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European Journal of Pharmacology 359 (1998) 41–48

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Neuroactive steroids exacerbate γ-hydroxybutyric acid-induced absence seizures in rats

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Received 18 May 1998; revised 17 August 1998; accepted 21 August 1998

Abstract

Certain naturally-occurring steroid metabolites and their synthetic analogs (neuroactive steroids) allosterically enhance $GABA_A$ receptor function and possess potent anticonvulsant properties. In the present study, the effect of two synthetic neuroactive steroids, alphaxalone (5α-pregnane 3α-ol-11, 20-dione) and tetrahydrodeoxycorticosterone was studied in a rat model of generalized absence seizures induced by γ-hydroxybutyric acid. Both steroids dose-dependently exacerbated γ-hydroxybutyric acid-induced absence seizures upon systemic administration and after focal administration into thalamic ventrobasal nucleus. However, alphaxalone and tetrahydrodeoxycorticosterone failed to potentiate γ-hydroxybutyric acid-induced absence seizures when injected into thalamic reticular nucleus. In all the doses of steroids tested in thalamic reticular nucleus, the duration of γ-hydroxybutyric acid-seizures was neither prolonged nor shortened. This nonresponsiveness of thalamic reticular nucleus to neuroactive steroids in modulating absence seizures may have arisen due to the molecular heterogeneity of $GABA_A$ receptor subunits within the thalamus.

Keywords: Absence seizure; Neuroactive steroid; Thalamus; γ-Hydroxybutyric acid; $GABA_A$ receptor

1. Introduction

Generalized absence seizures occur as highly synchronized thalamocortical oscillations (3 Hz) which evolve most readily from thalamic relay nuclei (e.g., ventrobasal nucleus) and the neocortex (Gloor et al., 1990). This oscillatory behavior in the thalamocortical network is regulated by thalamic reticular nucleus (McCormick, 1992), and it is believed that γ-aminobutyric acid (GABA)-ergic inhibition within the thalamus plays an important role in the generation and/or regulation of absence seizures. For example, focal administration of bicuculline (a $GABA_A$ receptor antagonist) into thalamic reticular nucleus has been shown to increase 3 Hz oscillation in thalamic slices (Huguenard and Prince, 1994), while in whole animal studies absence seizures are inhibited by direct injection of muscimol (a $GABA_A$ receptor agonist) into thalamic reticular nucleus. In contrast, focal injection of muscimol in thalamic relay nuclei exacerbates absence seizures (Liu et al., 1991). A similar exacerbation of absence seizures is observed after systemic administration of $GABA_A$ receptor agonists (King, 1979; Vergnes et al., 1984; Smith and Bierkamper, 1990). These findings together suggest that while a generalized increase in $GABA_A$ergic inhibition in the brain (after systemic injection of GABA-mimetics) tends to worsen absence seizures, a more selective increase in $GABA_A$ergic inhibition in thalamic reticular nucleus may attenuate absence seizures.

Low levels of 3α-hydroxy metabolites of progesterone and deoxycorticosterone (neurosteroids) are found in the brain (Paul and Purdy, 1992). These steroid metabolites are known to alter brain excitability, and cause sedation and anesthesia by allosterically enhancing the function of the $GABA_A$ receptors (Majewska et al., 1986; Turner et al., 1988; Morrow et al., 1990). There is some clinical evidence that these naturally-occurring steroid metabolites may possess anticonvulsant activities. For example, in women with partial focal epilepsy, the frequency of seizures during the luteal phase is usually low when the plasma progesterone levels increase (Mellon, 1994). The metabolism of progesterone to allopregnanolone (a 3α-hy-

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0014-2999/98/$ - see front matter
PII: S0014-2999(98)00629-3

droxy metabolite) in the brain has been suspected to be the underlying cause of this reduction in seizures (Mellon, 1994). Also, certain neurosteroids exhibit potent anticonvulsant activity in a variety of experimental seizure paradigms. For example, neurosteroids not only suppress seizures induced by pentylenetetrazole, bicuculline and picrotoxin ($GABA_A$ receptor antagonists), they antagonize *N*-methyl-D-aspartic acid (NMDA)-induced seizures (Belelli et al., 1990; Kokate et al., 1994; Budziszewska et al., 1998), and are also effective anticonvulsants in experimental limbic and status epilepticus seizure models (Kokate et al., 1996; Leskiewicz et al., 1997). Interestingly, neurosteroids have been found ineffective in suppressing maximal electroshock and strychnine-induced convulsions (Belelli et al., 1990). In the present study, we investigated the effect of two synthetic 3α-hydroxy neuroactive steroids, alphaxalone (5α-pregnane 3α-ol-11, 20-dione) and tetrahydrodeoxycorticosterone (5α-pregnane-3α, 21-diol-20-one, a deoxycorticosterone metabolite) in a rat model of absence seizures induced by γ-hydroxybutyric acid. Steroids were administered either systemically or directly into thalamic ventrobasal nucleus (a prominent relay nucleus) or into the neighbouring thalamic reticular nucleus. γ-Hydroxybutyric acid is a naturally-occurring metabolite of GABA which induces generalized absence-like seizures in rats and in a number of other animal species (Doherty et al., 1978; Snead, 1988).

