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Impaired acute and inflammatory nociception in mice lacking the p50 subunit of NF-κB

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Abstract

The transcription factor NF-κB is thought to play an essential role in inflammatory processes and pain. However, the *in vivo* function of individual NF-κB subunits in the development and processing of nociceptive responses is not clarified. In this study we investigated the role of the p50 subunit of NF-κB in models of acute and persistent nociception using NF-κB $p50^{-/-}$ mice. We found that these mice showed impaired basal responses to mechanical as well as thermal noxious stimulation in the dynamic plantar as well as the hot plate test, respectively, in comparison with wild-type mice. In the formalin test we observed a decreased nociceptive behavior in the first and the second phase in NF-κB $p50^{-/-}$ mice. In a model of persistent inflammatory hyperalgesia these mice also showed a reduced hyperalgesia to a thermal stimulus, which was in accordance with a lower cyclooxygenase-2 expression in the spinal cord after peripheral inflammatory stimulation. Taken together, our data indicate that the p50 subunit of NF-κB is of importance in acute and persistent inflammatory pain. The participation to persistent pain might rely on activation of NF-κB by inflammatory stimuli while the contribution to acute pain responses might be related to constitutive NF-κB activity in neurons of the nociceptive system.

Keywords: Nociception; Formalin assay; Zymosan; Paw inflammation

1. Introduction

The transcription factor nuclear factor kappa B (NF-κB) is composed of homo- and heterodimers of Rel family proteins (p65, RelB, c-Rel, p52, and p50) and plays an important role in immune responses, inflammatory diseases and cell death. In most unstimulated cells the majority of Rel/NF-κB dimers are localized in the cytoplasm and sequestered by binding to the inhibitory subunit I-κB. Upon activation by a variety of stimuli (reviewed in Kaltschmidt et al., 2005) I-κB is phosphorylated by I-κB kinases (IKK), subsequently ubiquitinylated and then degraded by a proteasome complex. Degradation of I-κB leads to the release of NF-κB from the trapping complex and translocation into the nucleus where it binds to the promoter region of various genes including cytokines, cyclooxygenase-2 (COX-2) and proteases thereby activating their transcription (Pahl, 1999). Dysregulations of NF-κB are associated with chronic inflammation (Kaltschmidt et al., 1999; Niederberger et al., 2001) and neurodegeneration (Kaltschmidt et al., 1999) and it has been shown that a blockade of NF-κB alters synaptic plasticity (Mattson and Camandola, 2001). Studies concerning the effects of NF-κB on nociception revealed that NF-κB inhibition attenuated the nociceptive response in models of neuropathic pain (Sakaue et al., 2001; Tegeder et al., 2004). In a model of inflammatory pain it has been observed that intrathecal pre-treatment of rats with NF-κB inhibitors reduced spinal NF-κB activation and subsequent expression of COX-2 mRNA thereby suppressing hyperalgesia following unilateral hind paw inflammation (Lee et al., 2004). Interestingly, some disease modifying antirheumatic drugs (DMARDs) such as glucocorticoids and gold salts (Jeon et al., 2000; Yang et al., 1995), as well as high doses of salicylate, inhibit NF-κB

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activation (Auphan et al., 1995; Kopp and Ghosh, 1994; Yin et al., 1998). However, the in vivo function of individual subunits in the development and processing of nociceptive responses remains to be elucidated.

Here, we investigated the role of the NF-κB p50 subunit in several behavioral models of acute and persistent nociception using NF-κB $p50^{-/-}$ mice. These mice are viable and fertile and show no obvious abnormalities in development, general activity and motor function but exhibit defects in immune responses (Kassed and Herkenham, 2004; Sha et al., 1995). In addition, we assessed the expression of cyclooxygenase-2 as a 'pain-relevant' NF-κB-dependent gene in $p50^{-/-}$ mice.

