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Stereoselective Inhibition of Rat Brain Cyclooxygenase by Dexketoprofen

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ABSTRACT Although it has been assumed that the effects of nonsteroidal antiinflammatory drugs (NSAIDs) are mainly the result of their action on local synthesis of prostaglandins, there is growing evidence to suggest that they may also exert a central analgesic action. Some authors have suggested that inhibition of prostaglandin synthesis in the brain could contribute to the analgesic action. The effect of dexketoprofen trometamol (tromethamine salt of the enantiomer (+)-S-ketoprofen) on prostaglandin synthesis was investigated in rat brain fragments and in cyclooxygenase preparations from rat brain microsomes. Effects of the (–)-R-enantiomer and the racemic mixture were also evaluated. Significant levels of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were synthesized in rat brain fragments after 10 min of incubation at 37°C. Dexketoprofen was found to be a potent inhibitor of this $PGF_{2\alpha}$ production in rat brain (IC_{50} = 6.2 n*M*), and it completely suppressed $PGF_{2\alpha}$ production at 1 μ*M* concentration. In addition, inhibition of $PGF_{2\alpha}$ synthesis by dexketoprofen was highly stereoselective since the enantiomer (–)-R-ketoprofen was significantly less potent (IC_{50} = 294 n*M*); with this enantiomer, even at high concentrations such as 1 μ*M*, less than 60% inhibition was achieved. These results correlated with those obtained in the study of racemic ketoprofen and its enantiomers on cyclooxygenase activity of rat brain microsomes, where dexketoprofen also inhibited enzymatic activity stereoselectively. IC_{50} values obtained for dexketoprofen, (–)-R-ketoprofen, and *rac*-ketoprofen were 3.5 μ*M*, 45.3 μ*M*, and 5.8 μ*M*, respectively. The above results could be related to the potent analgesic effect of dexketoprofen observed in vivo, which was also stereoselective. Taken together, these findings suggest that prostaglandin synthesis inhibition in rat brain by dexketoprofen could be associated, at least in part, with the analgesic effect of this NSAID. *Chirality 9:281–285, 1997.*

KEY WORDS: dexketoprofen; enantiomer; stereoselectivity; brain; cyclooxygenase; rat

Although several mechanisms have been proposed to explain the pharmacological effects of nonsteroidal antiinflammatory drugs (NSAIDs), the suppression of prostaglandin synthesis is believed to be of the greatest importance in the antiinflammatory and analgesic activities of these compounds.[1] In fact, it has been demonstrated in various experimental models that inhibition of cyclooxygenase correlates with the therapeutic effects as well as with some of the side effects of NSAIDs. However, whereas the antiinflammatory potency of NSAIDs can be correlated to the inhibition of this enzyme, the relation between their antinociceptive activity and their potency as cyclooxygenase inhibitors is more controversial. It has been assumed that the analgesic behavior of NSAIDs is the result of their effect on local synthesis of prostaglandins; in fact, the antinociceptive effect of NSAIDs is well correlated with their antiinflammatory activity.[2,3] However, there is growing evidence to suggest that NSAIDs may also exert a central action in addition to a peripheral action,[4–7] and some authors have suggested that prostaglandin synthesis inhibition in the brain could contribute to the analgesic effects.[8]

Previous studies have demonstrated that inhibition of cyclooxygenase by the NSAID ketoprofen is highly enantioselective and that this effect is caused by the enantiomer (+)-S-ketoprofen (dexketoprofen).[9–11] In addition, enantioselectivity was observed in vivo, and not only the antiinflammatory action but also the analgesic effect of ketoprofen in several animal models has been found to be predominantly due to (+)-S-ketoprofen.[12] Taking together all these findings and that prostaglandins synthesized in the brain are presumably involved in nociception, we have investigated the effect of the tromethamine salts of (+)-S-ketoprofen, the (–)-R-enantiomer, and racemic ketoprofen on prostaglandin synthesis in rat brain fragments and in the inhibition of cyclooxygenase activity isolated from rat brain microsomes. These in vitro studies have

