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$GABA_A$-receptor-mediated effects of progesterone, its ring-A-reduced metabolites and synthetic neuroactive steroids on neurogenic oedema in the rat meninges

Volker Limmroth, Won Suk Lee, & [1]Michael A. Moskowitz

Stroke and Neurovascular Regulation, Massachusetts General Hospital, Department of Neurosurgery, Department of Neurology, Harvard Medical School, Boston, MA 02114, U.S.A.

1 The effects of progesterone, its A-ring-reduced metabolites, allopregnanolone, tetrahydroxydeoxycorticosterone and the synthetic neuroactive steroid alphaxalone were evaluated in a rat model of plasma extravasation within the meninges following unilateral electrical stimulation (ES) of the trigeminal ganglion (0.6 mA, 5 ms, 5 min) or substance P administration (1 nmol kg^{-1}, i.v.).

2 When administered 55 min prior to electrical stimulation, progesterone ($\geqslant$500 μg, s.c.) dose-dependently decreased plasma extravasation within the meninges (ED_{50}: 650 μg) but not within conjunctiva and tongue. Promegestone (R5020), a non-metabolized progesterone agonist (1000 μg, i.p.) was ineffective. The administration of progesterone ($\geqslant$500 μg s.c.) 55 min prior to substance P partially suppressed plasma extravasation within the meninges (ED_{50}: 550 μg).

3 The $GABA_A$-antagonist, bicuculline (ED_{50}: 8.2 $\mu g\ kg^{-1}$, i.p) but not the $GABA_B$-antagonist, phaclofen (100 $\mu g\ kg^{-1}$, i.p.) attenuated the effects of progesterone after electrical stimulation and substance P administration.

4 The metabolites of progesterone, allopregnanolone (3α-hydroxy-5α-pregnan-20-one (THP); ED_{50}: 0.58 $\mu g\ kg^{-1}$, i.p.), tetrahydroxydeoxycorticosterone (3α,21-dihydroxy-5α-pregnan-20-one (THDOC); ED_{50}: 1.2 $\mu g\ kg^{-1}$, i.p.) as well as the synthetic steroid alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione; ED_{50}: 1.8 $\mu g\ kg^{-1}$, i.p.) suppressed plasma extravasation dose-dependently following ES, whereas the epimer of allopregnanolone, 3β-hydroxy-5α-pregnan-20-one (100 $\mu g\ kg^{-1}$, i.p.), did not. Extravasation caused by SP administration was partially suppressed by allopregnanolone ($\geqslant$1 $\mu g\ kg^{-1}$, i.p.) (ED_{50}: 2.1 $\mu g\ kg^{-1}$).

5 The effect of progesterone (1000 μg, s.c.) and allopregnanolone (100 $\mu g\ kg^{-1}$, i.p.) on neurogenic plasma extravasation was reversed by bicuculline (10 $\mu g\ kg^{-1}$, i.p.) or by a congener, bicuculline-methiodide (10 $\mu g\ kg^{-1}$, i.p.) which does not cross the blood brain barrier.

6 Progesterone (1000 μg, s.c.) had no effect on mean arterial blood pressure or heart rate when measured for 60 min after administration.

7 These results indicate that neurosteroid modulation of a $GABA_A$-receptor located outside the blood brain barrier suppresses neurogenic and substance P-induced plasma extravasation within the meninges. The findings are consistent with previously reported data showing that valproic acid and muscimol inhibit meningeal oedema by bicuculline-sensitive mechanisms. Drugs which activate $GABA_A$-receptors and its modulatory sites might be clinically effective in the treatment of migraine and cluster headache.

Keywords: $GABA_A$-receptors; neurogenic inflammation; progesterone; neurosteroids; headache; migraine

Introduction

The effects of hormones derived from cholesterol on the central nervous system have been recognized for over 50 years (Selye, 1941). At the intracellular level, progesterone and its metabolites (Rupprecht *et al.*, 1993) activate various genetic programmes by binding to receptors which can modulate, for example reproductive behaviour (McEwen, 1991). By contrast, progesterone via ring metabolites, modulates allosterically the $GABA_A$-receptor complex to enhance chloride ion conductance (Majewska *et al.*, 1986), an action which may modulate pain, anxiety and sleep (Crawley *et al.*, 1986; Kavaliers & Wiebe, 1987; Mendelson *et al.*, 1987). Receptors for $GABA_A$ are among the most abundant and have been identified within brain and peripheral tissues (Hobbiger, 1958).

