

# Placebo

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# The Biology of Deception: “Man’s Inhumanity to Man”

GB Stefano<sup>1\*</sup>, GL Fricchione<sup>2</sup>

<sup>1</sup>Neuroscience Research Institute, State University of New York at Old Westbury, Old Westbury, NY 11568, USA, <sup>2</sup>The Carter Center Mental Health Program, 1 Copenhill, Atlanta, GA 30307, USA

\*Correspondence: George B. Stefano, Neuroscience Research Institute, State University of New York at Old Westbury, NY 11568, USA.  
Email: [gstefano@sunynri.org](mailto:gstefano@sunynri.org)

## ABSTRACT

**Background:** Clearly, the evolvement of socialization leads to better survival chances for animals. Furthermore, organisms displaying even the smallest component of cognitive ability would have a trait with enormous survival advantage. Cognition would be an enhancement to social animals, in that, division of labor, aided by communication and tool building, would be enhanced as well. A protective social structure would provide for the long time required for the evolvement of cognitive abilities within populations. Historically, we know that man lived in small nomadic hunting groups. In part, the effectiveness of these groups was based on the strong behavioral and physical similarities of the individuals comprising them.

**Methods and Interventions:** The data and speculation for the present report was obtained from the scientific literature after a critical review.

**Results:** It would appear that the stress response is a real phenomenon that harnesses innate processes to promote health. However, if this response operates for extended periods of time it becomes harmful. The pathogenic effects

of stress are known to be buffered against by the support that comes from inclusion in a social group. Yet enlargement of the social in-group itself with newcomers from an out-group may lead to distress in the individual. Evolutionary reasons for this boundary condition exist and may serve as a rate-limiting step in the development of a more global human community unless cognitive change can continue apace with globalization.

**Conclusions:** Once our cognitive powers reached their present point of development our social structures, societies, got bigger. Yet we still have problems dealing with intra-group differences, both physical and behavioral, that come with this size increase propelled by global travel and communication between cultures. While we have developed technologically at a fantastically rapid rate, we have not shed the baggage of our gene-culture evolution as it pertains to our feelings of security and individuality in small homogeneous groups. In essence, in order for our evolution to be ultimately successful we must have further “cognitive evolution”.

**Key Words:** Socialization, stress, cognitive power, communication

## INTRODUCTION

Previously we hypothesized that the development of cognitive processes provided such endowed animals with an additional coping strategy in dealing with stress (1). It also provided an organism with a sense of unity and cause-effect linearity as a coping strategy, which is really a deception or illusion, in that it imposes perceived order and control. Our brains as organs evolved to assess sense data but also to maintain a healthy, allostatic, and calm internal milieu. Living life as a conscious cognitive being in our world of contingency and threat might itself present a danger to the individual. How then to build an organ in an organism with the ability to maintain keen awareness of stimuli in the environment while also modulating what this external sensory awareness will stimulate, namely the energy wasting hyper-awareness of emotion-laden internal stimuli?

Trivers, an anthropologist, has put forward an evolutionary theory of self-deception, by which he means the active misrepresentation of reality to the conscious mind. He suggests that there may be multiple sources of self-deception in our species and interactions between these sources (2). Self-deception may include denial of ongoing deception, self-inflation, ego-biased social theorizing, false narratives of intention and a conscious mind that works via denial to fashion a self-serving world. Such a world may be designed to reduce distress in the individual. Trivers

also analyzes self-deception in groups and highlights the inherent costs of deceptive differentiations as grouping mechanisms.

The neurologist V.S. Ramachandran has taken another approach to understanding the evolutionary origins of self-deception (3). Pathological denial of illness or anosagnosia, as it is called, occurs when a patient suffers a right parietal area stroke with resultant paralysis of the left side of the body. When asked to perform an action with his paralyzed left arm the patient will go to great lengths to point out that the arm is perfectly fine but he just doesn't feel like moving it at the moment. This is so striking that this form of denial is called a "right parietal syndrome". Ramachandran believes that in normal individuals the left hemisphere evolved to ordinarily dispense with small, local "anomalies" or discrepancies by trying to impose consistency as a way to preserve the status quo. However when the anomaly detected exceeds a certain threshold perhaps indicating a threat to the status quo, what he calls a 'devil's advocate' in the right hemisphere comes on line to create a paradigm shift that results in the construction of a new model employing the same data --one that demands attention and possible action because it is emotionally alarming. Indeed we know that the right hemisphere is more emotionally reactive and more tied in with the visceral nervous system. For example, when patients have a stroke in the left frontal region of the brain, patients are more

prone to depression, perhaps indicating that an unopposed and strong anomaly detector on the right is hyper-reactive to the illness experience. In its overreaction, the right hemisphere can itself cause a self-deception in the other direction, i.e., that all is lost and that the future is hopeless, which is usually not the case in reality.

In essence what we learn from the neurology of self-deception is that in a bi-hemispheric way, it works both ways. The left hemisphere is best equipped to perpetuate the status quo world-view. It deceives in the direction of safety, control and a good future. Life is sugar-coated and understandable. The right hemisphere is preferentially an anomaly detector. It deceives in the direction of danger, contingency fear and sadness about the future. Life is a bitter pill and inscrutable. Fortunately, the left hemisphere has evolved to dominate with enormous impact on human cognition and affect. However, one is left wondering whether this occurred because the left hemisphere was, after all is said and done, a better arbiter of reality and less deceptive or simply more pragmatic. Perhaps a dominant left hemisphere provides a survival advantage based on the cognitive trick of reducing our allostatic loading (our stress-related metabolic debt) through diminished fear conditioning, even if it takes deception to reduce it. Either way, what has occurred as a result is nothing short of an alteration of human life on earth, allowing for individual comfort, a longer lifespan, and

social groupings. The clear superiority of cultural evolution in terms of rapidity of effect over genetic evolution is a byproduct of this horizontal bi-hemispheric relationship and also of the vertical relationship between the neocortex and the limbic system. Whether or not it is based on deception about life, it is clear that approaching the world as presumably understandable and cognitively “user friendly” has been pragmatically successful. All science is based on this as a matter of fact. As William James would say, “It (this approach) has baked bread”.