2. Materials and methods

2.1. Animals

Three-month-old homozygous $p50^{-/-}$ mice (B6;129P2-Nfkb1^{tm1Bal}/J, Stock No. 002849) and $p50^{+/+}$ mice (B6129PF2/J, Stock No. 100903, parent strains: C57BL/6J-A^{w-J} and 129P3/J) were obtained from The Jackson Laboratory (Bar Harbour, ME). Animals had free access to food and water and were maintained in climate- and light-controlled rooms (24±0.5 °C, 12/12 dark/light cycle). In all experiments the ethic guidelines for investigations in conscious animals were obeyed and the procedures were approved by the local Ethics Committee for Animal Research. All efforts were made to minimize animal suffering and to reduce the number of animals used. All behavioral experiments were performed in a dedicated room with restriction on sound level and activity.

2.2. Mechanical sensitivity

Paw withdrawal latency to mechanical stimulation was assessed with an automated testing device consisting of a steel rod that is pushed against the plantar surface of the paw with increasing force until the paw is withdrawn (Dynamic Plantar Aesthesiometer, Ugo Basile, Varese, Italy). The maximum force was set at 5 g to prevent tissue damage and the ramp speed was 0.5 g/s. Mice were placed in test cages with a metal grid bottom. They were kept in the test cages for 2 days to allow accommodation. On day 3, the paw withdrawal latency was measured and taken to be the mean of six consecutive trials with at least 2 min in-between.

2.3. Hot-plate test

Animals were placed into a Plexiglas cylinder on a heated plate maintained at 52.5±0.2 °C (Ugo Basile), and the latency to jump or shake/flutter a hind paw was recorded. Each animal was tested only once since repeated testing in this assay leads to latency changes (Mogil et al., 1999). The cut-off time was 45 s.

2.4. Formalin test

The formalin test was performed as described (Dubuisson and Dennis, 1977; Hunskaar et al., 1985; Hunskaar and Hole, 1987). Mice were placed on a table top within a Plexiglas cylinder and were allowed to habituate for 60 min. Then 15 µl of a 5% formaldehyde solution (formalin) was injected subcutaneously into the dorsal surface of the right hind paw. The time spent licking the formalin-injected paw was recorded in 5-min intervals up to 45 min, starting right after formalin injection.

2.5. Thermal hyperalgesia following paw inflammation

Paw withdrawal latency to radiant heat was assessed according to Hargreaves et al. (1988), using a Plantar Test device (Ugo Basile). Mice were placed in test cages with a metal grid bottom. They were kept in the test cages for 2 days to allow accommodation. On day 3, each animal was tested several times to get the baseline paw withdrawal latency. On day 4, hind paw inflammation was induced by subcutaneous injection of 20 µl of a 3 mg/ml zymosan A (Sigma-Aldrich, Munich, Germany) suspension in phosphate buffered saline (0.1 M, pH 7.4) into the midplantar region of the right hind paw (Meller and Gebhart, 1997). The paw withdrawal latency was taken to be the mean of four consecutive trials with at least 10 s in-between and was assessed hourly up to 8 h after zymosan injection.

To estimate the extent of inflammation, animals were deeply anesthetized with isoflurane and then killed by cardiac puncture 8 h after zymosan injection. Subsequently, both hind paws were amputated exactly at the ankle. The paws were weighed and the difference between the inflamed and the non-inflamed control paw was calculated as a parameter for the scope of paw swelling.

2.6. Expression of cyclooxygenase-2

RNA was prepared from the lumbar spinal cords as described previously (Chomczynski, 1993). Investigation of

