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Effects of pre- or postoperative morphine and of preoperative ketamine in experimental surgery in rats, evaluated by pain scoring and c-fos expression.

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Introduction

Since Wall (1988) hypothesised a beneficial post surgical effect of preoperative analgesic treatment (so-called pre-emptive analgesic treatment) as a supplement to postoperative analgesic treatment, the concept has been subject to many scientific debates. According to the hypothesis, applying analgesics before the nociceptive stimulus is beneficial due to reduced wind-up and reduced central sensitisation resulting in diminished risk of postoperative hyperalgesia and allodynia *(Woolf and Chong, 1993).*

The scientific literature provides conflicting evidence for this theory. Beneficial effect of pre-emptive analgesic treatment has been reported after pre-emptive treatment with local analgesics, opioids and NSAID´s compared with placebo *(Woolf and Chong, 1993).* Some clinical settings have showed beneficial analgesic effect of pre-emptive analgesia, when the same pre-emptive and postoperative treatments with lidocaine *(Ejlersen et al., 1992; Doyle and Bowler, 1998)* or opioids *(Katz et al., 1992)* were compared. However, Dahl et al. (1992) and Elhakim et al. (1995) did not obtained supportive results in their clinical studies.

In the majority of studies using animal models addressing this concept, the nociceptive stimulus has been obtained by injection of irritating chemicals, in particular formalin. When somatic tissue is damaged or irritated, nociceptive receptors are activated by peripheral release of extracellular inflammatory mediators. The activated receptors lead the signal to the synapses in the dorsal horn of the spinal cord as a 2-phased signal. In the acute first phase, nociceptive stimuli are mediated centrally through Aδ fibres fibres. During the slower and long-lasting second phase, the nociceptive stimuli are mediated mainly through C-fibres *(Cross et al., 1994).* The release of extracellular inflammatory mediators increases the peripheral excitability, which leads to hyperalgesia *(Woolf, 1995*). Repetitive peripheral nociceptive impulses mediated through C-fibres result in an increased central excitability of dorsal appears to be in part mediated through N-methyl-horn neurones. This state is called wind-up and appears to be in part mediated through N-methyl-D-aspartate (NMDA) receptors on dorsal horn secondary nociceptive neurones. Transmission of multiple slow stimuli leads to release of glutamate, which removes the Mg^{++}-block in the NMDA receptor and allows substantial Ca^{++}-inflow *(Urban et al., 1994).* NMDA receptor antagonists bind to the same site as Mg^{++} and prevents Ca^{++}-inflow *(Hirota and Lambert, 1996; Kress, 1997).* NMDA-receptor antagonists can prevent wind-up but not the initial responses of the neurones, whereas the reverse is true for opioids *(Chapman and Dickenson, 1992).* NMDA-antagonists have no effect on pain of the acute first phase, but may act synergistic to the analgesic effect of opioids
(Chapman and Dickenson, 1992; Honoré et al., 1996).

Only few studies deal with a postoperative

experimental model in animals and those available are conflicting. Brennan et al. (1996) developed an elegant postoperative model in rats with surgical intervention on the plantar surface of the hind foot. In this study a relationship was found between behavioural pain observation scores and mechanical hyperalgesia. Ovariohysterectomized rats have also been used as animal models of postoperative pain *(Lascelles et al. 1995).*

A commonly used method of determining the nociceptive activity caused by a peripheral stimulus is to identify and quantify the nuclear protein Fos expressed in secondary nociceptive neurones in the spinal cord. c-fos is an immediate early gene (IEG), that encodes for Fos. IEG's are rapidly and transiently induced in neuronal cells within minutes of extracellular stimulation (*Sheng and Greenberg, 1990).* The c-fos mRNA accumulates, and reaches its peak after 30 to 40 minutes. The Fos level peaks approximately two hours after induction of c-fos *(Harris, 1998).* Since Hunt (1987) reported, that peripheral inflammation induced c-fos in neurones in the dorsal horn of the spinal cord, many studies have shown the relationship between nociception and c-fos expression.

