Endogenous Opioids and Stem Cells

Subjects: Cell Biology

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Opioids are considered the oldest drugs known by humans and have been used for sedation and pain relief for several centuries. Nowadays, endogenous opioid peptides are divided into four families: enkephalins, dynorphins, endorphins, and nociceptin/orphanin FQ. They exert their action through the opioid receptors (ORs), transmembrane proteins belonging to the su-per-family of G-protein-coupled receptors, and are expressed throughout the body; the receptors are the δ opioid receptor (DOR), μ opioid receptor (MOR), κ opioid receptor (KOR), and nociceptin/orphanin FQ receptor (NOP). Endogenous opioids are mainly studied in the central nervous system (CNS), but their role has been investigated in other organs, both in physiological and in pathological conditions. Here, it is presented a revision of their role in stem cell (SC) biology, since these cells are a subject of great scientific interest due to their peculiar features and their involvement in cell-based therapies in regenerative medicine. In particular, it will be focused on the endogenous opioids' ability to modulate SC proliferation, stress response (to oxidative stress, starvation, or damage following ischemia–reperfusion), and differentiation towards different lineages, such as neuro-genesis, vasculogenesis, and cardiogenesis.

endogenous opioid peptides opioid receptors stem cells differentiation stress response proliferation

1. Introduction

Opioids are considered the oldest drugs known by humans and have been used for pain relief and sedation for several centuries. They are a class of compounds related in structure to the natural plant alkaloids which are extracted from the resin of the poppy plant (Papaver somniferum) [1]. Among them, morphine is the most common, active compound, which exerts its action in the central and peripheral nervous systems (CNS and PNS, respectively) through binding to the opioid receptors (ORs) [2].

Nowadays, endogenous opioid peptides are divided into four families: enkephalins, dynorphins, endorphins, and nociceptin/orphanin FQ [3]. From a molecular point of view, each opioid peptide is synthesized as a prepro and a proform, creating functional peptides after precursor processing. All peptides share a common aminoterminal sequence, Tyr-Gly-Gly-Phe-(Met/Leu), namely, the opioid motif. For this reason, the same precursor may result in different opioid peptides (**Figure 1**) [4][5].