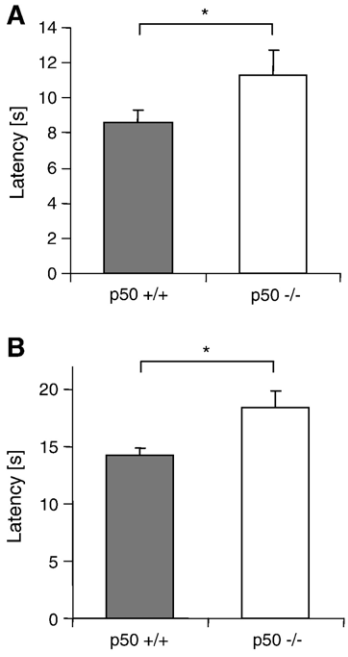

Fig. 1. Basal sensitivity towards thermal and mechanical stimulation. Comparison of the basal sensitivity of NF-κB $p50^{+/+}$ and NF-κB $p50^{-/-}$ mice in response to (A) mechanical stimulation using a Dynamic Plantar Aesthesiometer (n=8) and (B) noxious heat stimuli as measured with a hot plate (52.5 °C, n=10). * P<0.05.

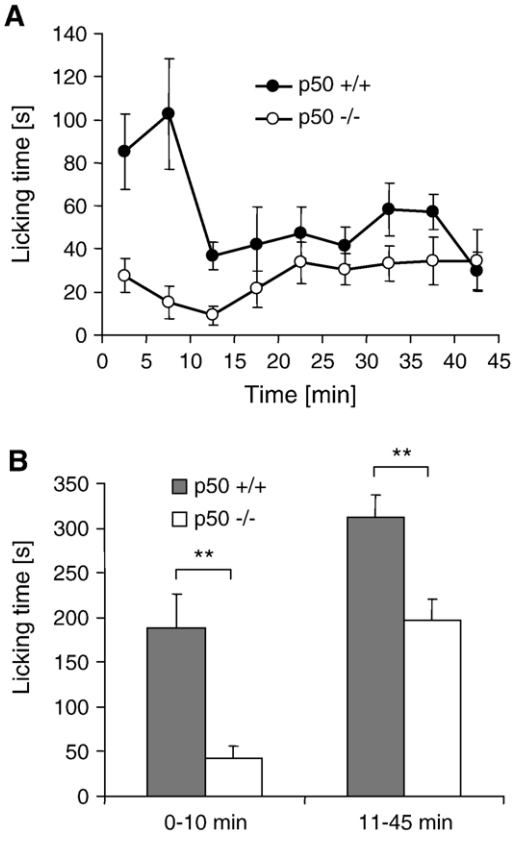

Fig. 2. Formalin test. (A) Time course (mean ± S.E.M.) of the licking behavior in NF-κB $p50^{+/+}$ ($n=8$) and NF-κB $p50^{-/-}$ ($n=6$) mice after injection of formalin (5%, 15 μl) into a hind paw. Formalin was injected at time '0', and the time spent licking the injected paw was measured in 5-min intervals for 45 min. (B) For statistical comparison the total licking time in the first (0–10 min) and second phase (11–45 min) of the formalin test was calculated (mean ± S.E.M.) $^{**}P<0.01$.

cyclooxygenase-2 mRNA expression was performed with a One-step RT-PCR Kit (Qiagen, Hilden, Germany). The following primers were used: COX-2 forward, 5′-AGAAGGAAATGGCTG-CAGAA-3′ and reverse, 5′-GCTCGGCTTCCAGTATTGAG-3′ and beta-actin forward 5′-CCGGATCCTCTTTGCTACTGAGA-CAGG-3′ and reverse 5′-CCGAATTCGGGATCTGAATG-CAATGTT-3′. Reverse transcription reaction was performed with specific primers at 50 °C for 30 min. PCR amplification was started with enzyme activation at 95 °C for 10 min. The samples were then denatured at 95 °C for 1 min, annealed at 58 °C for 1 min and extended at 72 °C for 1 min in 35 repetitive cycles. After a final extension at 72 °C for 10 min the PCR was stopped and the samples were analysed by agarose gel electrophoresis.

2.7. Data analysis

Statistical evaluation was done with SPSS 11.0.1 for Windows. Data were compared by Student's *t*-test and are presented as mean ± standard error of the mean (S.E.M.). For analysis of inflammatory hyperalgesia the areas under the paw withdrawal latency-versus-time curves over the 8 h observation period were calculated using the linear trapezoidal rule. For all tests, a probability value $P<0.05$ was considered as statistically significant.