The aim of the present investigation was to study the effect of pre-emptive versus postoperative opioid analgesic treatment by use of the surgical model of Brennan et al. (1996) and combine the pre-emptive and postoperative opioid treatment with pre-emptive ketamine. The effects were quantified by stereological estimation of the number of dorsal horn neurones expressing c-fos and pain scoring from the operated hind foot.

Materials and Methods

Animals

Forty-eight barrier raised male Mol:WIST Han rats health monitored in accordance with FELASA guidelines *(Kraft et al. 1994*) weighing 250 - 300 g were used in the study. The animals were allocated into eight treatment groups each of eight animals which again were divided into two time groups each of four animals (two and eight hours). The animals were allocated according to a randomisation scheme. They were kept in groups of two at 22±1 ^{0}C, at a relative humidity of 50 - 70 % and dark/light cycle of 12/12 hrs (ligths on from 6 a.m. to 6 p.m.) in transparent Macrolon type 3 cages (Scanbur Ltd, Lellinge, Denmark) on aspen bedding (Finn Tapvei, Kaavi, Finland).

The animals were fed Altromin 1314 diet (Altromin, Gentofte, Denmark) ad libitum and had free access of water. During the acclimatisation period of two weeks they were handled every second day to become familiar with the subsequent handling procedure in the experiment. During the acclimatisation period, animals with loss of body weight, skin wounds or abnormal behaviour were excluded from the study.

Study design

The study design for treatment is given in table 1. From each group four animals were killed two or eight hours after surgery.

The person dosing the animals, observing the animals and determining the number of c-fos positive neurones was blinded to the dosing regime. The dosing regime was disclosed when all results were collected.

Treatments

All animals were treated with isotonic saline, ketamine, (Ketaminol® Vet, Rosco), or morphine, (Morfin "DAK", Nycomed Danmark). Ketamine and morphine were diluted in 0,9 % saline to concentrations of 40 mg/ml and 5 mg/ml, respectively. The drug solutions were administered subcutaneously in the neck in a dose volume of 1 ml/kg. Pre-operative treatments were applied 15 min before anaesthesia for surgery. Post-operative treatment was applied 3 minutes after completion of surgery.

Anaesthesia

Anaesthesia was obtained by placing the rat in a transparent box ventilated with 5 % halothane (Halothane "halocarbon", Halocarbon) in oxygen,

Table 1: Study design

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Group	Pre-operative treatment	Post-operative treatment
1 – Saline treated control	Saline	Saline
2 – pre-operative morphine	Morphine, 5 mg/kg	Saline
3 – post-operative morphine	Saline	Morphine, 5 mg/kg
4 – pre-operative ketamine	Ketamine, 40 mg/kg	Saline
5 – pre-operative ketamine and morphine	Ketamine, 40 mg/kg Morphine, 5 mg/kg	Saline
6 – pre-operative ketamine and post-operative morphine	Ketamine, 40 mg/kg	Morphine, 5 mg/kg

using a high-flow vaporiser. Surgery was performed, when the rats reached surgical level of anaesthesia. This was evaluated by testing paw withdrawal reflex with a forceps. Anaesthesia was maintained with 2% halothane in oxygen applied using a facemask at a flow-rate of 300 ml/min until the end of surgery.

Surgical procedure

The method used was that described in detail by Brennan et al. (1996). In short, a skin incision was applied to the plantar part of the left hind foot, starting 0.5 cm from the proximal edge of the heel and extending 1 cm toward the toes. The underlying muscle (Musculus plantaris) was dissected by blunt dissection and isolated with a forceps. This caused strong distension of the muscle. An approximately 5 mm long incision was made in the muscle along the muscle fibres. The skin wound was subsequently closed with two horizontal mattress sutures of Dermalon 3-0 and the animal was allowed to wake. The mean duration of the surgical procedure was 7.2 min and the mean anaesthetic period (from placing the rat in the induction cage till the end of surgery) was 13.8 min. Potential loss of body temperature in this period was not prevented. The animals

were housed individually until observation for pain scoring and anaesthesia for perfusion fixation.