Thus, the biology of deception has been an important evolution leading to man as a cognitive creative being. We should be clear however, that emotion and cognition work hand in hand. A recent neuroimaging study for example used functional Magnetic Resonance Imaging (fMRI) to show that emotional engagement was required to make moral reasoning judgments (4,1,5). It is sometimes assumed that the cognitive process occurs by way of a “rational” mind alone, that is, the ability to evaluate many items of information and finally come up with a well thought out solution to a problem. As is implied, this only occurs after a complex weighing out of all details, considerations and facts in a situation. However, such a detailed cognitive process can lead, counter intuitively, to inactivity and/or major delays in response selection. Thus, for many circumstances an organism exhibiting unemotional “rationality” would not or

could not survive. From this premise we surmised that, in part, emotion can be viewed as a preserved aid or shortcut to rationalized action (5). Indeed the neuroscientist Paul MacLean and the behavioral neurologist Antonio Damasio have presented evidence that human emotion is a sine qua non for human reason (6,7). We have also surmised that man, as a cognitive being, did not just “appear” upon the top of the evolutionary tree with cognitive ability (8). In this last report, we discuss a logical argument for the apparent reluctance of man, in general, to accept cognitive attributes as being present in other animals. Indeed, this reluctance may be founded once more in the overall concept of the biology of deception, that is, a perceived distortion of reality, which favors survival.

In the present report, we will undertake the problem of “man’s inhumanity to man”, that is our apparent intolerance to accept physical, cultural and religious differences in peoples. Indeed, this too, may be an example of the biology of deception, which in the past had tremendous survival value.

## DISCUSSION

Throughout animal evolution we can find various forms of socialization involving single species that undoubtedly led to better survival chances for the individuals, and thus the species. In this regard, Man's evolution has been a prime example. We may also conclude that even organisms displaying the smallest component of cognitive

ability, since it did not just “jump” on the evolutionary scale of progress, would have an important survival advantage (1,5,8). This unique characteristic would be a further enhancement to social animals, in that division of labor, communication and tool building would be enhanced as well. It could even be predicted that a way for a “society”, and thus a species, to evolve would be to enhance intra-group communication. Furthermore, a protective social structure would provide for the long time required for the evolution of cognitive abilities within that population. Thus, cognitive evolution and socialization are mutually reinforcing.

We can assume that the time required for cognitive enhancements to evolve occurred over millions of years. Thus, social settings and structures, in which cognitive evolution has been nurtured, form its foundation. In this regard, from human history, we know that man lived in small nomadic hunting groups, as do other successful mammals. This situation, with a certain group size, most probably arose due to a limited supply of food within a given geographical region. These small group-like societies would also protect man in general from disease, since a disease, potentially viral in nature, would only “wipe out” a small segment of man. Thus, for most of man’s unwritten history, “we” had been nomadic hunters living in small, effective groups. In part, the effectiveness of these groups was based on the strong behavioral and physical similarities of

the individuals comprising them. The single purposefulness (or shall we say “mindfulness”) of the groups as well as their homogeneous composition led to their success. We speculate that variations in this composition or strangers entering the group with different forms of communication or physical characteristics were not tolerated in general. Indeed, these “strangers” were probably viewed simply as not similar, much like a simple immune antigenic challenge. This attitude, at that time, had great survival value in that it kept the groups tightly bonded from within, thus constantly protecting the “core” from external threats that would potentially disband the group thus making its individuals vulnerable to external assaults. In brief, maintaining a homogeneous in-group has great survival value for man and all other social animals.

Interestingly, once our cognitive powers reached their present point of development our social structures, societies, got larger. This was only possible due to our growing cognitive powers dominated by the left hemisphere, which imposed a sense of order based on our perceptions (1). However, since larger societies were a relatively recent development in our evolution (last 10,000 years), we have problems dealing with intra-group differences, both physical and behavioral, that come with larger more diverse societies as well as with global travel and communication between cultures. These last factors can clearly pit one group against another in modern times

even when separated by thousands of miles. Indeed, these conflicts may simply be based on a lag in cognitive understanding or evolution. While we have developed technologically at a fantastically rapid rate, we have not shed our gene-culture “baggage” regarding the feelings of security and individuality that small homogeneous groups provide. In short, our behavioral preferences still favor our earlier development (pre-human and human), which is understandable, given the long association of our cognitive development with man’s success and development in small groups.

In essence, in order for our evolution to be successful we must have further “cognitive evolution”. The fact that we have marveled in our own technological achievements and placed ourselves on a pedestal based on them, has in some ways hidden our Achilles’ heel. Namely, our intellectual creations are way ahead of our behavioral comprehension regarding our capacity for tolerance of other out-group peoples. In other words, we again are faced with a deception of reality from within. The human genome project has established that all human beings are essentially identical with virtually no racial differences, for example.

