REVIEW

Spinal Reflexes and Windup *In Vitro*: Effects of Analgesics and Anesthetics

Ivan Rivera-Arconada, Carolina Roza & Jose A. Lopez-Garcia

Department of Systems Biology, University of Alcalá, Alcalá de Henares, Madrid, Spain

Keywords

Biological markers; Electrophysiology; Pain.

Correspondence

J. A. Lopez-Garcia, Dpt. Biología de Sistemas,
Edificio de Medicina,
Universidad de Alcalá, Alcalá de Henares,
28871 Madrid, Spain.

Tel.: +34-91-8855106;

Fax: +34-91-8854807;

E-mail: josea.lopez@uah.es

Received 25 June 2015; revision 31 July 2015;

accepted 5 August 2015

SUMMARY

The spinal cord is the first relay center for nociceptive information. Following peripheral injury, the spinal cord sensitizes. A sign of spinal sensitization is the hyper-reflexia which develops shortly after injury and can be detected in the isolated spinal cord as a “memory of pain.” In this context, it is easy to understand that many analgesic compounds target spinally located sites of action to attain analgesia. *In vitro* isolated spinal cord preparations have been used for a number of years, and experience on the effects of compounds of diverse pharmacological families on spinal function has accumulated. Recently, we have proposed that the detailed study of spinal segmental reflexes *in vitro* may produce data relevant to the evaluation of the analgesic potential of novel compounds. In this review, we describe the main features of segmental reflexes obtained *in vitro* and discuss the effects of compounds of diverse chemical nature and pharmacological properties on such reflexes. Our aim was to compare the different profiles of action of the compounds on segmental reflexes in order to extract clues that may be helpful for pharmacological characterization of novel analgesics.

doi: 10.1111/cns.12446

Introduction

The interest of modern science for the spinal cord is mostly due to the comparative simplicity of its structure and its implication in somatosensory and motor events. In later years, the interest has grown due to the implication of the cord in nociceptive processing, central sensitization, and analgesia. *In vitro* preparations of the cord were first assayed in frogs and then in rats [1]. Later on, the *in vitro* spinal cord from rats and mice has been exploited in a number of laboratories around the world.

Technically speaking, the development of the preparation has been marked by the difficulties involved in maintaining a relatively thick piece of tissue adequately oxygenated. To overcome this difficulty, many laboratories use hemisected preparations obtained from neonate rats. Mice pups have a smaller cord that may survive without hemisection. The extraction process is performed under anesthesia in a cool saline environment, while the recording temperature is usually kept under 25°C. Although these conditions are not strictly physiological, intra- and extracellular recordings obtained from dorsal and ventral neurons still retain many traits of similar elements of the adult cord [2].

Segmental reflexes are obtained from a ventral root in response to electrical stimulation of the dorsal root corresponding to the same segment. It is very common to use lumbar segments which subserve motor and sensory functions related to the skin and muscles of the hind paws. Ventral root responses should reflect accurately the segmental integration of sensory inputs to produce

motor reflexes. In fact, segmental spinal reflexes should be considered as the basic neural substrate for somato-motor reflexes such as studied *in vivo*, that is, withdrawal reflex.

We believe that segmental reflexes can be used to study drug effects on spinal sensory processing. We are conscious that motor responses do not always reflect faithfully a sensory event and that certain compounds may depress sensory function without altering motor responses [3]. However, this is a relatively rare observation that can be detected by the use of specific electrophysiological indicators.

Structure of Segmental Reflexes

The motor response recorded from a ventral root to electrical stimulation of the dorsal root depends on stimulus intensity. Electrical stimuli of high intensity (C-fiber intensity) activate all fibers in the root including A- and C-fiber types. Low-intensity stimuli close to threshold activates large A fibers (A-fiber intensity). Ventral root responses to these stimuli have a characteristic and well-defined shape in which several components can be identified (see Figure 1). Responses from rats and mice are essentially similar although cords from mice are more active at producing spiking activity.

The first component to appear is the ventral root reflex or monosynaptic reflex (MSR; Figure 1A). This has a very short latency (5–15 ms), it is brief in time (4–5 ms) and it has high amplitude that may reach up to 10 or 12 mV under favorable

Figure 1 (A) shows the high amplitude and low latency monosynaptic reflex following the stimulus artifact (black dot). The amplitude of the MSR only increases slightly from A-fibre to C-fibre intensity stimuli. (B) shows the same responses in a slower time scale to show the ventral root potential. Note that both A-fibre and C-fibre stimuli elicit ventral root potentials of different magnitudes. Original recordings in (C) show responses elicited by repetitive stimulation of the dorsal root at intensities activating A and C fibers. With high-intensity stimulation (right), a cumulative depolarization can be observed in DC mode (lower panel), as well as a windup of action potentials in AC mode (upper panel). A-fibre intensity stimulation applied at the same frequency failed to elicit windup (left). Large vertical lines at regular intervals reflect stimulus artifacts.

recording conditions. This peak reflects the synchronous firing of a population of motor neurons driven by the activation of thick myelinated fibers of intrafusal origin. The MSR underlies the stretch muscle reflex which is not directly observable in small laboratory animals but is essential for a good motor performance. The MSR is elicited by A- and C-fiber intensity stimuli, and it usually reaches maximum amplitude at stimulus intensities within the A-fiber range. The MSR is insensitive to NMDA receptor blockade but very sensitive to AMPA receptor blockers [4,5].

