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Constitutive Cyclooxygenase-2 Is Involved in Central Nociceptive Processes in Humans

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Background: **Prostaglandins play a major role in inflammation and pain. They are synthesized by the two cyclooxygenase (COX) isoforms: COX-1, which is expressed constitutively in many cell types, and COX-2, which is induced at the site of inflammation. However, unlike peripheral tissues, COX-2 is expressed constitutively in the central nervous system and may play a role in nociceptive processes. The current study aimed to investigate the role of constitutive COX-2 in the spinal transmission of nociceptive signals in humans.**

Methods: **The authors used 12 healthy volunteers to compare the effects of the specific COX-2 inhibitor sodium parecoxib (1 mg/kg) or placebo, administered intravenously in a double-blind and crossover fashion, on the electrophysiologic recordings of the nociceptive flexion (RIII) reflex. The RIII reflex is an objective psychophysiologic index of the spinal transmission of nociceptive signals and was recorded from the biceps femoris after electrical stimulation of the sural nerve. Two experiments, 7 days apart, were conducted in each volunteer. On each experimental day, the effects of parecoxib or placebo were tested on (1) the RIII reflex threshold, (2) the stimulus–response curves of the reflex up to the tolerance threshold (frequency of stimulation: 0.1 Hz), and (3) the progressive increase of the reflex and pain sensations (*i.e.*, "windup" phenomenon) induced by a series of 15 stimulations at a frequency of 1 Hz (intensity 20% above RIII threshold).**

Results: **Parecoxib, but not placebo, significantly reduced the slope of the stimulus–response curve, suggesting a reduction in the gain of the spinal transmission of nociceptive signals. By contrast, the windup phenomenon was not significantly altered after administration of parecoxib or placebo.**

Conclusions: **This study shows that constitutive COX-2 modulates spinal nociceptive processes and that the antiinflammatory and antinociceptive actions of COX-2 inhibitors are not necessarily related.**

THE analgesic and antiinflammatory actions of nonsteroidal antiinflammatory drugs (NSAIDs) have traditionally been attributed to their inhibition of peripheral prostaglandins.[1,2] Prostaglandins are synthesized from arachidonic acids by two cyclooxygenase isoforms (COX-1 and COX-2) and play a major role in sensitizing nociceptors at the site of tissue injury.[3] COX-1 is constitutively expressed in many cell types, whereas COX-2 is induced at the site of inflammation.[4] Most conventional NSAIDs nonpreferentially inhibit both COX isozymes. Their analgesic effects are thought to be mostly due to their inhibiting the COX-2 isoform, and their adverse effects due to inhibiting COX-1. Therefore, the development of selective COX-2 inhibitors has contributed significantly to therapeutic progress as these molecules have similar antiinflammatory and analgesic properties but are better tolerated clinically.[5,6] However, recent data on acute cardiovascular toxicity has led to new recommendations for their use.[7–9] Therefore, there is active research into the mechanisms of NSAIDs action with the aim of improving their clinical use.

A growing body of experimental evidence suggests that, in addition to their well-established peripheral effects, NSAIDs may also exert their analgesic action directly within the central nervous system (CNS).[2,10] Both COX isoforms are constitutively expressed in rat brain and spinal cord.[11,12] COX-2 is the predominant isoform in the spinal dorsal horn and could play a role, not only in pathologic inflammatory pain, but also in normal physiologic pain (*i.e.*, without inflammation). There are few experimental data confirming the role of constitutive COX-2 in normal pain processing in animal models,[2,13] but no information in humans. More generally, there is no direct evidence for central antinociceptive effects of selective COX-2 inhibitors in humans. However, studies based on experimental models of inflammatory secondary hyperalgesia indirectly suggested a central action of parecoxib[14] and rofecoxib,[15] but not valdecoxib.[16]

In the current study, we analyzed the role of constitutive COX-2 in central nociceptive processes. We compared the effects on the nociceptive flexion (RIII) reflex of intravenous administration of parecoxib or placebo administered according to a double-blind, crossover design in healthy volunteers.

