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Attenuation by valproate of c-fos immunoreactivity in trigeminal nucleus caudalis induced by intracisternal capsaicin

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1 Valproic acid, useful in the treatment of migraine, is an inhibitor of γ-aminobutyric acid (GABA) aminotransferase and activator of glutamic acid decarboxylase. Its mechanism in migraine remains obscure. The effects of valproic acid (2-propylpentanoic acid) were examined on the number of cells expressing c-fos-like immunoreactivity (c-fos-LI), a marker of neuronal activation, within the trigeminal nucleus caudalis (lamina I, IIo; TNC) 2 h after intracisternal injection of the irritant, capsaicin (0.1 ml; 15.25 μg ml^{-1}), in urethane-anaesthetized Hartley guinea-pigs. Positive cells were counted in eighteen sections (50 μm) at three representative levels (rostral, middle and caudal) within lamina I, II$_o$ of the TNC in 90 animals.

2 Numerous cells were labelled after capsaicin instillation (244±25; 1 ml; 15.25 mM) but not after capsaicin vehicle (11±1). Positive cells were also found within the medial reticular nucleus, the area postrema and the nucleus of the solitary tract. A similar distribution has been demonstrated previously after application of intracisternal irritants such as autologous blood or carrageenin.

3 Valproate (⩾10 mg kg^{-1}, i.p.) reduced labelled cells by 52% ($P<0.05$) in lamina I, II$_o$ but not within the area postrema, the nucleus of the solitary tract or the medial reticular nucleus. A similar finding was obtained previously after administration of sumatriptan, dihydroergotamine or the NK$_1$ receptor antagonist RPR 100,893.

4 Pretreatment with bicuculline (30 μg kg^{-1}; i.p.), a GABA$_A$ antagonist, but not phaclofen (1 mg kg^{-1}) a GABA$_B$ antagonist, reversed the effect of valproate and increased c-fos positive cells within lamina I, IIo. Somewhat paradoxically, bicuculline by itself (30 μg kg^{-1} i.p.) decreased the number of labelled cells suggesting that more than a single GABAergic mechanism can suppress c-fos expression.

5 We conclude that the mechanism of action of valproate is mediated via GABA$_A$ receptors. Since valproate decreases both c-fos expression and as previously shown, neurogenic inflammation within the meninges, the GABA$_A$ receptor complex might provide an important target for drug development in migraine and related headaches.

Keywords: GABA; valproate; c-fos; trigeminal; headache; migraine; bicuculline

Introduction

Expression of the immediate early gene c-fos is an accepted marker of functional activity in neurones (Dragunow & Robertson 1987; Uemura *et al.*, 1991; Morgan & Curran, 1991). C-fos-like-immunoreactivity (c-fos-LI) appears in laminae I, II$_o$ of the dorsal horn after noxious peripheral stimulation (Hunt *et al.*, 1987; Menetrey *et al.*, 1989; Presley *et al.*, 1990). Tonic noxious stimuli are particularly effective. Similarly, c-fos expression is induced in the trigeminal nucleus caudalis (TNC) following noxious chemical stimulation of the meninges in rats (Nozaki *et al.*, 1992a,b) and guinea-pigs (Cutrer *et al.*, 1995a). We previously showed that the number of expressing cells following noxious meningeal stimulation can be significantly reduced by morphine, dihydroergotamine, sumatriptan, CP-93,129, a selective 5-hydroxytryptamine (5-HT)$_{1B}$ receptor agonist (Nozaki *et al.*, 1992b), the sumatriptan analogue CP-122,288 (Cutrer *et al.*, 1995b), and the NK$_1$ receptor antagonist RPR 100893 (Cutrer *et al.*, 1995a).

