

# **Screening for nociceptor specific genes**

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by

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## **ZUSAMMENFASSUNG**

Meiner Hypothese nach reguliert NGF (nerve growth factor) die Expression eines spezifischen Proteins bzw. einer Gruppe von Proteinen, welche möglicherweise unter normalen Bedingungen sensorischen Neuronen ihre Hitzesensitivität verleihen. Unterdrückung des NGF-Signalweges während der postnatalen Entwicklung führt, wie in der Literatur bereits beschrieben, zu teilweise veränderter Rezeptordifferenzierung, die myelinisierte und nicht myelinisierte Nozizeptoren einschließt. Die vorliegende Arbeit hat die Identifikation von Genen, die in nozizeptive Prozesse involviert sind und als Marker für die physiologische Unterscheidung von Subpopulationen von Nozizeptoren dienen könnten, wie die Untersuchung fehlenden NGF-Signalings in der frühen postnatalen Entwicklung zum Ziel.

Aus adäquaten Studien an Ratten, die mit anti-NGF-Antikörpern, welche die Funktion von NGF in der frühen postnatalen Periode blockieren sollen, behandelt wurden resultieren unsere Erwartungen, dass auch in der Maus die temporäre Blockierung von NGF während der frühen postnatalen Phase zu permanenten physiologischen Veränderungen in Nozizeptoren führt. Die detaillierte physiologische Analyse primärer sensorischer Neuronen in der Haut nach Entzug funktionellen NGF's während der frühen postnatalen Entwicklungsphase, ergab eine phänotypische Verschiebung in speziellen Subpopulationen von DRG-Neuronen. Außerdem wurde eine permanente Reduktion der Hitzesensitivität nicht myelinisierter Nozizeptoren beobachtet.

Die Temperaturreizschwelle für hitzesensitive polymodale C-Fasern in Mäusen, die neonatal mit anti-NGF behandelt worden waren, wurde ermittelt. Zudem zeigten diese Mäuse im Vergleich zu Kontrolltieren eine Hitze-Hyperalgesie im entsprechenden Verhaltensexperiment. Mittels Micro-Array-Experimenten wurden Gene, die in der Folge einer Behandlung mit anti-NGF in ihrer Expression reguliert sind identifiziert. Sogenannte „Whole-Mount-*in situ*-Hybridisierungen“ zeigten weiterhin, dass einige der Gene, die in den Micro-Array-Experimenten identifiziert wurden, ein spezifisches Expressionsmuster in den DRG's besitzen. Insofern wurden Kandidatengene gefunden, die spezifisch in solchen DRG-Neuronen mit kleinen Zellkörpern exprimiert werden.

Die vorliegende Arbeit untersucht eines dieser Gene, nämlich den c-Kit-Tyrosinkinase-Rezeptor, im Detail. Die Ausschaltung von *c-kit* in der Maus führte zu einer partiellen Phänokopie der mit anti-NGF behandelten Mäuse. Eine weiter verstärkte

Hitzeinsensitivität verglichen zu den mit anti-NGF behandelten Mäusen wurde in Tieren beobachtet. C-Faser-Nozizeptoren in *c-kit*- Null-Mutanten haben erhöhte Temperaturreizschwellen sowie reduzierte Feuerungsfrequenzen infolge schädlicher Hitzestimulation. Außerdem kontrolliert c-Kit die Mechanosensitivität in einer Subpopulation von „low threshold“-Mechanorezeptoren.

Die Blockierung des NGF-Signalweges während der postnatalen Entwicklungsphase ist insofern eine vielversprechende Möglichkeit, um nach Molekülen zu suchen, die in die Transduktion schädlicher Hitzestimuli involviert sind. Die mögliche Rolle anderer im oben beschriebenen Screen identifizierter Gene bleibt zum gegenwärtigen Zeitpunkt noch zu analysieren. Solche Moleküle könnten zum Beispiel Transduktionseigenschaften unterschiedlicher Typen von sensorischen Neuronen auf Temperatur oder mechanische Stimuli kontrollieren.

