LETTER

Opiate alkaloids and peptides in animals

Peter G. Fedor-Freybergh [1] and Richard M. Kream [2]

[1] Institute of Prenatal and Perinatal Psychology and Medicine, St. Elizabeth's University, Bratislava, Slovka Republic. [2] Neuroscience Research Institute, State University of New York Old Westbury, Old Westbury, NY 11568, USA.

Correspondence to: rmkream@sunynri.org

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Dr. Stefano has been awarded Dr. Honoris Causa from St. Elizabeth's University in Bratislava, Slovak Republic on October 16th (Photo) and on October 17th delivered an invited lecture at the Slovak Academy of Sciences, Institute of Normal and Pathological Physiology on Morphine Synthesis in Animal Tissues. On October 22, 2008 Dr. Stefano received the Gold Medal for Science at the First Medical Faculty, Division of Psychiatry of Charles University in Prague, Czech Republic. His invited lecture was Endogenous Morphine in Human Tissues.

Dr. Stefano's 30 year research career has been distinguished by its exceptional and unwavering dedication to the elucidation of opioid and opiate signaling mechanisms that extend throughout invertebrate and vertebrate/mammalian phyla. Throughout the 1980s, the Stefano laboratory demonstrated the expression of functional opioid receptors by invertebrate nervous tissues displaying many similarities to their mammalian counterparts. This work was complemented by the demonstration of related families of opioid peptides within these same tissues(Stefano *et al.*, 1993). In effect, the presence of an "endogenous" opioid system within invertebrate nervous tissues strongly indicated multiple regulatory roles and a biological continuum of opioid action spanning millions of years of positive evolutionary pressure.

Despite the important work completed during the 1980s, a serendipitous key branch point in Dr. Stefano's career arose in the early 1990's with the biochemical description of a novel μ opiate receptor, μ_3, expressed by different animal tissues such as human vascular endothelial cells, leukocytes and neural tissues (Cadet *et al.*, 2003). The μ_3 receptor's novel activation profile includes morphine and morphine-related morphinan alkaloids, and strictly excludes opioid peptides and synthetic opiates such as the phenylpiperdine analog fentanyl. Furthermore, the μ_3 receptor was found to be functionally coupled to constitutive nitric oxide synthase (cNOS) activation, supporting its essential role within a functionally distinct "morphinergic" signaling pathway existing in parallel to previously described endogenous opioid peptide systems.

The μ_3 receptor has been cloned and functionally expressed in mammalian cell lines. Importantly, expression cloning has preserved the novel coupling of μ_3 receptors to cNOS. Because it appeared that μ_3 receptors were strictly activated by morphine and morphine-related alkaloids, and selectively coupled to NO production and release, the implications of these observations were profound and stimulated the development of new hypotheses and research strategies. In effect, the golden age of discovery dedicated to research in endogenous opioid peptides and their cognate receptors was now overshadowed by a disturbing awakening to the possibility that the primordial opiate signaling molecule in animal systems may be morphine itself, chemically identical to the benzylisoquinoline alkaloid synthesized by the opium poppy.

The Stefano laboratory subsequently demonstrated that mammalian and invertebrate systems including human white blood cells (WBC) are capable of carrying out *de novo* synthesis of chemically authentic morphine from a related set of small precursor molecules derived from the aromatic amino acid L-tyrosine. Endogenous morphine production by animal cells had been previously believed to be a byproduct of random, unregulated, cellular events initially involving non-enzymatic condensation of dopamine (DA) and monoamine oxidase (MAO)-derived aldehyde metabolites of DA. Work from the Stefano laboratory critically dispelled this misconception pertaining to morphine expression by mammalian cells. Regulated production of biologically meaningful low concentrations of endogenous morphine is required for cellular metabolic stability mediated by activation of cognate μ_3 receptors coupled to cNOS activation. Accordingly, the discovery of a functionally linked "morphinergic", μ_3 receptor/NO coupled regulatory pathway indicates that positive evolutionary pressure has preserved a primordial, phylogenic broad, biochemical mechanism, including in human stem cells (Cadet *et al.*, 2007) by which the prototype opiate alkaloid morphine is expressed and utilized as a physiological regulator of relatively simple as well as significantly more complex cellular functions (Stefano & Scharrer, 1994). Highlighting the importance of endogenous morphine are recent studies from the Stefano laboratory, demonstrating that addictive properties of nicotine, alcohol and cocaine may arise from their ability to enhance endogenous morphine levels and its neuronal release, opening up a new level of understanding in substance abuse induced addiction and behavioral effects, as well as morphine regulation (Cadet, Mantione, Zhu, Kream, Sheehan, Stefano, 2007; Zhu *et al.*, 2006).

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