We previously reported that the GABAergic agent, valproic acid blocks neurogenic inflammation (NI) within the meninges (Lee *et al.*, 1995). NI is produced by the release of vasoactive peptides from primary afferent terminals and has been proposed to play an important part in the pathophysiology of migraine (Moskowitz *et al.*, 1979; Markowitz *et al.*, 1987; Moskowitz, 1991). In animals, activation of the trigeminovascular system following antidromic stimulation of the trigeminal ganglion leads to NI in the meninges with its typical characteristics: vasodilatation, plasma protein extravasation, endothelial activation, platelet aggregation and mast cell degranulation (Moskowitz *et al.*, 1989; Dimitriadou *et al.*, 1991). Abortive migraine drugs such as sumatriptan, ergotamine or non-steroidal antiinflammatory drugs (NSAID) such as ketorolac, are potent suppressors of NI (Buzzi *et al.*, 1989; Buzzi & Moskowitz, 1990). Since we provided evidence that $GABA_A$-receptors play a crucial role in the prevention of neurogenic and substance P-induced inflammation (Lee *et al.*, 1995), the modulation of $GABA_A$-receptors by metabolites of progesterone might constitute an important mechanism by which hormones modify headache.

In this study, we evaluated the effects of progesterone, its naturally occurring metabolites (neurosteroids) allopregnanolone (3α-hydroxy-5α-pregnan-20-one, THP), its epimer 3β-hydroxy-5α-pregnan-20-one, tetrahydroxydeoxycorticosterone

[1]Author for correspondence at: Massachusetts General Hospital, CNY-6403, 149, 13th Street, Charlestown, MA 02129, U.S.A..

(3α, 21-dihydroxy-5α-pregnan-20-one, THDOC) as well as the synthetic anaesthetic steroid, alphaxalone (3α-hydroxy-5α-pregnane-11,20-dione).

Methods

Animals

Female (160–180 g) and 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*. Six hours before the experiments, food but not water was restricted.

Electrical trigeminal ganglion stimulation (ES)

Ovariectomized female rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). Urethane (1.0–1.4 g kg^{-1}, i.p.) was used for all other experiments. Animals were placed in a stereotaxic frame (DKI 900, David Kopf Instruments, Tujunga, CA, U.S.A.). The right femoral vein was exposed and [^{125}I]-BSA (50 μCi kg^{-1}) was injected as a bolus. With the incisor bar set at −1.5 mm from the horizontal line, the calvarium was exposed by a midline incision. Symmetrical burr holes (2 mm in diameter) were drilled at 3.7 mm posterior to the bregma and 3.2 mm lateral to the sagittal suture. Bipolar electrodes (50 mm shaft, Rodes 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). In order to remove iodinated albumin completely from the lumen of blood vessels, animals were perfused via the left cardiac ventricle for 2 min with saline at a constant pressure of 100 mmHg. After opening the skull, the brain was removed. The dura mater was rinsed and dissected bilaterally. Radioactivity was 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).

Extracranial tissue dissection

In randomly selected animals, extracranial tissues (tongue, conjunctiva) were also studied. Conjunctiva and tongue were dissected bilaterally following perfusion and prior to dissection of the dura. Radioactivity was determined as mentioned for the dura mater.

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 (1 nmol kg^{-1}) was chosen based on previously published data showing plasma protein extravasation in dura mater was similar to that following a neurogenic stimulus (Markowitz *et al.*, 1987; Buzzi & Moskowitz *et al.*, 1990).

Ovariectomy

Female rats (160–180 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). After an incision (1 cm) in both flanks the underlying muscles were separated and ovary and fallopian tubes exposed. Both tubes were ligated with 4.0 surgical suture and the ovaries removed. Muscles and skin were adapted and closed with 4.0 surgical suture. The animals recovered from anaesthesia under a warming lamp and returned to the animal facility after 4 h. Female rats were ovariectomized 10 days before undergoing the experimental procedures.

