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Peripheral $GABA_A$ receptor-mediated effects of sodium valproate on dural plasma protein extravasation to substance P and trigeminal stimulation

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1 The GABA transaminase inhibitor and activator of glutamic acid decarboxylase, valproic acid is being used for the treatment of migraine. Its mechanism of action is unknown. We tested the effects of sodium valproate and $GABA_A$-agonist muscimol on dural plasma protein ([^{125}I]-bovine serum albumin) extravasation evoked by either unilateral trigeminal ganglion stimulation (0.6 mA, 5 ms, 5 Hz, 5 min) or substance P (SP) administration (1 nmol kg^{-1},i.v.) in anaesthetized Sprague-Dawley rats.

2 Intraperitoneal (i.p.) injection of sodium valproate or muscimol, but not baclofen ($\leqslant$10 mg kg^{-1}, i.p.) dose-dependently reduced dural plasma protein extravasation caused either by electrical trigeminal stimulation (ED_{50}: 6.6$\pm$1.4 mg kg^{-1}, i.p., and 58$\pm$18 μg kg^{-1}, i.p. for valproate or muscimol, respectively) or by intravenous substance P administration (ED_{50}: 3.2$\pm$1.4 mg kg^{-1}, i.p. and 385$\pm$190 μg kg^{-1}, i.p. for valproate or muscimol, respectively).

3 Valproate (6.6 mg kg^{-1}, i.p.) or muscimol (58 μg kg^{-1}, i.p.) had no effect on mean arterial blood pressure or heart rate when measured for 30 min after i.p. administration.

4 The $GABA_A$-antagonist bicuculline (0.01 mg kg^{-1}, i.p.) completely reversed the effect of valproate and muscimol on plasma extravasation following electrical stimulation or substance P administration, whereas the $GABA_B$-receptor antagonist, phaclofen (0.01–1 mg kg^{-1}, i.p.) did not. Bicuculline or phaclofen, given alone, did not alter the plasma extravasation response after either electrical stimulation or SP administration.

5 Valproate decreased plasma extravasation following substance P administration in adult animals, neonatally treated with capsaicin by a bicuculline-reversible mechanism. This suggests that $GABA_A$-receptors are not found primarily on those afferent neurones or fibres which are sensitive to capsaicin treatment in neonatal rats.

6 We conclude that sodium valproate blocks plasma extravasation in the meninges through $GABA_A$-mediated postjunctional receptors probably within the meninges. The dosages required are comparable to those used clinically. Agonists and modulators at the $GABA_A$ receptor may become useful for the development of selective therapeutic agents for migraine and cluster headache.

Keywords: Dura mater; GABA receptors; migraine; muscimol; neurogenic inflammation; sodium valproate

Introduction

Neurogenic inflammation (NI) within the meninges has been proposed as an important event in the pathogenesis of migraine headaches (Moskowitz *et al.*, 1979; Markowitz *et al.*, 1987; Moskowitz, 1992). NI develops following vasoactive neuropeptide release from perivascular trigeminal nerve fibres (Moskowitz *et al.*, 1983) and is characterized by plasma protein extravasation, platelet aggregation, mast cell degranulation and endothelial activation. Such changes have been visualized within the dura mater by light and electron microscopy (Dimitriadou *et al.*, 1991; 1992). In the dura mater, NI is blocked by abortive migraine drugs, including sumatriptan (Buzzi & Moskowitz, 1990), ergot alkaloids (Saito *et al.*, 1988), indomethacin and acetylsalicylic acid (Buzzi *et al.*, 1989), chronic corticosteroids (S. Moussaoui, personal comm.) and non-steroidal anti-inflammatory agents such as ketorolac (unpublished). Their site(s) of action might be peripheral, as sumatriptan penetrates the brain only poorly (Fowler *et al.*, 1991; Dixon *et al.*, 1993) and as the blood brain barrier is incomplete within the dura mater (Markowitz *et al.*, 1987).

