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Research report

The NSAID dexketoprofen trometamol is as potent as μ-opioids in the depression of wind-up and spinal cord nociceptive reflexes in normal rats

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Abstract

The aim of this study was to examine the potency of the antinociceptive effects of the non-steroidal antiinflammatory drug (NSAID), Dexketoprofen Trometamol (the active enantiomer of ketoprofen) on spinal cord nociceptive reflexes. These effects were compared with those of the μ-opioid receptor agonist fentanyl in normal animals. The experiments were performed in male Wistar rats anaesthetised with alpha-chloralose. The nociceptive reflexes were recorded as single motor units in peripheral muscles, activated by mechanical and electrical stimulation. Both dexketoprofen and fentanyl inhibited responses evoked by mechanical and electrical stimulation with doses in the same nanomolar range (dexketoprofen ID50s: 100 and 762 nmol kg^{-1} and fentanyl: 40 and 51 nmol kg^{-1}, respectively). Dexketoprofen and fentanyl also significantly inhibited wind-up. Since fentanyl has been shown to be some 1000 times more potent than morphine in this type of experiments, we conclude that dexketoprofen has central analgesic actions in normal animals and depresses nociceptive responses with a potency similar to that of μ-opioid agonists.

Keywords: Nociception; Withdrawal reflex; Pain; Noxious stimulation; C-fibre response; Single motor unit

1. Introduction

The inhibition of the synthesis of prostaglandins, by blocking the action of the enzyme cyclooxygenase (COX), is the mechanism of action of non-steroidal antiinflammatory drugs, as described by Vane in 1971 [23]. Although this family of drugs have in common a similar mechanism of action, it is a highly heterogeneous group of compounds, with different molecular structures and therefore different pharmacokinetics. The structure of a NSAID is critical for its rate of absorption and penetration into the central nervous system. This, in turn, will determine the site of the predominant antinociceptive and antiinflammatory effects as well as the intensity of unwanted side effects. In fact, the experimental evidence suggests that the blockade of prostaglandins synthesised in peripheral tissues does not fully explain the analgesic actions of the NSAIDs [6,16,17,24] and effects at central sites have been reported in several experimental preparations [16,17].

Although most NSAIDs cross the blood brain barrier to varying degrees, the search for NSAIDs with a preferential central site of action and an easy penetration to the central nervous system is currently an important challenge, since they are more likely to induce a potent analgesic action, especially in situations of hyperalgesia due to central sensitisation. An example of this is ketoprofen, a NSAID among the group of 2-arylpropionic acids (see, for example, Ref. [1]), that crosses the blood–brain barrier rapidly in humans [18] and that has antinociceptive actions in several models of pain [11,17]. We have previously reported that ketoprofen is effective in reducing nociceptive spinal cord reflexes activated by mechanical and electrical stimulation [10] in animals with inflammation, and that this effect is produced not only in the periphery but also centrally.

Furthermore, the tromethamine salt of dexketoprofen ((+)-(*S*)-ketoprofen, the active isomer of ketoprofen) has all the properties of the free acid ketoprofen but also, it is rapidly absorbed by the gastric mucosa and so the time to maximum plasma concentration is lower and it causes less gastric ulceration [14]. It is, therefore, a preferred compound for oral administration in humans.

Recently, it has been shown that the non-selective COX inhibitor indomethacin and the selective COX-2 inhibitor SC58125 reduce wind-up of nociceptive reflexes in ani-

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mals without peripheral inflammation. However, neither indomethacin nor meclofenamic acid (another non-selective COX inhibitor) modified responses of spinal cord neurones to mechanical stimulation [25].

The aim of this study was to assess the potency of the active isomer of ketoprofen: Dexketoprofen Trometamol (the tromethamine salt of (+)-(*S*)-ketoprofen) in reducing spinal cord nociceptive reflexes in animals without peripheral inflammation, comparing the effects with those of the μ-opioid selective agonist fentanyl, and to examine whether this type of NSAID that has high blood brain barrier penetration, has an effect on natural as well as electrical stimulation.

