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The effect of progesterone and its metabolite 5α-pregnan-3α-ol-20-one on focal epileptic seizures in the cat's visual cortex in vivo

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The acute effects of progesterone and its brain metabolite 5α-pregnan-3α-ol-20-one (3α-OH-DHP) on focal epileptic seizures in the cat's visual cortex was studied in vivo using an unanesthetized cervaux-isolé preparation. This model made it possible to study in parallel the effect of the drugs on ictal activity and synaptic transmission. A dose-dependent increase in seizure threshold was observed after i.v. injections of both 3α-OH-DHP and progesterone, 3α-OH-DHP being about 20 times as potent as the latter. I.v. injections of 3α-OH-DHP 1.0 mg/kg increased the median seizure threshold to 265% of baseline. While 3α-OH-DHP exerted an immediate effect on seizure thresholds, the maximal effect of progesterone was delayed about 20 min. Concerning the mechanisms underlying the antiepileptic effect, three changes occurred within the effective dose range: (1) a small, but significant reduction in the presynaptic nerve volleys, (2) a reduction in the postsynaptic excitatory field potentials in the dorsal lateral geniculate nucleus and cortex, and (3) an enhanced postsynaptic inhibition. Taken together, these observations point to both pre- and postsynaptic effects, supporting the hypothesis of a barbiturate-like mechanism of action of progesterone and its brain metabolites.

Introduction

The ability of ovarian steroids to influence seizure susceptibility is well documented. Progesterone and its metabolites decrease brain excitability[1,22,23,35,38] while estrogens exerts the opposite effect[25,31]. Clinically, these effects are believed to contribute to the commonly observed rhythmic variation in epileptic seizure frequency related to the menstrual cycle[20,34,42].

In vitro studies have shown that progesterone and its metabolites increase γ-aminobutyric acid (GABA)-ergic neurotransmission[5,16,30]. 5α-Pregnan-3α-ol-20-one (3α-OH-DHP), a progesterone metabolite produced within the brain (see Majewska[29]), is especially potent in this respect[13,21]. 3α-OH-DHP acts as a ligand to the $GABA_A$ receptor[12] and it stimulates the chloride uptake into isolated brain vesicles and increases the inward Cl^- current induced by GABA[30]. Other studies indicate that both progesterone and 3α-OH-DHP may depress glutamatergic activity[36,37] and neurotransmitter release[11,32,43].

In the present study we examined the acute effects of 3α-OH-DHP and progesterone on focal epileptic seizures in vivo. In the cat's primary visual cortex stable seizure thresholds can be established[18] in a neuronal circuitry that has been punctiliously characterized[8,9,10,14]. This model

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made it possible to study both excitatory and inhibitory mechanisms simultaneously with changes in seizure thresholds.

Methods

Experiments were performed on adult female non-estrous cats. Under short-acting anesthesia with alphaxalone/alphadolone (Saffan®, Glaxovet, 20 mg/kg i.m.) a tracheostomy was made and catheters placed in the femoral artery and vein. A craniotomy was performed behind the tentorium and the brainstem was transected at the ponto-mesencephalic border while sparing the basal arteries. This transection results in a comatose preparation (cervaux-isolé) and the experiment was subsequently continued without the use of anesthetics[18]. The experimental protocol was approved by the animal ethical committee of Gøteborg.

During recordings the animals were paralyzed with gallamine (5–7 mg/kg/h (Flaxedil®, May and Baker Ltd.)), and artificially ventilated with end-expiratory CO_2 adjusted to 3.5%. To reduce movements of the brain due to respiration, the animal was suspended by a clamp placed on the vertebrae at the mid-thoracic level. Central core temperature, heart rate and mean arterial blood pressure were continuously monitored and maintained within physiological ranges. For visual stimulation, the eyes were fitted with contact lenses and focused on a tangent screen on which visual stimuli were projected[8]. Unipolar stimulation electrodes were placed in the optic tract (OT) and the dorsal lateral geniculate nucleus (dLGN) in register with the cortical recording site. For seizure induction, a third electrode was inserted in the lower layer 6 of the primary visual cortex close to the area 17–18 border. Recordings were made by electrodes in the dLGN (i.e., the dLGN stimulation electrode was changed to a recording electrode by a switch), intracortically (lamina 2–6) and on the surface. After the placement of the electrodes, the cortical surface was covered by body-warm agar (3% in saline) to reduce pulsations. A simplified diagram of the neuronal circuitry involved and the experimental setup is given in Fig. 1.

Focal epileptic seizures were elicited by a brief train of electrical stimuli delivered through the intracortical electrode[18]. At least 1 h was allowed for recovery from the anesthesia before stimulation was started. During the next 2–3 h a stable threshold level for seizure initiation was established. The triggering stimulus was a 1.0–3.0 s long train of constant current pulses (0.2 ms, 50 Hz). The stimulation intensity was used as the dependent variable (threshold parameter). Control thresholds were typically 200–300 μA. Drug induced threshold changes were expressed in percent of the control level using the highest intensity that failed to elicit a seizure as a conservative estimate of the drug effect. The maximum intensity used was set to 2 mA to avoid tissue damage. Repeated stimulations were performed at 3-min intervals after seizures, and at 1-min intervals after non-convulsive responses.