Parecoxib is the sulfonamide-based prodrug of valdecoxib. It is a highly specific COX-2 inhibitor and is the only available parenterally administered coxib.[17] The RIII reflex is elicited by electrical stimulation of a cutaneous sensory nerve and is recorded from a flexor muscle on the ipsilateral limb. This polysynaptic spinal reflex is considered to be a reliable index of spinal nociceptive signal transmission because its threshold and amplitude are closely related to those of painful cutaneous sensations evoked by electrical stimulation.[18] RIII reflex recordings have been used in numerous pharmacologic

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studies related to analgesia in humans.[19,20] In particular, it was used to reveal the central action of conventional NSAIDs (*i.e.*, mixed COX-1–COX-2 inhibitors) on nociceptive processes.[21–23] In the current study, we tested the effects of parecoxib on the RIII reflex stimulus–response curves. We also analyzed the effects of parecoxib on the progressive increase of the reflex response and resulting sensation induced by repeated series of stimuli at relatively a high frequency (*i.e.*, 1 Hz) of fixed intensity. This "windup" phenomenon is due to the summation of nociceptive input over time (*i.e.*, temporal summation) in the spinal cord and is considered to be an experimental elementary form of central sensitization.[24]

Therefore, the goal of this electrophysiologic study was to demonstrate in humans that COX-2 inhibitors have central effects and act on nociceptive processes independently of inflammation.

Materials and Methods

The experiments were approved by a local ethics committee (Comité de Protection des Personnes, Hopital Ambroise Paré, Boulogne-Billancourt, France) and conducted in 12 paid healthy volunteers. The volunteers were carefully briefed about the experimental procedures and gave informed written consent for their participation in the study.

Electrophysiologic Recording of the RIII Reflex

During the recordings, the subjects sat comfortably reclined to ensure a state complete muscular relaxation (fig. 1). The RIII reflex was evoked and recorded with a computerised system (Notocord Systems, Croissy, France), using previously described techniques.[25,26] Briefly, the sural nerve was electrically stimulated at a rate of 0.1 Hz using a pair of surface electrodes placed 2 cm apart on the degreased skin overlying the nerve within its retromalleolar path. The electrical stimuli consisted of trains of six rectangular 1-ms pulses delivered over 12 ms from a constant current stimulator. Electromyographic responses were recorded from the ipsilateral biceps femoris muscle using a pair of surface electrodes placed 2 cm apart on the degreased skin over the muscle. The electromyographic responses were then amplified, digitized, and full-wave rectified, and the RIII response was quantified from the resulting integrals. The reflex responses were identified as multiphasic signals and integrated in a time window from 90 to 180 ms after stimulus onset. This time window restriction avoids any tactile (RII) reflex that can occur between 50 and 70 ms after stimulation or any artifacts produced by involuntary movements that can be observed as early as 250–300 ms after stimulation. Each individual experiment started with a control period during which the stimulus was applied at an intensity 20% higher than the threshold required for stable RIII reflex responses. This control period was considered to be a prerequisite before starting the pharmacologic procedure. The stimuli elicited slightly painful sensations similar to pinprick and were described by the subjects as originating from the stimulating electrodes and projecting into the distal cutaneous receptive field of the sural nerve on the lateral side of the foot.

