Reciprocal Evolution of Opiate Science from Medical and Cultural Perspectives

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Study Design A
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Over the course of human history, it has been common to use plants for medicinal purposes, such as for providing relief from particular maladies and self-medication. Opium represents one longstanding remedy that has been used to address a range of medical conditions, alleviating discomfort often in ways that have proven pleasurable. Opium is a combination of compounds obtained from the mature fruit of opium poppy, papaver somniferum. Morphine and its biosynthetic precursors thebaine and codeine constitute the main bioactive opiate alkaloids contained in opium. Opium usage in ancient cultures is well documented, as is its major extract morphine. The presence of endogenous opiate alkaloids and opioid peptides in animals owe their discovery to their consistent actions at particular concentrations via stereo select receptors. *In vitro* expression of morphine within a microbiological industrial setting underscores the role it plays as a multi-purpose pharmacological agent, as well as reinforcing why it can also lead to long-term social dependence. Furthermore, it clearly establishes a reciprocal effect of human intelligence on modifying evolutionary processes in papaver somniferum and related plant species.

MeSH Keywords: **Receptors, Opioid – History • Opium – History • Papaver – Drug Effects • Morphine – History • Morphine – Therapeutic Use • Opium – Therapeutic Use • Receptors, Opioid – Therapeutic Use**

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Opium Usage in Ancient Cultures

Opium represents the opiate-containing dried latex obtained from the mature fruit of opium poppy, papaver somniferum. Morphine and its biosynthetic precursors thebaine and codeine are the major bioactive opiate alkaloids contained in opium and its alcohol extract laudanum. Opium usage in ancient societies is well documented. For instance, the Sumerian clay tablet (about 2100 BC) is considered to be one of the oldest recorded lists of medical prescriptions in the ancient world and appears to reference opium use [1]. Interestingly, artifactual remains related to opium usage by humans were found in an Egyptian tomb from the 15th century BC [1]. The Ebers Papyrus from 1552 BC describes a blend of substances, including opium, which was used to sedate children, and it was noted that this was how the goddess Isis would sedate her son Horus [2]. Additionally, some researchers believe opium is referenced in the Bible as *rôsh* [2].

Hippocrates, the Father of Western Medicine, and Asclepiad priests apparently prescribed "meconium" (probably poppy juice) as a purgative, a narcotic and as a cure for leucorrhea [2]. The first recorded reference to utilizing the juice of the poppy as a medicinal agent was made by Theophrastus, a Greek scholar and philosopher in the third century BC [1], followed by first century references to opium and the opium poppy by Dioscorides, Pliny, the Roman encyclopaedist, Aulus Cornelius Celsus, and in the second century AD by the renowned Roman physician Galen [1,3]. It is believed that Galen popularized opium use in Rome, while also making note of its potential to be abused [4]. Interestingly, the image of an opium poppy was found on a coin issued during the last years of the Roman Empire [3].

From a more contemporary perspective the mixing of multiple substances for the treatment of ailments, many herbal preparations contained opium. Here, the ultimate goal was to develop a panacea for all diseases. A famous and expensive panacea was theriaca, containing up to sixty drugs, including opium [1]. During the 8th century, traders from the Near East brought opium to India and China, and between the 10th and 13th centuries, it was introduced to Europe. The Persian scholar, Avicenna (circa 1000 AD), is known to have recommended opium use for treatment of diarrhea and diseases of the eye [1,3]. In China, it is believed that people used this compound to control diarrhea for over a 1000 years [2].

The first uses of opium in combination with other plant-derived compounds for anesthetic purposes occurred in the 1st century AD, when Dioscorides recommended patients take mandrake (also containing scopolamine and atropine), mixed with wine, before limb amputation [1]. Celsus, in his *De Medicina*, suggested the use of opium before surgery [5]. Yet, its use as an agent for postoperative pain relief can only be found in records dating from the late 18th century onwards [6].

Opium Usage for Infant Care

For three millennia, opium has been employed for the treatment of ailments in infants, notably to diminish pain, excessive crying, and typically for treatment of diarrhea [7]. Following the recommendations of Galen and Avicenna, administration of opium for sedation of babies made its way into early medical treatises and pediatric instructions [7]. Interestingly, one route of opium administration entailed the dabbing of maternal nipples with bitter opiate-containing preparations that operationally hastened weaning [7]. Furthermore, in the 17th century, opium extracts were used for addressing discomfort from teething in foundling hospitals and by wet nurses [3].