2. Materials and methods

2.1. General

The experiments were performed in 10 Wistar male rats weighing 290–360 g. The preparatory surgery consisted in the cannulation of the trachea, one carotid artery and two superficial jugular veins and was performed under halothane anaesthesia (5% in 100% oxygen for induction and 1.5–2% for maintenance). After the surgery, the animals were transferred to an appropriate frame, the halothane was discontinued and the anaesthesia continued with alpha-chloralose (Sigma, 50 mg kg^{-1} initial dose and 20 mg kg^{-1} h^{-1} by perfusion pump for maintenance). The right hindlimb was fixed in inframaximal extension in a perpex block using plaster of paris. The core temperature was maintained at 37 ± 0.5°C by means of a feed-back controlled heating blanket and the blood pressure was continuously monitored. Adequate hydration was ensured by the continuous administration of saline (in which alpha-chloralose was diluted) in a rate of 1 ml h^{-1}. The preparation was left to rest for at least an hour before the experiment started.

2.2. Spinal cord reflex recording system

Withdrawal reflexes activated by either noxious mechanical or electrical stimulation were recorded from muscles as single motor units (SMU) following the technique described previously in detail [19]. Briefly, bipolar tungsten electrodes were inserted percutaneously into the muscles of the right hindlimb by means of a micromanipulator. The units were searched for by applying mild pressure to the paw and were isolated with the help of a window discriminator. Only units with a stable firing rate and summation of responses to constant intensity repetitive electrical stimulation (wind-up) were selected for the experiments. Fig. 1 shows an example of an original recording of one of these units. The threshold intensity for mechanical stimulation was studied by applying a ramp pressure on the most sensitive area of the cutaneous receptive field. The intensity considered as threshold was the minimum pressure required to evoke a constant firing rate after a few seconds of stimulation. Mechanical stimulation was applied over an area of 14 mm^2 by means of a computer controlled pincher, using a force of around 200

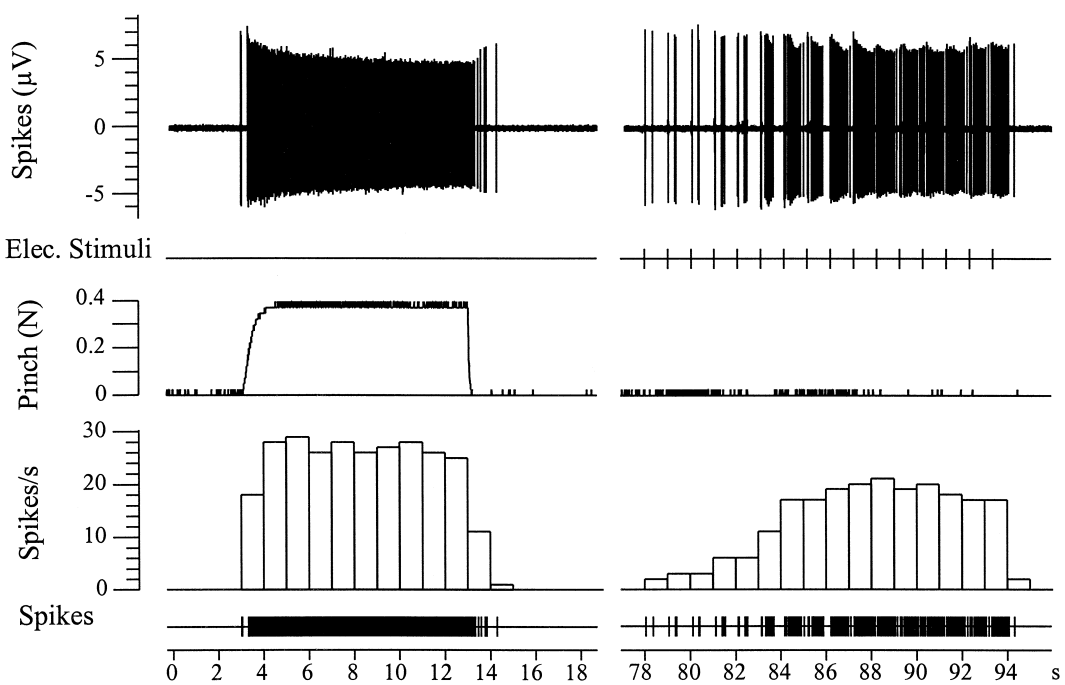

Fig. 1. Original record of an SMU activated by mechanical (left panel) and electrical stimulation (right panel). The top diagram shows the original action potentials evoked by 16, 1 Hz, 0.2 ms electrical pulses (next diagram) and pressure over a surface of 14 mm^2 (Pinch). The two diagrams in the bottom of the figure show the counted spikes expressed either as histograms or TTL pulses.