Valproic acid (2-propylpentanoic acid), commonly used for the treatment of epilepsy, is a GABA transaminase inhibitor (Godin *et al.*, 1969) and an activator of glutamic acid decarboxylase (Löscher, 1981). Following its administration, GABA levels increase. GABA is contained within dorsal horn neurones (Hunt *et al.*, 1981; Magoul *et al.*, 1987), and receptors for it localize to many regions including dorsal root ganglion cells and dorsal horn neurones, where at least some of them are located on capsaicin-sensitive primary afferent fibres (Price *et al.*, 1987; Persohn *et al.*, 1991). Interestingly, in the trigeminal ganglion, a major population of sensory neurones and some of their processes are immunopositive for GABA (Szabat *et al.*, 1992), while most trigeminal ganglion cells simultaneously express the mRNA for both the γ1 and γ2 $GABA_A$ receptor subunits (Kondo *et al.*, 1994). Although nerve fibres investing brain blood vessels possess the biosynthetic enzyme, glutamic acid decarboxylase (Imai *et al.*, 1991), the functional consequences of GABA on vascular permeability have not been reported to our knowledge, and the role of GABAergic mechanisms within peripheral tissues is not well understood (Ong & Kerr, 1990; Bowery, 1993).

In the past four years, several clinical studies have reported the usefulness of valproic acid for the prophylactic treatment of migraine (Hering & Kuritzky, 1992; Jensen *et al.*, 1994; Coria *et al.*, 1994), chronic daily headache (Mathew & Ali, 1991) and for the treatment of cluster headache (Hering & Kuritzky, 1989). Preliminary reports indicate that valproate

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might be effective acutely (Hering & Steiner, 1993; Kozubski & Sokolowski, 1994). Its mechanism of action is unknown. In this paper, we describe an effect of valproate on neurogenic inflammation, and suggest the importance of the GABA$_A$ receptor to this action.

Methods

Animals

Male Sprague-Dawley rats (200–250 g, Charles River Laboratories, Wilmington, MA, U.S.A.) were housed under diurnal lighting conditions and allowed food and water *ad libitum*

Electrical trigeminal ganglion stimulation and tissue dissection (Lee *et al.*, 1994).

Anaesthetized animals (pentobarbitone sodium, 60 mg kg^{-1}, i.p.) were placed in stereotaxic frame (DKI 900, David Kopf Instruments, Tujunga, CA, U.S.A.) with the incisor bar set at −1.5 mm from the horizontal line, and the calvarium was exposed by a midline incision. The right femoral vein was exposed and [^{125}I]-BSA (50 μCi kg^{-1}) was injected as a bolus. Symmetrical burr holes of 2 mm in diameter were drilled at 3.7 mm posterior to the bregma and 3.2 mm lateral to the sagittal suture for electrode placement. Bipolar electrodes (50 mm shaft, Rhodes Medical Instruments, Woodland Hills, CA, U.S.A.) were lowered into the trigeminal ganglia to a depth of 9.5 mm from the dura mater overlying the dorsal surface of the brain. The right trigeminal ganglion was stimulated for 5 min (0.6 mA, 5 ms, 5 Hz) (Pulsemaster A300 and Stimulus Isolator A365, World Precision Instruments, San Carlos, CA, U.S.A.; Oscilloscope V-134, Hitachi Densi, Tokyo, Japan). Immediately after stimulation, the animals were perfused with saline via the left cardiac ventricle for 2 min at a constant pressure of 100 mmHg in order to remove completely iodinated albumin from the lumen of blood vessels. The skull was then opened, the brain removed and the cranial cavity rinsed with saline. The dura mater was dissected bilaterally and the radioactivity determined on the two sides with a gamma-counter (Micromedic 4/600, Micromedic Systems Inc., Huntsville, AL, U.S.A.) as previously described (Markowitz *et al.*, 1987).

Substance P administration

Substance P (1 nmol kg^{-1}, i.v.) was administered 5 min after [^{125}I]-BSA injection. Animals were perfused transcardially 10 min after SP administration. The dosage of SP was chosen based on previously published data showing that 1 nmol kg^{-1} caused plasma protein extravasation in dura mater similar to that following an electrical stimulus (Markowitz *et al.*, 1987; Buzzi & Moskowitz, 1990).

Neonatal capsaicin pretreatment

Rats were treated with capsaicin (50 mg kg^{-1}, s.c. or vehicle s.c. for control animals) within the first 24 h of life as previously described (Jancso *et al.*, 1977). During capsaicin or vehicle injection, animals were kept in a tent containing an aerosol of isoprenaline (0.25 mg ml^{-1} for 10 min). After capsaicin or vehicle injection, neonates were returned to their dam. Three weeks later, they were maintained on a diurnal lighting cycle (4 per cage) and allowed access to food and water *ad libitum*. At > 8 weeks, those animals found to be insensitive to the ocular administration of topical capsaicin (wipe test, 0.1 mg ml^{-1} capsaicin) were studied.