Pain score

After two or eight hours the animals were observed for pain score by observation of the operated foot. During observation the animals were kept in a transparent cage with grid floor (grid size 6x6 mm, tread diameter 1 mm). For each animal the observation was made in triple and a sum calculated from the three observations for each animal. The observations were made in time intervals of approximately 2 minutes.

The following scoring was used:

0 – normal, full standing on the foot

1 - walking around with lifted heal and standing on the tip of the foot

2 - No or almost no standing on the foot

Perfusion fixation and collection of spinal cord

The animals were anaesthetised by i.p. injection of 1 ml/kg of pentobarbital (5%). A blunt canula connected to a perfusion pump was placed in the left heart chamber. A buffered formalin solution (4%, pH 7.0) was applied in a volume of 10 ml/min. The animals were bled from an incision in the right atria. The columna was isolated from the 10^{th} thoracic vertebra until the sacrum bone. The spinal cord was isolated by flushing the central spinal canal with isotonic saline and placed in formalin fixative the night over at 5^{o} C. After fixation the spinal cord samples were cryoprotected in 10% sucrose for 24 h and in 30% sucrose for 72 h at 5^{o} C.

Preparation for stereology:
An 18 mm piece of each spinal cord sample was used to obtain a sample of tissue sections. The caudal border of intumescentium lumbalis of the spinal cord samples was used as a landmark, and the 18 mm piece was cut 12 mm cranial and 6 mm caudal to that landmark. Before deciding which part of the spinal cord to examine, tissue samples of every 1 mm of the whole spinal cords of four rats, subjected to the same surgical procedure as used in the main study, were examined for c-fos expression. That pilot study showed that most of the c-fos expression after the surgical procedure could be identified within the above mentioned piece of spinal cord. The tissue sample was then obtained by slicing one 100 µm thick frozen cross-sections for every 1500 µm of spinal cord. All spinal cords were sliced in the cranio-caudal direction, and the beginning of the first piece was chosen at random between the first and fifthteenth slice. In this way 11 to 12 pieces of tissue were obtained per animal.

Immunohistochemistry.
The frozen tissue sections were collected in potassium phosphated-buffered saline (KPBS). The tissues were processed immunohistochemically as free-floating sections. All sections were immunostained for c-fos-like protein using an avidin-biotin-peroxidase method *(Hsu et al. 1981).*
The tissue sections were incubated for 10 min in a blocking solution of 1% H_2O_2 in KPBS and were then incubated for 20 min in 5% normal swine serum (NSS) in AB-buffer of 1% bovine serum albumin in KPBS. The tissue sections were then incubated over night in a solution of primary antibody (c-fos antibody, Oncogene, Calbiochem), 1:8000 in AB-buffer.
The incubated sections were washed three times in a washing buffer (0,25 % BSA in KPBS) and incubated for 60 min in biotinylated pig anti-rabbit IgG (DAKO). The sections were washed in wash buffer and in KPBS and then incubated for 1 h in avidin-biotin-peroxidase complex (Vectastain ABC kit, Vector Laboratories). Finally, the sections were washed in wash buffer, in KPBS, and in a TRIS-buffer before they were developed in a DAB-H_2O_2 solution for 20 min. The sections were washed twice in water to stop the staining process.
The sections were mounted on gelatine-subbed slides and air dried over night. Before the sections were cover slipped with eukit as glue, the tissue sections were stained with cresyle violet to enhance the contrast between c-fos positive neurones and c-fos negative neurones.

