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Assessment of neuroleptic-like properties of progesterone

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Abstract There is considerable evidence from epidemiological studies that the onset of psychiatric disorders may be related to changes in the secretion of gonadal hormones. For example, the postpartum period appears to be a vulnerable phase for the occurrence of psychiatric disturbances such as dysphoric mood and even severe psychotic disturbances. It has been suggested that a sudden drop in progesterone concentrations may contribute to the development of such disorders. Because the administration of this steroid might be of therapeutic value in psychiatric disturbances, we investigated the behavioral properties of progesterone in the rat to assess putative neuroleptic-like properties of this steroid. Progesterone administration dose-dependently increased the EEG activity during wakefulness in the 10- to 30-Hz frequency bands and decreased locomotor activity. While no anxiolytic activity could be detected in the plus maze, the highest dose of progesterone (90 mg/kg) exerted an inhibitory effect on the conditioned avoidance response. In contrast to haloperidol (0.5 mg/kg), progesterone neither produced catalepsy nor antagonized amphetamine-induced stereotypy. However, both progesterone (10, 30 and 90 mg/kg) and haloperidol (0.1 mg/kg) effectively restored the disruption of the prepulse inhibition (PPI) of the acoustic startle response (ASR) that was evoked by apomorphine (2 mg/kg). In contrast, allopregnanolone (10 mg/kg), one of the main metabolites of progesterone, did not significantly antagonize the effect of apomorphine on the PPI. This behavioral profile of progesterone is compatible with the sedative properties of its metabolite allopregnanolone via the $GABA_A$ receptor, but also with the possibility that progesterone itself shares some properties with atypical antipsychotics, which may be relevant for the development and treatment of psychotic disturbances.

Key words Progesterone · Neuroleptic · Psychosis · Neurosteroids · Schizophrenia

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Introduction

Considerable evidence accumulates indicating that the gonadal steroid progesterone, besides its role in reproductive endocrinology, has psychotropic properties. Administration of progesterone in various mammalian species has revealed analgesic, anxiolytic and sleep modulating effects (Kavaliers and Wiebe 1987; Bitran et al. 1993, 1995; Lancel et al. 1996). In humans, hypnotic (Merryman et al. 1954), sleep modulating (Friess et al. 1997) and anticonvulsant actions (Bäckström et al. 1984) have been found after intravenous or oral administration of progesterone. Some of the central nervous effects of progesterone have been attributed in part to the conversion of progesterone into neuroactive steroids that allosterically modulate the $GABA_A$ receptor (Paul and Purdy 1992; Lambert et al. 1995; Rupprecht 1997). Moreover, progesterone may act as a functional antagonist at the 5-HT_3 receptor (Wetzel et al. 1998). Thus, progesterone itself may also possess psychotropic properties.

Epidemiological studies suggest that the onset of psychiatric symptoms may be related to changes in the secretion of gonadal hormones (Häfner et al. 1993; Hallonquist et al. 1993). For example, the occurrence of clinical symptoms in schizophrenia has been shown to vary across the menstrual cycle (Hallonquist et al. 1993). Moreover, there is a difference between pre- and postmenopausal women, with an increased vulnerability for the onset of schizophrenic episodes after the menopause (Häfner et al. 1993). In addition, variations in the secretion patterns of progesterone may be associated with mood disturbances and impairment of physical well-being, alertness and sleep. The frequently reported fatigue during early pregnancy may be related to the elevated levels of progesterone (Biedermann and Schoch 1985), whereas the postpartum period appears to be an especially vulnerable phase for the occurrence of psychiatric disorders. Dysphoric mood and even severe psychotic disturbances may occur after delivery, which has been attributed to a postpartal "withdrawal" of progesterone secretion (Harris et al. 1994; Brockington and Meakin 1994). Thus, a sudden drop of progesterone concentrations may contribute to the development of such disorders.

Because the administration of progesterone might be of therapeutic benefit in psychotic disturbances, we evaluated the behavioral profile of progesterone to assess the putative neuroleptic-like properties of this steroid.

Materials and methods

Animals

In order to avoid interference of fluctuations of gonadal steroids throughout the menstrual cycle with the steroids administered, male Wistar rats (308 animals in total) (Charles River, Germany) weighing 250–300 g were used. They were housed in groups of six in plastic cages on a 12-h light/dark cycle (light on 7 a.m.) with water and food available ad libitum. For the EEG experiments, the animals were housed individually in a ventilated, sound-attenuated Faraday room. Ambient temperature and humidity were kept constant. The conditioned avoidance response was assessed in rats of the Roman High Avoidance (RHA) strain as described previously (Bartoszyk et al. 1996). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body.

EEG analysis

To assess the influence of progesterone on EEG activity during wakefulness, eight rats were implanted with EEG and EMG electrodes under deep anesthesia. At least 2 weeks were allowed for recovery from surgery and 4 days for adaption to the recording conditions. The animals received three randomized treatments consisting of 10 mg/kg, 30 mg/kg and 90 mg/kg progesterone dissolved in 35% hydroxypropyl-ß-cyclodextrin (Besins Iscovesco Laboratoires, Paris, France) administered IP at light onset. On the day preceding each treatment, vehicle consisting of 35% hydroxypropyl-ß-cyclodextrin was administered. The same progesterone and vehicle solutions were used in all other experimental conditions. EEG and EMG were continously recorded during the first 12 post-injection hours and subsequently analyzed in 10-s epochs. The EEG was subjected to a spectral analysis as described previously (Lancel et al. 1996). Average EEG power densities were computed over all 10-s epochs of wakefulness during the first 4 post-injection hours in two 2-h time intervals. For standardization, the 2-h data were expressed as percentage of the average power density in the same frequency band during wakefulness throughout the entire 12-h vehicle recording period and were then log transformed.