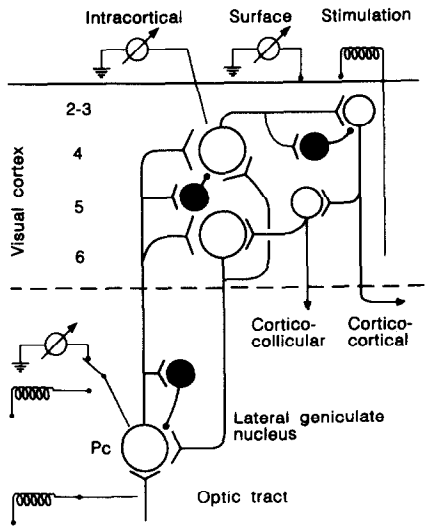

Fig. 1. Schematic diagram of experimental arrangements and major neuronal circuitry involved in focal seizure activity of the lateral geniculate nucleus and primary visual cortex. Open circles represent excitatory neurons and filled circles inhibitory interneurons.

Extracellular field potentials were simultaneously recorded in upper lamina A of the dLGN, on the surface of the primary visual cortex and within the cortex (lamina 2–6) after stimulation of OT fibers or dLGN neurons with negative constant current pulses (0.2 ms) at a rate of 2 Hz.

The effect on inhibitory processes in the dLGN and the cortex was studied by double stimulations of the OT. The interstimulus interval was 30–45 ms. At interstimulus intervals of this length, it is

mainly recurrent inhibitory mechanisms that modify the amplitude of the excitatory field potentials in the dLGN[24].

Recorded potentials were amplified, displayed with two different time bases on a double oscilloscope and photographed for subsequent analysis. Focal seizure activity was displayed on a chart recorder together with blood pressure and heart rate and stored on a tape recorder. All measured values are given as medians with ranges. Statistical analysis was performed using Wilcoxon rank sum test for independent samples and Wilcoxon signed rank test for paired samples.

3α-OH-DHP or progesterone (Sigma) was dissolved in glycofurol (polyethylene glycol monotetra-hydrofurfuryl ether; Agrar Soc., Italy) in concentrations of 0.5–10 mg/ml and 10–100 mg/ml, respectively. All solutions were made fresh on the day of the experiment. The drugs were given by a slow intravenous infusion (infusion time of about 3 min). The median volume injected was 0.15 ml/kg (range 0.13–0.4). The median volume injected of the control solution not containing 3α-OH-DHP was 0.20 ml/kg (range 0.15–0.4). Registration time after 3α-OH-DHP injections ranged from 30 to 100 min, with a median of 60 min. After progesterone injections, the median registration time was 80 min (range 40–90), and after control injections 33 min (range 20–50).

Results

The in vivo cervaux-isolé preparation used in this study allowed repeated focal seizures to be elicited in a limited region of the visual cortex without the use of anesthesia. With electrical induction the seizures could be initiated in a controllable and reproducible manner with stable and distinct seizure thresholds (Fig. 2A,B). In most preparations it was possible to maintain a basal electroshock seizure threshold (EST) within 20% of the original value for more than 10 h after an initial stabilizing period of 2–3 h. Typically, the seizures lasted 5–15 s and consisted of two phases. First, an initial 1–5 s long 'tonic' phase during which the asynchronous activity of cortical neurons became organized into 13–20-Hz synchronous bursts, followed by a more long-lasting 'clonic' phase with longer bursts at lower frequency (2–5 Hz)[18].

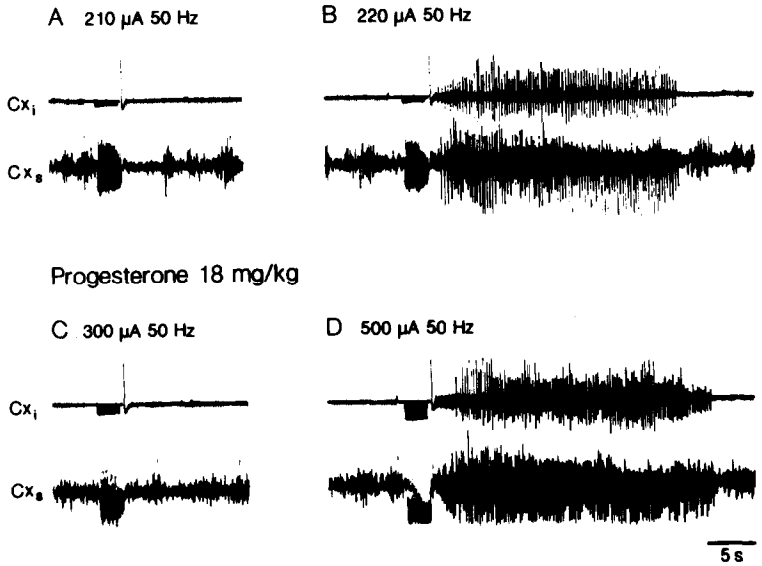

Fig. 2. Experimental focal seizures in the cat's visual cortex. Responses in A and B were obtained under control conditions and those in C and D about 20 min after progesterone (18 mg/kg). Note the distinct seizure threshold and the all-or-nothing character of the seizure activity. Following the progesterone injection the threshold intensity for seizure initiation more than doubled. The first spike-like component after the stimulation train in upper traces is a switch artefact. Cx_i: intracortical registration. Cx_s: cortical surface registration.