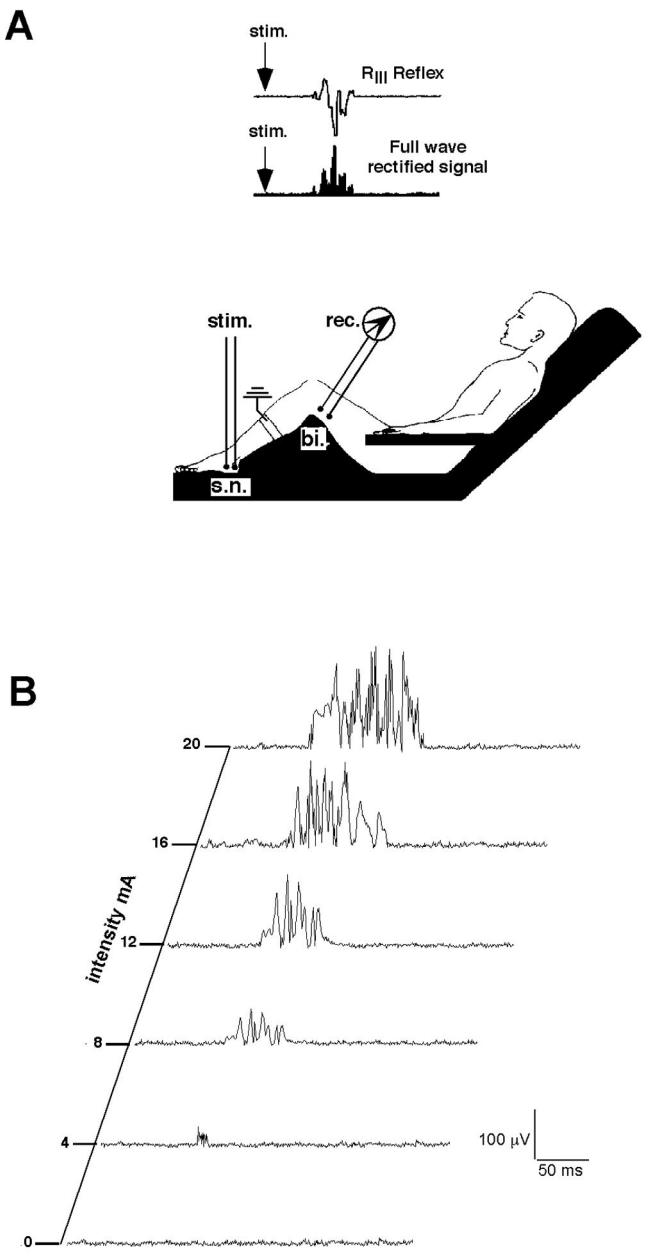

Fig. 1. (*A*) Experimental setup for recording the RIII reflex. The sural nerve (s.n.) was stimulated (stim.) behind the lateral malleolus, using a pair of surface electrodes. The electrical responses were recorded (rec.) from the ipsilateral biceps femoris muscle (bi.) using a pair of surface electrodes. An example of an RIII reflex response and the corresponding full-wave rectified signal are shown in the *upper part* of the figure. (*B*) Individual example of RIII reflex responses with increasing intensity of stimulation of the sural nerve at 0.1 Hz.

Experimental Procedures

This pharmacologic study was organized as a double-blind, crossover trial. We randomly assigned a placebo (saline) or 1 mg/kg parecoxib (Laboratoire Pfizer, Paris, France) in a volume of 10 ml, which was injected intravenously over a period of 10 min, with a maximum parecoxib dose of 80 mg. This dose was chosen because it was in the range used in clinical trials.[27,28] The maximum dose corresponds to the daily maximum dose authorized for clinical use in France. The experiments were conducted in the volunteers twice, with each experiment separated by an interval of 7 days. Six stimulation sequences were used on each experimental day: two before injection (*i.e.*, control period), and then at 20, 40, 60, and 80 min after administration of parecoxib or placebo. Each sequence consisted of (1) determining the RIII reflex threshold (defined as the average minimal current that elicited the reflex response) by four successive sequences of increasing and decreasing the stimulus intensity by steps of 0.5 mA, (2) building of the recruitment curve for the reflex as a function of stimulus intensity by increasing progressively the stimulus intensity by steps of 1 mA to the tolerance threshold at a frequency of 0.1 Hz (*i.e.*, 6 stimuli/min), and (3) applying a series of 15 stimuli at 1.2 times the threshold at a frequency of 1 Hz to analyze the windup phenomenon. After each windup sequence, the subjects were asked to use a 100-mm visual analog scale, graduated from 0 (no pain) to 100 (worst possible pain), to rate both the sensation evoked by the first stimulus in each series and the maximum pain produced by any of the stimuli. Blood pressure, heart rate, and arterial oxygen saturation were monitored during the experimental sessions. Side effects such as nausea, vomiting, sedation, dysphoria, and hallucinations were recorded when present. Sedation was scored using the following scale: 0 = patient fully alert; 1 = patient with intermittent sedation; 2 = patient sedated but responsive to verbal stimuli; 3 = patient unresponsive to verbal stimuli.

