Drop of the noxious heat threshold induced by surgical incision in the rat: mediators, pharmacological modulation by analgesics and by a novel peripheral neuroregulatory mechanism

PhD Thesis

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1 Introduction

Pain is an unpleasant sensation - a subjective concept - caused by intense stimuli that can lead to tissue damage. Nociception is the neural processes of encoding and processing of noxious stimuli (i.e. heat, mechanical, endogenous and exogenous chemical substances) resulting in a behavioral response. The reactions observed are called nocifensive behaviors, including avoidance of the noxious stimulus, flight and vocalization. Sensitization means an increase in responsiveness to noxious stimuli, thus the threshold for activation becomes lower and the phenomenon is called hyperalgesia. An antinociceptive effect means alleviation of nociception in hyperalgesic or non-hyperalgesic states by chemical agents or physical interventions. Encoding takes place in specific uni- or polymodal nociceptors. Nociceptors are specialized nerve endings of primary afferent neurons. These pseudounipolar neurons innervate the skin, mucous membranes and internal organs. Their peripheral axons form the sensory nerve fibers and the cell bodies are located in the dorsal root ganglia of the spinal cord or in the trigeminal ganglion. It is a characteristic feature of the polymodal nociceptors is that they express in their membrane the pharmacological receptor of capsaicin, the pungent agent of hot pepper. This receptor is called TRPV1 (transient receptor potential vanilloid 1). It can be activated by temperatures above 43 ℃, low pH, lipoxygenase products, cyclooxygenase (COX) products, nitric oxide (NO) and other chemical stimuli. From a neurochemical aspect non-peptidergic and can be distinguished. Peptidergic nociceptors contain neuropeptides, such as substance P, neurokinin A and B, calcitonin gene-related peptide and somatostatin, which are released upon activation. Peptidergic nociceptors, besides their afferent function have efferent functions in form of local tissue responses evoked by neuropeptides released.

At our department Pintér and Szolcsányi discovered that following bilateral transection of the dorsal roots or sciatic nerves stimulation of the peripheral stumps resulted in marked neurogenic inflammation of the ipsilateral hind paw skin which was strongly reduced by stimulation of the contralateral stump. They concluded that anti-inflammatory mediator(s) were also released from the sensory nerve endings and reached distant parts of the body via systemic circulation. It

was also observed that degeneration of capsaicin-sensitive fibers by perineural capsaicin pretreatment prevented the antiinflammatory effect of stimulation. Somatostatin-like immunoreactivity increased significantly in plasma samples, compared to the control animals and pretreatment with a polyclonal anti-somatostatin antibody prevented the remote anti-inflammatory effect, while systemic administration of exogenous somatostatin reduced neurogenic inflammation significantly. This study provided evidence for the first time that excitation of capsaicin-sensitive sensory nerve fibres elicited a systemic anti-inflammatory effect as well, via somatostatin release to the plasma from the activated nerve endings. This phenomenon was termed as the sensocrine function of peptidergic nociceptors.

An approach to measure thermonociception is based on measurement of the lowest temperature evoking a nocifensive reaction, the so-called noxious heat threshold. Recently an increasing-temperature water bath has been developed in our department. The equipment is suitable to apply an increasing-temperature stimulus to the animal’s hind paw, until it withdraws the paw from the water. The corresponding water temperature is considered the noxious heat threshold of the paw. The heat threshold shows little intra- and interindividual variability. This is advantageous, compared to classical methods, as reflex latencies determined in the hot plate or tail flick tests may vary upon repeated determinations.

2 Aims

1. The behavioral noxious heat threshold measured with an increasing-temperature water bath was previously shown to decrease in acute thermal hyperalgesia induced by heat injury. Surgical incision of the hind paw in rats and mice has been introduced as a new animal model of human postoperative pain which is a lasting hyperalgesic state. The aim of our first study was to examine whether the surgical incision-induced sustained hyperalgesia involves a drop of the heat threshold too, and to assess the effects of conventional opioid and non-opioid analgesics in this model.

2. The different time course of heat hyperalgesia induced by heat injury and surgical incision raised the possibility that different mediators take part in these two responses. Therefore the aim of the second study was to compare the peripheral mediator background of heat hyperalgesia in mild heat injury and surgical incision by locally applied test substances**.** We assessed the possible contribution of bradykinin B_2 and B_1, purinergic P2X and TRPV1 receptors as well as formation of lipoxygenase products and NO to these two types of thermal hyperalgesia by measurement of the noxious heat threshold.

