Eur J Clin Pharmacol (1997) 52: 359–364

PHARMACODYNAMICS

J. Lötsch · T. Hummel · H. Kraetsch · G. Kobal

The negative mucosal potential: separating central and peripheral effects of NSAIDs in man

Received: 19 May 1996 / Accepted in revised form: 14 November 1996

Abstract *Objective*: We wanted to test whether assessment of both a central pain-related signal (chemo-somatosensory evoked potential, CSSEP) and a concomitantly recorded peripheral signal (negative mucosal potential, NMP) allows for separation of central and peripheral effects of NSAIDs. For this purpose, experimental conditions were created in which NSAIDs had previously been observed to produce effects on phasic and tonic pain by either central or peripheral mechanisms.
Methods: According to a double-blind, randomised, controlled, threefold cross-over design, 18 healthy subjects (11 males, 7 females; mean age 26 years) received either placebo, 400 mg ibuprofen, or 800 mg ibuprofen. Phasic pain was applied by means of short pulses of CO_2 to the nasal mucosa (stimulus duration 500 ms, interval approximately 60 s), and tonic pain was induced in the nasal cavity by means of dry air of controlled temperature, humidity and flow rate (22 °C, 0% relative humidity, 145 $ml \cdot s^{-1}$). Both CSSEPs as central and NMPs as peripheral correlates of pain were obtained in response to the CO_2 stimuli. Additionally, the subjects rated the intensity of both phasic and tonic pain by means of visual analogue scales.
Results: As described earlier, administration of ibuprofen was followed by a decrease in tonic pain but – relative to placebo – an increase in correlates of phasic pain, indicating a specific effect of ibuprofen on the interaction between the pain stimuli under these special experimental conditions. Based on the similar behaviour of CSSEP and NMP, it was concluded that the pharmacological process underlying this phenomenon was localised in the periphery. By means of the simultaneous recording of interrelated peripheral and central electrophysiologic correlates of nociception, it was possible to separate central and peripheral effects of an NSAID. The major advantage of this pain model is the possibility of obtaining peripheral pain-related activity directly using a non-invasive technique in humans.

Key words Ibuprofen, Tonic pain, Phasic pain; peripheral nociception, trigeminal nociception, carbon dioxide, chemo-somato sensory event-related potential, non-steroidal anti-inflammatory drug (NSAID)

J. Lötsch (✉) · T. Hummel · H. Kraetsch · G. Kobal
Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nürnberg, Universitätsstrasse 22, D-91054 Erlangen, Germany
Tel. +49-9131-856897; fax +49-9131-856898;
e-mail jloetsch@pharmako.pharmakologie.uni-erlangen.de

Introduction

The pharmacological effects of non-steroidal anti-inflammatory drugs (NSAIDs) involve both peripheral and central mechanisms of action [1, 2]. The peripheral effects are mostly due to the inhibition of cyclooxygenases [3, 4]. Recently, effects of NSAIDs were also established at the spinal, thalamic, and cortical levels: spinal administration of several NSAIDs (indomethacin, flurbiprofen, ketorolac, zomepirac, ibuprofen, acetylsalicylic acid, and acetaminophen) significantly reduced behaviour associated with pain in the formalin test in rats [5, 6]. Sodium salicylate increased the nociceptive threshold in rats by stimulation of the hypothalamus [7]. Indomethacin and diclofenac, administered intracerebroventricularly, inhibited nociceptive responses in arthritic rats [8]. Microinjection of sodium acetylsalicylate into the preoptic anterior hypothalamic area produced dose-related analgesia in conscious monkeys [9]. Additionally, the demonstration of analgesic effects of NSAIDs which are only very weak inhibitors of cyclooxygenases, such as R-flurbiprofen or azapropazone, was interpreted as a possible central action [10–12].

In man, indications for a central action of NSAIDs are based on: 1. non-pain-specific effects on the spontaneous EEG activity [12]; 2. effects on late components of pain-related evoked potentials [13] believed to reflect central processing of nociceptive information; or 3.

simply the inconsistencies that arise when the effects are explained by known peripheral mechanisms.