Counting of c-fos labelled neurones.
An estimate of the number of c-fos positive neurones of lamina I-V of the ipsilateral side of dorsal horn was made, using the optical fractionator method. This method gives an estimate (N) of the total number of c-fos positive neurones, derived from the number of c-fos positive neurones in the sample fraction (N') and the inverse fraction (f) as in the formula: N=N'xf *(Pilegaard et 1996).* This was achieved by counting c-fos positive neurones in a known fraction of the thickness of the individual sections under a known fraction of the sectional area of the dorsal horn, on a known fraction of sections from a piece of spinal cord. The fraction of thickness and the fraction of the sectional area were selected by the use of a commercially available computerised program (C.A.S.T.-Grid, Olympus, Denmark). Estimates of the number of c-fos positive neurones were made with Microsoft® Excel 2000.

Statistics
Parametric data obtained are presented as mean ±SD. The c-fos counts were tested for homogeneity of variance by Forsyth-Brown's test. if the number of observations were equal in all groups and Levene's test, if the number of observations varied between the test groups. Unless noted, the variance was homogenous. Following this different test groups were compared by means of One-way ANOVA. The statistical analyses were made with STATISTICA for Windows, release 5.1, Statsoft Inc.
Ranked type data were tested by Wilcoxon-Mann-Whitney's test for non-parametric data using

StatXact®-4 for Windows, Cytel Software Corporation.

Results

Two hours after surgery the number of c-fos positive neurones was statistically significantly reduced in all groups treated with morphine (Fig. 1). The reduction was the same irrespective of whether morphine was applied before or after surgery.

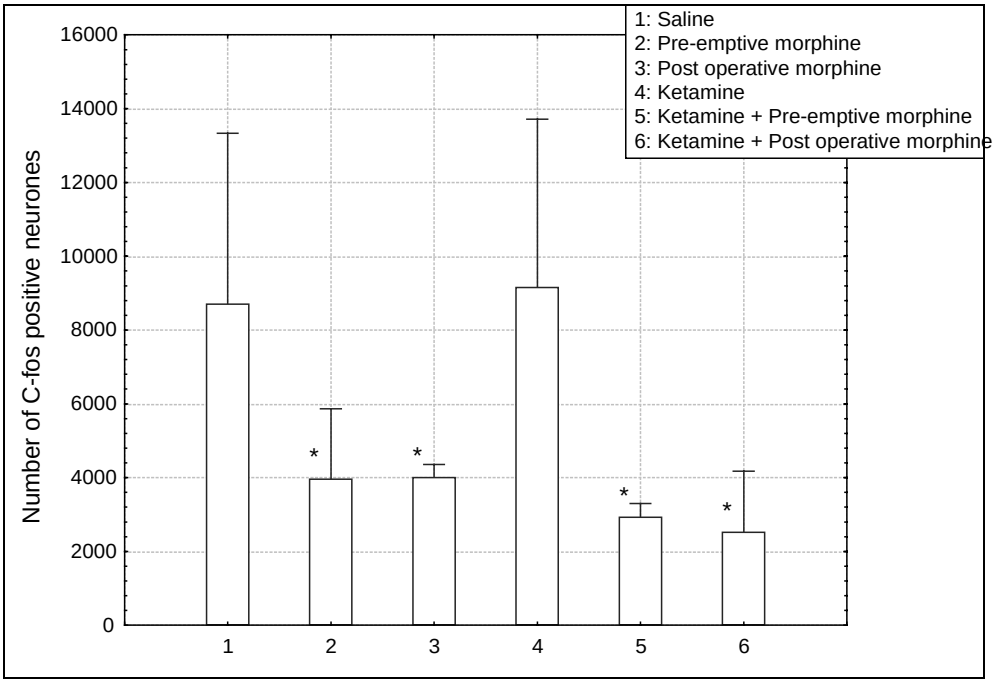

Figure 1. Number of c-fos positive neurones in spinal cord dorsal horn (mean +/- SD) two hours after surgery.
*: Statistically significant less c-fos positive neurones than groups 1, and 4 ($p<0,05$).