2. Materials and methods

2.1. Drugs

Alphaxalone, tetrahydrodeoxycorticosterone and γ-butyrolactone were obtained from Sigma (St. Louis, MO, USA). All other reagents were obtained from commercial sources and were of the highest-available purity. Stock solutions of steroids were made in dimethyl sulfoxide and diluted to final concentration with saline (pH 7.1) prior to systemic or intrathalamic administration. The final concentration of dimethyl sulfoxide was 0.1%, a concentration known not to interfere with $GABA_A$ receptor function (Nakahiro et al., 1992).

2.2. Surgery and EEG recording of spike–wave discharges induced by γ-hydroxybutyric acid

Adult male Sprague–Dawley rats (200–300 g) were used in all experiments. Animals were maintained on a 12 h light/dark cycle and given free access to food and water. Monopolar electroencephalographic (EEG) recording electrodes were surgically implanted on the surface of frontoparietal cortex under halothane anesthesia. The tips of these electrodes were aimed at frontal and parietal cortices bilaterally. Seven days after surgery, EEG recordings were made continuously with the animals freely moving in a heated shielded Plexiglas container. EEG recordings were made 30 min prior to, and for 3–6 h following the induction of γ-hydroxybutyric acid-seizures.

2.3. The γ-hydroxybutyric acid model of absence seizures

This model of absence seizures has been extensively studied in our laboratory and utilizes γ-butyrolactone, the prodrug of γ-hydroxybutyric acid (Snead, 1988). γ-Butyrolactone is converted to γ-hydroxybutyric acid by a circulating lactonase (Roth and Giarman, 1969) and produces a more rapid onset and predictable time-course of spike wave discharges than γ-hydroxybutyric acid itself. The first EEG change observed after intraperitoneal (i.p.) γ-butyrolactone at the dose of 100 mg/kg is a brief burst of spiking which quickly progresses to continuous spiking or hypersynchrony (Snead, 1988). This is associated with immobility, staring, facial myoclonus and vibrissal twitching. These behavioral and electrographic changes are responsive to antiabsence drugs such as ethosuximide (Snead, 1988).

2.4. Experimental design

Since earlier studies have shown that $GABA_A$-mimetics may produce opposing responses to absence seizures depending upon the site of drug administration (Liu et al., 1991), we administered the neuroactive steroids either systemically or directly into thalamic ventrobasal nucleus or in thalamic reticular nucleus, thalamic areas from which absence seizures evolve most readily (Gloor et al., 1990).

2.4.1. Systemic administration

Alphaxalone or tetrahydrodeoxycorticosterone was given i.p. at the dose of 1–5 mg/kg 20 min prior to γ-butyrolactone administration. Following γ-butyrolactone administration, absence seizures were quantitated as described below. Paired drug-naive controls were used in all experiments using 0.1% dimethyl sulfoxide in saline instead of steroids. In order to determine the effect of these steroids on ongoing γ-hydroxybutyric acid-seizures, we administered these steroids 10–15 min after the onset of seizures. The dose and the pre/posttreatment times of steroids were standardized during preliminary experiments.

2.4.2. Intrathalamic administration

Alphaxalone or tetrahydrodeoxycorticosterone (0.25–5 μg/side) was infused bilaterally into thalamic ventrobasal nucleus or into thalamic reticular nucleus, 10 min prior to the induction of γ-hydroxybutyric acid-seizures, or 10–15 min after the onset of seizures in order to determine their effect on ongoing γ-hydroxybutyric acid-induced absence seizures.

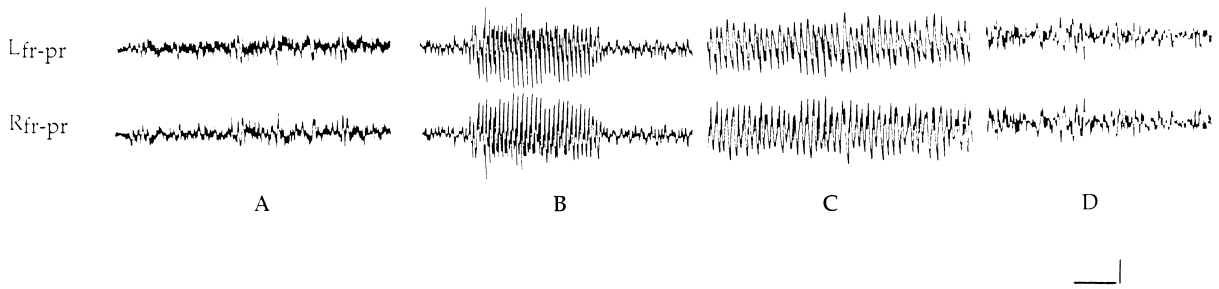

Fig. 1. EEG recordings from the right and left frontoparietal cortex ($R_{fr\text{-}pr}$ and $L_{fr\text{-}pr}$, respectively) of a rat. Panel A shows the background EEG activity. Within 5–10 min after systemic administration of γ-butyrolactone (100 mg/kg; i.p.), high voltage, 5–6 Hz rhythmic spike–wave discharges (SWD) evolved simultaneously from both the cortices (panel B). Panel C shows continuous SWD 1 h after the onset of first seizure. Panel D shows the normalization of EEG about 3 h after the administration of γ-butyrolactone. Calibration: 200 μV; 2 s.