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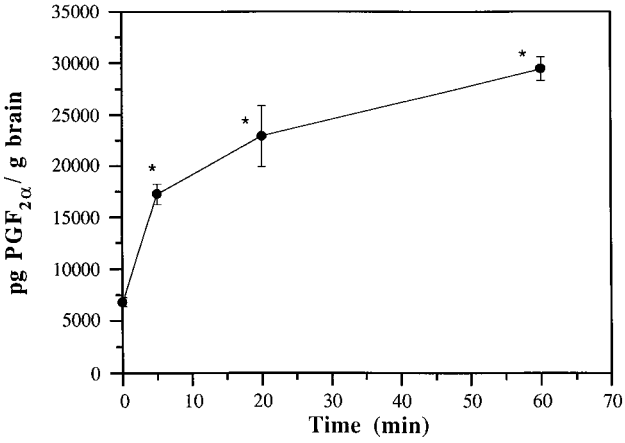

Fig. 1. Time course of $PGF_{2\alpha}$ (pg/g tissue) release by rat brain fragments. After extraction and excision, washed brain fragments were incubated for different times in oxygenated Tyrode solution (pH 7.4) at 37°C, in the absence of inhibitor, and levels of $PGF_{2\alpha}$ were determined by enzyme immunoassay (EIA) as described in "Material and Methods." Each value represents the mean ± SEM of at least $n = 4$ animals. $^*P < 0.05$ significant difference as compared to levels released at zero time.

been used in order to avoid the inversion process of (–)-R-ketoprofen in the rat,[13,14] which would be observed ex vivo.

MATERIALS AND METHODS

Chemicals

Tromethamine salt of (+)-S-ketoprofen dexketoprofen trometamol was synthesized at the Instituto Químico de Sarria (Barcelona, Spain). Tromethamine salts of *rac*-ketoprofen and (–)-R-ketoprofen were synthesized in Laboratorios Menarini, S.A. The optical purity of both enantiomers, measured by a direct enantiospecific high-performance liquid chromatographic (HPLC) method using a Chiracel-OJ™ column (Daicel Chemical Industries Ltd., Japan) was >99%.[15,16]

Bovine serum albumin (BSA) (fatty acid poor), ethylenediaminotetracetic acid (EDTA), epinephrine, reduced glutathione (GSH), arachidonic acid, as well as all of the inorganic salts employed in buffers were obtained from Sigma (St. Louis, MO). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was measured by the Prostaglandin $F_{2\alpha}$-Monoclonal Enzyme Immunoassay Kit from Cayman Chemical Company (Ann Arbor, MI).

Release of $PGF_{2\alpha}$ From Rat Brain Fragments

Inhibition of prostaglandin production in rat brain was determined as follows: Male Sprague-Dawley rats (180–230 g) were killed by ether anesthesia, and brains without cerebellum were rapidly excised. Organs were washed in ice-cold saline and then cut into small tissue fragments (about 1–3 mm^3). Fragments of one-half of the brain (500 mg) were incubated in 3.0 ml of oxygenated Tyrode solution (pH 7.4) at 37°C for 10 min in the presence of the inhibitor or vehicle. Incubations were terminated by brief centrifugation to remove tissue fragments. The resulting supernatants were stored at –80°C until levels of $PGF_{2\alpha}$ were determined by a specific competitive enzyme immunoassay (EIA). Baseline levels were the levels of $PGF_{2\alpha}$ (6824 ± 426 pg/g brain; Fig. 1) released from brain fragments at the zero time of incubation in the absence of inhibitor. Baseline was subtracted from all of the values obtained in the presence of different concentrations of compounds, after 10 min of incubation. Picograms of $PGF_{2\alpha}$/g brain were calculated from data obtained by EIA. These values were used to calculate the inhibition percentages with respect to the control group in the absence of inhibitor. Percent inhibition values were plotted versus compound concentration (Fig. 2), and IC_{50} for each treatment was estimated from the plot.