Following the ventral root reflex, there is a prolonged wave of smaller amplitude called the ventral root potential or polysynaptic reflex (PSR) which consists of two components. There is a slow wave starting at the decay of the MSR and lasting for 3–4 s when recording in DC mode (Figure 1B). This is polysynaptic in origin and depends on the activation of C fibers of various sizes as well as A fibers. When recording in AC mode with appropriate filtering settings, it is possible to detect the firing of asynchronous spikes typically in bursts during the first seconds of this wave. To elicit a strong slow wave, C-fiber intensity stimuli are required; however, a shorter and lower amplitude slow wave can be elicited by the activation of A fibers only. In clear contrast with the monosynaptic reflex, the slow wave is sensitive to NMDA receptor blockade [4,5].

The second wave of the ventral root potential is a very slow wave which is impossible to detect unless full DC recordings are used. This is a small amplitude tail potential that may add another 15–20 s to the duration of the response. It is not common to observe spike firing during this wave. These very slow waves only appear in response to high-intensity stimuli and have been shown to be sensitive to the blockade of NK receptors [6].

Windup in Response to Repetitive Stimulation

The repetitive application of high-intensity stimuli to the dorsal root at low frequency (0.5–2 Hz) tends to produce a summation of the response giving rise to the phenomenon of windup. This was first described by Mendell & Wall [7] in dorsal horn neurons using an acute preparation. It was soon realized that windup was also evident in ventral horn neurons, and reflexes but it took a while before the first studies on windup were obtained in the *in vitro* spinal cord with suction electrodes [8]. These first studies were performed with DC recordings which demonstrated a summation of slow waves during repetitive stimulation which was called “cumulative depolarization.” Our double AC and DC recordings showed that it was possible to identify a proper spike windup as well as the underlying cumulative depolarization from a ventral root in response to repetitive dorsal root stimulation (Figure 1C) [3]. Both the cumulative depolarization and the spike windup are signs of the same phenomenon, although the physiological relevance of spike windup is more evident.

Windup and central sensitization are not equivalent terms [9]. Windup may be a model for the first pretranscriptional events that may take place during the induction of sensitization. However, windup is a phenomenon exclusively dependent on the activation of C fibers. Application of repetitive A-fiber intensity stimulation tends to produce wind down or a conservation of the response but not windup (see Figure 1C).

The cumulative depolarization produced by repetitive stimulation is sensitive to the blockade of NMDA and NK receptors [10,11] suggesting that the long-lasting components of the single

reflexes are essential to build a summation and generate a windup response.

Treatments that induce inflammation and long-lasting pain may cause central sensitization which is seen in the form of large scale cumulative depolarization to standard C-fiber intensity or increased spike windup. These maneuvers as well as certain pharmacological compounds may amplify responses to A-fiber intensity stimuli to produce windup-like phenomena [12,13].

Physiological Modulation of Spinal Reflexes

As already explained, information transfer from primary afferents to spinal cord neurons is largely dependent upon glutamate release and the consequent activation of a variety of receptors: AMPAR, NMDAR, metabotropic glutamate receptors (see [4] for a review). In addition, a fraction of unmyelinated afferents may release other transmitters such as tachykinins either as a primary transmitter or as coadjuvants released during intense stimulation [14].

The spinal processing of peripheral signals involves the integration of afferent inputs, with descending modulating inputs mainly from brainstem and midbrain nuclei. The typical effectors of these descending pathways are the monoamines serotonin and norepinephrine. Less information is available about other transmitters of these pathways [15]. In addition to these substances, a number of other compounds of endogenous origin may modulate spinal processing, that is, GABA, neurotrophins, and opioids. The effects of such compounds on spinal processing have been intensively studied with a variety of techniques (see [15] for a review). It is interesting to note that many analgesics in use exploit mechanisms of action based on these basic compounds and their receptors.

Succinct data about the effects of endogenous modulators on segmental reflexes are given in Table 1 for comparative purposes.

Modulation of Spinal Reflexes by Analgesic Compounds

There are many compounds of different chemical origins that possess analgesic properties and are used for specific clinical conditions. Other compounds produce analgesia as well as other nonrelated effects such as sedation, anxiolytic, or myorelaxant actions. Here, we examine the effects of several well-characterized analgesic compounds with central effects on segmental reflexes. Table 1 summarizes the data of the major compounds presented here.

Opioids

Endogenous opioids are a family of peptide neurotransmitters that act via three different G protein-coupled receptors (μ -, δ - and κ -opioid receptors). All three receptors are expressed in the spinal cord where they can be found at pre- and postsynaptic loci. Opioids act by limiting neurotransmitter release from afferents and hyperpolarizing postsynaptic dorsal horn neurons [16].