During the industrial revolution, usage of opium within families was common among the working classes [7]. In German-speaking countries and regions, poppy extracts were administered to infants in soups and pacifiers. Opium formulations were sold as *soothers, nostrums, anodynes, cordials, preservatives*, and *specifics* at the doorstep or in shops [7]. Despite the observed toxicity of opium in infants, physicians continued to prescribe it for this particularly susceptible population [8].

Ingestion of Opium as an Oral Preparation

Ingestion of opium as an oral preparation has undergone many modifications over the years. Opium-based edibles typically consisted of a pasty mass composed of powdered opium that was mixed with syrup, honey, or other sweeteners. Thomas Dover (1660–1742), an English physician, developed a powdered form of edible opium that also was known to contain salt peter, tartar, licorice and ipecacuanha [9]. An ethanol tincture of opium, called laudanum, was created by the 17th century English physician, Thomas Sydenham, and was commonly paired with whiskey or rum [10]. Sydenham's recipe, "given his focus on dysentery in 1669, contained 1 pound sherry wine, 2 ounces opium, 1 ounce saffron, 1 ounce powder of cinnamon, and 1 ounce powder of cloves" [10]. As a means for preparing patients for surgery, Laudanum became widely used in Europe and North America into early 20th century [11].

Moreover, one of the first books dedicated to the uses and effects of opium, *Mysteries of Opium Reveal'd*, written by the Welsh physician, John Jones, was published in 1701 [12]. In particular, the book noted that adverse effects occurred with excessive use of opium and mixing it with its latent preparation residues [2]. Jones listed numerous adverse effects that include "gaity [sic] of humor at first", "alienation of the mind",

"loss of memory", "cold sweats" and "vomiting", as well as "death", and notes that many of these non-lethal outcomes are similar to "drinking a great quantity of wine in a short time" [12]. He also observed that "these effects do not happen to all [...] these effects are greater, or less, depending on the dose, constitution of the person, and other circumstances" [12]. His novel insights are even more important today regarding designer drugs and opiate abuse "cutting" designed to stretch amounts.

Opium Usage as a Surgical Analgesic/ Anesthetic

Before the development of general anesthesia, surgery was performed only in extreme necessity. It is probable that an analgesic such as opium would have been given during or following surgery; although, records of this type of usage in ancient times have proven elusive [6]. In this regard, in the 13th century, the monk and physician, Theodoric, described *spongia somnifera* as a mixture of several narcotic substances containing mandrake, henbane, mulberry, lettuce, and hemlock. According to Bergman (1998), the ingredients were boiled within a sponge that was then sniffed by the subject prior to surgery as an early form of anesthesia/analgesia. The Swiss-German physician, Paracelsus (1493–1541), referred to this drug as the "immortality stone" [2].

The first description of opium usage for postoperative analgesia is dated to 1784, when the Glasgow-born London surgeon, James Moore, stated: "Opium…is highly expedient to abate the smarting of the wound after the operation is over, and to induce sleep, but the strongest dose we dare venture to give has little or no effect in mitigating the suffering of the patient during the operation" (Moore 1784, Hamilton and Baskett 2000). The Scottish surgeon, Benjamin Bell, also noted: "In general they prove most useful when given immediately after, when they very commonly alleviate that pungent soreness of which patients at this time usually complain; and by continuing to give them in adequate doses from time to time, we are often enabled to keep the patient easy and comfortable" [13].

The Primacy of Morphine as the Main Bioactive Component of Opium

In 1805, Friedrich Wilhelm Adam Sertürner (1783–1841) obtained partially purified preparations of morphine from crude opium extracts, thereby initiating the ordered study of natural compounds as an acknowledged branch of pharmaceutical science [14]. Sertürner was the first to claim that the substance termed "mekonsaure", or "mohnsaure", was a sleep-inducing agent [6]. The biologically active purified substance first called

"principium somniferum" was later renamed "morphium" after the Greek god of dreams, Morpheus. Sertürner confirmed the hypnotic and analgesic properties of "morphium" and also acknowledged its potential negative side effects [14–16]. Subsequently, circa 1820, chemically pure morphine was manufactured from opium extracts and became commercially available in Europe and North America [2]. In 1831, William Gregory, a physician and chemist in Edinburgh, discovered a cheap method of isolating and purifying morphine salts for widespread pharmaceutical usage [17,18].