In einer komplementären Studie wurden die funktionellen Eigenschaften cutaner Mechanorezeptoren und Mechanonozizeptoren mittels einer *in vitro* Haut-Nerv-Präparation untersucht. Die charakteristische mechanische Latenz, d.h. das Zeitintervall, in dem ein mechanischer Stimulus in ein Aktionspotential transduziert wird, wurde für verschiedene Fasertypen bestimmt. Hierbei wurde speziell für nicht myelinisierte Nozizeptoren ein Zeitfenster von 150 ms als Minimum für die Transduktion eines mechanischen Stimulus in Aktionspotentiale ermittelt. Des Weiteren konnte eine starke Abhängigkeit der physiologischen Eigenschaften von der Temperatur speziell in C-Fasern belegt werden. Zudem konnte gezeigt werden, dass die Mechanosensitivität polymodaler C-Fasern im Vergleich zu hitzesensitiven C-Fasern geringer ist.

## **SUMMARY**

I hypothesized that nerve growth factor (NGF) regulates the expression of a specific protein or group of proteins that normally confer noxious heat sensitivity on sensory neurons. Deprivation of NGF signaling during postnatal development has been shown to lead to a phenotypic switch, influencing the myelinated and unmyelinated nociceptors. To identify genes involved in nociception, and possibly markers for physiologically distinct subpopulations of nociceptors, the effects of ablating NGF signaling in early postnatal development was studied.

Developing mice were deprived of NGF in the early postnatal period using a function-blocking antibody (anti-NGF). It was expected from previous studies in the rat that temporary block of NGF signaling during critical period would induce permanent changes in the physiology of nociceptors. The detailed physiological analysis of cutaneous primary sensory neurons after NGF deprivation during postnatal development revealed a phenotypic switch in DRG neuron subpopulations. In addition, the noxious heat sensitivity of unmyelinated nociceptors was permanently reduced.

Thus, the thermal threshold of heat sensitive polymodal C-fibers in anti-NGF treated mice was elevated and the mice display behavioral heat hypoalgesia compared to controls. Genes, altered in expression level following the neonatal anti-NGF treatment, were identified using gene chip arrays. Whole mount *in situ* hybridization showed a specific expression pattern for some of the genes that were identified in gene chip experiments. This screen revealed a few candidate genes specifically expressed in small diameter DRG cells.

The study was focused further on one of these gene – c-Kit tyrosine kinase receptor. Disruption of *c-kit* in mice led to a partial phenocopy of anti-NGF treated mice, inducing even more profound noxious heat insensitivity compared to anti-NGF treated mice. C-fibers nociceptors in *c-Kit* null mutants have elevated thermal thresholds and decreased firing rates upon noxious heat stimulation. In addition, c-Kit controls the mechanosensitivity of discrete subpopulation of low threshold mechanoreceptors.

The block of NGF signaling during postnatal development turned out to be promising approach in search of molecules that are regulated by its availability and involved in the transduction of noxious stimuli. The possible role of other candidate genes in nociception remains to be analyzed. These molecules might control the transduction properties of distinct types of sensory neuron to thermal and mechanical stimuli.

In a complementary study a detailed analysis of the functional properties of cutaneous mechanoreceptors and mechanonociceptors using the *in vitro* skin nerve preparation was conducted. The characteristic mechanical latency, period needed for the transduction of the mechanical stimulus into the action potential, was determined for different fiber types. A period of 150 ms was revealed as the minimum needed for the transduction of the mechanical stimulus into a train of action potentials in unmyelinated nociceptors. A strong dependence of physiological properties on temperature was found to be specific for C-fibers. It was also shown that the mechanosensitivity of polymodal C-fibers is lower compared to the mechanosensitivity of heat insensitive C-fibers.

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