Open field test

Rats were tested in an open field for forward locomotion following either vehicle or progesterone injection (10, 30, 90 mg/kg IP at 10 a.m.). The open field consisted of six activity chambers (each $60 \times 60 \times 40$ cm). A video motility system (TSE system, Bad Homburg, Germany) provided the activity measurement of six animals simultaneously at 10-min intervals (Shoaib et al. 1995). Motor activity was measured for 240 min and was expressed as the total distance (cm) travelled.

Elevated plus-maze test

The plus-maze consisted of a cross with two arms that were open to the environment and two arms that were enclosed by side and end walls, which were connected by a central area. At the start of the tests, each animal was placed in the central area facing a closed arm. The behavior of the animals was scored for 5 min via a camera. The entries into the open arms, expressed as percent ratio of entries in open arms/total number of entries in all arms, and the time spent in open arms, expressed as percent ratio of time in open arms/total time spent in all arms, as well as the overall activity shown by the total number of entries in closed arms were recorded. A reduced open-arm exploration served as an index of increased anxiety. Four hours prior to the behavioral testing the rats received an IP injection of either vehicle or progesterone (10, 30, 90 mg/kg) at 7 a.m. Diazepam (1.5 mg/kg IP) served as a reference substance in a separate experiment. In another set of experiments rats were first exposed to the social defeat paradigm. Reportedly, 10 min of social defeat is sufficient to increase anxiety-related behavior, and the effects of anxiolytic drugs are more pronounced in defeated rats (Liebsch et al. 1995). The procedure started by placing a rat in the home cage of a dominant male resident and its female, the male having been trained for several weeks to be aggressive to intruders. The intruder was attacked by the resident within the first minute of the encounter and continuously threatened and attacked during the following 10 min. Defeat was considered successful when the intruder had been attacked at least once and had shown at least two submissive body postures, e.g. lying on its back or defensive upright body posture. Each resident was only used twice to prevent a decrease of aggression intensity. Immediately after social defeat, the rats were tested in the plus-maze.

Inhibition of the conditioned avoidance response (CAR)

The inhibition of the CAR was assessed as described previously (Bartoszyk et al. 1996). Briefly, rats were trained and maintained 5 days a week to avoid electric foot-shock (0.5 mA for a maximum of 10 s) in an electromagnetically controlled two-way shuttle box. Then they were tested in four consecutive trials each consisting of an intertrial interval of 30 s followed by a 5-s conditioned stimulus (CS; light). Performance of two or fewer avoidance responses during CS was considered as a drug-induced inhibition of avoidance behavior. The rats were tested 1 h following intraperitoneal injection of vehicle, haloperidol (0.3 mg/kg) (RBI, Köln, Germany),

progesterone (10, 30, 90 mg/kg IP) or allopregnanolone (10, 15, 30 mg/kg IP) at 10 a.m. Allopregnanolone was dissolved in 35% hydroxypropyl-ß-cyclodextrin as described previously for progesterone.

Assessment of catalepsy

Rats were tested for catalepsy after IP injection at 10 a.m. of either 0.5 mg/kg haloperidol (RBI, Köln, Germany), progesterone (90 mg/kg) or vehicle alone. The degree of catalepsy was assessed 60, 90 and 240 min following injection in three tests performed in the following order: 1) bar: both forelegs were placed on a horizontal bar (9 cm above the surface), 2) podium: one foreleg was placed on a podium (3 cm high), 3) grid: the animal was clinged to a vertical wire grid. The latency from paw placement until complete removal of one paw from a support was measured (maximum 120 s) and termed descent latency.

Assessment of amphetamine-induced stereotypy

Animals were subcutaneously injected with 5 mg/kg *d*-amphetamine (Sigma, Deisenhofen, Germany) or saline. Amphetamine-induced stereotypy was measured by a rating scale according to Kelly et al. (1975). Rating was conducted by an observer, who was unaware of the treatment, at 15, 30, 45, 60 and 90 min following injection of vehicle, amphetamine, progesterone (90 mg/kg IP) at 10 a.m. or a combination of amphetamine and progesterone.

Prepulse inhibition experiments

R(-)-Apomorphine HCl (RBI, Köln, Germany) was dissolved in distilled water containing 0.1% ascorbic acid, and 2 mg/kg was injected IP immediately before testing. Haloperidol (RBI, Köln, Germany) was dissolved in distilled water containing 0.1% ascorbic acid and 0.1 mg/kg (IP) was injected 30 min before testing. Progesterone (3, 10, 30, 90 mg/kg) or allopregnanolone (10 mg/kg) was injected IP at 10 a.m., 60 min before testing. Separate experiments were performed for each progesterone dose.

Animals were tested on 6 days and received each of the following treatments in a counterbalanced way according to a within-subjects design: ascorbate/vehicle, apomorphine/vehicle, apomorphine/progesterone or allopregnanolone, apomorphine/haloperidol, haloperidol/vehicle, ascorbate/progesterone or allopregnanolone.