3. Previous studies demonstrated that stimulation of capsaicin-sensitive nerve endings resulted in a remote, systemic anti-inflammatory effect, mediated by somatostatin released into systemic circulation and referred to as sensocrine effect of peptidergic nociceptors. Somatostatin was shown to have antinociceptive effect in numerous experimental arrangements including ones in which a peripheral site of action can be assumed. The aim of the third study was to decide whether stimulation of polymodal nociceptors can evoke an antinociceptive effect in a distant part of the body, i.e. whether the sensocrine effect of peptidergic nociceptors involves an antinociceptive action as well. We assessed whether chemical stimulation of nociceptors can influence the incision-induced hyperalgesia in a remote part of the body.

3 Methods

The Ethics Committee on Animal Research of the University of Pécs approved the studies. Female Wistar rats weighing 150–200 g were used. Throughout all the experiments the same assistant handled all the animals. The observer was blind as to the drug treatment of animals.

To measure the noxious heat threshold an increasing-temperature water bath, developed in our department was used. It consists of a tap water-filled plastic container with a built-in heating and a controlling unit. The lightly restrained rats were held in an upright position above the water bath allowing free movement of the hind limbs. After one of the hind paws was immersed into the water the heating process was started. When the animal withdrew its paw, heating was immediately stopped and the corresponding temperature was recorded as the behavioral noxious heat threshold of the examined paw.

3.1 Induction and modulation of heat and mechanical hyperalgesia

In case of heat injury after determination of the control heat threshold of both hind paws, rats were anesthetized and the left paw was immersed into a constant, 51 °C water bath for 20 s. The acquired heat injury ensured a substantial drop of heat threshold without spontaneous nocifensive behavior. Following recovery from anesthesia, heat threshold determinations for the injured paw were repeated. In case of plantar incision the control noxious heat threshold of both hind paws was determined before the operation. Rats were anesthetized and 1 cm long incision was made in the midline of the plantar surface, intersecting the skin, fascia and plantar muscle. The skin was opposed with sutures. The animals recovered for 18 h. The duration of the recovery period was the shortest period of time needed to completely eliminate the behavioral consequences of general anesthesia.

In the series of experiments in which the effect of conventional analgesics on the plantar incision-induced hyperalgesia was investigated, after the postoperative noxious heat threshold measurement the drugs or their solvents were administered intraperitoneally or intraplantarly, followed by repeated heat

threshold measurements at 10 min intervals. The effect of each dose of drugs was examined by comparison to an actual solvent control.

To detect a potential remote antihyperalgesic effect we stimulated nociceptive nerve endings of the acutely denervated (by transection of both sciatic and saphenous nerves) hind limb of conscious rats, and measured incision-induced heat hyperalgesia on the contralateral hind paw. Denervation prevented peripheral impulses (generated by nociceptor stimulation) from entering the central nervous system. For chemical stimulation of peripheral nociceptors irritant substance (i.e. capsaicin intraplantarly or mustard oil percutaneously) or its solvent was administered to the denervated right hind paw.

The mechanonociceptive threshold of each hind paw was determined with a Randall–Selitto apparatus before mechanical hyperalgesia was induced with partial ligation of the sciatic nerve according to Seltzer. The operation was carried out under pentobarbital anesthesia. Seltzer' operation on the contralateral leg was carried out 48 h before acute denervation of the hind limb 18 h before the actual experiment.

3.2 Statistical analysis

One-way repeated measures analysis of variance (ANOVA) followed by Newman–Keuls post hoc test was used for comparison of thresholds determined upon repeated measurements in the same group of animals. Two-way ANOVA followed by Newman–Keuls post hoc test was used for comparison of threshold values determined in solvent and drug-treated animals at various time points. Student's t-test for unpaired samples was used for statistical comparison of the sums of threshold drops at repeated measurements. The overall effect of each drug was assessed on the basis of this parameter. The percentage inhibition of hyperalgesia was calculated according to the following formula:

$$[(Drop_{solv} - Drop_{drug})/Drop_{solv}] \times 100$$

where $Drop_{solv}$ and $Drop_{drug}$ refer to the average of the sum of threshold drops measured at the examined post-treatment time points in solvent- and drug-treated animals, respectively.