Data Analysis

Data are expressed as mean ± SEM. The reflex threshold was defined as the minimum intensity inducing an RIII response for at least 50% of the stimuli. The tolerance threshold was the maximum tolerable stimulation intensity defined by the volunteer during recording of the recruitment curve. Each reflex response was expressed as a percentage of the maximum response observed during recording of the control recruitment curve (*i.e.*, before the injection) to allow analysis of the group data. Recruitment curves were normalized between 0 and 20 mA according to the last observation carried forward method, such that when the tolerance threshold was less than 20 mA, the final value obtained for the reflex was assigned to all the higher intensities in the series. The windup phenomenon was analyzed by expressing each response during the sequence of 15 stimuli at 1 Hz as a percentage of the first response. We used Wilcoxon signed ranks test to compare paired data. The areas under the mean recruitment curves (AUCs) and the mean windup curves were calculated and used to compare the effects of the placebo and the parecoxib. A repeated-measures analysis of variance was used to test treatment, subject, sequence, and period effects. Results were considered to be significant at $P < 0.05$.

Results

Twelve volunteers (6 men, 6 women; aged 21–40 yr), completed the two sessions of the study. The mean dose of parecoxib administered intravenously was 57.0 ± 12.0 mg. No side effects were reported with either the placebo or parecoxib.

Effects of Parecoxib on the Recruitment Curve of the Nociceptive Flexion Reflex

The AUCs were similar before the injection of parecoxib or placebo (fig. 2). After administration of parecoxib, the AUC progressively and significantly decreased between 20 and 60 min, returning to baseline values at 80 min (fig. 2A). By contrast, we observed no significant change of AUC after administration of the placebo. The effects due to parecoxib were significantly different from those due to placebo ($P < 0.05$) at 40 and 60 min. The effects on the recruitment curve were not influenced by the sequence (parecoxib–placebo or placebo–parecoxib) or the period (first or second session).

As illustrated in figure 3, the decrease in AUC was due to the slope of the recruitment curve decreasing, with no significant change in the RIII reflex threshold at any time after the injection. Therefore, the mean RIII threshold, which was not significantly different at baseline between the parecoxib (7.8 ± 1 mA) and placebo (7.0 ± 0.7 mA) groups, was not significantly altered 60 min after treatment (7.5 ± 1.0 mA after parecoxib and 6.2 ± 0.6 mA after placebo). By contrast, the mean tolerance threshold (*i.e.*, the maximum stimulus intensity on the recruitment curve), which was not significantly different at baseline between the parecoxib (14.3 ± 2.7 mA) and placebo (15.0 ± 3.6 mA) groups, was significantly higher ($P < 0.05$) 40 and 60 min after administration of parecoxib (*i.e.*, 17.2 ± 2.4 and 17.3 ± 2.0 mA) than after the placebo (14.1 ± 1.1 and 14.0 ± 1.3 mA).

Effects of Parecoxib on the Windup Phenomenon

As previously described,[25,26] applying a series of 15 stimuli at 1.2 times the reflex threshold and at a frequency of 1 Hz progressively increased the reflex responses (up to 250% of the first response). This windup of the RIII reflex, due to the temporal summation of nociceptive stimuli, was similar during the two control

