Interactive report

Infusion of 3α,5α-THP to the pontine reticular formation attenuates PTZ-induced seizures

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Abstract

Whether progesterone (P₄) and its metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP) have anti-seizure effects through actions in the pontine reticular formation (PRF) was investigated. Concentrations of P₄ and 3α,5α-THP in the PRF were greater in proestrous and hormone-primed rats, that are typically more resistant to seizure-induction, than diestrous and males rats. Ovx, Long-Evans rats with unilateral microinjections into the PRF of 3α,5α-THP (5 μg/0.2 μl), but not P₄ (11 μg/0.2 μl) or vehicle (β-cyclodextrin), had a greater latency and lower incidence of tonic-clonic seizures induced by pentylenetetrazol (PTZ; 70 mg/kg, IP) administration. Infusions that missed the PRF were not effective. These data suggest 3α,5α-THP has anti-seizure effects in part through actions in the PRF. © 2000 Elsevier Science B.V. All rights reserved.

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The brain regions that are involved in progesterone’s (P₄) and its 5α-reduced metabolite, 5α-pregnan-3α-ol-20-one (3α,5α-THP) anti-seizure effects are unknown; however, the pontine reticular formation (PRF) may be a site of action. The PRF is activated during seizures [4] and infusions of the γ-aminobutyric acid (GABA)ₐ receptor (GBR) antagonist bicuculline to the PRF has anti-convulsant effects [21].

Progesterone has functionally relevant actions in the PRF. For example, infusions of P₄ decrease the latency to REM sleep in male and female rats [3]. Neurosteroids, such as 3α,5α-THP, also have robust sleep-enhancing effects [13]. The purpose of these experiments was twofold. First, to examine progestin levels in the PRF in hormonal and reproductive states associated with differences in seizure susceptibility. Second, to compare P₄’s and 3α,5α-THP’s ability to prevent seizures when infused into the PRF.

Sexually mature female (n=126; Experiment 1; n=40, Experiment 2) or male (n=42; Experiment 1) Long-Evans rats (Taconic Farms) were housed in polypropylene cages (25×23×20 cm). The rats were maintained on a 12/12 h reversed light cycle (lights off 8:00 am) with access to Purina Rat Chow and tap water in their home cages. The estrous cycle of some females was determined by daily lavage [7] for 2–3 cycles before inclusion in the experiment. Other rats had their ovaries removed (ovx) under Rhoempun (12 mg/kg IP) and Ketaset (80 mg/kg IP) anesthesia and a week later were hormone-primed with estradiol benzoate (10 μg SC in sesame oil at hour 0) and P₄ (500 μg SC in sesame oil at hour 44; tissues were collected 4 h later).

In Experiment 1, P₄ and 3α,5α-THP were measured by radioimmunoassay [8] in PRF. Tissue from PRF of 6 rats of the same condition were obtained at 1000 and later

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pooled for progestin measurement. There were 7 diestrous, 7 proestrous, 7 intact male, and 7 ovx hormone-primed pools. NB: Although the ovx rats were surgerized the cycling and male rats did not undergo sham surgery.

For Experiment 2, 40 rats were ovx and a week later were stereotaxically implanted with unilateral, 30 gauge, guide cannula aimed over the PRF (coordinates from bregma: AP: −9.7, ML: ±0.8, DV −9.0). Five days later rats were randomly assigned to be infused with 0.2 µl β-cyclodextrin vehicle [9] (n = 13), P₄ (11 µg; n = 13), or 3α,5α-THP (5 µg; n = 14), 30 min prior to pentylenetetrazol (PTZ; 70 mg/kg, IP) induction of seizures. Immediately after PTZ, ictal behaviors were recorded for 1 h [5]. Ictal behavior, recorded while rats were in a plexiglas arena (26×30×50 cm), was based on the latency and incidence of myoclonus, characterized by facial twitching and forelimb clonus, and tonic clonic seizures, identified by rearing and a complete loss of balance. Rats from each group (n = 2–3 per group) were also implanted with electrodes in the PRF and cortex so that we could monitor changes in seizures and electrical activity following steroid infusions and PTZ.