4 Results

4.1 Effect of plantar incision on the noxious heat threshold

Following plantar incision the noxious heat threshold of animals was measured daily for seven consecutive days. There was no significant change in the noxious heat threshold, compared to the baseline value, in case of uninjured hind paws; while in case of operated hind paws there was a marked decrease in the noxious heat threshold. This decrease was statistically significant, compared to the threshold values of the contralateral uninjured hind paw and the preoperative heat threshold, respectively. This heat hyperalgesia, manifesting itself as a drop of the noxious heat threshold, was sustained lasting for at least seven days. After the sixth postoperative day, the heat threshold started to increase approaching the baseline value. The experiment was terminated after the seventh postoperative day to reduce unnecessary animal discomfort.

4.2 Effects of conventional analgesics on the plantar incision-induced drop of heat threshold

After confirming the development of post-incision thermal hyperalgesia by measurement of the noxious heat threshold, morphine (0.1 to 3 mg/kg) or its solvent was administered intraperitoneally. Subsequently, threshold measurements were repeated at 10 min intervals. Morphine dose-dependently reduced the incision-induced drop of heat threshold as compared to the solvent-treated group. Its minimum effective dose was 0.3 mg/kg. The 3 mg/kg dose produced a reversal of hyperalgesia by about 60 % without affecting the overall behavior of the animals.

Diclofenac administered systemically in doses from 0.3 to 10 mg/kg i.p. was able to mitigate the postoperative drop of heat threshold in a dose-dependent manner. Its minimum effective dose was 1 mg/kg and its maximal effect ranged to about 40 % inhibition of hyperalgesia.

Systemically administered paracetamol also inhibited the incision-induced thermal hyperalgesia in a dose-dependent manner. The minimum effective dose was found to be 100 mg/kg. The maximum inhibition of thermal hyperalgesia was 52 % at the highest dose applied.

Based on the dose−response relationship for the antihyperalgesic effects of the reference analgesic drugs morphine proved the most potent and paracetamol the least potent in this experimental model as assessed on the basis of the minimum effective doses. Morphine and paracetamol showed similar efficacy, (i.e. maximum inhibition of the threshold drop) while diclofenac was less efficacious. In a series of experiments in previously anesthetized but uninjured animals it was clarified that the highest doses of morphine, diclofenac or paracetamol used in this study failed to alter the baseline noxious heat threshold or to have influence on the overall behavior of the animals.

The local effect of analgesics on the incision-induced heat hyperalgesia was examined by intraplantar administration of doses below the minimum systemic effective doses to avoid any systemic effect. The effect of each drug was compared to an actual solvent control. Drug or solvent was administered just after the first postoperative heat threshold measurement. Intraplantar injection of 10 µg morphine or 100 µg diclofenac significantly decreased subsequent drop of heat threshold. In contrast, locally administered paracetamol failed to have a statistically significant effect.

4.3 Mediators involved in the drop of noxious heat threshold induced by heat injury or plantar incision

Intraplantar administration of 10 µM HOE 140, a selective bradykinin B_2 receptor antagonist, significantly inhibited heat injury-induced noxious heat threshold drop 10 and 20 min after treatment. The overall effect of the drug proved to be significant. In the case of plantar incision, a statistically significant inhibition of the noxious heat threshold drop was observed at all time points of measurement in animals receiving intraplantar HOE 140 compared to the solvent-treated group.

Intraplantar treatment with 10 µM [des-Arg^{10}]-HOE 140, a bradykinin B_1 receptor antagonist, following heat injury resulted in a statistically significant difference in threshold drop between the substance- and solvent-treated groups at only one time point, 20 min after treatment, thus this antagonist failed to produce a significant overall inhibitory effect. In contrast, a local [des-Arg^{10}]-HOE 140 treatment after plantar incision evoked a marked and statistically significant inhibition of the noxious heat threshold drop at all time points of measurement compared to the solvent-treated group.

After heat injury, intraplantar treatment with the non-selective lipoxygenase inhibitor nordihydroguaiaretic acid led to a statistically significant overall inhibition of the heat threshold drop at both applied concentrations. Following plantar incision, intraplantar nordihydroguaiaretic acid treatment at the lower concentration (10 µM) caused no statistically significant difference compared to the solvent-treated group at any time points. With the higher concentration (30 µM) a statistically significant difference between heat threshold drop in the drug- and solvent-treated animals was observed only 10 min after treatment. The overall effect of the drug was not statistically significant at either concentration.