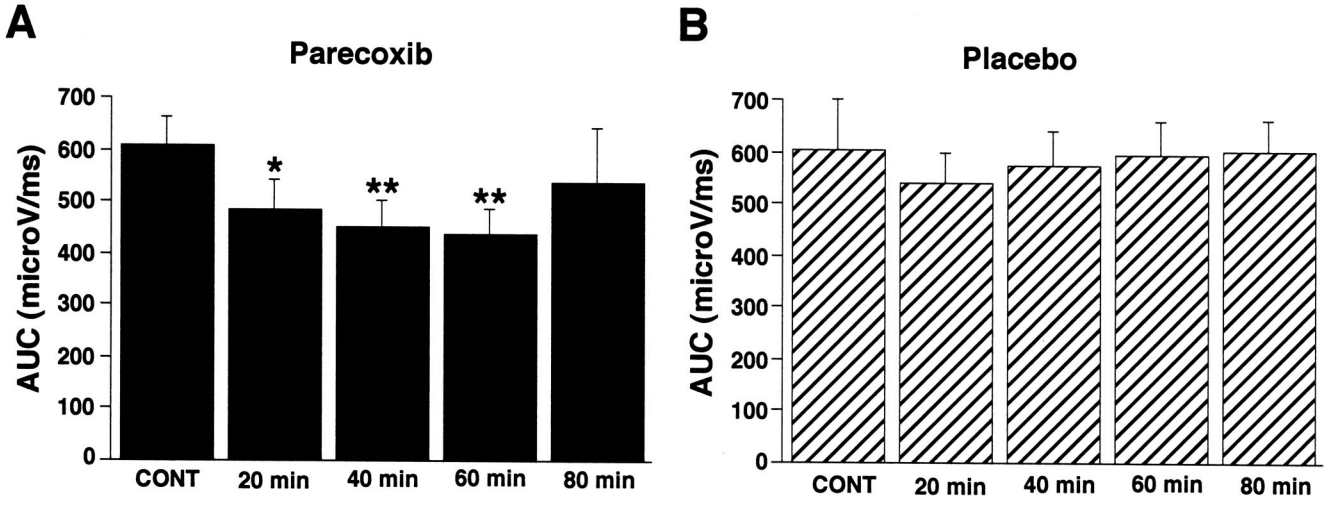

Fig. 2. Comparisons of the area under the recruitment curves (AUCs) from the control baseline period (CONT) and 20, 40, 60, and 80 min after the administration of parecoxib (*A*, *black columns*) or placebo (*B*, *hatched columns*). A significant reduction in the recruitment curve was observed from 20 to 60 min after the administration of parecoxib but not with the placebo. Data are mean ± SEM. * $P < 0.05$. ** $P < 0.01$.

periods and did not significantly change after administration of the placebo or parecoxib (fig. 4). Consistent with these electrophysiologic results, the progressive increase in pain sensation during the application of high-frequency stimulation was similar in the two treatment groups at baseline (visual analog scale score increased from 21.6 ± 8 to 52 ± 12 in the parecoxib group and from 23.6 ± 11 mm to 49 ± 15 in the placebo group) and was not significantly different after administration of parecoxib or placebo.

Discussion

The systemic administration of a clinically active dose of parecoxib significantly reduced the nociceptive flexion RIII reflex in healthy volunteers. This shows that COX-2 inhibitors act centrally and that the spinal transmission of nociceptive signals is affected by constitutively expressed COX-2 in the central nervous system. More generally, our results indicate that the antiinflammatory and antinociceptive actions of COX-2 inhibitors are not necessarily related.

Nonsteroidal antiinflammatory drugs have long been suspected to affect the CNS,[29] as numerous animals studies over the past 15 yr have suggested.[2,13] The finding that both COX isoenzymes are expressed in the CNS and that COX-2 is overexpressed in CNS neurons after peripheral inflammation, whereas COX-1 is overexpressed in the microglia,[30] and that prostaglandins, which are released in the spinal cord during nociceptive stimuli, could facilitate central nociceptive transmission strongly support this idea.[2,4,13,31] These results challenged the classic notion that the antinociceptive action of NSAIDs was due to a reduction of nociceptor sensitization. However, the peripheral and central modes of NSAID actions should not be considered as mutually exclusive but as complementary and possibly synergistic. In addition, the fact that COX-2 is also constitutively expressed in the CNS suggests that it could have a role in normal physiologic pain (*i.e.*, without inflammation).