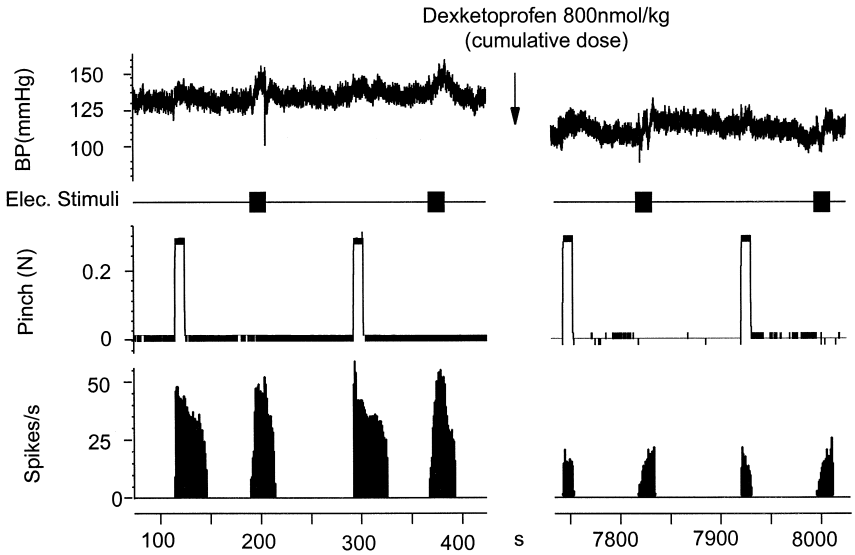

Fig. 2. Protocol of stimulation. The activation of SMUs was produced in 3-min cycles as shown in the figure. The left diagram shows the responses obtained in the two cycles of stimulation previous to the administration of the first dose of dexketoprofen. The right diagram shows the activity recorded after the last dose of the NSAID (800 nmol kg^{-1}). Note the minimal variation recorded in the blood pressure (top panel).

mN over the threshold of activation. The electrical stimulation was applied using two 0.2-mm needles inserted transcutaneously in the most sensitive area of the cutaneous receptive field, at an intensity of 2 × threshold, 2 ms pulse width, 16 pulses and 1 Hz. The intensity considered as threshold was the voltage needed to obtain long latency responses (C-volley) in 50% of the tests [7]. The stimulation sequence was repeated in 3-min cycles, and a sample of the protocol is illustrated in Fig. 2.

2.3. Drugs, collection of data and statistics

All drugs used were injected i.v. in a log 2 cumulative regime until one of the responses was reduced to at least 30% of the control response. The control was the mean of the three responses obtained before the first dose of each drug. Fentanyl (Fentanest, Syntex Latino) was injected every two cycles of stimulation (6 min) starting with 4 nmol kg^{-1} (2 μg kg^{-1}). Dexketoprofen Trometamol (provided by Laboratorios Menarini) was administered every seven cycles of stimulation (21 min), the initial dose being 25 nmol kg^{-1} (10 μg kg^{-1}) and at least 1 h after the last dose of fentanyl. The two drugs were dissolved in saline and injected in a constant volume of 0.3 ml. The animals were killed at the end of the experiment with an overdose of sodium pentobarbitone (Euta Lender, Normon).

The number of spikes counted in the two cycles between each dose of fentanyl and in the last two cycles of the inter-dose period for dexketoprofen were averaged and the means obtained used for the numerical analysis. The data from the electrical stimulation were analysed by counting the number of spikes evoked between 150 and 650 ms after each stimulus, (long latency responses, presumably C-fibre responses [7]). The data obtained from all the animals were pooled and the mean ± S.E.M. are expressed as percentage of control.

