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Institutet**

Institutionen för fysiologi och farmakologi

Insights into the role of spinal mTOR in the modulation of inflammation and nociception

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
Institutet offentligen försvaras i Farmakologens föreläsningssal,
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Fredagen den 25 maj, 2012, kl 9.00

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Stockholm 2012

ABSTRACT

Pain, especially chronic pain, is a major clinical problem. Pain therapy has proven to be complicated and the results are often unsatisfactory. Despite many years of research, the number of available pain therapeutics remains relatively small. The available therapeutics are often not efficient enough and associated with adverse effects, which may be problematic when treating pain both in the short and long term. In order to develop new and improved pain therapeutics the identification of novel pharmaceutical drug targets is of crucial importance. This thesis investigates the protein mTOR as a new potential drug target to treat inflammatory pain. In addition, methodological approaches to obtain reliable in vitro spinal astrocyte cell cultures were studied.

To determine whether spinal mTOR plays a role in inflammatory pain processing, activation of spinal mTOR in rats was measured following the induction of a peripheral inflammation. An increased activation of the mTOR pathway was seen in spinal dorsal horn neurons following injection of carrageenan into the hindpaw. Inhibition of spinal mTOR with rapamycin resulted dose-dependently in decreased nociceptive behavior, further supporting a role for mTOR in inflammatory pain signaling.

In vitro model systems are important tools to study cellular signaling events. In order to obtain reliable cell cultures from rodent tissue with properties similar to human tissue cultures, the culture conditions are of vital importance. Using a variety of specific markers to identify astrocytes, the culture conditions were studied in astrocyte cultures generated from spinal cord samples taken from human tissue, as well as two genetically different rat substrains. Culturing rat astrocytes in medium optimized for astrocytes, results in lower contamination and cells with a more astrocyte-like phenotype. In addition, the choice of rat strain can also affect the characteristics of the cultured astrocytes.

Microglia and astrocytes have received much attention in central pain processing research. Preclinical studies have demonstrated an important role for these cells in pain signaling. To examine the possible role of mTOR for astrocyte activation, two glia inhibitors, the methyl xanthines pentoxifylline and propentofylline, were used. Both substances inhibited astrocyte activation in vitro as demonstrated by reduced astrocyte proliferation and growth, and an altered morphology. Further, pentoxifylline and propentofylline counteracted TNF-induced mTOR activation in cultured astrocytes but did not affect EGF-induced mTOR activation. These data suggest that pentoxifylline and propentofylline act in an mTOR- and stimulus-dependent fashion to inhibit astrocyte activation.

In addition to targeting mTOR directly, pharmacological interference with factors associated with mTOR signaling, that can indirectly regulate activation of mTOR and other signaling pathways may prove useful as potential drug targets. One such factor that may be linked to mTOR regulation is caveolin-1, a protein that can act as a regulatory factor in several disease states. Although the importance of caveolin-1 in pain signaling is not yet clarified, links to TNF signaling indicated in the present thesis, suggest a possible role in inflammatory pain processing and further studies are required to determine if caveolin-1 is an important regulator of mTOR signaling. As a whole, this thesis provides insight into the role of mTOR in spinal pain processing. Further studies of the molecular function of mTOR in neurons and glial cells in the dorsal horn may provide a basis for the development of new therapeutics for the treatment of inflammatory pain.