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REVIEW

The μ-opioid receptor: an electrophysiologist's perspective from the sharp end

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Morphine, the prototypical opioid analgesic drug, produces its behavioural effects primarily through activation of μ-opioid receptors expressed in neurones of the central and peripheral nervous systems. This perspective provides a historical view of how, over the past 40 years, the use of electrophysiological recording techniques has helped to reveal the molecular mechanisms by which acute and chronic activation of μ-opioid receptors by morphine and other opioid drugs modify neuronal function.

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Abbreviations
DAMGO, [D-Ala^2,NMe-Phe^4,gly-ol^5]-enkephalin; GIRK, G protein-activated potassium conductance; GRK, G protein-coupled receptor kinase; INRC, International Narcotics Research Conference; LC, locus coeruleus; Met Enk, methionine enkephalin; PAG, periaqueductal grey region; VTA, ventral tegmental area

This perspective is based on the author's Founders' Lecture delivered at the 2013 International Narcotics Research Conference (INRC). The aim was to review the contribution that electrophysiological recording techniques have made over the past 40 years to elucidating the actions of opioid drugs on neurones of the CNS. This is not intended to be a comprehensive review of μ-opioid receptor (receptor nomenclature conforms to Alexander *et al.*, 2013) pharmacology rather it reflects somewhat the author's scientific journey and so apologies are due to those whose work is not cited.

μ-Opioid receptor activation

Interaction with potassium and calcium channels

In the early 1970s experiments using extracellular recording from brain neurones *in vivo* led to reports such as the following – 'Out of 76 neurones studied, morphine [applied by iontophoresis] increased the firing rate of 33 and depressed that of 17. . . . The remaining 26 neurones were unaffected' (Bradley and Dray, 1974, p. 48). It was the introduction of intracellular recording that enabled more sophisticated analysis of opioid action, first with the use of sharp electrode recording of membrane potential and single-electrode voltage clamp then with patch clamp recording of whole-cell and single-channel currents. In the mid-1970s in Aberdeen, the late Hans Kosterlitz, one of the founders of INRC, with great foresight encouraged Alan North and myself to study opioid action by recording from opioid-sensitive neurones. This led to the observation that activation of μ-opioid receptors resulted in membrane hyperpolarization through opening of potassium channels in guinea pig myenteric plexus neurones (North and Tonini, 1977) and guinea pig and rat locus coeruleus (LC) neurones (Figure 1A; Pepper and Henderson, 1980; Williams *et al.*, 1982).

The opioid-activated potassium conductance in LC neurones was subsequently characterized as inwardly rectifying (North and Williams, 1985) and, as the coupling from receptor to channel is through pertussis toxin-sensitive G-proteins, is now referred to as a G-protein-activated inwardly rectifying potassium conductance (GIRK). We now know from studies in other types of neurones that μ-opioid receptors can couple