The heat injury-induced drop of the noxious heat threshold was significantly decreased by intraplantar treatment with 100 µM L-NOARG, a nonselective nitric oxide synthase inhibitor, at all time points of measurement except for the last one, when the difference was marked but not statistically significant. In case of plantar incision, the intraplantar administration of 100 µM L-NOARG reduced the drop of noxious heat threshold significantly at all time points of measurement compared to the solvent treated group.

Heat injury-induced drop of noxious heat threshold was significantly attenuated at all time points of measurement by an intraplantar treatment with 0.3 µM TNP-ATP, a P2X purinoceptor antagonist In the case of plantar incision-induced thermal hyperalgesia, the drop of noxious heat threshold was significantly diminished by intraplantar TNP-ATP treatment (0.3 µM) at the first three time points of measurement. The overall inhibitory effect of the substance was significant.

The heat injury-induced drop of noxious heat threshold was diminished by intraplantar treatment with the TRPV1 receptor antagonist AMG9810 in a concentration-dependent manner. The overall inhibitory effect of the lower concentration proved non-significant but that of the higher one was significant. In the case of plantar incision-induced thermal hyperalgesia, intraplantar treatment with the TRPV1 receptor antagonist SB-366791 produced no statistically significant decrease in the noxious heat threshold drop at 10 μM, while at 100 μM it had a significant inhibitory effect at all time points of measurement. Accordingly, the overall inhibitory effect of the lower concentration was not significant but that of the higher one was significant.

4.4 Investigation of a remote antinociceptive effect induced by chemical stimulation of nociceptors

In these experiments, peripheral nociceptors of the acutely denervated hind paw were stimulated by chemical agents applied intraplantarly or percutaneusly. The effect of this procedure on thermonociception in the contralateral, hind paw was investigated. Because intraplantar administration of 1 μg, 10 μg or 100 μg capsaicin into the acutely denervated right hind paw failed to alter the baseline noxious heat threshold of the contralateral hind paw, thermal hyperalgesia was induced by plantar incision in order to detect subtle changes in thermonociception. Intraplantar injection of increasing doses of capsaicin into the acutely denervated right hind paw diminished the incision-induced noxious heat threshold drop of the contralateral hind paw in a dose dependent manner i.e. it evoked a remote thermal antihyperalgesic effect. The lowest capsaicin dose was without a significant effect while the highest dose had an antihyperalgesic effect comparable to that of 3 mg/kg morphine administered i.p.

To clarify the role of capsaicin sensitive nerve endings in the observed effect we exploited that chronic denervation leads to irreversible destruction of nerve endings including capsaicin-sensitive ones rendering the denervated area unresponsive to capsaicin (and any other stimulus) but sparing possible systemic effects of capsaicin. Five days after denervation performed by section of both the sciatic and saphenous nerves intraplantar administration of highest dose of

capsaicin into the denervated hind paw failed to have an effect on plantar incision-induced at hyperalgesia of the contralateral hind paw. Similarly, a sustained exposure to high concentration of capsaicin can cause desensitization of the TRPV1-expressing polymodal nociceptive nerve endings rendering them unable to respond to further stimulation by capsaicin and other stimuli. Pretreatment with intraplantar injection of a single high dose of capsaicin (100 μg) into the intact hind paw was carried out 3 days prior to the experiment. After this capsaicin desensitization, intraplantar injection of capsaicin failed to have any effect on plantar incision-induced heat hyperalgesia of the contralateral hind paw. Pretreatment with the solvent of capsaicin did not alter the inhibitory effect of subsequent capsaicin administration on contralateral thermal hyperalgesia.
Percutaneous treatment with 5 % mustard oil of the denervated hind paw significantly reduced the plantar incision-induced heat threshold drop of the contralateral hind paw. The extent of the mustard oil effect was comparable to that of capsaicin.

Based on previous data obtained at our department we supposed that somatostatin may play role in the remote anthyperalgesic effect. To test this hypothesis systemic pretreatment with cyclosomatostatin 20 min before intraplantar administration of capsaicin or mustard oil anointment was applied. It inhibited its remote antihyperalgesic effect as it diminished the capsaicin-evoked reduction of the incision-induced drop of the heat threshold in the contralateral hind paw. Its solvent did not have any effect on the heat threshold In order to further clarify the role of somatostain in the remote antihyperalgesic effect of nociceptor stimulation, the effect of exogenous somatostatin on incision-evoked heat hyperalgesia was studied. Compared to actual solvent treatment, i.p. applied somatostain significantly reduced the plantar incision-induced noxious heat threshold drop at all measurement points. This effect could be prevented by cyclosomatostatin pretreatment.