These hypotheses were based mostly on animal data since few studies were performed in humans. Investigation of the central effects of COX-2 inhibitors in humans has relied on the analysis of primary and secondary hyperalgesia induced by cutaneous ultraviolet B irradiation injury,[15] electrical stimulation,[14] or capsaicin application.[16] However, these models allow only an indirect approach to the central analgesics effects of treatment. In addition, the results seem to depend on the type of experimental pain model, because negative results were reported with the capsaicin model.[16] Our data based on recordings of the nociceptive flexion RIII reflex, which represents an objective and quantifiable electrophysiologic correlate of the spinal transmission of nociceptive signals, show more directly the central action of COX-2 inhibitors. This methodology is particularly interesting because the cutaneous electrical stimulation of the sural nerve at the ankle bypasses the peripheral nociceptor. Therefore, changes in the RIII reflex after administration of pharmacologic agents, which do not act on the nerve conduction or muscular contraction, can be attributed to a central action of the drug. This methodology allows analysis of the pharmacologic effects on the pain threshold and also over a wide range of suprathreshold stimulus intensities. The central effects of various mixed COX-1–COX-2 inhibitors (ketoprofen, ibuprofen, indomethacin) have been confirmed in healthy volunteers using this methodology.[21–23] Our finding that a more selective COX-2 inhibitor modulates the RIII reflex complements these results and suggests that the central action of conventional NSAIDs involves the inhibition of