There is heartening research just published that suggests that out-group bias is not hard-wired (9). The researchers report experiments using a tool called the memory confusion protocol, which unobtrusively reveals whether subjects are categorizing target individuals into groups and

whether they are using dimensions such as race to do so. Their results suggest that categorizing individuals by race is not inevitable. Rather, another hypothesis emerges. Encoding by race may be a reversible byproduct of cognitive machinery that evolved to detect coalitional alliances. Coalitional affiliations are indeed encoded as part of normal representations of persons encountered. However, when cues of coalitional affiliations no longer correspond to race, subjects greatly reduce the extent to which they mark others by race and may entirely cease to use racial categorization. It is of interest that facial recognition is mediated in the same right parietal brain region we discussed above, so that certain cues of coalitional affiliation may also be lost in a right parietal stroke. Such loss of facial recognition is called prosopagnosia. Here, too, there would be loss of "anomaly" detection on a gross level but the above experiment suggests that exposure to an "alternate social world" (setting up affiliations with racially integrated basketball teams) for 4 minutes of their experiment was enough to dilute the tendency to categorize by race.

## CONCLUSION

In conclusion, we surmise that the discomfort individuals may experience upon first meeting someone new or being in physically different cultures may, in fact, be due to a primitive behavior imbedded in our gene-culture evolution reflecting our desire to exist

in a homogeneous group, one that the individual has been conditioned to exist within as part of a coalitional affiliation. While this discomfort, during man's evolution, led to a measure of his successful attempt to survive and "prosper", in and of itself, it is really a deceptive behavioral mechanism since it implies that the individual's group is the only anomaly free safe one. As such, one might hope that this behavior will become outdated given the advances achieved by man's intellect. Man's cognitive ability must evolve to include the understanding that all men are basically the same, and their differences are really small compared to their similarities on all levels. But this is not the only task that lies ahead. Human response selection always involves an emotional valence interpenetrating the cognitive choice. How we evolve the ability to broaden our emotional affiliations may hold the key to the future of the species.

## ACKNOWLEDGMENTS

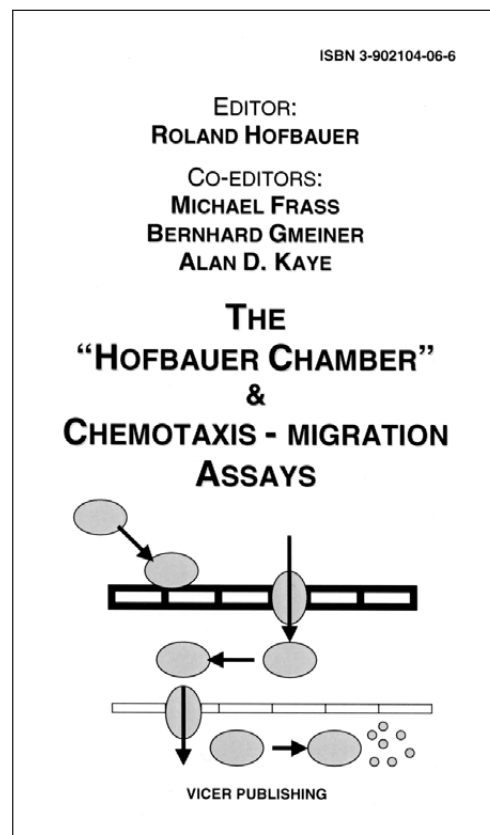
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# Neuroimmune modulation by non-opioid peptides family through the animal kingdom

**M Salzet**

Laboratoire de Neuroimmunologie des Annelides, Université des Sciences et Technologies de Lille, France

Correspondence: Laboratoire de Neuroimmunologie des Annelides, UMR CNRS 8017, SN3, IFR 17 INSERM, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France. Tel : +33 3 2043 6839, Fax : +33 320041130. E-mail:michel.salzet@univ-lille1.fr

## ABSTRACT

**Background:** Opioid are schematically divided into three different classes of active peptides (enkephalins, dynorphins, endorphins), which been found in all metazoans. These opioid peptides like almost all neuropeptides, are synthesized via the proteolytic processing by prohormone convertases of larger inactive precursor molecules i.e. proenkephalin (proEnk), prodynorphin (proDyn) and proopiomelanocortin (POMC).

**Method and Investigations:** The data and the speculation for the present report were obtained from our results and scientific literature.

**Results:** Regardless to opioid peptide sequence, these peptides shared the common N-terminal sequence i.e. the YGGFX sequence which is responsible of their activity. Beside this family, a number of other endogenous opioid peptides have been found and they share in common the YP dipeptide N-terminal sequence. These peptides constitute a novel family of peptides, called non-classical opioid peptides

**Conclusions:** The two families are synthesized by both nervous and immune system and are implicated as signalling molecules in their cross-talk.

**Key Words:** Placebo, Neuroimmunity, non-classical opioids, metallopeptidases

## INTRODUCTION

It has long been thought that all physiological functions, including immune reactions were exclusively under brain control. Pioneer studies of Metchnikoff and Pasteur have unrav-

eled the key role of the immune system, which is acting as «mobile brain», in the organism's defense mechanisms. It is now clear that the central nervous and the immune systems are indeed cross-talking and are both involved in immune response. Since 1980, numer-

ous studies have demonstrated that mental variations, which are subsequent to stress or hypnosis, are modulating immune response and are implicated in the organism's defense mechanisms. In contrast, it has also been shown that immune system modulates both the peripheral and the central nervous system and is involved in fever production which is resulting from an infection (1). These interactions are mediated via different molecules including peptides such as CRH and ACTH, monoamines (epinephrine, norepinephrine and dopamine), glucocorticoids, free radicals, cytokines such as IL1, IL6 and TNF $\alpha$ , and opioid peptides and opiates (2). In this way, Smith and Blalock have demonstrated the existence of communications between endocrine and immune system, which are mediated via intercellular messengers (3). Moreover, they demonstrated, for the first time, that peptides such as ACTH are indeed produced by immune cells. Reciprocally, it has also been shown that some cytokines are synthesized in the central nervous system following peripheral or central infections, ischemy or neurodegenerative diseases (4).