After administration of the drugs the rats were placed in the test chamber and the test session started following a 5-min acclimation period during which they received no stimuli with the exception of a continuous white background noise of 55 dB sound pressure level (SPL). The acoustic startle response (ASR) was measured in a wire mesh cage ($20 \times 10 \times 12$ cm) provided with a steel-plate floor mounted on a piezoelectric accelerometer inside a sound-attenuated chamber (Koch and Bubser 1994). The voltage output of the accelerometer caused by the rat's motion was amplified, digitized, and fed into a computer for further analysis. Acoustic stimuli were generated by a computer using a function-synthesizer (Hortmann, Neckartenzlingen, Germany) and were delivered through a loudspeaker mounted at a distance of 40 cm from the test cage. All intensity measurements were performed with a 1/2 inch condenser microphone and a measuring amplifier (Brüel & Kjaer, Copenhagen, Denmark) after bandpass filtering outside the hearing range of the rat (lower cutoff: 250 Hz, upper cutoff: 80 kHz). The whole-body ASR amplitude was calculated from the difference between the maximum voltage output of the accelerometer during 80 ms after, and 80 ms before the onset of the acoustic startle stimulus. The test session included an initial startle stimulus followed by four different trial types given in a pseudorandom order: 1) pulse alone (100 dB SPL broad band noise bursts, 20 ms duration), 2) prepulse (70 dB SPL 10 kHz tone pulse, 20 ms duration, including 0.4 ms rise/fall times) followed by a noise pulse 100 ms after prepulse-onset, 3) prepulse alone and 4) no stimulus. Background noise intensity was 55 dB SPL. A total of five presentations of each trial type was given with an interstimulus interval of 30 s. Prepulse inhibition (PPI) was measured as the difference between the pulse-alone trials and the prepulse-pulse trials and expressed as percent PPI [100 × (mean ASR amplitude on pulse-alone trials – mean ASR amplitude on prepulse-pulse trials/mean ASR amplitude on pulse-alone trials)]. The response to the single pulse at the beginning of the test session was discarded.

Determination of progesterone and allopregnanolone in rat brain and plasma

All injections were given IP at the indicated concentrations at lights on. Plasma and brain tissue was collected as described previously (Lancel et al. 1996) at baseline and then 15 min, 1, 3, 5 and 9 h following progesterone injection. Progesterone was quantified by a radioimmunoassay after HPLC separation and liquid-liquid extraction from the biological matrix as described in detail elsewhere (Lancel et al. 1996). The lower detection limit was 0.12 ng/ml for plasma and 2.8 ng/g for brain tissue. The coefficient of variation was 2% for plasma and 8% for brain tissue. Quantification of allopregnanolone was performed by combined gas chromatography/mass spectrometry as described previously (Lancel et al. 1996). The lower detection limit was 0.5 ng/ml for plasma and 2.5 ng/g for brain tissue.

Statistics

EEG data and locomotor activity were analyzed with a two-way analysis of variance (ANOVA) with repeated measures followed by post-hoc testing with a two-sided paired *t*-test. Plus-maze behavior was analyzed using the Mann-Whitney *U*-test. The results of the CAR were analyzed using Fisher's exact test. The effects of the different treatments on catalepsy, amphetamine-induced stereotypy, on % PPI and on the ASR amplitude in the absence of a prepulse were analyzed with a one-way ANOVA with repeated measures followed by Tukey´s *t*-test for post-hoc comparisons.

Results

Effects of progesterone administration on EEG power density during wakefulness

For a first screening of the potential psychopharmacological properties of progesterone, we used the spectral analysis of EEG recordings. There was a dose-dependent increase in the EEG power densities in the frequency bands between 10 and 30 Hz following progesterone administration, which was most pronounced after the highest progesterone dose (Fig. 1). This increase in EEG activity was most prominent in the first 2 post-injection hours.

Effects of progesterone administration on locomotor activity

The open field test was employed to study the effects of progesterone on the locomotor activity of the rats.

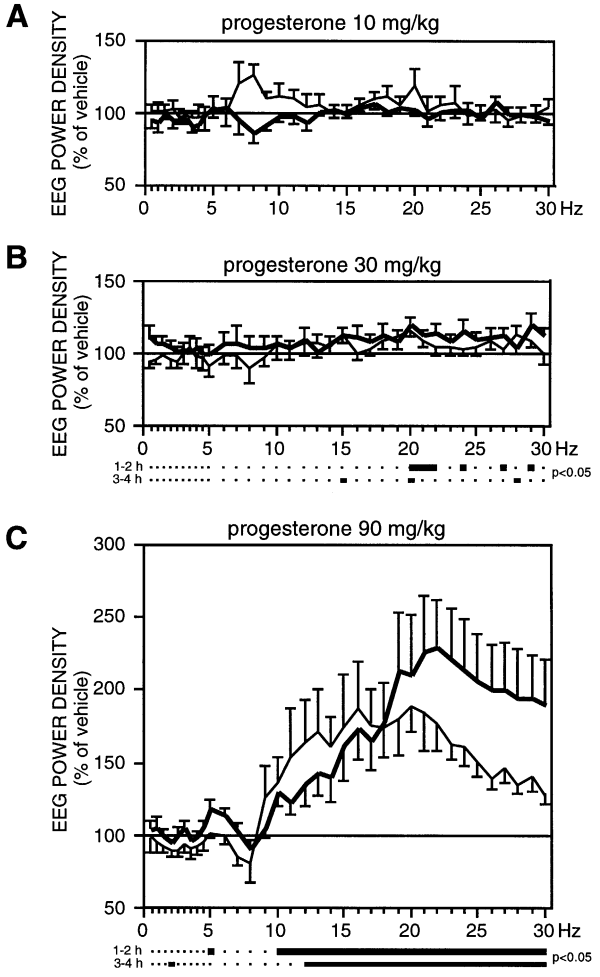

Fig. 1A–C EEG power densities during wakefulness over the first two 2-h intervals (*thick lines*: hours 1 and 2, *thin lines*: hours 3 and 4) following administration of 10 mg/kg progesterone IP **A**, 30 mg/kg progesterone IP **B**, and 90 mg/kg progesterone IP **C**. *Curves* connect mean values ($n = 8$, mean ± SEM). For plotting purposes, the data were expressed as percentage of the corresponding vehicle values (vehicle = 100%). *Bar*s below the *abscissa* indicate frequencies for which ANOVA for repeated measures and the two-sided, paired *t*-test yielded significant effects ($P < 0.05$, run on standardized and log transformed values)