3. Results

3.1. Sensitivity to mechanical and thermal stimuli

Differences in the sensitivity towards mechanical stimulation and acute noxious heat were investigated in naive $p50^{-/-}$ and $p50^{+/+}$ mice using the dynamic plantar aesthesiometer and the hot plate test, respectively. The latency time for evoking a paw withdrawal reflex upon mechanical stimulation was significantly increased in $p50^{-/-}$ mice as compared to wild-type mice (Fig. 1A), indicating a reduced nociception. $p50^{-/-}$ mice also exhibited a significantly reduced nociceptive response to noxious heat in the 52.5 °C hot plate test (Fig. 1B).

3.2. Chemically induced nociceptive behavior

The formalin test was performed to assess nociceptive responses during tonic noxious stimulation. In wild-type mice, injection of formalin into a hind paw induced the typical biphasic nociceptive behavior consisting of licking the injected paw. The first phase started immediately after the injection and lasted for 10 min. The second phase was observed 11–45 min after formalin injection and peaked at 30–40 min. $p50^{-/-}$ mice

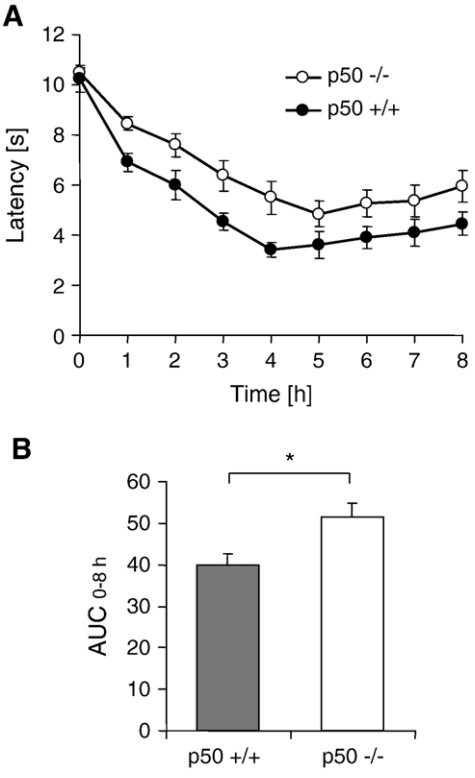

Fig. 3. Zymosan-induced thermal hyperalgesia. (A) Time course of thermal hyperalgesia in NF-κB $p50^{+/+}$ and NF-κB $p50^{-/-}$ mice following injection of 60 μg zymosan into a hind paw. The diagram shows the paw withdrawal latencies (mean ± S.E.M.) in response to radiant heat as assessed with the Hargreaves model ($n=6$). (B) Area under the curve (AUC) over the 8-h observation period. $^{*}P<0.05$.

spent significantly less time licking the formalin-injected hind paw than wild-type mice. These differences occurred in both phases of the formalin test but were more pronounced in the first phase (Fig. 2A). The sum of licking time in the first and the second phase showed significant differences between $p50^{+/+}$ and $p50^{-/-}$ in both phases of the formalin assay (Fig. 2B).

3.3. Thermal hyperalgesia following paw inflammation

The development of thermal hyperalgesia in $p50^{-/-}$ mice was assessed by using the Hargreaves model. At baseline, the paw withdrawal latency to radiant heat did not differ between $p50^{-/-}$ and $p50^{+/+}$ mice. After induction of unilateral hind paw inflammation by injection of zymosan A, the paw withdrawal latencies decreased in both groups. However, in $p50^{-/-}$ mice the decrease of latency was less pronounced than in wild-type mice indicating a reduced hyperalgesic response to a thermal stimulus (Fig. 3A). The statistical comparison of the area under the paw withdrawal latency-versus-time curves from 0 to 8 h after zymosan injection revealed significant differences between $p50^{-/-}$ and wild-type mice ($P=0.02$) (Fig. 3B). As a parameter for peripheral paw inflammation we measured the weight of the zymosan-injected inflamed paw and the untreated control paw. The difference between both paws is a marker for the extent of the inflammation-induced paw swelling. No significant differences were detectable in the paw edema between knockout (inflamed paw − control paw = 1.59 ± 0.06 g, $n=8$) and wild-type animals (inflamed paw − control paw = 1.60 ± 0.05 g, $n=7$) indicating that the reduced nociceptive response is not correlated with the swelling of the paw.

