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Methadone and its usage in the treatment of opioid drug addiction

Metadon a jeho využití při léčbě pacientů závislých na opioidech

BAKALÁŘSKÁ PRÁCE

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze dne 15. 5. 2017 **Kembe Chona**

Poděkování

Tímto bych velmi rád poděkoval především panu docent Petru Svobodovi, vedoucímu mé práce, za jeho trpělivý a i přes mé prodlevy velmi ochotný přístup. Dále bych též rád poděkoval panu doktoru Petru Popovovi, primáři pražské kliniky adiktologie, za jeho čas a velmi obahacující rozhovor, jež mi nastkytl zajímavý náhled na problematiku metadonové terapie z klinického prostředí. V neposlední řadě bych samozřejmě chtěl poděkovat své drahé matce za značnou morální podporu.

Abstrakt

Metadon, syntetický opioid vytvořený ve 40. Letech, je silným agonistou mu opioidních receptorů. Opioidní receptory jsou podskupinou super-rodiny GPCR. Jejich nejdůležitější funkcí je inhibice nervových přenosů pomocí regulace activity iontových kanálů a efektorových proteinů. μ-opioidní receptory jsou hlavním místem působení heroinu, metadonu a jiných klasických opioidních agonistů. Díky distribuci opioidních receptorů v centrální nervové soustavě a periferních tkáních je metadon schopen působit na širokou škálu funkcí v organismu. Metadon vyvolává mnoho s běžných účinků opioidů jako jsou analgeze, seadace, deprese dýchacího centra, euforie aj. I přes původní záměr syntézy metadonu jakožto analgetika byly velmi brzy objeveny jeho vlastnosti použitelné pro jiné účely. Výzkum metadonové substituční terapie byl započat v roce 1963 profesorem Vincentem P. Dolem a jeho týmem. Velice rychle se prokázalo, že substituční terapie poskytuje z dlouhodobého hlediska zdaleka největší úspěchy rehabilitace pacientů závislých na opiátech. Díky vysoké biodostupnosti, relativní účinnosti a dlouhému poločasu rozpadu je metadon nejvíce využívanou látkou pro substituční terapii. Metadon podávaný v přiměřených dávkách vyvolává pouze slabé nežádoucí účinky, a zároveň je schopen znovu nastolit fyziologickou rovnováhu rozrušenou užíváním heroinu a potlačit touhu po droze.

Klíčová slova: metadon, opioidní receptory, heroin, substituční terapie

Abstract

Methadone, a synthetic opioid created in the 1940s is a potent mu opioid receptor agonist. Opioid receptors form a sub-group of the GPCR super-family. Their most significant role is the inhibition of neural pathways by regulating the activity of ionic channels and effector proteins. μ-opioid receptors are the site of action of heroin, methadone and other classical opioid agonists. Due to the opioid receptors distribution in both the central nervous system and peripheral tissues, methadone affects a wide variety of functions in the organism. Methadone induces many of the effects of classical opioids including analgesia, respiratory suppression, sedation, euphoria. While originally being developed as an analgesic it had soon shown potential for other therapeutic methods. Methadone maintenance therapy was introduced in 1963, by professor Vincent P. Dole and his team. It quickly became clear that methadone substitution therapy is indeed very effective and shows the highest ability to retain patients. Thanks to its high oral bioavailability, higher intrinsic efficacy and long terminal half-life methadone is the first choice drug for opioid substitution therapy. Methadone, used in appropriate doses produces only mild adverse effects and has the ability to normalize physiological homeostasis disrupted by heroin abuse and slash drug-craving.

Keywords: methadone, opioid receptors, heroin, maintenance therapy

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List of abbreviations

AAG – α 1-acid glycoprotein
AC – Adenylyl cyclase
BA 2/3 – β-arrestin 2/3
BARK2 - β-arrestin kinase 2
cAMP – cyclic Adenosinmonophosphate
CNS – central nervous system
CYP – cytochrome P450
DOP/DOR – δ(delta)-opioid receptor
EDDP - 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EMDP - 2-Ethyl-5-methyl-3,3-diphenylpyrroline
GABA – γ(gamma)-aminobutyric acid
GDP/GTP – gunosindiphosphate/triphosphate
GIRK – G-protein coupled inwardly rectifying potassium channel
GPCR – G-protein coupled receptor
GRK – GPCR kinase
Gα – G-protein alfa subunit
Gβγ – G-protein beta-gamma
IUPHAR – International Union of Basic and Clinical Pharmacolgy
K+ - potassium ion
KOP/KOR – κ(kappa)-opioid receptor
LAAM – levacetylmethadol
MAPK – mitogen activated protein kinase
MMT – methadone maintenance treatment
MOP/MOR - μ(mu)-opioid receptor
NOP/NOR/ORL-1 – nociception receptor/opioid receptor like-1
OR – opioid receptor
PAG – periaqueductal grey
PI3K – phosphatidylinositol-3 kinase
PLC – phospholipase C
TM – transmembrane domain of GPCR
UNODC – United Nations Office on Drugs and Crime VGCC

1 Introduction

Despite the continuous efforts of governments, religious groups and social activists alike, illicit drug abuse remains a major issue in societies worldwide. According to the World Drug Report (2016), the United Nations Office on Drugs and Crime (UNODC) estimates that there are around 29 million people worldwide with drug use disorders, out of which 12 million inject them intravenously, and of whom 14 % are living with HIV. In 2014, there had been an estimated 207,400 drug related deaths. Death due to drug overdose contributes to approximately one third to one half of drug related deaths and can mostly be attributed to opioids. It is very clear that the illicit use of opioids (heroin in particular) can be considered to be the cause of the most severe drug related problems. Heroin, morphine and other related drugs are typically administered intravenously, and therefore carry the highest potential risk of health issues. As opposed to normal population, opioid addicts are the highest risk group for acquiring infectious diseases like HIV, hepatitis and many other. The drug-seeking behaviour also leads them to engage in various criminal activities, such as theft and prostitution. The UCOD states that the number of opiate users has changed very little in recent time. However, for example, in North America there has been a considerable increase in opiate use over the past two decades. As for the Czech Republic, according to the Annual Report on Drug Situation (Mravčík, 2016), there are approximately 12,300-13,200 opioid addicts (3,300-4,700 on heroin and 6,800-7,300 on buprenorphin). The focus of this work will be to make a detailed review of the properties of methadone, a synthetic opioid used clinically as a medicinal substitute for persons striving to rehabilitate from their addiction. This cannot be done without dedicating a great part to the function of opioid receptors. The first part of this work will focus on the incredibly diverse and complex system of opioid receptors and their function in opioid metabolism. The second part will be dedicated to methadone itself and its interaction with the opioid receptors in place.

1.1 Opioids

The pharmacological term opioid as it is recognised today describes a group of chemical substances that share a common ability to bind onto opioid receptors (ORs) and induce morphine-like effects. This term includes the whole variety of drugs of both natural or synthetic origin, as well as the naturally occurring endogenous peptides that serve as ligands of the ORs. The older term used to this day - *opiate* - is used to describe a group of naturally occurring alkaloid drugs that are contained in opium or are its semi-synthetic derivatives. Opium has been used for centuries in many regions of the Asian continent. It is typically obtained from the immature pods of the opium poppy plant *(Papaver somniferum)* by small incisions into the pods which release a thick white latex. The latex is then collected and dried to form what is called *raw opium.* Raw opium is further processed by boiling and filtering to obtain the final product, although the specific methods used can differ. Opium contains a rich spectrum of chemical substances whose concentration can vary significantly depending on the preparation method employed.

However, the most active alkaloid components are typically morphine (around 10-16%) and codeine (around 3%). Other components include thebaine, noscapine or papaverine (Kalant, 1997).

Due to the role of the ORs in the central nervous system, opioids exhibit a wide range of effects, including euphoria, analgesia, sedation and smooth muscle relaxation resulting in respiratory depression and cough suppression. They also have a peripheral effect on the gastrointestinal tract by modulating its endocrine and autonomic functions, or can alter its motility, causing symptoms such as constipation. The isolation of morphine by F. W. A. Sertürner in 1806 marked a beginning of modern pharmacology and led to the study and isolation of practically all opioid compounds used to date.