Compared with vehicle, the administration of 10 mg/kg progesterone did not result in significant changes in spontaneous activity. However, the higher doses of progesterone prominently and dose-dependently decreased locomotor activity. This decrease started 20 min following progesterone injection and persisted over the entire observation period (data not shown). Fig. 2 depicts the total distance travelled by the animals following either vehicle or progesterone injection.

Effects of progesterone administration on the behaviour in the elevated plus-maze test

The putative anxiolytic properties of progesterone administration were assessed in the elevated plus-maze.

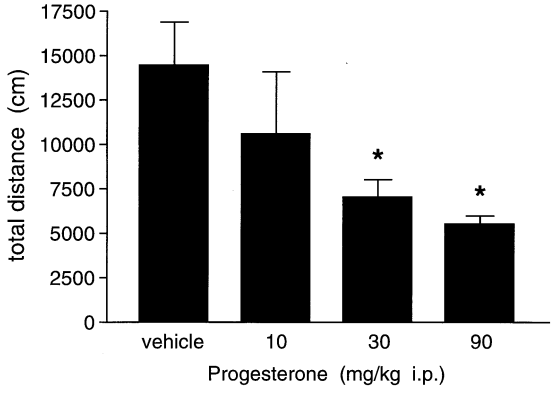

Fig. 2 Effects of progesterone (10, 30, 90 mg/kg IP) on locomotor activity in the open field test. The total distance travelled was cumulated over 240 min. Mean ± SEM ($n = 8$ – 14) are presented. *Asterisks* indicate significant differences ($P < 0.01$) from the vehicle group

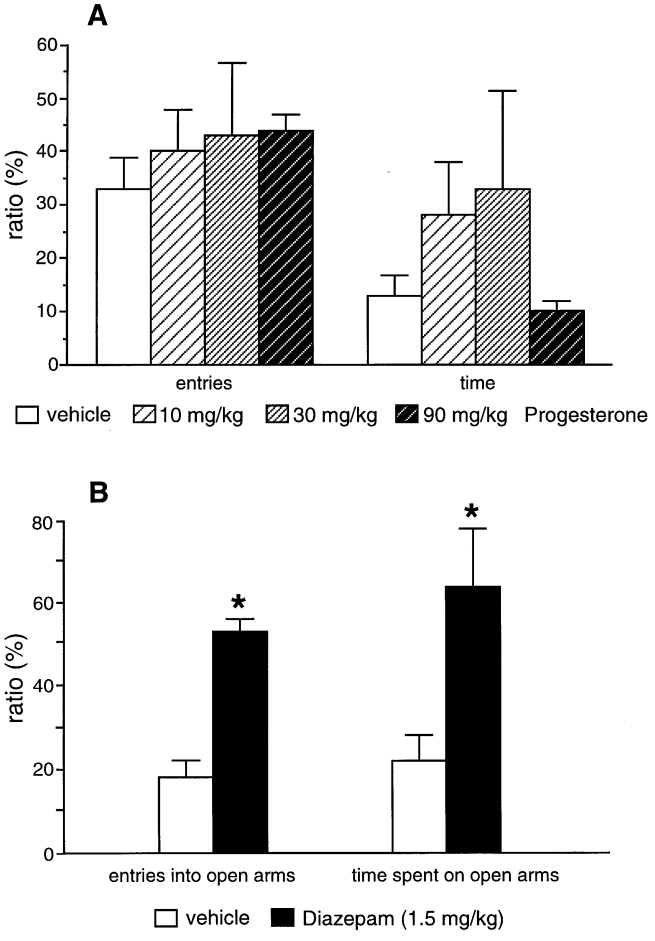

Fig. 3A,B Effects of progesterone administration (vehicle, *open bars*; 10 mg/kg, *hatched bars*; 30 mg/kg, *shaded bars*; 90 mg/kg, *dark bars*) **A** and diazepam (1.5 mg/kg, *dark bars*) **B** in the elevated plus-maze test. All groups were subjected to 10 min of social defeat prior to testing. The *bars* indicate the percent ratio of the time spent in the open arms from the total time spent in all arms and the percent ratio of the entries the made into the open arms from the total number of entries made in all arms. Mean ± SEM ($n = 8$) are presented. *Asterisks* indicate a significant difference ($P < 0.05$) from the vehicle group

No significant effect could be demonstrated for any of the three progesterone doses when the animals were tested in the elevated plus-maze only (data not shown). We therefore investigated the effects of progesterone administration in the elevated plus-maze after the animals had been exposed to a social defeat that has been shown to be more sensitive in detecting anxiolytic effects of drugs than the elevated plus-maze test alone (Liebsch et al. 1995). However, no anxiolytic effects of progesterone could be shown in this test paradigm either whereas the reference substance diazepam exerted a significant anxiolytic activity in the same test paradigm (Fig. 3).

