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Carbamazepine exerts anti-inflammatory effects in the rat

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Abstract

In a first set of experiments, we evaluated the effects of different doses (5.0, 10, 20 and 40 mg/kg p.o.) of carbamazepine on nociceptive thresholds to thermal and mechanical stimuli, and on paw inflammatory hyperalgesia induced by the injection of brewer's yeast. Moreover, we studied the effect of carbamazepine on paw inflammatory edema by plethysmometry. Carbamazepine did not modify nociceptive latencies, but dose dependently reduced the hyperalgesia and the edema induced by the brewer's yeast injection in the rat hindpaw. In a second set of experiments, we studied the effects induced by the same doses of the drug on subcutaneous carrageenin-induced inflammation. Carbamazepine dose dependently reduced the inflammatory exudate, the prostaglandin E_2-like activity in the exudate, and the substance P concentrations in the exudate. Our results demonstrate that carbamazepine is able to inhibit the development of different types of inflammation in the rat.

Keywords: Carbamazepine; Edema; Inflammatory hyperalgesia; Nociception; Prostaglandin; Substance P

1. Introduction

Carbamazepine is an anticonvulsant drug used also for the treatment of trigeminal neuralgia and neuropathic pain in humans (Maciewicz et al., 1985). The mechanism by which carbamazepine exerts its analgesic action is largely unknown, as are its biochemical effects. In the last years, evidence has been accumulating that demonstrates the efficacy of other psychotropic drugs (e.g. tricyclic antidepressants) in the treatment of human deafferentation pain (Panerai et al., 1990), and in preventing brain peptide modifications induced by peripheral nerve deafferentation in rats (Panerai et al., 1988). We have previously shown that chlomipramine, an antidepressant drug that, similarly to carbamazepine, has a tricyclic molecular structure, can induce an antinociceptive effect (Bianchi et al., 1988) and reduce inflammatory edema and hyperalgesia in the rat (Bianchi et al., 1994).

Moreover, we have observed that only the antidepressant drugs with a tricyclic molecular structure are able to affect the chemotaxis of immune cells in vitro (Sacerdote et al., 1994). It has been recently suggested that periaxonal inflammatory or immunological processes might be involved in the increase in excitability recorded in the soma of sensory neurons whose axons are in ligated nerves in Aplysia (Clatworthy et al., 1994), and they play a role in the hyperalgesia evoked by nerve injury in rats (Clatworthy et al., 1995).

For all these reasons, and in order to better characterize carbamazepine as analgesic drug, we considered it of interest to evaluate the effects of carbamazepine in different models usually employed for the study of analgesic and anti-inflammatory drugs in rats.

2. Materials and methods

Sprague-Dawley CD male rats (Charles River, Calco, Italy), ten in each experimental group, were used in all experiments. The animals, 200–250 g body weight, were housed at 22°C with a light : dark cycle of 14:10 h, with food and water ad libitum. All experiments began between 09:00–09:30 a.m.

The tail-flick test was used to assess nociceptive thresholds. The noxious stimulus consisted of radiant heat from a lamp focused on a 2.0 × 2.0 mm area of

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the dorsal surface of the rat's tail. The stimulus was applied 3 cm from the distal end of the tail. The timer of the tail-flick apparatus, which was precise to 0.1 s, was stopped by a photocell when a tail-flick occurred. Basal values were in the range of 3.0–4.0 s and the cut-off time was 8 s. Treatments were administered only to the rats in which the basal tail-flick latency had remained stable for three subsequent measurements. Afterwards, in order to prevent tissue damage, only one tail-flick response was measured per time point.

Paw inflammation was induced by the injection of a 10% suspension of brewer's yeast in the plantar part of the left hindpaw, in a volume of 0.1 ml. Inflammatory edema was evaluated by the measurement of the hindpaw swelling induced by the injection of yeast suspension. We used a 7150 Plethysmometer (Basile, Comerio, Italy); the hindpaw was submerged to the tibiotarsal joint into the water-filled cell of the instrument. The volume of displacement, which is equal to the paw volume, was then read on a digital display.