Morphine has been reported to produce consistent inhibitory effects on ventral root responses evoked by C-fiber intensity stimulation of the corresponding dorsal root at concentrations in the

nM range [17–22]. Similarly, the firing associated to the polysynaptic reflex was maximally reduced by 1 μ M morphine [23]. The MSR was mainly unaffected in these studies although small amplitude reductions are reported for high concentration applications of morphine [19–21]. Morphine applied in the nM range produces clear depressant effects of the cumulative depolarization and the associated windup [21].

The endogenous opioids Met5-enkephalin and dynorphin have been shown to reduce the PSR [17]. Other endogenous μ -opioid ligands, such as endomorphin EM-2, reduced significantly the C-fiber-dependent PSR although EM-1 was ineffective [23]. Different κ -opioid agonists such as enadoline and U69-593 reduced the PSR applied in the nM range [18,20]. Enadoline had no effect on MSR nor on the A-fiber induced PSR [20]. The synthetic and short-lasting opioid alfentanil had no effect on MSR but depressed the slow depolarization with an EC₅₀ of 2 nM [24]. Synergic effects of these compounds with NMDA antagonists, benzodiazepines, and anesthetics have been reported [18,24,25].

Clonidine and Other Alpha-2 Adrenergic Agonists

In the spinal cord, clonidine mimics the actions of descending inhibitory fibers by acting through alpha-2 adrenergic receptors. Epidural administration of clonidine is used for postoperative and intractable pain. Clonidine had an inhibitory effect in both the A- and C-fiber-mediated ventral root responses applied in the nM range, but the amplitude of the MSR was stable even at μ M concentrations [19,21,26,27]. Applied at low concentrations, clonidine had a strong depressant effect on windup [21].

Dexmedetomidine, an alpha-2 adrenergic agonist, inhibited the C-fiber induced depolarization at ~10 nM, but at high concentrations produced a reduction in the MSR [26,28]. Tizanidine also reduced the C-fiber induced depolarization (EC₅₀ 135 nM) without affecting the MSR [29].

Pregabalin

Pregabalin interacts with the $\alpha 2\delta$ accessory subunits of voltage-dependent calcium channels. It decreases neurotransmitter release from the central terminals of primary afferents. Besides its anticonvulsant effects, it has become a first-line drug for certain types of neuropathic pain such as postherpetic neuralgia and diabetic neuropathy [30]. Pregabalin applied at μ M concentrations had an inhibitory effect on both the A- and C-fiber-mediated ventral root slow wave, but the amplitude of the MSR was unaffected even at the largest concentrations used. It also has inhibitory effects on windup which is obvious from very low concentrations [21].

Duloxetine

Duloxetine acts as a reuptake inhibitor for serotonin and norepinephrine. Besides its role as an antidepressant, it has become a first choice treatment for diabetic neuropathy and fibromyalgia [31]. When tested on spinal cord reflexes recorded from neonatal mice, it reduced the A- and C-fiber-mediated PSR and caused a concentration-dependent inhibition on windup. These effects occurred in the absence of MSR depression; however, large

Table 1 Summary of effects of key compounds on the components of segmental reflexes

Drug/Compound	Mechanism of action	Category	MSR	A-fiber induced slow wave	C-fiber induced slow wave	Windup/cumulative depolarization	Refs
Serotonin	5-HT1A,B 5-HT2,3	Endogenous modulator	Depressant	Depressant	Depressant	n.t.	[55]
Noradrenalin	α 1A; α 2	Endogenous modulator	No effect	n.t.	Depressant	Depressant/Excitatory	[3]
GABA	GABA-A, GABA-B and GABA-C receptors agonist	Endogenous modulator	No effect (up to 500 μ M)	Depressant 500 μ M	Depressant 500 μ M	n.t.	[56]
BDNF	Trk-B receptor agonist	Endogenous modulator	n.t.	Slight increase 2 – 1000 ng/mL	Increase 2 – 1000 ng/mL	n.t.	[57,58]
Morphine	Opioid receptors (μ , δ , κ)	Analgesic	Depressant ($\geq$ 10 μ M)	Depressant EC ₅₀ 99 nM	Depressant EC ₅₀ 79 nM EC ₅₀ 90 nM 200 nM	Depressant ~EC ₅₀ 10 nM	[17–21,23]
Clonidine	α 2-adrenergic receptor	Analgesic/Hypertension/Withdrawal symptoms from narcotics	No effect	Depressant EC ₅₀ 9 nM	Depressant EC ₅₀ 25 nM	Depressant ~EC ₅₀ 10 nM	[19,21,26,27]
Duloxetine	5-HT and NE reuptake	Analgesic/Antidepressant	Depressant ($>$ 10 μ M)	Depressant 10 μ M	Depressant 10 μ M	Depressant ~EC ₅₀ 10 μ M	[21]
Pregabalin	Ca _v channels	Analgesic/ Anticonvulsant	No effect	Depressant 10 μ M	Depressant 10 μ M	Depressant ~EC ₅₀ 10 μ M	[21]
Ketoprofen	COX-2 inhibitor	Antiinflammatory/ Mild Analgesic	No effect	Depressant (~300–500 μ M)	Depressant (~300–500 μ M)	n.t.	[34,35]
Indomethacin	COX-1 inhibitor	Antiinflammatory/Mild Analgesic	n.t.	No effect	No effect	No effect	[33]
Meloxicam	COX-2 inhibitor	Antiinflammatory/Analgesic	n.t.	No effect	Depressant 100 μ M	Depressant 100 μ M	[33]
Diazepam	GABA-A receptor benzodiazepine site modulator	Anxiolytic/Sedative	No effect (up to 3 μ M)	No effect (up to 3 μ M)	Depressant 1–2 μ M	n.t.	[27,49,59]
Sevoflurane	GABA-A R Glyc R NMDA R K ⁺ Channels	Anesthetic	Depressant EC ₅₀ 0.2–1.2 mM	n.t.	Depressant EC ₅₀ 72 μ M	Depressant EC ₅₀ 98 μ M	[37,38,42]
Propofol	GABA-A R Na ⁺ channels	Anesthetic	No effect (up to 10 μ M)	Depressant 0.5–10 μ M	Depressant 0.5–10 μ M	Depressant 1–10 μ M	[38,43]
Lidocaine	Na ⁺ channel blocker	Local/regional anesthetic	Depressant EC ₅₀ 175 μ M	No effect (up to 60 μ M)	Depressant 3–60 μ M	Depressant 60 μ M	[46–48]
Retigabine	Neuronal Kv7 channels opener	Anticonvulsant/ Analgesic (PHN)*	No effect (up to 10 μ M)	Depressant 10 μ M	Depressant 1–10 μ M	Depressant 1–10 μ M	[51]