Valproate (2-propylpentanoic acid), an effective prophylactic agent for migraine treatment (Hering & Kuritzky, 1992; Jensen *et al.*, 1994; Coria *et al.*, 1994), increases endogenous γ-aminobutyric acid (GABA) levels by blocking degradation and enhancing synthesis (Godin *et al.*, 1969; Loescher, 1981). GABA is a neutral amino acid which binds to one of at least 2 receptor subtypes (Bonanno & Ratieri, 1993; MacDonald & Olsen, 1994). While the mechanism of valproate's effect in migraine is not known, one possible explanation might lie in a GABA-mediated suppression of the transmission of nociceptive information from meningeal afferents to the superficial laminae of the medullary trigeminal nucleus caudalis. To investigate this possibility, we examined the effect of valproate on c-fos expression in laminae I, II$_o$ of the TNC after noxious meningeal stimulation. We now demonstrate that valproate decreases capsaicin-induced c-fos-LI within laminae I, II$_o$, in clinically-relevant doses, by a bicuculline reversible mechanism.

Methods

Animal preparation

Male Hartley guinea-pigs (200-300 g; Charles River Laboratories, Wilmington, MA, U.S.A.) ($n=90$) were anaesthetized with urethane (1.3 g kg^{-1} i.p.) and supplemented (130 mg kg^{-1} i.p.) to suppress the withdrawal response to hindpaw stimulation. A soft catheter (PE-10, 0.28 mm internal diameter; Intramedic, Clay Adams, Parsippany, NJ, U.S.A.) was introduced into the cisterna magna through the atlantooccipital membrane after a midline skin incision was made from the occipital protuberance to the cervical area. In the valproate experiments, the guinea-pigs were placed prone for 5.5 h as previously described (Nozaki *et al.*, 1992a). At ap-

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proximately 5 and a half hours after catheter placement, animals received drug treatment as described below. Six hours after catheter placement, capsaicin solution (100 μl of 15.25 μg ml^{-1} unless otherwise indicated) was injected into the cisterna magna over 1 min with a tuberculin syringe.

To facilitate capsaicin distribution within the subarachnoid space, animals were placed in the reverse Trendelenberg position (−30°) for 30 min and in prone position for the next 90 min. Core temperature was maintained at 36–37°C by a homeothermic blanket (Harvard Apparatus No. 551, South Natick, MA, U.S.A.). An overdose of pentobarbitone (80 mg kg^{-1}; i.p.) was used for euthanasia 2 h after capsaicin instillation.

Animals were perfused via the ascending aorta with saline (0.9%, 200 ml), and then by formaldehyde (4%, 500 ml) in 0.1 M phosphate buffer (pH 7.3). Perfused brainstems with attached spinal cords were postfixed in 4% formaldehyde overnight and then placed in cryoprotectant solution (20% sucrose, 30% ethylene glycol in 0.1 M phosphate buffer) for 48 h before sectioning. The upper cervical spinal cord and medulla (from the level of C2 to 1 mm rostral to the obex) were serially sectioned by using a freezing microtome (Reichert-Jung; 2000 Leica, U.S.A.) (190 ± 10 sections). Of these 18 selected sections were saved and processed immunohistochemically.

Protocols

Vehicle (n = 13), or valproate [1 (n = 3), 3 (n = 4), 10 (n = 13) or 20 mg kg^{-1} (n = 5)] was injected i.p. 5 h and 30 min after catheter placement and 2 h and 30 min before death. At 6 h after catheter placement and 2 h before death, capsaicin was injected intracisternally.

In a separate series of experiments, bicuculline, phaclofen or vehicle was administered 5 h and 20 min after catheter placement. Ten minutes later, either vehicle or valproate (10 mg kg^{-1}) was administered. Capsaicin was injected (i.c.) at 6 h after catheter placement. Seven treatment groups included: (1) Vehicle + vehicle (n = 8), (2) vehicle + valproate (n = 6), (3) bicuculline (10 μg kg^{-1}) + vehicle (n = 5), (4) bicuculline (30 μg kg^{-1}) + vehicle (n = 3), (5) bicuculline (10 μg kg^{-1}) + valproate (n = 7), (6) bicuculline (30 μg kg^{-1}) + valproate (n = 7) and (7) phaclofen (1 mg kg^{-1}) + valproate (n = 5).

