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Cross-over evaluation of electrically induced pain and hyperalgesia

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ABSTRACT

Background: A new experimental protocol of electrically induced pain and hyperalgesia was established to examine orally administered drugs. In a randomized, double-blind, placebo-controlled cross-over study this experimental protocol was used to assess the effects of paracetamol.
Methods: Twenty-four subjects were enrolled in this study. The magnitude of pain, axon reflex flare, and areas of pin-prick hyperalgesia and touch-evoked allodynia were assessed in two consecutive sessions; prior to, and 2 h after drug administration. This protocol was repeated after 1 week. Subjects were randomized to receive either paracetamol (2 g) or a placebo.
Results: In comparison to the placebo arm there were no significant effects of paracetamol on pain, hyperalgesia, allodynia, or axon reflex flare. Pain and flare responses were highly reproducible on the same day ($r = 0.77$ and $r = 0.79$, respectively), and after 1 week ($r = 0.6$ and $r = 0.71$, respectively). The correlation between areas of hyperalgesia and allodynia was, however, significantly improved when the protocol was repeated on the same day ($r = 0.8$ and $r = 0.75$), as opposed to after a week ($r = 0.54$ and $r = 0.53$).
Discussion: The electrical pain model is a well established method for the assessment of intravenously applied analgesics. In order to assess effects of orally applied drugs the model had to be modified: for the assessment of hyperalgesia and allodynia a protocol repeating the model within 1 day proved to have advantages over repetition after 1 week.

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1. Introduction

Central sensitization plays a key role in the development and maintenance of many chronic pain conditions [9,13,20]. A previous study established an electrical stimulation protocol as an experimental model to investigate central sensitization processes as well as acute pain in human beings [10]. A recent study, however, demonstrated a continuous decline of pain ratings and areas of hyperalgesia within one session of investigation. These findings were most likely due to the constant stimulation paradigm [11]. Repetition of the stimulation protocol in a 1-week-interval revealed a successive decline of hyperalgesia, whereas pain ratings remained at a constant level. This psycho-physical pattern was observed in two different models of acute pain and central sensitization [8,11]. The effects of intravenous analgesic drugs on experimentally evoked axon reflex flare, pain, and hyperalgesia have already been investigated using various electrical stimulation protocols [3,10,18]. Due to the fast onset of analgesia after intravenous application the drug, the effects could easily be tested in one session. Placebo sessions were usually performed at 1- or 2-week intervals. Such stimulation paradigm was not applicable to orally administered drugs. In the present study the stimulation protocol was modified to explore the efficacy of orally administered drugs. Two stimulation sessions were performed on 1 day at an interval of 4 h, and were repeated after a week. Thereby we aimed for a lower variation of pain and hyperalgesia.

Koppert et al. could already demonstrate reduced hyperalgesia and allodynia following intravenous application of 1 g paracetamol on [12]. In the present study the effects of 2 g of orally administered paracetamol on pain, axon reflex flare, pin-prick hyperalgesia, and touch-evoked allodynia were investigated hypothesizing that the antihyperalgesic effect of *oral* paracetamol could be confirmed using the modified experimental protocol due to lower variability of hyperalgesia.

2. Methods

2.1. Subjects and study design

The study was conducted at the University Hospital of Erlangen-Nuremberg. The study protocol was approved by the Ethics Committee of the University according to the Good Clinical Practice guidelines and the Declaration of Helsinki.

Twenty-four healthy volunteers (12 males and 12 females) were recruited and introduced to the stimulation procedures described below. Each subject provided written informed consent prior to the participation in the study. The subjects underwent a routine medical and physical examination and were screened for drug abuse. Women were screened for pregnancy.

A double-blind, placebo-controlled, cross-over design was utilised. Each subject received the electrical stimulation protocol on 2 different days, separated by 1 week. The previously described stimulation protocol [10] was performed twice on the same day, with the first session being in the morning and the following session 4 h later in the afternoon. The afternoon session was performed on the contra-lateral forearm (Fig. 1).

2.2. Treatment and dose rationale

Two hours prior to each afternoon session tablets containing either 2 g of paracetamol, or sucrose as placebo were swallowed with 150 ml still mineral water.

