due to a lack of a functioning system, i.e., immune, over an extended period of time. Thus, desensitization sets in and allows the various processes to be stimulated and operational once more. Indeed, there is a rebound into excitation from inhibition that also occurs with morphine [24–26,45]. Clearly, the timely 'rebound' of the immune and nervous processes involved with opiate actions provides for a successful mechanism to ensure survival as has been demonstrated (see [23]). Since tolerance has been demonstrated in invertebrates and vertebrates**,** the use of this strategy becomes even more evident.

Dependence/addiction

In drug dependence, one can see tolerance develop with a decreased response to the actions of a drug. Thus, in order to achieve the same effect one has to take a larger dose. The phenomenon of dependence occurs upon the withdrawal of the drug, which produces behavioral signs opposite of those desired. Furthermore, associated psychological dependence involves compulsive drug-seeking behavior (i.e., craving) as well as the diminishing of normal motivators, i.e., health issues [12]. In all animals there are normal behaviors which can be said to be based on a compulsive 'foundation'. It

Table 1. Phenomena that may trigger or represent the basis of addiction. Immune cells in malignant histyocytosis where found to be devoid of opiate receptors and did not respond to morphine, that is, becoming down regulated [50]. Exercise has the ability to increase endogenous plasma levels of morphine [51].

Addiction
1. Morphine Insufficiency Syndrome: Individuals may not be making enough morphine and/or opiate receptors may be faulty or not present, and when they first experience it they feel normal. This may induce normal behaviors that augment endogenous morphine processes/levels, i.e., exercise. In time, tolerance may set in, causing an increase in this behavior, which may become compulsive. The other possibility is that after experiencing external morphine they directly seek it, restoring normality. However, tolerance and dependency will eventually set in because this external source cannot be fine tuned.
2. External sampling of morphine, i.e., for pleasure or analgesia, which bypasses the normal and appropriate reward and/or pain circuitry, triggering the cycle of tolerance and finally dependency upon continuous use.

would be interesting to speculate that "addiction" emerges from tolerance if the presence and level of endogenous opiates do not or cannot return to their previous or pre-stimulation low levels. In this scenario, once tolerance occurs, the opiate molecules, i.e., morphine, remain relatively elevated. In this event, in order to further lower the threshold of activation, e.g., immunosuppression, one would require even greater increases in morphine levels due to tolerance. It would be to an organism's benefit to have such an immune mechanism, since this would allow for a more dynamic response to antigenic challenge, for example. Thus, an animal would have several levels of immune responsiveness to call upon. Indeed, we surmise, the lack or dysfunction of such a system may lead to various pathologies, i.e., hyperactive cell disorders, autoimmune disorders, etc.

However, given the above, the dynamics of passing through many levels of tolerance may adversely affect the organism. Clearly, operating at different morphine levels may force a "system/process" to continually re-establish its threshold for excitability due to tolerance setting in. We surmise that at critical developmental periods, this process may also leave a permanent change in neurological and immune systems [12]. This endogenous trauma may manifest itself, as noted above, in the display of opposite "behaviors" i.e., excitability of nerve or immune cells. Indeed, one may associate immune stimulation with morphine actions due to the fact the process has become tolerant and thus supersensitive when morphine availability ceases [23]. In a concurrent neuronal morphine receptor supersensitive state, if the stimuli/state became cognitive, one would actively seek out opiates. In this regard, addictive or compulsive behavior may be viewed as a process indicative of morphine insufficiency. A subpopulation of individuals seeking for continuously higher levels of opiates (i.e., those addicted) may be operating from a morphine insufficiency status. Additionally, these individuals may also exhibit lower concentrations or densities of mu receptors, since tolerance and other factors possibly induce a downregulation of related opioid receptors, leading to the need for higher opiate concentrations, i.e., morphine, for a normal physiology to operate. Therefore, addictive behavior may be viewed as a phenomenon that may reflect opiate insufficiency in an organism with attendant alterations in neuro- and immunoregulation. Indeed, what may initiate the cascade of tolerance in a potential addict, an individual who may have an endogenous morphine biosynthesis and/or excessive degradation problem, is the very first experience with such a substance (Table 1). For in this experience, a subpopulation of individuals may for the first time 'feel' normal. This might reflect the 'neurological susceptibility' to drug addiction referred to by Dole and Nyswander in their 1967 metabolic theory of addiction [46]. Normal is defined as being in the sense a diabetic feels being given insulin [47]. However, in the morphine insufficiency scenario, as morphine is administered, tolerance develops because it is the normal way endogenous morphine's presence is down regulated. Thus, persons enter into this cycle of dependence as they continually seek to regain and maintain the 'normal' feeling – often overshooting into a state of intoxication. This hypothesis offers the explanation for the phenomenon that some individuals treated for drug addiction are not 'cured'. In this specific case, the reason may simply be that the endogenous opiate insufficiency has not been addressed, and by providing the substance one compromises the existing regulatory processes, i.e., tolerance.

Conclusions

Taken together, endogenous morphine appears to exert positive effects on health by limiting the degree of excitation. Supporting this view is the fact that the mu3 opiate receptor subtype is coupled to constitutive nitric oxide release, which also appears to have this down regulating action in neural, immune, vascular and gastrointestinal tissues [16,48,49]. Thus, morphine down regulates tissue processes in addiction, an action/function that it may normally perform when the situation calls for this appropriate function and by so doing in this natural setting, sustain life.

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