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Commentary

Duration of opioid receptor blockade determines biotherapeutic response

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ABSTRACT

Historically, studies on endogenous and exogenous opioids and their receptors focused on the mediation of pain, with excess opiate consumption leading to addiction. Opioid antagonists such as naloxone and naltrexone blocked these interactions, and still are widely used to reverse drug and alcohol overdose. Although specific opioid antagonists have been designed for mu, delta, and kappa opioid receptors, the general antagonists remain the most effective. With the discovery of the opioid growth factor (OGF)-OGF receptor (OGFr) axis as a novel biological pathway involved in homeostasis of replicating cells and tissues, the role of opioid receptor antagonists was expanded. An intermittent OGFr blockade by low dosages of naltrexone resulted in depressed cell replication, whereas high (or sustained) dosages of naltrexone that conferred a continuous OGFr blockade resulted in enhanced growth. More than 3 decades of research have confirmed that the duration of opioid receptor blockade, not specifically the dosage, by general opioid antagonists determines the biotherapeutic outcome. Dysregulation of the OGF-OGFr pathway is apparent in a number of human disorders including diabetes, multiple sclerosis, and cancer, and thus opioid antagonist disruption of interaction prevails as a therapeutic intervention. We review evidence that the duration of opioid receptor blockade is correlated with the magnitude and direction of response, and discuss the potential therapeutic effectiveness of continuous receptor blockade for treatment of diabetic complications such as corneal defects and skin wounds, and of intermittent receptor blockade by low dosages of naltrexone for treatment of autoimmune diseases and cancer.

1. Introduction

Opioid receptors were identified first followed by the discovery of endogenous opioids that acted as ligands. Concomitantly, pharmacologists began designing opioid antagonists that blocked the neurotransmitter function of these receptors in brain and gut. Nearly five decades have elapsed since the initial identification of opioid receptors, and two of the original antagonists, naloxone and naltrexone, remain on the forefront of treatment for cancer pain, addiction, drug overdose, alcoholism, and other psychosomatic disorders. The discovery of the non-classical opioid receptor, opioid growth factor receptor (OGFr), that shares several pharmacological properties with mu, delta, and kappa opioid receptors, has led to research that broadens the usefulness of opioid antagonists. Depending on the duration of opioid receptor blockade, opioid antagonists such as naloxone and naltrexone are effective therapies for cancer, autoimmune diseases, and complications associated with diabetes. Understanding the dysregulation of the OGF-OGFr axis in each disease dictates whether continuous blockade to enhance cellular proliferation or intermittent blockade to inhibit cellular proliferation is warranted.

1.1. Opioid receptors

1.1.1. Classical G-protein coupled opioid receptors

Research to discover opioid receptors commenced in the early 1970s when biochemical studies reported that certain drugs

Abbreviations: OGF, opioid growth factor; OGFr, opioid growth factor receptor; NTX, naltrexone; LDN, low dose naltrexone; EAE, experimental autoimmune encephalomyelitis; DAMGO, [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin; DPDPE, d-Pen^2, d-Pen^5-enkephalin; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2; CTAP, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2; VEGF, vascular endothelial growth factor; FGF-2, fibroblast growth factor -2; α-SMA, alpha smooth muscle actin; DMSO, dimethyl sulfoxide; MOG, myelin oligodendrocytic glycoprotein; PLP, proteolipid protein; imp, importin; Kd, dissociation constant; Bmax, maximal binding indicating total concentration of receptors; NLS, nuclear localization signal.

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interacted with specific molecules within different regions in the central nervous system [1–3]. Radiolabeled binding of exogenous opiate agonists such as levorphanol were used to locate and isolate specific binding proteins [4]. Pert and Snyder published a seminal paper on the identification of the binding site for radiolabeled naloxone [1], and eventually identified the mu opioid receptor. Many of the investigations involved nervous tissue, and in rapid succession, mu, delta, and kappa opioid receptors were identified and characterized in the brain or enteric nervous system [2,3]. Two decades later, the molecular structure of these classical opioid receptors was revealed [5–7]. Cloning of the mu, delta, and kappa opioid receptors illustrated that all three receptors are G protein-coupled transmembrane proteins that are members of the subfamily of rhodopsin receptors [7]. The receptors share 60% identity with more than 70% identity in the transmembrane domains and intracellular looping regions. The N terminus is least similar among the 3 receptors, but all have an extracellular domain in the N terminus with glycosylation sites and intracellular loops with multiple amphiphatic α-helixes. All three classical opioid receptors stimulate cAMP accumulation and are blocked by pertussis toxin [8].