These data allowed the emergence of a new research field which is called neuroimmunology or even psychoneuroimmunology and which includes endocrinology, immunology and neurobiology (5). However, because of the complexity of nervous and immune system in mammals, the study of these interactions is a difficult task. Invertebrate models have been particularly

useful to understand the fundamental physiological mechanisms involved in development, apoptosis, aging and immunity. Between the numerous hormonal messengers produced in immune cells (2), this review is dealing with non-classical opioid peptides, and it will compare the role of these peptides to those of classical opioids in the modulation of the immune system both in vertebrate and in invertebrate species (6-9).

## I- STRUCTURE AND BIOSYNTHESIS OF NON-CLASSICAL OPIOIDS PEPTIDES

Opioid are schematically divided into three different classes of active peptides (enkephalins, dynorphins and endorphins), which been found in mammals (10), and more recently in invertebrates (7). These opioid peptides like almost all neuropeptides, are synthesized via the proteolytic processing by prohormone convertases (11), of larger inactive precursor molecules i.e. proenkephalin (proEnk), prodynorphin (proDyn) and proopiomelanocortin (POMC) (7,12). Regardless to opioid peptide sequence, these peptides shared the common the N-terminal sequence i.e. the YGGFX sequence which is responsible of their activity.

Since the discovery of enkephalins (7), a number of other endogenous opioid peptides have been found and they share in common the YP dipeptide N-terminal sequence. These peptides are able to bind to sigma or mu

**Table 1. Non-classical Opioids peptides family found in vertebrates.**

Name	Sequence
MIF-1	PLGamide
Tyr-MIF-1	YPLGamide
Tyr-W-MIF-1	YPWGamide
Tyr-K-MIF-1	YPKGamide
Hemorphin-4	YPWT
Hemorphin-7	YPWTQRF
VV-Hemorphin-7	LVVYPWTQRF
Valorphin	VVYPWTQ
Spinorphin	LVVYPWT
$\beta$ -casomorphin-5	YPFVE
$\beta$ -Casomorphin-8	YPFVEPIP
$\beta$ -Casomorphin-5	YPFPG
$\beta$ -Casomorphin-7	YPFPGPI
Cytochromorphin-4	YPFT
Endomorphin-1	YPTFamide
Endomorphin-2	YPPFamide
morphiceptin	YPPFamide

opiate receptors (Table 1) (13-16) with a high selectivity e.g. endomorphin-1 has 4000 and 15000-fold binding selectivity to the  $\mu$ -opioid receptor over  $\delta$  and  $\kappa$ -opioid receptors. This allowed Zadina and colleagues to propose that these peptides are natural endogenous ligands for  $\mu$ -opioid receptors and belongs to a novel family, the non-classical opioids peptides one (13-16). Most of the peptides of this new family are hemoglobin or casein-

**Table 2. Non-classical Opioids peptides family found in invertebrates.**

Name	Sequence
MIF-1	PLGamide
LORF	IPEPYVWD, IPEPYVWDamide
Hemorphin-like	SVVYPWTQTF

derived opiate peptides i.e. hemorphin-4, endomorphins (16,17), are present in nervous tissues and have endocrine effects (13-17). They have been found both in vertebrates and in invertebrates (18-22). Several in vitro and in vivo studies revealed that hemoglobin, casein are processed into bioactive peptides such like neokytorphin, VV-hemorphin-4, a bradykinin-potentiating peptide, casomorphins, exomorphins (23). LVV-hemorphin-7, derived from beta-, gamma-, delta- or epsilon- chains of human hemoglobin has been found in cerebrospinal fluid (CSF) of patients suffering from cerebrovascular bleedings, but not in CSF from healthy individuals (18). Hydrolysis of hemoglobin by aspartyl protease such like pepsin, cathepsin D will allow producing in the course of the peptic hydrolysis, LVV-haemorphin-7, VV-haemorphin-7 and VV-haemorphin-4 (24). In leeches, fragment of beta hemoglobin has recently been found and it shares at its C-terminal side, a hemorphin-like peptide (Salzet, Unpublished data) (Table 2). Casein degradation generates small peptides acting like hemorphins as opioid peptides (23, 24). By contrast, MIF-1 (PLGamide), Tyr-MIF-1 (YPLGamide), Tyr-W-MIF-1 (YPWGamide), Tyr-K-MIF-1 (YPKGamide) are endogenous peptides not deriving from the cleavage of larger proteins (16). MIF-1 has also been found in leeches like another non-classical opioid, the leech osmoregulator factor (IPEPYVWDamide) and its cleavage products i.e. IPEP, YVW-