Following testing all rats were infused with indigo blue and then intracardially perfused with 0.9% saline and 10% formalin. Fixed frozen brains were sliced at 40 µM so that infusion and electrode placement could be determined. Of the 40 rats that had infusions, 30 (n = 10 per group) were localized to the PRF. The 10 rats that had infusions localized to the dorsal medial tegmental nucleus were not included in the analyses because these infusions had no impact on ictal activity. All rats with ‘missed’ infusions had tonic clonic seizures within 75 s of infusion irrespective of whether vehicle (n = 3), P₄ (n = 3), or 3α,5α-THP (n = 4) was infused. Statistical analyses consisted of multiple one-way analysis of variances (ANOVAs) to examine effects of hormone condition on progestin levels or effects of PRF infusion condition on ictal activity. Where appropriate, ANOVA’s that revealed significant differences at the P < 0.05 level were followed by Duncan’s post-hoc tests.

Progesterone (F₃,₄₄ = 9.734, P = 0.002) and 3α,5α-THP (F₃,₄₄ = 5.867, P = 0.003) concentrations in the PRF varied significantly across groups. Although concentrations of P₄ were very low in the PRF the P₄ levels were highest in the hormone-primed (1.38 ± 0.20 ng/g) and proestrus (1.27 ± 0.24 ng/g) rats; however, 3α,5α-THP levels were nearly 10 fold higher than P₄ (see Fig. 1).

The significant differences across groups in latency (F₂,₂₇ = 3.277, P = 0.05) and incidence of tonic clonic convulsions (F₂,₂₇ = 3.76, P = 0.03), were largely due to 3α,5α-THP’s anti-seizure effects. Infusion of 3α,5α-THP to the PRF produced longer latencies (see Fig. 2a) and fewer tonic clonic seizures (see Fig. 2b) than did P₄ or vehicle infusions. Only one of ten rats with 3α,5α-THP infusions to the PRF had seizures, while seven of ten of the rats with P₄ or vehicle to the PRF had tonic clonic seizures. Notably, the ten rats that had infusion sites in the dorsal medial tegmental nucleus, that is those with ‘missed’ PRF infusions, all had tonic clonic seizures.

3α,5α-THP infusions to the PRF also tended to enhance the latency to myoclonic seizures (F₂,₂₇ = 2.66, P = 0.08; 75.1 ± 9.7 s), versus that seen in vehicle (60.3 ± 7.2 s) or P₄ infused (48.4 ± 6.8 s); however, there were no differences in incidence of myoclonus across groups.

Electrographic recordings from the PRF and motor cortex were examined from rats in each PRF infusion condition. Progesterone did not modify the electrographic recording prior to PTZ administration but 3α,5α-THP reduced EEG. The latter markedly reduced ictal events induced by PTZ. Fig. 3 shows a representative recording during the most intense ictal activity after PTZ administration of rats previously infused with 3α,5α-THP, P₄, or vehicle to the PRF. 3α,5α-THP infusions produced fewer spikes in the PRF, compared to that observed in rats infused with vehicle or P₄; however, P₄ infusions also reduced epileptiform activity compared to vehicle. In the motor cortex both 3α,5α-THP and P₄ reduced ictal activity (see Fig. 3).

These data demonstrate that 3α,5α-THP is in high concentrations in the PRF and is greater in proestrous and hormone-primed rats, that are typically less seizure susceptible [6,7] than are diestrous and males rats. Infusion of 3α,5α-THP into the PRF of ovx, Long-Evans rats administered PTZ markedly diminished the percentage of animals with seizures, and produced a longer latency and lower incidence of tonic-clonic seizures compared to vehicle or P₄ infusions. These data suggest that 3α,5α-THP has anti-
seizure effects in part through actions in the PRF. These findings are intriguing because of the importance of localizing the CNS site at which progestins may have anti-seizure effects and also because of implications regarding the putative mechanism of 3α,5α-THP’s action.