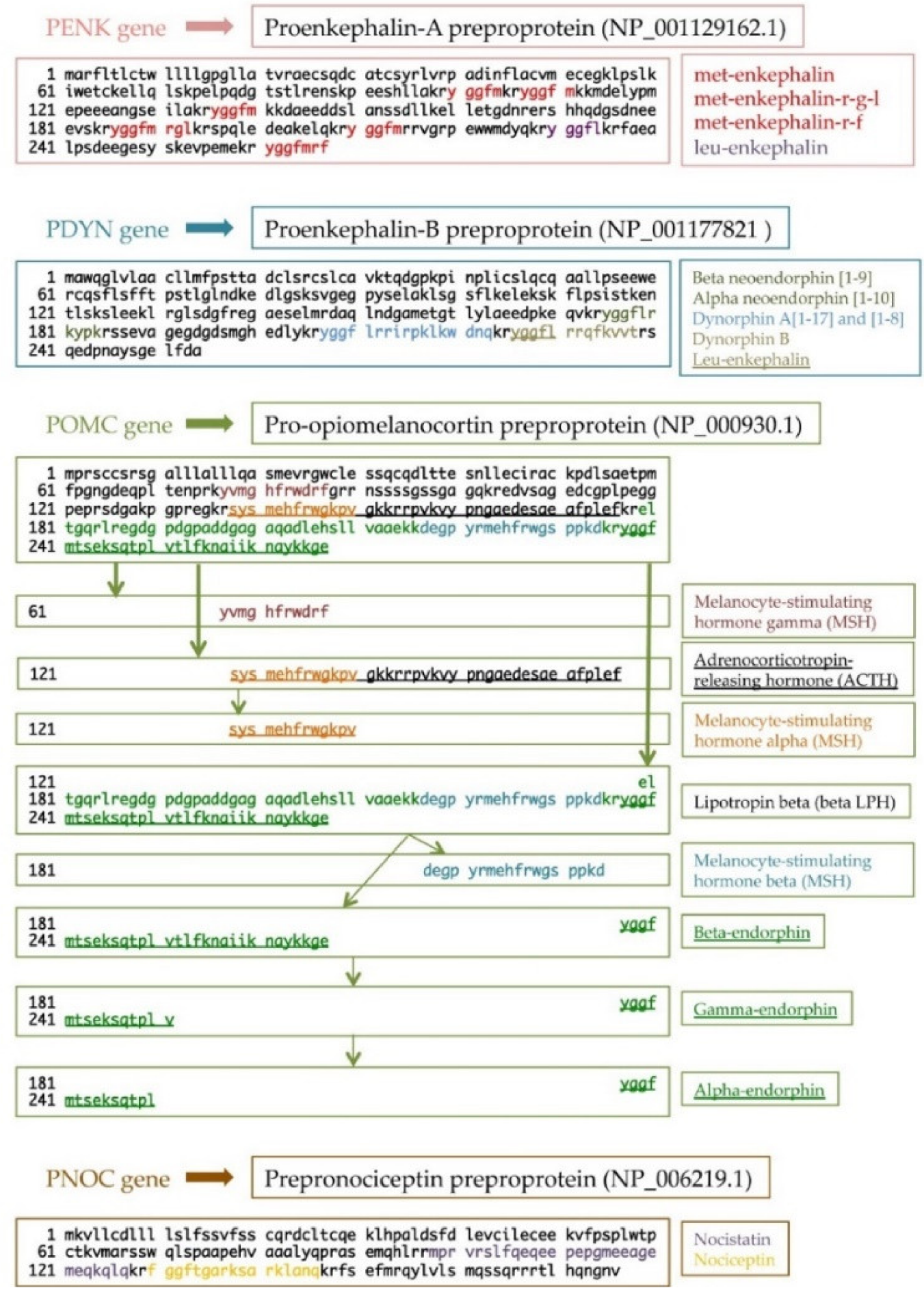
PENK gene
Proenkephalin-A preproprotein (NP_001129162.1)
1 marfltlctw llllgpglla tvraecsqdc atcsyrlvrp adinflacvm ecegklpslk
61 iwetckellq lskpelpqdg tstlrenskp eeshllakry ggfmkryggf mkkmdelypm
121 epeeeangse ilakryggfm kkdaeeddsl anssdllkel letgdnrers hhqdgsdnee
181 evskryggfm rglkrspqle deakelqkry ggfmrrvgrp ewwmdyqkry ggflkrfaea
241 lpsdeegesy skevpemekr yggfmrf
met-enkephalin
met-enkephalin-r-g-l
met-enkephalin-r-f
leu-enkephalin
PDYN gene
Proenkephalin-B preproprotein (NP_001177821)
1 mawqglvlaa cllmfpstta dclsrcslca vktqdgpkpi nplicslqcq aallpseewe
61 rcqsflsfft pstlglndke dlgsksvgeg pyselaklsg sflkeleksk flpsistken
121 tlsksleekl rglsdgfreg aeselmrdaq lndgametgt lylaeedpke qvkryggflr
181 kypkrsseva gegdgdsmgh edlykryggf lrrirpklkw dnqkryggfl rrqfkvvtrs
241 qedpnaysge lfda
Beta neoendorphin [1-9]
Alpha neoendorphin [1-10]
Dynorphin A[1-17] and [1-8]
Dynorphin B
Leu-enkephalin
POMC gene
Pro-opiomelanocortin preproprotein (NP_000930.1)
1 mprsccsrsg alllalllqa smevrgwcle ssqcqdltte snllecirac kpdlsaetpm
61 fpgngdeqpl tenprkyvmg hfrwdrfgrr nssssgssga gqkredvsag edcgplpegg
121 peprsdgakp gpregkrsys mehfrwgkpv gkkrrpvkvy pngaedesae afplefkrel
181 tgqrlregdg pdgpaddgag aqadlehsll vaaekkdegp yrmehfrwgs ppkdryggf
241 mtseksqtpl vtlfknaiik naykkge
61 yvmg hfrwdrf
Melanocyte-stimulating hormone gamma (MSH)
121 sys mehfrwgkpv gkkrrpvkvy pngaedesae afplef
Adrenocorticotropin-releasing hormone (ACTH)
121 sys mehfrwgkpv
Melanocyte-stimulating hormone alpha (MSH)
121 el
181 tgqrlregdg pdgpaddgag aqadlehsll vaaekkdegp yrmehfrwgs ppkdryggf
241 mtseksqtpl vtlfknaiik naykkge
Lipotropin beta (beta LPH)
181 degp yrmehfrwgs ppkd
Melanocyte-stimulating hormone beta (MSH)
181 yggf
241 mtseksqtpl vtlfknaiik naykkge
Beta-endorphin
181 yggf
241 mtseksqtpl v
Gamma-endorphin
181 yggf
241 mtseksqtpl
Alpha-endorphin
PNOC gene
Prepronociceptin preproprotein (NP_006219.1)
1 mkvllcdlll lslfssvfss cqrdcltcqe klhpaldsfd levcilecee kvfpsplwtp
61 ctkvmarssw qlspaapehv aaalyqpras emqhlrrmpr vrslfqeqee pepgmeeage
121 meqkqlqkrf ggftgarksa rklanqkrfs efmrqylvls mqssqrrrtl hqngnv
Nocistatin
Nociceptin