*Postherpetic Neuralgia, phase IIa study 2011. n.t. nontested.

concentrations of the compound may lead to a complete abolition of this reflex [21].

NSAIDs

All members of the large family of nonsteroidal anti-inflammatory drugs (NSAIDs) exert their actions by inhibiting the cyclooxygenase (COX) enzyme and hence reducing prostaglandin production. While both isoforms (COX-1 and COX-2) are constitutively expressed in the spinal cord, COX-2 levels are upregulated by peripheral inflammation [32].

Meloxicam, selective for the COX-2 isoform, has both analgesic and antiinflammatory effects. When tested in the neonatal rat spinal cord, the responses to low-intensity stimulation were unaffected by the compound in naïve- and carrageenan-inflamed rats, but the responses to high threshold stimulation were significantly reduced in both groups. The windup was concentration dependently inhibited to the same degree in spinal cords from naïve and inflamed neonatal rats. On the other hand, indomethacin, a COX-1 preferring NSAID, had no significant effects on responses to either low- or high-intensity stimuli or windup in either type of preparation [33].

Ketoprofen inhibits both COX-1 and COX-2 isoforms. It has been shown to depress responses to low- and high-intensity stimuli to the same extent and with similar EC_{50} with only a minor reduction in the MSR [34,35].

Modulation of Spinal Reflexes by Anesthetic Compounds

Anesthetics form a heterogeneous group of compounds with activity at different targets like glutamate receptors, GABA receptors, and ion channels. General anesthetics produce a set of complex and reversible effects which include hypnosis or loss of consciousness, analgesia, amnesia, and akinesia. Spinal cord mechanisms may contribute toward analgesia and akinesia.

Ketamine

The general anesthetic ketamine is a noncompetitive blocker of NMDA receptors that also shows activity on several ionic channels. As expected for an NMDA receptor blocker, ketamine depresses the slow ventral root potentials with pronounced effects on the NMDA-mediated slow wave, although the very slow components of the ventral root potential were also depressed [34,36]. Depressant effects on the monosynaptic reflex were only reported for specific A-fiber-activating stimuli, but not for supramaximal stimuli.

Volatile Anesthetics

The effects of volatile anesthetics such as halogenated cyclobutanes and halogenated ethers (including halothane, isoflurane, enflurane, and sevoflurane) on spinal cord reflexes have been studied by several groups [28,37–41]. In general terms, volatile anesthetics strongly and reversibly depressed all the components of the ventral root responses, with higher affinity for the slow components mediated by NMDA and NK receptors than for the

monosynaptic reflex. Interestingly, reduction in slow potentials was achieved at subanesthetic concentrations, whereas the IC_{50} for MSR depression was similar to the minimum alveolar concentration (MAC) required for anesthesia [42]. Among volatile anesthetics sevoflurane has been the compound studied in more detail using the *in vitro* spinal cord preparation. Different IC_{50} values have been reported for MSR reduction in the literature, probably reflecting experimental differences such as the recording temperature [37,42]. Slow wave responses to single and repetitive stimuli of high intensity were also depressed by sevoflurane in a concentration-dependent manner [37]. Action potential windup recorded from ventral roots was also very sensitive to sevoflurane, which depressed or completely abolished the response [38]. Maximal effects of sevoflurane on spinal reflexes and windup occurred at concentrations well below its reported anesthetic EC_{50} .

Propofol

Propofol is an intravenous anesthetic agent that acts mainly by potentiating GABA-A receptor function. Propofol has been shown to produce a reversible reduction of slow ventral root potentials at concentrations that do not affect the MSR [38,43]. These effects were reversed by bicuculline indicating that they are mediated by GABA-A receptors [43]. Responses to repetitive stimulation at C-fiber intensity were also reduced by propofol, showing a stronger depressant effect on spike windup than on cumulative depolarization [38].