Cyclooxygenase Activity in Rat Brain Microsomes

The enzymatic generation of $PGF_{2\alpha}$ resulting from the catalytic activity of the fatty acid cyclooxygenase was quantified in microsomal preparations from rat brain, according to the method described by Lysz et al.,[17] with minor modifications.

Preparation of microsomes Brains from Sprague-Dawley rats (200–250 g) were dissected and hand-homogenized in 4 vol (1.4 g brain/7 ml) of cold 0.1 *M* potassium phosphate buffer containing 1% BSA (essentially fatty acid-free) and 10 m*M* EDTA, (pH 7.4). EDTA and BSA were used to reduce the activity of calcium-dependent phospholipase A and to protect the cyclooxygenase from autocatalytic destruction by released fatty acids. Homogenates were centrifuged at 8000 *g* for 15 min at 4°C and the resulting supernatant fraction at 100,000 *g* for 60 min at 4°C. The pellet (microsomes) was washed three times with 0.1 *M* potassium phosphate buffer (pH 7.4) and resuspended in the same buffer. The protein concentration was determined using the method of Bradford[18] with BSA as standard.

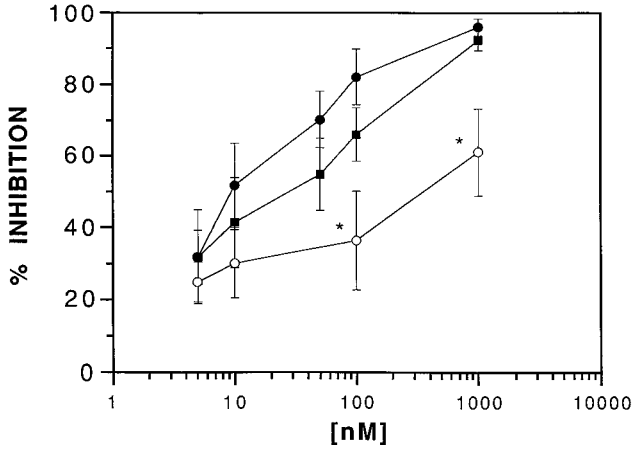

Fig. 2. Concentration-response curves for inhibition of $PGF_{2\alpha}$ synthesis in rat brain fragments by dexketoprofen (●), (–)-R-ketoprofen (○), and *rac*-ketoprofen (■). Compounds were incubated with brain fragments for 10 min at 37°C in oxygenated Tyrode solution (pH 7.4), as described in "Materials and Methods." Basal levels of prostaglandin production (zero time) were subtracted from all of the values. Results (mean ± SEM of n = 4–10 animals) are expressed as percentage inhibition with respect to the control group in the absence of inhibitor. Statistically significant differences from percent inhibition caused by dexketoprofen: $^*P < 0.05$.

Cyclooxygenase activity: synthesis and determination of $PGF_{2\alpha}$ Inhibitors were first dissolved in a small volume of ethanol and diluted before use. Compounds were incubated with microsomal preparations (0.3 mg protein) for 5 min at room temperature. Then, the enzymatic reaction was started by addition of substrate, exogenous arachidonic acid (24 μ*M*), and cofactors—epinephrine (1 m*M*) and GSH (1 m*M*)—in 200 μl final volume. After 60 min at 37°C, the reaction was terminated by freezing at –80°C. $PGF_{2\alpha}$ production was determined by EIA. Baseline was determined in reaction mixture without enzyme preparations. Inhibitory potencies, expressed as percent inhibition with respect to the control group in the absence of inhibitor, were calculated from EIA data and plotted versus tested concentrations. IC_{50} for each treatment was estimated from the plot.

Statistics

Results obtained in studies with COX from rat brain microsomal preparations were shown as the means ± SD. The *n* values indicate the number of experiments. In the case of rat brain fragments, results were plotted as the mean with standard error of the mean (SEM), where *n* is the number of animals used. Statistical analysis of individual percent inhibition values was performed by Student's unpaired *t* test, with a probability value of $P < 0.05$ regarded as significant. IC_{50} values were estimated from the plot of log drug concentration versus percent inhibition by a simple regression analysis, and 95% confidence limits were given.