Traditionally P₄ is understood to work via passive diffusion into cells, binding with specific intracellular receptors in target cells to form a steroid receptor complex, that in the nucleus binds to an acceptor site on the DNA, and enhances transcriptional and translational processes. However, the actions of progestins at these intracellular receptors do not fully account for their effects on seizure activity. There is a great disparity in affinity for intracellular progestin receptors between progestins, such as P₄ and 3α,5α-THP [14]. That P₄ has a high affinity for intracellular progestin receptors, and 3α,5α-THP is devoid of activity at intracellular progestin receptors, and both can have anti-seizure effects, suggest that P₄’s and 3α,5α-THP’s effects on seizure threshold are not the sole consequence of actions at traditional intracellular progestin receptors.

Many anti-epileptic drugs used therapeutically have their effects by increasing the activity of the primary inhibitory neurochemical in the brain, GABA; progestins can also enhance activity of this system through actions at GBRs. Progestins effects on GBRs accounts for their rapid analgesic [9], anxiolytic [2], and anesthetic [1] actions, and may also underlie some of progestins’ anticonvulsant effects. Progesterone itself only binds weakly to GBRs [16] but is metabolized by the 5α-reductase and 3-hydroxysteroid dehydrogenase enzymes to 3α,5α-THP, which is 500 times more potent than P₄ [15] and 50 times more potent than valium at modulating GBRs [20]. Anti-seizure effects of some progestins like 3α,5α-THP, which can directly activate the GABA-chloride channel [18], may be partially understood through its actions at GBRs.

In order to elucidate the CNS substrates through which progestins have anti-seizure effects, it is important to consider progestin metabolism because P₄ and 3α,5α-THP typically have different mechanisms of action. Previous findings suggest that P₄ has anti-seizure effects [12] but that metabolites of P₄ may mediate these effects. Systemic administration of either P₄ or 3α,5α-THP prevent seizures; however, the latency for P₄’s anti-convulsant effects are longer than 3α,5α-THP’s and more P₄ than 3α,5α-THP is required to produce similar anti-seizure effects [10]. Blocking P₄’s metabolism to 3α, 5α-THP attenuates its anti-seizure actions [11]. In people and rats, decreased seizure susceptibility is better correlated with increases in 3α,5α-THP than P₄ [6,19]. In the present study P₄’s metabolism to 3α,5α-THP in the PRF may have been limited and precluded P₄’s anti-seizure effects. The decrease in ictal activity in the PRF and cortex following P₄ infusions to the PRF suggest that P₄ possesses some anticonvulsant effects. It is possible that the molecular encapsulization vehicle or the 30 min latency may have diminished P₄’s conversion to 3α,5α-THP and thereby precluded P₄’s anti-seizure effects. Another possibility is there may not have been enough P₄ to metabolize to 3α,5α-THP.

In summary, these studies reveal promising findings regarding the CNS site through which progestins may prevent tonic clonic seizures and extend previous findings about the importance of the PRF in mediating tonic clonic
Fig. 3. Representative electrographic recordings from the motor cortex and pontine reticular formation (PRF) from a rat in each condition during the most intense ictal activity. Note that commensurate with the lower ictal activity are fewer spikes in the PRF of the rat infused with 3α,5α-THP compared to the rats infused with vehicle or P₄.

seizures [4,17]. Concentrations of 3α, 5α-THP were high in the PRF of proestrous and hormone-primed rats that are typically less susceptible to seizures. Infusions of 3α,5α-THP to the PRF markedly diminished the number of animals with seizures, produced longer latencies and lower incidences of tonic clonic seizures than did vehicle or P₄ infusions. Because of the important implications of these findings, our laboratory will continue to examine the mechanisms and role of progestins in the PRF to prevent ictal activity.

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References

[7] C.A. Frye, L.E. Bayon, Cyclic withdrawal from endogenous and