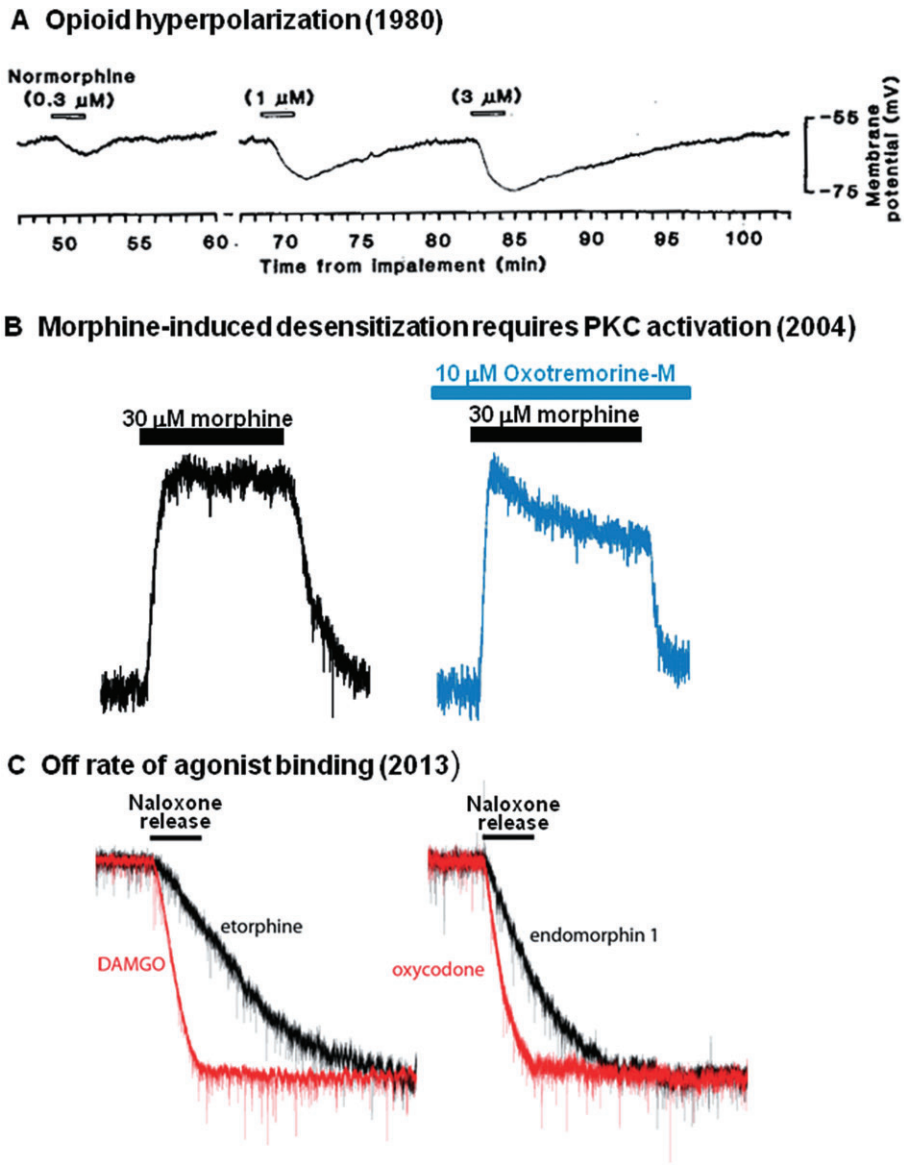

Figure 1

Milestones in electrophysiological studies of μ-opioid receptor function in LC neurones over 33 years (1980–2013). (A) The first published membrane hyperpolarization in response to opioid activation of the μ-opioid receptor in an LC neurone. Reproduced with permission from Pepper and Henderson (1980). (B) Rapid morphine-induced desensitization of μ-opioid receptor-induced GIRK current in LC neurones requires concomitant PKC activation by stimulation of M_3 muscarinic receptors. Reproduced with permission from Bailey *et al.* (2004). (C) Off rate of agonist binding from μ-opioid receptors on LC neurones measured by the decrease in each opioid-evoked GIRK current following local, flash release of naloxone from a caged derivative. Traces supplied by J.T. Williams, Vollum Institute; experimental details are as in Banghart *et al.* (2013). The amplitudes of currents in (B) and (C) have been normalized to facilitate comparison.

to a variety potassium channels including calcium-activated, inwardly rectifying, dendrotoxin-sensitive and M-type channels (for review, see Williams *et al.*, 2001). The relative importance of each opioid-sensitive potassium channel in behavioural responses to opioids remains to be elucidated fully.

Around the same time as studies on the opioid-activated GIRK current were progressing, it was reported that in cultured dorsal root ganglion cells opioids reduced the calcium component of the action potential without activating a potassium conductance (Mudge *et al.*, 1979). However, it was not until some time later that two groups, using whole-cell patch clamp recording, demonstrated that μ-opioid receptor activation resulted in G-protein-mediated inhibition of voltage-activated N-type calcium channels (Schroeder *et al.*, 1991; Seward *et al.*, 1991). P-type and Q/R-types of voltage-activated calcium channel have also now been shown to be inhibited.

Other $G_{i/o}$-coupled receptors couple to the same ion channels as μ-opioid receptors. The question that arose was whether in a cell expressing more than one type of $G_{i/o}$-

coupled receptor do the different receptor types share pools of G-protein and ion channels or do they function independently? This was a long time before we knew of GPCR dimerization and scaffolding proteins. North and Williams (1985) observed in LC neurones that the GIRK currents activated by μ-opioid receptors and α_2-adrenoceptors were non-additive above the maximum μ-opioid receptor response. They did not, however, determine whether it was the pool of G-protein or the pool of GIRK channels that was limiting. More recently, this question has been re-addressed by John Traynor's laboratory studying inhibition of adenylyl cyclase rather than potassium or calcium channels (Levitt *et al*., 2011). They used the 'neuronal' cell line, SH-SY5Y, in which a number of endogenously $G_{i/o}$-coupled receptors, including μ-opioid receptors, δ-opioid receptors and α_2-adrenoceptors, are expressed and share a common pool of adenylyl cyclase and concluded that the limiting factor is the G-protein rather than adenylyl cyclase because δ-opioid receptor activation did not increase GTPγS binding after it had been maximally stimulated with the μ-opioid receptor agonist [D-Ala^2,NMe-Phe^4,gly-ol^5]-enkephalin (DAMGO).