RESULTS

In order to elucidate if prostaglandins were synthesized after extraction and fragmentation of the rat brain, a preliminary experiment was conducted. Levels of $PGF_{2\alpha}$ released from rat brain fragments after different periods of incubation in oxygenated Tyrode solution (pH 7.4) at 37°C (0–60 min) were determined in the absence of compound. Results (Fig. 1) demonstrated that the synthesis of $PGF_{2\alpha}$ was time-dependent in brain fragments. Although the production of $PGF_{2\alpha}$ continued throughout the 60 min incubation period, significant amounts were detected after 10 min of incubation (16245 ± 2077 pg $PGF_{2\alpha}$) compared with levels released at zero time (6824 ± 426 pg $PGF_{2\alpha}$/g brain). Therefore, the inhibitory effect of dexketoprofen and other NSAIDs on prostaglandin production in rat brain fragments can be quantified.

Inhibition of $PGF_{2\alpha}$ synthesis in fragments of rat brain by the tromethamine salts of ketoprofen enantiomers was then assessed. In all cases, amounts of $PGF_{2\alpha}$ synthesized at zero time of incubation were subtracted from values obtained after 10 min of incubation in the presence of compound. Results, plotted in Figure 2, show that dexketoprofen markedly inhibited $PGF_{2\alpha}$ production in rat brain fragments under our experimental conditions, with IC_{50} = 6.2 n*M*. Moreover, the generation of $PGF_{2\alpha}$ was completely suppressed when 1 μ*M* dexketoprofen was incubated with portions of tissue. This inhibition of $PGF_{2\alpha}$ production is a highly stereoselective process. Thus, dexketoprofen was four times as active as the racemate (IC_{50} = 24.7 n*M*), and the (–)-R-enantiomer was significantly less potent (IC_{50} = 294 n*M*) and did not reach 60% inhibition at concentrations such as 1 μ*M* (Table 1).

TABLE 1. Inhibitory potencies, expressed as IC_{50}, of dexketoprofen, (–)-R-ketoprofen, and *rac*-ketoprofen, on $PGF_{2\alpha}$ production by rat brain fragments

Compound	IC_{50} (n*M*)	95% Confidence limits
Dexketoprofen	6.2	1.18–18.24
(–)-R-etoprofen	294	N.D.
rac-ketoprofen	24.7	7.48–55.8

Compounds were incubated with brain fragments for 10 min at 37°C as described in "Materials and Methods." IC_{50} values were estimated from the plot of Figure 2 by a simple regression analysis, and 95% confidence limits were given.
N.D., not determined.

These results correlate with those obtained after studying the effect of dexketoprofen, (–)-R-ketoprofen, and *rac*-ketoprofen on cyclooxygenase activity purified from rat brain microsomes (Fig. 3). Dexketoprofen was a potent inhibitor of rat brain cyclooxygenase ($PGF_{2\alpha}$ synthesis), with IC_{50} = 3.5 μ*M* (Table 2), and it entirely blocked enzymatic activity above 100 μ*M*. Cyclooxygenase activity of rat brain microsomes was also stereoselectively inhibited by dexketoprofen since (–)-R-ketoprofen was 13 times less active than the cyclooxygenase inhibitor, its IC_{50} being 45.3 μ*M*.

DISCUSSION

In the present study, we have shown that the in vitro generation of $PGF_{2\alpha}$ in rat brain is markedly and stereoselectively inhibited by dexketoprofen ([+]-S-ketoprofen) in two different systems. First, in fragments of tissue incubated under appropriate conditions, prostaglandins being formed from endogenous substrate and second, when exogenous arachidonic acid was added and converted into prostaglandins by cyclooxygenase-enriched preparations of rat brain microsomes.