3.4. Expression of cyclooxygenase-2

To determine the impact of the NF-κB p50 deletion on the expression of 'pain-relevant' NF-κB dependent genes, we performed RT-PCR analysis of cyclooxygenase-2 mRNA in the spinal cord. Basal COX-2 expression was similar in spinal cords of $p50^{-/-}$ mice as compared to $p50^{+/+}$ mice. After treatment with zymosan the COX-2 mRNA levels significantly increased in $p50^{+/+}$ mice while no significant change was detectable in $p50^{-/-}$ mice (Fig. 4).

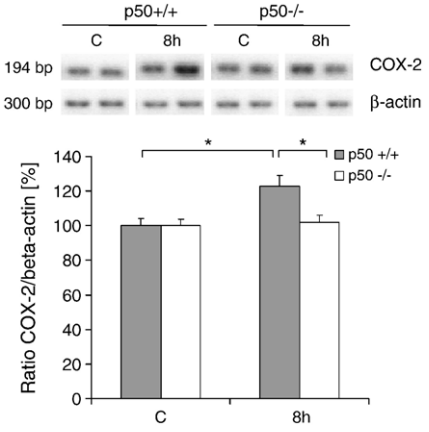

Fig. 4. mRNA expression of cyclooxygenase-2 in the spinal cord. RT-PCR of cyclooxygenase-2 mRNA in the spinal cords of naive $p50^{-/-}$ and $p50^{+/+}$ mice and 8 h after zymosan injection into a hind paw. β-actin was used as internal standard. Representative results of two independent RT-PCRs. The diagram shows the cyclooxygenase-2/β-actin ratio, which has been calculated from the densitometric analysis of the gels ($n=5$ for wild-type control, $n=4$ for wild-type 8 h post-zymosan, $n=5$ for knockout control, $n=4$ for knockout 8h post-zymosan). C: Control, $^*P<0.05$.

4. Discussion

In the present study we have shown that mice lacking the p50 subunit of NF-κB display decreased nociceptive behavior compared with wild-type mice in models of (i) acute mechanical and thermal stimulation, (ii) acute and 'tonic' chemical stimulation, and (iii) persistent inflammatory hyperalgesia. These data indicate that the p50 subunit of NF-κB is involved in acute as well as persistent inflammatory pain.

Previous *in vivo* studies revealed that NF-κB activation contributes to inflammatory pain hypersensitivity. It has been shown that NF-κB is activated in the 'chronic' pain model of Complete Freund's adjuvant-induced hyperalgesia (Chan et al., 2000) and that intrathecal pretreatment with different NF-κB inhibitors reduced the nociceptive behavior upon thermal or mechanical stimulation in this model (Lee et al., 2004). Inhibition of NF-κB activation also decreased the nociceptive behavior after zymosan-induced paw inflammation (Tegeder et al., 2004) and in a model of proinflammatory cytokine-mediated pain (Ledeboer et al., 2005). Moreover, in a model of neuropathic pain inhibition of NF-B activation by pyrrolidinedithiocarbamate alleviated dynorphin-induced allodynia (Laughlin et al., 2000). These data suggest that NF-κB is activated upon noxious stimulation and contributes to pain hypersensitivity by increasing the transcription of 'pain-related' genes such as cyclooxygenase-2 and proinflammatory cytokines. In good agreement with these considerations, we have observed a reduced inflammatory hyperalgesia in the $p50^{-/-}$ mice following injection of zymosan into a hind paw. The zymosan model has been used since it reliably produces persistent hyperalgesia and leads to the induction of numerous genes that are involved in nociceptive processing (Kunz et al., 2005; Niederberger et al., 2003; Sweitzer et al., 1999). Furthermore, zymosan injection evokes an edema of the injected paw (Meller and Gebhart, 1997). NF-κB expression is mainly described in inflammatory cells such as monocytes and macrophages. Therefore, one might suppose that the lack of NF-κB produces antinociceptive effects by inhibition of the expression of proinflammatory proteins in the periphery and a thereby reduced inflammation. However, since no difference in paw swelling could be detected between wild-type and knockout animals we hypothesize that the reduced nociceptive response includes signal transduction mechanisms in the central nervous system. Accordingly, in this study induction of cyclooxygenase-2 is clearly inhibited in the spinal cord of the p50 knockout mice after injection of zymosan.