1.2 Classification of opioids

Opioids can be divided based upon several attributes. As mentioned above, the three basic groups are the natural alkaloid opioids found in plants, endogenous opioid peptides and synthetic opioids. The synthetic opioids can be divided based on their basic chemical structure and the compounds they are derived from. The nomenclature used to classify opioids varies between sources, but generally speaking the most relevant groups are phenanthrenes which contain a distinct 6-hydroxyl group (morphine, codeine, thebaine and naloxone), semi-synthetic morphine derivatives (diacetylmorphine=heroin, oxycodone, oxymorphone, hydromorphone), phenylpiperidines (meperidine), benzomorphans (pentazocine, phenazocine), diphenylheptanes (methadone, propoxyphene) and morphinans (levorphanol) (Pathan, 2012).

Opioids are also classified by the action they induce upon binding onto the ORs. The generally accepted classification divides opioids into three groups: full agonists, partial agonists and antagonists. Agonists possess a very high affinity to the ORs and bind with very high efficacy to and induce the full cascade of the physiological downstream effects (such as the analgesia produced by the administration of morphine, heroin or methadone). The antagonists bind to the ORs without producing any functional response, while also blocking the receptors' availability to the agonists (naloxone). Partial agonists bind to the Ors, producing only a partial physiological response regardless of the amount or saturation of receptors (buprenorphine). Some opioids can also prevent a full agonist from binding to the receptor through the mechanism of competitive action, and therefore circumstantially act as antagonists. These are sometimes classified as mixed partial agonists/antagonists. (Pathan, 2012)

The endogenous peptides also known as endorphins have a very similar, mostly analgesic, effect as morphine. They serve as neuromodulators, neurotransmitters and hormones, and are therefore mostly present in the central nervous system (CNS) and the peripheral secretory glands. They occur naturally in the organism and are formed by post-translational cleavage of their much larger protein precursors. The precursors are categorized as three prohormonal formulas: proenkephalins, prodynorphins,

proopiomelanocortin and pre-pro-nociceptin. With respect to the mentioned precursors the cleavage than gives rise to DOP agonists, leu- and met-enkephalins (Hughes et al., 1975), KOP agonists dynorphines A and B (Goldstein et al., 1979), MOP agonists β-endorphin (Li and Chung, 1976) and endomorphines (Zadina et al., 1997; Goldberg et al., 1998) and NOP ligand nociception/FQ orphanin (Meunier et al., 1995; Reinscheid et al., 1995). All of the above-mentioned peptides (with the exception of nociception and endomorphins) contain an N-terminal pentapeptide sequence TyrGlyGlyPheMet/Leu (YGGFM/L). The Tyrosine N-terminal sequence is crucial in the peptides' ability to bind into the binding sites of the ORs and its function is comparable to that of the hydroxyl group of morphinans. Despite their existence and isolation, these naturally occurring OR ligands are not the popular choice for the research of ORs function, the main reasons being that the exogenous opioids generally possess a higher affinity and selectivity to the individual types of ORs and are also more convenient for reasons such as their improved ability to cross the hematoencephalic barrier.

1.3 Opioid receptors and their mechanism

Opioid receptors belong to a large super-family of G protein-coupled receptors (GPCR's). GPCR's are membrane bound integral proteins and their structure comprises of 7 helical trans-membrane domains (TM1-7) joined by three extracellular and three intracellular linking domains, an extracellular N-terminal domain and an intra cellular C-terminal domain (Birnbaumer, 1990).

As members of the GPCRs, their function is associated with heterotrimeric pertussis toxin sensitive G-proteins (Gilman, 1987), namely those of the G_i/G_0 family. The individual subunits of the G-protein complex coupled with the ORs have been shown to exist in several subtypes: the $G\alpha_i$ (three forms), the Gαo (A and B forms) and Gαz. The β and γ subunit heterodimer is formed by combining one of five different β and twelve different γ protein products (Hurowitz et al., 2000). In the inactive state, G-protein exists as a hetero-trimeric complex consisting of Gα and Gβγ subunits.

The mechanism of the downstream signalling of OR follows the steps typical for all other members of GPCR family. The agonist binds to the specific, high-affinity ligand binding site of OR and induces a conformational change of the receptor molecule (**R (inactive) → R* transition (active)**) (De Lean, 1980). The conformational change is mostly attributed to the upward movement of TM3 and the rotation of TM6 (Tehan, 2014). This conformational change is transmitted into the cell interior, i.e., from the extracellular to the intracellular side of the receptor molecule, and onto the trimeric G-protein complex. In the resting state, Gα subunits contain a tightly bound GDP molecule. The interaction

with an agonist-activated receptor **R*** results in the opening of a guanine-nucleotide binding pocket of the G-protein and the exchange of GDP for GTP. (Rodbell, 1980) The GTP-bound G-protein complex subsequently dissociates into the free Gα and Gβγ sub-units, and these subunits transfer the hormonal signal further down-stream to effector proteins.

The **R→ R*** is a simplified model. Based on results from Tehan (2014), the available data for crystal structures of GPCRs seem to suggest that there are in fact three conforma-tional states: 1) an inactive state in which the receptor is crystallized in a complex with its antagonist/inverse agonist, 2) an agonist-bound state lacking the coupled G-protein, and 3) a fully active state, a formed ternary complex consisting of the receptor, an ago-nist and an associated G-protein.

In the case of ORs, the most important effector proteins are represented by family of adenylcyclases I-X (ACs I-X), the inwardly rectifying potassium channels (GIRKs) and voltage gated calcium channels (VGCCs). The free Gαi subunits inhibit AC activity caus-ing a decrease of intracellular concentrations of cAMP (Reisine, 1996). Contrarily, free Gβγ subunits increase the activity of ACII in the brain (Sunhara, 20012). The free Gβγ subunits also interact with GIRKs (G-protein activated, inwardly rectifying potassium channels) causing a drastic outflow of K+ ions. This results in hyperpolarisation of the plasma (cell) membrane causing an inhibitory effect of the signal transmission. The free Gβγ subunits also inhibit the permeability of L- and N-type calcium channels (VGCCs) in the plasma membrane thus restricting calcium influx into the cell and further stimulat-ing the GIRKs (Al-Hasani & Bruchas, 2011). Several other effector proteins are stimu-lated by Gβγ including phospholipase C (PLC), GPCR kinases 2/3 (GRK), phosphatidy-linositol-3-kinase (PI3K) and many more (Dupré, 2009).

Figure 1.

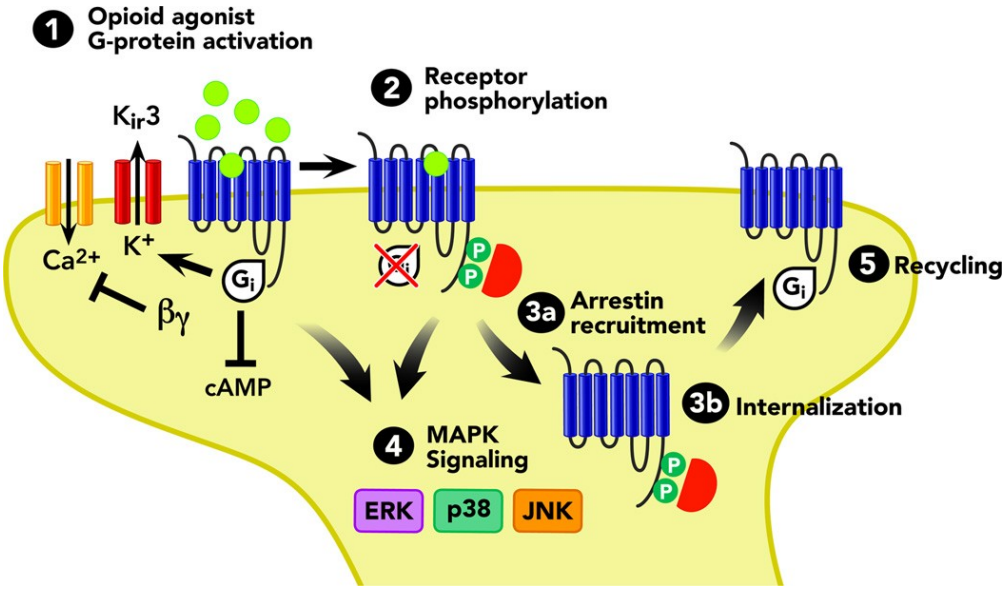

Fig. 1 Classical signalling pathway of opioid receptors and their effector proteins (adopted from Al-Hasani & Bruchas, 2011)

The regulatory modes of interactions between different subtypes of G-protein subunits and the various effectors, primarily represented by the ACs and the ionic channels, are incredibly complex, and have been shown to vary significantly among different cell types and regional tissues. These interactions are summarized in Table 1.