Effects of progesterone and allopregnanolone on the conditioned avoidance reaction (CAR)

As a reduction in locomotor activity can be induced also by antipsychotic agents, we used the conditioned avoidance reaction (CAR), which is frequently used to screen for neuroleptic effects of psychopharmacological drugs, to evaluate possible neuroleptic-like properties of progesterone. While none of the animals treated with vehicle showed an inhibition of the CAR, the injection of haloperidol (0.3 mg/kg) inhibited the CAR in 15 of 16 animals (93.7%) ($P < 0.01$). There was an inhibition of the CAR in ten of 16 animals (62.5%) ($P < 0.05$) following administration of 90 mg/kg progesterone, whereas the inhibition of the CAR was not significant with the lower progesterone doses. Moreover, the CAR was inhibited in seven of ten animals (70%) ($P < 0.05$) following injection of 30 mg/kg allopregnanolone, while the lower doses did not significantly affect the CAR.

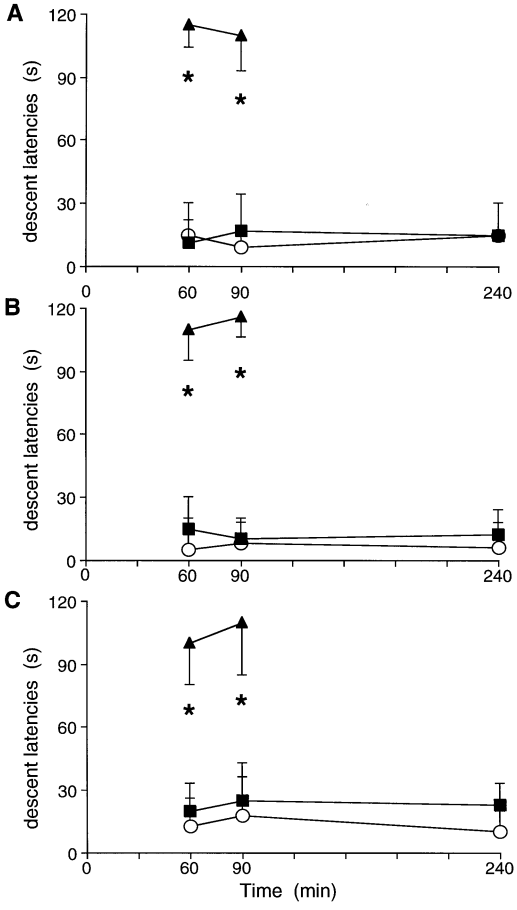

Fig. 4A–C Effects of haloperidol (0.5 mg/kg, *closed triangles*) progesterone (90 mg/kg, *closed squares*) and vehicle (*open circles*) on descent latencies at different time points. The time until complete removal of one paw was measured (descent latency) from *horizontal bar* **A**, podium **B** and vertical wire grid **C**. Mean ± SEM ($n = 8$) are presented. The *asterisks* indicate signifcant differences ($P < 0.01$) from the vehicle control

Effects of progesterone administration on catalepsy and on amphetamine-induced stereotypy

Because the effect of progesterone on the CAR may be caused by its sedative properties we studied whether progesterone is also effective in other test paradigms used for screening of putative antipsychotic agents. Classical neuroleptics like haloperidol produce extrapyramidal side effects that can be predicted by the induction of catalepsy and an antagonism of the amphetamine-induced stereotypy in animal studies. Accordingly, the reference substance haloperidol was effective in producing catalepsy as assessed by descent latencies in various tests (Fig. 4). Descent latencies following injection of progesterone (90 mg/kg IP) did not differ from those after a vehicle injection at all time points of observation. Thus, progesterone did not induce cataleptic effects.

The administration of amphetamine resulted in a significant increase in stereotype behavior compared to saline injection ($P < 0.01$). The amphetamine-induced stereotypy, however, was unaffected by progesterone administration (90 mg/kg IP) (Fig. 5).

Effects of progesterone and allopregnanolone administration on acoustic startle response (ASR) and prepulse inhibition (PPI)

In contrast to classical neuroleptics, atypical neuroleptics like clozapine are nearly devoid of extrapyramidal side effects and therefore ineffective in the respective test paradigms. Therefore, we used the prepulse inhibition of the acoustic startle response and its disruption by apomorphine as an animal model because both classical and atypical neuroleptics have been shown to be effective in antagonizing the effects of apomorphine in this behavioral paradigm. A one-way repeated measures ANOVA revealed a significant effect of treatment on the PPI [$F_{(5,55)} = 10.7$, $P < 0.01$ for testing

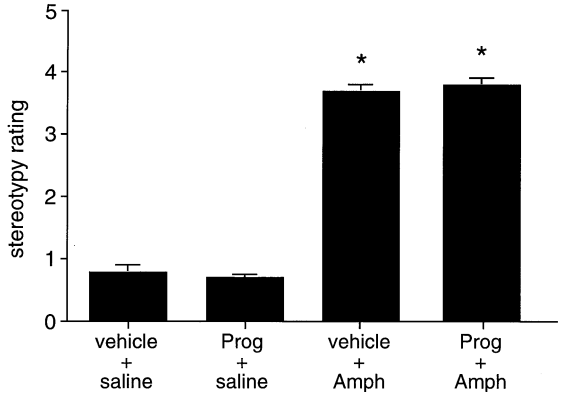

Fig. 5 Effects of progesterone (90 mg/kg IP, *Prog*) on amphetamine (*Amph*)-induced stereotypy. The *asterisks* indicate significant differences ($P < 0.01$) from the vehicle control. Mean ± SEM ($n = 8$) are presented