Immunohistochemistry and cell counting

Tissues were processed as free floating sections with the avidin-biotin procedure by use of commercially available kits (Vectastain ABC; Vector Labs Burlingame, CA) as described previously (Uemura *et al.*, 1991), with slight modifications (Cutrer *et al.*, 1995a,b). The primary c-fos antiserum, a rabbit polyclonal antiserum directed against an *in vitro* translated product of the c-fos gene, was kindly provided by Dr Dennis Slamon, the Department of Hematology and Oncology at the University of California, LA. The staining pattern and distribution of labelled cells were similar to that of a commercially available antibody raised against residues 4-17 of the c-fos protein (Microbiological Associates, Inc., Bethesda, MD). After immunohistochemical processing, tissue sections were serially arranged on gelatin slides, air dried overnight and coverslipped.

C-fos positive cells (i.e., stained nuclei) were counted by an observer naive to the treatment group (F. M. C.) and confirmed in randomly selected sections by a second investigator (G. A.) also blinded to the treatment groups, as described previously (Cutrer *et al.*, 1995a).

Weighted average counting method

Total c-fos expression in the TNC was estimated as previously described (Cutrer *et al.*, 1995b). Based on the observation that c-fos expression was maximal at −2.25 to −2.55 mm and decreased linearly both rostrally and caudally, six 50 μm sections (every third section) were processed immunohistochemically and counted at each of three levels: (+0.15 to −0.60 (midpoint −0.225); −2.10 to −2.85 (midpoint: −2.475); and −6.60 to −7.35 (midpoint: −6.975). The mean of each of these samples was determined (x_1, x_2 and x_3 respectively). The area under the curve was obtained by use of the trapezoid rule, which in this instance reduces to $7.5x_1 + 22.5x_2 + 15x_3$. The weighted average was then calculated by dividing this sum by 45 (the number of 50 μm sections sampled every 150 μm from −0.225 to −6.975). These data are expressed as cells per section.

Qualitative estimate of c-fos LI

A qualitative estimate of c-fos staining in the area postrema (AP, 1–2 sections), the nucleus of the solitary tract (NTS, 12 sections), the medial reticular nucleus (MRN, 8 sections), the lateral reticular nucleus (LRN, 6 sections) and Rexed laminae III-V, VII and X of the cervical spinal cord (6 sections) was performed by use of a 4 point scale: 3+ intense expression (>30 cells per section); 2+ moderate expression (11–30); 1+ (1 to 10 cells) and 0 indicating no cells. This assessment was performed in animals treated with intracisternal capsaicin plus valproate (10 mg kg^{-1}, n = 8), bicuculline (30 μg kg^{-1}, n = 3), phaclofen (1 mg kg^{-1}, n = 7), drug vehicle (n = 15) or after capsaicin vehicle alone (n = 3).

Drugs

Capsaicin solution (Polyscience Inc. Warrington, PA, U.S.A.) was prepared fresh for each experiment. Capsaicin (3.05 mg) was diluted in 1 ml of saline:ethanol:Tween 80 (8:1:1) and sonicated for 5 min. The solution was further diluted (1:200) in normal saline and kept at room temperature. Urethane (7.5 g) (Sigma, St. Louis, MO, U.S.A.) was diluted in 50 ml of distilled water. Sodium pentobarbitone was obtained from Anthony Products Co. Arcadia, CA, U.S.A. Valproic acid (Sigma, St. Louis, MO, U.S.A.) was diluted in normal saline. (+)-Bicuculline and phaclofen (Research Biochemicals Inc., Natick, MA, U.S.A.) were dissolved in 0.1 N HCl, adjusted to a pH of 5.0 with a few drops of 0.1 N NaOH.

Statistics

Data are expressed as a weighted average ± s.e.mean. Statistical comparisons of weighted average values were made between groups by use of Analysis of Variance plus Bonferroni/Dunnett *post hoc* tests. Qualitative data were analysed by the Mann-Whitney unpaired t test.

Results

C-fos expression after capsaicin instillation

Four ± 1 positive cells were counted per section in the TNC in urethane anaesthetized animals with cisternal catheter (n = 2) whereas 11 ± 1 positive cells were found after catheterization and instillation of capsaicin vehicle (n = 3). In the latter group, fewer than 10 positive cells per section (0 or 1+ on the qualitative scale) were present in the AP, NTS, or in the MRN. Pretreatment with valproate (10 mg kg^{-1}; n = 2) or bicuculline (30 μg kg^{-1}; n = 2) did not decrease these numbers.

