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The periaqueductal gray is the site of the antinociceptive action of carbamazepine as related to bradykinin-induced trigeminal pain

Foo-Wah Foong[1] & Masamichi Satoh[2]

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan

1 Using freely moving and conscious rats, the antinociceptive effects of microinjections of carbamazepine, into the periaqueductal gray (PAG), nucleus reticularis paragigantocellularis (NRPG) and nucleus raphé magnus (NRM) on the biting-like responses induced by bradykinin applied to the tooth pulp, were investigated to determine the primary site of action of this drug.

2 Microinjections of carbamazepine into the PAG ipsi- and contralateral to the stimulated tooth pulp produced dose-dependent suppressive effects on the biting-like responses within 1 min. The ED_{50} was 1.57 μg per rat, that is about 1,500 times less than that for carbamazepine administered systemically.

3 The antinociceptive effect of carbamazepine administered into the PAG was inhibited by pretreatment with bicuculline but not by phentolamine, propranolol and haloperidol.

4 Microinjections of carbamazepine into the NRPG and NRM were rarely effective in the production of antinociception at doses used (up to 3 μg per rat).

5 These results suggest that the PAG is one of the primary target sites for the antinociceptive activity of carbamazepine, and that GABAergic systems are involved this action of carbamazepine.

Introduction

Carbamazepine is used clinically for the treatment of trigeminal neuralgia but, until recently, the antinociceptive potency of this drug was only detectable in animals (tail-pinch and tail-flick tests) after huge doses had been administered (Theobald *et al.*, 1968). This was overcome by devising a method in which inhibition of the biting-like response, induced by application of bradykinin onto the tooth pulp, was used as a measure of antinociceptive activity (Foong *et al.*, 1982). Further, this method was shown to be feasible for evaluating the activity of not only atypical analgesic drugs, such as carbamazepine and phenytoin, but also for gerneral ones including morphine and pentazocine (Foong & Satoh, 1983).

We have demonstrated that the suppressive effect of systemic carbamazepine on the bradykinin-induced biting-like response can be significantly inhibited by systemically injected bicuculline (a GABA antagonist), phentolamine (an α-adrenocepter blocker), propranolol (a β-adrenocepter blocker) and haloperidol (a dopamine receptor blocker) but not by naloxone (an opioid antagonist), methysergide (a 5-hydroxytryptamine receptor blocker) or atropine (a muscarinic receptor blocker), thereby suggesting the involvement of GABAergic, noradrenergic and dopaminergic systems in the production of antinociception by carbamazepine (Foong & Satoh, 1984). Fromm *et al.*, (1981) noted that systemically administered carbamazepine resulted in inhibition of the neuronal responses in the spinal trigeminal nuclei of cats to electrical stimulation of the maxillary nerve. We showed that intraperitoneally administered carbamazepine depressed single neuronal responses of the medullary dorsal horn cells following application of bradykinin onto the tooth pulp of rabbits (Satoh & Foong, 1983). However, neither of these publications specify the primary target site(s) for the antinociceptive activity of carbamazepine.

[1] Present address: Department of Pharmacology, John Curtin School of Medical Research, The Australian National University, Canberra, A.C.T. 2601, Australia. [2] To whom correspondence should be addressed.

Investigations using microinjection techniques have shown that morphine and other opioids act primarily on several loci of the brain stem, such as the periaqueductal gray (PAG), nucleus reticularis paragigantocellularis (NRPG), nucleus reticularis gigantocellularis (NRGC) and nucleus raphe magnus (NRM) (Tsou & Jang, 1964; Takagi *et al.*, 1977; Yaksh & Rudy, 1978; Takagi, 1980). In the present experiments, carbamazepine was microinjected into the PAG, NRPG, NRM in order to determine the primary site of antinociceptive action of carbamazepine, as evidenced by the suppression of bradykinin-induced biting-like responses of rats.

Methods

All experiments were carried out on freely moving and conscious, male Sprague-Dawley rats (180–200 g) supplied from Keari Co. Ltd (Osaka) and housed in a temperature-, humidity- and light-controlled room (22–23°C, 53–55% and with 12 h of illumination starting at 07 h 00 min). Food and water were available *ad libitum*. The rats were implanted with a catheter for local application of bradykinin to the tooth pulp plus a guide-cannula for microinjection of carbamazepine into a target brain stem region.