The collection of data and stimulation protocols were performed by computer using commercial software (CED, UK; Spike 2). Statistical analysis was performed with different tests: one-way analysis of variance (ANOVA) for repeated measures, with post-hoc Dunnett's test, for the analysis of wind-up curves; the non-parametric Mann–Whitney *U*-test for comparisons between each dose and the control; and the non-parametric Wilcoxon matched pairs test for comparison between mechanical and electrical stimulation. The analysis were performed using commercial software (GraphPad-Prism and GraphPad-Instat for Windows 95). All the experiments performed in this study were carried out in accordance with European Union legislation regarding the use of animals for experimental protocols.

3. Results

3.1. Effects on responses evoked by mechanical and electrical stimulation

The administration of dexketoprofen produced a dose-dependent reduction of SMU responses to both mechanical and electrical stimulation (Fig. 3) with minimum effective doses (MED) of 100 and 200 nmol kg^{-1}, respectively. The dose needed to reduce at least one of the two types of responses below 30% of control was either 400 nmol kg^{-1} (160 μg kg^{-1}, five animals) or 800 nmol kg^{-1} (320 μg kg^{-1}, three animals). Such reduction was only observed in the responses to mechanical stimulation (16.8 ± 5% of control), the responses to electrical stimulation were only

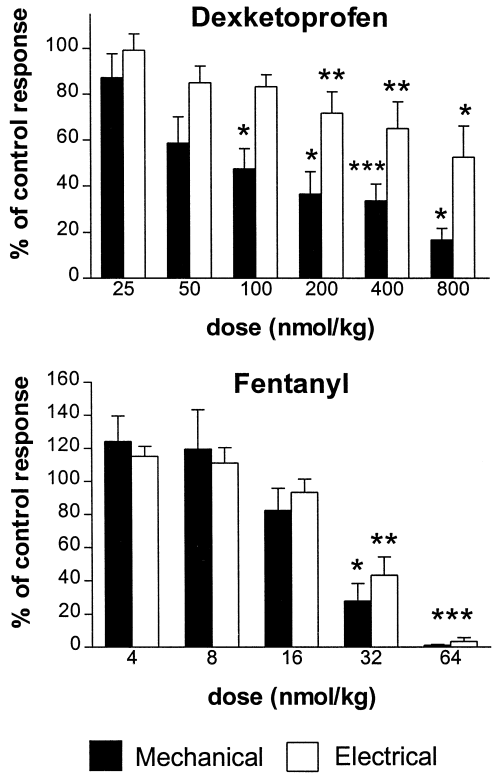

Fig. 3. Effect of dexketoprofen and fentanyl on SMUs. Pooled data from all the experiments performed showing the effect of dexketoprofen and fentanyl on SMUs activated by mechanical and electrical stimulation. Both drugs were very effective in the reduction of responses although dexketoprofen was more potent on responses evoked by mechanical stimulation ($p < 0.05$). Statistical significance was calculated with Mann–Whitney *U*-test respect to control; control being the mean of the responses observed in the three cycles previous to the first dose. ($*$: $p < 0.05$, $**$: $p < 0.01$, $***$: $p < 0.001$).

reduced to $52.6 \pm 13\%$ of control. The calculated ID50s were 100 and 762 nmol kg^{-1} for mechanical and electrical stimulation, respectively. The different potency observed in the two types of stimulation was significant ($p < 0.05$). In every case, the inhibition of responses observed after the last dose of dexketoprofen did not recover after at least 30 min of observation.

Fentanyl also induced a dose-dependent reduction of SMUs activated by mechanical and electrical stimulation (Fig. 3), with a MED of 30 nmol kg^{-1}. Similar reductions were observed for mechanical and electrical stimulation. The ID50s were 40 nmol kg^{-1} for mechanical stimulation and 51 nmol kg^{-1} for electrical stimulation. The effect observed after the administration of fentanyl recovered very quickly, with an apparent half-life of 4 min.