Opioid excitation

One might predict that if opioids activate potassium conductances (which will lead to membrane hyperpolarization) and inhibit calcium entry during action potential firing, which would be expected to inhibit neurotransmitter release, then the predominant response to μ-opioid receptor activation should be inhibitory. Such inhibition is seen at the level of the dorsal horn of the spinal cord and in the LC. However, Nicoll *et al*. (1977) reported that opioids excite hippocampal pyramidal neurones. This gave rise to a flurry of activity as well as to several divergent, contradictory hypotheses on how such excitation was produced (for historical review, see Henderson, 1983). At the time it was difficult to rationalize the various theories because the results reported from different laboratories were often contradictory, a common theme in opioid research. With the passage of time, however, things have become clearer and there is now general agreement that the predominant mechanism by which μ-opioid receptor activation results in excitation, not only in the hippocampus but also in other brain regions important in the analgesic and euphoric actions of opioids [i.e. the periaqueductal grey region (PAG) and ventral tegmental area (VTA)], is by disinhibition whereby opioids act on μ-opioid receptors located on inhibitory interneurones (usually GABAergic interneurones) and reduce inhibitory tone resulting in apparent excitation of the output neurone (Johnson and North, 1992; Vaughan and Christie, 1997).

Opioid inhibition of transmitter release

μ-Opioid receptor activation results in inhibition of the release of numerous neurotransmitters from nerve terminals in both the peripheral and central nervous systems. There has been much debate about the relative importance of potassium channel activation and inhibition of voltage-activated calcium channels in presynaptic $G_{i/o}$-coupled receptor-mediated inhibition of neurotransmitter release (see e.g. Shen and Surprenant, 1990; Vaughan and Christie, 1997; Vaughan *et al*., 1997). It would appear that μ-opioid receptor activation can also directly inhibit the neurotransmitter release machinery independent of any effect on membrane conductances (Capogna *et al*., 1993) given that μ-opioid receptor activation reduced the miniature GABAergic inhibitory synaptic currents evoked by the calcium ionophore ionomycin, that is, by direct calcium entry into the nerve terminals (Capogna *et al*., 1996; Bergevin *et al*., 2002).

Endogenous opioid peptide activation of μ-opioid receptors

In many areas of the CNS opioid peptide-containing nerve terminals can be seen to form axo-dendritic as well as axo-axonic synapses. In the LC methionine enkephalin (Met Enk)-containing nerve terminals form synapses on to tyrosine hydroxylase-containing dendrites (Pickel *et al*., 1979; Van Bockstaele *et al*., 1995). In addition the LC receives a β-endorphin-containing input from neurones whose cell bodies lie in the arcuate nucleus. Although it has been reported that stimulation of the arcuate nucleus *in vivo* produces a naloxone-sensitive inhibition of neuronal firing in the LC (Strahlendorf *et al*., 1980), several investigators studying synaptic transmission in LC slices have failed to observe any endogenous opioid-mediated inhibitory postsynaptic responses (see e.g. Egan *et al*., 1983). This has been mirrored in studies of other opioid peptide containing brain regions.

In contrast, in a number of brain regions including hippocampal CA1 region and dentate gyrus (see Simmons and Chavkin, 1996) and the amygdala (E. Bagley, pers. comm.), endogenous opioid peptides released on nerve stimulation have been shown to act at presynaptic μ-opioid receptors as well as δ- and κ-opioid receptors to inhibit the release of other neurotransmitters. This is not to say that endogenous opioids only mediate presynaptic inhibition but, similar to nicotinic and P2X ligand-gated ion channels in the CNS, it would appear that presynaptic effects may predominate (Khakh and Henderson, 2000)

Tolerance and dependence

Following two decades of studying primarily the acute actions of opioids on brain neurones, the focus of much electrophysiological research on opioids moved on to studying adaptive responses that occur as a result of long-term activation of the μ-opioid receptor. Such studies have gone some way to elucidating the adaptive changes that underlie opioid tolerance and physical dependence, but many questions remain unanswered.