In case of analgesic effects it is plausible that endogenous opioids and cannabinoids may be involved. To test this hypothesis systemic pretreatment with the opioid antagonist naloxone or CB_1 cannabinoid receptor antagonist AM 251, was applied, before administration of capsaicin into the denervated hind paw. This

treatment significantly reduced, but did not abolish the analgesic effect of intraplantar capsaicin treatment on the heat threshold drop of the contralateral, incised hind limb. Pretreatment with peripherally acting naloxone methiodide did not lead to reduction in the antihyperalgasic effect of the capsaicin treatment.

While studying another pain modality, i.e. mechanical hyperalgesia, administration of capsaicin into the denervated hind paw significantly diminished the mechanical hyperalgesia of the contralateral paw induced by partial sciatic nerve ligation (Seltzer's operation) at both time points of measurement with a Randall–Selitto apparatus. Similar effect was noted following mustard oil anointment.

Intraplantar injection of the lower of the two effective doses of capsaicin (10 µg) into the intact (not denervated) right hind paw reduced the noxious heat threshold drop induced by plantar incision on the contralateral, left hind paw (Fig. 32). Capsaicin injection induced an intense nocifensive reaction consisting of paw licking and shaking, which lasted for approximately 5 minutes. The higher capsaicin dose was not tested for ethical reasons. The antihyperalgesic effect was found to be significant upon all measurements following treatment.

5 Discussion

In our experiments a novel approach to the study of thermonociception was employed: measurement of the noxious heat threshold temperature with an increasing-temperature water bath. With this method, heat hyperalgesia manifests itself as a drop of heat threshold.

In our first series of experiments surgical incision resulted in a marked, relatively constant drop of heat threshold lasting for several days. The heat threshold drop had a similar time course as the withdrawal latency time reduction measured with the standard plantar test indicating that heat threshold measurement is as reliable as latency determination for revealing incision-induced thermal hyperalgesia.

Due to the excellent reproducibility of heat threshold measurement even with short intervals it was possible to assess the degree and time course of the effect of conventional analgesics within the same animal. All analgesics studied exerted a dose-dependent inhibitory effect on incision-evoked heat threshold drop. As the heat threshold of the intact, uninjured hind paw was not altered even by the highest tested doses of the analgesics, the heat threshold changes were considered true antihyperalgesic effects not secondary to a direct threshold-elevating action. Our findings indicate that the noxious heat threshold is an at least as sensitive parameter as the paw withdrawal latency for revealing the thermal antihyperalgesic action of morphine in the incision model.

The non-selective COX inhibitor diclofenac had a dose-dependent inhibitory effect on the heat hyperalgesia following plantar incision. Paracetamol a non-opioid analgesic was the least potent (i.e. requiring the highest administered dose) among the examined analgesics, but its maximum effect was higher than that of diclofenac but less than that of morphine. The antihyperalgesic action of either morphine or diclofenac administered intraplantarly was similar to that observed in our previous studies examining the heat injury- or resiniferatoxin-induced heat threshold drop. The locally applied doses were substantially lower than the respective systemic minimum effective doses, ascertaining that the drugs acted locally without any systemic effect. The local antinocicptive effect of diclofenac can be explained by inhibition of the formation of nociceptor-sensitizing prostaglandins in inflamed or damaged tissues while in case of opioids a peripheral site of action

at the level of peripheral nociceptors was revealed, especially under inflammatory conditions.