Microinjections of carbamazepine into the PAG, NRPG and NRM were given according to the methods described by Akaike *et al.*, (1979). In brief, under pentobarbitone anaesthesia, a guide-cannula (stainless steel tubing of 0.7 mm o.d.) was unilaterally implanted into the cerebellum, and positioned 3 mm above the caudal PAG at a level of the interauricular line in antero-posterior axis or 4 mm above the NRPG and NRM, one week before commencing the experiment. For microinjection, an injection cannula made of glass tubing (90–140 μm o.d.) was inserted into the guide cannula and placed just 3 or 4 mm beyond the end of the guide cannula so that the tip was introduced into the PAG, the NRPG or the NRM. The 0.5 μl of drug solution was injected through the injection cannula at a rate of 0.1 μl $10\,s^{-1}$.

Implantation of a cannula containing a solution of bradykinin onto the exposed tooth pulp, fixation of it to the lower incisor surfaces and of a protector, were carried out under ether anaesthesia on the day when the experiment was done. At least 2 h were allowed to elapse between the discontinuation of the anaesthetic and the beginining of the experiment. Bradykinin (Protein Research Foundation, Mino, Japan) dissolved in distilled water was applied to the tooth pulp, in a dose of either 0.63 or 1.25 ng in a volume of 0.5 or 1.0 μl and at intervals of 60 min. Details of the procedures have been described previously (Foong *et al.*, 1982).

Application of bradykinin onto the tooth pulp of either one of the lower incisors produced a biting-like response and other signs of aversion, with a latency of 1 min or less. The biting-like response was reproducible and hence, was used for assaying the antinociceptive effect of carbamazepine. Only rats which showed this biting-like response for 20 min or more before drug application were used for further experimentation. When the duration of the biting-like response induced by bradykinin was reduced to 5 min or less by carbamazepine (observed time: at least 10 min), the effect of the drug was considered to be antinociceptive. Recovery was regarded as positive when rats showed the biting-like response for more than 5 min after bradykinin application.

In some experiments, bicuculline (1.0 or $1.2\,mg\,kg^{-1}$, subcutaneously), phentolamine ($5.0\,mg\,kg^{-1}$, intrapentoneally (i.p.), propranolol ($2.0\,mg\,kg^{-1}$, i.p.) and haloperidol (2.0 mg kg, i.p.), which have been shown to be effective in inhibiting

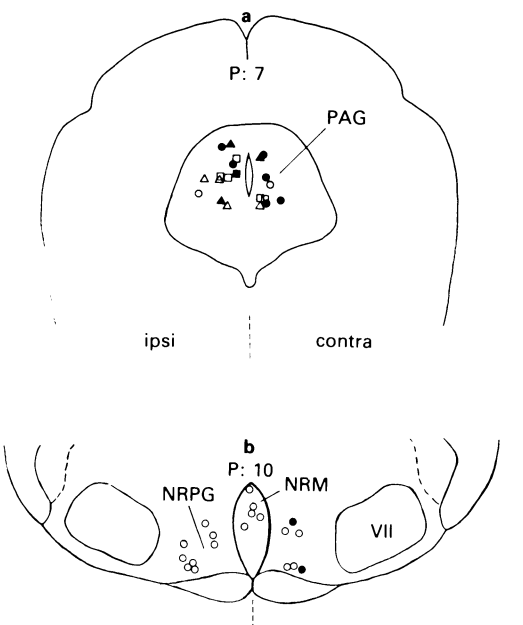

Figure 1 Effects of microinjections of carbamazepine into (a) the periaqueductal gray (PAG) and (b) nucleus reticularis paragigantocellularis (NRPG) and nucleus raphe magnus (NRM) of the rat on the bradykinin-induced biting-like responses. (a and b) Represent the dorsal part of P: 7 and ventral part of P: 10 plane of the atlas of Fifková & Maršala (1967), respectively. Closed and open symbols indicate that microinjection produced or did not produce suppression, respectively. In (a), squares, triangles and circles show the doses of 1.0, 1.5 and 2.0 μg per rat, respectively. In (b), circles represent the dose of 3.0 μg per rat, in both NRPG and NRM. Ipsi and contra mean microinjection sites ipsi- and contralateral to the stimulated tooth pulp.

the antinociceptive effect of carbamazepine administered systemically (Foong & Satoh, 1984), were administered 30 min before the microinjection of carbamazepine to reveal neurotransmitters involved in the antinociceptive effect of carbamazepine applied via the PAG.

Each rat was used only once. At the end of the experiments, the animal was deeply anaesthetized with chloroform and thionine blue solution was microinjected in a volume of 0.5 μl into the target site, using the same method as that for carbamazepine. The microinjection site was histologically verified to be in the PAG, NRPG or NRM, according to the atlas of Fifková & Maršala (1967). If the microinjection site was outside the three regions, the data were discarded.