To date, there is a lack of an experimental pain model which allows simultaneous assessment of effects in the periphery and the central nervous system in man. The problem might relate to the unavailability of a peripheral specific nociceptive correlate which can be assessed with non-invasive methods. Such a signal was recently described: the negative mucosal potential (NMP) can be non-invasively recorded from the nasal mucosa after stimulation of nasal nociceptors [14–17]. This response was shown to correlate with pain-related chemo-somatosensory evoked potentials (CSSEP), which have been used in numerous investigations of analgesic drug effects [18–21].

The working hypothesis of the present study was that the combined assessment of a central pain-related signal (CSSEP) and a concomitantly recorded peripheral signal (NMP) allows for the separation of central and peripheral effects of NSAIDs. Similar changes in the periphery and the CNS would indicate a peripheral effect, while changes observed only in the cortical response would indicate a central nervous effect. This hypothesis was tested by creating experimental conditions in which an NSAID (ketoprofen) produced characteristic effects on phasic and tonic pain [22]. Specifically, when phasic (short pulses of CO_2 [14, 23, 24]) and tonic (an airstream of controlled humidity and temperature [12]) painful stimuli were applied to the same nostril, ketoprofen decreased tonic pain, but increased phasic pain [22]. Both peripheral and central mechanisms were used to explain this phenomenon: Peripheral actions may relate to inflammatory processes induced by the tonic stimulus. These processes may have led to changes in composition and amount of the mucus, to alterations of the microcirculation or to changes in the local production of inflammatory mediators and the NSAID might have intervened at this level. Central mechanism of action may relate to a gate-control mechanism localised in the spinal cord. Transmission of tonic pain via C-fibres [25] might have opened or closed a "gate" for transmission of phasic pain via Aδ-fibres [25, 26]. It is conceivable that administration of NSAIDs changed the balance between the two systems [22].

Thus, the aim of the study was to assess the suitability of the experimental pain model to differentiate between peripheral and central effects of NSAIDs.

Methods

Subjects and experimental design

Eighteen healthy subjects (11 male, 7 female; mean age 26 years) participated in the study. All subjects gave written informed consent. The local ethics committee approved the study performed in accordance to the Declaration of Helsinki on biomedical research involving human subjects (Tokyo amendment). According to a double-blind, randomised, controlled threefold cross-over design, the subjects participated in three experiments, separated by at least 5 days, and received either placebo or 400 or 800 mg ibuprofen (Aktren, Bayer, Germany) orally, with 200 ml water. Subjects were requested to abstain from solid food for at least 8 h before commencement of measurements. The experiments consisted of five sessions taking place before administration of the medication, and at 30, 60, 90, and 120 min after. Each session lasted approximately 30 min. During the experiments, subjects were comfortably seated in an air-conditioned room. White noise of approximately 50 dB HL (ERA stimulator, Tönnies, Germany) was used to mask the switching clicks of the chemical stimulator. In an additional training session prior to the actual experiments, subjects became acquainted with the experimental procedures and, specifically, with a breathing technique which avoids respiratory flow inside the nasal cavity during stimulation (velopharyngeal closure).

Pain-related parameters

Stimulation procedures

Tonic and phasic painful stimuli were applied homotopically to the left nostril.

Phasic pain was induced by short pulses of CO_2 (stimulus duration 500 ms, interval approximately 60 s). As described previously [14], CO_2 stimuli specifically excited nasal nociceptors. During sessions, 16 stimuli of two concentrations (52% and 59% v/v CO_2) were applied in a randomised order.

Tonic painful stimulation was produced by means of dry air of controlled temperature, humidity and flow rate (22 °C, 0% relative humidity, 145 ml $\cdot$ s^{-1}). The airstream was delivered throughout sessions, starting 5 min before the beginning of each session. Subjects reported a dull or burning pain reaching its steady state within a few minutes. As a rule, both the slight swelling and the pain induced by this procedure decreased immediately after termination of the stimulation and disappeared within 1 h.

Chemo-somatosensory evoked potentials

CSSEPs were obtained from EEG recordings (bandpass 0.2–30 Hz) from 9 positions of the international 10/20 system (Fz, F3, F4, Cz, C3, C4, Pz, P3 and P4) and referenced to linked earlobes (A1 + A2). Eye-blinks were monitored from an additional site (Fp2/A1 + A2). After analogue-to-digital conversion (sampling rate 250 Hz, CED 1401, UK), stimulus-linked EEG segments of 2048 ms were averaged off-line to yield pain-related late nearfield event-related potentials (for review see [27]). All single responses contaminated by artefacts were discarded from the average. The base-to-peak amplitudes N1 and P2, their latencies and the peak-to-peak amplitude N1P2 were analysed (Fig. 1).