μ-Opioid receptor desensitization

One advantage of electrophysiological recording is that it provides real-time readout of receptor–effector coupling during prolonged agonist application (Figure 1B). Another is that it allows comparison between different, sometimes small populations of neurones that would be difficult with a technique such as GTPγS binding. However, unlike GTPγS binding assays, changes in μ-opioid receptor coupling to ion channels

can occur at the receptor, G-protein or ion channel level and care must be taken to determine which of these components has been altered.

Numerous kinases have been implicated in neuronal μ-opioid receptor desensitization and opioid tolerance including G protein-coupled receptor kinases (GRKs), PKC isoforms, JNK and ERK. The exact roles of each kinase and the mechanisms by which they contribute to μ-opioid receptor desensitization have still to be worked out. For a detailed discussion of the evidence for the involvement of each of these kinases in μ-opioid receptor desensitization and opioid tolerance, readers are referred to the extensive review by Williams *et al*. (2013). Here I will focus primarily on two: PKC and GRK.

Although highly effective in producing analgesia and respiratory depression, in the whole animal morphine has lower agonist intrinsic efficacy at μ-opioid receptor than drugs such as methadone, fentanyl and DAMGO (McPherson *et al*., 2010). In LC neurones *in vitro* morphine induced much less μ-opioid receptor desensitization than higher efficacy opioid agonists (Alvarez *et al*., 2002; Bailey *et al*., 2003). The level of morphine-induced desensitization could be enhanced by concomitant activation of PKC either indirectly by stimulation of G_q-coupled M_3 muscarinic receptors on LC neurones (Figure 1B) or directly with a phorbol ester (Bailey *et al*., 2004). In both trigeminal and nucleus accumbens neurones morphine activation of μ-opioid receptors results in enhanced PKC activity (Chen and Huang, 1991; Martin *et al*., 1997), but in LC neurones there was no such direct μ-opioid receptor-mediated enhancement of PKC (Oleskevich *et al*., 1993) and to observe morphine-induced μ-opioid receptor desensitization PKC activity had to be increased by other means. This may be due to differential expression of PKC isoforms in different neuronal populations – the isoform responsible for μ-opioid receptor desensitization in LC neurones is PKCα (Bailey *et al*., 2009a) – or PKC activity being low in LC neurones when they are in a brain slice, rather than the brain *in vivo*. It would be of interest to determine whether morphine induces a PKC-dependent desensitization of μ-opioid receptors in trigeminal and nucleus accumbens neurones without the requirement for additional PKC activation. Whether PKC directly phosphorylates the μ-opioid receptor to enhance desensitization or acts indirectly to facilitate some other desensitization mechanism still needs to be determined (see Bailey *et al*., 2006; Johnson *et al*., 2006).

For high-efficacy μ-opioid receptor agonists, there has been a tendency in the literature to conflate the processes of μ-opioid receptor desensitization and trafficking (both internalization and reinsertion into the plasma membrane) by assuming (i) that desensitization and internalization will occur sequentially by the same mechanism and (ii) that GRK phosphorylation and arrestin binding induces μ-opioid receptor desensitization as well as internalization. Although there is little doubt that a GRK- and arrestin-dependent mechanism is involved in μ-opioid receptor-mediated ERK signalling as well as in its trafficking in response to occupancy by high-efficacy agonists such as DAMGO and Met Enk in recombinant expression systems, the role of GRKs and arrestins in μ-opioid receptor desensitization in neurones by such drugs is still contentious (see Williams *et al*., 2013). In electrophysiological studies of CNS neurones, various experimental approaches have been used by different investigators to inhibit GRK activity (e.g. intracellular perfusion with peptide and small-molecule inhibitors, viral overexpression of dominant negative mutant GRKs and transgenic modification of specific GRKs to render them sensitive to chemical inhibition) and these have provided contradictory results. Endomorphin-2, which has similar low-agonist efficacy to morphine in GTPγS binding assays (McPherson *et al*., 2010), induces μ-opioid receptor desensitization in LC neurones in the absence of PKC activation (Rivero *et al*., 2012), suggesting that agonist efficacy for G-protein activation is not the determinant of which desensitization pathway an agonist will induce. That endomorphin-2 is an arrestin-biased opioid agonist (Rivero *et al*., 2012) could be taken to indicate that arrestin binding is involved in μ-opioid receptor desensitization. Unconditional arrestin3 knockout, however, has been reported to leave DAMGO-induced μ-opioid receptor desensitization unaffected in sensory neurones (Walwyn *et al*., 2007) and LC neurones (Arttamangkul *et al*., 2008), but there may be other confounding effects of arrestin3 knockout (Mittal *et al*., 2012). Also, knockout of only one form of arrestin may not be sufficient to attenuate desensitization if both arrestins can bind to agonist-activated μ-opioid receptors (Groer *et al*., 2011). Furthermore, Dang *et al*. (2009) have suggested that there are in fact two mechanisms underlying high-efficacy agonist-induced μ-opioid receptor desensitization in LC neurones, a GRK component and an ERK component. Both need to be inhibited concomitantly to reduce Met Enk-induced desensitization (i.e. there is redundancy).