Another hint for the involvement of NF-κB activation in persistent inflammatory nociception is the reduced nociceptive behavior of $p50^{-/-}$ mice in the second phase of the formalin test. This phase involves inflammatory processes that result in sensitization of nociceptive spinal cord neurons associated with

a prolonged nociceptive behavior (Coderre et al., 1994). In contrast, the first phase following formalin injection can be attributed to acute chemical stimulation of peripheral nociceptors leading to intense neuronal activity in the spinal cord (Hunskaar and Hole, 1987). Interestingly, this phase of acute nociception was also strongly inhibited in the $p50^{-/-}$ mice. Moreover, $p50^{-/-}$ mice showed a reduced nociceptive behavior in two other models of acute nociception using mechanical (dynamic plantar aesthesiometer) or thermal (hot plate test) stimuli. These observations are not due to motoric dysfunctions because the motor functions are not impaired in NF-κB $p50^{-/-}$ mice (Kassed and Herkenham, 2004). However, both genotypes exhibited nearly identical baseline paw withdrawal responses in the Hargreaves test before zymosan injection, which also measures acute thermal nociception. It is of interest to note that different modalities of thermal stimulation may involve separate mechanisms to modulate nociception (Le Bars et al., 2001). In the hot plate test, the thermal stimulus is uniformly applied on all paws and the behavioral responses (i.e., jumping or shaking/fluttering a hind paw) are considered to be both spinally and supraspinally integrated. On the other hand, the stimulus in the Hargreaves model comprises a radiant heat source with relatively weak caloric power affecting only a small part of a hind paw and the paw-withdrawal response mainly represents a spinal reflex. Thus, the lack of NF-κB does obviously not impair the reflex response upon stimulation by weak radiant heat, but it has impact on the more complex nociceptive behavior evoked by the hot plate. The interpretation is somewhat puzzling, but similar discrepancies in the pain response to different types of thermal stimuli have also been found in other knockout mice (Blakeman et al., 2003; Chen et al., 2002; Kim et al., 2001; Lichtman et al., 2004).

The fact that $p50^{-/-}$ mice exhibit decreases in pain behavior in a variety of *acute* nociceptive assays is quite remarkable since it suggests that the p50 subunit of NF-κB has a constitutive role in nociception. Interestingly, there are several reports about a constitutive activity of NF-κB in neurons. It has been suggested that constitutively activated NF-κB in a subset of neuronal nuclei participates in normal brain function and reflects a distinct state of neuronal activity or differentiation (Kaltschmidt et al., 1994). Immunohistochemical studies identified NF-κB subunits in neuronal processes and at synapses implicating their participation in synaptic plasticity (Guerrini et al., 1995; Kaltschmidt et al., 1993). Moreover, in a recent study it was shown that spontaneous basal synaptic transmission activates NF-κB, which could be further enhanced by glutamate application and suppressed by pharmacological inhibitors of synaptic transmission (Meffert et al., 2003). Therefore, it is conceivable that the lack of this basal NF-κB activity in nociceptive neurons might be responsible for the reduced nociceptive behavior of the NF-κB p50 knockouts in models of acute nociception.