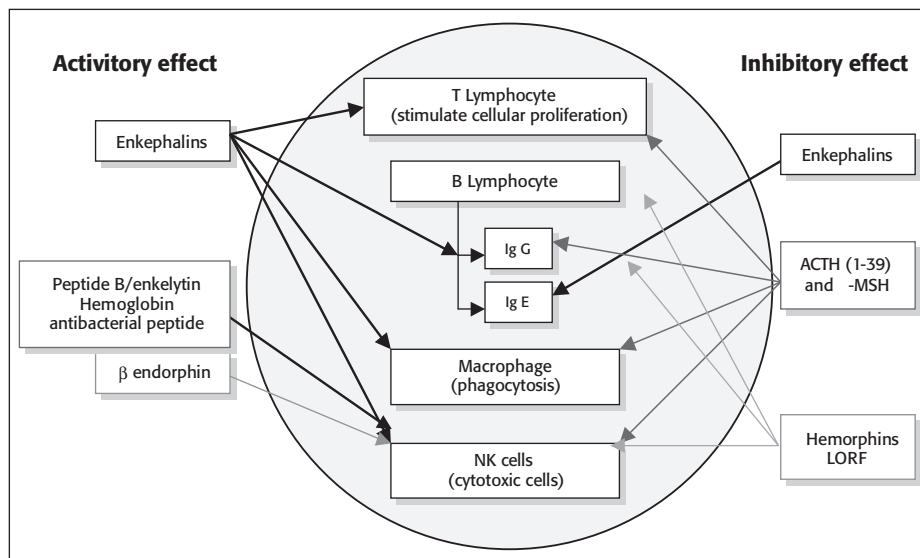
Damide (20-22). However, its precursor is still unknown. Nevertheless, two possibilities can be envisaged either LORF is derived from non-hemic oxygen binding protein N-cleavage product, the hemerythrin (25) or from a completely novel precursor. Recently, we demonstrated that hemerythrin gene is present in leech brain (Deloffre, unpublished data). In this context, this sustains the first hypothesis that LORF derived from an aspartyl protease action on the hemerythrin protein to generate it like for hemorphins. It has to be noted that LORF is also present in mammals' brain tissues (22).

## II- NEUROIMMUNE ACTIONS

These peptides have been found in the nervous systems of mammals and in vitro they like hemorphin-7 regulate the release of growth hormone and prolactin in the male rat (26). It also mimics the effects of the ones previously found with morphine and other  $\mu$ -opioid peptides receptor agonists (26). Endomorphins like morphine are able to produce short acting anti-nociception (27) and exhibit vasodilatory actions in rat and rabbit through  $\mu_1$  or  $\mu_2$  receptors like opioid peptides (27). However, little is known about their distributions or concentrations in tissues as cardiovascular or gastro-intestinal. First isolated from bovine spinal cord, whose sequence matches a conserved region of beta-hemoglobin, spinorphin inhibits enkephalin-degrading enzymes and is analgesic. Recently, spinorphin was reported to

block neutrophil activation induced by the chemotactic N-formylpeptide N-Formylmethionyl-leucylphenylalanine (fMLF), suggesting a potential role as an endogenous negative regulator of inflammation (28). Spinorphin induced calcium flux in normal mouse neutrophils, but was inactive in neutrophils from mice genetically deficient in the fMLF receptor subtype FPR (N-formylpeptide receptor). Consistent with this, spinorphin induced calcium flux in human embryonic kidney 293 cells transfected with mouse FPR, but had no effect on cells expressing the closely related fMLF receptor subtype FPR2. Despite acting as a calcium-mobilizing agonist at FPR, spinorphin was a weak chemotactic agonist and effectively blocked neutrophil chemotaxis induced by fMLF at concentrations selective for FPR (28). Spinorphin did not affect mouse neutrophil chemotaxis induced by concentrations of fMLF that selectively activate FPR2. Thus, spinorphin blocks fMLF-induced neutrophil chemotaxis by acting as a specific antagonist at the fMLF receptor subtype FPR (28).

Recently, Jessop et al. (29) have demonstrated the presence of endomorphins in mammalian immune tissues (spleen, thymus, 29). Moreover, as opioid peptides (Fig. 1), these peptides seem to be implicated in neural, neuroimmune and autoimmunoregulatory signaling (6, 29). These phenomena have been supported by studies documenting the presence of stereospecific opioid receptors on specific leuko-



**Figure 1.** Inflammation modulation by classical opioids peptides and non-classical opioids peptides. ACTH: adercorticotrophin hormone, MSH: melanostimulating hormone, LORF: leech osmoregulator factor

cytes, i.e., granulocytes, and nerve cells (6). Additionally, these cells express the actual signaling molecules used for this chemical signaling, including the expression of mRNA (6). Opioid peptides induce immunocyte chemotaxis as well as initiate the release of cytokines (6) and methionine-enkephalin is now considered as a cytokine (30). In invertebrates, these same signaling peptides induce chemotaxis and the release of mammalian-like cytokines (6), including interleukin-1,-6 and tumor necrosis factor- $\alpha$ . Furthermore, invertebrate immunocytes contain both  $\delta 1$  and  $\delta 2$  opioid receptors, which also occur on human granulocytes (6), supporting their presence and significance in immune signaling in invertebrates.

In recent years hemorphin structures have been identified as naturally

occurring peptides in brain, plasma, and cerebrospinal fluid (18). In vitro release of these peptides revealed that the major lysosomal enzyme cathepsin D constitutes a good candidate for the in vivo release of two hemorphins: LVV-hemorphin-7 and VV-hemorphin-7 (31). Moreover, Hemorphin generation by mice peritoneal macrophages has been recently reported. Because lysosomes are believed to be the main site of degradation in the endocytic pathway, a study on their potential implication in the generation of hemorphins from Hemoglobin was recently investigated (32). When this protein is submitted to purified rat liver lysosomes, an early generation of hemorphin-7-related peptides, detected by a radioimmunoassay, was observed. These peptides seemed to be

relatively stable during the first hours of hydrolysis. This confirms the fact that immune cells are able to generate these molecules, which can be released in the circulation during phagocytosis.