TABLE I

The effect of 3α-OH-DHP, progesterone and glycofurol (solvent) on focal seizure thresholds in the cat's visual cortex

Substance	n	Dose (mg/kg)	Max. EST % control	Time to max. EST (min)
3α-OH-DHP	3	0.1	133	< 5
			(125–240)	(all)
	2	0.25	184	< 5
			(168–200)	(all)
	6	1.0	265	< 5
			(250–400)	(all)
Progesterone	1	1.0	133	16
	4	18.0	212	19
			(200–250)	(17–25)
Glycofurol	7	–	120	7
			(100–125)	(0–18)

All values are given as medians with ranges. EST, electroshock seizure threshold, calculated in percent of control value obtained immediately before injection (= 100%); n, number of infusions.

Focal seizure thresholds

A progressive dose-dependent increase in seizure threshold was observed after i.v. injections of both 3α-OH-DHP and progesterone (Table I). Representative examples of the effect are shown in Fig. 3. With 3α-OH-DHP, a minimal threshold change was observed with a dose of 0.1 mg/kg, while two subsequent injections of 1.0 mg/kg both resulted in more than a doubling of the seizure threshold. Similar results, with a median threshold increase to 265% of baseline, were obtained in all six experiments with the higher dose of 3α-OH-DHP

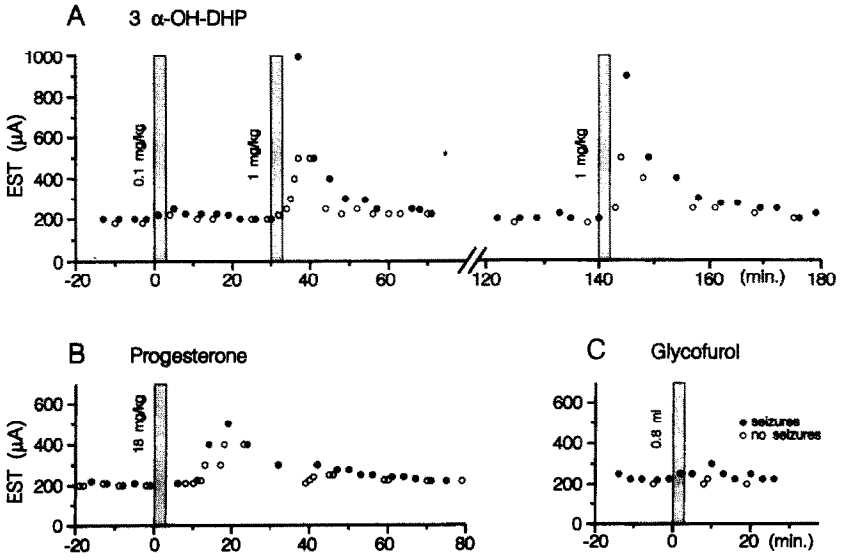

Fig. 3. Changes in electroshock seizure thresholds (EST) after i.v. injections of 3α-OH-DHP (A), progesterone (B) and the solvent glycofurol (C). Vertical bars represent injection periods. Open circles represent stimulation trials that were subthreshold for seizure initiation, filled circles trials followed by seizures. 3α-OH-DHP at 0.1 mg/kg had a minimal effect while 1 mg/kg more than doubled the seizure threshold in a reversible and reproducible manner. A clear threshold increase was also seen after progesterone, but at a much higher dosage (B). Note the difference in time course of the threshold change after 3α-OH-DHP and progesterone.

(1.0 mg/kg). In all cases there was a rapid onset of the effect with the maximal threshold level reached within the first 5 min after the end of the infusion. Recovery to within 20% of baseline level was obtained after 30–40 min. Control injections of the solvent (glycofurol) produced only small threshold changes (125% or less; Fig. 3B, Table I).

Progesterone produced comparable seizure threshold increases but at higher doses (Figs. 2 and 3C; Table I). With 18 mg/kg the threshold increased to a median of 212% of baseline while only a small effect was seen with 1.0 mg/kg. In contrast to the effect of 3α-OH-DHP, the progesterone effect evolved more slowly with a maximal threshold increase after about 20 min. The recovery process was also slower and thresholds within 20% of the control level were not reached until after 60–70 min.

It should be mentioned that the reported threshold changes are, if anything, underestimates. To calculate the drug induced threshold changes we used the highest stimulation intensity that failed to elicit a seizure, rather than the intensity of the effective stimulus. Furthermore, to avoid tissue damage, maximum stimulation intensity was set to 2 mA. In some experiments, this intensity was below the actual seizure threshold, but was nevertheless taken as the real threshold value.

Excitatory synaptic responses

Recordings of extracellular field potentials in the dLGN and the visual cortex, evoked by OT stimulations, were used to study the effects of 3α-OH-DHP and progesterone on excitatory synaptic transmission. A typical response in the dLGN (Fig. 4, lower traces) consisted of an initial positive-negative potential (t_1) representing the presynaptic volley in fast conducting Y-type optic tract fibers followed by a negative-positive potential (r_1) representing the associated monosynaptic excitatory field potential[4]. In the simultaneously recorded cortical response a presynaptic volley (p) and mono- (m) and disynaptic (d) excitatory field potentials (Fig. 4, upper traces) could be discerned. In each experiment the optic tract was stimulated at several different intensities, but for the statistical analysis only intensities giving a maximal dLGN monosynaptic (r_1) response were used. Each value represents the median of 12–16 individual responses obtained just before the drug injection and at about the peak of the antiepileptic effect as judged from seizure threshold determinations.