Figure 1. Schematic representation of human endogenous opioid families and their main functional peptides after precursor processing. For each family of peptides, the following information is reported: (i) the names of the genes (*PENK*, *PDYN*, *POMC*, and *PNOC*); (ii) the amino acid sequence of the preforms (NCBI Reference Sequence is reported in brackets next to the proform names); (iii) on the right, the names of the main functional peptides highlighted with a corresponding colour in the preform peptide sequence and in the isolated peptide sequence when it is required.

Endogenous opioid peptides (and exogenous opioids) exert their action through the opioid receptors. ORs are transmembrane proteins belonging to the super-family of G-protein-coupled receptors (GPCRs), which are widely studied due to their key role in mood disorders, drug abuse/addiction, and pain management [6][7][8]. They are expressed not only in the CNS but also in many other districts. There are four subtypes of OR: δ opioid receptor (DOR), μ opioid receptor (MOR), κ opioid receptor (KOR), and nociception/orphanin FQ (NOP) receptor.

Here it will be presented the effect od endogenous opioids on stem cells. Among all the cell types forming the body's tissues, stem cells (SCs) are the subject of great scientific interest due to their peculiar features. In fact, they are characterized by two important properties: the ability to self-renew and the ability to differentiate into different cell types. Although the mechanisms orchestrating the biology of SCs are not completely understood, it is suggested that their fate strongly depends on the interactions with their microenvironment, called the niche. Increasing evidence states that the niche, consisting of other non-SCs, the extracellular matrix, and signaling factors, in combination with the intrinsic characteristics of SCs, consistently defines their properties and potential. Within this frame, SCs represent a particularly attractive tool for therapeutic applications and regenerative medicine.

2. Endogenous Opioids Modulate Stem Cell Proliferation and Cell Stress Response

The opportunity to modulate SC proliferation and stress response represents one of the main goals of biological SC research aimed at improving the efficiency of SC transplantation. The following Table shows the major outcomes of studies committed to evaluating the role of endogenous opioid peptides on these SC features (**Table 1**)

Table 1. Effects of endogenous opioids on stem cell proliferation and stress response.