In this experimental model it had been shown previously that 1 g of paracetamol administered intravenously attenuates hyperalgesia and allodynia [12]. After application of 2 g of paracetamol administered intravenously in healthy subjects the maximum plasma concentration lies between 235 and 521 mmol/l, which is far below the 1000 mmol/l threshold for potential hepatotoxity [16]. In the present study, therefore, 2 g of paracetamol was applied,

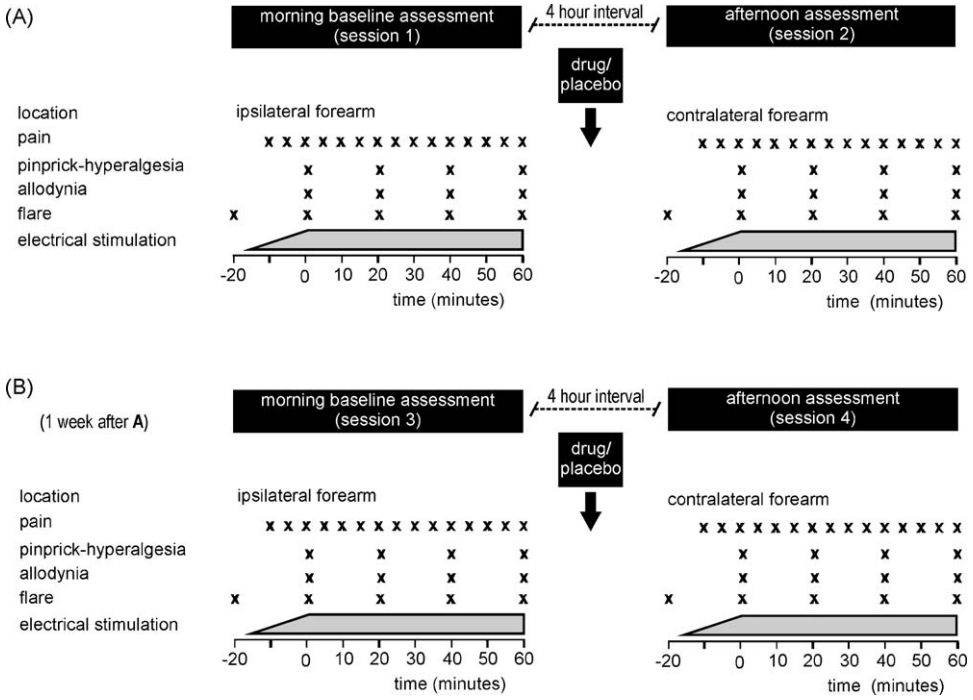

Fig. 1. Schematic view of experimental protocol. (A) Each subject was investigated twice a day repeating the electrical stimulation in the morning of the first day (session 1, baseline) in the afternoon (session 2). These two sessions were separated by a 4-h interval. Two hours prior to the afternoon assessment tablets containing either 2 g of paracetamol or placebo (arrow, "drug/placebo") were administered in a randomised fashion. (B) The identical protocol (sessions 3 and 4) was repeated at a 1-week time interval in a cross-over fashion.

instead of the recommended 1 g, in order to achieve better analgesia without jeopardising safety.

2.3. Study procedures

As described previously [10] intradermally delivered constant current stimuli were used to induce an axon reflex flare, ongoing pain, pin-prick hyperalgesia and touch-evoked allodynia. Two intradermal electrodes (Dermal Dialysis, Erlangen, Germany) were inserted in the central volar forearm. Current pulses (pulse width 0.5 ms, 2 Hz) of alternating polarity were delivered via a constant current stimulator (DS7A mod, Digitimer, Hertforshire, United Kingdom). The current intensity was gradually increased targeting a pain rating of 5–6 on the 11-point numeric rating scale (NRS) with the endpoints 0 (no pain) and 10 (maximum pain imaginable). Current intensity was halted at this level for 2 min, during which the perceived pain gradually declined. After the 2-min stimulation period the estimated pain was documented. The current was then adjusted to gain a pain rating of 5–6 NRS again, kept constant for another 2 min, and followed by another pain rating of the subject. This procedure was repeated over 16 min, after which the current was kept constant for further 60 min. The stimulation pattern of consecutive current increase was recorded for each subject and was used in all subsequent sessions.