2.5. Intrathalamic infusion of steroids

Stainless steel guide cannula (i.d. 0.19 mm) were implanted bilaterally into thalamic ventrobasal nucleus or reticular nucleus using bregma as reference point (Paxinos and Watson, 1986). Intrathalamic microinfusion was performed as described earlier (Banerjee and Snead, 1995). Briefly, seven days after surgery, alphaxalone or tetrahydrodeoxycorticosterone was infused in freely moving animals by introducing stainless steel injection cannula (i.d. 0.05 mm) into implanted guide cannula so that they extend 1 mm beyond the tip to the guide cannula. The injection cannula was connected with a 1 μl Hamilton syringe through propylene tubing. Alphaxalone or tetrahydrodeoxycorticosterone as 0.1% dimethyl sulfoxide in 1% Direct Blue solution (pH 7.1) was infused bilaterally at the rate of 0.2 to 0.3 μl/min. Total volume of infusion was 0.2 μl/side. The injection cannula were kept in position for an additional 60 s after the cessation of infusion in order to reduce the likelihood of injected compound being drawn back by capillary forces. Control animals received comparable volume of 0.1% dimethyl sulfoxide in 1% Direct Blue (pH 7.1). EEG recordings were made 30 min prior to start of infusion and then 5–6 h after the administration of γ-butyrolactone.

2.6. Histology

After completion of each infusion experiment, animals were killed and their brains chilled in isopentane (−40°C) for 60 s. 20 μm coronal brain sections were stained with

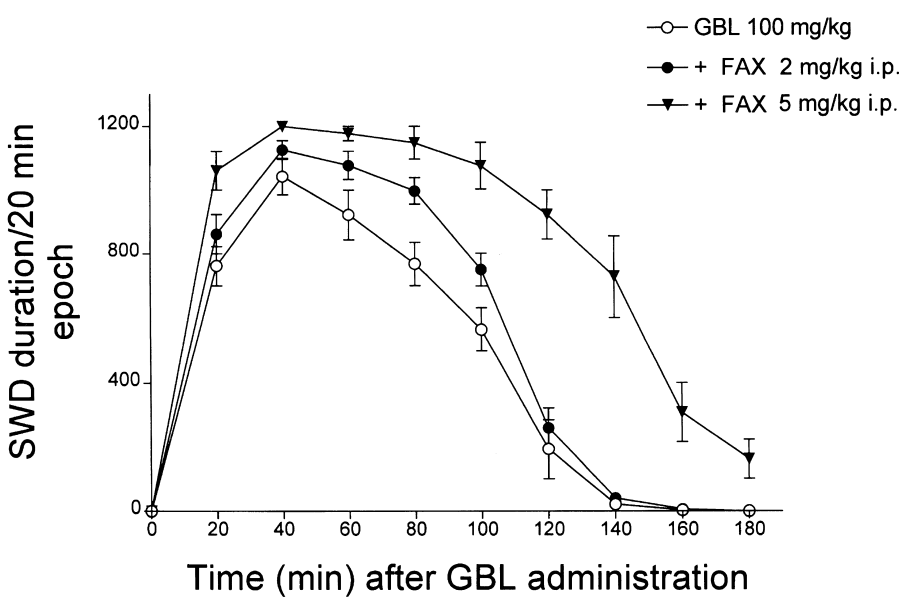

Fig. 2. Dose-dependent exacerbation of γ-hydroxybutyric acid-induced spike–wave discharges (SWD) by systemic administration (i.p.) of alphaxalone (FAX). Values are mean duration of γ-hydroxybutyric acid-seizures or SWD (s) in each 20 min epoch ± S.E.M. from 5–6 rats. A score of 1200 ± 0 was assigned when rats seized continuously for 20 min. At 5 mg/kg dose, the potentiation was significant at all time points ($P < 0.001$) as compared to the respective γ-butyrolactone (GBL)-alone groups (One-way ANOVA, Dunnett's test).

cresyl violet and the cannula tract and the infusion site (marked with Direct Blue) was identified under light microscope.

2.7. Data analysis

The experimental data are quantitated as described earlier (Banerjee and Snead, 1995). Total duration of absence seizures (in seconds) in each 20 min epoch was scored. All data are expressed as arithmetic mean ± S.E.M. In the infusion studies, only those rats exhibiting correct placement of the injection cannula (as assessed by histology) were incorporated in the data analysis. The number of animals in each group of experiments varied from 4–7. The data were analyzed using one-way analysis of variance (ANOVA), and multiple group means were simultaneously compared with the control mean using Dunnet's post hoc test.