Protocols

Except for the neuropeptide SP, all drugs were administered intraperitoneally. In preliminary experiments, we determined that sodium valproate exhibited maximum inhibitory effect on the leakage of [^{125}I]-BSA within dura mater after electrical trigeminal stimulation when given as a single dose (10 mg kg^{-1}) 30 min before stimulation; when given 60 min or 120 min before stimulation, the drug was without effect (data not shown). Therefore, we tested dosages of sodium valproate, muscimol or baclofen 30 min before electrical trigeminal stimulation or SP administration and 25 min before [^{125}I]-BSA injection.

To examine the ability of either bicuculline or phaclofen to reverse the effect of valproate or muscimol, bicuculline (0.01, 0.1 and 1 mg kg^{-1}) or phaclofen (0.01, 0.1 and 1 mg kg^{-1}) was administered 5 min before sodium valproate (6.6 mg kg^{-1}) or muscimol (58 μg kg^{-1}) treatment and 30 min before electrical stimulation.

Systemic parameters

Arterial blood pressure and heart rate were continuously recorded through a femoral arterial catheter for 30 min following the administration of valproate (6.6 mg kg^{-1}, i.p.) or muscimol (58 μg kg^{-1}, i.p.) in pentobarbitone anaesthetized rats. Data were recorded, digitized and stored by a data-acquisition and analysis system (MacLab/8-System, AD Instruments, Australia).

Drugs

^{125}I-labelled bovine serum albumin (BSA, New England Nuclear, Boston, MA, U.S.A.) was diluted in saline as were substance P (SP), sodium valproate (Sigma Chemicals Inc., St. Louis, MO. U.S.A.) and muscimol hydrobromide (Research Biochemicals Inc., Natick, MA, U.S.A.). Capsaicin (Polyscience Inc., Wilmington, PA, U.S.A.) was dissolved in saline-ethanol-Tween 80 (8:1:1). (±)-Baclofen, (+)-bicuculline and phaclofen (Research Biochemicals Inc., Natick, MA, U.S.A.) were dissolved in 0.1 N HCl, adjusted to pH 5.0 with a few drops of 0.1 N NaOH. The highest concentration of baclofen (10 mg kg^{-1}) was dissolved in 45% 2-hydroxypropyl-cyclodextrin (Research Biochemicals Inc., Natick, MA, U.S.A.).

Data analysis

Data are given as mean ± s.e.mean. [^{125}I]-BSA extravasation is expressed as the ratio of c.p.m. mg^{-1} of wet weight (stimulated side)/c.p.m. mg^{-1} of wet weight (unstimulated side). Results with substance P are expressed as a percentage of c.p.m. mg^{-1} of tissue in the substance P- versus vehicle-treated animals. ED_{50} value (the dose at which [^{125}I]-BSA extravasation was inhibited by 50%) was determied by regression analysis using Graft (Sigma, St. Louis, MO, U.S.A.). Student's t test was used for statistical analysis (unpaired t test for comparisons between control and drug-treated groups and paired t test for comparison between stimulated and unstimulated sides). Two way analysis of variance was used to determined the effects of antagonists on valproate dose/response curve. Changes in systemic parameters were analysed by one way analysis of variance. Probability values (P) of less than 0.05 were considered significant.

Results

Systemic parameters

Mean arterial blood pressure (MAP) did not change significantly when measured before and continuously for 30 min following treatment with valproate or muscimol (Figure 1). There was no change in heart rate during this time as well (data not shown).

Electrical trigeminal stimulation

Unilateral electrical trigeminal ganglion stimulation increased the leakage of [^{125}I]-BSA within the ipsilateral dura mater of rats treated with vehicle from 35±4 to 60±6 c.p.m. mg^{-1} wet weight ($P<0.001$, $n=4$). The ratio between the stimulated and unstimulated sides was 1.71±0.07 and was similar to previously reported values after saline-vehicle administration (Buzzi *et al.*, 1991).

Sodium valproate, muscimol and baclofen

Valproate (1, 3, 10, 30 and 100 mg kg^{-1}, $n=4$ in each group) dose-dependently decreased plasma protein extravasation when administered i.p. 30 min before electrical stimulation (Figure 2). Threshold dosage and ED_{50} were 3 mg kg^{-1} and 6.6±1.4 mg kg^{-1}, respectively. Complete blockade was achieved at a dosage of 100 mg kg^{-1}. Leakage of plasma protein did not differ on the unstimulated side between the treated and untreated groups.