2.4. Psycho-physical assessment

During the electrical stimulation protocol the subjects were asked to estimate the perceived intensity of ongoing pain on the NRS at 5-min intervals.

The area of pin-prick hyperalgesia was measured at 20-min intervals using a hand-held von Frey filament, delivering a force of 450 mN. The borders of hyperalgesia were delineated by stimulating along four linear paths in radial orientation to the stimulation site (proximal vs. distal and lateral vs. medial). The measurements were started in an area of normal skin sensation and were continued in 0.5 cm steps towards the stimulation site. Subjects were instructed to report instantly any changes of sensation in terms of increased pain evoked by the filament.

The area of touch-evoked allodynia was determined in 20-min intervals by gently stroking the skin surface with a hand-held cotton wool bud. The subjects were asked to report any new perception of pain or an unpleasant sensation. The areas of hyperalgesia and allodynia were indicated using a marker. The areas were calculated from the recorded distances, as described previously [2].

2.5. Recording and analysis of the axon reflex flare

Axon reflex vasodilatation was quantified by laser Doppler imaging (MoorLDI Ltd., Axminster, United Kingdom). A baseline image was recorded 20 min prior to the experimental protocol and at 20-min intervals after onset of the stimulation. Off- line analysis of skin blood flow was performed using dedicated software (MoorLDI Research Version 5.0, Axminster, United Kingdom), as described previously [5]. Mean flux and standard deviation were determined in a baseline image. Areas of axon reflex flare were determined as flux increase above the baseline scan exceeding 2 folds of the standard deviation. Flare area was assessed in each image of the sequence.

2.6. Statistical analysis

All data were evaluated with Statistica7.1 software package (StatSoft, Tulsa, USA). Pain ratings, axon reflex flare, and areas of hyperalgesia and allodynia were calculated by analysis of variance (ANOVA). Variances of the mean (s^2) between the sessions (j_1 and j_2) were calculated as follows: $s^2(j_1 j_2) = i - \text{sum}[(\text{AUC}(i,j_1) - \text{AUC}(i,j_2))^2]/n$. Variances of the mean squares (r^2) between the sessions were calculated as follows: $r^2(j_1 j_2) = s^2(j_1 j_2)/s^2(1,3)$, with AUC = area under curve; i = panelist-index; j = session-index; n = number of panelists (=24).

p-Values less than 5% were considered as significant. All values were given as mean ± standard error of the mean (SEM).

3. Results

3.1. Gender variability and paracetamol effects

There were no significant gender differences regarding flare development ($p = 0.26$, ANOVA), pain ($p = 0.36$), touch-evoked allodynia ($p = 0.30$) or pin-prick hyperalgesia ($p = 0.91$). Stimulation of the right or the left arm revealed no significant difference for flare ($p = 0.85$, ANOVA), pain ($p = 0.22$), allodynia ($p = 0.89$) and hyperalgesia ($p = 0.80$). A pre-treatment with paracetamol revealed no significant effect on flare ($p = 0.96$), pain ($p = 0.7$), allodynia ($p = 0.77$), or pin-prick hyperalgesia ($p = 0.83$) (data not shown).

3.2. Variability of axon reflex flare, pain, pin-prick hyperalgesia, and touch-evoked allodynia

Pain ratings ($r = 0.86$ and $r = 0.68$ for paracetamol and placebo, respectively), and axon reflex flare areas ($r = 0.72$ and $r = 0.87$ for paracetamol and placebo, respectively) highly correlated between the two sessions performed on the same day within a 4-h interval (Fig. 2, left column). When pain ratings and flare areas were compared between the sessions repeated after 1 week there was a slightly higher variability for pain ratings ($r = 0.6$) and axon reflex flare areas ($r = 0.71$) (Fig. 2, right column).