3. Results

3.1. γ-Hydroxybutyric acid-induced absence-like seizures

In control rats, following systemic γ-butyrolactone (100 mg/kg, i.p.) administration, bursts of bilaterally synchronous 4–6 Hz spike–wave discharges appeared in the EEG as recorded from the surface of the frontoparietal cortex. These EEG changes occurred within 5–10 min of γ-butyrolactone administration and lasted for more than 2 h. About 3 h after γ-butyrolactone administration, the

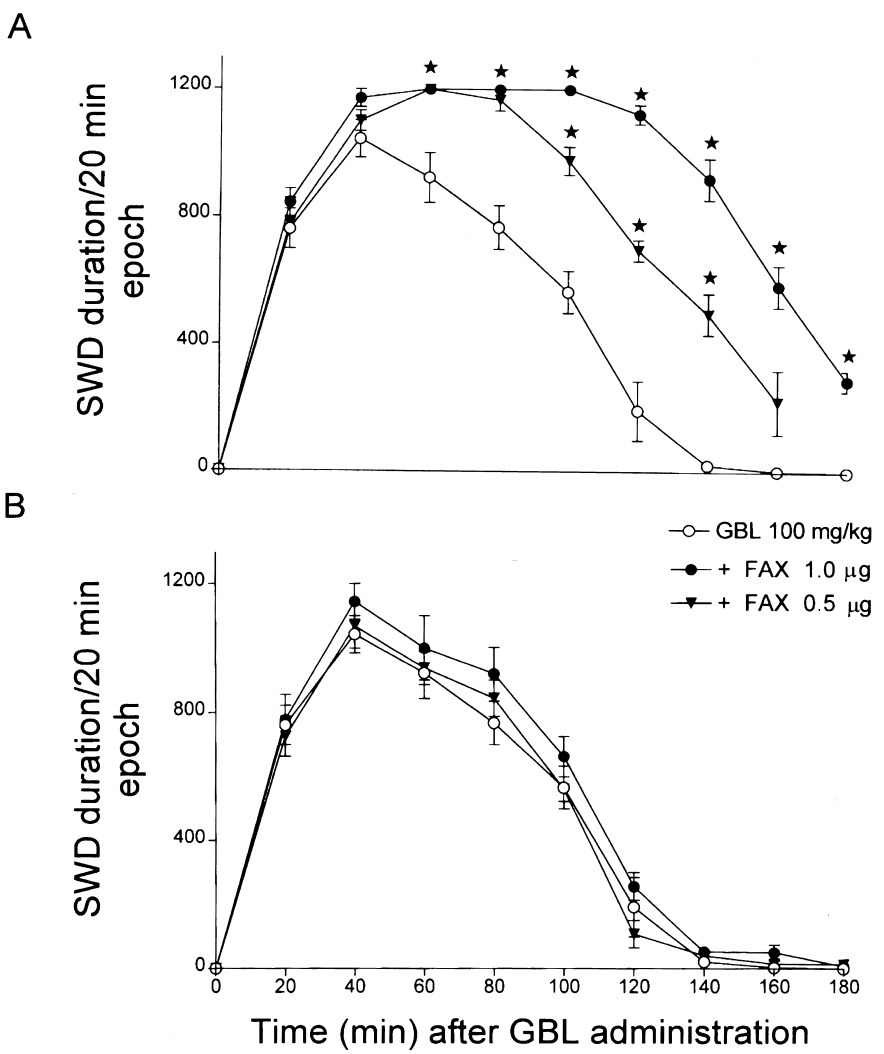

Fig. 3. Effect of bilateral microinfusion of alphaxalone (FAX) in thalamic ventrobasal nucleus (A) and in thalamic reticular nucleus (B) on γ-hydroxybutyric acid-induced spike–wave discharges (SWD). Alphaxalone was infused 10 min before the administration of γ-butyrolactone (GBL). Alphaxalone exacerbated γ-hydroxybutyric acid-seizures when administered into ventrobasal nucleus but not in thalamic reticular nucleus. Values are mean duration of γ-hydroxybutyric acid-induced SWD (s) in each 20 min epoch ± S.E.M. from 5–6 rats. A score of 1200 ± 0 was assigned when rats seized continuously for 20 min. $* P < 0.001$ as compared to the respective γ-butyrolactone-alone groups (One-way ANOVA, Dunnett's test).