Thus involvement of these peptides in immune response seemed to be interesting. In fact, sensory nerves mediate peripheral inflammation via the release of sensory peptides at the site of tissue injury. Using a blister model of inflammation, Sanderson et al. (33) have previously documented that endogenous opioids modulate chronic but not acute inflammation. By contrast, hemorphins might play a role in inhibiting the inflammatory response in acute, but not in recurrent or chronic injury conditions via a pre-terminal action on opioid receptors (33, 34). This reflects that different endogenous inhibitory mechanisms may operate under different injury conditions; endogenous hemorphins and opioid peptides may modulate acute and chronic inflammation, respectively.

In invertebrates, displacement studies of endomorphins or MIF-1 for  $^3\text{DHM}$  binding to  $\mu_3$  specific morphine receptor present in immunocytes revealed a lack of affinity. Amperometric determination of nitric oxide release (NO) in real-time, demonstrated that morphine stimulates in leeches NO release, supporting earlier observations (35) whereas endomorphins or PLGamide does not (19,36), suggesting that these peptides are really near from opioids family than the opiates ones. At the contrary, LORF heptapeptide is able to inhibit

immunocytes activation through NO release like morphine does (22).

Interestingly, antifungal and antibacterial activities were detected in the hemolymph and gut contents of the cattle tick, *Boophilus microplus* (37). This peptide is identical to a fragment of the bovine alpha-hemoglobin. A synthetic peptide based on the sequence obtained showed characterization data identical to those of the isolated material, confirming its structure. The synthetic peptide was active in micromolar concentrations against Gram-positive bacteria and fungi. These data suggest that this antibacterial activity detected in tick gut contents is the result of enzymatic processing of a host protein, hemoglobin. Ticks may use this activity as a defense against microorganisms (37). In clear this reflects that hemoglobin during inflammation can process like a neuropeptide precursor in antibacterial peptide and non-classical opioid peptides. We recently demonstrate the same things with other oxygen binding proteins e.g. hemerythin in Annelids (25) and in mollusks for hemocyanin (Bachere et al., personal communication). These molecules are processed to generate antibacterial, antifungal peptides and bioactive peptides that modulate the inflammation (37, 38).

These results have to be linked to the presence of antibacterial peptides on proenkephalin A precursor i.e. synenkephalin, enkelytin, peptide B (8,38-42). Indeed, these antibacterial peptides, with their high antibacterial activities further associate opioid pep-

tides with immune related activities. We surmise, that immune signaling may lead to enhanced proenkephalin proteolytic processing freeing both opioid peptides and antibacterial peptides (8,38-42). In this scenario the opioid peptides would stimulate immunocyte chemotaxis and phagocytosis as well as the secretion of classical cytokines. During this process the simultaneously liberated proenkephalin fragment having antibacterial activities would attack bacteria immediately, allowing time for the immune stimulating capabilities of opioid peptides to manifest itself. This hypothesis is supported by the presence of specific Met-enkephalin receptors. Moreover, antibacterial peptides inactivation release in both invertebrates and human, new powerful immune stimulatory factors i.e. Met-Enk Arg Phe and Met-Enk (38, 43).

Taken together, these demonstrate that classical opioids precursors like the non-classical ones are immune modulators of inflammation.

## VASCULAR ACTIONS

Stefano and colleagues (44) demonstrate that the confinement of opiate receptors to the nervous and immune systems has been broadened to several other cell types. In fact, endothelial cells may represent a target for the opiate substances. Endothelial cells (human arterial and rat microvascular) contain a high-affinity, saturable opiate binding site presumed to mediate the morphine effects, that is stereo selectively and characteristically antago-

nized by naloxone (44). Experiments with endothelial tissue and aortic ring of rats cultured in vitro demonstrate that morphine exerts direct modulatory control over the activities of endothelial cells, which leads to vasodilatation. It induces the production of nitric oxide, a process that is sensitive to naloxone antagonism and nitric oxide synthase inhibition (44). In contrast with that of opiates, the administration of opioid peptides does not induce nitric oxide production by endothelial cells. In other reports, they demonstrate that this induced endothelial down-regulation also manifests itself in lowering immunocyte activity, i.e., adhesive actions see (6,45), demonstrating a dynamic communication of immune cells with the endothelium. In this scenario, we can now include the non-classical opioid peptides family. In fact, we demonstrate that LORF is coupled to nitric oxide (NO) release in human saphenous vein in a manner, which is inhibited by a nitric oxide synthase inhibitor (22). Moreover, like hemorphins or casomorphins (46). LORF inhibits the action of vasopressic enzyme, the angiotensin-converting enzyme (ACE) in intravenous injection conducting to a high hypotension (47).

The decapeptide hemorphin was isolated in high yield (1.5 nmol/ml) from bronchoalveolar lavage (BAL) fluid from a patient with an adenocarcinoma of the lung (48). This peptide, termed LVV-hemorphin-7 represents residues 32-41 of the beta-chain of hemoglobin and has been shown to be an endogenous ligand for opioid recep-

tors. The N-terminal flanking peptide of LVV-hemorphin-7 [residues (1-31) of hemoglobin beta-chain] was also isolated in high yield. Neither peptide was detected in BAL fluid from the tumor-free lung of the same patient or from patients with non-neoplastic inflammatory lung disease. LVV-hemorphin-7 was not identified in BAL fluid from seven additional patients with non-small cell lung cancer, indicating that the formation of the peptide is unlikely to be of any diagnostic significance. However, the ability of LVV-hemorphin-7 to inhibit angiotensin-converting enzyme suggests that its formation may be of pathophysiological significance in the regulation of tumor blood flow in certain patients (48).