Pain scores obtained two hours after surgery tended to reflect the pattern of c-fos expression (Table 2). However, only in group 5 and 6 the pain score reduction was statistically significant.

Eight hours after surgery the number of c-fos positive neurones was reduced in the group treated with ketamine before surgery and in the group treated with ketamine before surgery and with morphine after surgery (Fig. 2). In contrast the number was not reduced in the group treated with

ketamine and morphine before surgery.
Pain scores obtained eight hours after surgery tended to reflect the pattern of c-fos expression (Table 3). However, there were no differences at a statistically significant level.

Discussion

The expression of c-fos has previously been shown to reach its peak approximately 2 hours after application of an acute nociceptive stimulus (*Herdegen et al., 1991; Honoré et al., 1995*). Eight hours after an acute noxious stimulus, the c-fos expression in the dorsal horn has been shown to approach the basic level *(Herdegen et al. 1991).* Therefore the time points of c-fos quantification were chosen at two and eight hours after surgery. The treatment dosages were chosen after pilot studies showing, that 5 mg/kg morphine decreased pain related behaviour, and that 40 mg/kg ketamine induced a short lasting sedation, and finally, that the animals tolerated the combination of the two drugs. Halothane was chosen as anaesthetic agent as this has been shown not to suppress c-fos expression significantly (*Sun et al., 1996).*

The present study has shown that the surgical model used resulted in c-fos expression in roughly 10.000 dorsal horn neurones two hours after surgery. Eight hours after surgery roughly 3.500 neurones expressed c-fos. The eight hour expression may be a combination of neurones activated at surgery, neurones activated by the inflammatory processes subsequently caused by the surgery and neurones activated by the nociception elicited while the rats were walking on the wound.

The experimental procedure used in this study has been developed by Brennan et al. (1996) to evaluate postoperative pain. Rats subjected to the surgical procedure suffered from primary hyperalgesia for 2 hours up to 7 days after surgery, and suffered from secondary hyperalgesia for 2 hours to 2 days after surgery. The results were obtained from behavioural observations and withdrawal thresholds to a variety of mechanical stimuli *(Brennan et al., 1996*). This surgical procedure and the pain score models have been used to evaluate different therapies of postoperative pain. Administration of morphine subcutaneously or intrathecally immediately after wound closure have been found to provide analgesia for up to two hours (*Zahn et al., 1997*).

Table 2: Pain scores two hours after surgery.
*: Significantly lower pain score than group 4 ($p<0,05$).

	Score						
	0	+	++	+++	++++	+++++	Total
Group 1	0	1	1	0	1	1	4
Group 2	1	1	0	2	0	0	4
Group 3	1	2	0	1	0	0	4
Group 4	0	0	0	4	0	0	4
Group 5*	0	3	1	0	0	0	4
Group 6*	0	1	3	0	0	0	4
Total	2	8	5	7	1	1	24