Table 1
Effect of intrathalamic administration of tetrahydrodeoxycorticosterone (THDOC) on γ-hydroxybutyric acid-induced absence seizures

Drug	Duration (s) of γ-hydroxybutyric acid-seizures/each 20 min epoch					
	20	40	60	80	100	120
γ-Butyrolactone (alone)	761 ± 90	1042 ± 126	922 ± 106	768 ± 91	566 ± 80	192 ± 41
+ THDOC (1 μg/side; in VB)	826 ± 92	1200 ± 0	1200 ± 0[a]	1200 ± 0[a]	1100 ± 119[a]	985 ± 121[a]
+ THDOC (1 μg/side; in nRt)	800 ± 81	1000 ± 102	1056 ± 121	855 ± 90	612 ± 44	266 ± 41

Values are mean duration of γ-hydroxybutyric acid-induced seizures (s) in each 20 min epoch ± S.E.M. from 4–7 rats. A score of 1200 ± 0 was assigned when rats seized continuously for 20 min.
[a] $P < 0.001$ when compared to the respective γ-butyrolactone-alone groups (one-way ANOVA, Dunnett's test). VB, thalamic ventrobasal nucleus; nRt, thalamic reticular nucleus.

animals appeared normal both electrographically and behaviorally (Fig. 1).

3.2. Neuroactive steroids exacerbated γ-hydroxybutyric acid-induced absence seizures upon systemic administration

Alphaxalone or tetrahydrodeoxycorticosterone (1–5 mg/kg; i.p.) when administered either prior to the onset of, or after the onset of γ-hydroxybutyric acid-induced absence seizures, significantly increased the duration of absence seizures. In steroid-treated rats, γ-hydroxybutyric acid-seizures lasted for more than 4–4.5 h, while in the absence of steroids γ-hydroxybutyric acid-seizures continued for about 2.5 h (Fig. 2). Steroids alone produced no discernible EEG or behavioral effects.

3.3. Effect of bilateral microinfusion of steroids in thalamic ventrobasal nucleus and in thalamic reticular nucleus

Bilateral administration of alphaxalone or tetrahydrodeoxycorticosterone (0.25–1 μg/side) into thalamic ventrobasal nucleus, 10 min prior to the administration of γ-butyrolactone, resulted in a significant dose-dependent prolongation in the duration of γ-hydroxybutyric acid-induced absence seizures (Fig. 3A and Table 1, at 1 μg/side: $P < 0.001$ at all time points except 20 min and 40 min). Similarly, bilateral infusion of these steroids into thalamic ventrobasal nucleus, 10 min after the onset of γ-hydroxybutyric acid-seizures, also potentiated γ-hydroxybutyric acid-seizures (data not shown). However, similar doses of these steroids failed to prolong the duration of absence seizures when infused bilaterally into thalamic reticular

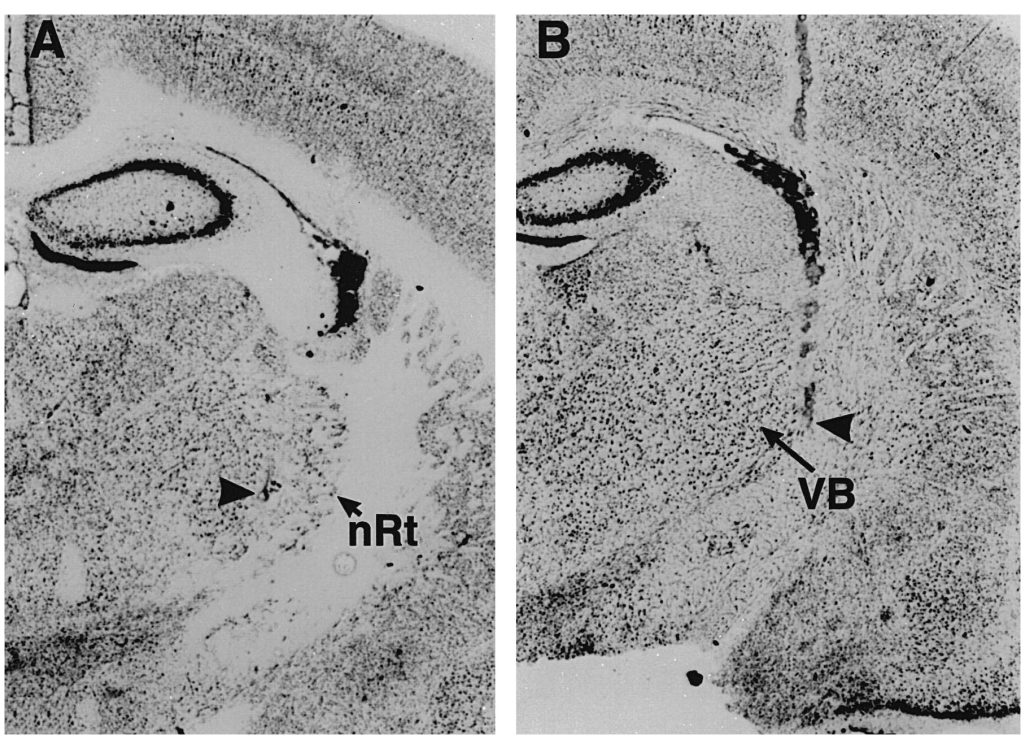

Fig. 4. Photomicrographs of rat brain coronal sections showing the cannula tract and the infusion site in thalamic ventrobasal nucleus, VB (A) and in thalamic reticular nucleus, nRt (B). Arrowheads indicate the infusion site.