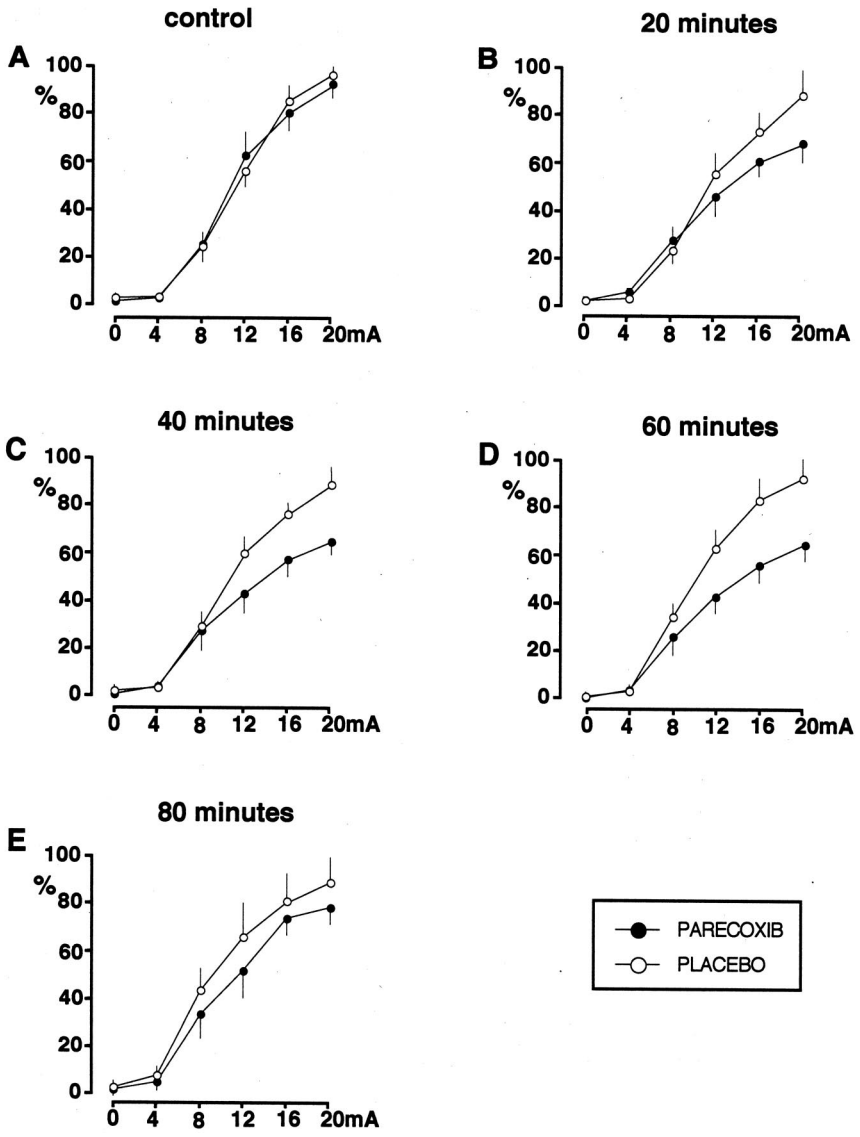

Fig. 3. Effects of administration of placebo or parecoxib on the shape of the recruitment curves for the RIII reflex at baseline (*A*) and 20 (*B*), 40 (*C*), 60 (*D*), and 80 (*E*) minutes after the administration of placebo (*white circles*) or parecoxib (*black circles*). Reflex responses are expressed as a percentage of the maximum response obtained when building the control curve. A progressive reduction of the slope of the recruitment curve was observed from 20 min after the administration of parecoxib.

central COX-2. This result, however, does not exclude that central COX-1 has also a role in analgesia, as suggested by experimental studies.[30,32]

The preferential effects of parecoxib on the RIII reflex recruitment curve (*i.e.*, intensity–response) may indicate that it more specifically acts on the "gain" of nociceptive signal transmission in the spinal cord. Consistent with this, COX-2 has been shown to be the predominant constitutive isoform expressed in the CNS and particularly in the spinal cord.[12,13] In accordance with electrophysiologic and behavioral data in animals,[13] our results indicate that constitutive COX-2 and probably prostaglandins are involved in normal nociceptive processing in humans. However, because in our study parecoxib was injected intravenously, we cannot exclude that it also acts supraspinally and the modulation of the RIII reflex involved descending controls. Interestingly, Willer *et al.*[33] showed that the effects of ketoprofen on the RIII reflex were reduced in paraplegic patients with a complete spinal cord transection, suggesting that mixed inhibitors could act supraspinally.

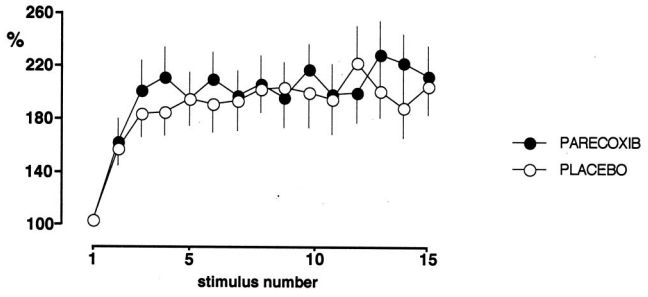

Fig. 4. Effects of placebo and parecoxib on the windup of the RIII reflex. Each reflex response was expressed as a percentage of the first response in the series.

A series of animal studies have suggested that COX-2 and prostaglandins, especially prostaglandin E2, are involved in central sensitization (*i.e.*, hyperexcitability of spinal nociceptive neurons) and hyperalgesia developing after peripheral inflammation and probably also nerve

injury.[2,30,32,34,35] The fact that COX-2 inhibitors reduced secondary hyperalgesia also suggests an action on central sensitization.[14,15] In the current study, we investigated the effects of parecoxib on the progressive increase (*i.e.*, windup) of the RIII reflex induced by the temporal summation of the nociceptive inputs, which may be related to central sensitization.[24,36] Consistent with the results of animal studies, it has been shown previously, in humans, that the windup of the RIII reflex involves activation of *N*-methyl-D-aspartate receptors, because it is selectively reduced by low doses of ketamine.[25] In the current study, the windup phenomenon was not altered by parecoxib. This is consistent with the results of animal electrophysiologic studies of the effects of NSAIDs on windup,[37,38] although contradictory results have also been reported.[39] In any case, the opposite effects of parecoxib and ketamine on windup of the RIII reflex suggest that *N*-methyl-D-aspartate and COX-2 play different roles in the central nociceptive mechanisms.

In conclusion, our results provide the first electrophysiologic experimental evidence showing that constitutive COX-2 can modulate central nociceptive processes in humans independent of peripheral inflammation. Therefore, the development of centrally acting COX-2 inhibitors may be of interest in the treatment of pain. The current study also shows that recordings of RIII reflex and analysis of its recruitment curve are a reliable experimental model for analyzing the analgesics effects new COX-2 inhibitors.

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