Table 1

AC Isoform[a,b]	Chromosomal Location	Tissue Distribution	Regulation by G Protein Subunits	Protein Kinases	Calcium Effects	Putative Function
AC1	7p12 (154)[c]	Brain adrenal medulla	Stimulated by $G\alpha_s$ Inhibited by $G\beta\gamma$ Inhibited by $G\alpha_o$	PKC: weak stimulation CaMKIV: inhibition	Stimulated by Ca^{2+}-CaM inhibition	Learning, memory, LTP Synaptic plasticity
AC2	5p15 (155, 156)	Brain, skeletal muscle, lung, heart	Stimulated by $G\alpha_s$ Stimulated by $G\beta\gamma$[d]	PKC: stimulation		
AC3	2p22-p24 (157)	Brain, olfactory epithelium	Stimulation by $G\alpha_s$	PKC: weak stimulation CaMKII: inhibition	Stimulated by Ca^{2+}-CaM	Olfaction
AC4	14q11.2 (156)	Brain heart, kidney, liver, lung, BAT, uterus	Stimulation by $G\alpha_s$ Stimulated by $G\beta\gamma$[d]	PKC: inhibition		
AC5	3q13.2-q21 (157)	Heart, brain, kidney, liver, lung, uterus, adrenal, BAT	Stimulation by $G\alpha_s$ Inhibited by $G\beta\gamma$[e] Inhibited by $G\alpha_i$[f]	PKA: inhibition PKCα,ξ: stimulation	Inhibited	
AC6	12q12-q13 (157)	Ubiquitous	Stimulation by $G\alpha_s$ Inhibited by $G\beta\gamma$[e] Inhibited by $G\alpha_i$[f]	PKA: inhibition PKC: inhibition	Inhibited	
AC7	16q12-q13 (158)	Ubiquitous, highly expressed in brain	Stimulation by $G\alpha_s$ Stimulated by $G\beta\gamma$[d]	PKC: stimulation		Drug dependency
AC8	8q24 (155, 156)	Brain, lung (testis, adrenal, uterus, heart)	Stimulation by $G\alpha_s$		Stimulated by Ca^{2+}-CaM	Learning, memory, LTP, Synaptic plasticity
AC9[g]	16p13.3 (159)	Brain, skeletal muscle	Stimulation by $G\alpha_s$			
SAC[h]	1q24 (24)	Testis	Not regulated by G protein subunits			Sperm capacitation

BAT, brown adipose tissue; LTP, long-term potentiation.

[a] All isoforms except sAC are inhibited by P-site inhibitors.

[b] Forskolin stimulates human AC1-AC8, whereas AC9 is weakly stimulated by forskolin. sAC is not affected by forskolin.

[c] Cited reference numbers.

[d] $G\beta\gamma$ stimulation of AC isoforms is conditional upon $G\alpha_s$ co-activation.

[e] Inhibition determined by transfection only and could be an indirect $G\beta\gamma$ effect.

[f] Denotes $G\alpha_i$ family members $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_z$.

[g] Inhibited by calcineurin.

[h] Stimulated by bicarbonate.

Table 1. Adopted from Sunahara (2012)

To date, four groups of opioid receptors have been cloned successfully, with each being coded by a single gene. All the recognized receptors were cloned in the early 1990s with the DOP being cloned from mice in 1992 (Evans, 1992), followed by KOP cloned from rats in 1993 (Li et al., 1993), MOP in 1993 (Fukuda, 1993), and finally the NOP in 1994 (Mollereau et al., 1994). The DOP, KOP and MOP display an approximate 60 % sequence homology. The nomenclature for naming these opioid receptors has been changed several times over the course of the past decades, and although the most recent agreement of the International Union of Pharmacology (IUPHAR, 2016) classifies 4 groups: the μ-opioid receptor (mu-OR, MOP), δ-opioid receptor (delta-OR, DOP), κ-opioid receptor (kappa-OR, KOP) and the most recently discovered nociceptin receptor also known as

opioid receptor-like 1 (NOP/ORL1), we often encounter different names. (McDonald & Lambert, 2005) Despite the recommended terminology, many researchers continue using the older naming system and refer to the receptors as MOR, DOR, KOR and NOR, respectively. The evolution of the nomenclature is summarized in Table 2. There is a substantial amount of evidence pointing to the existence of various phenotypes, and this creates an obvious disparity, considering the apparent existence of only four opioid genes. The mechanism of different mRNA splice variants, possible scaffolding with other proteins, association of the existing ORs into multimeric bodies and the regional tissue difference of the ORs all point to a much more complex system of sub-divisional phenotypes (Waldhoer, 2004). However, it is important to note that the division of the receptors into subclasses may only reflect their differences in the pharmacological effect, and not necessarily in their genetic origins, and thus the division and classification of ORs remains a matter of great controversy (Dietis, 2011). ORs also interact with a vast array of endogenous proteins forming protein-protein interactions that significantly alter their function in relation to opioid ligands, such us the recruitment of β-arrestin2 (BA2) which had been identified as the key component in receptor desensitization and internalization, and the development of MOP mediated antinociceptive tolerance in BA2 knockout mice. (Bohn, 2000)

Table 2

Changes in nomenclature of classical opioid receptors overtime

Pre-cloning	Post cloning	IUPHAR 1996	IUPHAR 2000
Δ	DOR	OP1	DOP
K	KOR	OP2	KOP
μ	MOR	OP3	MOP

Table 2. adopted from Pathan (2012)

1.3.1 Delta-opioid receptors

Compared to the other types, DOPs are less widely distributed receptors. Two subtypes have been identified (δ_1 and δ_2) with met/leu-enkephalin being their endogenous agonist. Highest densities have been localized in the amygdala, olfactory bulb, deep cortex and nuclei accumbens, as well as the afferent peripheral sensory neurons where they

inhibit the release of neurotransmitters (Mansour et al., 1995; Peng, 2012). Much like MOPs, they have been shown to reduce the motility of the gastrointestinal tract and cause respiratory depression.

1.3.2 Kappa-opioid receptors

The KOPs are by far the least studied of the three major OR types. The endogenous ligand of this OR is dynorphin-A/B. Three isoforms of the KOP gene are recognized (κ_1, κ_2, κ_3). They are localized in the PAG, the hypothalamus as well as the substantia gelatinosa of the spinal cord (Mansour et al., 1995; Peng, 2012). Intriguingly enough the KOPs agonists not only create no respiratory depression but have also shown an ability to attenuate the analgesic effect of MOP agonists and reduce hypoxia (Zeynalov et al., 2007).

1.3.3 Nociceptin receptor

The last member of the OR family also known as opioid receptor like-1 is coded by the OPRL-1 gene. Its endogenous ligand is the nociceptin heptadecapeptide/orphanin FQ(N/OFQ). The structure of this ligand is very similar to that of the other endorphins. The NOP is the only member of the OR group without any affinity to the μ, δ, κ – agonist naloxone, or to other classical opioids. NOP activation affects a vast range of functions, including food intake, memory processes, depression and anxiety, cardiovascular functions, gastrointestinal motility and neurotransmitter release at peripheral and central sites.