It is well known that the peripheral inhibition of prostaglandin synthesis in inflammatory cells and tissues may explain the analgesic effect of NSAIDs, but the role of these mediators in their central actions is still under discussion. NSAIDs such as ketoprofen cross the blood-brain barrier and can reach the brain structures involved in the regulation of pain sensation.[19] In addition, the existence of a central component in the analgesic action of ketoprofen has been evidenced, and supraspinal structures appear to be crucial in the mechanisms of this central effect.[20] We found that rat brain portions, under appropriate conditions, are able to generate prostaglandins ($PGF_{2\alpha}$) in a time-dependent manner, as was previously observed by Peskar et al.[8] in several rat organs. These authors demonstrated that prostaglandin production in several organs, including brain, was inhibited by flurbiprofen. Although brain PGE_2 levels have been measured by several authors, the time course of $PGF_{2\alpha}$ biosynthesis paralleled the rate of PGE_2 formation in the microsomes prepared from the rat brain, as previously shown by Lysz et al.[17] These authors also observed that rat brain PGE_2 and $PGF_{2\alpha}$ production were

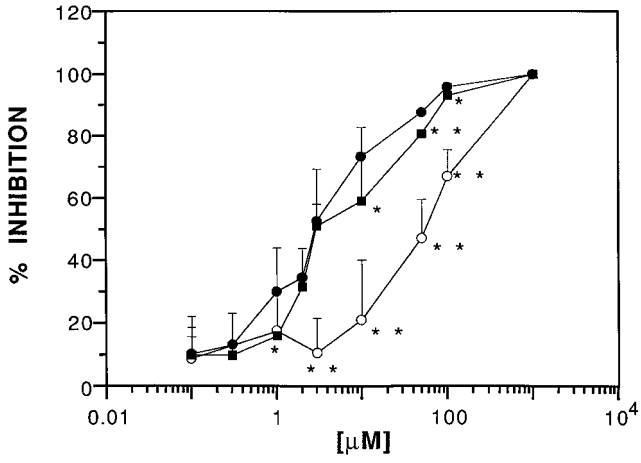

Fig. 3. Concentration-response curves for inhibition of rat brain cyclooxygenase activity ($PGF_{2\alpha}$ synthesis) from microsomal preparations by dexketoprofen (●), (–)-R-ketoprofen (○), and *rac*-ketoprofen (■). Compounds were incubated with enzyme preparations for 5 min. After this period, enzymatic reaction was started by addition of substrate and cofactors as described in "Materials and Methods." After 60 min incubation at 37°C, the reaction was stopped by freezing at –80°C. Results represent percentage inhibition with respect to the control group in the absence of inhibitor, and values are the mean ± SD of at least three experiments performed on different days. Statistically significant differences from percent inhibition caused by dexketoprofen: $*P < 0.05$ and $**P < 0.005$.

inhibited to the same extent in response to NSAID treatment. To compare the effects of the pure enantiomers of ketoprofen on prostaglandin release from brain, ex vivo experiments cannot be performed in the rat since a high degree of bioinversion is observed when the (–)-R-enantiomer is administered orally.[13,14,21] Thus, for better comparison we assessed the effects of these compounds when brain portions were incubated in oxygenated Tyrode solution in the presence or absence of compounds at 37°C for 10 min, according to a procedure previously described elsewhere.[8] At this time point, $PGF_{2\alpha}$ production in the absence of inhibitor was significantly different from production at zero time, and inhibition by dexketoprofen was also significant. Under our conditions, dexketoprofen stereoselectively inhibited prostaglandin release from rat brain. These findings demonstrate that the ability of ketoprofen to inhibit prostaglandin biosynthesis in rat brain is mainly due to the (+)-S-enantiomer as it occurs in other cells and tissues.[9–11] In fact, dexketoprofen is 47 times more active than (–)-R-ketoprofen when IC_{50} values were compared.