In conclusion, we were able to show for the first time that the p50 subunit of the transcription factor NF-κB plays an important role in acute and persistent nociception and may therefore provide a novel specific target for pharmacological intervention in pain.

References

Auphan, N., DiDonato, J.A., Rosette, C., Helmberg, A., Karin, M., 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. Science 270, 286–290.

Blakeman, K.H., Hao, J.X., Xu, X.J., Jacoby, A.S., Shine, J., Crawley, J.N., Iismaa, T., Wiesenfeld-Hallin, Z., 2003. Hyperalgesia and increased neuropathic pain-like response in mice lacking galanin receptor 1 receptors. Neuroscience 117, 221–227.

Chan, C.F., Sun, W.Z., Lin, J.K., Lin-Shiau, S.Y., 2000. Activation of transcription factors of nuclear factor kappa B, activator protein-1 and octamer factors in hyperalgesia. Eur. J. Pharmacol. 402, 61–68.

Chen, C.C., Zimmer, A., Sun, W.H., Hall, J., Brownstein, M.J., Zimmer, A., 2002. A role for ASIC3 in the modulation of high-intensity pain stimuli. Proc. Natl. Acad. Sci. U. S. A. 99, 8992–8997.

Chomczynski, P., 1993. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15, 532–534, 536–537.

Coderre, T.J., Yashpal, K., Henry, J.L., 1994. Specific contribution of lumbar spinal mechanisms to persistent nociceptive responses in the formalin test. NeuroReport 5, 1337–1340.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174.

Guerrini, L., Blasi, F., Denis-Donini, S., 1995. Synaptic activation of NF-kappa B by glutamate in cerebellar granule neurons in vitro. Proc. Natl. Acad. Sci. U. S. A. 92, 9077–9081.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J., 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77–88.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Methods 14, 69–76.

Jeon, K.I., Jeong, J.Y., Jue, D.M., 2000. Thiol-reactive metal compounds inhibit NF-kappa B activation by blocking I kappa B kinase. J. Immunol. 164, 5981–5989.

Kaltschmidt, C., Kaltschmidt, B., Baeuerle, P.A., 1993. Brain synapses contain inducible forms of the transcription factor NF-kappa B. Mech. Dev. 43, 135–147.

Kaltschmidt, C., Kaltschmidt, B., Neumann, H., Wekerle, H., Baeuerle, P.A., 1994. Constitutive NF-kappa B activity in neurons. Mol. Cell Biol. 14, 3981–3992.

Kaltschmidt, B., Uherek, M., Wellmann, H., Volk, B., Kaltschmidt, C., 1999. Inhibition of NF-kappaB potentiates amyloid beta-mediated neuronal apoptosis. Proc. Natl. Acad. Sci. U. S. A. 96, 9409–9414.

Kaltschmidt, B., Widera, D., Kaltschmidt, C., 2005. Signaling via NF-kappaB in the nervous system. Biochim. Biophys. Acta 1745, 287–299.

Kassed, C.A., Herkenham, M., 2004. NF-kappaB p50-deficient mice show reduced anxiety-like behaviors in tests of exploratory drive and anxiety. Behav. Brain Res. 154, 577–584.

Kim, C., Jun, K., Lee, T., Kim, S.S., McEnery, M.W., Chin, H., Kim, H.L., Park, J.M., Kim, D.K., Jung, S.J., Kim, J., Shin, H.S., 2001. Altered nociceptive response in mice deficient in the alpha(1B) subunit of the voltage-dependent calcium channel. Mol. Cell. Neurosci. 18, 235–245.

Kopp, E., Ghosh, S., 1994. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 265, 956–959.

Kunz, S., Tegeder, I., Coste, O., Marian, C., Pfenninger, A., Corvey, C., Karas, M., Geisslinger, G., Niederberger, E., 2005. Comparative proteomic analysis of the rat spinal cord in inflammatory and neuropathic pain models. Neurosci. Lett. 381, 289–293.

Laughlin, T.M., Bethea, J.R., Yezierski, R.P., Wilcox, G.L., 2000. Cytokine involvement in dynorphin-induced allodynia. Pain 84, 159–167.