Nitrous Oxide and Xenon

Other gaseous anesthetics, such as N_2O and xenon, also depress ventral root reflexes *in vitro* [44]. Application of 30% xenon depressed MSR by 64% of control, whereas both the slow and the early components of the response were reduced to about a third of control responses. Nitrous oxide (30%) showed only a marginal (<25% of reduction) effect on all three components.

Modulation of Spinal Reflexes by Exogenous Ion Channel Modulators

Intrinsic modulation of ionic conductances is a potential mechanism for controlling neuronal excitability [45]. In addition, the use of specific exogenous modulators may be considered as a valid strategy to produce analgesia and anesthesia. Many of these compounds act at peripheral terminals; however, there are data on a discrete number of ion channel modulator's effects on spinal segmental reflexes.

Voltage-Dependent Sodium Channels

Voltage-dependent sodium channels are responsible for the generation of action potentials and transmission in excitable cells. A complete blockade of sodium channels leads to the abolition of synaptic transmission and network function and eventually to death if applied systemically to a living animal. Lidocaine is used to produce fast peripheral nerve blockade and pain relief in odontology. Lidocaine and the typical sodium channel blocker tetrodotoxin have been shown to depress spinal reflexes when

applied to the cord at low concentrations which do not block impulse transmission in nerves. TTX produced a concentration-dependent depression of both the MSR and the slow ventral root potentials [46]. At very low concentrations, lidocaine specifically reduced the slower components of the ventral root potential, whereas the MSR were unaffected [46,47]. However, higher concentrations of lidocaine can also affect the monosynaptic reflex [48]. Lidocaine applied at low concentration also depressed the cumulative depolarization evoked by repetitive high-intensity stimulation of the dorsal root [47].

The effects of other sodium channel blockers with central muscle relaxant action were studied on responses to activation of non-nociceptive primary afferents. Eperisone, tolperisone, and other related compounds produced a concentration-dependent depression of the MSR and A-fiber-mediated responses, causing complete blockade of all components at sufficient concentrations [48–50]. Other compounds such as riluzole, lamotrigine, and carbamazepine showed similar effects [50].

Potassium Channels

Voltage-dependent potassium Kv7 channels yield a low-threshold slowly activating voltage-dependent potassium current (M-current) that has a wide distribution in peripheral and central neurons including the spinal cord. The Kv7 channel opener retigabine applied to the spinal cord strongly reduced ventral root responses to single and repetitive high-intensity stimulation of the dorsal root in a concentration-dependent manner [51]. Cumulative depolarization and spike windup were strongly depressed or completely abolished. Retigabine did not produce clear depressant actions on the MSR elicited by supramaximal stimuli, but it increased the threshold stimulus required to evoke a ventral response. Retigabine effects were prevented by application of XE-991, a Kv7 channel blocker.

Small conductance calcium-activated potassium channels (SK) are also expressed in sensory pathways, including the spinal cord, where they may modulate synaptic transmission. The presence of these channels has also been related to the occurrence of postspike afterhyperpolarization (AHP) in several neuronal types. Application of the SK enhancer I-EBIO (1 mM) reduced all the components of ventral root responses with partial reversion on washout [52]. However, the slow C-fiber-mediated component was affected to a greater extent (87% reduction) than mono- and polysynaptic reflexes mediated by A fibers (18 and 32%, respectively).

Discussion

From the published literature, it seems evident that the endogenous systems that regulate spinal reflex activity are very flexible in that they can modulate particular components of the responses either to increase them or to inhibit them. This is the great advantage of the synaptic delivery of modulators at specific points containing specific receptors. In contrast, volume conduction or pharmacological application of compounds affects all targets available in the tissue and simple effects are expected unless the compound interacts with targets of different nature. This is the case reported for noradrenalin with a mixture of inhibitory effects in sensory pathways and excitatory effects on motor pathways

which may resemble the simultaneous analgesia and hyper-reflexia experienced under stress conditions by living animals and humans.

Analgesics and anesthetics were created to fulfill specific functions and all of them use endogenous targets, mechanisms, and pathways, which at least in part, involve the spinal cord. The interference of these compounds with segmental reflex activity gives clues about the targets and mechanism used. Furthermore, compounds belonging to a given class may exhibit specific profiles of action on spinal reflexes. If such patterns are found, then it should be possible to use them to evaluate the potential analgesic or anesthetic value of new compounds.

In this context, it is revealing to see that the four major analgesics reviewed here (morphine, clonidine, duloxetine, and pregabalin) tend to produce a partial reduction of windup, a reduction of the slow wave associated to A- and C-fiber intensity stimuli, and have no effect on the MSR [21]. We will refer to this pattern of effects on segmental reflexes as the “analgesic pattern.” The analgesic pattern of action exhibited by analgesic compounds on segmental reflexes is in good agreement with what should be expected for spinally mediated analgesia, that is, reducing activity in nociceptive pathways without affecting muscle reflexes that are essential to maintain motor control. Other compounds reviewed here share this pattern. One of them is retigabine, an M-current activator which reduces nociceptive behaviors in experimental models of chronic pain, being therefore in good agreement with our proposal [53]. However, phase II studies in patients with postherpetic neuralgia were regarded as inconclusive.