1.3.4 Mu-opioid receptors

MOPs are by far the best studied group of the four OR types as they are the primary sites of action for most of the opioid drugs. Even though only one gene of the MOP has been identified (OPRM1 (Fukuda, 1993)), many studies have suggested the existence of several splice variations. To date, three isoforms of the MOP have been characterized (μ_1, μ_2, μ_3) and several more splice variants described in humans (Pan et al., 2005), rodents and several other species (Pan, 2005). They are the main agents of action and interaction with the exogenous opioids, as most of these initiate the physiological response by binding to this very type. The importance of MOPs was confirmed by experiments with MOP gene knockout mice in which the administration of morphine failed to induce the analgesic effects (Matthes et al., 1996). MOPs play the most crucial role in pain modulation, and are thus found in large densities in areas responsible for the control of nociceptive transmission. These attributes are mostly credited to μ_1-OR, as high densities have been identified in the CNS in both pre- and post-synaptical areas, particularly in the periaqueductal grey (PAG) and the afferent neurons of the dorsal horn within the spinal cord (Mansour et al., 1995; Peng, 2012). They are believed to affect nociception by disinhibiting the pain responsive neurons via direct inhibition of the oncoming GABAergic inhibitory cellbodies and synapses (Williams et al., 2001). The proposed inhibitory effect of MOPs on neuronal synapses responsive for nociceptive transmission is schematized in Fig. 1 adopted from Al-Hasani (2011).

The other two subtypes are mostly active in peripheral regions and are mostly credited for causing respiratory depression, altering gastrointestinal endocrine secretion. There is also evidence of effect on the cardiovascular system, immune function and thermo-regulation (Mansour et al., 1995).

Figure 2

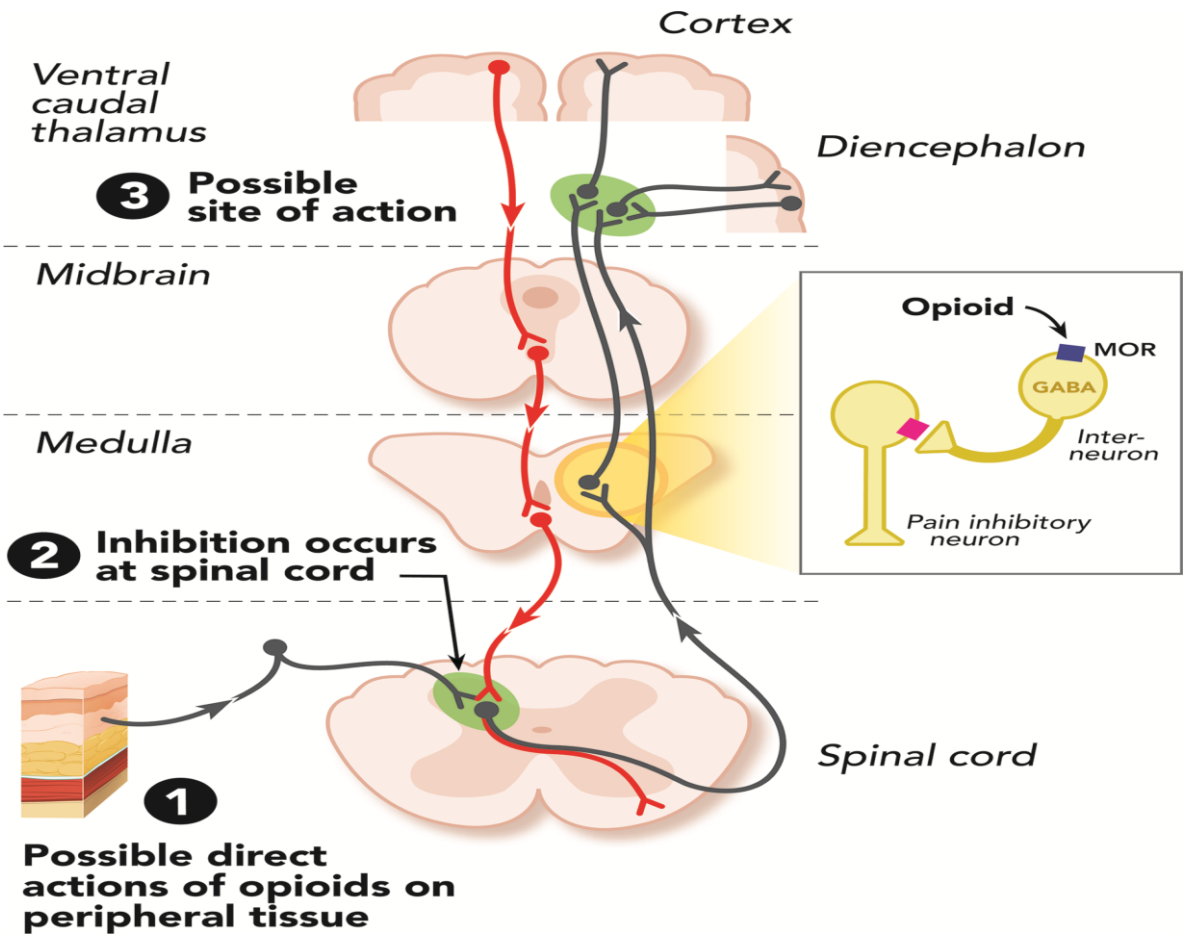

Fig. 2 Sites of inhibition mediated by opioid MOP in pain modulating pathways in the CNS and PNS (adopted from Al-Hasani & Bruchas, 2011) GABA=γ-aminobutyric acid, the major inhibitory neurotransmitter in the CNS

The opioid receptor types along with their coding genes, and endogenous ligands and their precursors are summarized in Table 2.

Table 3

OR type	Coding gene	Precursor	Endogenous ligand
MOP	ORPM1	POMC	β-endorphin
		unkown	Endomorphin-1
			Endomorphin-2
DOP	OPRD1	Pro-enkephalin	[Met]-enkephalin
			[Leu]-enkephalin
KOP	OPRK1	Pro-dynorphin	Dynorphin-A
			Dynorphin-B
NOP	OPRL1	Pre-pro-nociceptin	N/OFQ

Table 3 adopted from Pathan (2012)

1.4 Mechanism of action of opioids at MOPs

The administration of opioids and their subsequent binding to the ORs does not only produce a cascade of signals but also greatly influences their overall function, levels of expression and many other properties. The focus will be oriented towards the MOPs which play the central role in opioid mediated effects, as mentioned above. There are several terms that need to be explained in order to further explore their function. These include: tolerance, dependence, downregulation, internalization and receptor desensitization, some of which are often used interchangeably. It is a well known fact that chronic exposure to opioids leads to the development of tolerance and subsequent dependence.

Tolerance can be broadly defined as a decrease of the responsiveness to the presence of an agonist after continuous exposure. There are several approaches to studying the mechanism of the development of tolerance and dependence. Either in vitro, by using cultured cell lines stably expressing the MOP receptors exposed to the opioid while their actions are observed, or as in vivo experiments with animal subjects that are first chronically exposed to opioids until tolerance is established, and then the resulting changes in their organisms are observed (Williams et al., 2013). Another important attribute of tolerance is that it leads to the need to increase the dosage of the agonist to achieve the desired effect. Strictly speaking, tolerance can be observed after long term exposure (days, or even weeks) to the agonist but is often used to describe the short term acute response to the presence of the agonist.

Receptor desensitization is a much more rapid process, typically occurring within minutes after agonist exposure, and is not associated with an actual change in the net numbers of the receptors. It is in fact a decrease in the rate of receptor signalling resulting

from molecular alteration of the receptors (Williams, 2013). This process can also be reversed within a short period of time by removing the agonist.

The ORs desensitization occurs after the agonist activation-induced phosphorylation of the protein by β-arrestin receptor kinase2 (BARK2). Subsequently the affinity of the OR for the recruitment of β-arrestin2/3 (BA2/BA3) proteins rises which also interferes with its ability to couple with the signalling G-protein (Tsao and von Zastrow, 2000). However, it is important to note that the inactivation of the G-protein mediated signalling pathway does not necessarily mean that the receptor itself is fully inactivated. According to Lefkowitz (2005), β-arrestins coupled with receptors can also act as important signal transducers and act in signalling associated with MAP-kinases and several other effectors. Desensitization can also be divided into two types: homologous (reduced effect of a single agonist on a specific receptor type) and heterologous (reduced effect of an agonist acting on multiple sites sharing a common component of their signalling cascade) (Williams, 2013).