3 mg/kg progesterone, $F_{(5,65)} = 14.2$, $P < 0.01$ for testing 10 mg/kg progesterone, $F_{(5,65)} = 23.1$, $P < 0.01$ for testing 30 mg/kg progesterone and $F_{(5,65)} = 8.1$, $P < 0.01$ for testing 90 mg/kg progesterone]. Post-hoc comparisons showed a significant decrease of the PPI after apomorphine ($P < 0.01$), which was effectively antagonized by pretreatment with both progesterone (30 mg/kg IP: $P < 0.01$; 10 mg/kg, 90 mg/kg IP: $P < 0.05$) and haloperidol ($P < 0.01$) (Fig. 6). However, progesterone failed to antagonize the apomorphine-induced disruption of the PPI at a dose of 3 mg/kg. No effects of progesterone or haloperidol on the PPI itself were detected (Fig. 6). The ASR amplitude in the absence of a prepulse (pulse-alone trials) was significantly increased by treatment with apomorphine ($P < 0.05$) and this effect was antagonized by pretreatment with haloperidol ($P < 0.01$), but not with progesterone. In contrast, progesterone (90 mg/kg) reduced the ASR amplitude in the absence of apomorphine ($P < 0.05$). To assess the effects of allopreganolone on the PPI and its disruption by apomorphine we injected 10 mg/kg allopregnanolone IP in the same experimental setting as described previously for progesterone. A one-way repeated measures ANOVA revealed a significant treatment effect on the PPI [$F_{(5,55)} = 5.4$, $P < 0.01$]. Post-hoc comparisons showed again a significant decrease of the PPI after apomorphine ($P < 0.01$) that was effectively reversed by haloperidol ($P < 0.05$) but not by allopregnanolone (Fig. 7). Moreover, similar to progesterone, allopregnanolone did not significantly affect the PPI itself.

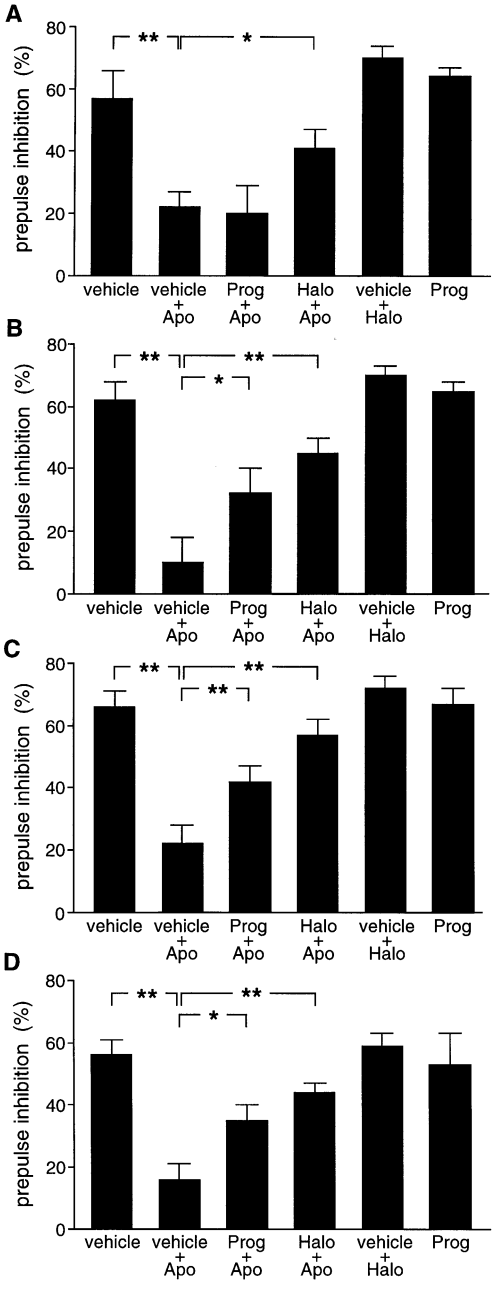

Fig. 6A–D Effects of progesterone administration (*Prog*) in relation to haloperidol (0.1 mg/kg, *Halo*) on the PPI and its disruption by apomorphine (2 mg/kg, *Apo*). **A** Progesterone 3 mg/kg IP ($n = 12$). **B** 10 mg/kg progesterone IP ($n = 14$). **C** Progesterone 30 mg/kg IP ($n = 14$). **D** 90 mg/kg Progesterone IP ($n = 14$). Mean ± SEM are presented. The *asterisks* indicate significant differences from the vehicle plus apomorphine condition (*$P < 0.05$, **$P < 0.01$)

Pharmacokinetics of progesterone and allopregnanolone

The administration of progesterone resulted in a sharp increase in both progesterone and allopreganolone concentrations in plasma and brain tissue with a maximum at 15 min following progesterone injection (Fig. 8). Progesterone and allopregnanolone concentrations rapidly declined thereafter but remained elevated over baseline levels for several hours. This increase was dose dependent for both progesterone and allopregnanolone.