Protocols

A *Progesterone* (1) To evaluate the effects of hormones on NI, progesterone (250, 500, 1000 μg, s.c.) or vehicle was given s.c. 55 min before ES. (2a) In order to investigate the involvement of a specific GABA-receptor subtype, the $GABA_A$-receptor antagonist, bicuculline (10 μg kg^{-1}, i.p.) or $GABA_B$-antagonist, phaclofen (100 μg kg^{-1}, i.p.) was given 25 min following the administration of progesterone (250, 500, 1000 μg, s.c.) and 30 min before ES. (2b) To evaluate the effect of bicuculline in progesterone-treated animals, bicuculline (1, 10, 100 μg kg^{-1}, i.p.) was injected 25 min after progesterone (ED_{50}-dose, s.c.) and 30 min before ES. (3) To examine the effects of a non-metabolized progesterone receptor agonist which is without allosteric modulatory action at the $GABA_A$-receptor site, promegestone (R5020, 1000 μg, i.p), a progesterone receptor agonist, was given i.p. 30 min before ES in an additional experiment.

B *Progesterone metabolites* (1) Allopregnanolone (0.1, 1, 10, 100 μg kg^{-1}, i.p.) was given 30 min before ES. THDOC (0.1, 1, 10, 100 μg kg^{-1}, i.p.), or the synthetic neurosteroid alphaxalone (0.1, 1, 10, 100, 1000 μg kg^{-1}, i.p.), was also given 30 min before ES. In a single experiment 6 male rats received THDOC (100 μg kg^{-1}, i.p.) 5 and 2 min before electrical stimulation intravenously in order to evaluate an early onset of drug action. (2) One group of animals treated with allopregnanolone was pretreated with bicuculline (10 μg kg^{-1}, i.p.) 5 min before administration of allopregnanolone (10, 100, 1000 μg kg^{-1}, i.p.). (3) The epimer of allopregnanolone, 3β-hydroxy-5α-pregnane-20-one (100 μg kg^{-1}, i.p.) was given 30 min before ES.

C *Progesterone, allopregnanolone and the blood brain barrier (BBB)* To determine the relative importance of central and peripheral central nervous system receptor sites, the hydrophilic compound (−)-bicuculline methiodide (10 μg kg^{-1}), which does not cross the BBB (Pong & Graham, 1972), was given 25 min after the administration of progesterone (1000 μg, s.c.) and 30 min before ES or 5 min before allopregnanolone (100 μg kg^{-1}) and 35 min prior to ES.

D *Progesterone, its metabolites and substance P induced extravasation* (1) Substance P was administered 60 min after progesterone (250, 500, 1000 μg, s.c.) and 5 min following [^{125}I]-BSA injection. Bicuculline (10 μg kg^{-1}) was given 30 min after progesterone administration and 30 min before SP-administration in order to confirm the involvement of the $GABA_A$-receptor. (2) The effects of allopregnanolone (0.1, 1, 10, 100 μg kg^{-1}, i.p.) on substance P-induced extravasation were evaluated as well when given 25 min before [^{125}I]-BSA injection and 30 min before SP administration.

Six experiments have been performed with ovariectomized female rats: progesterone-dose-response following electrical stimulation, substance P administration, progesterone plus bicuculline and phaclofen, allopregnanolone-dose-response, allopregnanolone plus bicuculline. To reveal possible gender differences progesterone-dose-response and the allopregnaolone-dose-response were repeated in male rats. After obtaining similar results in both groups subsequent experiments were performed with male rats only (**A**(2b), **A**(3), **B**(1), **B**(2) and **B**(3), C, **D**(2)).

Systemic parameters

Arterial blood pressure and heart rate were continuously recorded through a femoral arterial catheter for 60 min following the administration of progesterone (1000 μg, s.c.) in pentobarbitone and urethane-anaesthetized rats. Data were recorded, digitized and stored by a data-acquisition and analysis system (MacLab/8-System, AD Instruments, Australia).