Internalization is a component of receptor trafficking and can be considered the key process in receptor recycling from desensitization. (Williams, 2013) However, even though it typically follows the administration of many MOP agonists, including methadone (Celver, 2004), there are some that do not necessarily induce internalization like morphine and are capable of recycling without it (Whistler et al., 1999). Internalization follows up the cascade of desensitization (phosphorylation and recruitment of BA2/3), and the receptor is sequestered into a clathrin coated vesicle and into the endosomal compartment (Celver, 2004; Williams et al. 2001; Tsao and von Zastrow, 2000). The steps are shown in Fig. 3.

Figure 3

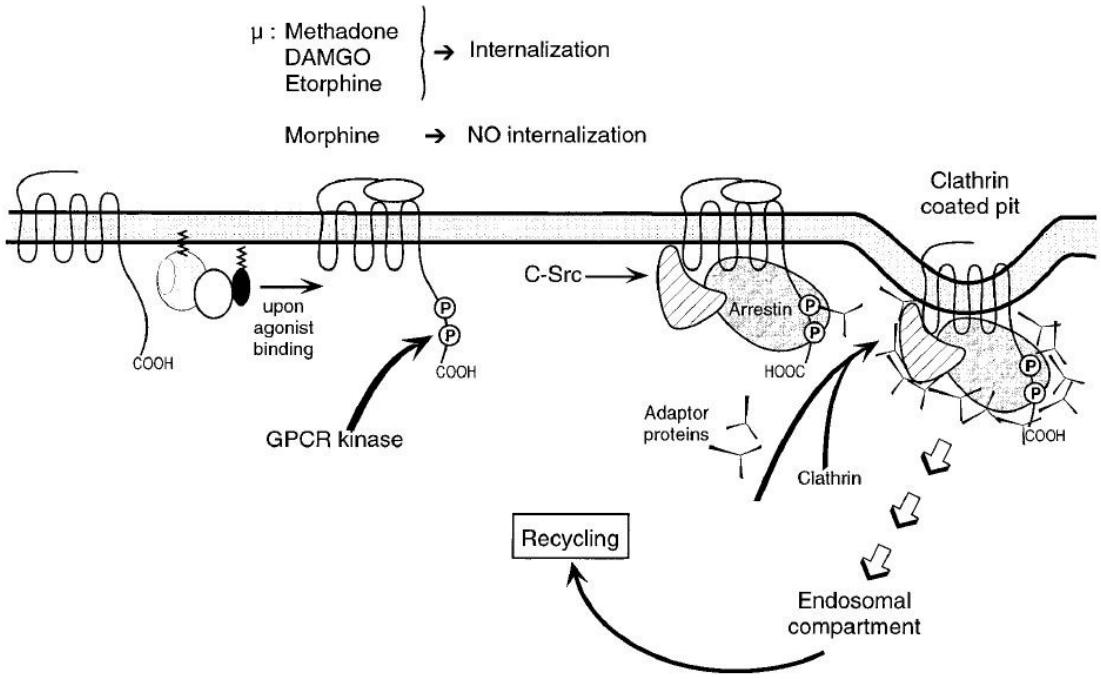

Fig. 3 Agonist induced internalization (Adopted from Williams et al.; 2001)

From there on, the receptor can either be recycled or follow the pathway to downregulation. Although these mechanisms almost certainly precede dependence, they are mostly involved in the acute response to opioids.

Downregulation is a gradual attenuation of receptor signalling activity, is typically described as the reduction of the net amount of membrane receptors present in the cell, and is usually detected by the reduced amount of radioligand binding sites in a specific tissue (Williams, 2013). It is mediated both through specific degradation and reduced biosynthesis of the receptor proteins (Tsao and von Zastrow, 2000).

The long-term tolerance that develops during chronic opioid exposure is of a compensatory nature. In other words, the chronic stimulation of MOPs leads to a shift of the homeostasis, and the signalling system requires the presence of the agonist in order to function normally. Contrarily to the short-term effects, long term stimulation leads to an increased activity of the AC, and therefore an increase of intracellular cAMP levels. This has been observed in a study by Avidor-Reiss et al. with morphine treated Chinese hamster ovary cells (1995). This phenomenon is referred to as AC hyper-sensitization or super-activation. The adaptive responses and changes observed in the given cells and synapses are proposed to be the main source of opioid withdrawal (Williams et al., 2001). However, the exact mechanisms are still not fully understood.

There had also been some efforts to link single nucleotide polymorphisms (SNPs) within the OPRM1 gene to the development of tolerance and addiction. Bond et al. (1998) conducted a sequencing study with 113 ex-heroin addicts in MMT and 39 control subject

without prior opioid abuse. They identified five SNPs with A118G substitution in exon 1 of the OPRM1 gene as the most prevalent (10%). The MOP with this substitution had an approximately 3-fold higher binding affinity to the endogenous opioid β-endorphin *in vitro*, implicating the possibility of altering the development of addiction. However, this claim had since been dismissed as having little to no effect on heroin addiction (Shi et al., 2002). Currently the focus on SNPs and their potential to alter addiction is predominantly aimed on studying alterations within the non-coding regions of the OPRM1 gene. Shi's group (2002) has implicated the possibility of involvement of an intronic SNP IVS2 + 31G -> A as individuals carrying this variation tended to consume larger amounts of drugs compared to other addicts. The effect of genomic variations of OPRM1 on the biological functions still remains poorly understood, and a lot of the available data is contradictory.

2 Methadone

2.1 Chemical structure

Methadone is a synthetic opioid analgesic commonly used in the form of its hydrochloride, much like the case of morphine and heroin. Therefore, these terms have become synonymous and are used interchangeably. It acts primarily as a mu-opioid agonist and displays actions similar to those of morphine. *(From NCIB's PubChem compound database, see also Martindale, The Extra Pharmacopoeia, 30th ed, p1082-3).* The chemical formula of methadone is 6-(dimethylamino)-4,4-diphenylheptan-3-one according to the IUPAC nomenclature (Fig. 4).

Figure 4

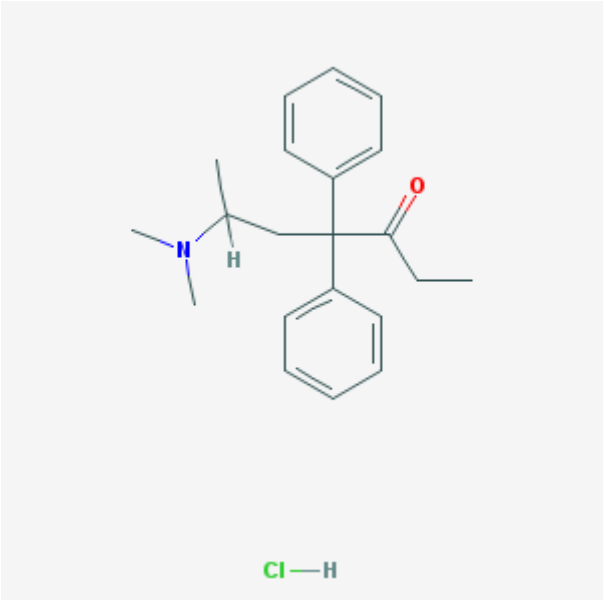

Fig. 4 2D structure of methadone hydrochloride taken from NCIB's PubChem molecular database

The structure of methadone contains a central asymmetrical carbon atom which results in its 2 enantiomeric forms, the R- and the S-methadone. Although R-methadone displays a much higher affinity the common clinical practice uses the racemic mixture (R-(-)-methadone and S-(+)-methadone or D-L-methadone) as a 50/50 ratio (Inturrisi, 2005). R-methadone has approximately 10-fold higher affinity for the MOPs (Kristensen, 1995). A study by Scott et al. with human subjects has concluded that R-methadone has about 50 times higher analgesic potency and effectiveness in preventing opioid withdrawal than the S-enantiomer (Scott et al., 1948). Even though purification of only the R-enantiomer is relatively expensive it is still rarely manufactured in this form for example in Germany (Inturrisi, 2005).