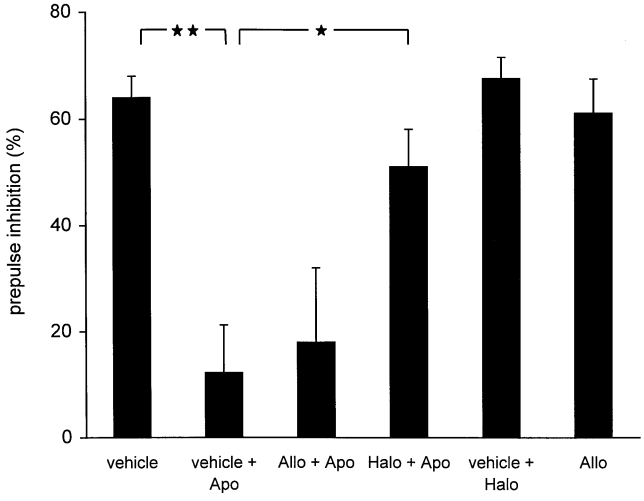

Fig. 7 Effects of allopregnanolone administration (10 mg/kg, *Allo*) in relation to haloperidol (0.1 mg/kg, *Halo*) on the PPI and its disruption by apomorphine (2 mg/kg, *Apo*). Mean ± SEM ($n = 12$) are presented. The *asterisks* indicate significant differences from the vehicle plus apomorphine condition (*$P < 0.05$, **$P < 0.01$)

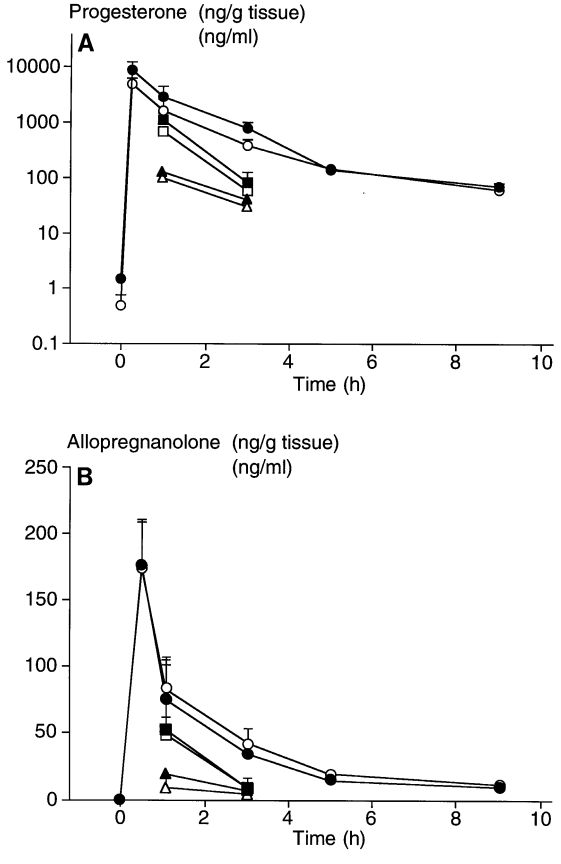

Fig. 8A,B Pharmacokinetics of progesterone **A** and allopregnanolone **B** in plasma (*open symbols*) and brain (*closed symbols*) following progesterone administration (*triangles*: 10 mg/kg, *squares*: 30 mg/kg, *circles*: 90 mg/kg). Mean ± SEM ($n = 6$) are presented

Discussion

Following progesterone administration, a dose-dependent enhancement of high-frequency (≥ 10 Hz) EEG activity during wakefulness, a dose-dependent decrease of spontaneous activity, a lack of anxiolytic and cataleptic activity, an inhibitory effect on the CAR at the highest progesterone dose, the absence of an antagonizing effect on the amphetamine-induced stereotypy and a restoration of the apomorphine-induced disruption of the PPI could be observed.

An increase in EEG power densities within the respective frequency range is frequently observed after administration of a variety of psychopharmacological drugs, including benzodiazepines and conventional and atypical neuroleptics (Krijzer et al. 1993). Although sleep studies with progesterone revealed EEG patterns similar to those obtained with agonistic modulators of the $GABA_A$ receptor that are probably due to a bioconversion of progesterone into allopregnanolone (Lancel et al. 1996, 1997), these effects do not exclude additional neuroleptic-like effects of progesterone itself.

Surprisingly, the administration of progesterone did not produce anxiolytic effects in the elevated plus-maze test despite a rise in allopregnanolone levels and a sleep-EEG pattern comparable to that obtained with agonistic modulators of the $GABA_A$ receptor (Lancel et al. 1996, 1997). This contrasts with previous reports in ovariectomized Sprague-Dawley rats (Bitran et al. 1993, 1995) that showed an anxiolytic activity in a similar test setting 4 h following progesterone injection. Therefore, we investigated the effects of progesterone in the elevated plus-maze test in combination with a preceding social defeat paradigm that together are more sensitive to the anxiolytic effects of drugs than the elevated plus-maze test alone (Liebsch et al. 1995). Again no anxiolytic effect of progesterone could be detected. These apparent discrepancies cannot yet be explained. Possibly the sedative properties of progesterone may have interfered with the test results to some extent. Moreover, there was already a considerable decline in allopregnanolone levels at the time of the testing. However, it may also be that the anxiolytic effects of progesterone differ between rat strains and are gender specific as an anxiolytic activity of progesterone has been shown in female Sprague-Dawley rats but not in male animals (Rodriguez-Sierra et al. 1986).

The observed decrease in locomotor activity evoked by progesterone may be explained by a sedative effect of progesterone metabolites mediated via the $GABA_A$ receptor (Wieland et al. 1995) or by a potential neuroleptic-like activity, because a reduction in locomotion is observed with both classical and atypical neuroleptics (Kinon and Lieberman 1996). Although we observed an inhibitory effect of progesterone on the CAR at the highest progesterone dose, it has to be considered that this impairment of the CAR may also reflect the sedative and analgetic properties of this

steroid or its GABAergic metabolites, e. g. allopregnanolone. This is also supported by the observation that allopregnanolone itself impairs the CAR at a lower dose when compared with progesterone. Moreover, an inhibition of the CAR is not specific for neuroleptic drugs because it is also observed with anxiolytic agents such as benzodiazepines (Treit 1985).