Le Bars, D., Gozariu, M., Cadden, S.W., 2001. Animal models of nociception. Pharmacol. Rev. 53, 597–652.

Ledeboer, A., Gamanos, M., Lai, W., Martin, D., Maier, S.F., Watkins, L.R., Quan, N., 2005. Involvement of spinal cord nuclear factor kappaB activation

in rat models of proinflammatory cytokine-mediated pain facilitation. Eur. J. Neurosci. 22, 1977–1986.

Lee, K.M., Kang, B.S., Lee, H.L., Son, S.J., Hwang, S.H., Kim, D.S., Park, J.S., Cho, H.J., 2004. Spinal NF-kB activation induces COX-2 upregulation and contributes to inflammatory pain hypersensitivity. Eur. J. Neurosci. 19, 3375–3381.

Lichtman, A.H., Shelton, C.C., Advani, T., Cravatt, B.F., 2004. Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 109, 319–327.

Mattson, M.P., Camandola, S., 2001. NF-kappaB in neuronal plasticity and neurodegenerative disorders. J. Clin. Invest. 107, 247–254.

Meffert, M.K., Chang, J.M., Wiltgen, B.J., Fanselow, M.S., Baltimore, D., 2003. NF-kappa B functions in synaptic signaling and behavior. Nat. Neurosci. 6, 1072–1078.

Meller, S.T., Gebhart, G.F., 1997. Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. Eur. J. Pain 1, 43–52.

Mogil, J.S., Wilson, S.G., Bon, K., Lee, S.E., Chung, K., Raber, P., Pieper, J.O., Hain, H.S., Belknap, J.K., Hubert, L., Elmer, G.I., Chung, J.M., Devor, M., 1999. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain 80, 67–82.

Niederberger, E., Tegeder, I., Vetter, G., Schmidtko, A., Schmidt, H., Euchenhofer, C., Brautigam, L., Grosch, S., Geisslinger, G., 2001. Celecoxib loses its anti-inflammatory efficacy at high doses through activation of NF-kappaB. FASEB J. 15, 1622–1624.

Niederberger, E., Tegeder, I., Schafer, C., Seegel, M., Grosch, S., Geisslinger, G., 2003. Opposite effects of Rofecoxib on nuclear factor-kappaB and activating protein-1 activation. J. Pharmacol. Exp. Ther. 304, 1153–1160.

Pahl, H.L., 1999. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853–6866.

Sakaue, G., Shimaoka, M., Fukuoka, T., Hiroi, T., Inoue, T., Hashimoto, N., Sakaguchi, T., Sawa, Y., Morishita, R., Kiyono, H., Noguchi, K., Mashimo, T., 2001. NF-kappa B decoy suppresses cytokine expression and thermal hyperalgesia in a rat neuropathic pain model. NeuroReport 12, 2079–2084.

Sha, W.C., Liou, H.C., Tuomanen, E.I., Baltimore, D., 1995. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. Cell 80, 321–330.

Sweitzer, S.M., Colburn, R.W., Rutkowski, M., DeLeo, J.A., 1999. Acute peripheral inflammation induces moderate glial activation and spinal IL-1beta expression that correlates with pain behavior in the rat. Brain Res. 829, 209–221.

Tegeder, I., Niederberger, E., Schmidt, R., Kunz, S., Guhring, H., Ritzeler, O., Michaelis, M., Geisslinger, G., 2004. Specific Inhibition of IkappaB kinase reduces hyperalgesia in inflammatory and neuropathic pain models in rats. J. Neurosci. 24, 1637–1645.

Yang, J.P., Merin, J.P., Nakano, T., Kato, T., Kitade, Y., Okamoto, T., 1995. Inhibition of the DNA-binding activity of NF-kappa B by gold compounds in vitro. FEBS Lett. 361, 89–96.

Yin, M.J., Yamamoto, Y., Gaynor, R.B., 1998. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 396, 77–80.