2.2 A brief history of methadone discovery

The history of methadone is surrounded by a vast amount of myths and popular misconceptions spread among both the professional circles and the general public. The substance known today as methadone was first synthesised in the year 1939 at the pharmaceutical laboratories of the renowned German Hoechst company, by then a subsidiary of the petrochemical titan I. G. Farbenkonzern. The discovery was an outcome of a long stretch of research focused on the synthetic antipyretics and analgesics dating back to the 1880s. The continuous efforts of the researchers, most notably of Hoescht chemists Eisleb and Schummann, led to the discovery and synthesis of several opioid analgesic drugs, some of which had been launched commercially, such as pethidine, also known as meperidine (1-methyl-4-phenyl-4-carbaethoxypiperidin), launched under the trade name Dolantin ® (Galcher, 2004). Later research joined by two fellow Hoescht chemists Bockmühl and Ehrhart led to the discovery and synthesis of hundreds of other basically substituted diphenylmethanes that showed both analgesic and spasmolytic activity. One of these created by the addition of a ketone side chain to the diphenyl methane radical had been named Va 10820. According, to the reports, made by the aforementioned Hoescht chemists, Va 10820 was further tested on animals, and the test confirmed that it had 5-10 times higher analgesic potency than pethidine. Va 10820 had been given a generic name “amidon” (Defalque, 2007). After the patents were confiscated from the I. G. and brought to the US and in 1947 amidon was named methadone by the Council on Pharmacy and Chemistry of the American Medical Association. The patent rights for methadone synthesis were sold for a token sum of one dollar, and the first company to commence commercial production under the trade name Dolphine (from Latin dolor = pain and finis = end) was the firm Eli-Lilly. Many other companies from the USa and abroad followed suit soon after, and methadone was sold under many trade names, some of which are used to date: Adanon®, Adolan®, Althose®, Amidone®, AN-148®, Dolophine®, Methadone® and many others. (Galcher, 2004) The pioneers in the use of methadone as means of maintenance therapy were Drs. Vincent Dole and Marie Nyswander of the Rockefeller institute in New York City who started the first methadone maintenance program (MMT) in 1964. Joined by Dr. Mary Jane Kreek, they hypothesized that opioid drug abuse is not simply a deviation, or a sign of weak will, but a complex

behavioural and physiological disease of the brain. Their hypothesis shook the ideas behind psychogenic theories of addiction, their main argument being that the behavioural patterns typically associated with drug addiction, such as involvement in crime and other antisocial activities, have diminished to an astonishing degree, or ceased altogether in a majority of the MMT admitted patients just months after entering the programme. With many of them successfully reintegrating themselves into society (finding jobs, re-establishing family ties etc.)

This led them to believe that the addiction associated behaviours are not necessarily deviant character traits present in the individual before the addiction but are in fact a consequence of the metabolic dysfunction resulting in the actions being fuelled by drug hunger (Dole and Nyswander, 1967). Their team also observed that methadone, when used in appropriate moderate to high doses (ranging from 60-150 mg/day), is an ideal agent capable of preventing all symptoms of narcotic abstinence and drug hunger, and can be used effectively for rehabilitation efforts. Over the course of following decades this hypothesis continues to be proven right as methadone remains the first choice in substitution therapeutic treatment for opiate addicts and seems to be very effective in allowing truly successful rehabilitation (Kreek, 2000).

2.2.1 Maintenance treatment

Today maintenance treatment is believed by many to be the most successful therapeutic method for rehabilitation from heroin addiction. Aside from methadone, two other alternative substances are currently used for maintenance treatment: the even longer acting agonist L-α-acetylmethadol (LAAM) and the combination of partial agonist/antagonist buprenorphine/naloxone. Heroin (and other related opiate) addiction is typically defined as the compulsive abuse of drugs (multiple daily self-administrations for months or even years) despite the obvious negative consequences to self and others. From a biological perspective, it is believed to be caused by the adaptive changes in the neuronal system (especially involved in the reward system) (Williams et al., 2001). A key component of opioid addiction is the development of physical dependence which occurs after prolonged exposure to opioids and the development of tolerance. A major problem for addicts trying to abstain from opiates is the subsequent withdrawal syndrome which can consequently lead to drug-induced craving, dangerous drug seeking behaviour, and relapse. Methadone effectively slashes the craving and helps re-establish the physiological homeostasis (Kreek, 2000). A study with rats by Martin et al. which compared the effects of heroin and methadone on selected regions of the brain concluded that methadone displays a higher intrinsic efficacy while also inducing greater MOP desensitization than heroin (2007). Celver (2004) also demonstrated greater desensitization and receptor internalization using *Xenopus laevis* oocytes and AtT20 cells. Both stereoisomers of methadone have also been shown to also act as NMDA receptor agonists in a study with rats by Gorman et al. (1997). Gorman's group suggested that methadone may have an ability to attenuate the development of tolerance when co-

administered with other opiates like morphine. These findings indicate why methadone used in appropriate dosing creates the desired effects.

Another beneficial trait of methadone is that, even when administered daily in relatively high doses, it does not induce hepatotoxicity (Kreek et al., 1972). A majority of heroin addicts entering MMT have serological evidence of exposure to hepatitis B, C (and even delta). These diseases are typically linked to some form of liver dysfunction. Aside from that, heroin addicts are also often heavy alcohol abusers, and liver damage is therefore very common. (Kreek, 2000) Methadone being primarily metabolised in the liver therefor does not hold any significant risks to users even when their liver function is impaired.

The strategy of maintenance treatment is to establish the optimal dose. The optimal dose is characterized by its ability to: 1) sufficiently relieve narcotic craving, 2) supress the withdrawal symptoms for an appropriate amount of time until the next dose, 3) create a narcotic blockade of other opioids, and finally, 4) not induce euphoria, analgesia and other side effects that could impair the patients functioning and perception (Renner, 1984; Kreek, 2000). Usually an initial daily dose of around 20-40 mg is administered to the patient, in order not to exceed the individual degree of tolerance and dependence. The doses are then titrated and gradually increased, while the patients are under careful clinical observation, until they reach the desired effects. This is typically achievable by around 80-120 mg; however, in rare cases some patients will require doses even exceeding 150mg/day. Despite the interindividual differences in opioid tolerance/dependence, the results of most clinical trials seem to favour the use of a moderate to high doses exceeding 80 mg/day. Patients receiving such doses seem to have a much lower inclination to use illicit opioids, contrary to the ones maintained on low doses below 60 mg/day (Amato et al., 2005; D'Aunno & Vaughn, 1992). According to D'Aunno's (2014) extensive US national study of the changes in dosing from 1988-2011, there had been a very favourable shift from lower dosing (below 60mg/day) to the higher dosing (over 80 mg/day) which resulted in a much higher percentage of patients being retained in treatment for longer periods of time. Ultimately it is safe to state that patients who successfully manage to find their optimal dose remain in MMT for long periods of time and realize the improved quality of life (D'Aunno & Vaughn, 1992).

2.3 Methadone pharmacokinetics

Pharmacokinetics describes the fate of active substances in the (both endo and exogenous) organism and can be divided into four basic steps: absorption, distribution, metabolism and excretion. Distribution, metabolism and excretion are often collectively referred to as elimination (IUPHAR, 2017). To fully appreciate pharmacokinetics, we must also establish four basic parameters used to describe the states of the drug within the body. These parameters are: 1) clearance, a measure of the body's ability to fully

eliminate the drug, 2) bioavailability, the fraction of the absorbed drug in an unchanged form that reaches the systemic circulation, 3) volume of distribution, a portion of available space in the body capable of containing the drug, and finally 4) half-life, the amount of time required for the drug to change from the initial concentration to be reduced by half (Benet, 1995).

Absorption is the phase during which the substance makes its way into blood circulation. The rate of absorption depends on the method used for application (oral or parenteral administration). Methadone is most commonly administered orally, and therefore the absorption takes place in the gastrointestinal tract almost entirely in the liver microsomes where it undergoes a relatively mild first pass effect. In contrast, when administered intravenously, methadone shunts this effect and the effects of related enzymes, and therefore displays a higher concentration to dose ratio of the active (R)-enantiomer by up to 23% (Felder et al., 1999).