With respect to the dLGN, the injection of 3α-OH-DHP 1.0 mg/kg produced a small but significant decrease in the presynaptic volley (median 90%) and of the monosynaptic excitatory field potential (median 75%; Table II; Fig. 4). The effect occurred without a detectable change in threshold for electrical stimulation of OT fibers. The decrease in the dLGN potentials was fully reversible and their amplitudes had returned to control values at the time of the normalization of the seizure thresholds.

Similar effects were observed at the cortical level. After 1.0 mg/kg of 3α-OH-DHP, the presynaptic volley evoked by OT stimulation was reduced to 83%, and the mono- and disynaptic excitatory field potentials to 76% and 72% respectively (Table II; Fig. 4). These results were based on measurements from surface recordings, but qualitative-

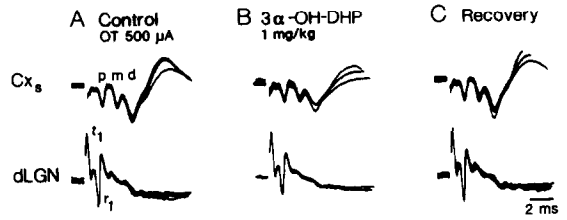

Fig. 4. Effect of 3α-OH-DHP on evoked potentials in the primary visual cortex (Cx_s) and the lateral geniculate nucleus (dLGN). The responses were evoked by electrical stimulation of the ipsilateral optic tract (OT) at 2 Hz and 500 μA. Each pair of records is composed of three superimposed responses, simultaneously recorded in the dLGN and from the cortical surface. Different components of the evoked potential are indicated by letters in A. For the cortical response, p indicates the incoming presynaptic nerve volley, m and d the mono- and disynaptic field potentials respectively; for the dLGN, t_1 is the presynaptic nerve volley and r_1 the monosynaptic field potential evoked by fast conducting (Y type) optic tract fibers. According to conventions positivity is indicated by a downwards deflection in the recordings from the cortical surface and upwards in the recordings from the dLGN. An i.v. injection of 3α-OH-DHP (1 mg/kg) caused a small but significant decrease in all components of the evoked potentials in the dLGN and the cortex (cf. A and B). The effect was fully reversible as shown by the records in C, obtained about 70 min after the injection of 3α-OH-DHP.

TABLE II

Effects of 3α-OH-DHP, progesterone and glycofurol on extracellular field potentials evoked by optic tract stimulation

Substance dose (mg/kg)	n	dLGN volley		dLGN field potential		Cx volley		Cx mono		Cx di	
		median	sign.	median	sign.	median	sign.	median	sign.	median	sign.
3α-OH-DHP 0.1	1	97	NS	106	NS	100	NS	98	NS	92	NS
3α-OH-DHP 1.0	6	90 (82–100)	5/6	75 (53–83)	6/6	83 (67–104)	5/6	76 (63–91)	5/6	72 (42–80)	6/6
3α-OH-DHP 1.0, dLGN stimulation	1	–	–	–	–	92	0/1	81	1/1	66	1/1
Progesterone 18	3	90	1/1	74	1/1	89	1/1	88 (83–94)	2/3	74 (72–83)	2/3
Glycofurol	3	102 (94–108)	1/2↑	104 (98–110)	0/2	105 (100–106)	0/3	111 (108–113)	0/3	100 (100–103)	0/3

All values are given as medians with ranges and calculated in percent of control values. 'sign.' denotes number of infusions with significant reduction ($P<0.05$, Wilcoxon rank sum test) compared to the total number of infusions. ↑, significantly increased in one of two experiments. Cx, cortex; Mono/di, mono- and disynaptic component of the cortically evoked field potential; n, number of infusions.

ly similar changes were observed with intracortical registrations. In one experiment stimulation was performed in the dLGN with comparable results. There was no change in the dLGN or the cortical field potentials after the smallest dose of 3α-OH-DHP (0.1 mg/ml; Table II). Likewise, there was no significant effect of the solvent alone.

Progesterone at a dose of 18 mg/kg exerted very similar effects as 1.0 mg/kg of 3α-OH-DHP with a reduction of the dLGN presynaptic volley to 90% and of the excitatory field potential to 74% (Table II). At the cortical level, the incoming volley was reduced to 89%, and the mono- and disynaptic components of the excitatory field potential to 88 and 74%, respectively. Also, these changes were reversible within the period of the seizure threshold increase.

Since the amplitudes of presynaptic potentials were reduced by both 3α-OH-DHP and progesterone there remained the possibility that the decrease in synaptic potentials was secondary to a reduced afferent input. To eliminate this factor as a possible error, we also calculated the changes in synaptic potentials after corrections for the reduced presynaptic volley. The correction relied on the finding that with stimulation of the OT at different intensities, there was an approximately linear relation between the amplitudes of the pre- and postsynaptic potentials both in the dLGN and the cortex (not illustrated, see Bishop and Davis[4]). Accordingly, control values reduced in proportion to the drug-induced change in the presynaptic volley were used in these calculations.

After such corrections, the injections of 3α-OH-DHP 1 mg/kg reduced the dLGN excitatory field potential in all six experiments to a median value of 84% (Table III). The reduction was significant in five of six individual experiments. Cortically, the monosynaptic component was only minimally reduced, but on a group basis also this effect was significant ($P<0.05$, Wilcoxon signed rank test). The disynaptic component was reduced to 88%. With progesterone 18 mg/kg the dLGN excitatory field potential was reduced to 79%, and the mono- and disynaptic components of the cortical response to 97 and 79%, respectively (Table III).