nucleus (Fig. 3B, Table 1). We increased the dose of steroids up to 3 μg/side in thalamic reticular nucleus and found that thalamic reticular nucleus was resistant to the facilitatory effects of steroids on absence seizures. In all the above doses of steroids tested in thalamic reticular nucleus, the duration of absence seizures was neither prolonged nor shortened. Also, infusion of alphaxalone and tetrahydrodeoxycorticosterone in thalamic reticular nucleus 10 min after the onset of γ-hydroxybutyric acid-seizures produced no significant change in seizure-duration as compared to the γ-hydroxybutyric acid-alone group. Increasing further the dose of steroids (to 5 μg/side in thalamic reticular nucleus) produced slowing of the baseline EEG activity, which quickly progressed to a state that resembled EEG burst-suppression (characterized by alternating periods of low voltage activity or isoelectric period disrupted by bursts of spikes) after the administration of γ-butyrolactone. Due to this steroid-induced disturbance in the baseline EEG activity we did not test higher doses of steroids in thalamic reticular nucleus.

Microinjection of steroids precisely into thalamic reticular nucleus was difficult. Histologic verification of the injection sites revealed that only in about 30–40% of the rats where the injection was intended in thalamic reticular nucleus the drug was found largely restricted within this nucleus (see Fig. 4).

Bilateral infusion of 0.1% dimethyl sulfoxide in 1% Direct Blue (the vehicle) in either ventrobasal thalamus or thalamic reticular nucleus did not produce any significant effect on the duration of γ-hydroxybutyric acid-seizures (data not shown).

4. Discussion

Our data suggest that both alphaxalone and tetrahydrodeoxycorticosterone, which are known to enhance $GABA_A$ receptor function in the brain (Paul and Purdy, 1992), potentiated γ-hydroxybutyric acid-induced absence seizures upon systemic administration and after focal administration into thalamic ventrobasal nucleus (a thalamic area from which absence seizures evolve most readily). However, we found that thalamic reticular nucleus was resistant to the effects of steroids. This nonresponsiveness of thalamic reticular nucleus to steroids in modulating absence seizures was surprising because there is evidence that absence seizures are attenuated by increasing $GABA_A$ergic inhibition selectively in thalamic reticular nucleus. For example, focal administration of muscimol (a $GABA_A$ receptor agonist) in thalamic reticular nucleus of GAERS (genetically absence epilepsy-prone rats of Strasbourg) decreases the duration of absence seizures (Liu et al., 1991), while application of bicuculline (a $GABA_A$ receptor antagonist) in this structure has been shown to exacerbate or strengthen absence-like rhythms in thalamic slices (Huguenard and Prince, 1994). In contrast, increasing $GABA_A$ergic inhibition in thalamic relay nuclei (e.g., ventrobasal nucleus) is known to exacerbate absence seizures (Liu et al., 1991). These data suggest that absence seizures or absence-like thalamocortical oscillations could be differentially regulated by modulating $GABA_A$ergic inhibition within thalamic reticular nucleus and thalamic relay nuclei. However in the present study, we did not observe any significant effect of these steroids on γ-hydroxybutyric acid-seizures when the steroids were infused in thalamic reticular nucleus, although both alphaxalone and tetrahydrodeoxycorticosterone dose-dependently facilitated γ-hydroxybutyric acid-seizures after focal administration into thalamic ventrobasal nucleus. This suggested that these steroids (in doses used in the present study) increased $GABA_A$ergic inhibition in thalamic ventrobasal nucleus, but failed to do so in thalamic reticular nucleus. We suspect that this heterogeneity in neurosteroid response to absence seizures within thalamus may have arisen due to known molecular heterogeneity of $GABA_A$ receptor subunits in thalamus (Wisden et al., 1992).

The $GABA_A$ receptor is a heteropentameric protein that contains specific binding sites for GABA, benzodiazepines, barbiturates, picrotoxin and steroids (Macdonald and Olsen, 1994). Molecular cloning has identified several genes of $GABA_A$ receptor subunits. The α, β, γ and δ subunits constitute brain $GABA_A$ receptors (Macdonald and Olsen, 1994). Also, $GABA_A$ receptor subunits exhibit variable tissue-specific expression in mammalian brain. For example, the thalamic relay nuclei contain high levels of α4 and δ subunits, while these subunits are not well expressed in thalamic reticular nucleus (Wisden et al., 1992). Such differential distribution of $GABA_A$ receptor subunits in different brain regions may represent functionally distinct receptor subtypes. Indeed, the $GABA_A$ receptor subunit heterogeneity within the thalamus (and in the cerebral cortex) has provided some explanation as to why benzodiazepines attenuate absence seizures while barbiturates aggravate them (Browne and Penry, 1973; Penry and So, 1981), despite the fact that both benzodiazepines and barbiturates allosterically enhance $GABA_A$ receptor function (Macdonald and Olsen, 1994). It is known that the presence of a γ subunit in the $GABA_A$ receptor is required for benzodiazepine (but not barbiturate) sensitivity (Pritchett et al., 1989), and γ subunits are more abundant in the cortex than in the thalamus (Wisden et al., 1992). Due to this differential distribution of γ subunits, benzodiazepine (but not barbiturate)-induced $GABA_A$ergic inhibition may be expected to be more pronounced in the cortex than in the thalamus. Indeed, benzodiazepines have been shown to increase $GABA_A$ receptor function more effectively and potently in the cortex than in thalamus (Oh et al., 1995; Gibbs et al., 1996). Further, since cortical neurons possess much smaller proportional low-threshold Ca^{2+} current (T-current) as compared to thalamic neurons, cortical neurons are not paradoxically activated by GABAergic inhibition as do the thalamic neurons (Oh et al., 1995).