Distribution is the process during which the drug binds onto specific plasma proteins and disperses throughout the bodily fluids and surrounding tissue compartments (IUPHAR, 2017). Thanks to its high lipid solubility, 98% of methadone that makes its way into the central compartment quickly disperses into other tissues, particularly the liver, kidneys, lungs, and a small fraction even travels to the CNS (Dole & Kreek, 1973).

About 1-2% remains in the bloodstream (Merasaar, 1981), 60-90% is bound to plasma proteins: the acidic α1-globulins (like the α1-acid glycoprotein (AAG)) (Garrido et al., 2000), and only a small part (13.4-17.4%) to γ-globulins (Olsen, 1973). Methadone has a quite large and variable volume of distribution (2,1-9,2 l/kg) and differences have been observed between the R/S enantiomers (Meresaar, 1981; Dole & Kreek, 1973). Based on this large volume of distribution, it has been assumed that there is in fact a large tissue compartment operating in a dynamic equilibrium with a proportionally smaller central compartment. The important result of this tissue accumulation is that when on MMT, a temporary decrease of methadone concentration in the blood is usually not associated with any evident withdrawal symptoms (Ferrari, 2004; Plummer, 1988).

That being said, methadone blood concentrations are also greatly affected by the AAG, an acute phase glycoprotein that has a high binding affinity to methadone and can render it inactive (Garrido et al., 2000). It has been shown that the up regulation of AAG negatively correlates with methadone's bioavailability, and can therefore lead to withdrawal symptoms as only unbound methadone is active (Behan et al., 2013).

Metabolism involves the recognition of the foreign substance by the organism and its biotransformation into daughter metabolites. This step includes alteration of the parent compound that is typically hydrophobic/lipid-soluble (as in the case of methadone) into metabolites that are water-soluble enough to be excreted via biliary tract in urine or faeces. This step involves N-demethylation and subsequent cyclisation into 2-ethylidene-1,5-dimethyl-3,3-dipenylpyrrolidine (EDDP). EDDP can be further altered by

a second demethylation, forming 2-ethyl-5-methyl-3,3-diphenylpyrolidine (EMDP). Neither of the metabolites have shown any significant pharmacological activity (Ferarri, 2004). These steps are catalysed by a group of enzymes from the cytochrome P450/CYP superfamily which will be further discussed.

Excretion is the final step of elimination during which the compound leaves the body either as the metabolite, or in an unchanged form through the biliary tract (IUPHAR, 2017). Elimination of methadone occurs mainly through the kidneys in unchanged form, as EDDP and as EMDP. Urine is therefore usually used to analyse the rate of elimination. Interestingly, the rate of unchanged methadone elimination is very dependent on the pH of urine, as it increases with urine acidification and decreases with its alkylation (Dinis-Oliveira, 2016; Ferarri, 2004).
Methadone is a long-acting opioid, and compared to other common morphine like opioids, it has a very slow metabolism. Like many pharmaceutics, methadone's elimination in the organism is described by a bi-exponential curve consisting of 2 phases: the quick initial α-phase corresponding the drugs transfer from the liver compartment into the surrounding tissue compartments, and the beginning of elimination followed by a more gradual β-phase corresponding with the actual elimination (Ferarri, 2004). Another important property is the amount of time it takes during the initial α-phase for methadone to reach its maximum plasma concentration, T_{max}. The amount of time can range but is usually somewhere in between 2.5 and 4.4 hours (Dale, 2004; Eap, 1990; Merasaar, 1981). It possesses a very high oral bioavailability usually exceeding 80%. However, several studies have concluded that the rate of bioavailability varies significantly among individuals and the mean values can therefore often be misleading. In a study conducted with opiate users, bioavailability in subjects either exceeded 90% or ranged from 41 to 76 % (Meresaar, 1981). While a study healthy opioid naive patients concluded with the bioavailability ranging from 75-97% (with a mean value of 86%) (Dale, 2004).
The elimination half-life was shown to be the most variable property of methadone ranging from 8.5 to 47hours (Meressar, 1981).

2.3.1 Cytochrome P450

The metabolism of methadone is mediated by specialized enzymes belonging to the cytochrome P450 family, also known as CYPs. Induction or inhibition of these CYP enzymes causes changes in the rate of methadone metabolism and its elimination from the organism. The CYP class of enzymes represents proteins containing heme as the prosthetic group.

The CYP family is divided into sub-families and smaller subgroups based on the identity of their amino acid sequence. The families have an identity of at least 40% and are identified by an Arabic number. The subfamilies have an identity of at least 55% and are designated by a capital letter. The last Arabic number represents the individual coding gene (Nelson et al., 1996).

In general, induction of an enzyme (up-regulation) occurs after repeated administration of the drug which represents its substrate, and may result in an increase of both expression of the given enzyme and its overall activity. This alteration consequently increases

the degradation of methadone and finally results in a decrease of concentration of methadone in blood and tissues. For this process to develop, usually at least 1-2 weeks are needed.

In contrast, enzymatic inhibition develops rather quickly as it requires a small amount of time for the drug to bind to the enzyme and elevate its activity. Pharmacokinetics can also be altered when two drugs that act as substrates to the same enzymes which are present in the system. In this situation the drug that has the higher affinity usually prevails in binding to the enzyme over the other, and can therefore partially prevent the metabolism of the given substance. This is another useful ability of methadone which can be used for effective blockade of the effects of other opioids like morphine or heroin, if the patient was exposed to them while on methadone substitution therapy. Obviously, this case is not rare among opiate addicts attempting rehabilitation.

There are several isoforms of the CYP enzymes that are involved in methadone metabolism, most notably CYP3A4, CYP2B6, CYP2D6 and CYP2C19. There are other isoforms that are probably involved in the metabolism but, based on the up to date results, their function is either minimal or not sufficiently understood. These include CYP2C9, CYP2C8, CYP1A2 (Ferrari, 2004; Chang et al., 2011).

The primary pathway in methadone metabolism involves two consecutive N-demethylations which are mainly catalysed by CYP3A4. After the initial demethylation of the secondary ammine, the metabolite spontaneously cyclizes to form EDDP which can then be further demethylated to form EDMP (Gerber, 2004). Neither of these metabolites are active at opioid receptors; however, they have been shown to be very effective blockers of α3β4 and α4β2 neuronal nicotinic acetylcholine receptors (nAChRs) in vitro with human cell lines (Xiao, 2001).
Figure 5

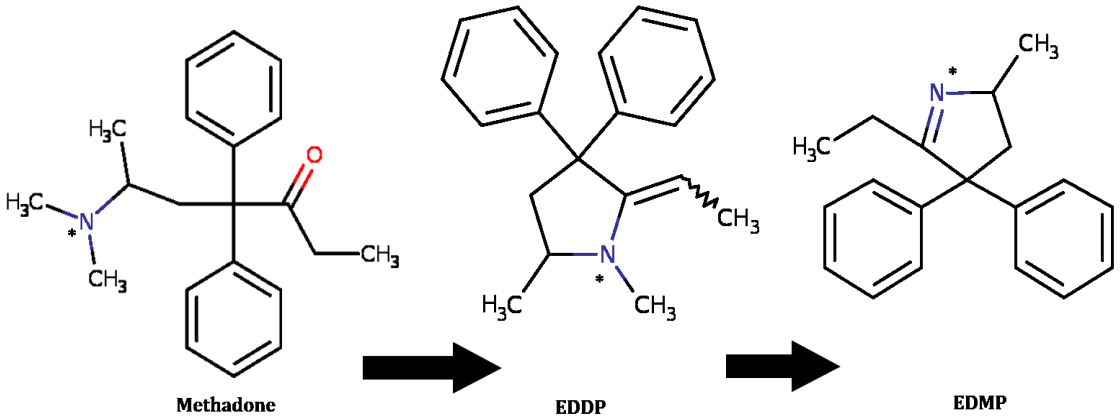

Fig. 5 N-demethylation of methadone into EDDP and subsequent demethylation into EDMP (created using images from HMDB database)

Based on the results of several research groups, it is now generally believed that CYP3A4's enzymatic activity with racemic methadone is non-stereoselective (Chang et al., 2011; Wang, 2003; Gerber, 2004). Interestingly, some of these groups presented contrasting results when studying methadone depletion (Kamal et al., 2013). Wang and De Vane concluded that CYP3A4 depleted R-methadone at a much higher rate (about 4-

fold) whereas Gerber's group concluded that there was now significant difference between enantiomers (Gerber, 2004).