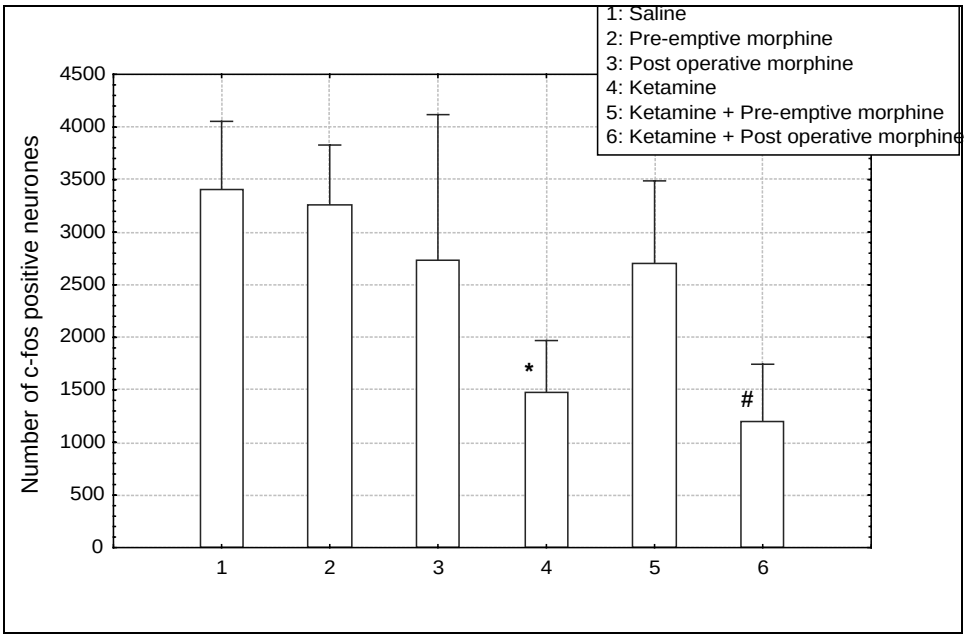

Fig. 2: Number of c-fos positive neurones in spinal cord dorsal horn (mean +/- SD) eight hours after surgery.
*: Statistically significantly less c-fos positive neurones than groups 1, 2 and 3 ($p<0,05$).
#: Statistically significantly less c-fos positive neurones than groups 1, 2, 3, and 5 ($p<0,05$).

Table 3: Pain scores eight hours after surgery.

	Score						Total
	0	+	++	+++	++++	+++++	
Group 1	0	2	1	1	0	0	4
Group 2	0	2	1	1	0	0	4
Group 3	0	0	4	0	0	0	4
Group 4	0	0	2	2	0	0	4
Group 5	0	3	1	0	0	0	4
Group 6	0	0	3	0	0	1	4
Total	0	7	12	4	0	1	24

A similar result was obtained after administration of intrathecal morphine 15 min before the

beginning of surgery, and no differences were seen between the pre-emptive and postsurgical therapy *(Brennan et al., 1997*). The reduction of pain score and of c-fos expression seen in the present study match these conclusions, which suggests that both simple pain score models and c-fos expression are valid tools in this experimental model. The pain score also states, that morphine administered subcutaneously before or immediately after surgery provides similar short lasting postoperative analgesia.

Effect of ketamine in this surgical model has not previously been evaluated. However, other competitive and non-competitive NMDA-receptor antagonists administered intrathecally to rats 30 min after they were subjected to this surgical procedure, did not reduce pain-related behaviour for up to four hours after wound closure *(Zahn et al. 1998*). This conclusion matches the present study, as ketamine alone had no effect on pain scores or c-fos expression 2 hours after surgery.

Equal analgesic effect of pre-emptive or postoperative administration of morphine has been demonstrated in inflammatory pain models *(Abram et al., 1993; Chapman et al., 1994*) and in clinical trials *(Dahl et al., 1992).* However, other studies have shown improved postoperative analgesia of pre-emptive administered analgesic treatment. In a study where female rats were subjected to ovariohysterectomy, pre-emptive intramuscular administration of pethidine significantly reduced tail flick latency when compared to pethidine given postsurgically *(Lascalles et al. 1995*). Rats subjected to intraplantar formalin injection before or after treatment with a μ-agonist significantly reduced pain-related electrophysiological activity *(Dickenson & Sullivan, 1987b*) and pain-related behaviour (*Yamamoto & Yaksh, 1992*). Tölle et al. (1994) showed that pre-emptive administration of morphine reduced c-fos expression after heat stimuli, whereas posttraumatic morphine administration did not. These results and the results of the present study suggest, that the effect of morphine used as analgesia depends on the type of noxious stimuli, the timing of the administration of morphine and the methods used for assessment of the posttraumatic pain.