Drugs

[^{125}I]-bovine serum albumin (BSA; New England Nuclear, Boston, MA, U.S.A.) was diluted in saline, substance P (SP), (Sigma Chemicals Inc., St. Louis, MO, U.S.A.) was dissolved in saline. Progesterone (Steraloids Inc. Wilton, NH, U.S.A.) was dissolved in sesame oil (Sigma Chemicals Inc., St. Louis, MO, U.S.A.). Promegestone (R5020, Dupont, Boston, MA, U.S.A.) was dissolved in 45% 2-hydroxypropyl-cyclodextrin (Research Biochemicals Inc., Natick, MA, U.S.A.). Allopregnanolone (3α-hydroxy-5α-pregnan-20-one), tetrahydrodeoxycorticosterone (THDOC or 3α, 21-dihydroxy-5α-pregnan-20-one) and alphaxalone were dissolved in 45% 2-hydroxypropyl-cyclodextrin (Research Biochemicals Inc., Natick, MA, U.S.A.) and further diluted in saline. The stereo epimer 3β-hydroxy-5α-pregnan-20-one (Sigma Chemicals Inc., St. Louis, MO, U.S.A.) was dissolved in 45% 2-hydroxypropyl-cyclodextrin. (+)-Bicuculline (Research Biochemicals Inc., Natick, MA, U.S.A.) was dissolved in 0.1 N HCl, adjusted to pH 5.0 with a few drops of 0.1 N NaOH. (−)-Bicuculline-methiodide (Sigma Chemicals Inc., St. Louis, MO, U.S.A.) was dissolved in saline.

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} wet weight (stimulated side/unstimulated side). In the substance P experiments, results are expressed as c.p.m. mg^{-1} of tissue (substance P)/(vehicle-treated animals). ED_{50} values were determined by regression analysis. ANOVA followed by Bonferoni/Dunnett's-test for *posthoc* analysis was used to evaluate statistical significance. Systemic parameters were analysed by one way ANOVA. Probability values (P) of less than 0.05 were considered significant. Charts were generated using a curve fitting programme (grafit).

Results

Systemic parameters

Mean arterial blood pressure (MAP) did not change significantly following treatment with progesterone, when measured continuously for 60 min after administration ($n=4$). Prior to treatment the MAP was 91 ± 6 mmHg. Fifteen, 30, 45 and 60 min after treatment the changes in MAP were −4 ± 3 mmHg, −2 ± 4 mmHg, −3 ± 2 mmHg and −1 ± 2 mmHg, respectively. There was no change in heart rate during this time as well (data not shown). Also in urethane-anaesthetized rats, MAP did not change significantly after administration of progesterone (1000 μg). Before treatment, MAP was 106 ± 4 mmHg and +1 ± 5, +9 ± 7, +8 ± 10 and +6 ± 10 after 15, 30, 45 and 60 min, respectively. There was also no change in heart rate (data not shown).

Electrical stimulation

Progesterone and promegestone Progesterone dose-dependently suppressed plasma-extravasation in female ovariectomized rats (see Figure 1) from 1.68 ± 0.04, (vehicle) to 1.11 ± 0.04 at 1000 μg ($P<0.01$). The lowest dose tested achieving a statistically significant reduction of plasma-extravasation and ED_{50}s were 500 μg and 650 μg, respectively. Progesterone had the same effect when given to male Sprague Dawley rats 30 min before ES: vehicle, 1.72 ± 0.10 ($n=4$); 250 μg, 1.52 ± 0.03 ($n=4$); 500 μg, 1.42 ± 0.07, ($n=5$, $P<0.05$); 1000 μg, 1.11 ± 0.05 ($n=4$, $P<0.01$). Progesterone (1000 μg, $n=4$) suppressed plasma extravasation with a similar potency when given to rats under urethane anaesthesia: 1.09 ± 0.04. The extravasation on the untreated side did not differ significantly between groups. Promegestone (1000 μg i.p., 30 min before ES) was inactive (Figure 1).

As previously reported (Markowitz *et al.*, 1987), plasma extravasation in extra-cranial tissues is significantly higher than in the dura mater after electrical stimulation (c.p.m. mg^{-1} wet weight). Progesterone (500 μg/rat, $n=6$ per group) did not decrease the ratio in extracranial tissues: tongue, 2.18 ± 0.24 versus 2.18 ± 0.23 for vehicle and progesterone, respectively; conjunctiva, 3.39 ± 0.38 versus 3.52 ± 0.34 for vehicle and progesterone, respectively.

Progesterone and GABA-antagonists The effect of progesterone was blocked by the $GABA_A$-antagonist, bicuculline (10 μg kg^{-1}) but not by the $GABA_B$-antagonist phaclofen (100 μg kg^{-1}) (see Figure 1). When administered at the ED_{50} dose (650 μg), bicuculline dose-dependently reversed the effects of progesterone (see Figure 2). The threshold and ED_{50} were 2.2 μg kg^{-1} and 8.2 μg kg^{-1}, respectively.