Negative mucosal potentials

The NMP was recorded from the nasal septum by means of a tubular electrode filled with 1% Ringer-agar containing a chlorided silver wire (impedance 1–5 kΩ at 1 kHz in 0.9% NaCl). A chlorided silver EEG-electrode was attached to the bridge of the nose and served as reference. The signals were recorded using DC-amplifiers (Tönnies; lowpass 30 Hz). After analogue-to-digital conversion (sampling rate 125 Hz, CED 1401, UK), stimulus-linked NMP segments of 16384 ms duration were obtained (prestimulus period 4096 ms). Additionally, the subjects were observed via a video camera to control for movements during the recording period. Records that were affected by movements or eye blinks were excluded from further analysis. The remaining records were averaged separately for the two stimulus concentrations (52% and 59% v/v CO_2). Both amplitudes and latencies of the peaks, P1 and N1, were then measured in relation to stimulus onset (Fig. 1). Following the maximum amplitude of the NMP (amplitude N1), we observed variable rates of decay of the signal in different subjects.

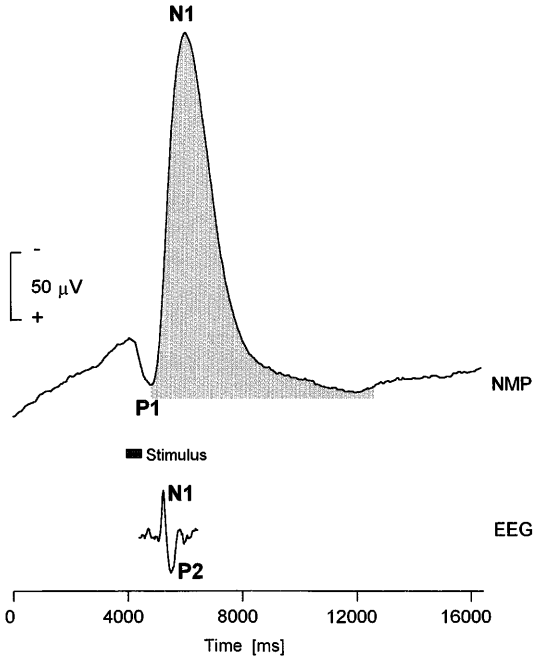

Fig. 1 NMP (*top*) and CSSEP (*EEG*, *bottom*) after painful stimulation of the nasal mucosa with CO_2. The onset of the NMP is observed immediately after application of the painful stimulus, preceding the onset of the CSSEP. Both amplitudes and latencies of the peaks P1 and N1 of the NMP were measured. Additionally, the area under an 8-s segment of the NMP starting from P1 was calculated (*shaded area*). To quantify the CSSEP, the base-to-peak amplitudes N1 and P2, their latencies and the peak-to-peak amplitude N1P2 were analysed

Therefore, we calculated the area under the curve starting from P1 for a period of 8 s (Fig. 1).

Intensity estimates of painful stimuli

After presentation of a phasic stimulus, subjects estimated its intensity in relation to a standard (52% v/v CO_2) which had been applied at the beginning of the first session of each experiment. The intensity of pain was rated by means of a visual analogue scale (VAS) displayed on a computer monitor [19]. The intensity of the standard was defined as 100 estimation units (EU). The tonic painful sensation was rated in a similar manner at the end of each session. Subjects had been instructed to relate their ratings to the intensity of tonic pain experienced during the session before drug administration (100 EU). Since the study was placebo-controlled, possible sequence effects due to sensitisation phenomena were eliminated.

Non-pain related parameters

Tracking performance

During intervals between phasic painful stimuli, subjects were required to perform a tracking task on a video screen [19]. Using a joystick they had to keep a small square inside a larger one which moved around at random. By checking for how long the subjects lost track of the independently moving square, it was possible to detect changes in the state of vigilance and/or motor co-ordination (expressed as percentage of successful tracking performance). The data were averaged separately for each session.