Kissin (1996) suggested that effective analgesia must cover the entire duration of the high intensity noxious stimuli. Intraplantar inflammation provoked by chemical irritant gives almost instantly high intensity noxious stimuli, whereas surgical procedures create noxious stimuli from the incision and from the releasing of inflammatory mediators caused by damage of tissue. In the present study no difference was found between pre-emptive or postoperative treatments with morphine suggesting that the majority of nociception came from postoperative inflammatory reaction. Alternatively it suggests that the nociceptive input during this short lasting surgical procedure is so intense that the pre-surgical dose of morphine applied was incapable of reducing the input.

Ketamine and other NMDA antagonists have been proved to provide analgesia by lowering or inhibiting wind-up induced by repetitive electrical shocks or heat pulses (*Dickenson & Sullivan, 1987a, Price et al., 1994)*. However, the analgesic effect of ketamine and other NMDA-receptor antagonists has been tested with conflicting results.

In inflammatory animal models pre-emptive administration of NMDA-receptor antagonists MK801 (*Yamamoto and Yaksh, 1992; Zhang et al., 1998*) and (+)-HA966 *(Chapman et al., 1995*) reduced c-fos expression, spinal DYN mRNA production (DYN is an immediate-early-gene, related to c-fos) and pain-related behaviour. The analgesic effect of (+)-HA966 was less potent given after injection of the inflammatory substance *(Chapman et al., 1995*). In a hot plate test, no effect of three different NMDA-antagonists was seen *(Grass et al., 1996).* In a long-term study of hyperalgesia after ligation of n. ischiadicus treatment with NMDA-recptor antagonist MK-801 reduced hyperalgesia compared to untreated rats *(Munglani et al., 1995).*

In clinical trials, ketamine has been used successfully as single analgesic drug in prevention of postoperativ pain *(Kee et al., 1997; Stubhaug et al., 1997).* However, other studies could not demonstrate this effect *(Kucuk et al., 1997; Wong et al., 1998).* Also in clinical trials, ketamine has

been found to act synergistic in combination with morphine *(Javery et al., 1996)* or morphine and lidocaine *(Wong et al., 1998),* whereas Choe et al. (1997) and Kucuk (1997) could not obtain such effect. Combinations of morphine and ketamine in the present study did not provide a synergistic reduction in c-fos expression or in pain score when comparing groups treated with morphine. However, pain scores obtained two hours after surgery showed, that animals treated with ketamine and morphine had a significant analgesic effect compared to animals treated with ketamine alone. The above mentioned clinical trials all used different surgical procedures, why it is difficult to compare those results with the data obtained in the present study.

The above mentioned conclusions suggest that the experimental model is crucial to obtain analgesic effect of NMDA-receptor antagonists. The results of the inflammatory pain models also suggest that NMDA-receptor antagonists are most effective when given before the nociceptive stimuli, hence before the wind-up is evident. In the present study the NMDA receptor blockade was obtained by pre-operative ketamine and its subsequent inhibition of wind-up phenomenon. Therefore it is understandable that the number of neurones expressing c-fos was reduced by ketamine eight hours after surgery. This reduction was seen after ketamine alone and ketamine plus post-operative morphine. Ketamine alone did not reduce c-fos expression two hours after surgery, suggesting that no or only little wind-up was probably evident two hours after surgery. The use of ketamine seems to decrease nociception several hours after the surgical procedure. Correlation between pain score and c-fos expression was not seen eight hours after surgery. This suggests, that c-fos expression may be more sensitive than the simple pain scoring used in this study.

The results of c-fos expression eight hours after surgery of this study show a remarkable difference between the ketamine treated groups. Considering the low number of animals in the test groups, it is a possibility that this may be an incidental result. However, in the group treated with ketamine and pre-emptive morphine 2022 to 3707 c-fos positive neurones were estimated, which is a higher c-fos expression than in any other ketamine-treated animal. In the group treated with ketamine alone 799 to 1907 c-fos positive neurones were found and in the group treated with ketamine and postoperative morphine the number of c-fos positive neurones ranged from 434 to 1708. The individual c-fos expressions and the fact that the study was blinded for the observers that performed the experimental work indicate that the result of the group treated with ketamine and pre-emptive morphine is valid. However, there is no immediate explanation to this result, which need to be further investigated.