Inhibition

Double stimulations of the OT with interstimulus intervals of 30–45 ms were used to estimate 3α-OH-DHP and progesterone effects on postsynaptic inhibitory processes in the dLGN and the cortex. As seen in Fig. 5, a conditioning stimulation of the OT produced a clear depression of the postsynaptic test responses both in the dLGN and in the cortex (cf. Fig. 5A and B). At this long interstimulus interval the depression is primarily due to postsynaptic inhibitory processes, in the case of the

TABLE III

Effects of 3α-OH-DHP, progesterone and glycofurol on extracellular field potentials after correction for changes in the incoming volley

Substance dose (mg/kg)	n	dLGN field potential		Cx mono		Cx di	
		median	sign.	median	sign.	median	sign.
3α-OH-DHP 0.1	1	106	NS	98	NS	92	NS
3α-OH-DHP 1.0	6	84 (61–93)	5/6	97 (88–99)	2/6	88 (63–117)	3/6
3α-OH-DHP 1.0, dLGN stimulation	1	–	–	81	1/1	67	1/1
Progesterone 18	3	79	1/1	97 (84–106)	1/3	79 (78–96)	2/3
Glycofurol	3	105 (104–106)	0/2	111 (108–113)	0/3	100 (100–103)	0/3

Details as in Table II.

dLGN mediated by a recurrent inhibitory pathway (see Discussion). The depression was more pronounced after 3α-OH-DHP 1 mg/kg than in the control situation (cf. Fig. 5B and D). This change was taken to indicate an enhanced inhibitory effect. A similar enhancement of inhibition was found also after the injection of progesterone 18 mg/kg. In both cases the effects were fully reversible with about the same time course as for the antiepileptic effect.

The enhanced inhibition by 3α-OH-DHP and progesterone is illustrated graphically in Fig. 6. The graphs show the suppression of the dLGN and cortical postsynaptic test responses by conditioning stimulations at different intensities (A, C, D) or relative amplitudes (B). After both 3α-OH-DHP and progesterone injections, the inhibitory effect was more pronounced at all levels of conditioning stimulations while there was no effect of the solvent alone.

The quantitative analysis of the drug-induced enhanced inhibition was complicated by the fact that both the test and the conditioning responses were directly affected by the drugs. For this reason, two sets of measurements were taken. First, the same combination of conditioning and test stimulation intensities was used before and after injection, ignoring the drug-induced reduction in the synaptic

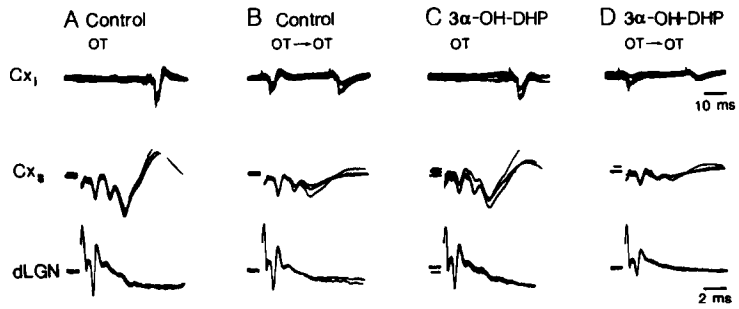

Fig. 5. Effect of 3α-OH-DHP on postsynaptic inhibition in the lateral geniculate nucleus and the visual cortex. The lower pair of records are evoked potentials recorded in the dLGN and from the cortical surface (Cx$_s$) after optic tract stimulation (OT) at 2 Hz and 500 μA (as in Fig. 4). Upper responses (Cx$_i$) are from lamina 2 of the cortex, recorded simultaneously with the lower traces but with a slower sweep speed. Recordings in A and C are unconditioned test responses, those in B and D test responses conditioned by a preceding stimulation of OT at 300 μA. The time interval between conditioning and test stimuli was 30 ms, as seen in the upper records. The conditioning stimulation suppressed the excitatory field potentials both in the dLGN and in the cortex, more so after i.v. injection of 3α-OH-DHP than in the control condition.