Neurosteroids Allopregnanolone (3α-hydroxy-5α-pregnan-20-one, THP), decreased plasma extravasation in male rats (see Figure 3). The threshold, ED_{50} and maximum inhibition were 0.1 μg kg^{-1}, 0.58 μg kg^{-1} and 100 μg kg^{-1}, respectively. The epimer of allopregnanolone, 3β-hydroxy-5α-pregnan-20-one (100 μg kg^{-1}) was inactive. THDOC decreased the ratio in a dose-dependent manner as well. The threshold, ED_{50} and maximum inhibition was 1 μg kg^{-1}, 1.12 μg^{-1} and 100 μg kg^{-1}, respectively. Alphaxalone showed comparable potency. The threshold, ED_{50} and maximum inhibition were 1 μg kg^{-1}, 1.8 μg kg^{-1} and 100 μg kg^{-1}, respectively (see Figure 3).

The onset of activity was rapid. When given 2 min before ES, THDOC completely suppressed plasma extravasation (1.69 ± 0.06, vehicle versus 1.07 ± 0.05, $n=3$, $P<0.01$).

Neurosteroids and $GABA_A$-antagonists Bicuculline (10 μg kg^{-1}, i.p.) reversed the inhibition of plasma extravasation (Figure 4), when administered 5 min before allopregnanolone injection (100 μg kg^{-1}).

Blood brain barrier (−)-Bicuculline-methiodide (10 μg kg^{-1}, i.p.) blocked the effects of both progesterone (1000 μg, s.c.)

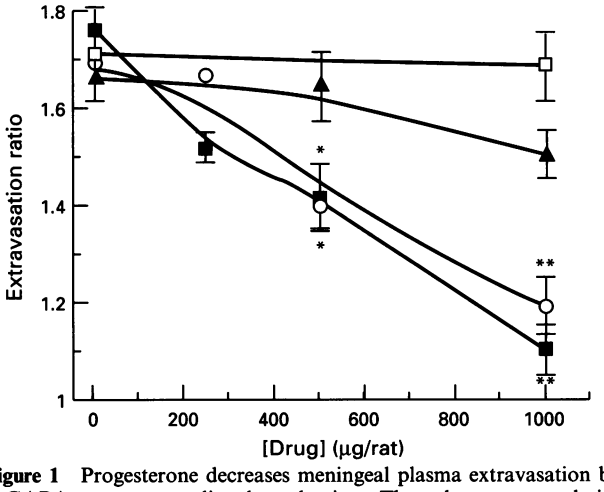

Figure 1 Progesterone decreases meningeal plasma extravasation by a $GABA_A$-receptor-mediated mechanism. The subcutaneous administration of progesterone (■, $n=4$, dosages ≥ 500 μg, $P<0.05$) 55 min before electrical stimulation, dose-dependently decreased plasma extravasation in ovariectomized female rats. Promegestone (□, $n=5$) was inactive. Progesterone's effect was suppressed by bicuculline (▲, 10 μg kg^{-1}, i.p., $n=3$ in each group, $P<0.05$ comparing progesterone alone vs. pretreatment with bicuculline), but not by phaclofen (○, 100 μg kg^{-1}, $n=5$ in each group) when given 25 min after progesterone administration and 30 min before electrical stimulation. Phaclofen plus progesterone (250 μg) did not differ from progesterone alone. Results are expressed as c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean ± s.e.mean). *$P<0.05$ or **$P<0.01$ as compared to progesterone alone or progesterone plus phaclofen versus vehicle (=0).