Capsaicin increased positive cells in the TNC [12, 23, 244 and 425 cells per section after 0.305, 3.05, 15.25 and 30.5 μg ml^{-1}, respectively (n = 3 per group)]. Five nmol (15.25 μg ml^{-1}; 100 μl) of capsaicin were injected for all subsequent experiments.

Spatial distribution

C-fos-LI was present bilaterally and most prominently within laminae I and II_o of the TNC after capsaicin instillation. Expression was intense in both the dorsal and ventral segments at

each sampled level. Many labelled cells were present at −2.475 (361±35) and −0.225 (339±43). At −6.975, 40±5 cells per section were labelled ($n=13$).

In the area postrema, 3+ labelling was observed whereas in the MRN and the NTS 2+ was present at −0.225. Also at −0.225, the LRN contained 1+ staining. At −2.475, the NTS and MRN contained few cells (1+). At −6.975, laminae III, IV, V and X of cervical spinal cord also exhibited 1+ staining. There were no positive cells in lamina VII (see Figure 1).

Drug treatment

Valproate Valproate dose-dependently reduced labelling within lamina I, II_o. The number of positive cells (244±22 cells per section after i.c. capsaicin) was decreased by 26%, 36%, 52%($P<0.01$) and 54% ($P<0.01$), after 1, 3, 10 and 20 mg kg^{-1}, respectively (Figure 2). After 10 and 20 mg kg^{-1}, significant reductions were found at −0.225 and −2.475 ($P<0.01$) (see Figure 3) but not at −6.975. It was our impression that cell numbers were decreased in both dorsal and ventral aspects of TNC to a similar extent.

Bicuculline or phaclofen plus valproate Bicuculline dose-dependently reversed the suppression by valproate. Bicuculline (10 or 30 μg kg^{-1}) plus valproate (10 mg kg^{-1}) decreased cell labelling by 35% and 16% respectively (see Figure 4a) whereas valproate 10 mg kg^{-1} decreased positive cells by 65% (375±37 versus 133±26 positive cells per section with valproate). The number of c-fos positive cells after bicuculline plus valproate did not differ statistically from vehicle-treated controls. C-fos-LI was significantly higher in animals receiving bicuculline (30 μg kg^{-1}) plus valproate (10 mg kg^{-1}) than in those receiving valproate alone ($P<0.01$). Cell labelling was not modified by phaclofen (1 mg kg^{-1} i.p.) 10 min before valproate (see Figure 4b).

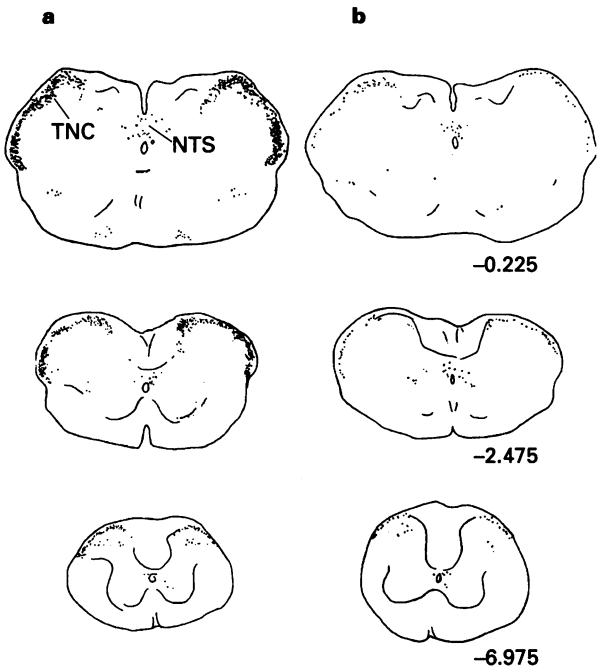

Figure 1 Camera lucida drawings showing the location of c-fos immunostained cells (dots) in coronal brain stem sections after intracisternal capsaicin. Guinea-pigs pretreated with vehicle (a) or valproate (10 mg kg^{-1}, i.p.) (b), were given capsaicin (0.1 ml, 15.25 μg ml^{-1}) were killed 120 min later. The distance (mm) caudal to the obex is given to the right. The findings from these 2 animals are representative of the results in 13 animals per group. Not drawn are the c-fos protein-like immunoreactive cells in pia mater, arachnoid and ependyma. TNC, trigeminal nucleus caudalis; NTS, nucleus of the solitary tract.