The second series of experiments aimed at comparing peripheral mediators of thermal hyperalgesia evoked by mild heat injury and plantar incision. All test substances were applied intraplantarly at doses that have previously been employed in several other studies making likely that their effects were restricted to the periphery. Activation of the B_2 bradykinin receptor, P2X purinoceptors and the TRPV1 receptor as well as formation of NO were observed in both models. In mild heat injury-induced heat hyperalgesia formation of lipoxygenase products was shown as well, while incision-evoked heat hyperalgesia involved activation of B_1 bradykinin receptors, too. An involvement of both B_2 and B_1 receptor activation in the incision-evoked drop of heat threshold was revealed in our experiments. In our experiments the incision-evoked heat hyperalgesia was tested 18 h after injury when B_1 receptor induction is likely to have already occurred. In case of heat injury-evoked heat hyperalgesia only B_2 receptor activation was found possibly because the measurements took place early, 20–50 min after injury when induction of B_1 expression was not yet likely. During the validation process of the increasing-temperature water bath, it was shown that intraplantar application of COX inhibitors exerted an antihyperalgesic effect following heat injury or plantar incision, in both models decreasing the injury-evoked heat threshold drop. These results also provide evidence for an involvement of COX products i.e. prostanoids in both experimental paradigms. In accordance with the known pro-nociceptive role of lipoxygenase products, our results suggest that activation of the lipoxygenase pathway takes place following both heat injury and plantar incision, but its effect is more pronounced following heat injury. Our results are in accordance with findings of other research groups revealing the mediator role of locally formed NO in both models because an intraplantarly applied NOS inhibitor diminished the heat threshold drop evoked by burn injury or incision. Involvement of P2X receptor activation was demonstrated both in plantar incision and heat injury model by local administration of TNP-ATP, a selective P2X receptor antagonist.The contribution of TRPV1 to the heat threshold drop in these models was expected as thermal hyperalgesia was shown to critically depend on activation of this ion channel in several models of acute/subacute inflammation.

Considering that the sum of the percentage inhibition values obtained with the various mediator blockers exceeds 100 %, it is unlikely that these mediators act independently in a parallel fashion, rather, their action may converge on common pathway(s). Such a role can be proposed for TRPV1.

In summary, relatively small differences were revealed between the examined peripheral mediators of heat hyperalgesia evoked by mild heat injury and plantar incision despite the different nature and time course of hyperalgesia in these conditions.

In the third series of experiments we examined a remote antihyperalgesic effect induced by chemical stimulation of peripheral endings of nociceptive primary afferent neurons. The experiments were designed according to a previous model by Pintér and Szolcsányi in which they revealed a novel neuroregulatory mechanism by discovering a remote, systemic anti-inflammatory effect induced by chemical or electrical stimulation of peripheral nociceptive nerve endings. They showed that activation of the peripheral nociceptors leads to release of somatostatin that is absorbed into systemic circulation and exerts anti-inflammatory actions in different parts of the body behaving as a hormonal agent derived from sensory nerve endings. As somatostatin is known to have antinociceptive action, our hypothesis was that the above-described systemic, sensocrine effect of peptidergic polymodal nociceptors involves a systemic antinociceptive action, too. It explains why the chemically stimulated hind paw had to be acutely denervated in order to exclude possible neural compensatory mechanisms in response to propagation of action potentials to the central nervous system. It should be also noted that acute denervation does not impair the mediator-releasing ability of peptidergic nociceptors. The injection of capsaicin into the denervated hind paw resulted in a dose-dependent reduction in the noxious heat threshold drop induced by prior surgical incision of the contralateral hind paw. This remote antihyperalgesic effect was was comparable to that seen with morphine. As capsaicin is known to act on a subset of primary afferent neurons we hypothesized that neuronal elements are involved. To test our hypothesis capsaicin challenge was repeated following chronic denervation of the stimulated right hind paw. Administration of capsaicin to the chronically denervated paw failed

to alter the noxious heat threshold of the incised left hind paw, thus we concluded that neuronal elements of the paw should be involved in the phenomenon. In addition, this finding argues against an involvement of a possible systemic antinociceptive effect of capsaicin. As the only type of nerve endings known to be responsive to capsaicin are the TRPV1-expressing polymodal nociceptors. We decided to test their involvement by performing local capsaicin desensitization. An intermediate dose of capsaicin was injected into the right hind paw of the animal three days before the actual experiment to allow sufficient time for inhibition of capsaicin-sensitive nerve endings. As capsaicin injected after local desensitization failed to alter the heat threshold on the contralateral incised paw, we concluded that capsaicin-sensitive nerve endings participate in the remote effect of nociceptor stimulation. An activator of TRPA1 receptor mustard oil applied to the denervated hind paw was able to exert a similar remote antihyperalgesic effect, we concluded that the examined phenomenon is not restricted to TRPV1 receptor activation. As somatostatin is known to exert a peripheral antinociceptive effect it was plausible to examine its role in the remote antihyperalgesic effect, too. Following systemic pretreatment with the somatostatin receptor antagonist cyclosomatostatin, the reduction in noxious heat threshold drop of the contralateral incised hind paw was prevented. In a further experiment the animals went through the same operations but received systemic somatostatin treatment instead of peripheral nerve ending stimulation. The effect of i.p. administered somatostatin on the noxious heat threshold drop of the incised hind paw was similar to the antihyperalgesic effect of local capsacin administration into the denervated hind paw. These findings support the role of somatostain release from peripheral nerve endings into the systemic circulation in the remote antihyperalgesic effect. As somatostatin is known to be unable to penetrate the blood brain barrier the likely site of action for the antihyperalgesic effect are the somatostatin receptors located on peripheral nociceptors. Denervation of the stimulated hind paw prevented neuronal impulses from primary afferents from reaching the spinal cord, suggesting that release of SOM from capsaicin sensitive nerve endings is possible without generation of antidromic impulses.