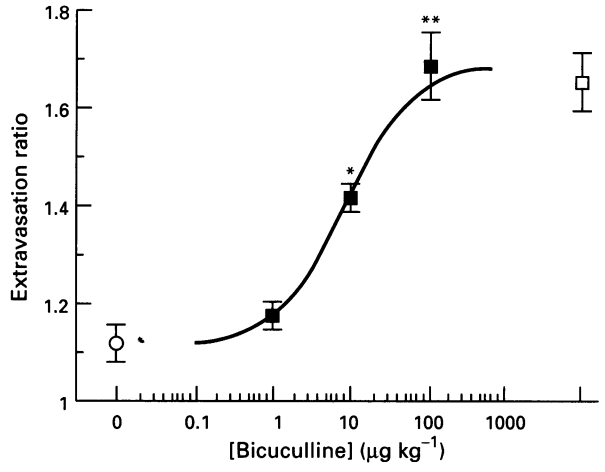

Figure 2 Bicuculline blocked the effect of progesterone. Intraperitoneal administration of bicuculline plus progesterone (■, $n=4$ per group) dose-dependently suppressed the effects of progesterone alone (=0, ○, ED_{50} dose, s.c.) when given 35 min before ES. Results are expressed as ratio of c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean). *$P<0.05$ or **$P<0.01$ as compared progesterone alone versus progesterone plus bicuculline or versus vehicle (□).

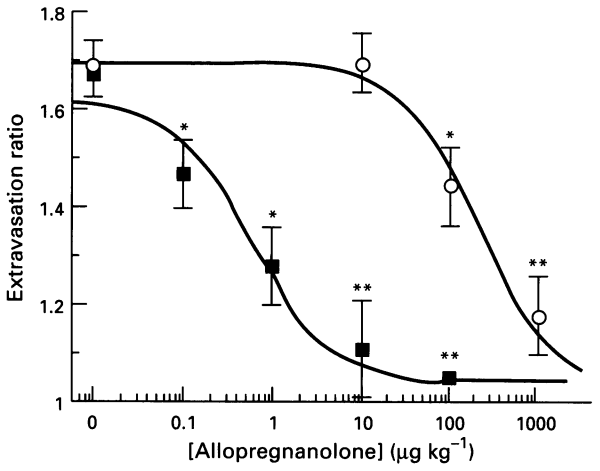

Figure 4 $GABA_A$-receptors mediate the effects of allopregnanolone. Bicuculline (10 μg kg^{-1}, i.p.) blocked the effects of allopregnanolone (■, i.p., $n=5$) and shifted the dose-response curve by a factor of 350 to the right when given 5 min before allopregnanolone to male rats. Bicuculline alone did not affect plasma extravasation. Results are expressed as ratio of c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean). *$P<0.05$ or **$P<0.01$ as compared allopregnanolone versus vehicle (□=0) or allopregnanolone plus bicuculline versus vehicle (○=0).

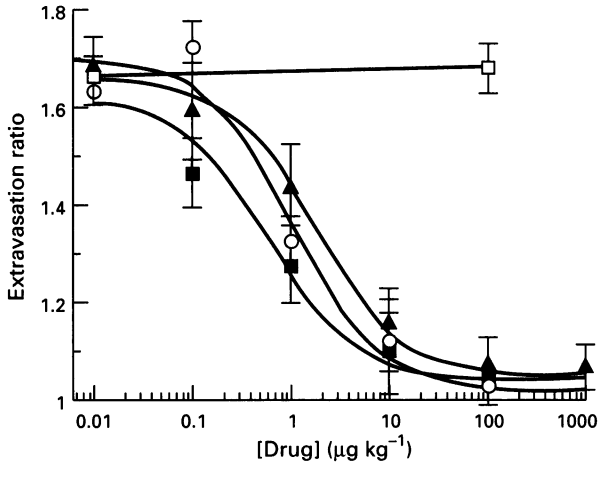

Figure 3 Neurosteroids suppress meningeal plasma protein extravasation. Allopregnanolone (■, i.p. $n=5$ per group), allotetrahydrodeoxycorticosterone (○, i.p., $n=5$ per group) and alphaxalone (▲, i.p., $n=5$ per group) dose-dependently decreased plasma-protein extravasation ($P<0.05$). The ED_{50} values were 0.58 μg kg^{-1}, 1.2 μg kg^{-1} and 1.8 μg kg^{-1}, respectively. 3β-hydroxy-5α-pregnan-20-one (□, 100 μg kg^{-1}, i.p., $n=5$), the epimer of allopregnanolone was inactive when administered 30 min before ES. Results are expressed as ratio of c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean±s.e.mean).