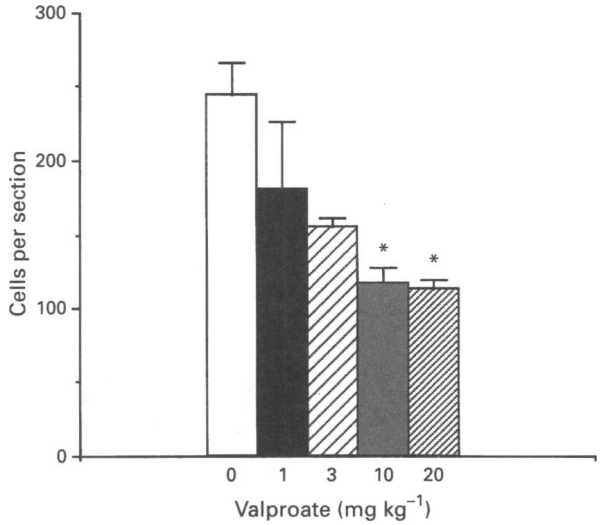

Figure 2 Pretreatment with valproate dose-dependently decreases the number of c-fos-like immunoreactive cells evoked by intracisternal capsaicin injection. Cell numbers are given per 50 μm section within lamina I, II_o of the trigeminal nucleus caudalis (TNC) as determined by a weighted average method (see Methods). Vehicle ($n=13$), valproate 1 ($n=3$), 3 ($n=4$), 10 ($n=13$) or 20 mg kg^{-1} ($n=5$) was injected 30 min before capsaicin and the animals killed 120 min later. *$P<0.01$ as compared to vehicle-treated group.

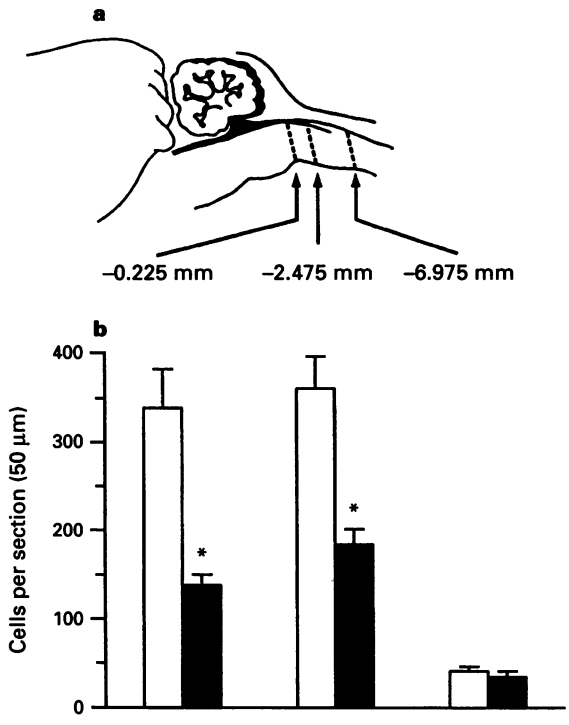

Figure 3 (b) Distribution of c-fos protein-like immunoreactive cells at three different levels within trigeminal nucleus caudalis (TNC; lamina I, II_o) in guinea-pigs that received capsaicin injection (0.1 ml, 15.25 μg ml^{-1}) into the cisterna magna 30 min prior to vehicle ($n=13$, open columns) or valproate (10 mg kg^{-1}, $n=13$, solid columns) and killed 120 min later. Cells were counted in 50 μm sections sampled every 150 μm at −0.225 (6 sections), −2.475 (6 sections) and −6.975 mm (6 sections). *$P<0.05$ compared with vehicle-treated animals. (a) Shows a midsagittal section through brain stem and cerebellum. The arrows refer to the brain stem levels from which the analysis was performed.