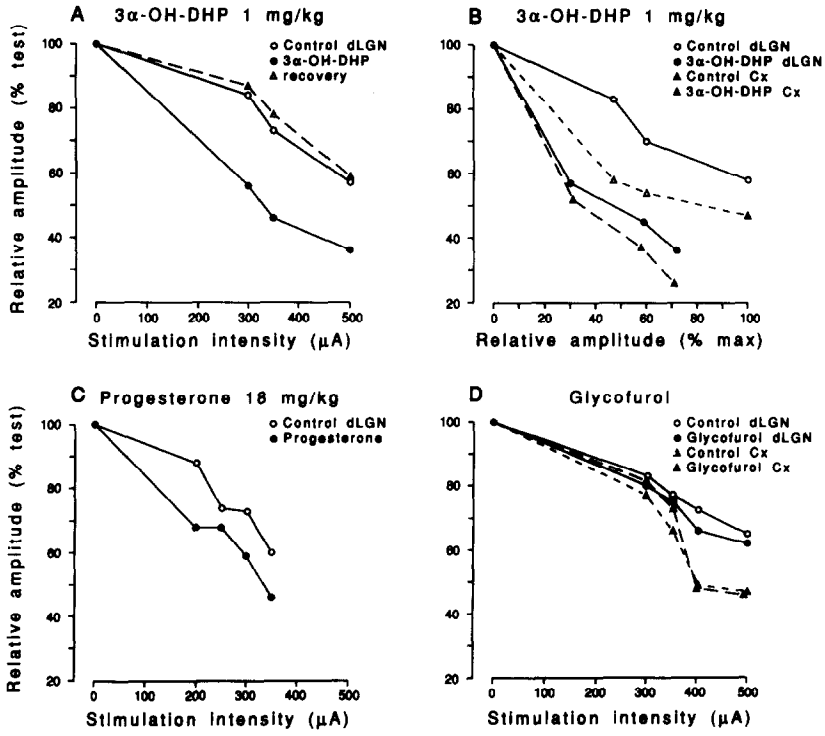

Fig. 6. Effect of 3α-OH-DHP, progesterone and glycofurol on postsynaptic inhibition induced by different intensities of conditioning stimulation. Diagram in A shows the relative amplitude of a test monosynaptic (r_1) field potential in the dLGN plotted against the intensity of conditioning OT stimulation. Each point is the median value of at least 12 individual traces. (B) Relative amplitude of monosynaptic field potentials in the dLGN (r_1) and the cortex (m) plotted against the relative amplitude of the conditioning response, before (open symbols) and after (filled symbols) 3α-OH-DHP. Same experiment as in A. (C, D) Changes in monosynaptic dLGN field potentials induced by conditioning OT stimulation at different intensities (as in A), but before and after i.v. injection of progesterone (18 mg/kg; C) or glycofurol (0.8 ml; D). The diagram in D also includes effects on the monosynaptic field potential in the cortex. 3α-OH-DHP and progesterone enhanced the conditioning effect at all intensities or amplitudes while the solvent glycofurol was without effect.

responses and assuming linearity in the inhibitory effect. The intensities were adjusted to give a 20–40% inhibition of the dLGN monosynaptic response in the control situation before the drug injection. With this procedure, the inhibition in the dLGN was significantly increased from a median value of 24% to 36% of the test responses after 3α-OH-DHP (Table IV). As for the excitatory responses, these values were based on measurements from a large number of individual responses of each category per experiment (typically 12–16). The corresponding values for the mono- and disynaptic components of the cortical response were 36% to 51% and 65% to 93%, respectively. Similar effects were seen at both the dLGN and cortical levels after progesterone 18 mg/kg. Neither a low dose of 3α-OH-DHP (0.1 mg/kg) nor the solvent had any effect on the inhibition.

In the second procedure we adjusted the stimulation intensities so that the amplitudes of the postsynaptic responses evoked by the test and conditioning stimulation were the same before and after drug injections. The aim of this compensation was to ascertain the same level of neuronal activity in the excitatory and inhibitory pathways in the two situations. Thus, this procedure does not require linearity in the interaction between the conditioning and test responses. Also with this matching procedure, the inhibitory effect was significantly increased after the higher doses of 3α-OH-DHP and progesterone (Table IV).

TABLE IV

Effect of 3α-OH-DHP, progesterone and glycofurol on postsynaptic inhibition, uncompensated and matched comparisons (see Results)

Substance dose (mg/kg)	n	dLGN mono		Cortex mono		Cortex di	
		control	drug	control	drug	control	drug
3α-OH-DHP	1	37	34	45	41	–	–
0.1							
3α-OH-DHP	6	24	36*	36	51*	65	93*
1.0		20–42	34–55	25–59	40–71	30–100	64–100
Matched	4	28	50#	42	50$	40	67#
1.0		21–35	39–52	29–52	49–71	22–59	67–75
Progesterone	3	24	38	17	32	39	52
18		–	–	17–19	30–39	33–40	46–56
Matched	2	12	42#	26	41#	34	62#
18		–	–	24–29	35–47	22–47	61–62
Glycofurol	3	25	28	37	30	53	52
		25–44	19–43	33–42	28–37	45–62	41–63

All values are given as medians with ranges and calculated as percent inhibition of unconditioned control values. Mono/di: mono- and disynaptic component of the field potential.

*Significant increase, all experiments, $P < 0.05$, Wilcoxon signed rank test, paired differences.

#Significant increase in all individual experiments, $P < 0.01$, Wilcoxon rank sum test.

$$P = 0.02$$ in one of two experiments.

n = 2 when calculating the matched values for cortex mono- and disynaptic component after 3α-OH-DHP exposure.

Electrocorticography (ECG), blood pressure and heart rate

A visual evaluation of the ECG revealed that the higher doses of both 3α-OH-DHP (1 mg/kg) and progesterone (18 mg/kg) induced a reversible increase in amplitude of an ongoing slow-wave activity. With 3α-OH-DHP the onset was prompt with a clear change in ECG activity occurring already before the infusions were completed, while after progesterone the changes evolved more slowly over several minutes. The recovery was also faster after 3α-OH-DHP as was the case for the antiepileptic effect. There was no obvious ECG effects of the lower doses of 3α-OH-DHP and progesterone or of the solvent. The functional significance of these ECG findings is difficult to evaluate since the cervaux-isolé brainstem transection isolates the brain from major components of the reticular activation system.

There was no systematic change in mean arterial blood pressure or heart rate with the present slow infusions of 3α-OH-DHP or progesterone. Body temperature and respiration were no variables since any change was prevented by a feed-back controlled heating device and artificial respiration.