Similar to the results obtained for pain and axon reflex flare, areas of pin-prick hyperalgesia ($r = 0.86$ and $r = 0.75$ for paracetamol and placebo, respectively) and touch-evoked allodynia ($r = 0.76$ and $r = 0.75$ for paracetamol and placebo, respectively) were highly reproducible when electrical stimulation was repeated after 4 h (Fig. 3, left column). When electrical stimulation was repeated after 1 week there was still a significant correlation between the areas ($r = 0.54$ and $r = 0.53$ for pin-prick hyperalgesia and touch-evoked allodynia, respectively). The correlation, however, was considerably less tight in comparison to the repetition of the test on the same day (Fig. 3, right column).

There was no significant difference between pain ratings and areas of mechanical hypersensitivity when assessments were performed on the same day. When comparing pain and hyperalgesia induced in the morning sessions at a 1-week interval no significant differences for pain ($p = 0.44$), hyperalgesia ($p = 0.11$), and allodynia ($p = 0.82$) were assessed, albeit there was a tendency to decline. Only the electrically induced flare area was slightly smaller when repeated at a 1-week interval (AUC session 1: 84.5 ± 21.9, AUC session 3: 71.0 ± 23.7, $p = 0.045$).

In order to evaluate the differences in variability between different sessions, the variance for repetitions at 1-week interval was calculated (Table 1). Variances for pain ratings, flare area and areas of mechanical hypersensitivity were lower when repetitions were performed on the same day. A particular reduction was observed for hyperalgesia that reached only 37–50% of the variation calculated for the 1-week interval. In contrast, variances of flare response were already pretty low for the 1-week interval and did only decrease to 57–80% when calculated for the repetition on the same day (Table 1).

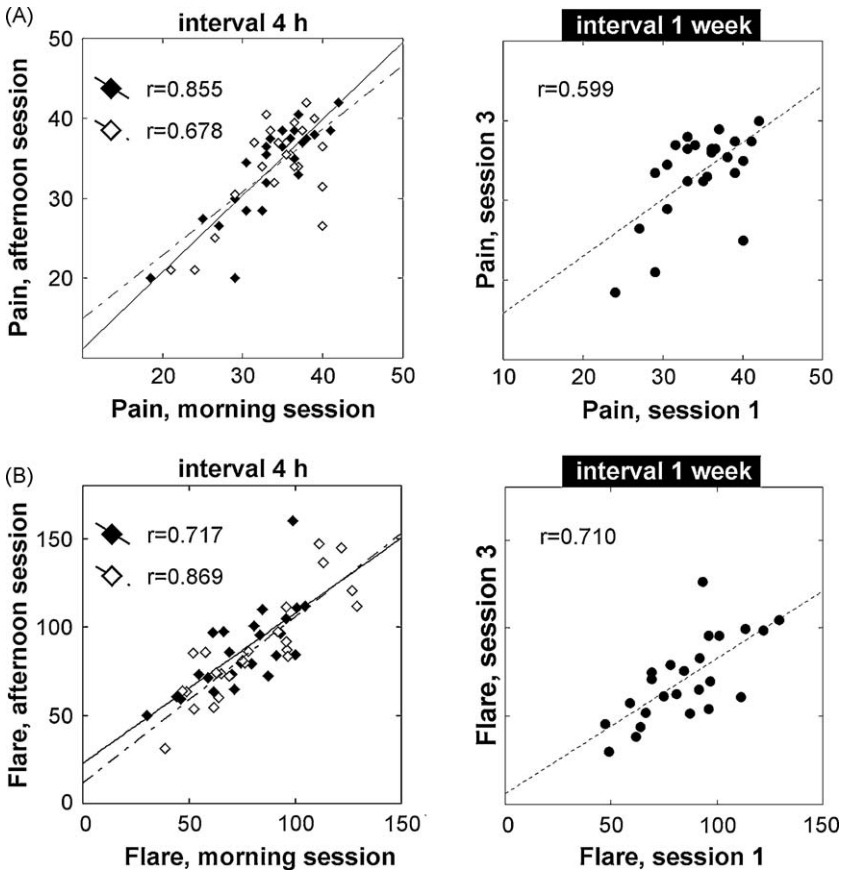

Fig. 2. Correlations of pain (A) and flare (B) for repetitions at the same day (left columns) and at a 1-week interval (right columns). Pain ratings correlated well between the morning and afternoon sessions (upper left panel) both, for placebo (open symbols) and paracetamol (filled) application. There was a less tight correlation of pain ratings, when comparing the baseline morning sessions ("session 1" and "session 3") that were assessed at a 1-week interval (upper right panel). (B) Similarly, flare areas correlated well between the morning and afternoon sessions (lower left panel) for both placebo (open symbols) and paracetamol (filled) application. The correlation between flare areas was not reduced when assessed at an interval of 1 week (lower right panel).