Muscimol (0.1, 1, 10, 100, 1000 μg kg^{-1}, $n=6$ or 7 in each group) reduced the leakage in dura mater in a dose-dependent manner (Figure 3). The threshold dosage was 10 μg kg^{-1} ($P<0.05$) and the ED_{50} was 58±18 μg kg^{-1}. Increasing muscimol dosages seemed to yield a shallow dose-response curve (i.e. with a slope < 1), but the limited number of dosages precluded a more detailed analysis. Baclofen (10, 100, 1000 μg kg^{-1}, $n=6$ in each group), even at high concentrations (4 mg kg^{-1} [$n=5$]; 10 mg kg^{-1} [$n=3$]), did not affect plasma protein extravasation (Figure 3).

GABA receptor antagonists

At the doses used, bicuculline and phaclofen did not by themselves affect the plasma protein extravasation response (data not shown). Bicuculline (0.01 mg kg^{-1}, $n=4$ or 5 for each valproate-dose) inhibited the effect of increasing doses of sodium valproate shifting the dose-response curve by a factor of 40 (Figure 2). Bicuculline (0.01, 0.1 and 1 mg kg^{-1}, $n=3$ in each group) when administered 5 min before muscimol (58 μg kg^{-1}) completely reversed the effect of muscimol in a dose-dependent manner (see Figure 4).

Phaclofen (0.01, 0.1 and 1 mg kg^{-1}) did not attenuate the effect of valproate (6.6 mg kg^{-1}). Even at the highest dose (1 mg kg^{-1}) the ratio remained at 1.27±0.1 ($n=3$ in each group). The ratio was also not influenced when phaclofen was given at dosages of 0.01, 0.1 and 1 mg kg^{-1} in muscimol (58 μg kg^{-1})-treated animals ($n=3$) (Figure 4).

SP-induced plasma protein leakage

Valproate and muscimol SP increased the amount of iodinated albumin within dura mater by 161±5% (from 71±4 to 115±4 c.p.m. mg^{-1}; $P<0.001$, $n=7$ per group). Sodium valproate (1, 3, 10, 30 and 100 mg kg^{-1}, $n=6$ or 7 in each group), when administered i.p. 30 min before SP, dose-dependently decreased the plasma protein extravasation (Figure 5). The ED_{50} for the valproate response was 3.2±0.5 mg kg^{-1}.

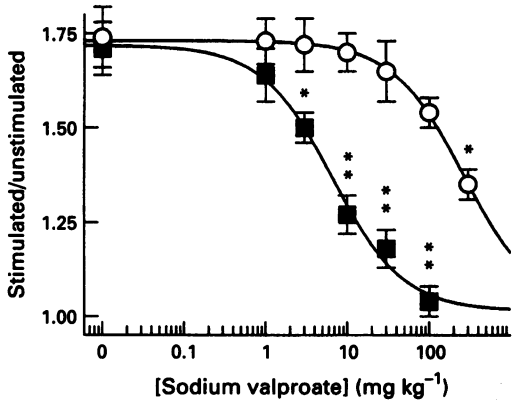

Figure 2 Sodium valproate (■) dose-dependently (≥3 mg kg^{-1}, $n=4$ in each group, $^*P<0.05$, $^{**}P<0.01$) decreased plasma protein ([^{125}I]-BSA) extravasation in rat dura matter when administered 30 min before electrical trigeminal stimulation (see Methods). The valproate effect was reversed by the GABA$_A$ receptor antagonist, bicuculline (0.01 mg kg^{-1}; ○) and shifted the curve to the right. Bicuculline was given i.p. 5 min before sodium valproate. Results are expressed as the ratio of the c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean).