CYP3A4 is mainly expressed in the intestine and liver, affecting metabolism in both tissues, is inducible, and no genetic polymorphism has been identified. However, the activity of CYPA4 varies greatly inter- and intraindividually. This is credited to different levels of expression among individuals and tissue regions. According to Chang et al., the 3A4 isoform has a lower ability to generate EDDP (compared to 2B6) but is responsible by means of differential expression generates about 63-74% of EDDP (Chang et al., 2011).

The importance of CYB2B6's function compared to 3A4 is still somewhat controversial. Even though 2B6's EDDP formation rate is the highest out of the CYP isoforms, it only generates about 12-32% of overall EDDP. (Chang, 2011; Gerber, 2004) Unlike 3A4 CYP2B6 is highly stereoselective preferential for S-methadone, and is also very polymorphic. The SNPs occurring within the coding genes for this isoform result in different levels of metabolism rate, and can thus lead to very poor or very rapid metabolism, leading some to believe that its function is in fact superior to that of 3A4 (Kharasch, 2015; Gadel, 2015). The most common and clinically significant allele is the CYP2B6*6, which is often linked to methadone associated deaths. Higher S-methadone concentrations indicate that individuals possessing this allele are very poor metabolisers. Therefore, it would appear, that carriers of this allele require smaller doses of methadone as they are more prone to risks of methadone toxicity (Eap et al. 2007, Levran, 2011). Another notable trait of the 2B6 variant is its known involvement in efavirenz metabolism related to HIV treatment. Interestingly, this allele is quite frequent among some ethnic groups, particularly African and African-American (33-50%), Asian (10-21%) and Hispanic populations (Kharasch, 2015; Gadel, 2015). The frequency of this allele interestingly correlates the results of D'Aunno (2014) who observed that a majority of patients in MMT who receive the lower doses (<60mg/day) belong to these ethnic groups. A contrasting result was observed with carriers of the less frequent CYP2B6*4 variant. CYP2B6*4 carriers had a much faster elimination and clearance, and lower methadone concentration compared to the wild type and other alleles (Kharasch, 2015). This further supports the assumption that some individuals in MMT require higher doses (>150 mg/day), in order to prevent withdrawal symptoms.

CYP2C19 and CYPD6 are also expected to play a minor role in methadone metabolism but their exact function is still poorly understood. According to Chang et al. (2011) 2C19 is responsible for generating 1.4-14% of overall EDDP and is stereoselectively specific for the R-enantiomer while the 2D6 isoform is preferential for the S-enantiomer. The activity of the isoforms was ordered accordingly: CYP-2B6>3A4>2C19>2D6>2C18.

3 Adverse effects of methadone use

Despite the positive outcomes that can be directly attributed to the use of methadone in MMT, chronic use of methadone carries a variety of adverse side effects. Thanks to the MMT patient's tolerance to opioids, they are usually affected by only mild adverse effects. These side effect affect a variety of bodily functions and usually diminish after stable dosing is achieved. Although methadone is relatively safe, it does possess some very severe and potentially lethal risks which arise from its potential toxicity when combined with additional substances. Aside from the obvious disadvantages, such as the continuation of dependence and tolerance to opioids, this section will focus on some of the other cons of MMT.

The milder symptoms which have been reported to persist in patients for periods exceeding 6 months include: excessive sweating (persistent after 3 years of treatment), constipation (particularly during the first year of treatment), insomnia, sexual dysfunction and loss of libido, loss of appetite, nausea, drowsiness and overall tenseness (Kreek, 1978). The symptoms are summarized in Table 4.

Side effects observed in long-term MMT patients

Treatment duration for 6 months or more; moderate doses 40-80 mg/day		Treatment duration for 3 years or more; high doses 80-120 mg/day
(%)	**Symptoms ad signs**	**(%)**
47	Excessive sweating	48
57	Constipation	17
26, -	Libido abnormalities, sexual dysfunction	22, 14
23	Insomnia	16
25	Nausea	-
23, 21	Drowsiness, tenseness	-, -

Table 4. Side effects observe in longterm patients in MMT, adopted from (Kreek, 1978)

Due to their distribution in the CNS, as well as the peripheral tissues of the GI tract, the activated MOPs mediate their effect on the GI tract in a very complex way by affecting (in a both stimulatory and inhibitory fashion) the neural pathways within the CNS and the enteric nervous system (Leppert, 2012). Constipation is mainly caused by the depression of peristaltic contractions, increased muscle tone of GI smooth muscle cells and also

a decrease in electrolyte and water secretion into the lumen of the gut (Sobczak et al., 2014).

Despite the precautions taken by clinicians, methadone possesses a high potential for both abuse and overdose related death. A majority of methadone-related deaths occur during the initial induction phase of MMT, when correct dosing is being established (Srivastava and Kahan, 2006). Concurrent abuse of other substances acting as CNS depressants (particularly alcohol, other opiates and benzodiazepine) is of frequent occurrence among victims of methadone toxicity (Drummer et al., 1992). Respiratory depression and subsequent cardiac arrest is believed to be the main cause of methadone-related deaths.

Respiratory depression along with sedation usually appear after methadone accumulation. It develops about 12-14 hours after ingestion of methadone, particularly in those with only a weak tolerance to the drug (Drummer et al., 1992). The drug acts by altering the function of the brain stem's respiration control centre. This results in decreased sensitivity of CNS chemoreceptors to CO2 and hypoxia (Corkery et al., 2004; Kreek, 1978).

In addition to its effects on respiration, methadone like other classical opioids is known to affect cardiac activity (Kreek, 1978), specifically causing QTc prolongation. The QTc interval is an ECG measure of time describing the hearts deporalization (Q wave) and repolarization (T wave) (Modesto-Lowe et al., 2010). This had been further supported by *in vitro* experiments of Katchman's group (2002). In their study cell lines, transfected with the humanether-a-go-go related gene (HERG) that gives rise to a subunit of a ventricular GIRK, were treated with methadone which in turn blocked the ionic channels activity. There is a possible link between chronic high dose methadone use resulting in Torsades de Pointes (TdP). TdP is a potentially life threatening ventricular arrhythmia. Based on Cruciani's extensive review of several studies, it is safe to say that although there are indeed indications of methadone induced QTc prolongation, it however cannot as of now be directly linked to TdP (2008).

Ultimately the major problem of determining the exact source of methadone potential toxicity is the frequent polysubstance abuse among MMT patients. The drug-drug interactions that resolve from concomitant use of various substances make it fairly unpredictable. In spite of that, methadone is still deemed as relatively safe when dosed appropriately , and the positive effects greatly outweigh the adverse ones.

4 Conclusion

Despite the extensive research of opioid receptors there are still many unanswered questions surrounding the exact mechanism of this incredibly complex system. The neurophysiological aspects of addiction are yet to be fully understood. The effects of methadone are mostly identical with those of classical opioids; however, the traits that represent its key advantage are its high oral bioavailability and intrinsic efficacy. The benefits of MMT compared to other therapeutic approaches are undeniable. Over the decades since its induction, methadone maintenance therapy has been proven to greatly reduce the severe harms associated with heroin abuse, greatly reducing drug related criminal behavior, as well as the transmission of deadly infectious diseases. It also successfully normalizes the physiological homeostasis and immune activity (both of which are greatly impaired in addicts) while causing only minor adverse side effects. Of course, it remains a matter of controversy whether this method is ideal. Aside from the potential adverse effects, the main argument of MMT's disadvantage is the fact that it does not tackle the problem of dependence and tolerance. Be that as it may, given the severity of opioid withdrawal syndrome and addiction, a method including complete detoxification which would be more persistent is yet to be found and MMT remains by far the most successful method of long term rehabilitation.

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