The Randall-Selitto paw-withdrawal test was used to measure mechanical nociceptive thresholds in inflamed and non-inflamed hindpaws (Randall et al., 1957). The stimulus was applied with an analgesymeter (Basile, Comerio, Italy), which generates a linearly increasing mechanical force, applied by a conical piece of plastic with a dome-shaped tip on the dorsal surface of the rat's hindpaw. The results represent the maximal pressure (expressed in grams) tolerated by the animal.

In a second set of experiments, sterile polyester sponges ($4 \times 1.5 \times 0.5$ cm) were implanted subcutaneously as previously described (Berti et al., 1987). Briefly, the sponges were sterilized and soaked in 2% carrageenin (Sigma, St. Louis, Mo, USA). Dorsal incisions were made under light ether anesthesia, two sponges were implanted, the skin flaps were closed

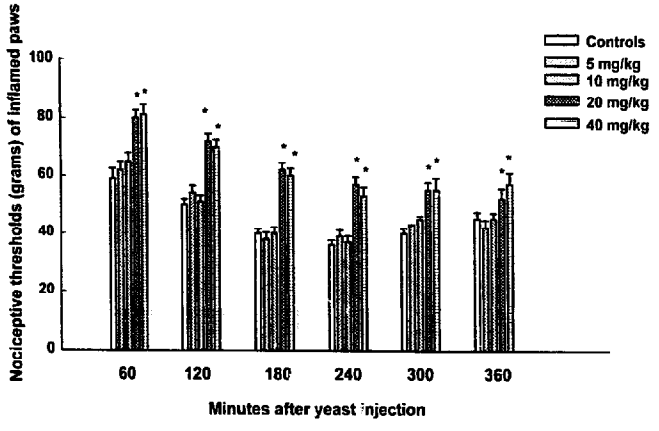

Fig. 1. Effect of carbamazepine (5, 10, 20, and 40 mg/kg p.o.) on paw hyperalgesia induced by the injection of brewer's yeast. Hyperalgesia was measured in the Randall-Selitto test. Values are grams, means ± S.E.M. $^{*}P < 0.005$ vs. controls (rats with inflamed left hindpaw).

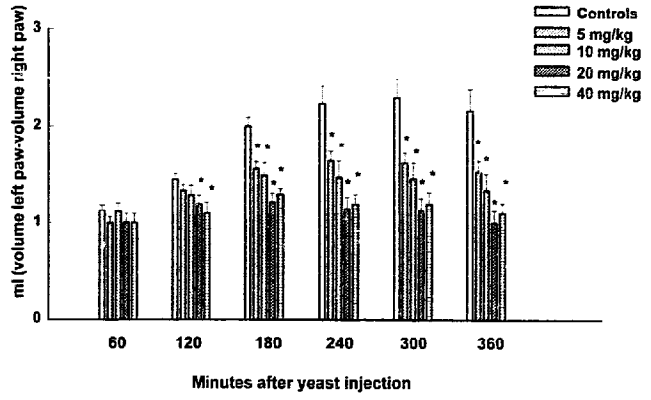

Fig. 2. Effect of carbamazepine (5, 10, 20, and 40 mg/kg p.o.) on paw edema induced by the injection of brewer's yeast. Values are means ± S.E.M. of the algebraic difference between the volume of treated and untreated paws. Edema was measured by plethysmometry. $^{*}P < 0.005$ vs. controls (rats with inflamed left hindpaw).

with suture clips, and the animals were replaced in their cages. The rats were killed 6 h after sponge implantation; the sponges were removed and immediately immersed in 10 ml heparinized saline contained in 50 ml glass centrifuge tubes and gently squeezed. The sponges were then centrifuged for 15 min at 1000 rpm. After centrifugation, the dry sponges were removed and the volume of the remaining fluid was measured.

Acid lipids were extracted into chloroform after dilution of the inflammatory exudate with 50% ethanol and acidification to pH 3 with formic acid. The chloroform was evaporated to dryness and the residue was taken up in Krebs-Henseleit solution for prostaglandin bioassay and substance P measurement.

Prostaglandin activity was estimated by parallel bioassay on the superfused rat stomach strip, using a prostaglandin E_2 standard for calibration. In order to increase the tissue sensitivity and to prevent endogenous prostaglandin generation, the tissues were superfused with Krebs-Henseleit solution containing a mixture of antagonists (atropine 10^{-6} M, methysergide 2×10^{-8} M, pirilamine 10^{-6} M, propranolol 10^{-6} M, phenoxybenzamine 3×10^{-6} M, and indomethacin 3×10^{-6} M).