Materials used were: carbamazepine and phentolamine (gifts from Ciba-Geigy (Japan), Ltd., Takarazuka, Japan), (+)-bicuculline methiodide (Pierce Chem. Co., Ltd, Rockford, U.S.A.), propranolol HCl and haloperidol HCl (gifts from Sumitomo Chem. Co. Ltd, Osaka, Japan). Carbamazepine was dissolved in a mixture of polyethylene glycol 200: polyoxyethylene hydrogenated castor oil: physiological saline (0.9% w/v NaCl solution) in a ratio of 1:1:8, which has been successfully used to dissolve diazepam (Kawasaki & Matsushita, 1981). The maximum concentration attained at 38°C was 6 mg ml^{-1}, that is 3 μg 0.5 μl^{-1} (microinjection volume into the PAG, NRPG and NRM). When the maximum concentration of carbamazepine was used, the temperature of the solution was kept at 38°C or slightly above. The other drugs were dissolved in physiological saline or distilled water.

Results

Microinjections into the PAG (Figure 1a)

Microinjections of the vehicle (solution used for dissolving carbamazepine) in a volume of 0.5 μl into the PAG ipsi- or contralateral to the stimulated tooth pulp, had no effect on the biting-like responses induced by bradykinin in the 6 rats tested.

Carbamazepine, when microinjected in doses of 1.0, 1.5 and 2.0 μg per rat into the ipsilateral PAG produced a dose-related suppression of the biting-like responses induced by bradykinin, in 1 out of 4 rats tested, 2 out of 5 and 2 out of 3, respectively. Similar microinjections of the same doses into the contralateral PAG produced an antinociceptive effect in neither of 2 rats examined, 1 out of 2 and 4 out of 5, respectively. The suppressive effect of carbamazepine appeared within 1 min after microinjection and disappeared within 60 min. No notable abnormalities in motor performance were observed following microinjections of the drug into either side of the PAG. As there were no gross differences in the effectiveness of microinjected carbamazepine between the ipsi- and contra-lateral PAG, the data from both sides were combined: hence, the antinociceptive effects were observed in 1 out of 6 rats examined (17%), 3 out of 7 (43%) and 6 out of 8 (75%) at doses of 1.0, 1.5 and 2.0 μg per rat, respectively (Figure 2). The ED_{50} value (95% confidence limits) calculated from the corrected data was 1.57 (1.16–2.12) μg per rat.

In another series of experiments, 3 μg of carbamazepine microinjected into the ipsilateral PAG produced an inhibitory effect on the bradykinin-induced biting-like responses, in all 5 rats examined. In 7 rats pretreated with bicuculline (1.0 or 1.2 mg kg^{-1}, s.c.), however, the same dose of carbamazepine showed no such antinociceptive effect, in any rat. On the other hand, pretreatment with phentolamine (5.0 mg kg^{-1}, i.p.), propranolol (2.0 mg kg^{-1}, i.p.) or haloperidol (2.0 mg kg^{-1}, i.p.) did not influence the antinociceptive effect of carbamazepine applied to the PAG, in all of 5, 4 and 5 rats tested, respectively.

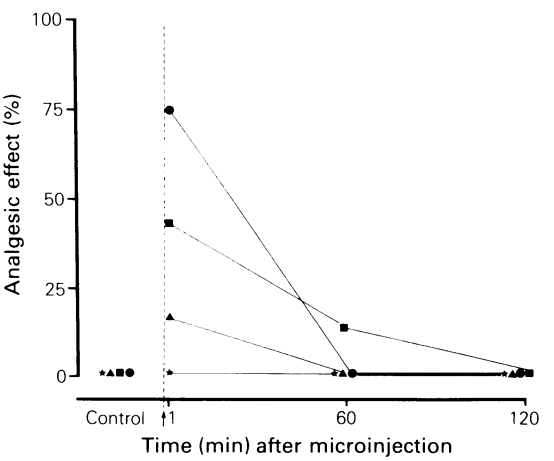

Figure 2 Dose-dependent antinociceptive effect of carbamazepine microinjected into the periaqueductal gray as shown by the inhibition of bradykinin-induced biting-like responses in the rat. (▲) Carbamazepine 1.0 μg per rat (8 rats), (■) 1.5 μg per rat (7 rats), (●) 2.0 μg per rat (6 rats) and (★) vehicle alone in a volume of 0.5 μl per rat (6 rats). The ordinate scale indicates the percentage equivalent to the number of rats showing antinociception, over the total number of rats used for a particular dose, and the abscissa scale shows the time after drug microinjection in min.