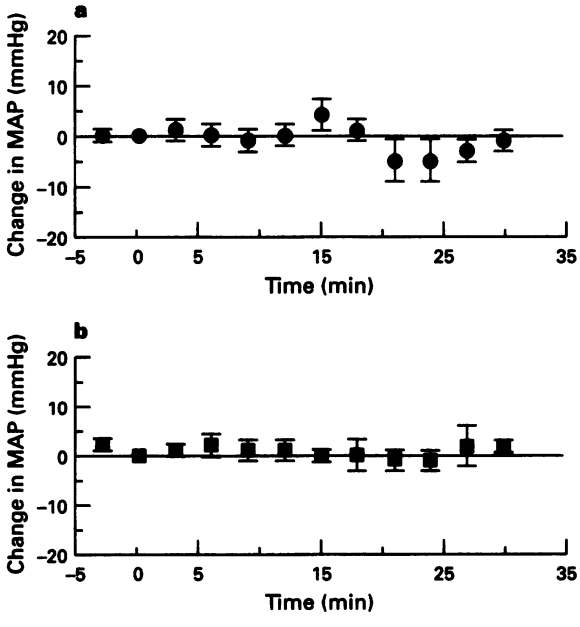

Figure 1 Mean arterial blood pressure did not change significantly after treatment with (a) muscimol (●, $n=4$) or (b) valproate (■, $n=4$) when measured before and continuously for 30 min after administration. Changes in MAP were calculated from baseline-MAP (time = 0). MAPs at baseline were 113±3 and 106±6 for muscimol and valproate, respectively.

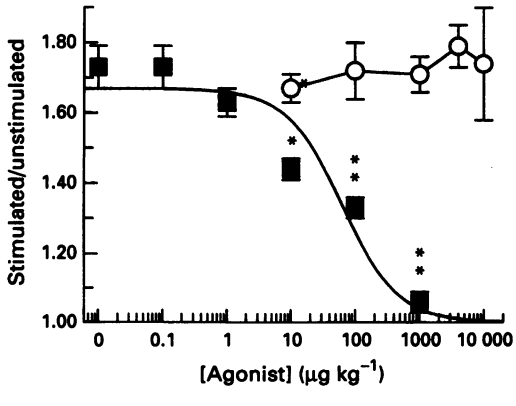

Figure 3 Muscimol (■, ≥10 μg kg^{-1} = $^*P<0.05$ or $^{**}P<0.01$) but not baclofen (○) reduced plasma protein extravasation caused by trigeminal stimulation in a dose-dependent manner. In a dosage of 1000 μg kg^{-1}, muscimol caused a complete blockade of plasma extravasation. Muscimol ($n=6$ or 7 in each group) or baclofen ($n=6$ in each group) was administered i.p. 30 min before electrical trigeminal stimulation and 25 min before [^{125}I]-BSA injection. In the presence of vehicle only, the ratio was 1.67±0.04. Results are expressed as the ratio of the c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean).

However, the highest dose of valproate (100 mg kg^{-1}) did not completely reverse the plasma leakage caused by SP administration (123±3%). Muscimol (1, 10, 100, 300, 1000 $n=6$, 3000 μg kg^{-1}, $n=3$) reduced SP-induced plasma protein extravasation in a dose-dependent manner (Figure 5). The threshold was 300 μg kg^{-1}, which reduced the plasma extravasation from 177±4% to 137±8% [ED_{50} value was 385±190 μg kg^{-1}]. The highest tested muscimol concentration was also not able to reverse completely the SP effect (120±6%)

Valproate plus bicuculline As shown in Figure 6, pretreatment with bicuculline (0.01 mg kg^{-1}, $n=3$) completely blocked the inhibitory effect of sodium valproate (10 mg kg^{-1}) on SP-induced extravasation. In the absence of receptor antagonist, valproate decreased the extravasation ratio to 130±7% ($n=9$), while the ratio was significantly higher (157±4%; $P<0.05$, $n=3$) when rats were pretreated with bicuculline. The latter ratio was not significantly different from that obtained when SP was administered alone ($P>0.05$). Phaclofen (0.1 mg kg^{-1}, $n=3$) did not reverse the effects of sodium valproate on SP-induced plasma protein extravasation.

Neonatal treatment with capsaicin In adult rats, SP caused the same amount of dural plasma protein extravasation whether capsaicin, vehicle or no treatment was given during the neonatal period. Valproate decreased the plasma extravasation following substance P administration in adult rats treated as neonates with either capsaicin or vehicle (saline-ethanol-tween 8:1:1). Bicuculline (0.01 mg kg^{-1}) reversed the effect of valproate (100 mg kg^{-1}) completely in both groups as well (see Figure 7).