Presynaptic μ-opioid receptors

Until very recently, intracellular recordings were invariably made from relatively large and easily imaged cell somata rather than from small nerve terminals as the somata were more readily accessible to sharp and patch electrodes. This has made the study of μ-opioid receptors located on nerve terminals, the ones important for inhibition of neurotransmitter release, more difficult. Recording the characteristics of spontaneous and evoked synaptic responses in the postsynaptic cell does, however, give a measure of neurotransmitter release, which can be used to study the effect of activating presynaptic μ-opioid receptors. In elegant studies of opioid inhibition of synaptic transmission, several groups have observed that presynaptic μ-opioid receptors on GABAergic terminals in the PAG (Fyfe *et al*., 2010) and VTA (Lowe and Bailey, 2015) as well as those on the terminals of β-endorphin-containing arcuate neurones (Pennock and Hentges, 2011; Pennock *et al*., 2012) do not desensitize in response to acute agonist activation, whereas those on the somata of the same neurones do desensitize. Why this should be is still unknown but a likely explanation would seem to be expression of essential components of the acute desensitization mechanism(s) in the soma but not in nerve terminals. Although μ-opioid receptors on nerve terminals do not exhibit desensitization on acute exposure to morphine, there is a loss of μ-opioid receptor function following chronic morphine exposure (i.e. tolerance develops). The very recent description of a technique by which patch clamp recordings can be made from nerve terminals of cultured neurones (Novak *et al*., 2013) is an exciting development that will facilitate studies of nerve terminal μ-opioid receptor function.

Morphine tolerance

On repeated or prolonged exposure to opioids, tolerance develops. This can be observed not only in intact animals with μ-opioid receptor-mediated antinociception and respiratory depression but also at the level of individual neurones.

Building on results from *in vivo* experiments that had demonstrated that morphine antinociceptive tolerance was reversed by PKC inhibition (Smith *et al.*, 2002; 2007), we observed that cellular tolerance in LC neurones either following prolonged exposure of brain slices to morphine *in vitro* or in brain slices taken from morphine-treated animals was reversed by PKC inhibition (Bailey *et al.*, 2009b). Cellular tolerance, unlike rapid morphine-induced μ-opioid receptor desensitization (see above), did not require PKC activity to be enhanced. The PKC-mediated cellular tolerance was due to a loss of μ-opioid receptor function indicating that μ-opioid receptor desensitization contributes to opioid tolerance. More recently, Levitt and Williams (2012) have shown that there are in fact two components to cellular tolerance to morphine in LC neurones, a PKC-mediated, rapidly reversible component and a second component that does not reverse rapidly on removal of morphine. The second component of cellular tolerance may be responsible for the tolerance observed in nerve terminals. The mechanism underlying this second component of tolerance has not yet been elucidated. In a historical context, it is interesting to note that back in 1975 Brian Cox observed that tolerance to the antinociceptive effect of morphine *in vivo* consisted of a large, rapidly reversing component and a second, smaller and more sustained component (Cox *et al.*, 1975).