1.1.2. Non-classical nuclear membrane-associated opioid receptor

A non-classical opioid receptor, OGFr, was first recognized in the 1980s, and subsequently characterized in both murine neural cancer cells [9] and normal rodent brain tissue [10,11]. The isolated protein was originally termed zeta (ζ) to maintain consistent naming with the Greek symbols of mu, delta, and kappa, and was appropriately called "zeta" for the Greek word zoe", loosely defined as "growth". Concomitantly, other studies were conducted to determine the endogenous opioid involved with this binding protein, and the ligand [Met^5]-enkephalin was identified to have inhibitory growth properties when binding to this receptor. The endogenous peptide was termed opioid growth factor (OGF), to distinguish the neurotransmitter function from that of being an inhibitory growth factor, and the zeta receptor was renamed OGFr. The cDNA for the rat OGFr was cloned by searching expression libraries [12], and subsequently the sequence was identified in human and mouse [13]. Based on extensive biochemical characterization, and cloning, the similarities of classical mu, delta, and kappa opioid receptors with OGFr were in the pharmacology, and not at the molecular level. The open reading frame for human OGFr is 697 amino acids with 8 imperfect repeats of 20 amino acids each at the C terminus. The human OGFr is located on chromosome 20q13.3 [13]. Thus, the molecular and protein structure of OGFr has no resemblance to classical opioid receptors. Based on NMR studies as well as confirmation from websites such as FoldIndex [14], OGFr is an intrinsically unstructured protein with approximately 78% amino acid identity between mouse, rat, and human. In studies on subcellular localization of OGFr using COS-7 African green monkey kidney cells, it has been documented that the receptor has three nuclear localization signals (NLS) within its sequence, two mono-partite NLS$_{383\text{-}386}$ and NLS$_{456\text{-}460}$, and one bi-partite NLS$_{267\text{-}296}$ [15]. Studies utilizing site directed mutagenesis demonstrated when NLS$_{383\text{-}386}$ and NLS$_{456\text{-}469}$ were both mutated the nuclear localization was decreased by 80%, and the regulatory effects of OGF were diminished indicating that the OGF-OGFr action on proliferation is dependent on the ability of OGFr to translocate into the nucleus requiring the presence of NLS, karyopherin β and Ran [15]. Transport of fluorescein-labeled naltrexone was not temperature dependent, and was observed in the nucleus for 48 h (Fig. 1) [15]. Export of OGFr from the nucleus is CRM-1 dependent.

Subcellular fractionation studies using developing rat brain and cerebellum revealed that OGFr binding is associated with the nucleus [9,11,13]. These biochemical studies were confirmed by confocal microscopy studies in the rat cornea that demonstrated immunogold labeling of OGFr in the paranuclear cytoplasm, within the nucleus, and adjacent to heterochromatin in corneal epithelial cells [16]. Colocalized immunog old labeling of OGFr and OGF was detected on the outer nuclear envelope and inside the nucleus [16]. Collectively, these data suggest that the receptor is located on or near the outer nuclear envelope and functions by translocating inside the nucleus with its cargo, the endogenous [Met^5]-enkephalin ligand.

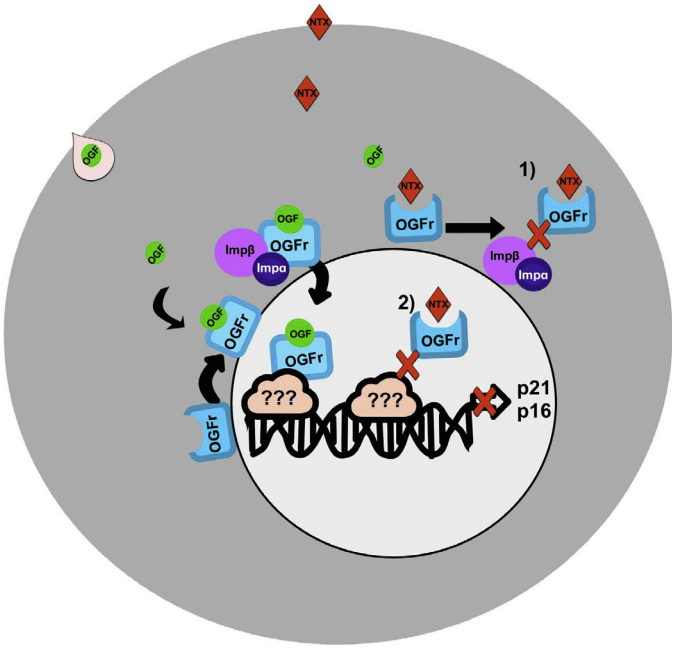

Fig. 1. Nuclear interactions of naltrexone with OGFr in the cytoplasm as well as within the nucleus. Naltrexone binds to OGFr, trafficks into the nucleus and blocks OGF interactions that upregulate p21 and p16 cyclin-dependent inhibitory kinases. Impα = importin α; impβ – importin β; NTX = naltrexone.

The gene and protein for OGFr have been identified in cells and tissues arising from all 3 dermal derivatives [13]. Gene expression for OGFr has been documented in human fetal tissues including brain, liver, lung, and kidney as well as in adult heart, brain, liver, skeletal muscle, kidney, and pancreas [13]. Binding assays on adult and fetal rat brain have quantitated OGFr binding [17], and studies conducted in adult mice demonstrated RNA levels in brain, heart, lung, liver, kidney and skeletal muscle. Additionally, OGFr has been detected in neoplasia, as well as in cell lines derived from human cancers [18–20].

1.2. Opioid receptor antagonists

Opioid antagonists are compounds that competitively bind to opioid receptors with affinity greater than that of specific agonists. However, antagonists have no function other than to block this interfacing. In the case of opioid receptors, agonists are both exogenous compounds such as morphine, codeine, congeners of morphine, and endogenous molecules such as endorphins and enkephalins. The general antagonists were synthesized first to block exogenous opiate interactions, and later were instrumental in research on the isolation of opioid receptors [2].

1.2.1. General opioid receptor antagonists

Opioid receptor antagonists are either general and bind to all classical opioid receptors, or are specific and selective. The two most widely studied opioid receptor antagonists, naloxone and