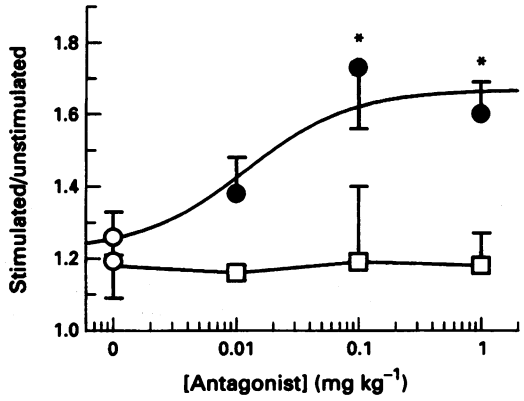

Figure 4 Bicuculline (●), but not phaclofen (□) given in dosages of 0.01, 0.1 and 1 mg kg^{-1}, i.p., reversed the effects of muscimol in a dose-dependent manner, when administered 5 min before muscimol (60 μg kg^{-1}, i.p., $n=3$ for each dose in each group). Results are expressed as the ratio of the c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean). Muscimol alone (○) decreased plasma extravasation to a ratio of 1.25±0.06. *$P<0.05$ when comparing the effect of muscimol alone with pretreatment of bicuculline at a dose of 0.1 and 1 mg kg^{-1}.

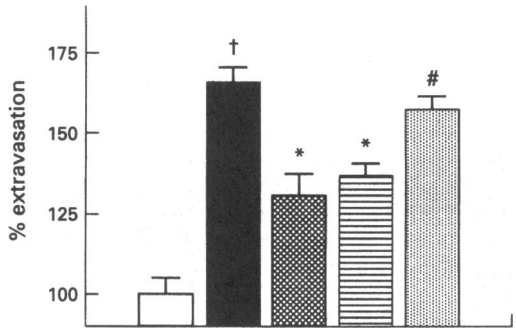

Figure 6 SP-induced (1 nmol kg^{-1}, i.v.) plasma protein extravasation (solid column) was attenuated by valproate (cross-hatched column) and the effect of valproate was reversed by bicuculline (0.01 mg kg^{-1}, $n=3$; stippled column) but not by phaclofen (0.1 mg kg^{-1}, $n=3$; horizontally-hatched bar). The receptor antagonists were given i.p. 5 min before sodium valproate treatment (10 mg kg^{-1}, $n=9$) and 35 min before SP injection. †$P<0.001$ as compared to vehicle-treated group (open column; $n=9$); *$P<0.01$ as compared to SP alone ($n=9$; solid columns); #$P<0.05$ as compared to SP plus valproate group (cross-hatched column) and no significant difference with the group given SP alone.

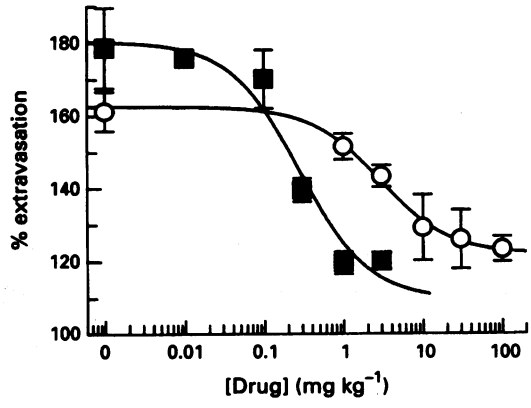

Figure 5 Sodium valproate (○) partially decreased substance P - induced (1 nmol kg^{-1}) plasma protein extravasation in a dose-dependent manner (dosages ≥3 mg kg^{-1} = $P<0.05$). Sodium valproate ($n=6$ or 7 in each group) was administered i.p. 30 min before SP injection. Similar results were obtained with muscimol (■, $n=6$ in each group), although the baseline-extravasation was slightly higher in the muscimol-group (no statistical difference). Dosages > 300 μg kg^{-1} significantly ($P<0.05$) reduced SP-induced plasma extravasation. Data are expressed as percentage of c.p.m. mg^{-1} of tissue in the substance P- (i.e. 0 mg kg^{-1} drug) versus vehicle-treated animals (i.e. 100%; $n=7$).