Moreover, interestingly, recently it has been demonstrated that Angiotensin IV (Ang IV), the 3-8 fragment of angiotensin II (Ang II), binds to a distinct receptor designated the AT(4) receptor. The peptide elicits a range of vascular and central actions including facilitation of memory retention and retrieval in several learning paradigms. The human neuroblastoma cell line SK-N-MC cells express a high-affinity Ang IV binding site with a pharmacological profile similar to the AT(4) receptor: (125)I]-Ang IV and (125)I]-Nle(1)-Ang IV bind specifically to the SK-N-MC cell membranes ( $K(d) = 0.6$  and  $0.1$  nM) in a saturable manner ( $B(max) = 1.2$  pmol/mg of protein). AT(4) receptor ligands, Nle(1)-Ang IV, Ang IV and LVV-haemorphin 7 (LVV-H7), compete for the binding of [(125)I]-Ang IV or

[(125)I]-Nle(1)-Ang IV to the SK-N-MC cell membranes with rank order potencies of Nle(1)-Ang IV > Ang IV > LVV-H7 with IC(50) values of 1.4, 8.7 and 59 nM ([[(125)I]-Ang IV) and 1.8, 20 and 168 nM ([[(125)I]-Nle(1)-Ang IV), respectively. The binding of [(125)I]-Ang IV or [(125)I]-Nle(1)-Ang IV to SK-N-MC cell membranes was not affected by the presence of GTP gamma S. Both Ang IV and hemorphin stimulated DNA synthesis in this cell line up to 72 and 81% above control levels, respectively. The AT(4) receptor in the SK-N-MC cells is a 180-kDa glycoprotein; under non-reducing conditions a 250-kDa band was also observed (49).

Finally, several studies in cardiology have shown that hemorphins appeared to be released when patients are subjected to open-heart surgery using a heart-lung machine. In these conditions, immunocytes can be lysed by mechanical contact. In these circumstances, the level of hemorphin-7 increased in patient plasma (18). These data have to be reliable with the increase levels of  $\beta$ -endorphin, enkephalins (43). This allow to hypothesis that endocrine or paracrine pathways linked non-classical opioids peptides to opioid ones through brain and immune systems activation.

## CONCLUSION

There is a growing interest on studies that based on detailed analysis of the immunity mechanisms in invertebrates that are directly used in verte-

brates. Two examples reflect such remark. The first one is the panoply of anti-bacterial peptides found in invertebrates that constitutes the major element of the innate immunity (50). Some similar peptides are present in vertebrates and in invertebrates and also in plant e.g. the defensin. These molecules seem to be well adapted to offer a first defense line against pathogens. The second one is illustrated by the recent work of Medzhitov (51). This team has demonstrated in human the presence of a Toll receptor, initially discovered in Insects (52). Thus, the discovering of such receptor implicated in the initiation of the innate response reflects the usefulness of simple models. Such results have suggested that the innate response is conserved in course of evolution and this type of immunity would allow combating pathogens during take place of the specific immune response. Nevertheless, this will occur if different messengers are produced by nervous and/or immune systems informing of the presence of pathogens on the organisms or modulator factors of the inflammatory immune response. Such chemical signaling molecules are opioids and now we can add non-classical opioids peptides playing in such bi-directional information exchanges, so called neuroimmunity.

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# Redefinition of fundamental postulate of classical endocrinology

**M Salzet**

Laboratoire de Neuroimmunologie des Annélides, Université des Sciences et Technologies de Lille, France

Correspondence: Laboratoire de Neuroimmunologie des Annélides, UMR CNRS 8017, SN3, IFR 17 INSERM, Université des Sciences et Technologies de Lille, 59655 Villeneuve d'Ascq, France. Tel : +33 3 2043 6839, Fax : +33 320041130. E-mail:michel.salzet@univ-lille1.fr

## ABSTRACT

**Background:** Since the last century, Claude Bernard supposed that not only endocrine glands but many organs in the organism have the ability for "internal secretion". Moreover, evidences that identical biogenic amines and peptides hormones were found in neurones and in amine precursor and uptake decarboxylation cells located in different organs firstly confirmed his visions of the endocrinology. The existence of a diffuse neuroendocrine system (DNES) concept was born. This discovery was completed since this last decade by the evidence of a cross-talk between nervous and endocrine system involved in the stability of the organism. The psychoneuroimmunity field knocked to the door of many endocrinologists as well as of immunologists.

**Method and Investigations:** The data and the

speculation for the present report was obtained from our results and scientific literature.

**Results:** Evidence are now given that immune expressed endocrine markers like neuropeptides, biogenic amine, neuropeptide processing enzymes, regulated secretion pathway. In clear, immune cells expressed like the brain an endocrine phenotype during pathogen attacks.

**Conclusions:** It is now time to suggest some revisions of the fundamental postulate of classical endocrinology. One of the new definitions is now to consider that we can speak about endocrine phenotype not only in the brain, the DNES but also in the immune system. This phenotype must be under the control of master genes which are over expressed during insult, pathogenesis or aging. These genes have now to be identified.