Discussion

The present in vivo observations confirm and extend earlier findings with respect to the antiepileptic effect of 3α-OH-DHP and progesterone. The main advantage with our model was that it allowed the effects of the drugs on ictal activity to be studied in parallel with their possible mechanisms of action. The test situation may thus be clinically more relevant than experiments in vitro[16,30] or earlier studies in vivo with interictal or seizure activity induced by GABA antagonists[3,22,23,35].

Both progesterone and 3α-OH-DHP markedly increased the seizure threshold in a dose-dependent manner with 3α-OH-DHP being about 20 times as potent as progesterone. Earlier studies have yielded very different results concerning the relative potencies of progesterone and 3α-OH-DHP, although it is generally agreed that pregnanolones are more potent than progesterone. Landgren et al.[23] found that 3α-OH-DHP was about three times as potent as progesterone in reducing penicillin-induced interictal spike activity in vivo. On the other hand, Gee et al.[13] showed 3α-OH-DHP to be nearly 300 times as potent as progester-

one in terms of affinity for the $GABA_A$/benzodiazepine receptor complex in vitro. Our observation is more in agreement with Gyermek et al.[15] who found the 5β analog of 3α-OH-DHP (5β-pregnan-3α-ol-20-one) to be about 10 times as potent as progesterone in producing sleep spindles in the EEG of cats. This metabolite exerts about the same effect as 3α-OH-DHP on interictal spike activity[23].

With intravenous injections 3α-OH-DHP exerted an immediate effect on seizure thresholds while the maximal effect of progesterone was delayed about 20 min. Comparable time differences were found by Smith et al.[36,37] for drug augmentation of GABA-induced inhibitory responses in cerebellar Purkinje cells. The best measure of a rapid onset of the 3α-OH-DHP effect was provided by Landgren et al.[23] who observed a profound depression of interictal epileptiform activity within seconds after intraarterial injections. Such a rapid onset precludes that 3α-OH-DHP acts via 'classical' intracellular hormonal receptors and points to a direct effect on the neuronal membrane. The existence of progesterone plasma membrane receptors within the brain was proposed by Towle and Sze[48] and later a specific 3α-OH-DHP binding site at or near the $GABA_A$/benzodiazepine receptor complex has been characterized[12,45]. The brain contains the necessary enzymes to convert progesterone to 3α-OH-DHP and production of 3α-OH-DHP from progesterone has also been demonstrated (see Majewska[29]). It is thus possible that progesterone serves as a precursor for the more potent 3α-OH-DHP in the brain. The time difference in onset and the relatively high potency of progesterone in vivo compared to the in vitro receptor affinity[13] may suggest that such a conversion is important for the antiepileptic effect of progesterone.

A limitation with the present study was that we were unable to measure the relevant intracerebral concentrations of 3α-OH-DHP or progesterone. A rough estimate would suggest, however, that the effective doses of both substances resulted in brain concentrations well above the physiological range (reported to be 9 and 0.8 ng/g brain tissue in the rat for 3α-OH-DHP and progesterone, respectively[2]). The high threshold doses should not be taken to exclude a role of 3α-OH-DHP in catamenial epilepsy. Note that healthy non-estrous animals with intact, non-epileptic brains and normal inhibitory mechanisms were used for the study. It is quite possible that patients with epilepsy and possibly impaired inhibitory systems might be much more susceptible to changes in brain 3α-OH-DHP levels.

Although the effective dose of 3α-OH-DHP may be high in physiological terms, it is low when compared to most antiepileptic drugs in clinical use[27]. For instance, 3α-OH-DHP was about five times as potent as carbamazepine concerning the effect on seizure thresholds in the present model[41]. Furthermore, it is about 20 times as potent as phenobarbital on inhibitory mechanisms in hippocampal slices in vitro[40]. With such a potency, 3α-OH-DHP or related neurosteroids might have a future in the therapeutic arsenal against catamenial epilepsy[13,23].

A considerable drawback with 3α-OH-DHP as an antiepileptic drug is its extremely short duration of action. To be clinically useful a more long-lasting derivative or a slow-release preparation would have to be developed. Another problem might be a possible sedative effect. In the present study, we observed a clear increase in amplitude of the slow-wave EEG activity at effective doses and similar changes have been reported for cats with the 5β analog (5β-pregnan-3α-ol-20-one) in the same dose range[15,19]. Since these experiments involved preparations with reduced afferent inflow (cervaux-isolé and/or paralyzed) the findings cannot be directly extrapolated to intact animals. However, the related steroid 3α-hydroxy-5α-pregnane-11,20-dione (alphaxalone) produce a brief period of sleep in cats after 1 mg/kg i.v.[6]. Possibly, the sedative effect may be less pronounced with more long-lasting derivatives, as is the case for barbiturates.

Three main observations may explain the antiepileptic effect of progesterone and 3α-OH-DHP: (1) a small, but significant reduction in the presynaptic nerve volleys, (2) a reduction in the postsynaptic excitatory field potentials in dLGN and cortex, and (3) an enhanced postsynaptic inhibition. All these changes appeared simultaneously within the same dose and time intervals as the drug-induced increase in seizure thresholds, indicating a causal

relationship. While an increased inhibition could be anticipated from several other studies, the effect on the presynaptic volley and the excitatory field potentials was unexpected. In the following, we will discuss each of these findings separately.