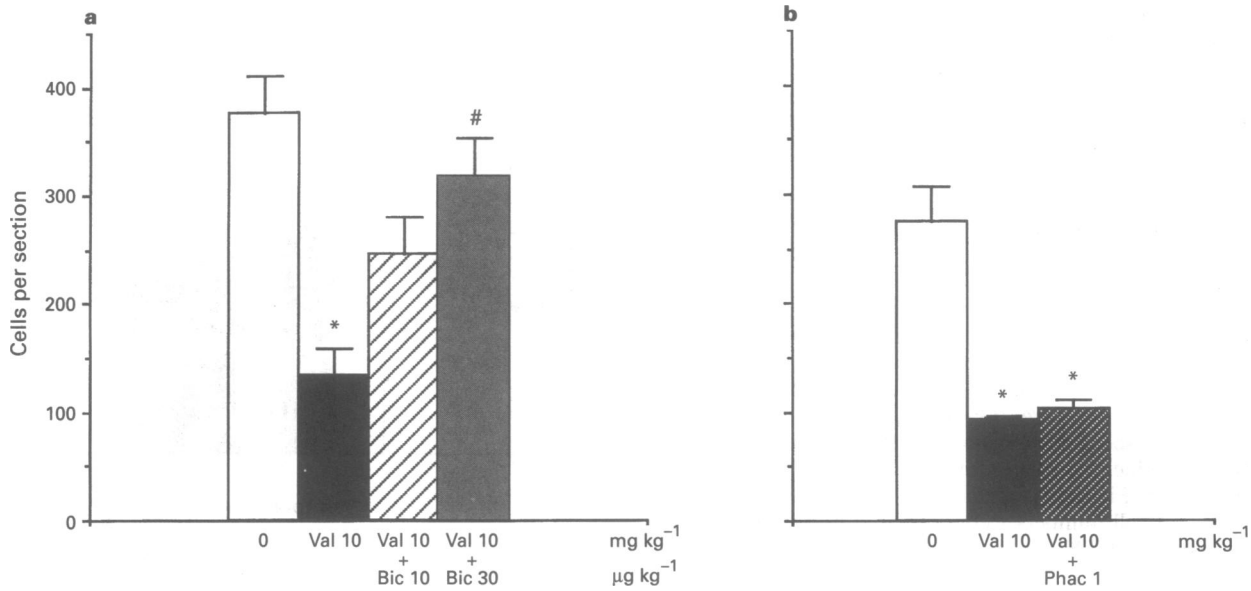

Figure 4 (a) Bicuculline dose-dependently reverses valproate induced suppression of c-fos immunoreactive cells within trigeminal nucleus caudalis (TNC) (lamina I, II_o) after intracisternal (i.c.) capsaicin injection. C-fos-LI was significantly higher after bicuculline (Bic, 30 $\mu g\,kg^{-1}$) plus valproate (Val, 10 $mg\,kg^{-1}$) than in animals receiving valproate alone. Cell numbers are given as the weighted average (see Methods) per 50 μm section within the TNC. Vehicle ($n=4$) open column, valproate 10 $mg\,kg^{-1}$ i.p. ($n=4$), bicuculline 10 $\mu g\,kg^{-1}$ i.p. + valproate 10 $mg\,kg^{-1}$ ($n=7$) or bicuculline 30 $\mu g\,kg^{-1}$ i.p. + valproate 10 $mg\,kg^{-1}$ ($n=7$) were injected intraperitoneally 40 (bicuculline) or 30 (vehicle or valproate) min before i.c. capsaicin and the animals killed 120 min later ($^*P<0.05$). Only valproate 10 $mg\,kg^{-1}$ was significantly different from vehicle-treated controls ($^{\#}P<0.01$). (b) Pretreatment with the $GABA_B$ antagonist, phaclofen (Phac), does not reverse valproate induced suppression of c-fos within the TNC. Vehicle ($n=3$), valproate 10 $mg\,kg^{-1}$ ($n=3$) or phaclofen 1 $mg\,kg^{-1}$ (40 min before) + valproate 10 $mg\,kg^{-1}$ i.p. (30 min before) ($n=5$) were administered intraperitoneally before intracisternal injection of capsaicin. $^*P<0.01$ as compared to vehicle-treated animals.