3.3. Adverse events

All study treatments and procedures were tolerated well and no subject withdrew from the study. Each subject cooperated fully during the assessments.

4. Discussion

The electrical stimulation used in the current study activates a subpopulation of mechano-insensitive C-nociceptors (silent nociceptors). This class of nociceptors is involved in the induction of axon reflex flare and secondary mechanical hyperalgesia [19]. The activation of the axons of the nociceptors using an electrical stimulation leads to a well controlled firing frequency of the nociceptors. The resulting action potentials are transmitted to the central synapses as well as to the peripheral terminals. At the peripheral terminals they induce the release of neuropetides, which in turn cause the development of an axon reflex flare. The assessment of this axon reflex flare is a valuable and an objective tool to test the activation of nociceptors. In addition to the peripheral effects, the central sensitization following continuous electrical stimulation can be assessed psycho-physically, measuring the development of hyperalgesia. Common analgesic drugs have shown to differential efficacy to attenuate electrically induced pain and hyperalgesia [2,10,17].

In addition to the development of secondary hyperalgesia around the stimulation electrodes, electrical stimulation or capsaicin injection also causes spreading mechanical hypesthesia [7] that can also be measured contra-laterally [4] and thus is assumed to be of central origin. In contrast to clinical pain, there is no evidence for contra-lateral spread of hyperalgesia in human pain models [4,7].

In the present study there was no significant effect of 2 g orally applied paracetamol on the magnitude of the estimated pain. This was in accordance with previous studies, in which pain thresholds to acute, noxious mechanical or thermal stimulation were not affected by this class of drugs [1,6,15]. Koppert et al. could demonstrate a significant attenuation of secondary hyperalgesia following intravenous administration of 1 g paracetamol [12]. This finding is of particular interest as it provides evidence for a spinal effect of paracetamol that is confined to a reduction of secondary mechanical hyperalgesia, but not to pain intensity. This pattern of antihyperalgesic effect has also been shown for gabapentin in the electrical human pain model [2,17]. In contrast to our expectations there was no significant reduction of hyperalgesia or allodynia

Table 1
Variances of pain ratings (area under the curve for the numeric rating scale values), flare area, area of hyperalgesia and allodynia (area under the curve values) are given for the repetition of the pain model at 1-week interval (column 1). Variances for the repetition on the same day with 4 h interval were calculated and depicted in column 2 (sessions 1 and 2) and column 3 (sesssions 3 and 4).

	Variance 1-week interval (100%)	Relative variance (sessions 1 and 2)	Relative variance (sessions 3 and 4)
Pain	22.1	13.8 (63%)	14.1 (64%)
Flare	474	270 (57%)	380 (80%)
Hyperalgesia	10,957	5348 (49%)	4054 (37%)
Allodynia	6490	4977 (77%)	1784 (27%)