Therefore, it is likely that upon oral or systemic administration, benzodiazepines (but not barbiturates) preferentially increase cortical GABAergic inhibition and thereby reduce excitability in the entire thalamocortical network and produce antiabsence effect. Barbiturates, in contrast, aggravate absence seizures by effectively enhancing $GABA_A$ergic inhibition both in the thalamus and cortex (Oh et al., 1995).

Although it is not known whether the functional modulation of $GABA_A$ receptors by neuroactive steroids is subunit-dependent, several lines of evidence suggest that the sensitivity of $GABA_A$ receptors to neurosteroids is somewhat dependent on the α isoform present in the receptor. For example, $GABA_A$ receptors containing $\alpha 6$ subunits ($\alpha 6\beta 2\gamma 2$) are modulated more effectively and potently by steroids than those containing $\alpha 1$ subunits (Shingai et al., 1991; Puia et al., 1993). Since $\alpha 4$ (which is abundant in thalamic ventrobasal nucleus but not in the reticular nucleus) and $\alpha 6$ subunit (in the cerebellum) share a high degree of amino acid identity (Tyndale et al., 1995), and produce benzodiazepine-insensitive $GABA_A$ receptors in thalamus (and other forebrain regions) and cerebellum, respectively (Knoflach et al., 1996), it is possible that the $\alpha 4$ subunit is the 'steroid-sensitive' subunit in the thalamus. The near absence of this 'steroid-sensitive' $\alpha 4$ subunit in thalamic reticular nucleus may explain why neuroactive steroids failed to produce any effect in the present study upon infusion into the reticular nucleus.

In conclusion, the present findings suggest that neurosteroids, which exhibit potent anticonvulsant actions against limbic seizures and status epilepticus, aggravate absence seizures. This may be due to the presence of a key α subunit isoform ($\alpha 4$) in thalamic ventrobasal nucleus and other relay nuclei (from which absence seizures evolve most readily) but not in thalamic reticular nucleus.

Acknowledgements

This work was supported by the Program in Brain and Behavior, Hospital for Sick Children (Toronto, Canada).

References

Banerjee, P.K., Snead, O.C., 1995. Thalamic NMDA receptors in γ-hydroxybutyrate model of absence seizures: a cerebral microinjection study in rats. Neuropharmacology 34, 43–53.

Belelli, D., Lan, N.C., Gee, K.W., 1990. Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. Neurosci. Biobehav. Rev. 14, 315–322.

Browne, T.R., Penry, J.D., 1973. Benzodiazepines in the treatment of epilepsy. Epilepsia 14, 277–310.

Budziszewska, B., Siwanowicz, M., Leskiewicz, M., Jaworska-Feil, L., Lason, W., 1998. Protective effects of neurosteroids against NMDA-induced seizures and lethality in mice. Eur. Neuropsychopharmacol. 8, 7–12.

Doherty, J.D., Hattox, S.E., Snead, O.C., Roth, R.H., 1978. Identification of endogenous γ-hydroxybutyrate in human, guinea pig and rhesus monkey brain. J. Pharmacol. Exp. Ther. 20, 130–139.

Gibbs, J.W., Schroder, G.B., Coulter, D.A., 1996. $GABA_A$ receptor function in developing rat thalamic reticular neurons: whole cell recordings of GABA-mediated currents and modulation by clonazepam. J. Neurophysiol. 76, 2568–2579.

Gloor, P., Avoli, M., Kostopoulos, G., 1990. Thalamocortical relationships in generalized epilepsy with bilaterally synchronous spike–wave discharge. In: Avoli, M., Gloor, P., Kostopoulos, G., Naquet, R. (Eds.), Generalized Epilepsy: Neurobiological Approaches. Birkhauser, Boston, MA, pp. 190–212.

Huguenard, J.R., Prince, D.A., 1994. Clonazepam suppresses $GABA_B$-mediated inhibition in thalamic relay neurons through effects in nucleus reticularis. J. Neurophysiol. 71, 2576–2581.

King, G.A., 1979. Effect of systemically applied GABA agonists and antagonists on wave–spike ECoG activity in rat. Neuropharmacology 18, 47–55.