Bicuculline alone (10 or 30 $\mu g\ kg^{-1}$) decreased capsaicin-induced labelling cells by 31% and 45% respectively (Figure 5) compared to drug vehicle.

Other brainstem nuclei Valproate, bicuculline or phaclofen did not change the estimation of positive cells in the area postrema (AP), the medullary reticular nucleus (MRN), the lateral reticular nucleus (LRN) or the nucleus of the solitary tract (NTS).

Within each experimental series, the variance was less than 15% and treatment effects were quite consistent. However, some variability was observed in the intensity of c-fos staining among capsaicin-treated control animals between experiments performed on different days (e.g. 244 ± 22 cells versus 423 ± 41). This variability may have been due to the loss of staining with reuse of the primary antisera (up to three times). To minimize variability, appropriate control animals were included in each experiment and comparisons were made only within each experimental series.

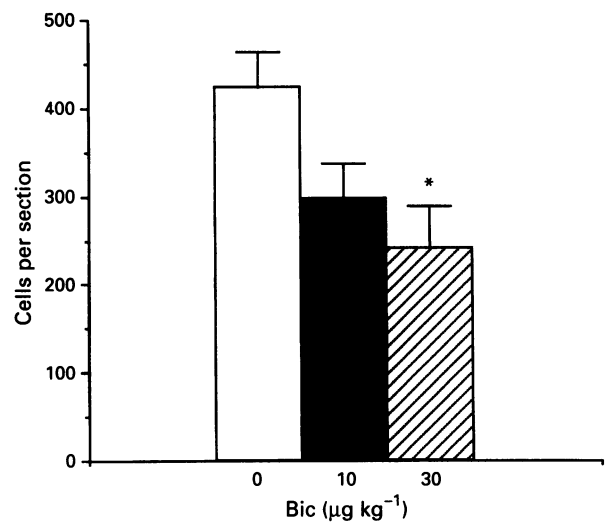

Figure 5 Pretreatment with bicuculline (Bic) dose-dependently decreases c-fos immunoreactive cells evoked within trigeminal nucleus caudalis (TNC) by intracisternal capsaicin. Cell numbers are weighted average values (see Methods) within lamina I, II_o of TNC. Vehicle (open column, $n=5$), bicuculline 10 $\mu g\,kg^{-1}$ ($n=4$), or 30 $\mu g\,kg^{-1}$ ($n=3$) was injected 40 min before capsaicin and the animals killed 120 min later. $^*P<0.05$ as compared to vehicle-treated group.

Discussion

As in previous studies (Cutrer *et al.*, 1995a,b), intracisternal capsaicin evoked c-fos-LI over the rostro-caudal extent of the TNC as well as within the AP, LRN, NTS and MRN. Pretreatment with valproate (≥ 10 mg kg^{-1}, i.p.) significantly and dose-dependently suppressed cell staining specifically within the TNC. The effect of valproate was reversed by bicuculline in a dose-dependent manner but not by phaclofen pretreatment, suggesting that $GABA_A$ receptors mediate the valproate response. Valproate via GABAergic mechanisms probably inhibited neurotransmission rather than c-fos genomic expression inasmuch as the blockade was observed selectively within the TNC and not other brainstem nuclei. Changes in vascular haemodynamics probably did not mediate valproate's effect because rodents injected with valproate and monitored for 30 min show no alteration in heart rate or blood pressure (Lee *et al.*, 1995).

C-fos-LI within the TNC

As in previous studies, (Strassman & Vos 1993; Cutrer *et al.*, 1995a,b), capsaicin proved to be an effective stimulus for c-fos activation within the trigeminal system. Meningeal fibre activation rather than direct irritation of trigeminal roots or gangion cells may be responsible because induced c-fos-LI is markedly decreased in animals that have undergone selective nasociliary nerve transection before intracisternal autologous

blood injection (Nozaki *et al.*, 1992a). We remain uncertain about the integrity of the blood brain barrier after capsaicin instillation. Hence, no firm conclusions can be drawn about the site of activity of valproate and related drugs in the experiments described.