Opioid withdrawal mechanisms

Early in the 1970s the first observations were made of the μ-opioid receptor being negatively coupled to AC resulting in decreased production of cAMP. Thereupon the late Harry Collier, another founder of INRC, postulated that opioid withdrawal might result from the opposite of the acute response, that is, a rebound increase in the production of cAMP. This was some time before the discovery of forskolin, and so to increase cAMP levels in the brain, he and his colleagues used theophylline to inhibit cAMP breakdown by PDE. They observed that in naïve rats (i.e. non-morphine treated) theophylline administration reproduced some of the symptoms associated with morphine withdrawal (Collier *et al.*, 1974). Soon after, using an *in vitro* neurochemical approach, Sharma *et al.* (1975) demonstrated in NG108-15 cells that morphine withdrawal resulted in a rebound increase in cAMP production that resulted from superactivation of AC. It might appear churlish to some to point out that the NG108-15 cells used in this latter study express only δ-opioid receptors and so the effect observed, which has had a major impact on our understanding of the mechanisms underlying physical dependence, was actually made examining morphine's actions on δ- not μ-opioid receptors. The authors of the original article were not to know that, however, as the δ-opioid receptor was not discovered until 2 years after the publication of their paper.

Over the years it had been observed *in vivo* that opioid withdrawal resulted in increased neuronal excitability and enhanced neurotransmitter release, but it took 30 years for the link between opioid withdrawal-induced AC superactivation and increased neuronal excitability and enhanced neurotransmitter release to be revealed at the molecular level. Recording from PAG neurones in brain slices from mice chronically treated with morphine, Bagley *et al.* (2005) observed that opioid withdrawal increased the GABA transporter-1 cation current and that this resulted from enhanced PKA activity.

Is there a future?

Readers may wonder whether after 40 years there are still scientific questions about μ-opioid receptor function to be answered using electrophysiological recording techniques. What a stupid idea, of course there are! The combination of electrophysiological recording with fluorescence and confocal imaging as well as optogenetic techniques provides an even greater experimental power with which to study opioid responses on mature neurones. Also, being able to record from small neuronal entities such as nerve terminal varicosities and dendritic spines will provide fascinating insights into receptor function.

Even the LC, which has been very extensively studied with regard to μ-opioid receptor function, still has its uses. Recently, Banghart *et al.* (2013) reported on the off rate of binding of opioid agonists from μ-opioid receptors endogenously expressed on intact LC neurones in an extracellular environment designed to replicate the brain *in vivo* (Figure 1C). No longer do we have to extrapolate from studies performed on membrane fragments in Tris buffer lacking sodium ions.

An exciting area that I have not even touched on in this perspective has been the adaptive changes in synaptic efficacy that occur in response to drugs of abuse such as stimulants and opioids. Acute and chronic drug administration alters both long-term potentiation and long-term depression in various areas of the brain including the VTA (for review, see Lüscher and Malenka, 2011). Such changes are likely to contribute to the intensity of the memory of the drug experience and relate to craving and relapse (Xia *et al.*, 2011; Van den Oever *et al.*, 2012). There is much still to be understood in this area.

Opioid addicts are notorious polydrug users often taking alcohol, benzodiazepines, cocaine and other drugs in addition to opioids. The interaction between opioids and these other drugs is an under-researched area. We have recently observed that relatively low amounts of ethanol can reverse tolerance to morphine at both the cellular and whole animal level (Hull *et al.*, 2013; Llorente *et al.*, 2013), a finding that may have significance in regard to the frequency with which ethanol and opioids are found in the bloodstream of subjects who have died from acute overdose.

How changes in gene expression induced by chronic opioid exposure alter neuronal function and contribute to psychological and physical dependence as well as to tolerance are subjects that are only beginning to be investigated. Adaptive changes at the level of synaptic remodelling and neurone–glia interactions might be refractory to access by electrophysiological techniques alone – an important illustration to any budding electrophysiologist of the limitations of

the single experimental technique approach in today's science.

It was an honour for me to deliver the INRC Founders' Lecture in 2013. I attended my first opioid scientific meeting in Aberdeen in 1971 as a young graduate student and had the pleasure of meeting several of the venerable scientists who founded the INRC a few years later. Perusing the proceedings of that meeting (Kosterlitz *et al.*, 1971) reminded me of just how far our understanding of opioid pharmacology has advanced since that time. It would be a brave person who would try to predict what new discoveries will be made over the next 40 years.

Ultimately, such discoveries need to be harnessed and used to solve the clinical and social problems surrounding opioids by facilitating the development of new therapeutics. Remifentanil, tapentadol, alvimopan and Subutex®, although useful additions to the pharmacopeia, hardly represent quantum advances or an adequate return for the time, effort and resources that has been invested. Heterodimer-selective ligands, biased ligands and allosteric modulators offer new hope. The elucidation of the crystal structures of the opioid receptors represents a new dawn that will provide further stimulus for research and drug development.

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Conflict of interest

The author has no conflicts of interest.

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