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
Met-enkephalin Morphine (10^{-6} M)		Naloxone (3 × 10^{-6} M)	DOR MOR	NPCs (from EGL of postnatal 5- and 6-day-old mice)	Morphine significantly reduced DNA content; this effect was attenuated by naloxone co-administration. Met-enkephalin did not	[9]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					alter DNA synthesis. Opioids did not affect cell viability.	
Met-enkephalin (10^{-6} or 10^{-5} M)			MOR	hCB-$CD34^+$ and hPB-$CD34^+$ cells	hCB-$CD34^+$ expressed MOR more than hPB-$CD34^+$ cells. In treated hCB-$CD34^+$ cells, phospho-MAPK was increased by 4.7- to 6.1-fold compared to the untreated cells; the increase of phospho-p38 was moderate. In hCB-$CD34^+$, met-enkephalin did not reducethe apoptosis induced by irradiation.	[10]
Dynorphin-A[1–17] Dynorphin-A[2–17] U50,488 (10^{-14} to 10^{-8} M)		Nor-BNI (10^{-6} M)	KOR	NPCs (from 7- to 9-week-old human fetal brain tissue)	Dynorphin-A[1–17] and U50,488 stimulated cell proliferation and migration in a dose-dependent manner.	[11]
Morphine			MOR	NSCs	Theoretical hypothesis: since morphine reduces testosterone levels, increases DHT levels, andover-expresses *p53* gene, it might prevent NSC proliferation.	[12]
Morphine sulfate (10^{-6} to 1.3×10^{-5} M)		Naloxone	MOR	NPCs (from 14-day-oldmouse embryos)	Morphine decreased proliferation of NPCs and induced the caspase-3 activity in a dose-dependent manner. Morphine induced neuronal differentiation of NPCs.	[13]
Nociceptin			NOP	Mouse SSCs and spermatocytes	Nociceptin is an upstream Sertoli cell transcription factor	[14]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					that regulates SSC self-renewal and spermatocyte meiosis.	
Morphine (10^{-4} M)		Naloxone (5×10^{-5} M)	MOR	Rat NSCs	Morphine decreased NSC growth and increased apoptosis. Morphine reduced the secretion of insulin and insulin-like growth factors and downregulated insulin receptor expression.	[15]
DADLE (10^{-7} M)	Serum deprivation	Naltrindole	DOR	hUCB-MSCs	DADLE increased anti-apoptotic Bcl-2, decreased pro-apoptotic Bax/Bad, decreased the activated caspase-3, upregulated PI3K subunit p110γ, and activated Akt. DADLE upregulated the release of anti-inflammatory cytokines (IL-4, IL-10, and TGF-β) and downregulated the secretion of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1).	[16]
DADLE (10^{-7} M)	H_2O_2 (6×10^{-4} M)		DOR	hUCB-MSCs	DADLE increased cell viability, upregulated the anti-apoptotic protein Bcl-2,and suppressed the pro-apoptotic proteins Bax/Bad. DADLE reduced intracellular ROS levels and AP sites. DADLE downregulated UPR genes: *IRE-1α*, *BiP*, *PERK*, *ATF-4*, and *CHOP*.	[17]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
DADLE (10^{-7} M)	H/R induced by $CoCl_2$ (7.5 × 10^{-4} M)	Naltrindole	DOR	hUCB-MSCs	DADLE increased cell viability and reduced intracellular ROS levels. DADLE suppressed mitochondrial complex 1 activity. DADLE upregulated the anti-apoptotic gene *Bcl-2* while downregulating the pro-apoptotic gene *Bax* and UPR genes *PERK*, *IRE-1α*, *BiP*, *PERK*, and *ATF-6*. DADLE upregulated the release of anti-inflammatory cytokines (IL-4, IL-10, and TGF-β) and downregulated the secretion of pro-inflammatory cytokines (TNF-α, IL-6, IFN-γ, and IL-1β).	[18]

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γ, interferon gamma; IL-1β, interleukin 1 beta.

2. Endogenous Opioids modulate Stem Cell Differentiation

The ability to differentiate is one of the most important properties of SCs. The following Table summarizes the results obtained from the studies demonstrating the involvement of endogenous or synthetic opioids in SC commitment and/or differentiation, in particolar neural, hematopoietic, vascular and cardiac stem cell differentiation (**Table 2**).

Table 1. Effects of endogenous opioids on stem cell proliferation and stress response.

