ABSTRACT

The spinal cord is the first relay centre for somatosensory inputs from peripheral receptors. These include nociceptors that detect harmful or potentially harmful stimuli. Spinal neurons carry out the first processing of nociceptive information and therefore, acting at this early level can determine nociceptive transmission.

Modulation of spinal circuits has become a very important strategy to design analgesic and anaesthetic compounds. In this Thesis we have tested the possibility of regulating spinal circuits using modulators of M type potassium current. M currents emerge from assemblies of KCNQ (Kv7) protein subunits. Due to its functional characteristics, M-currents have been shown to act as a brake on neuronal excitability.

The aims of this Thesis were (1) to study the presence of functional M-currents in the different components of the spinal cord and (2) to determine how modulation of the M-current affects the functioning of these components.

To these purposes several electrophysiological and pharmacological techniques were used in a hemisected spinal cord preparation obtained from rat pups (7 to 12 days old). The spinal cord was maintained under *in vitro* conditions pinned down to a sylgard-bottom recording chamber. Primary afferent fibers were electrically activated using suction electrodes located on the dorsal root. Different intensity stimuli were used to activate specifically non-nociceptive fibers or all fibers present in the dorsal root (nociceptive and non-nociceptive). Spinal reflexes and dorsal root responses were recorded using extracellular electrodes. Single dorsal horn and motor neurons responses were recorded using intracellular electrodes.

Pharmacological assays were performed using retigabine, a M-channel opener, and XE-991, a M-channel blocker. Both compounds were bath applied to the entire spinal cord at known concentrations.

Results indicate the expression of functional M-currents in the neonatal rat spinal cord using extracellular recordings from ventral roots. Retigabine depressed spinal reflexes in response to nociceptive primary afferents activation. XE-991 showed opposite effects and reverted those of retigabine, indicating a specific modulation of M-currents.

Using intracellular recordings we obtained evidence of the presence of functional M-currents on dorsal horn and motor neurons of the spinal cord. Modulation

of M-currents located in these neurons altered their ability to transmit synaptic inputs. Retigabine hyperpolarized the resting potential and reduced the excitability of spinal neurons. XE-991 depolarized the resting potential of spinal neurons, however the effect of this compound on the excitability of motor neurons was stronger than that observed in dorsal horn neurons. We believe that differences in the sensitivity to XE-991 expressed by dorsal and motor neurons may reflect differences in the structure of channels located in these neurons.

Extracellular recordings from dorsal roots indicated that M-currents are also expressed by primary afferent fibers. Retigabine application hyperpolarized primary afferent fibers. Retigabine produced this effect acting on M-currents present in primary afferent terminals. Results also suggest the expression of M-currents in the interneurons that mediate dorsal root reflexes. We believe that the existence of M-currents in primary afferents could partially explain the depressing effect of retigabine on somatosensory transmission.

In conclusion, the present Thesis contains experimental results that show the expression of functional M type potassium currents in the main components of the spinal cord, i.e. primary afferents, dorsal horn neurons and motor neurons. M-current activation leads to a strong reduction in the excitability of spinal circuits. This property of retigabine may be useful to reduce the neuronal hyperexcitability that characterize hyperalgesic states.