Adverse reactions/cardiovascular parameters

Subjects reported all possible physical or psychological effects related to the medication. Additionally, after each session, they estimated the intensity of four symptoms (“tiredness”, “headache”, “drowsiness”, and “vertigo”) by means of VAS, ranging from 0 (“no such symptom”) to 100 (“symptom experienced at maximum”). Additionally, all spontaneous reports of the subjects were noted, and their blood pressure and heart rate were recorded in the sitting position before and 120 min after drug intake.

Plasma concentrations of ibuprofen

In order to control for absorption of ibuprofen, blood samples were drawn through an intravenous catheter at the end of each experiment, 120 min after administration of the medication. Racemic ibuprofen and S-ibuprofen concentrations were assayed by HPLC [28]. The limit of quantification, consistent with a precision of 10% or less, was 0.1 $\mu g \cdot ml^{-1}$. The coefficient of variation over the calibration range of 0.1–50 $mg \cdot ml^{-1}$ of racemic ibuprofen was less than 6%.

Statistical analyses

SPSS PC+ programs were employed for statistical evaluation. To allow comparison of the data obtained in different subjects on different days, differences were computed between data recorded after (sessions 2, 3, 4, and 5) and before (session 1) administration of the drugs. Subsequently, these data were submitted to univariate analyses of variance for repeated measures (MANOVA; within subject factors “drug” and “session”). Trend analyses were performed only when the MANOVA detected significant effects of the factor “drug”. Regression analyses were performed to demonstrate relations (1) between tonic and phasic pain and (2) between NMP and CSSEP data. The α-level was set to 0.05 .

Results

Pain-related parameters

Chemo-somatosensory evoked potentials (CSSEPs)

Significant effects of the medication (MANOVA factor “drug”) were observed for amplitude N1P2 at recording position Cz in response to the stronger CO_2 stimuli (59% v/v CO_2; $P < 0.05$; $F = 3.97$). This amplitude decreased after administration of placebo (linear trend $P < 0.05$). In contrast, the amplitude N1P2 remained unchanged after administration of ibuprofen (Fig. 2). For amplitudes N1 and P2, no statistically significant effects of the medication were observed. Similarly, no statistically significant effects of the medication on the latencies N1 and P2 were observed. However, at recording position Cz in response to the stronger CO_2 stimuli, the latency of N1 tended to increase after administration of placebo, while it remained unchanged after administration of ibuprofen. In contrast to CSSEP amplitudes, for latencies N1 and P2 there was no significant effect of the medication.

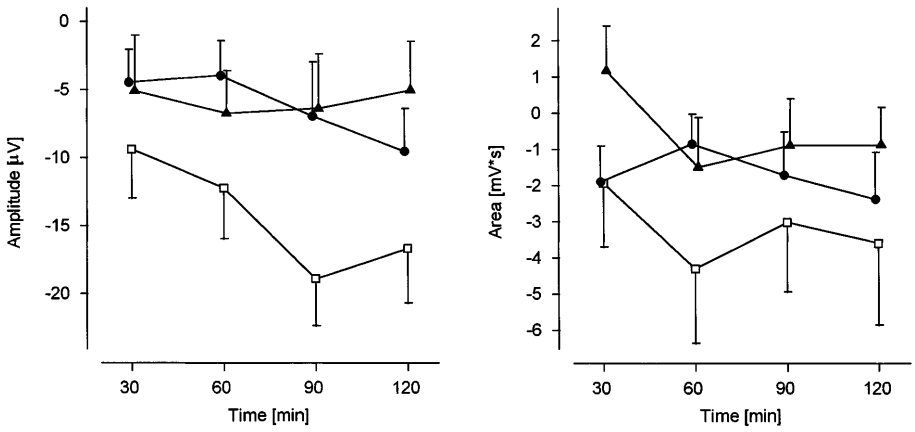

Fig. 2 Means ($n = 18$) and standard errors of means of both CSSEP amplitudes N1P2 (*left*) and areas under the 8-s NMP curve segment starting with P1 (*right*) in response to phasic stimulation with 59% v/v CO_2, after oral administration of 0 (□), 400 (●), and 800 (▲) mg ibuprofen. Data are related to measurements obtained before drug administration. After administration of placebo both CSSEP amplitudes and NMP areas decreased, while after administration of ibuprofen they remained unchanged regardless of the dose