The stereoselective inhibition of prostaglandins released from rat brain was confirmed with preparations of cyclooxygenase from rat brain microsomes, to which exogenous arachidonic acid was added as substrate. COX activity purified from rat brain microsomes was quantified as $PGF_{2\alpha}$ generation. We found that COX activity was stereoselectively inhibited by dexketoprofen, the (+)-S-enantiomer being 13 times more potent than the (–)-R-enantiomer. In this system, (–)-R-ketoprofen inhibited COX activity, but this effect was significant only at high concentrations (>100 μ*M*), suggesting that this inhibitory action was due to nonspecific effects rather than to direct enzymatic interaction. These results parallel the steroselectivity observed in cells and are well correlated with the effect of other NSAID enantiomers.[8]

Although inhibitory potencies (IC_{50}) found using microsomal preparations were very different from those obtained in brain fragments, the stereoselective effect of (+)-S-ketoprofen was evident in both systems. The fact that *rac*-ketoprofen and its enantiomers were more potent in inhibiting $PGF_{2\alpha}$ production in rat brain fragments than microsomal COX may well be due to the fact that the brain fragments utilize endogenous sources of arachidonate, whereas exogenous arachidonic acid was added to microsomes. Similar differences were previously observed by several authors when the effect of COX inhibitors, such as NSAIDs, were studied on intact cells and purified enzymes.[22–24] These authors suggested that results from intact cell and tissue preparations may correlate better with biological activity in animals and humans.

It is well known that the inducible form of cyclooxygenase (COX-2) is expressed in rat brain, and its distribution has been mapped.[25–27] However, the role of this isoenzyme in the central nervous system remains unclear. In addition, not only has the effect of *rac*-ketoprofen on COX-2 been well described[28–30] but it has been reported[11,31] that COX-2 activity, evaluated in several biological systems, was stereoselectively inhibited by (+)-S-ketoprofen in a similar way to COX-1, suggesting that inhibition of the inducible enzyme by *rac*-ketoprofen should be attributed to its (+)-S-enantiomer. It appears to be very difficult to distinguish between the inhibition of two rat brain cyclooxygenases in our in vitro systems, without the previous isolation of the isoenzymes. Moreover, since the enantioselective effect of (+)-S-ketoprofen on COX-1 paralleled the blockade of COX-2 activity, no significantly different behavior would be expected when comparing the effects of separate isoforms with the results obtained in brain fragments or microsomal preparations. Therefore, in this work using microsomal preparations, COX activities were not studied individually. However, it would be very interesting to determine in further studies whether isoforms of cyclooxygenase purified from rat brain are inhibited in a similar way to COX-1 and COX-2 from other cells or tissues.

On the other hand, the analgesic effect of several NSAIDs of the 2-arylpropionic acid class have been de-

TABLE 2. Inhibitory potencies, expressed as IC_{50}, of dexketoprofen, R-(–)-ketoprofen, and *rac*-ketoprofen, on cyclooxygenase activity ($PGF_{2\alpha}$ synthesis) by rat brain microsomes

Compound	IC_{50} (μ*M*)	95% Confidence limits
Dexketoprofen	3.5	1.3–5.3
(–)-R-ketoprofen	45.3	21.6–78.3
rac-ketoprofen	5.8	2.2–10.8

Compounds were incubated with enzyme preparations for 5 min, and the reaction was initiated by substrate and cofactor addition. Reaction was terminated after 60 min at 37°C by freezing at –80°C as described in "Materials and Methods." IC_{50} values were estimated from the plot of Figure 3 by a simple regression analysis, and 95% confidence limits were given.

scribed in several animal models. In all cases, the (+)-S-enantiomers were more potent than the (–)-R-enantiomers.[12,32,33] Therefore, taking together the stereoselective antiociceptive action in vivo and the preferential inhibition of brain prostaglandin production by dexketoprofen, it could be suggested that the inhibition of cerebral prostaglandin synthesis by dexketoprofen could be related, at least in part, to its analgesic effect.