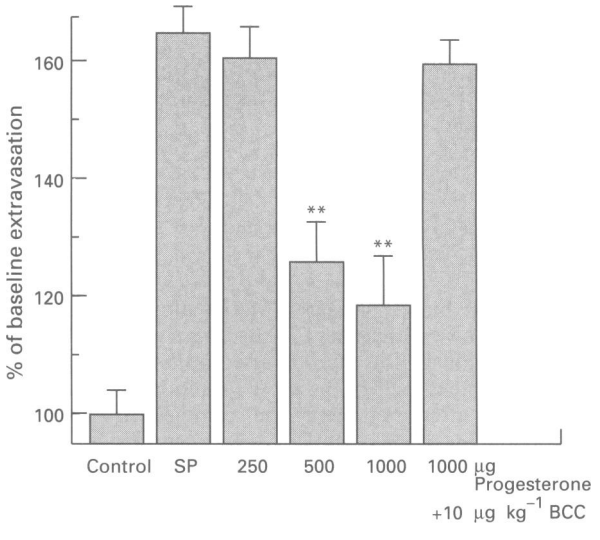

Figure 5 Progesterone suppresses substance P (SP)-induced plasma protein extravasation. SP (1 nmol kg^{-1}, i.v., $n=5$) increased plasma extravasation within the meninges. Progesterone (250, 500, 1000 μg, s.c., $n=5$ per group) when administered 55 min before SP-injection, decreased SP-induced plasma protein extravasation dose-dependently. This effect was reversed when bicuculline (10 μg kg^{-1}, i.p., $n=4$) was given 25 min after progesterone (1000 μg, s.c.) administration and 30 min before SP administration (BCC). Data are expressed as percentage of c.p.m. mg^{-1} of tissue in the SP versus vehicle or SP plus drug-treated animals. **$P<0.01$ as compared to SP alone.

(1.12±0.07, progesterone alone versus 1.62±0.05 after progesterone plus bicuculline, $n=5$) and allopregnanolone (100 μg kg^{-1}, i.p., $n=5$, from 1.05±0.07, allopregnanolone versus 1.64±0.06, bicuculline-methiodide plus allopregnanolone, $P<0.01$).

SP-induced plasma protein extravasation

SP increased the leakage of iodinated albumin within dura mater of ovariectomized female rats as compared to vehicle-treated animals. Progesterone partially blocked this leakage in a dose-dependent manner (threshold, 500 μg; ED_{50}, 550 μg). This effect was reversed by bicuculline (see Figure 5). Allopregnanolone also decreased SP-induced extravasation (threshold, 1.0 μg kg^{-1}; ED_{50}, 2.9 μg kg^{-1}) (see Figure 6). Unlike after electrical stimulation, complete blockade was not achieved at the highest doses (116% of baseline extravasation at a dose of 100 μg kg^{-1}).

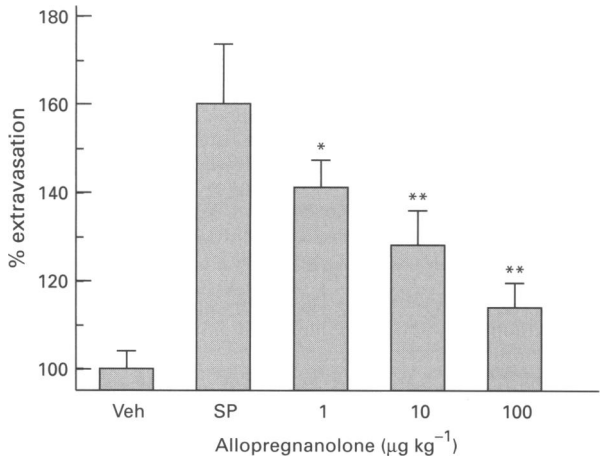

Figure 6 Allopregnanolone suppresses substance P (SP)-induced plasma protein extravasation. SP (1 nmol kg^{-1}, i.v., $n=5$) increased plasma extravasation within the meninges. Allopregnanolone (1, 10, 100 $\mu g\,kg^{-1}$, i.p., $n=5$ per group) when administered 30 min before SP-injection decreased SP-induced plasma protein extravasation dose-dependently. Data are expressed as percentage of c.p.m. mg^{-1} of tissue in the substance P versus vehicle or SP plus drug-treated animals. $*P<0.05$ or $**P<0.01$ as compared to SP alone.