While studying endogenous antinociceptive mechanisms the role of endogenous opioids was inevitable to examine. In another experiment animals received a

systemic pretreatment with the opioid receptor antagonist naloxone before stimulation of the denervated hind paw's nerve endings. This pretreatment resulted in inhibition of the decrease in noxious heat threshold drop of the contralateral, incised hind paw supporting a mediator role of opioids in the remote antihyperalgesic effect. The peripherally acting naloxone methiodide did not have any effect on the antihyperalgesic action of capsaicin, therefore a central site of action of the released endogenous opioids was confirmed. The source of the opioids cannot be determined on the basis of our results.

As pretreatment with a selective CB_1 cannabinoid receptor antagonist resulted in a similar decrease in the remote antihyperalgesic activity as in case of naloxone, we can conclude that endocannabinoids also contribute to the remote antihyperalgesic activity. Although there is evidence for the presence of CB_1 receptors on peripheral terminals of primary afferent neurons a central site of action for them cannot be excluded on the basis of the present results.

According to the findings gathered with specific antagonists we can conclude that at least three mediators (somatostatin, endogenous opioids, endocanabinoids) contribute to the remote antihyperalgesic effect of stimulation of the peripheral nerve endings following acute denervation. The cellular source(s) of these mediators and their possible interconnections cannot be determined on the basis of the present results necessitating further investigations in this direction.

A plausible question was whether the remote antihyperalgesic effect is operational against non-thermal e.g. mechanical hyperalgesia. As neuropathic conditions are often accompanied by a marked mechanical hyperalgesia, we decided to examine whether chronic, neuropathic mechanical hyperalgesia elicited by partial sciatic nerve ligation (Seltzer's operation) can be influenced by the above-described remote antinociceptive mechanism. The denervation was carried out as previously but instead of plantar incision, partial sciatic nerve ligation (48 h prior) was performed on the contralateral side to induce a decrease of the mechanonociceptive threshold as measured with the Randall–Selitto method. Stimulation of the denervated hind paw either with capsaicin i.pl. or mustard oil percutaneously resulted in a reduction of neuropathy-induced mechanical

hyperalgesia. These results indicate that the discovered remote antihyperalgesic is effective not only against heat hyperalgesia of traumatic origin but against neuropathic mechanical hyperalgesia too, suggesting that it can be exploited in a variety of painful conditions including neuropathic pain.

To simulate a more physiological and clinically more relevant situation, in the last experiment we stimulated by capsaicin the nerve endings of the right hind paw without denervation. The hyperalgesic state on the contralateral side was induced by incision. To avoid unnecessary animal discomfort, only the lowest effective dose of capsaicin (producing nocifensive behavior and such as lifting and licking) was administered i.pl. The reduction in noxious heat threshold drop was similar to that found in animals with a denervated hind leg suggesting that denervation, as expected, is not a "sin equa non" of the remote antihyperalgesic effect. Nevertheless, denervation was an important experimental tool for revealing the mechanism of the phenomenon.

The experimental setup used in our study can be considered as a specialized animal model of counter-irritation. Although the precise mechanism of the counter-irritation-evoked analgesia is not known, most authors explain it by a central nervous system processing of the neural impulses. Our present results raise the possibility that counter-irritation-evoked analgesia may be mediated by a peripheral mechanism, too. Moreover, it can also be hypothesized that a sensed irritation is not a prerequisite for the phenomenon.