In 1836, the first subcutaneous administration of morphine was described by Lafargue, a French physician [6]. His "inoculation" method placed morphine under the epidermis via lancet [6]. In 1845, the Irish physician, Francis Rynd, was the first to administer subcutaneous liquid morphine [19]. In 1863, Sir James Paget employed subcutaneous morphine administration for postoperative pain relief. Following amputation of a leg, Paget administered 20 mg morphine to the patient by subcutaneous while under chloroform anesthesia [20]. During the 19th century, the American Civil War, Crimean and Prussian Wars fuelled the widespread usage of oral and subcutaneous morphine for soldiers wounded in combat [21]. Consequently, associated medical records documented the development of physical dependence in severely wounded surviving soldiers chronically treated with morphine [21]. As such, large segments of the population became familiar with the potentially debilitating effects of chronic morphine usage.

The development of the modern syringe fitted with a hypodermic needle engendered the widespread use of morphine as a parenterally administered pharmaceutical agent [6]. Nonetheless, intravenous injection of opium preceded subcutaneous administration of morphine by almost two centuries. Sir Christopher Wren, the 17th century English architect who designed St. Paul's Cathedral, was the first to inject opium into a vein. Wren, along with chemist Robert Boyle (1627–1659), injected opium into a dog, resulting in the animal being stupefied but remaining alive [6].

In addition to oral and injectable methods, rectal administration of morphine was investigated by Shoemaker in the late 19th century. In A Practical Treatise on Materia Medica and Therapeutics, he states: "Opium, morphine, and codeine may also be introduced into the rectum in the form of suppositories, in painful conditions of the bowel and neighboring organs, and also, for their general effects, in producing sleep and quieting cough or restlessness" [22]. In 1886, Rudolph Matas, a vascular surgeon working in New Orleans, injected morphine directly into the spinal cord, thereby presaging the specialty of regional analgesia. Matas' preparation contained both cocaine and morphine in a saline solution and was intended to provide sedation [6]. In 1979, Behar and colleagues injected

morphine into the epidural space of the spinal cord for pain relief, initiating a widely employed clinical procedure for management of postoperative pain [23]. Finally, in 1991 Stein and colleagues observed that intra-articular administration of morphine provided postoperative analgesia following arthroscopic knee surgery, an effect mediated by inhibition of peripheral nociceptive systems [24].

Discovery of Opiate Receptors, Opiate-Dependent Signaling Pathways, and Endogenous Morphine

Prior to the advent of biochemical and molecular science, the stereotypic biological effects of administered morphine suggested that specific mechanisms of action existed. In 1858, a London surgeon, Charles Hunter, published a report indicating that an injection of morphine at a site remote from the painful area produced equivalent pain relief to that achieved by a proximal injection. Hunter concluded: "The idea that the relief results from localization of the remedy in the painful part is erroneous – equal relief being afforded in either case" [6]. Accordingly, a systemic functional basis for a unified mechanism of action was outlined by Hunter's work.

In 1977, Snyder and Pert provided compelling empirical evidence supporting the hypothesis that opioids exert their actions through selective CNS membrane proteins, designated opioid receptors, enriched in the limbic system, periaqueductal gray area of the midbrain and brainstem, and spinal cord, in the substantia gelatinosa of the spinal cord [25–28].

The seminal discovery of multiple classes of opioid receptors including the Mu (μ), Delta (δ) and Kappa (κ) [27,29] and nociceptin/orphanin FQ (NOR) subtypes [30] subsequently facilitated the elucidation and characterization of their cognate, endogenously-expressed, opioid ligands. They included enkephalins [25,31–33], beta-endorphin [34–36], dynorphin [37,38], nociceptin/orphanin FQ [30] and endomorphins [39,40], which due to their peptidic nature were called opioids. Moreover, invertebrate nervous tissues were found to express multiple subtypes of opioid receptors in addition to their cognate peptidergic ligands, thereby demonstrating evolutionary conservation of fundamental opioid mechanisms [41–45].

In contrast, chemically authentic morphine has also been identified and extensively characterized as an endogenous chemical messenger at CNS and peripheral sites [46–49]. Indeed, Mavrojannis speculated in 1903 that endogenous morphine was present based on the mimicry of pharmacological actions that was produced by plant-derived morphine [50]. During the 1970s, multiple reports were published that hypothesized the presence of endogenous morphine in mammalian tissues

(see [51–53]. Although, some reports were skewed with the issue of contamination or a dietary origin of morphine's presence in tissues. Identification of the four endogenous opiates from bovine brain and adrenal was performed in 1985 by Goldstein and collaborators [54]. Yet, at this time, contamination via glassware was an issue. The possibility of endogenous production of morphine was confirmed by its detection using high-density polypropylene tubes *in vivo* in invertebrates that were maintained for at least two weeks under sterile and fasting conditions [55,56].