Negative mucosal potentials

The area under the curve decreased after administration of placebo (quadratic trend $P < 0.05$). In contrast, it remained constant after administration of ibuprofen (Fig. 2). However, these changes did not reach statistical significance. A main effect of the factor "drug" could not be established, and only a tendential interaction between MANOVA factors "drug" and "session" ($P = 0.073$, $F = 2.00$) was observed. Changes of the area under curve correlated significantly with the CSSEP amplitude N1P2 (all study medications: $r = 0.75$, $P < 0.001$; placebo: $r = 0.86$, $P < 0.001$). Thus, although showing higher variance than CSSEPs, the NMPs exhibited behaviour that corresponded to changes of CSSEPs (Fig. 2).

Intensity estimates of tonic pain

There was a significant effect of the medication on the ratings of tonic pain ($P < 0.05$, $F = 5.28$). Estimates decreased after administration of 800 mg ibuprofen (linear trend $P < 0.05$). In contrast, estimates remained unchanged after administration of 400 mg ibuprofen or placebo (Fig. 3).

Intensity estimates of phasic pain

The ratings of phasic pain were not significantly influenced by the medication (Fig. 3).

Non-pain related parameters

Tracking performance

Ibuprofen did not produce significant changes in tracking performance, indicating that it did not produce major changes in the subject's vigilance.

Adverse reactions/cardiovascular parameters

Ibuprofen produced no serious adverse reactions. It had no significant effects on cardiovascular parameters, and there was no effect of the factor "drug" on "headache", "drowsiness", and "vertigo" or "tiredness".

Plasma concentrations of ibuprofen

There was a relatively large variability in plasma concentrations between subjects (Fig. 4). Average plasma

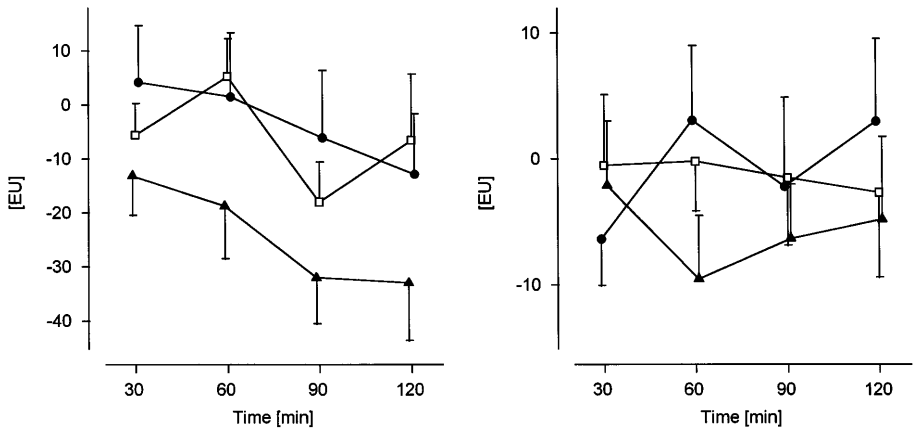

Fig. 3 Means ($n = 18$) and standard errors of means of intensity estimates of both tonic (*left*) and phasic (52% v/v CO_2; *right*) stimuli after oral administration of 0 (□), 400 (●), and 800 (▲) mg ibuprofen. Data are related to measurements obtained before drug administration. Estimates are given in estimation units (*EU*). After administration of 800 mg ibuprofen, ratings of tonic pain decreased, while ratings of phasic pain tended to increase