3.2. Effects on wind-up

Dexketoprofen was effective in the depression of SMU wind-up and induced a dose-dependent reduction of the curve (Fig. 4), with a MED of 50 nmol kg^{-1}. No significant reduction was observed in the initial level of responses counted in the C-fibre volley-evoked response, even with the highest dose of dexketoprofen tested (2–4 spikes in average). Also, the shape of the curve was not modified except by the highest dose given. The saturation of the responses occurred at pulse number 4, and, (except with the last dose) no significant differences were seen thereafter. Though fentanyl was also able to reduce SMU wind-up, its effect was different. The saturation point was modified and occurred later with progressive doses of fentanyl (Fig. 4), the maximum firing rate being observed with the stimulus number 16 with doses above 15 nmol kg^{-1}. The initial level of excitability was also reduced, and as a consequence, the wind-up curve was not only depressed but also shifted to the right. The depression was significant from doses of 30 nmol kg^{-1} (16 μg kg^{-1}).

3.3. Effects on blood pressure

The effects of dexketoprofen on blood pressure were minimal. The maximal (not significant) depression of the

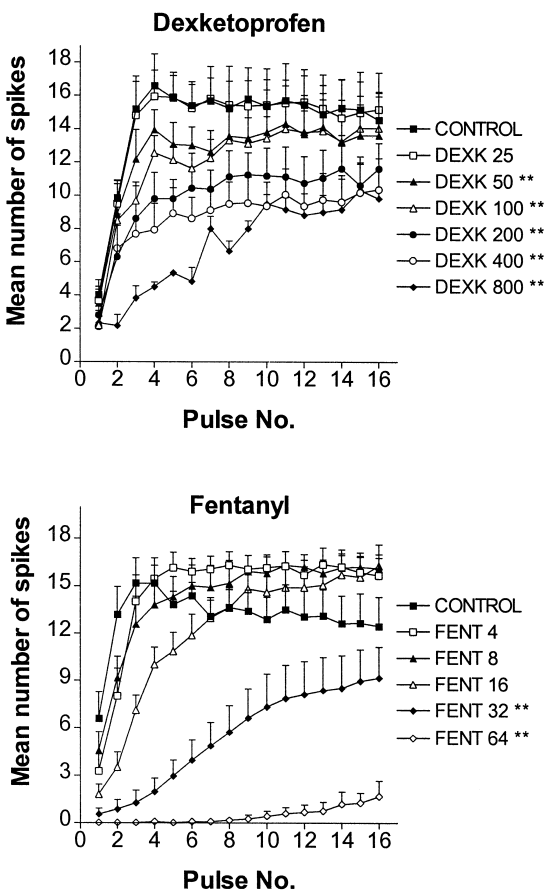

Fig. 4. Effect of dexketoprofen and fentanyl on SMU wind-up. A significant reduction of wind-up was observed after 50 nmol kg^{-1} of dexketoprofen and 32 nmol kg^{-1} of fentanyl. Note that whereas fentanyl produced a decrease in the initial number of spikes, and a consequent shift of the curve to the right, dexketoprofen did not reduce the initial excitability. Statistical significance was calculated with one-way ANOVA for repeated measures, with post-hoc Dunnett's tests. (p values, the same in Fig. 3).

mean arterial pressure was 15 ± 5 mmHg with the highest dose used. Fentanyl, however, induced a strong depression of blood pressure during the administration of each dose, minimised as much as possible with a very slow administration. Nevertheless the reduction observed when measured 5 min after the administration of each dose (after the initial depression had recovered) was dose-dependent, the maximum being 58 ± 12 mmHg ($p < 0.001$).

4. Discussion

Three main observations have been made from the experiments performed in the present study: (i) Dexketoprofen trometanol is an effective depressor of spinal cord nociceptive reflexes in normal, non-inflamed anaesthetised rats; (ii) The potency of dexketoprofen trometamol in SMUs is very high and comparable to that of μ-opioids, and (iii) The effect of this NSAID was observed not only on responses evoked by natural stimulation but also on those evoked by electrical stimulation, including wind-up.

It is not new that NSAIDs are effective as antinociceptive drugs in SMU recording experiments. In fact, we have previously shown that other NSAIDs [9,13], including racemic ketoprofen [10] have different potency in the depression of SMUs activated by natural or electrical stimulation, acting at peripheral and/or central sites. The fact, however, that dexketoprofen trometamol was very effective in normal non-inflamed animals is new, and may suggest that its action over the COX1 or constitutive cyclooxygenase is very potent. Nevertheless, an action both on COX1 and COX2 in the central nervous system, probably in the spinal cord, is also possible since intense COX2-like immunoreactivity has been found in the grey matter of the spinal cord and in dorsal root ganglion cell bodies in normal animals [25].