The present study shows, that c-fos expression and simple pain score tests are valid methods to evaluate postoperative pain of this particular surgical model. Treatment of postoperative pain with morphine administered before or after the surgery provides analgesia for at least two hours after surgery. Pre-emptive therapy was not found superior to postoperative treatment. Ketamine was found to decrease nociception eight hours after surgery, but conflicting results concerning the effect of a combination with morphine were seen. This suggests that the timing in combination therapy is crucial. Further investigations of combination therapy of opioids and NMDA-receptor antagonists are needed to obtain optimal analgesic therapy with these classes of drugs.

Summary

Pre-emptive analgesic treatment as a supplement to postoperative analgesic treatment should be beneficial by reducing wind-up and central sensitisation. Supporting evidence has been obtained in studies using animal models. However, results obtained from postoperative animal models and from human clinical studies are limited and conflicting. The purpose of this study was to evaluate the effect of pre-emptive or postoperative administration of morphine with or without pre-emptive ketamine in a rat model for postoperative pain. Rats were treated with saline, morphine, ketamine or morphine and ketamine, 15 min before anaesthesia with halothane. Saline or morphine was administered about 3 min after closure of the surgical wound. Postoperative pain was measured by scoring pain behaviour and by

quantification of the number of dorsal horn neurones expressing c-fos at two and eight hours after the surgical procedure.
Two hours after surgery morphine irrespective of treatment regimen reduced the number of c-fos positive neurones to about 30 % of that of untreated rats. Ketamine had no effect. Trends reflecting the pattern of c-fos expression were obtained by pain scoring. Eight hours after surgery ketamine and ketamine + postoperative morphine reduced the c-fos expression to about 30 % of that of saline treated rats. Other treatments had no effect at this time point. The results do not support the theory, that pre-emptive analgesia is superior to postoperative analgesia in reducing postoperative pain.

Resumé
Visse studier har vist, at præ-emptiv analgesi er effektivt til at reducere wind-up og øget excitabilitet i dorsal horns neuroner. Emnet er imidlertid undersøgt i begrænset omfang, specielt i kirurgiske modeller. De tilgængelige data fra smerteundersøgelser i såvel laboratoriemodeller som i kliniske studier med mennesker er til en vis grad modstridende. Formålet med dette forsøg var at undersøge effekten af henholdsvis præ-emptiv (præ-operativ) og postoperativ administration af morfin, med og uden præ-emptiv administration af ketamin i en kirurgisk model med rotter. Det kirurgiske indgreb var en incision gennem hud- og muskelvæv plantart i den højre bagpote, udført i anæstesi med halothan. Dyrene blev behandlet 15 min før induktion af anæstesi med saltvand, morfin, ketamin eller morfin + ketamin. Tre minutter efter operationens afslutning blev rotterne behandlet med saltvand eller morfin. Den postoperative smerte blev bestemt ved at score smerterelateret adfærd og ved at kvantificere antallet af dorsal horns neuroner med c-fos ekspression henholdsvis 2 og 8 timer efter operationen.
To timer efter operation var c-fos ekspressionen i alle grupper behandlet med morfin reduceret til ca. 30 % af ekspressionen i gruppen behandlet med saltvand. Behandling med ketamin alene havde ingen effekt på c-fos ekspressionen på dette tidspunkt. Scorerne as smerterelateret adfærd viste samme tendens som c-fos ekspressionen. Otte timer efter operation var c-fos ekspressionen i dyr behandlet med ketamin eller ketamin + postoperativ morfin reduceret til ca. 30 % af ekspressionen i dyr behandlet med saltvand. De øvrige behandlinger havde ingen påviselig effekt.
De opnåede resultater antyder, at præ-emptiv analgesi med morfin ikke er mere effektiv end postoperativ analgesi i en operationsmodel med rotter.

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