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LITERATURE CITED

1. Vane, J.R., Botting, R. Inflammation and the mechanism of action of antiinflammatory drugs. FASEB J. 1:89–96, 1987.
2. Preston, S.J., Arnold, M.H., Beller, E.M., Brooks, P.M., Buchanan, W.W. Comparative analgesic and antiinflammatory properties of sodium salicylate and acetylsalicylic acid (aspirin) in rheumatoid arthritis. Br. J. Clin. Pharmacol. 27:607–611, 1989.
3. Ferreira, S.H., Nakamura, M. Prostaglandin hyperalgesia, a cAMP/Ca^{2+} dependent process. Prostaglandins 18:179–190, 1979.
4. Urquhart, E. Central analgesic activity of nonsteroidal antiinflammatory drugs in animal and human pain models. Semin. Arthritis Rheum. 23:198–205, 1993.
5. Malmberg, A.B., Yaksh, T.L. Spinal actions of non-steroidal antiinflammatory drugs: evidence for a central role of prostanoids in nociceptive processing. Prog. Pharmacol. Clin. Pharmacol. 10:91–110, 1993.
6. Neugebauer, V., Schaible, H.G., He, X., Lücke, T., Güdling, P., Schmidt, R.F. Electrophysiological evidence for a spinal antinociceptive action of dipyrone. Agents Actions 41:62–70, 1994.
7. Neugebauer, V., Geisslinger, G., Rümenapp, P., Weiretter, F., Szelenyi, I., Brune, K., Schaible, H.G. Antinociceptive effects of R-(–)- and S-(+)-flurbiprofen of rat spinal dorsal horn neurons rendered hyperexcitable by an acute knee joint inflammation. J. Pharmacol. Exp. Ther. 275:618–628, 1995.
8. Peskar, B.M., Kluge, S., Peskar, B.A., Soglowek, S.M., Brune, K. Effects of pure enantiomers of flurbiprofen in comparison to racemic flurbiprofen on eicosanoid release from various rat organs ex vivo. Prostaglandins 42:515–531, 1991.
9. Hayball, P.J., Nation, R.L., Bochner, F. Enantioselective pharmacodynamics of the non-steroidal antiinflammatory drug ketoprofen: in vitro inhibition of human platelet cyclooxygenase activity. Chirality 4:484–487, 1992.
10. Suesa, N., Fernández, M.F., Gutiérrez, M., Rufat, M.J., Rotllan, E., Calvo, L., Mauleón, D., Carganico, G. Stereoselective cyclooxygenase inhibition in cellular models by the enantiomers of ketoprofen. Chirality 5:589–595, 1993.
11. Carabaza, A., Cabré, F., Gutiérrez, M., Rotllan, E., Gómez, M., García, M.L., Mauleón, D.J. Stereoselective inhibition of inducible cyclooxygenase by chiral nonsteroidal antiinflammatory drugs. J. Clin. Pharmacol. 36:505–512, 1996.
12. Calvo, L., Fernández, M.F., Ferrer, X., Ruíz, A., Cama, C., Mauleón, D., Carganico, G. Analgesic action of ketoprofen enantiomers in animal models. Pharmacol. Res. 31(Suppl.):257, 1995.
13. Carabaza, A., Suesa, N., Tost, D., Pascual, J., Gómez, M., Gutiérrez, M., Ortega, E., Montserrat, X., García, A.M., Mis, R., Cabré, F., Mauleón, D., Carganico, G. Stereoselective metabolic pathway of ketoprofen in the rat: incorporation into triacylglycerols and enantiomeric inversion. Chirality 8:163–172, 1996.
14. Yasui, H., Yamaoka, K., Nakagawa, T. Moment analysis of stereoselective enterohepatic circulation and unidirectional chiral inversion of ketoprofen enantiomers in rat. J. Pharm. Sci. 85:580–585, 1996.
15. Palomer, A., Cabré, M., Ginesta, J., Mauleón, D., Carganico, G. Resolution of *rac*-ketoprofen esters by enzymatic reactions in organic media. Chirality 5:320–328, 1993.
16. Carganico, G., Mauleón, D., García, L. New arylpropionic derivatives, procedure for its preparation and use as analgesic. World Patent WO 9411332.