Among the reviewed literature, we can also find examples of false positive to our proposed analgesic pattern. An example of this is propofol which, being an anesthetic, displays an analgesic pattern of action on spinal reflexes. It is evident that propofol has cerebral effects not shared by analgesics. In the other extreme, some antiinflammatory agents have analgesic effects but do not comply with the analgesic pattern. For these, it is likely that analgesia is secondary to the alleviation of peripheral inflammation and not directly related to their spinal effects.

All of these exceptions to the proposal of the analgesic pattern of action on spinal reflexes warn against simple interpretation of results. Compounds may have actions in peripheral and supraspinal structures which modulate or complement their spinal effects. Furthermore, detailed analysis has to be made about concentrations required to produce analgesia and those required to produce the analgesic pattern.

Anesthetics present more variable effects on segmental reflexes than analgesics. For example, sevoflurane shows a very potent reduction of C-fiber-mediated reflexes and windup to the point of nearly complete abolition. In addition, sevoflurane blocks the MSR. These potent effects on segmental transmission may be indicative of the strong analgesic and akinesic effects of the compound. Propofol (or the local anesthetic lidocaine) causes less intensive depressant effects on C-fiber-mediated reflexes, and it has no effect on the MSR. Interestingly, this compound is administered together with opiates during surgical anesthesia and has smaller effects on the H reflex as compared to sevoflurane [54] although both compounds are good anesthetics. Certainly, anesthetics have profound effects on cerebral nuclei some of which may cooperate with spinal actions.

Conclusion

In view of the available data, we conclude that *in vitro* recordings of segmental reflexes may be used as a valuable tool to investigate the analgesic potential of novel compounds. Compounds that depress windup and other C-fiber-mediated events without acting on the MSR may be considered good candidates for analgesia. Of course, other peripheral and cerebral effects of compounds have to be considered.

Authors Contribution

J.A.L. conceived and designed the manuscript. All authors contributed to data gathering, drafting, revising the manuscript and approved its final version.

