
Preface

Opioid receptors, beyond their involvement in pain transmission, play a number of relevant physiological roles in the central nervous system and in peripheral organs. Opioid receptors can be considered a crossroads where endogenous opioid peptides and foreign opioids and opiates meet the cell and transmit their messages to another vast array of stimulus–response mechanisms. In recent years, studies on their emerging roles have been favored by numerous and fruitful techniques that have opened new avenues of preclinical and clinical research that demands multidisciplinary approaches.

The post-genomic era has opened up novel opportunities for the exploitation of these novel technologies. As an increasing number of investigators seek to harness the fruits of knowledge in these emerging fields, it is essential that well-tested protocols are made available to researchers. With this in mind, it is apposite to provide a collection of protocols to favor innovative studies on opioid receptors written by experts who are routinely employing these techniques in their laboratories. This book presents the protocols in the stepwise “cookbook” style of this well-known book series along with summaries of state-of-the art methods that have been utilized for understanding opioid receptor functionality at a molecular, cellular, structural, and organism level. *Opioid Receptors: Methods and Protocols* is, hence, an invaluable guide for researchers in the fields of neuroscience, biochemistry, pharmacology, and molecular and structural biology.

Part I of this book (Chapters 1–5) focuses on procedures to evaluate genetics and structural biology of opioid receptors as well as their transcriptional and posttranscriptional regulation. An overview of genetic analysis of opioid receptors with the latest sequencing methods is included (Chapter 1). The recent publication of crystal structures of all the three opioid receptors has been instrumental to the development of computational protocols, designed to estimate thermodynamic and kinetic parameters describing the receptor binding of small molecule ligands and the formation of supramolecular complexes (Chapter 2). Furthermore, techniques for the epigenetic and posttranscriptional analysis of opioid receptor genes are presented (Chapters 3 and 4). Finally, a protocol is dedicated to the use of DNA microarrays and next-generation sequencing methodologies to obtain a transcriptional profile of genes influenced by activation of opioid receptors (Chapter 5).

Part II (Chapters 6–12) illustrates methods for the cellular detection and analysis of opioid receptors. Techniques aimed to monitor the trafficking and interaction of opioid receptors and related signaling molecules are described. Total internal reflection fluorescence microscopy has been used to investigate, in real-time, surface trafficking events of opioid receptors at the single molecule level (Chapter 6). An innovative protocol aimed at investigating opioid receptor internalization and trafficking events in vivo is reported (Chapter 7). Techniques for monitoring heteromerization between opioid receptors and the interaction of opioid receptors and beta-arrestin in living cells by bioluminescence resonance energy transfer are illustrated in Chapters 8 and 9. The study of protein–opioid receptor interactions assists the understanding of biological functions and elucidation of biochemical pathways, and Chapter 10 details procedures to assay the interaction between

protein 14-3-3 zeta and the human kappa opioid receptor by co-immunoprecipitation, pull-down assay, and fluorescence microscopy. Two separate procedures to detect opioid receptors by immunoblot assays in brain areas (Chapter 11) and in peripheral tissues (Chapter 12) are presented.

Part III (Chapters 13–16) covers strategies for the analysis of signaling events modulated by opioid receptors activated by agonist ligands. Following opioid receptor activation, GTP will replace GDP on the α -subunit of the G-protein, leading to a dissociation of the $\beta\gamma$ -subunit. The [^{35}S]GTP γ S autoradiography assay, described in Chapter 13, is useful to monitor opioid receptor activation in discrete brain areas. Chapter 14 describes two real-time fluorescence-based assays of mu-opioid receptor activation by agonists monitoring cell membrane hyperpolarization in AtT-20 cells. These assays may be scaled up for high-throughput screening. The use of imaging assays (Chapter 15) and of the whole cell patch clamp (Chapter 16) to investigate the activation of inwardly rectifying potassium channels and calcium channels by mu- and delta-opioid receptor agonists in cultured mouse dorsal root ganglion neurons are described.

Part IV (Chapters 17–23) covers experimental techniques to investigate opioid receptor-mediated functions at organismal level in a physiological or pathological context. An in vitro skin-saphenous nerve preparation to test the modulatory effects of opioids on the function of cutaneous sensory neurons in experimental models of pain is discussed (Chapter 17), whereas Chapter 18 reports the analysis of cutaneous stimulation-induced sensory input by Von Frey hairs. A protocol to detect drug-stimulated intracellular zinc release in rodent brain slices using time-lapse microscopy and fluorescence imaging is presented in Chapter 19. Chapter 20 provides detailed procedures to measure activation of retinal opioid receptors and to assess their roles in retina neuroprotection by electroretinogram. The immunosuppressive effects mediated by opioids are central to the in vivo activation of opioid receptors, and Chapters 21 and 22 explain strategies towards attaining this objective. Finally, a technique to evaluate the role of opioid receptors in migration and wound recovery in cultured human keratinocytes and fibroblasts is presented in Chapter 23.

Part V (Chapters 24–27) showcases methods for the analysis of behavioral effects induced by opioids. Chapter 24 is an overview on a reinstatement animal model that has contributed to disentangling the mechanisms underlying relapse to opioid-seeking in laboratory animals. This procedure is useful to investigate the neurobiology of relapse. Analysis of heroin-seeking reinstatement in the rat is useful to study the mechanisms underlying relapse to heroin and vulnerability factors that enhance the resumption of heroin-seeking behavior. This protocol is described in Chapter 25. Chapter 26 presents a procedure to investigate the role of opioid receptors in alcoholism by adopting a model that combines chronic ethanol exposure procedures with voluntary ethanol drinking in rodents. Behavioral tests designed to evaluate in pups the activity and involvement of opioid receptors are described in Chapter 27.

I sincerely hope that these protocols will help both experienced and new entrants in this field to carry out their experiments successfully. Finally, I would like to thank all the authors for their outstanding contributions and the series editor, John M. Walker, for valuable editorial help.

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