Discussion

Progesterone and its active A-ring metabolites block neurogenic and SP-induced plasma extravasation by modulation of a $GABA_A$-receptor located within dura mater (but not within facial tissues) in pentobarbitone or urethane anaesthetized rats. Male rats and ovariectomized females showed a similar response. Hence, male rats were used for subsequent experiments. The findings are consistent with a prior report showing that muscimol, a $GABA_A$-agonist and valproic acid block oedema formation in the meninges (Lee *et al.*, 1995). The data do not support a genomically-mediated neurosteroid mechanism because promegestone, which activates the progesterone-receptor but not the GABA allosteric modulatory site, was inactive in this model, and because progesterone metabolites block plasma extravasation after only a 2 min pretreatment.

$GABA_A$-receptors possess modulatory binding sites for benzodiazepines, picrotoxin, and barbiturates in addition to neurosteroid binding sites. Benzodiazepines blocked meningeal oedema in preliminary studies, whereas pentobarbitone does not appear to affect plasma extravasation (Limmroth & Moskowitz, unpublished observation). It remains to be determined whether the barbiturate results reflect a particular $GABA_A$-receptor subtype or low binding affinity.

Progesterone, allopregnanolone as well as muscimol and valproate (Lee *et al.*, unpublished observations) completely blocked neurogenic plasma extravasation and partially suppressed SP-induced plasma protein leakage. Since SP mediates neurogenic oedema following trigeminal stimulation, it seems unlikely that neurosteroids inhibit leakage by blocking neuropeptide release from trigeminal primary afferent fibres, as demonstrated for other antimigraine drugs such as sumatriptan or dihydroergotamine (Buzzi *et al.*, 1991). Experiments in adult rats treated as neonates with capsaicin support this conclusion (Lee *et al.*, 1995). However, we have no explanation as to why SP-induced plasma leakage is only partially blocked by neurosteroids. (Complete blockade was reported with NK_1 antagonists Lee *et al.*, 1994). Additional experiments will be needed to address this point. Consistent with a $GABA_A$-receptor site distinct from the $5\text{-}HT_{1D}$-receptor, preliminary studies indicate that inhibition of oedema formation by drugs acting at GABA receptors is completely blocked by transection of meningeal parasympathetic axons (Limmroth & Moskowitz, unpublished observations). For these reasons we have tentatively assigned the $GABA_A$-receptor complex to a locus outside the BBB and within the meninges.

Significance of neurosteroids

Excluding the inactive epimer of allopregnanolone, all tested neuro-steroids were potent in the high ng–low μg. The threshold and ED_{50}-dosages suggest the following order of decreasing potency: allopregnanolone > THDOC > alphaxalone. Neurosteroid modulation of [^{35}H]-muscimol binding to rat cortex (Goodnough & Hawkinson, 1995) showed a similar rank order of potency as did neurosteroid effects on GABA-evoked currents (Lambert *et al.*, 1991). Thresholds were similar to those which decreased pain sensitivity (Freye & Duncan, 1994).

The possibility that endogenous neurosteroids modulate meningeal inflammation and affect the clinical course of migraine remains an open question. Plasma and brain allopregnanolone levels correlate with progesterone levels in rats as well as in human subjects (Purdy *et al.*, 1990) with high concentrations during the menstrual cycle, pregnancy or stress (Paul & Purdy, 1992). Therefore, it has been suggested that neuro-active steroids via central $GABA_A$-receptors mediate analgesic and/or anxiolytic effects in pregnancy or stress (Purdy *et al.*, 1991; Majewska, 1992). Since pregnancy, menopause and stress affect the clinical presentation of migraine, hormones and their metabolites in particular may be important in endogenous headache modulation.

Clinical trials and hormone treatment

First attempts to treat headache with steroid hormones date back to the early 1930s (Blakie & Hossack, 1932). Progesterone or closely related steroids were effective when administered in acute as well as in prophylactic treatment (Gray, 1941; Singh *et al.*, 1947; Lundberg, 1968; Bradley *et al.*, 1968). Twelve clinical trials in more than 500 patients confirmed these findings. Negative results were found in only one study of 6 patients (Sommerville, 1971). Metabolites of progesterone have not been tested clinically, to our knowledge. More recently, oestrogen-preparations were tested in the prophylactic treatment of menstrual migraine with mixed results (de Lignieres *et al.*, 1986; Pfaffenrath, 1993). Central nervous system side effects of progesterone and oestrogen might be circumvented by developing neurosteroids which do not cross the BBB. However, the rapid onset of neurosteroid action might also be beneficial where sedation or anxiolytic effects are desired together with pain relief.

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