Conventional neuroleptics such as haloperidol act through the blockade of D_2 receptors, which is also responsible for extrapyramidal side effects, e.g. the induction of catalepsy and the antagonism of amphetamine-induced stereotypy (Kinon and Lieberman 1996). Whereas haloperidol is effective in such paradigms, progesterone failed to induce catalepsy and did not antagonize amphetamine-induced stereotypy. However, atypical neuroleptics such as clozapine are also ineffective in such test paradigms, although they are highly potent in clinical trials (Brunello et al. 1995). Therefore, we used the prepulse inhibition (PPI) of the acoustic startle response (ASR) and its disruption by apomorphine (Davis et al. 1990; Rigdon 1990; Swerdlow et al. 1994) as an animal model for antipsychotic drug action, as the apomorphine-induced disruption of the PPI can be effectively restored by both classical (Swerdlow and Geyer 1993; Swerdlow et al. 1994) and atypical (Swerdlow and Geyer 1993; Swerdlow et al. 1994; Varty and Higgins 1995) neuroleptics. Apomorphine increased the ASR amplitude alone and this effect was reversed by haloperidol, but not by progesterone or allopregnanolone. The disruption of the PPI and the enhancement of the ASR by apomorphine are effects that have been reported repeatedly from several laboratories, which, however, are dependent on the stimulus characteristics and the rat strain used (Swerdlow et al. 1986; Davis et al. 1990; Rigdon 1990). Both haloperidol and progesterone administration were effective in antagonizing the disrupting effect of apomorphine on PPI. The effects of progesterone administration in the PPI paradigm seem to be mediated by progesterone itself as significant effects could be detected with a 10 mg/kg doses, whereas GABAergic sleep-EEG patterns required a 90 mg/kg dose (Lancel et al. 1996). This is also supported by the pharmakokinetic study which revealed much higher concentrations of progesterone than allopregnanolone. Moreover, the administration of 10 mg/kg allopregnanolone did not significantly antagonize the disruption of the PPI induced by apomorphine. Thus, the effects of progesterone in the PPI paradigm cannot be attributed only to the sedative effects of allopregnanolone. The plasma and brain concentrations of progesterone attained after the injection of the 10 mg/kg dose at the time of the behavioral tests were about 300 nM. Although peripheral plasma concentrations of progesterone throughout the menstrual cycle are in the low nanomolar range, plasma levels of about 300 nM are achieved during pregnancy, and substantially higher concentrations may occur in tissues due to the lipophilic properties of this steroid (Su et al. 1989). Thus, progesterone concentrations that are effective in counteracting the apomorphine-induced disruption of the PPI physiologically occur in vivo during pregnancy.

In conclusion, the administration of progesterone produced a behavioural profile that probably in part reflects the sedative properties of allopregnanolone via the $GABA_A$ receptor, but does not exclude the possibility that progesterone itself shares some properties with atypical antipsychotics in view of the effects of this steroid in the PPI paradigm. In this context it should be considered that drugs with a pharmacological profile different from those of already approved classical or atypical neuroleptics, which usually have antidopaminergic properties, may produce a distinct behavioral profile and the currently available animal models, which mostly reflect antidopaminergic activity, do not allow a definite conclusion whether a substance will be a clinically effective antipsychotic or not.

The potential molecular mechanisms underlying the psychopharmacological properties of progesterone appear to be highly complex. Progesterone may activate intracellular progesterone receptors that act as transcription factors in the regulation of gene expression (Evans 1988). Moreover, progesterone has also antimineralocorticoid and antiglucocorticoid properties (Rupprecht et al. 1993b). Thus, via its genomic mechanism of action progesterone may influence the expression of dopamine receptors (Di Paolo 1994; Guivarc´h et al. 1995) and of enzymes involved in dopamine synthesis, e.g. the tyrosine hydroxylase (Lewis et al. 1987; Kohama and Bethea 1995). Besides their genomic effects (Rupprecht et al. 1993a), progesterone or its metabolites may also interact with various ligand-gated ion channels. In addition to the GABA-potentiating effects of its metabolites (Paul and Purdy 1992), progesterone itself may exert functional antagonistic effects at sigma receptors (Monnet et al. 1995), at nicotinic acetylcholine receptors (Valera et al. 1992) and 5-HT_3 receptors (Wetzel et al. 1998). Moreover, progesterone has also been shown to modulate the release of dopamine (Dluzen and Ramirez 1990; Ramirez and Zheng 1996). Currently, it has not yet been investigated whether progesterone or related steroids may interact directly with dopamine receptors. Nevertheless, there is evidence that steroids may affect also G-protein coupled mechanisms (ffrench-Mullen et al. 1994). Thus, both the various genomic and nongenomic properties of progesterone are potential mechanisms responsible for the behavioral properties of progesterone. However, the high progesterone doses needed and the rather short time interval between the injection of progesterone and the behavioral effects are in favor of non-genomic mechanisms of actions of progesterone underlying the behavioral changes observed in this study.

More than 50 years ago, it was suggested in a single case-report that administration of progesterone might prevent the relapse of postpartum psychosis related to the recurrence of the menstrual cycle (Schmidt 1943). Although extrapolations to a putative clinical relevance have to be made with caution in view of the limited usefulness of animal models for the understanding of psychosis and the use of male animals in this study, our data on PPI are compatible with the idea that a sharp drop in progesterone concentrations may contribute to the development and course of psychotic symptoms and that an administration of progesterone as an additive therapy to neuroleptic drugs might be beneficial in psychotic disturbances.

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