Postsynaptic inhibition involving GABA as the transmitter and $GABA_A$ postsynaptic receptors plays an important role for the normal function of both the dLGN and the primary visual cortex. It is well established that a weakening of these inhibitory mechanisms, as may be obtained experimentally by local application of the $GABA_A$ antagonists bicuculline and penicillin, lowers the seizure thresholds in vivo. Conversely, enhanced GABAergic inhibition results in seizure threshold increases. In this study, an indirect method with double stimulations and extracellular recordings of field potentials was used to assess changes in the strength of these inhibitory mechanisms. Admittedly, the indirect procedure is rather complicated since a decrease in the conditioned test response may result from several processes in addition to postsynaptic inhibition, i.e., relative refractoriness of presynaptic fibers, temporal suppression of transmittor release, afterhyperpolarization in the postsynaptic cells. For the dLGN, these processes are rather short-lasting and a long interstimulus interval was therefore used to avoid these complications. At intervals of 30–45 ms, a depression of the conditioned test response can safely be ascribed to postsynaptic inhibition. Furthermore, it can be concluded that the inhibition involves a recurrent inhibitory mechanism since the dLGN feed-forward inhibition has a much shorter duration[24]. The same argument seems to be valid for the mono- and disynaptic components of the cortical field potentials although the inhibitory mechanisms at this level are primarily of the feed-forward type[8].

In our study the conditioned test responses, both in the dLGN and in the cortex, were more depressed after injections of 3α-OH-DHP or progesterone. This enhanced inhibition was observed simultaneously with an effect on seizure thresholds. The increase was of the same order of magnitude whether a matched test procedure or a simpler one assuming linearity of interaction was used (see Results). Cortically, the inhibitory effect was most pronounced at the disynaptic component of the field potential, emphasizing the importance of a cumulative effect in a polysynaptic neuronal chain.

Several in vitro studies have demonstrated that progesterone and 3α-OH-DHP enhance the inhibitory effect of topically applied GABA[16,30,33,36,37]. This enhancement was first described by Majewska et al.[30] who found that 3α-OH-DHP increased both the peak amplitude and duration of a GABA-activated inward Cl^- current in rat hippocampal neurons in culture. Recently, these observations have been extended to studies of synaptically released transmitter. In interaction tests analogous to the present one, both Luntz-Leybman et al.[26] and Taubøll and Gjerstad[40] found that 3α-OH-DHP enhanced postsynaptic inhibition in hippocampal neurons in vitro (see also Landgren[21]).

Also the reduction of unconditioned postsynaptic excitatory field potentials in the dLGN and the cortex by 3α-OH-DHP and progesterone might be due to an enhanced GABAergic inhibition. These field potentials are generated by the current flow at excitatory synapses and by spike activity in postsynaptic neurons. The latter component might be reduced by an enhanced tonic inhibition that prevents the spike initiation. Such tonic inhibition would be expected from the ongoing spontaneous activity (without visual stimulation) of retinal ganglion cells and dLGN neurons. In addition, it is well known that the excitation of both dLGN and cortical neurons increases after bicuculline blockade of GABAergic inhibition.

For several reasons we do not believe that an enhanced GABAergic inhibition is the only explanation for the reduced excitatory field potentials. This reduction mimicks the effect of barbiturates in the same system. But barbiturates have, in addition to an effect on $GABA_A$ receptors, presynaptic actions. They reduce the transmitter release, presumably by affecting the presynaptic calcium entry[7,17,28,39,46]. In a study of monosynaptic excitatory transmission in the spinal cord Løyning et al.[28] found that the barbiturate-reduced transmitter release was associated with a small decrease in the presynaptic nerve volley. A similar decrease in the presynaptic nerve volley was found after both 3α-OH-DHP and progesterone in the present study. The change was small, but clearly signifi-

cant and reversible within the same time course as the antiepileptic effect of the drug. This change could not be explained by a reduced number of activated afferent fibers since the threshold for electrical stimulation of the optic tract fibers remained the same. In vitro, similar presynaptic effects of 3α-OH-DHP have recently been described in the hippocampus with reduced presynaptic volley and field excitatory postsynaptic field potential after 3α-OH-DHP exposure[21]. Thus, it is tempting to suggest that 3α-OH-DHP and progesterone reduced the dLGN and cortical field potential in a barbiturate-like manner by affecting both postsynaptic GABAergic inhibition and the presynaptic transmitter release.

All synaptic changes induced by 3α-OH-DHP and progesterone were quite moderate compared to their pronounced effect on seizure thresholds. Remember, however, that these measurements refer to only one or a few synaptic steps. For a focal seizure to be initiated polysynaptic excitatory loops have to be activated. In the visual cortex, these loops are well characterized and consist of at least four excitatory neurons in series with associated inhibitory neurons[18] (Fig. 1). When the excitation spreads around such polysynaptic loops, small changes at each level will accumulate and add up to a substantial change in the overall excitability of the neuronal network. Thus, it is easy to understand that the modifications induced by 3α-OH-DHP and progesterone can lead to a marked increase in the seizure threshold by preventing the initiation of reverberating epileptic activity in these loops.

In conclusion, progesterone and its brain metabolite 3α-OH-DHP exerted potent antiepileptic effects in vivo. At effective doses, they enhanced GABAergic inhibitory mechanisms in the dLGN and the visual cortex. They also depressed excitatory evoked potentials, possibly by a presynaptic effect on the transmitter release. Electrophysiologically, these effects closely resemble those of barbiturates. The strong antiepileptic effect and low toxicity[3] of brain active progesterone metabolites may give such steroids a future as a new class of antiepileptic drugs.

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