Substance P concentrations in the inflammatory exudate were measured by radioimmunoassay, using antiserum and methods previously described in detail (Panerai et al., 1988). The antibody was raised in rabbit against synthetic substance P, and it is directed towards the *C*-terminal of the peptide. ^{125}I-substance P was purchased from Amersham Italia, Milano, Italy. The sensitivity of the radioimmunoassay was 10 pg/tube and the intraassay and interassay coefficients of variation were 8% and 11%, respectively.

In all experiments, carbamazepine (Ciba-Geigy,

Basel, Switzerland) was dissolved in methocel 0.25% in 0.9% NaCl and administered orally at the doses of 5.0, 10, 20 and 40 mg/kg, 1 h before the induction of the inflammation. The control animals received an equal volume of the vehicle.

The data were analyzed by means of analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons.

3. Results

Fig. 1 shows that, at higher doses, carbamazepine significantly reduced the paw inflammatory hyperalgesia induced by the yeast injection. In contrast, the nociceptive thresholds to mechanical stimulation of the non-inflamed hindpaws were not affected by carbamazepine administration (data not shown). Consistently, the drug did not modify nociceptive thresholds to a thermal stimulus applied to the tail (data not shown). A comparable pattern of observations was obtained by considering the effects of the drug on the development of paw edema. Also in this case, carbamazepine exerted an evident anti-inflammatory action. However, the edema was reduced also after the administration of the lowest dose of carbamazepine (Fig. 2). Therefore, the anti-hyperalgesic and anti-inflammatory effects of carbamazepine seem to be partially dissociated.

In rats with subcutaneous inflammation induced by the implantation of carrageenin soaked sponges, the pretreatment with carbamazepine (10, 20, and 40 mg/kg) significantly reduced the volume of inflammatory exudate (Fig. 3) and the prostaglandin E_2-like activity in the inflammatory exudate (Fig. 4). Finally, all the tested doses of the drug significantly reduced the amount of substance P in the inflammatory exudate (Fig. 5). Carbamazepine caused also a significant reduction of the concentrations of both PGE-like material and substance P in the inflammatory exudates.

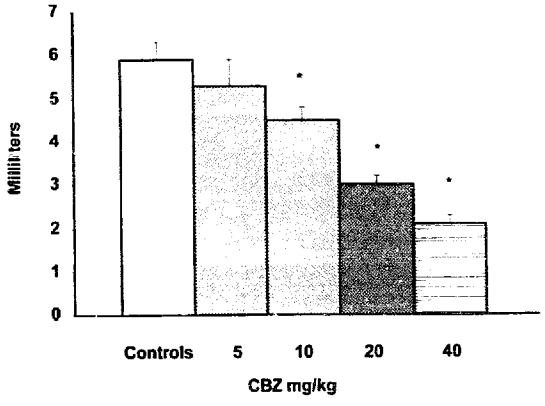

Fig. 3. Effect of carbamazepine (5, 10, 20, and 40 mg/kg p.o.) on the volume of inflammatory exudate induced by the subcutaneous implantation of carrageenin-soaked sponges. ED_{50} = 27.5 mg/kg. $^*P <$ 0.005 vs. controls (rats with subcutaneous inflammation).

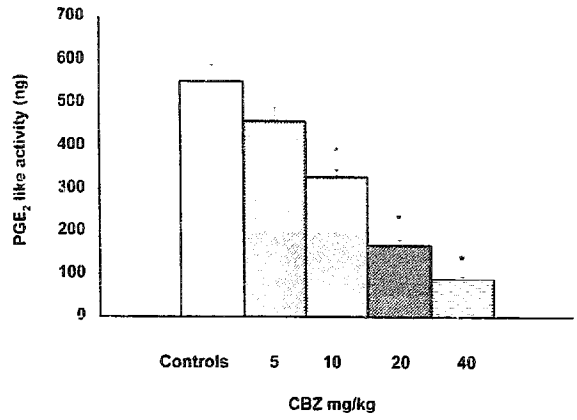

Fig. 4. Effect of carbamazepine (5, 10, 20, and 40 mg/kg p.o.) on the prostaglandin E_2-like activity in the inflammatory exudate induced by subcutaneous implantation of carrageenin-soaked sponges. ED_{50} = 17.2 mg/kg. $^*P < 0.001$ vs. controls (rats with subcutaneous inflammation).