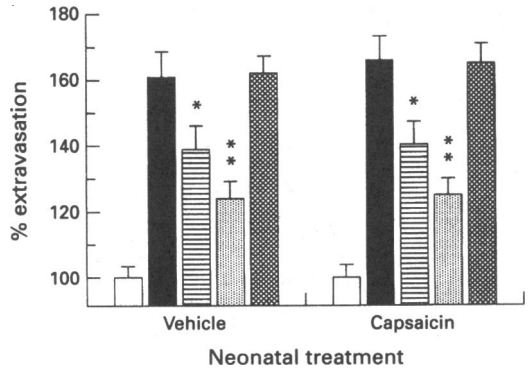

Figure 7 Substance P (1 nmol kg^{-1}) induced plasma extravasation in adult rats treated as neonates with either vehicle or capsaicin. Sodium valproate (30 mg kg^{-1} and 100 mg kg^{-1}, horizontally hatched and stippled column respectively) decreased SP-induced plasma protein extravasation in dura mater of adult rats after neonatal treatment with vehicle or capsaicin ($n=5$ in all groups, $P<0.05$). Bicuculline (0.01 mg kg^{-1}; cross-hatched columns) reversed the valproate effect when administered i.p. 5 min before sodium valproate pretreatment in both groups. *$P<0.05$ comparison of SP alone (solid column) to valproate treatment at dose of 30 mg kg^{-1}; **$P<0.01$ SP compared to valproate (100 mg kg^{-1})-treated animals.

Discussion

These results establish the importance of GABA receptors for the actions of valproic acid in a model of neurogenic and SP-induced inflammation within dura mater. Valproate ($\geqslant$3 mg kg^{-1}) blocks plasma protein extravasation caused by trigeminal ganglion stimulation or by intravenous substance P. This effect is mimicked by the GABA$_A$ receptor agonist, muscimol, a structural analogue of GABA, but not by high concentrations of the GABA$_B$ receptor agonist, baclofen (4 and 10 mg kg^{-1}, i.p.). The effects of valproate and muscimol were reversed completely by the competitive GABA$_A$ receptor antagonist, bicuculline but not by the GABA$_B$ antagonist, phaclofen. Preliminary data show that benzodiazepines (e.g. diazepam or zolpidem) or progesterone metabolites (e.g. allopregnanolone) which both activate the GABA$_A$-receptor modulatory sites, suppressed both the SP and neurogenic inflammatory response (Limmroth *et al.*, unpublished observations). Together these data support the conclusion that GABA$_A$ receptors are the major mediators of the action of valproate in the meninges.

It seems likely that the effects of valproate, which activates glutamate-decarboxylase (GAD) and inhibits GABA-transaminase (GABA-T), are mediated through peripheral GABAergic mechanisms within dural vessel wall. Fibres immunoreactive for GAD and GABA-T innervate cerebral blood vessels in several species (Hamel *et al.*, 1983; Imai *et al.*, 1991). GAD immunoreactivity is also present in the endothelium of cerebral arteries (Imai *et al.*, 1991). Fujiwara *et al.* (1975) and others (Edvinsson & Krause, 1979; Alborch *et al.*, 1984) have shown that GABA$_A$ agonists relax isolated cerebral vessels through a bicuculline-sensitive mechanism and increase regional cerebral blood flow (rCBF) (Edvinsson *et al.*, 1980). Because decreased vascular resistance, increased endothelial surface area and increased rCBF augment vascular permeability, it seems possible that valproate and GABA$_A$ receptor-mediated effects may be mediated by local meningeal events. Previous work showing hypotensive effects of muscimol (Giuliani *et al.*, 1986) are probably irrelevant to this paper because dosages for that study were twenty times higher and the drug was administered intravenously and not intraperitoneally as here. Furthermore, our own data have established that muscimol and valproate do not alter heart rate or blood pressure when monitored for 30 min after intraperitoneal administration. We have recently shown that parasympathectomy (sphenopalatine ganglionectomy) completely blocks the effects of valproate and muscimol on plasma extravasation caused by electrical trigeminal stimulation and substance P administration suggesting that GABA$_A$ receptors may be expressed by parasympathetic fibres innervating the meninges (Limmroth & Moskowitz, unpublished).

The fact that plasma protein extravasation is completely blocked by valproate and muscimol following trigeminal stimulation, whereas leakage caused by exogenous SP is only partially blocked suggests that a subpopulation of relevant GABA$_A$ receptors might be expressed on primary afferent fibres in a manner similar to that described for the 5-HT$_{1D\alpha}$ receptor subtype, a putative target of the antimigraine drug, sumatriptan (Moskowitz, 1992; Rebeck *et al.*, 1994). Heterogeneity among valproate targets is also suggested by the shallow muscimol dose-response curve on electrically-induced extravasation (Figure 2), which might indicate an action with different affinities at 2 sites in this model. Interestingly, the effect of muscimol on SP-induced extravasation was well-fitted with a one site model (Figure 3), which could correspond to its low affinity site in the electrical stimulation paradigm.