In more recent decades, morphine precursors and intermediate reactions in animal tissues were identified [45,55–68]. These biochemical findings supported an earlier hypothesis forwarded by ethanol abuse researchers that biosynthesis of chemical authentic morphine in mammals is highly similar to the enzymatic pathway previously described in opium poppy [69]. Confirmation of morphine synthesis *in vivo* in animals was initially suggested by the discovery of endogenous morphine and codeine in mouse urine following ingestion of ethanol and a report using an *in vivo* model in precursor treatments to enhance endogenous morphine levels see [55,56].

In our group, the discovery of the μ3 opiate receptor, which was opioid peptide insensitive and opiate alkaloid selective, constituted additional validation for morphine as an endogenous substance [44]. This receptor has been cloned and found to be a novel 6 trans-membrane domain GPCR with signal coupling to nitric oxide synthase [70–73]. Subsequently, an attempt to provide validating proof that biosynthesis of morphine in mammals occurs by *de novo* mechanisms utilizing dopamine and L-DOPA as essential precursors was presented by Zenk and colleagues [74,75] and Stefano and colleagues [55,56,68]. The empirical work by Zenk and colleagues employed cultures of a human neuroblastoma cell line that were exposed to isotopically labelled forms of dopamine and L-DOPA linked to isolation of isotopically enriched intermediate precursors, including tetrahydropapaveroline, reticuline, and salutaridine [74–76]. Despite its widespread dissemination throughout the neurobiological community, the overall biological impact of the work was diminished by the very low recovery rate of isotopically labelled intermediate precursors, and by the phenomenon of phenotypic revision observed in neuroblastoma cell lines.

Work from our laboratory has provided compelling *in vitro* and *in vivo* evidence supporting the biological expression of endogenous morphine by mammalian cells [66,68,72,73,77–79]. Experiments included the administration of early and intermediate morphine precursors including dopamine, L-DOPA, tetrahydropapaveroline, reticuline, and salutaridine to primary cultures of human leukocytes and *in vivo* administration of these same compounds to nervous tissues of the marine mollusk μ.

edulis. In all instances, levels of newly synthesized morphine were significantly enhanced, thereby providing conclusive evidence of the biological importance of endogenous morphine. Concurrently, our laboratory has provided key biochemical and molecular studies of the unique 6-transmembrane domain cognate GPCR, the µ3 opiate receptor, that recognizes and transduces the physiological effects of endogenous morphine within a nitric oxide-linked signaling pathway, including mitochondrial modulation [44,73,80–84].

Conclusions

Opiate usage has a longstanding history in cultures across the world. Indeed, it can be viewed as a body of knowledge that has developed over centuries, tried and tested in terms of its range of medicinal uses in various temporal and sociocultural contexts. Significantly, however, empirical research examining the developmental and regulatory roles of endogenously expressed, and chemical authentic, morphine in the course of biological evolution of lower and higher animal phyla is still in its infancy. Recent work from our laboratory has helped to establish a working hypothesis with respect to morphine and its cognate µ3 receptor playing a key regulatory role in intermediate metabolic processes via their ability to modulate

mitochondrial respiratory complexes through nitric oxide recruitment [81,83].

Over the last two decades, plant biochemists and molecular biologists have successfully identified, characterized, cloned, and functionally expressed all of the enzyme-encoding genes within the morphine biosynthetic pathway in papaver somniferum. Subsequently, advances in recombinant DNA technology have facilitated stable genetic expression of the complete morphine biosynthetic pathway from papaver somniferum in bacteria [85–88]. Ongoing fine tuning of the recombinant biosynthetic pathway in bacteria will facilitate high yield *in vitro* production of pharmaceutical grade morphine, thebaine, and codeine, thereby alleviating major sociological concerns relating to the illicit drug trade emanating from foreign poppy fields. As an important corollary, the large scale production of morphine and its pharmacologically valuable congeners, i.e., thebaine and codeine, within a microbiological industrial setting reinforces the long-term clinical commitment and dependence on opiate therapy for post-operative and chronic pain management. From a speculative teleological perspective, genetic replication of a plant biosynthetic pathway within a eukaryotic host clearly suggests a higher order design of human intelligence to selectively engineer evolutionary progression of papaver somniferum genes for widespread medical purposes.

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