Our third study has provided evidence for a conceptually novel peripheral neuroregulatory mechanism of pain. Chemical stimulation of the peripheral endings of peptidergic capsaicin-sensitive nociceptive primary afferent neurons can exert a remote, most likely systemic antinociceptive effect that is mediated by release from the stimulated nerve endings of somatostatin (and other mediator(s) which after entering the systemic circulation exerts an antinociceptive action throughout the body. The novelty of this mechanism is that peripheral nociceptors, so far thought to be developed for only detecting painful stimuli, are also a source for antinociceptive mediator(s) functioning as hormone-like agents.

6 Novel findings

1. Using an increasing-temperature water bath, a sustained decrease (lasting for days) of the noxious heat threshold following incision of the plantar surface of the rat hind paw was revealed as a part of heat hyperalgesia.

2. In the incision model, the antihyperalgesic actions of systemically and/or locally applied morphine, diclofenac and paracetamol could be demonstrated making the paradigm suitable for preclinical testing of conventional analgesics.

3. Relatively small differences between peripheral mediators of heat hyperalgesia evoked by mild heat injury and plantar incision were revealed despite the different time course of heat threshold drop. B_1 bradykinin receptors were shown to be involved in the incision-evoked drop of heat threshold whereas an involvement of lipoxygenase products was revealed only in the heat injury evoked hyperalgesia. The B_2 bradykinin receptor, P2X purinoceptors and the TRPV1 receptor activation as well as NO formation were revealed in both models.

4. Chemical stimulation of peripheral endings of capsaicin-sensitive nociceptors in the acutely denervated rat hind paw not connected to the central nervous system can evoke a remote thermal antihyperalgesic effect manifesting itself as an inhibition of incision-induced heat threshold drop in the contralateral hind paw. This novel manifestation of counter irritation-evoked antinociception is peripherally initiated and is mediated by somatostatin, endogenous opioids and endocannabinoids acting on CB_1 receptors.

5. Chemical stimulation of peripheral endings of capsaicin-sensitive nociceptors in the acutely denervated rat hind paw can also lead to a diminishment of mechanical allodynia induced by partial sciatic nerve ligation on the contralateral side demonstrating the broad spectrum of the sensocrine antinociceptive mechanism revealed in the present studies.

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8 Publications related to the dissertation

8.1 Published papers

Furedi R, Bolcskei K, Szolcsanyi J, Petho G

Effects of analgesics on the plantar incision-induced drop of the noxious heat threshold measured with an increasing-temperature water bath in the rat.

EUROPEAN JOURNAL OF PHARMACOLOGY 605:(1-3) pp. 63-67. (2009)

IF: 2.778

Independent citations: 5, All citations: 9

Furedi R, Bolcskei K, Szolcsanyi J, Petho G

Comparison of the peripheral mediator background of heat injury- and plantar incision-induced drop of the noxious heat threshold in the rat.

LIFE SCIENCES 86:(7-8) pp. 244-250. (2010)

IF: 2.704

Independent citations: 2 All citations: 4

8.2 Posters

Furedi R, Bolcskei K, Petho G, Szolcsanyi J.

Somatostatin-mediated, remote thermal antihyper-algesic effect evoked by counter-irritation of the dener-vated hindpaw in rats. Poster presented at: Neuropeptides 2007 – Function, Dysfunction and Therapeutic Options; Santorini, Greece

Furedi R, Bolcskei K, Petho G, Szolcsanyi J. Remote thermal antihyperalgesic effect evoked by counter-irritation of the denervated hindpaw in rats.. Poster presented at: FENS Forum 2008; Geneva, Switzerland

9 Publications not releated to the dissertation

9.1 Published papers

Varga E, Simon M, Tényi T, Schnell Z, Hajnal A, Orsi G, Dóczi T, Komoly S, Janszky J, Füredi R, Hamvas E, Fekete S, Herold R.
Irony comprehension and context processing in schizophrenia during remission - A functional MRI study.
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Muhl D, **Furedi R**, Gecse K, Ghosh S, Falusi B, Bogar L, Roth E, Lantos J
Time course of platelet aggregation during thrombolytic treatment of massive pulmonary embolism.
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IF: 1.248
Independent citations: 1 All citations: 2

Muhl D, **Furedi R**, Cristofari J, Ghosh S, Bogar L, Borsiczki B, Gasz B, Roth E, Lantos J
Evaluation of oxidative stress in the thrombolysis of pulmonary embolism.
JOURNAL OF THROMBOSIS AND THROMBOLYSIS 22:(3) pp. 221-228. (2006)
IF: 1.985
Independent citations: 3 All citations:: 7