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
Neural Differentiation						
DAMGO U69,593 (10^{-7}–10^{-6} M)	RA neuralinduction		KOR-1 MOR-1	ESCs (from mouse blastocyst) ESCs (from ICM of 3.5-day-old mouse)	MOR-1 and KOR-1 were expressed in undifferentiated ESCs and in RA-induced ESC-derived NPCs. Both opioids induced ESC neuronal	[19]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					differentiation activating ERK pathway.	
DAMGO U69,593 (10^{-6} M)	RA neural induction		KOR MOR	ESCs (from mouse blastocyst)	Opioids reduced neurogenesis and astrogenesis in RA-induced ESC-NPCs through p38 MAPK and ERK pathways, respectively. Opioids stimulated oligodendrogenesis via both ERK and p38 signaling pathways.	[20]
DAMGO SNC80 U50,488H (10^{-7}–3×10^{-5} M)			DOR KOR MOR	MEB5 (from 14.5-day-old mouse forebrains)	Only the DOR agonist SNC80 promoted neural differentiation.	[21]
	Neural induction			Human USSCs and BM-MSCs	Neural induction increased enkephalinergic markers (Ikaros, CREBZF, and PENK), especially in USSC-derived neuron-like cells. PDYN expression was enhanced in USSC-derived neuron-like cells.	[22]
Dynorphin-A U50,488H (10^{-6} M)	Neural induction with opioid/ agonist	Nor-BNI (10^{-5} M)	KOR	NSCs (from 8-week-old mouse hippocampus)	NSCs expressed high levels of KOR. Opioid treatment decreased neurogenesis by modulating Pax6/Neurog2/NeuroD1 activities via upregulation of miR-7a expression. Opioid treatment did not alter astrogenesis and oligodendrogenesis. Opioid treatment did not	[23]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					affect proliferation and apoptosis.	
Morphine (10^{-5} M)	Neural induction with opioid			NSCs (from postnatal p0 mouse hippocampus)	Morphine promoted neurogenesis, increased apoptosis, and decreased total cell number during the later stages of differentiation. Morphine increased glutathione/glutathione disulfide ratio and decreased S-adenosylmethionine/S-adenosylhomocysteine ratio.	[24]
Hematopoietic and Vascular Differentiation						
Beta-endorphin (1 to 1000 ng/mL) Dynorphin (1 ng/mL) Leu-enkephalin Met-enkephalin (100 ng/mL)	EP (0.4 U/mL) induced erythropoiesis with opioid			Mouse BM progenitor cells	In the presence of EP, opioids enhanced BM progenitor differentiation into CFU-e.	[25]
TRK820 U50,488H (10^{-5} M)	Vascular induction		KOR	ESstA-ROSA (engineered mouse ESCs)	KOR agonists inhibited EC differentiation and 3D vascular formation in ESC-derived vascular progenitor cells. KOR agonists decreased the expression of Flk1 and NRP1 through inhibition of cAMP/PKA signaling in vascular progenitor cells.	[26]
Met-enkephalin (10^{-14} to 10^{-8} M)			KOR DOR	Mouse BM progenitor cells	Met-enk upregulated the expression of KOR and DOR in BM-derived DCs. Met-enk induced BM-derived DCs to differentiate mainly towards the mDC	[27]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					subtype. Met-enk increased the expression of MHC class II molecules and the release of pro-inflammatory cytokines (IL-12p70, TNF-α).	
Hematopoietic and Vascular Differentiation						
Morphine (10^{-4} M)		Naloxone (10^{-4} M)		Rat NSCs	Morphine reduced survival and clonogenicity, negatively affecting tubulogenesis properties of NSCs by the inhibition of neuro-angiogenesis trans-differentiation.	[28]
Cardiac Differentiation						
Dynorphin-B (10^{-9} to 10^{-6} M)	DMSO 1%		KOR	Mouse ESCs	DMSO increased *PDYN* gene expression and dynorphin-B synthesis and secretion. Dynorphin-B elicited *GATA-4* and *Nkx-2.5* gene transcription and enhanced gene and protein expression of α-MHC and MLC-2V.	[29]
Dynorphin-B (10^{-8} to 10^{-6} M)	Cardiac induction		KOR	GTR1-ESCs (engineered mouse ESCs)	ESC plasma membranes and nuclei expressed KOR-specific opioid binding sites. ESC-derived cardiomyocytes showed an increase in dynorphin-B around the nucleus. Dynorphin-B induced an increase of *GATA-4*,	[30] [31]