**Key Words:** Placebo, Endocrine definition, Neuroimmunity, Hormones, Immunocytes, Processing enzymes

One of the major foresights of the last century was Claude Bernard (1855). He was the first to suppose that not only endocrine glands but many organs in the organism have the abili-

ty for "internal secretion" (1). The evidences that identical biogenic amines and peptides hormones are found in neurones and in amine precursor and uptake decarboxylation (APUD) cells

located in different organs firstly confirmed his visions of the endocrinology (2). The existence of a diffuse neuroendocrine system (DNES) concept was born (2). Recently, weigent and Blalock demonstrate that communication and reciprocal regulation between the nervous, endocrine and immune systems are essential for the stability of the organism, these three systems use the same signalling molecules. (3) Among others, cytokines, hormones and neuropeptides have been identified as messengers mediating the communication between the three systems (4, 5). Moreover, in the last few years various animal models have served to study neuroimmune mechanisms confirming the view of communication between the neuroendocrine and immune systems via neuropeptide signalling and through specific receptors (6-8). These include neuropeptides such as corticotrophin releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), monoamines (epinephrine, norepinephrine and dopamine), glucocorticoids, free radicals, cytokines such as interleukin (IL)-1, IL-6 and Tumor Necrosis Factor (TNF), opioid peptides, opiates and endocannabinoids (4-8).

Another emerging function of neuropeptides within the immune system is their direct roles in defence. Peptides with antibacterial properties have been shown to be derived from neuropeptide precursors such as proenkephalin and chromogranin B (8-14). The role of neuropeptide precursors in immunity, through the release of antibacter-

ial peptides, is an entirely novel concept. The biosynthetic pathway that leads to the production of biologically active neuropeptides begins with the synthesis of large inactive precursor proteins which are cleaved at specific paired or single basic residues within the Golgi secretory pathway (15). It is a family of subtilase-like pro-protein convertases (SPCs) (15) that is largely responsible for these processing events that activate precursor proteins into neuropeptides. The SPCs have been extensively studied in both neural and endocrine systems. However, much less is known concerning their expression, regulation and role within the immune system at the basal level (15, 16) or their function during microbial challenge (4). We recently demonstrate that SPC functions are important since differential expression of SPCs and the resulting cleavage patterns determine the nature and biological activity of the peptide products. Thus, depending on the pattern of SPC expression, a single protein precursor can give rise to different peptides with diverse biological activities like antimicrobial substances (secretolytin, enkelytin/peptide B, dermaseptins (9-13, 17) or chemoattractant factors (Methionine-enkephalin) (7, 8). Moreover, processing of "true" proantibacterial peptide precursors like prodefensins by neuropeptide processing enzymes is also novel (18-22). Alpha-defensins are a class of defensins expressed in human and other mammalian neutrophils and Paneth cells of the small intestine. They are synthesized as 90-100 amino

acid preprodefensins, with a 19 amino acid signal sequence, a ~45 amino acid anionic propeptide and a 29-40 amino acid C-terminal mature cationic defensin (18). The removal of the anionic propeptide is an activation step that converts the inert prodefensin to antimicrobial mature defensin (20-22). Serine protease, aspartyl-protease, metalloproteinase are the enzyme(s) yet known implicated in the process of prodefensins to defensins in neutrophil (18-22). The processing of neutrophil defensins bears resemblance to that of various peptide hormones (1-3). Therefore, enzymatic processing, including differential processing events, is a key mechanism to generate an antimicrobial defence in tissues like neuropeptides in the neuroendocrine system.

Evidences are also given that at least four different types of granules are formed during maturation of neutrophil granulocytes in the bone marrow (23). Functional properties of release from the different granule populations differ in several respects from characteristics of neurotransmitter release; the best understood secretory process in mammals. To gain direct access to the secretory machinery and study the regulation, mechanisms, and effectors of  $\text{Ca}^{2+}$ -dependent neutrophil secretion, method of plasma membrane permeabilization using streptolysin O was developed by Ligeti and collaborators (23). The authors confirmed previous studies that permeabilized neutrophils secrete in response to calcium alone, but as a

biphasic  $\text{Ca}^{2+}$  dose-response. Secretion is detectable at  $<1.0 \mu\text{M}$   $\text{Ca}^{2+}$  and reaches a plateau between 1.0 and 60 to 80  $\mu\text{M}$ . When stimulated with  $>80 \mu\text{M}$   $\text{Ca}^{2+}$ , secretion is two- to threefold greater than at lower  $[\text{Ca}^{2+}]$ , suggesting that two distinct mechanisms of  $\text{Ca}^{2+}$ -dependent secretion that differ in their affinity for  $\text{Ca}^{2+}$  exist in neutrophils. Although permeabilization allows 100% leak of lactate dehydrogenase, maximum secretion from permeabilized cells is 80% that of f-met-leu-phe-stimulated intact cells, indicating that the essential components of the  $\text{Ca}^{2+}$ -dependent secretory apparatus are predominantly, if not entirely, membrane bound. Permeabilization causes leakage of 100% of annexins V and VI, but 41% of annexin I and 12% of annexin III are retained. Immunofluorescence microscopy revealed that retained annexins I and III are associated with granule membranes. Addition of soluble annexins I and III to permeabilized cells increased  $\text{Ca}^{2+}$ -induced secretion up to 15% and 90%, respectively, implying that both annexins participate in this secretory pathway (23). Recent data support the concept that the (lysosomal type) azurophil granules, but not the specific granules, are secreted through the endosomal pathway (24). However, in a human disease (Chediak-Higashi syndrome), the defect of the secretory pathway affects mainly the cells of the haemopoietic lineage; Taken together, these data suggest that regulated exocytosis from neutrophil granulocytes (or perhaps also from

other haemopoietic cells) may represent a specific case of the general mechanism of secretion.

Considering all the above data, the aim of this letter is to now suggest some revisions of the fundamental postulate of classical endocrinology. One of the new definition is now to consider that we can spoken about endocrine phenotype not only in the brain, the DNES but also in the immune system. This phenotype must be under the control of master genes which are over expressed during insult, pathogenesis or aging. These genes have now to be identified but one of the major must be the one which is implicated in granule secretion formations.

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