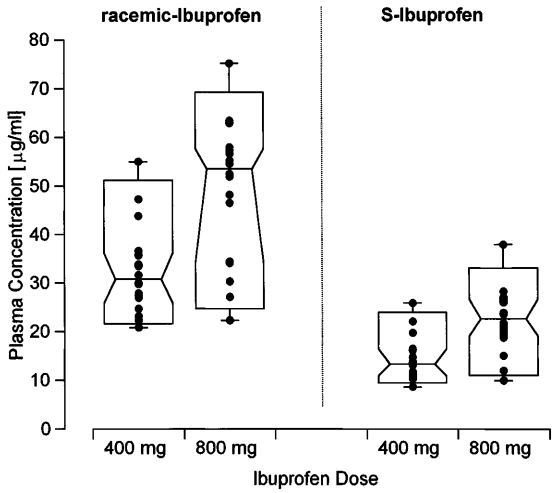

Fig. 4 Plasma concentrations of racemic ibuprofen and S-ibuprofen, measured at the end of each experiment, 120 min after administration of the medication. Box-whisker chart: Sample data are marked with circles (●). Boxes on each side of the notched area include data from the 10th to the 25th percentile and the 75th to the 90th. Whiskers show data from each box end to the 5th and 95th percentiles, respectively. The horizontal lines at the notches represent the median of the data.

concentrations were higher after administration of 800 mg than after 400 mg ibuprofen. However, doubling of the dose was not followed by doubling of ibuprofen plasma concentrations. That is, after 800 mg, individual plasma concentrations (both racemic ibuprofen and S-ibuprofen) increased, on average, only by a factor of 1.6 compared with 400 mg ibuprofen.

Discussion

Phasic and tonic painful stimuli were applied to the same nostril. Tonic pain was assessed with psychophysical methods (pain ratings), while phasic pain was also quantified by means of electrophysiological (CSSEP and NMP) methods.

After administration of an NSAID, under the special experimental conditions of concomitant phasic and tonic painful stimulation of the same nostril, parameters of phasic pain increased (CSSEP and NMP), while parameters of tonic pain (pain ratings) decreased. These findings confirm previous results [22] and suggest a specific interaction between tonic and phasic pain in this situation which is modulated by NSAIDs. From the similar behaviour of CSSEP and NMP, it could be concluded that the pharmacological process underlying this phenomenon is localised in the periphery. If the process were localised in the central nervous system, the changes should have been observed only for the CSSEPs, the NMPs remaining unchanged.

In all probability, this phenomenon is based on local inflammatory processes induced by the tonic stimulus [29]. It may be hypothesised that these inflammatory processes might have affected the characteristics of mucus secretion. These changes might have created a barrier for the gaseous CO_2 stimuli which in turn led to a decrease of correlates of phasic pain when placebo was administered. NSAIDs might have reduced the production of mucus and thus eliminated the barrier for the CO_2 stimuli. This might explain the relative increase in electrophysiological parameters related to phasic pain after administration of ibuprofen in comparison with placebo. However, since mucus production was not measured, other effects might apply as well, e.g. changes in microcirculation or the liberation of inflammatory mediators.

However, the interaction between tonic and phasic pain and its modulation by NSAIDs makes the interpretation of the data difficult in terms of analgesic drug effects. Therefore, in contrast to heterotopic application of phasic and tonic pain stimuli to the nasal mucosa [12, 21], homotopic application seems not to qualify for the reliable assessment of analgesic drug effects. The aim of this study, however, was not to find out whether ibuprofen has analgesic effects, since this has been sufficiently demonstrated previously [21, 30]. The homotopic application of phasic and tonic pain stimuli to the nasal mucosa was utilised in the present study only to test the hypothesis that the simultaneous assessment of peripheral and central nociceptive effects allows for the separation of peripheral and central effects of NSAIDs.

The area under NMP correlated highly with the amplitude N1P2 of the pain-related evoked potentials. This confirms previous work demonstrating the NMP to be a peripheral neurogenic signal, generated by nocisensors, that represents the peripheral nociceptive input signal [14–16]. The NMPs, however, exhibited a greater variance than the CSSEPs. This higher variability might have been caused by the placement of the electrode without endoscopical control. Thus, the variance seen in the NMP might reflect different nociceptor densities at different areas of the nasal mucosa. Under endoscopic control, reproducible electrode placement can be performed at the same region of the nasal mucosa, whereby the intertrial variability of the NMP is reduced. This technique has meanwhile been used successfully [17].

To summarise, by means of concomitant recording of interrelated peripheral and central electrophysiologic correlates of nociception it was possible to differentiate between central and peripheral effects of NSAIDs. A major advantage of the present pain model is the possibility of directly recording peripheral pain-related signals with a non-invasive technique in humans. This represents a direct approach to peripheral and central nociceptive effects of analgesics in man.

Acknowledgement This study was supported by Bayer AG, Leverkusen, Germany.

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