Opioids/Agonists	Pre-Treatment	Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
					Nkx-2.5, and *PDYN* gene expressions and promoted cardiogenesis by PKC signaling.	
	HBR cardiac induction (0.75 mg/mL)			GTR1-ESCs (engineered mouse ESCs)	HBR-induced ESC-derived cardiomyocytes enhanced *GATA-4*, *Nkx-2.5*, and *PDYN* gene transcriptions and the intracellular level of dynorphin-B.	[32]
	ELF-MF exposition during cardiac induction (50 Hz, 0.8 m Trms)			GTR1-ESCs (engineered mouse ESCs)	ELF-MF spontaneously induced cardiogenesis, upregulating GATA-4, Nkx-2.5, and PDYN gene expression and enhancing intracellular levels and secretion of dynorphin-B.	[33]
Cardiac Differentiation						
	REAC exposition during cardiac induction (MF of 2.4 and 5.5 GHz)			Mouse ESCs and human ASCs	Both SCs committed to cardiac lineage and exposed to REAC increased the expression of *GATA-4*, *Nkx-2.5*, and *PDYN* gene.	[34] [35]
Dynorphin-B (10^{-7} M)	Cardiac induction			CPCs (from 11.5-day-oldembryonic mouseventricles)	Dynorphin B promoted CPC differentiation into cardiomyocytes.	[36]
Dynorphin-A Dynorphin-B Met-enkephalins Leu-enkephalins (10^{-5} M)	Cardiac induction		DOR KOR	Mouse ESCs	Both DOR and KOR increased during ESC differentiation. Dynorphin-B inhibited *Oct-4* and increased *Nkx-2.5* gene expression. Dynorphin-A, met-enkephalins, and leu-	[37]

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Opioids/Agonists Pre-Treatment Antagonists	Opioid Receptor	Cell Type	Biological Effects	Ref.
			enkephalinsdid not affect ESC differentiation.	

DAMGO, [D-Ala2,MePhe4,Glyol5]-enkephalin; U69,593, N-methyl-2-phenyl-N-[(5R,7S,8S)-7-(pyrrolidin-1-yl)-1-oxaspiro[4.5]dec-8-yl]acetamide; RA, retinoic acid; KOR-1, κ opioid receptor isoform 1; MOR-1, μ opioid receptor isoform 1; ESCs, embryonic stem cells; ICM, inner cell mass; NPCs, neural progenitor cells; ERK, extracellular signal-regulated kinase; p38 MAPK, p38 mitogen-activated protein kinase; SNC80, [(+)-4-[(alphaR)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide]; U50,488H, (–)-trans-(1S,2S)-U-50488 hydrochlo-ride; Nor-BNI, nor-binaltorphimine; DOR, δ opioid receptor; MEB5, multipotent neural stem cells; USSCs, unrestricted somatic stem cells; BM-MSCs, bone mar-row mesenchymal stem cells; Ikaros, IKAROS family zinc finger 1; CREB2, cAMP response element-binding protein 2; ATF, activating transcription factor; ENK, proenkephalin; PZ, PN, prodynorphin; NSCs, neural stem cells; Pax6, paired box 6; Neurog2, neurogenin 2; NeuroD1, neuronal differentiation 1; leu-enkephalin, leucine-enkephalin; met-enkephalin, methionine-enkephalin; EPO, erythropoietin; CFU-e, colony-forming unit-erythroid; TRK820, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[N-methyl-trans-3-(3-furyl) acrylamido]morphinan hydrochloride; EC, endothelial cell; Flk1, fetal liver kinase 1/VEGF receptor 2; NRP1, neuropilin 1; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; DCs, dendritic cells; mDCs, myeloid dendritic cells; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor alpha; IL-12p70, active heterodimer of interleukin 12; p53, tumor protein p53; DMSO, dimethyl sulfoxide; GATA-4, GATA binding protein 4; Nkx-2.5, Nkx homeobox 5; α-MHC, α-myosin heavy chain; MLC-2V, myosin light chain; PKC, protein kinase C; HBR, hyaluronan mixed esters of butyric and retinoic acids; ELF-MF, extremely low frequency magnetic fields; REAC, radio electric asymmetric conveyer; ASCs, adipose-derived mesenchymal stem cells; SCs, stem cells; CPCs, cardiac progenitor cells; Oct-4, octamer-binding transcription factor 4.

3. Conclusion

Overall, opioidergic systems encompass a wide-ranging variety of bioactive peptides, providing multi-layered control of major determinants in cell and SC biology. Compounding their biological complexity, opioid peptides were found to act as “one component–multiple target conductors”, which often led to the observation of opposite effects on the same outcome (i.e., proliferation or differentiation) depending on the specific SC target towards which activity was probed.

Nevertheless, deciphering the complexity of the informational cues associated with opioid peptide-mediated responses may hold promise for intriguing future developments. These future perspectives involve the potential for the timely and synergistic use of naturally occurring and synthetic opioids for the fine tuning of remarkable develop-ments in regenerative medicine, including differentiation, proliferation, multicellular cross talk, inflammation, and tissue remodelling.

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