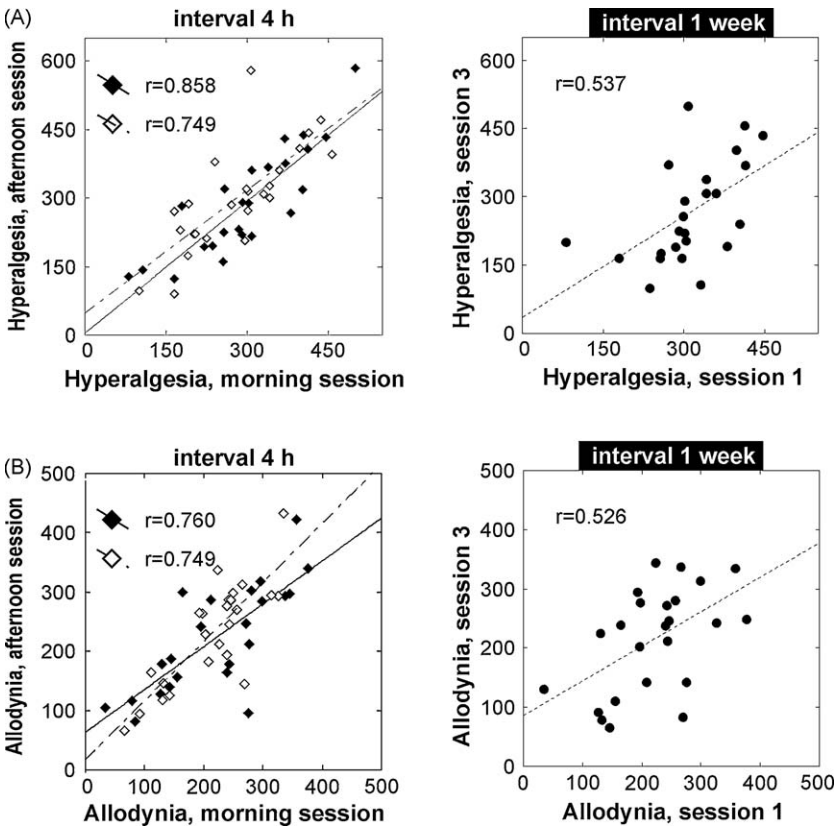

Fig. 3. Correlations of areas of hyperalgesia (A) and allodynia (B) for repetitions at the same day (left columns) and at a 1-week interval (right columns). Areas of hyperalgesia correlated well between the morning and afternoon sessions (upper left panel) both, for placebo (open symbols) and paracetamol (filled) application. There was a marked less tight correlation of hyperalgesia areas, when comparing the baseline morning sessions ("session 1" and "session 3") that were assessed at a 1-week interval (upper right panel). (B) Similarly, areas of allodynia correlated well between the morning and afternoon sessions (lower left panel) for both placebo (open symbols) and paracetamol (filled) application. Again, the correlation between areas of allodynia was markedly reduced when assessed at an interval of 1 week (lower right panel).

after oral administration of 2 g paracetamol in the present study. The different result could be attributed to either pharmacokinetic differences or the experimental set up.

Higher peak plasma concentrations upon intravenous injection might lead to higher central concentrations and hence more efficient antihyperalgesic activity. Higher central levels of paracetamol following intravenous injection as compared to oral application can be assumed regarding the higher occurrence of central side effects for the i.v. routes [14].

The onset of an analgesic effect can be expected by few minutes if paracetmol is applied intravenously [14]. An internal control without a drug infusion can therefore be easily performed within the same experimental session using the pre-injection baseline as a control level. This experimental set up can, however, not be easily used for the assessment of orally administered drugs for the following two reasons: (1) from an ethical point of view the duration of the experimental protocol may exceed the tolerance level of the recruited subjects; (2) a continuous stimulation leads to a gradual decline of the investigated parameters, in particular pain and hyperalgesia [11].

A similarly declining area of hyperalgesia was observed in the present study. This decline could be partly due to an endogenous opioid release, since in a previous work it could be shown that naloxone could prevent it [11].

In the present study, therefore, a cross-over design with two sessions on the same day, followed by two sessions a week later was chosen for the investigation of orally administered drugs. This experimental setting ensured an internal control between the sessions, revealed a high reproducibility of the analyzed parameters within 1 day, and, to a lesser extent, within 1 week. This study protocol could serve as an improved experimental setting for the analysis of the efficacy of analgesics on pain and hyperalgesia.

5. Conclusion

A new experimental protocol of electrically induced pain and hyperalgesia was established to examine orally administered drugs. This experimental setting ensured an internal control between the sessions, revealed a high reproducibility of pain intensity and areas of mechanical hypersensitivity within 1 day and within 1 week. This study protocol could serve as an improved experimental setting for the analysis of the efficacy of analgesics on pain and hyperalgesia.

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Declaration of financial relationship

There are no relationships to be declared.

Conflict of interest

None of the authors has any actual or potential conflict of interest concerning this work.

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