References

- Otsuka M, Konishi S. Electrophysiology of mammalian spinal cord in vitro. *Nature* 1974;**252**:733–734.
- King AE, Lopez-Garcia JA. Excitatory amino acid receptor-mediated neurotransmission from cutaneous afferents in rat dorsal horn in vitro. *J Physiol* 1993;**472**:443–457.
- Hedo G, Lopez-Garcia JA. Alpha-1A adrenoceptors modulate potentiation of spinal nociceptive pathways in the rat spinal cord in vitro. *Neuropharmacology* 2001;**41**:862–869.
- Evans RH. The pharmacology of segmental transmission in the spinal cord. *Prog Neurobiol* 1989;**33**:255–279.
- King AE, Lopez-Garcia JA, Cumberbatch M. Antagonism of synaptic potentials in ventral horn neurones by 6-cyano-7-nitroquinoxaline-2,3-dione: A study in the rat spinal cord in vitro. *Br J Pharmacol* 1992;**107**:375–381.
- Otsuka M, Yanagisawa M. Effect of a tachykinin antagonist on a nociceptive reflex in the isolated spinal cord-tail preparation of the newborn rat. *J Physiol* 1988;**395**:255–270.
- Mendell LM, Wall PD. Responses of single dorsal cord cells to peripheral cutaneous unmyelinated fibres. *Nature* 1965;**206**:97–99.
- Boxall SJ, Thompson SW, Dray A, Dickenson AH, Urban L. Metabotropic glutamate receptor activation contributes to nociceptive reflex activity in the rat spinal cord in vitro. *Neuroscience* 1996;**74**:13–20.
- Woolf CJ. Windup and central sensitization are not equivalent. *Pain* 1996;**66**:105–108.
- Thompson SW, King AE, Woolf CJ. Activity-dependent changes in rat ventral horn neurons in vitro; summation of prolonged afferent evoked postsynaptic depolarizations produce a d-2-Amino-5-phosphonovaleric acid sensitive windup. *Eur J Neurosci* 1990;**2**:638–649.
- Barbieri M, Nistri A. Depression of windup of spinal neurons in the neonatal rat spinal cord in vitro by an NK3 tachykinin receptor antagonist. *J Neurophysiol* 2001;**85**:1502–1511.
- Hedo G, Laird JM, Lopez-Garcia JA. Time-course of spinal sensitization following carrageenan-induced inflammation in the young rat: A comparative electrophysiological and behavioural study in vitro and in vivo. *Neuroscience* 1999;**92**:309–318.
- Thompson SW, Dray A, Urban L. Injury-induced plasticity of spinal reflex activity: NK1 neurokinin receptor activation and enhanced A- and C-fiber mediated responses in the rat spinal cord in vitro. *J Neurosci* 1994;**14**:3672–3687.
- Jessell TM. Substance P in nociceptive sensory neurons. *Ciba Found Symp* 1982;**91**:225–248.
- Millan MJ. Descending control of pain. *Prog Neurobiol* 2002;**66**:355–474.
- Yaksh TL, Noueihed R. The physiology and pharmacology of spinal opiates. *Annu Rev Pharmacol Toxicol* 1985;**25**:433–462.
- Nussbaumer JC, Yanagisawa M, Otsuka M. Pharmacological properties of a C-fibre response evoked by saphenous nerve stimulation in an isolated spinal cord-nerve preparation of the newborn rat. *Br J Pharmacol* 1989;**98**:373–382.
- Feng J, Kendig JJ. The NMDA receptor antagonist MK-801 differentially modulates mu and kappa opioid actions in spinal cord in vitro. *Pain* 1996;**66**:343–349.
- Faber ES, Chambers JP, Brugger F, Evans RH. Depression of A and C fibre-evoked segmental reflexes by morphine and clonidine in the in vitro spinal cord of the neonatal rat. *Br J Pharmacol* 1997;**120**:1390–1396.
- Faber ES, Chambers JP, Evans RH. Selective depression of dorsal root-evoked high threshold synaptic excitation by the selective kappa opioid receptor agonist enadoline in the neonatal rat hemisectioned spinal cord in vitro. *Neuropharmacology* 1998;**37**:745–750.
- Mazo I, Roza C, Zamanillo D, Merlos M, Vela JM, Lopez-Garcia JA. Effects of centrally acting analgesics on spinal segmental reflexes and wind-up. *Eur J Pain* 2014;**19**:1012–1020.
- Feng J, Kendig JJ. Selective effects of alfentanil on nociceptive-related neurotransmission in neonatal rat spinal cord. *Br J Anaesth* 1995;**74**:691–696.
- Tao PL, Lai YS, Chow LH, Huang EY. Effects of morphine and endomorphins on the polysynaptic reflex in the isolated rat spinal cord. *Naunyn Schmiedeberg Arch Pharmacol* 2005;**371**:72–80.
- Feng J, Kendig JJ. Synergistic interactions between midazolam and alfentanil in isolated neonatal rat spinal cord. *Br J Anaesth* 1996;**77**:375–380.
- Smith C, McEwan AI, Jhaveri R, et al. The interaction of fentanyl on the Cp50 of propofol for loss of consciousness and skin incision. *Anesthesiology* 1994;**81**:820–828; discussion 26A.
- Kendig JJ, Savola MK, Woodley SJ, Maze M. Alpha 2-adrenoceptors inhibit a nociceptive response in neonatal rat spinal cord. *Eur J Pharmacol* 1991;**192**:293–300.
- Siarey RJ, Long SK, Evans RH. The effect of centrally acting myorelaxants on NMDA receptor-mediated synaptic transmission in the immature rat spinal cord in vitro. *Br J Pharmacol* 1992;**107**:628–633.
- Savola MK, Woodley SJ, Maze M, Kendig JJ. Isoflurane and an alpha 2-adrenoceptor agonist suppress nociceptive neurotransmission in neonatal rat spinal cord. *Anesthesiology* 1991;**75**:489–498.
- Siarey RJ, Long SK, Tulp MT, Evans RH. The effects of central myorelaxants on synaptically-evoked primary afferent depolarization in the immature rat spinal cord in vitro. *Br J Pharmacol* 1994;**111**:497–502.
- Bockbrader HN, Radulovic LL, Posvar EL, et al. Clinical pharmacokinetics of pregabalin in healthy volunteers. *J Clin Pharmacol* 2010;**50**:941–950.
- Sultan A, Gaskell H, Derry S, Moore RA. Duloxetine for Pharmacological properties of a C-fibre response evoked painful diabetic neuropathy and fibromyalgia pain: by saphenous nerve stimulation in an isolated spinal Systematic review of randomised trials. *BMC Neurol* 2008;**8**:29–36.
- Beiche F, Scheuerer S, Brune K, Geisslinger G, Goppelt-Strube M. Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. *FEBS Lett* 1996;**390**:165–169.
- Lopez-Garcia JA, Laird JM. Central antinociceptive effects of meloxicam on rat spinal cord in vitro. *NeuroReport* 1998;**9**:647–651.
- Lizarraga I, Chambers JP, Johnson CB. Depression of NMDA-receptor-mediated segmental transmission by ketamine and ketoprofen, but not L-NAME, on the in vitro neonatal rat spinal cord preparation. *Brain Res* 2006;**1094**:57–64.
- Lizarraga I, Chambers JP, Johnson CB. Synergistic depression of NMDA receptor-mediated transmission by ketamine, ketoprofen and L-NAME combinations in neonatal rat spinal cords in vitro. *Br J Pharmacol* 2008;**153**:1030–1042.
- Brockmeyer DM, Kendig JJ. Selective effects of ketamine on amino acid-mediated pathways in neonatal rat spinal cord. *Br J Anaesth* 1995;**74**:79–84.
- Matute E, Lopez-Garcia JA. Characterisation of sevoflurane effects on spinal somato-motor nociceptive and non-nociceptive transmission in neonatal rat spinal cord: An electrophysiological study in vitro. *Neuropharmacology* 2003;**44**:811–816.
- Matute E, Rivera-Arconada I, Lopez-Garcia JA. Effects of propofol and sevoflurane on the excitability of rat spinal motoneurons and nociceptive reflexes in vitro. *Br J Anaesth* 2004;**93**:422–427.
- Wong SM, Cheng G, Homanics GE, Kendig JJ. Enflurane actions on spinal cords from mice that lack the beta3 subunit of the GABA(A) receptor. *Anesthesiology* 2001;**95**:154–164.
- Kendig JJ, Kodde A, Gibbs LM, Ionescu P, Eger EI2nd. Correlates of anesthetic properties in isolated spinal cord: Cyclobutanes. *Eur J Pharmacol* 1994;**264**:427–436.
- Takenoshita M, Takahashi T. Mechanisms of halothane action on synaptic transmission in motoneurons of the newborn rat spinal cord in vitro. *Brain Res* 1987;**402**:303–310.
- Tsutahara S, Furumido H, Ohta Y, Harasawa K, Yamamura T, Kemmotsu O. Effects of halothane,