4. Discussion

This study gives the first demonstration of a marked anti-inflammatory action of the anticonvulsant drug carbamazepine under experimental conditions. Our observations that other antiepileptic drugs, such as phenytoin (150 mg/kg p.o.) and sodium valproate (200 mg/kg p.o.), did not interfere with the development of inflammatory edema and hyperalgesia in rats (unpublished data) suggest that the anti-inflammatory activity of carbamazepine is not necessarily related to its anti-

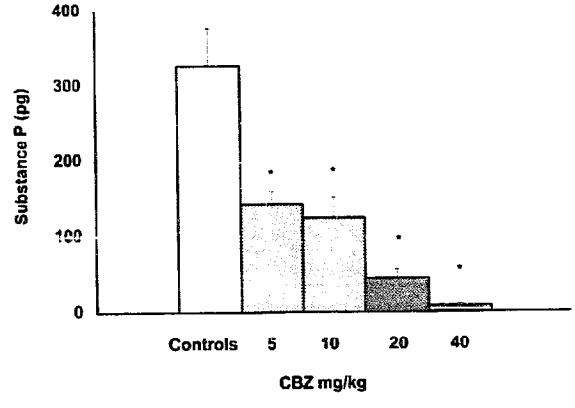

Fig. 5. Effect of carbamazepine (5, 10, 20, and 40 mg/kg p.o.) on the substance P levels in the inflammatory exudate induced by the subcutaneous implantation of carrageenin-soaked sponges. ED_{50} = 2.8 mg/kg. $^*P < 0.001$ vs. controls (rats with subcutaneous inflammation).

convulsant properties. Although all inflammatory parameters that we considered were affected by carbamazepine administration, this drug was particularly effective in reducing the substance P levels in the exudate. Substance P plays an important role in the induction of 'neurogenic inflammation' in the skin (Foreman, 1987), and it has been shown to exert potent pro-inflammatory actions such as vasodilatation, increased capillary permeability, and the secretion of prostaglandin E_2 (Scott et al., 1994). Therefore, it can be postulated that the anti-inflammatory action of carbamazepine is mainly mediated by the inhibition of substance P. However, our results do not allow us to completely clarify the mechanism of the anti-inflammatory effect of carbamazepine. Both central and peripheral actions of the drug are conceivable. Concerning the possible central mechanisms, there is evidence that brain monoamines may be involved in the anti-inflammatory activity of other psychotropic drugs such as antidepressants in laboratory animals (Arrigoni Martelli et al., 1967). Since it has been demonstrated that carbamazepine can enhance noradrenergic transmission both in vitro (Purdy et al., 1977) and in vivo (Olpe and Jones, 1983), we cannot exclude that such a mechanism might be involved also in the anti-inflammatory effects of this anticonvulsant drug.

In considering the possible involvement of peripheral mechanisms, we consider it important to focus attention on the molecular structure of carbamazepine. This drug, in fact, is structurally related to tricyclic antidepressant drugs. We have previously shown that the tricyclic antidepressant drug chlomipramine exerts anti-inflammatory effects in the rat (Bianchi et al., 1994), and that the tricyclic structure might be relevant for the inhibition of the immune responses exerted by antidepressant drugs (Sacerdote et al., 1994). Moreover, it has been demonstrated that different psychotropic drugs inhibit prostaglandin synthetase in vitro, and that the tricyclic compounds chlomipramine and chlorpromazine are the most potent in doing this (Krupp and West, 1975).

Therefore, we suggest that it could be of interest to further evaluate the role of the tricyclic structure in the peripheral modulation of inflammatory responses exerted by different drugs, including carbamazepine.

Finally, since it has been recently suggested that peri-axonal inflammation is critical in the development of hyperalgesia in a model of neuropathic pain (Clatworthy et al., 1994), these findings might contribute to our understanding of the analgesic effect of carbamazepine in clinical situations of neuropathic pain.

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