17. Lysz, T.W., Zweig, A., Keeting, P.E. Examination of mouse and rat tissues for evidence of dual forms of the fatty acid cyclooxygenase. Biochem. Pharmacol. 37:921–927, 1988.
18. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248–254, 1989.
19. Netter, P., Lapicque, F., Bannwarth, B., Tamisier, J.N., Thomas, P., Royer, R.J. Diffusion of intramuscular administered ketoprofen into the cerebrospinal fluid. Eur. J. Clin. Pharmacol. 29:319–321, 1985.
20. Willer, J.C. A supraspinal mechanism is involved in the central analgesic effect of ketoprofen, a non-steroidal anti-inflammatory drug, in man. Prog. Pharmacol. Clin. Pharmacol. 10:11–22, 1993.
21. Aberg, G., Ciofalo, V.B., Pendleton, R., Ray, G., Weddle, D. Inversion of (R)- to (S)-ketoprofen in eight animal species. Chirality 7:383–387, 1995.
22. Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J., Vane, J.R. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc. Natl. Acad. Sci. USA 90:11693–11697, 1993.
23. Glaser, K.B. Cyclooxygenase selectivity and NSAIDs: cyclooxygenase-2 selectivity of etodolac (lodine). Inflammopharmacology 3:335–345, 1995.
24. Engelhardt, G., Bogel, R., Schnitzer, C., Utzmann, R. Meloxicam: influence on arachidonic acid metabolism. Part 1. In vitro findings, Biochem. Pharmacol. 51:21–28, 1996.
25. Yamagata, K., Andreasson, K.I., Kaufmann, W.E., Barnes, C.A., Worley, P.F. Expression of amitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. Neuron 11:371–386, 1993.
26. Breder, C.D., DeWitt, D., Kraig, R.P. Characterization of inducible cyclooxygenase in rat brain. J. Comp. Neurol. 355:296–315, 1995.
27. Chen, J., Marsh, T., Zhang, J.S., Graham, S.H. Expression of cyclooxygenase-2 in rat brain following kainate treatment. Neuroreport 6:245–248, 1995.
28. Grossman, C.J., Wiseman, J., Lucas, F.S., Trevethick, M.A., Birch, P.J. Inhibition of constitutive and inducible cyclooxygenase activity in human platelets and mononuclear cells by NSAIDS and COX-2 inhibitors. Inflamm. Res. 44:253–257, 1995.
29. Brideau, C., Kargman, S., Liu, S., Dallob, A.L., Ehrich, E.W., Rodger, I.W., Chan, C.C. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. Inflamm. Res. 45: 68–74, 1996.
30. Young, M., Panah, S., Sachawatcharaphong, C., Cheung, P.S. Human whole blood assays for inhibition of prostaglandin G/H synthase-1 and -2 using A23187 and lipopolysaccharide stimulation of thromboxane B_2 production. Inflamm. Res. 45:68–74, 1996.
31. Panara, M.R., Greco, A., Santini, G., Sciulli, M.G., Rotondo, M.T., Padovano, R., Di, G.M., Cipollone, F., Cuccurullo, F., Patrono, C., Patrignani, P. Effects of the novel anti-inflammatory compounds, N-(2- (cyclohexyloxy)-4-nitrophenyl) methanesulphonamide (NS-398) and 5-methanesulphonamido-6(2,4-difluorothiophenyl)-1-indanone (L-745,337), on the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. Br. J. Phramacol. 116:2429–2434, 1995.
32. Malmberg, A.B., Yaksh, T.L. Antinociceptive actions of spinal nonsteroidal antiinflammatory agents on the formalin test in the rat. J. Pharmacol. Exp. Ther. 263:136–146, 1992.
33. Neugebauer, V., Geisslinger, G., Rümenapp, P., Weiretter, F., Szelenyi, I., Brune, K., Schaible, H. Antinociceptive effects of R-(–)- and S-(+)-flurbiprofen on rat spinal dorsal horn neurons rendered hyperexcitable by an acute knee joint inflammation. J. Pharmacol. Exp. Ther. 275:618–628, 1995.