GABA in the trigeminal system

Valproate, which increases GABA by enhancing glutamate-decarboxylase (GAD) activity (Godin *et al.*, 1969) and by GABA transaminase (GABA-T) inhibition (Loescher, 1981), may affect c-fos expression via central and/or peripheral sites of action. For example, GABA is present in high concentrations in the dorsal horn of the spinal cord (Barber *et al.*, 1982; Magoul *et al.*, 1987; Carlton & Hayes, 1990). A relatively high density of $GABA_A$ and $GABA_B$ receptors are present within the superficial laminae of dorsal horn in both rodents (Palacios *et al.*, 1981; Bowery *et al.*, 1987) and man (Waldvogel *et al.*, 1990). Within the trigeminal ganglion, a large proportion of neuronal cell bodies and occasional axons exhibit GABA immunoreactivity (Szabat *et al.*, 1992). GAD and GABA-T immunoreactive fibres invest cerebral blood vessels (Hamel *et al.*, 1983; Imai *et al.*, 1991).

There is increasing evidence that GABA modulates trigeminovascular activity. Valproate pretreatment decreases plasma extravasation within the dura mater induced by substance P or electrical trigeminal ganglia stimulation (Lee *et al.*, 1995) at doses similar to those required to attenuate c-fos expression. Similarly, bicuculline (10 $\mu g\ kg^{-1}$ i.p.) but not phaclofen reversed valproate's effect on extravasation. The $GABA_A$ allosteric modulators, allopregnanolone, tetrahydrocorticosterone and alphaxalone as well as the benzodiazepines, diazepam and zolpidem blocked plasma protein extravasation as well (Limmroth, unpublished data).

Evidence for central modulation

Bicuculline appears to attenuate the c-fos response to capsaicin when given alone. This seemingly paradoxical finding is consistent with the results of Sluka *et al.* who showed a decrease in paw withdrawal latency and joint circumference after spinal cord administration of bicuculline (Sluka *et al.*, 1994) in kaolin and carrageenan-induced arthritis. The authors demonstrated the presence of a dorsal root reflex which could be blocked by bicuculline but not by the $GABA_B$ antagonist CGP35348. Our findings suggest that the trigeminovascular system possesses more than a single GABAergic mechanism. The observed GABA effect may be determined by the vector sum of these different mechanisms.

GABA and nociception

The complex neuronal and synaptic mechanisms underlying GABA induced analgesia are not well understood. There is evidence for both presynaptic (Hiura *et al.*, 1991) and post-synaptic modulation (Liu *et al.*, 1992). $GABA_B$ binding sites (Price *et al.*, 1987) as well as GABA and GAD-T immunoreactive terminals are presynaptic to primary afferent fibres and to dendrites within the trigeminal nucleus caudalis (Basbaum *et al.*, 1986). Conversely, Liu *et al.* (1992) showed that presynaptic GABA immunoreactive fibres terminate on enkephalin-immunoreactive cell bodies and dendrites within laminae I, II_o and V of cat dorsal horn. They propose that disinhibition of opioid-containing neurones by GABA may increase post-synaptic hyperpolarization of nociresponsive circuits and thereby affect pain. Further investigation will be required to determine whether the observed reduction in c-fos-LI in the TNC results from GABA modulation of presynaptic or postsynaptic activity, or both.

Drugs which alter GABA receptor activity modulate pain. In rodents, intrathecal $GABA_A$ receptor agonists, muscimol and isoguvacine increase nociceptive threshold in behavioural tests (Hammond *et al.*, 1984) whereas, bicuculline and picrotoxin lower nociceptive threshold (Roberts *et al.*, 1986). The $GABA_B$ agonist baclofen inhibits pinch and heat induced c-fibre depolarization in single unit spinal cord recordings (Dickenson *et al.*, 1985). Observed increases in tail flick latency after intrathecal (-)-baclofen are dose-dependently antagonized both by phaclofen (Aran & Hammond 1991) and CGP 35348 (Hammond & Washington, 1993).

Our data indicate that transmission of nociceptive information within the trigeminal system may be influenced via $GABA_A$ receptors. Because of the multiple modulatory sites and potential allosteric interactions at this receptor complex, the $GABA_A$ receptor may provide an important target for the development of novel drug therapies in migraine and related headache disorders.

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