Westlund and colleagues have recently provided evidence that blockade of dorsal horn GABA$_A$-receptors significantly decreases oedema formation in a rodent model of arthritis (Sluka *et al.*, 1993). In these studies, the infusion of bicuculline into the dorsal horn decreased the severity of joint inflammation and prevented the development of heat hyperalgesia by blocking the development of dorsal root reflexes (Rees *et al.*, 1994). Results from the two models suggest that blockade of central GABA$_A$-receptors plus activation of peripheral GABA$_A$-receptors together may provide a powerful mechanism for decreasing pain and inflammation.

Our studies cannot exclude the possibility that GABA inhibitory mechanisms within the cerebral cortex also contribute to the action of valproate in migraine. There is little experimental data to address this possibility, and certainly valproate and GABA possess prominent central inhibtory effects. Valproate is used most commonly as a prophylactic agent (Hering & Kuritzy, 1989; Mathew & Ali, 1991; Hering & Kuritzy, 1992; Jensen *et al.*, 1994), although two preliminary reports describe positive results after acute administration (Hering & Steiner, 1993; Kozubski & Sokolowski, 1994). In preliminary studies, we observed that acute valproate adminstration blocked neurogenic plasma protein leakage for the subsequent 60 min whereas chronic administration inhibited the response for at least 3 h (10 mg kg^{-1}, i.p. twice a day for 10 days; n=6 per group).

We also have preliminary data showing that valproic acid (10 mg kg^{-1}, i.p.) blocks the expression of the immediate early gene c-fos (c-fos immunoreactivity) with lamina I, IIo of trigeminal nucleus caudalis in response to noxious meningeal stimulation produced by intracisternal capsaicin administration (Cutrer *et al.*, unpublished). We (Nozaki *et al.*, 1992; Cutrer *et al.*, 1995) have used the c-fos response after noxious stimuli as an indicator of functional activity within nociceptive neurones. Modifications of the c-fos response in this region point to an effect on peripheral and/or central neurones mediating nociception (Chi *et al.*, 1993; Cutrer *et al.*, 1995). Suppression of the c-fos response within lamina I, IIo of trigeminal nucleus caudalis was reported previously after injecting abortive antimigraine agents such as sumatriptan and dihydroergotamine, and also following the analgesic, morphine (Nozaki *et al.*, 1992).

Valproate is administered to human subjects in doses ranging from 400 to 2000 mg per day (Mathew & Ali, 1991; Hering & Kuritzky, 1992; Jensen *et al.*, 1994; Coria *et al.*, 1994). Tremor and drowsiness caused by central nervous system actions may appear at higher dosages. Based on the fact that valproate inhibits plasma leakage produced by exogenous substance P in intact animals as well as after destruction of unmyelinated C fibres, it can be assumed that a peripheral GABA$_A$ receptor probably mediates the valproate inhibition of plasma leakage. Thus valproate may not need to cross the blood brain barrier in order to reduce the inflammatory response within meninges. This appears true for actions of sumatriptan as well (Fowler *et al.*, 1991; Dixon *et al.*, 1993) and suggests that brain impermeant GABA agents devoid of central side effects might be useful in migraine.

While the effects of valproate are probably mediated by an increase in GABA concentration, GABA$_A$ receptors are known to possess modulatory sites for molecules other than GABA, including barbiturates, alcohol, benzodiazepines, metabolites of progesterone and other neurosteroids (MacDonald & Olsen, 1994). Some of these sites may also be important for the genesis of migraine and other headaches because changes in sex hormones and the ingestion of alcohol reportedly trigger headaches (Lance & Anthony, 1966; Kudrow, 1975) and alcohol may precipitate withdrawal headaches. Also, plasma protein extravastion in this study was measured by use of a standard protocol including pentobarbitone anaesthesia. The level of protein extravasation under baseline conditions may have been underestimated due to direct activation of GABA$_A$ receptors by high doses of barbiturates (Owen *et al.*, 1986). However, studies comparing animals undergoing pentobarbitone anaesthesia with those anaesthetized with urethane did not reveal any differences in plasma extravasation (Limmroth, unpublished). Further experiments will address this issue. Although GABA$_A$ receptors are present in most neuronal populations and several peripheral tissues, the existence of multiple subtypes for the different subunits, the various combinations thereof and the potential for modulatory site modification should increase the possibilities for developing antimigraine drugs which specifically inhibit neurogenic inflammation.

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