Acknowledgment

Research was supported by the Spanish Ministry of Economy and Competitiveness (Grant No. BFU2012-37905) and the University of Alcalá (Grant No. CCG2014/BIO-020).

Disclosure

This manuscript has been neither published nor considered for publication elsewhere. The authors declare no conflict of interest.

- isoflurane, enflurane, and sevoflurane on the monosynaptic reflex response in the isolated spinal cord of newborn rats. *Masui* 1996;**45**:829–836.
43. Jewett BA, Gibbs LM, Tarasiuk A, Kendig JJ. Propofol and barbiturate depression of spinal nociceptive neurotransmission. *Anesthesiology* 1992;**77**:1148–1154.
 44. Watanabe I, Takenoshita M, Sawada T, Uchida I, Mashimo T. Xenon suppresses nociceptive reflex in newborn rat spinal cord in vitro; comparison with nitrous oxide. *Eur J Pharmacol* 2004;**496**:71–76.
 45. Rivera-Arconada I, Lopez-Garcia JA. Changes in membrane excitability and potassium currents in sensitized dorsal horn neurons of mice pups. *J Neurosci* 2010;**30**:5376–5383.
 46. Jaffe RA, Rowe MA. Subanesthetic concentrations of lidocaine selectively inhibit a nociceptive response in the isolated rat spinal cord. *Pain* 1995;**60**:167–174.
 47. Nagy I, Woolf CJ. Lignocaine selectively reduces C fibre-evoked neuronal activity in rat spinal cord in vitro by decreasing N-methyl-D-aspartate and neurokinin receptor-mediated post-synaptic depolarizations; implications for the development of novel centrally acting analgesics. *Pain* 1996;**64**:59–70.
 48. Kocsis P, Farkas S, Fodor L, et al. Tolperisone-type drugs inhibit spinal reflexes via blockade of voltage-gated sodium and calcium channels. *J Pharmacol Exp Ther* 2005;**315**:1237–1246.
 49. Ishizuki M, Yanagisawa M. Antinociceptive effects of tizanidine, diazepam and eperisone in isolated spinal cord-tail preparations of newborn rat. *Pain* 1992;**48**:101–106.
 50. Than M, Kocsis P, Tihanyi K, et al. Concerted action of antiepileptic and antidepressant agents to depress spinal neurotransmission: Possible use in the therapy of spasticity and chronic pain. *Neurochem Int* 2007;**50**:642–652.
 51. Rivera-Arconada I, Martinez-Gomez J, Lopez-Garcia JA. M-current modulators alter rat spinal nociceptive transmission: An electrophysiological study in vitro. *Neuropharmacology* 2004;**46**:598–606.
 52. Bahia PK, Suzuki R, Benton DC, et al. A functional role for small-conductance calcium-activated potassium channels in sensory pathways including nociceptive processes. *J Neurosci* 2005;**25**:3489–3498.
 53. Rivera-Arconada I, Roza C, Lopez-Garcia JA. Enhancing m currents: A way out for neuropathic pain? *Front Mol Neurosci* 2009;**2**:10.
 54. Baars JH, Mager R, Dankert K, Hackbarth M, von Dincklage F, Rehberg B. Effects of sevoflurane and propofol on the nociceptive withdrawal reflex and on the H reflex. *Anesthesiology* 2009;**111**:72–81.
 55. Lopez-Garcia JA. Serotonergic modulation of spinal sensory circuits. *Curr Top Med Chem* 2006;**6**:1987–1996.
 56. Rozzo A, Ballerini L, Nistri A. Antagonism by (1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid of synaptic transmission in the neonatal rat spinal cord in vitro: An electrophysiological study. *Neuroscience* 1999;**90**:1085–1092.
 57. Kerr BJ, Bradbury EJ, Bennett DL, et al. Brain-derived neurotrophic factor modulates nociceptive sensory inputs and NMDA-evoked responses in the rat spinal cord. *J Neurosci* 1999;**19**:5138–5148.
 58. Rivera-Arconada I, Benedet T, Roza C, et al. DREAM regulates BDNF-dependent spinal sensitization. *Mol Pain* 2010;**6**:95–109.
 59. Akagi H, Yanagisawa M. GABAergic modulation of a substance P-mediated reflex of slow time course in the isolated rat spinal cord. *Br J Pharmacol* 1987;**91**:189–197.