The effect of ketoprofen on the inhibition of prostaglandins, especially prostaglandin F2 alpha (PGF2) is certainly very important [12,22], and this effect has been shown to be stereoselective, due to the dextrorotatory enantiomer ((+)-(*S*)-ketoprofen, or dexketoprofen). In those experiments, dexketoprofen inhibited the synthesis of PGF2 with an IC50 of 6.2 nM in rat brain fragments [2] and the synthesis of prostaglandin E2 in different cultured cells with IC50 values between 0.1 nM and 0.8 μM [20]. The antinociceptive potency of dexketoprofen observed in the present experiments is therefore in the range of those needed to inhibit prostaglandins in in vitro preparations, but it also matches the doses used in humans [14], though in that case, the administration of the drug was oral. The mechanism of action of dexketoprofen seems therefore specific for the inhibition of the synthesis of prostaglandins. However, since Willingale et al. [25] have shown that other different NSAIDs, either non-selective COX inhibitors or COX2 selective inhibitors, only inhibit spinal cord neurones in situations of hyperexcitability, it is possible that dexketoprofen has also an additional mechanism of action, especially in animals without inflammation. In fact, the doses needed to produce a depression of SMU responses in the present experiments were much lower than those expected according to the experiments performed previously with the racemic mixture in animals with inflammation [10].

Dexketoprofen had in the present experiments a potent effect in the depression of wind-up, an attribute of spinal nociceptive neurones first described by Mendell in 1966 [15], and shown to be dependant on the NMDA receptor [3,5] and on NK1 receptors [4]. It is therefore clear that dexketoprofen was very active at central sites, probably at the spinal cord level, in agreement with previous results obtained with ketoprofen in animals with inflammation [10] and with the results observed with other NSAIDs [25].

We have previously shown in similar experiments that fentanyl is around 1000 times more potent than morphine [8]. Therefore, in SMU experiments, dexketoprofen trometamol shows an antinociceptive efficacy in the range of μ-opioids, but probably with less side-effects, as shown by the lack of change in blood pressure. It is clear, however, that the mechanism of action is different to that of opioids, not only because of the well-documented action of the NSAID on the inhibition of prostaglandins at the doses used (see above), but also because of the different depression of SMU responses, especially wind-up, when compared to that observed with fentanyl. Fentanyl depressed the responses to both mechanical and electrical stimulation with the same potency, as shown by the similar IC50s obtained. Dexketoprofen, however, was more effective in reducing responses evoked by mechanical stimulation. This type of stimulation is natural, and therefore detected by nociceptors, whereas electrical stimulation bypasses nociceptors and stimulates sites above them (see Ref. [10] for further discussion). In this case, a summated action, central and peripheral, would help to explain the greater potency on responses to mechanical stimulation, although another additional mechanism of action is also possible, as mentioned above.

It is also possible that the two different modalities of stimulation were not produced at the same intensity and that this discrepancy was the cause for a different action of the drugs. We have addressed this problem before using different intensities of electrical stimulation [21] and observed that the effect of fentanyl and other drugs was different depending on the intensity used. Since in the present experiments we observed a similar potency of fentanyl on electrical and mechanical stimulation, it is logical to attribute the differences of the drug potency to a different drug behaviour rather than to a different intensity of stimulation. Furthermore, we tried to produce a similar maximum firing rate when using different types of stimuli (see histograms in Figs. 1 and 2) and so the level of excitability of the SMU should be similar.

In conclusion, the results observed in the present study show that the NSAID dexketoprofen trometamol is a po-

tent antinociceptive agent when tested on SMU responses, activated by both natural and electrical stimulation. The action of this NSAID was observed in normal animals without the presence of inflammation. The potency was in the range of that observed in in vitro experiments for the inhibition of the synthesis of prostaglandins and that of μ-opioids tested in similar experiments, but with less cardiovascular side-effects. Dexketoprofen also significantly inhibited wind-up, showing that it has important central effects in addition to its peripheral actions.

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