Microinjections into the NRPG and NRM (Figure 1b)

Microinjections of carbamazepine, 3.0 μg per rat, into the NRPG ipsilateral to the stimulated tooth pulp did not suppress the biting-like responses induced by bradykinin, in any of the 8 rats tested. The same dose of the drug applied to the contralateral NRPG produced an antinociceptive effect in 2 out of the 6 rats examined. Carbamazepine, when microinjected in a dose of 3.0 μg per rat into the NRM, did not produced any antinociceptive effect, in any of the 5 rats investigated. Higher doses or larger volumes could not be used because of the limited solubility of carbamazepine and a slight inhibition of the biting-like response seen following microinjections of the vehicle, into the NRPG or NRM, in a volume of 1.0 μl.

Discussion

This study using intracerebral microinjection techniques is the first to reveal the primary site of action for the analgesic effect of carbamazepine. The drug, when microinjected into the PAG, produced a dose-dependent suppressive effect on the bradykinin-induced biting-like responses, while the vehicle alone showed no such effect. The ED_{50} value was 1.57 μg per rat, that is, 8.3 $\mu g\,kg^{-1}$, which is about 1,500 times smaller than that previously observed (13.1 $mg\,kg^{-1}$) following systemic administration of carbamazepine (Foong *et al.*, 1982). Furthermore, the suppressive effect of carbamazine appeared within 1 min after the microinjection. These results indicate that the PAG is one of the primary sites for the antinociceptive action of carbamazepine.

Electrical stimulation of the PAG produces a potent inhibitory effect on nociceptive transmission in the medullary dorsal horn following electrical stimulation of tooth pulp (Sessel *et al.*, 1976; Yokota & Hashimoto, 1976). In preliminary experiments using rabbits, we found that single neuronal responses of the medullary dorsal horn cells induced by application of bradykinin onto the tooth pulp were markedly inhibited by microinjection of carbamazepine into the PAG (unpublished data). These findings suggest that activation by carbamazepine of the inhibitory system originating from the PAG to the medullary dorsal horn contributes to the antinociceptive action of this drug when microinjected into the PAG (as shown in the present experiments) and to the inhibitory effect of systemic carbamazepine on the activity of single neurones in the medullary dorsal horn (Fromm *et al.*, 1981; Satoh & Foong, 1983).

Microinjections of carbamazepine into the PAG were bilaterally effective on the suppression of bradykinin-induced biting-like responses. This in accordance with the observations of Figueiras *et al.*, (1983), that unitary responses of the medullary dorsal horn cells evoked by electrical stimulation of the canine detine are inhibited by electrical stimulation of the PAG ipsi- and contralateral to the stimulated canine. This bilaterality of effectiveness may be the result of intrinsic connections between both sides of the PAG (Beitz, 1982).

Bicuculline, a GABA antagonist, but not phentolamine, propranolol or haloperidol (catecholamine receptor blockers), inhibited the antinociceptive action of carbamazepine microinjected into the PAG, although all of these blockers have been shown to be effective in inhibiting the action of the drug administered systemically (Foong & Satoh, 1984). These results indicate that an activation of the GABAergic systems is necessary for the production of the antinociceptive effect seen following the microinjection of carbamazepine into the PAG. A local GABAergic network in the PAG and nucleus raphé dorsalis of the rat, as shown by Belin *et al.*, (1979) might be activated by carbamazepine. The primary sites of action of carbamazepine resulting in activation of catecholaminergic systems remain to be elucidated.

Carbamazepine injected into the NRPG and NRM did not produce a suppressive effect on the bradykinin-induced biting-like responses, although both regions seem to be involved in the production of antinociceptive effects of morphine, as revealed by conventional antinociception tests such as the tail-pinch method (Akaike *et al.*, 1978; Dickenson *et al.*, 1979). We have previously demonstrated that the antinociceptive effect of carbamazepine administered systemically is not inhibited by naloxone (Foong & Satoh, 1984). Hence, it is conceivable that carbamazepine produces its antinociceptive action through different sites and mechanisms from those related to the effects of morphine.

In conclusion, one of the primary sites of antinociceptive action of carbamazepine appears to be the PAG, as microinjections of the drug into this region produced a dose-dependent suppressive effect on the biting-like responses induced by application of bradykinin onto the tooth pulp. The GABAergic system almost certainly has a role in the production of the antinociceptive effects of carbamazepine in the PAG.

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