Knoflach, F., Benke, D., Wang, Y., Scheurer, L., Luddens, H., Hamilton, B.J., Carter, D.B., Mohler, H., Benson, J.A., 1996. Pharmacological modulation of the diazepam-insensitive recombinant γ-aminobutyric $acid_A$ receptors $\alpha 4\beta 2\gamma 2$ and $\alpha 6\beta 2\gamma 2$. Mol. Pharmacol. 50, 1253–1261.

Kokate, T.G., Svensson, B.E., Rogawski, M.A., 1994. Anticonvulsant activity of neurosteroids: correlation with γ-aminobutyric acid-evoked chloride current potentiation. J. Pharmacol. Exp. Ther. 270, 1223–1229.

Kokate, T.G., Cohen, A.L., Rogawski, M.A., 1996. Neuroactive steroids protect against pilocarpine- and kainic acid-induced limbic seizures and status epilepticus in mice. Neuropharmacology 35, 1049–1056.

Leskiewicz, M., Budziszewska, B., Jaworska-Feil, L., Lason, W., 1997. Effects of neurosteroids on kainate-induced seizures, neurotoxicity and lethality in mice. Pol. J. Pharmacol. 49, 411–417.

Liu, Z., Vergnes, M., Depaulis, A., Marescaux, C., 1991. Evidence for a critical role of GABAergic transmission within the thalamus in the genesis and control of absence seizures in the rat. Brain Res. 545, 1–7.

Macdonald, R.L., Olsen, R.W., 1994. $GABA_A$ receptor channels. Annu. Rev. Neurosci. 17, 569–602.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of GABA receptors. Science 232, 1004–1007.

McCormick, D.A., 1992. Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. Prog. Neurobiol. 39, 337–388.

Mellon, S.H., 1994. Neurosteroids: biochemistry, modes of action, and clinical relevance. J. Clin. Endocrinol. Metab. 78, 1003–1008.

Morrow, A.L., Pace, J.R., Purdy, R.H., Paul, S.M., 1990. Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: evidence for multiple steroid recognition sites. Mol. Pharmacol. 37, 263–270.

Nakahiro, M., Arakawa, O., Narahashi, T., Ukai, S., Kato, Y., Nishinuma, K., Nishimura, T., 1992. Dimethyl sulfoxide (DMSO) blocks GABA-induced current in dorsal root ganglion neurons. Neurosci. Lett. 138, 5–8.

Oh, K.S., Lee, C.J., Gibbs, J.W., Coulter, D.A., 1995. Postnatal development of $GABA_A$ receptor function in somatosensory thalamus and cortex: whole-cell voltage-clamp recordings in acutely isolated rat neurons. J. Neurosci. 15, 1341–1351.

Paul, S.M., Purdy, R.H., 1992. Neuroactive steroids. FASEB J. 6, 2311–2322.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, Orlando, FL.

Penry, J.K., So, E., 1981. Refractoriness of absence seizures and phenobarbital. Neurology 31, 158.

Pritchett, D.B., Sontheimer, H., Shivers, B.D., Ymer, S., Kettenman, H.,

Schofield, P.R., Seeburg, P.H., 1989. Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature 338, 582–585.

Puia, G., Ducic, I., Vicini, S., Costa, E., 1993. Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition?. Recept. Chann. 1, 135–142.

Roth, R.H., Giarman, N.J., 1969. Conversion in vivo of γ-aminobutyric acid to γ-hydroxybutyric acid in the rat. Biochem. Pharmacol. 18, 247–250.

Shingai, R., Sutherland, M.L., Barnard, E.A., 1991. Effects of subunit types of the cloned $GABA_A$ receptor on the response to a neurosteroid. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 206, 77–80.

Smith, K.A., Bierkamper, G.G., 1990. Paradoxical role of GABA in a chronic model of petit mal (absence)-like epilepsy in the rat. Eur. J. Pharmacol. 176, 44–55.

Snead, O.C., 1988. γ-Hydroxybutyrate model of generalized absence seizures: further characterization and comparison with other absence models. Epilepsia 29, 361–368.

Turner, D.M., Ransom, R.W., Yang, J.S.J., Olsen, R.W., 1988. Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. J. Pharmacol. Exp. Ther. 248, 960–966.

Tyndale, R.F., Olsen, R.W., Tobin, A.J., 1995. $GABA_A$ receptors. In: North, R.A. (Ed.), Handbook of Receptors and Channels: Ligand- and Voltage-gated Ion Channels. CRC Press, Ann Arbor, MI, pp. 265–290.

Vergnes, M., Marescaux, C., Micheletti, G., Depaulis, A., Rumbach, L., Warter, J.M., 1984. Enhancement of spike and wave discharges by GABAmimetic drugs in rats with spontaneous petit mal-like epilepsy. Neurosci. Lett. 44, 91–94.

Wisden, W., Laurie, D.J., Seeburg, P.H., 1992. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain: I. Telencephalon, diencephalon, mesencephalon. J. Neurosci. 12, 1040–1062.