CCD-3693: An Orally Bioavailable Analog of the Endogenous Neuroactive Steroid, Pregnanolone, Demonstrates Potent Sedative Hypnotic Actions in the Rat

DALE M. EDGAR, WESLEY F. SEIDEL, KELVIN W. GEE, NANCY C. LAN, GEORGE FIELD, HAIJI XIA, JON E. HAWKINSON, SCOTT WIELAND, RICHARD B. CARTER, and PAUL L. WOOD

Sleep Research Center, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford (D.M.E., W.F.S.); Department of Pharmacology, College of Medicine, University of California, Irvine (K.W.G.); and CoCensys Inc., Irvine, California (N.C.L., G.F., H.X., J.E.H., S.W., R.B.C., P.L.W.)

Accepted for publication March 5, 1997

ABSTRACT

An endogenous neuroactive steroid, pregnanolone, and an orally available synthetic analog, CCD-3693, were administered to rats at the middle of their circadian activity phase (6 hr after lights off). Electroencephalogram-defined sleep-wake states, locomotor activity and body temperature were concurrently measured 30 hr before and after treatment. Identical procedures were used to test triazolam and zolpidem. Triazolam (0.1–1.6 mg/kg), zolpidem (2.5–10 mg/kg) and the neuroactive steroids (10–30 mg/kg) produced dose-dependent increases in non-rapid eye movement (NREM) sleep. At this dose and time of day (in which the rats were predominantly awake during the 6 hr before treatment) the neuroactive steroids appeared more intrinsically efficacious in promoting NREM sleep than the benzodiazepine ligands. The neurosteroids did not, however, significantly interfere with rapid eye movement sleep and were more selective in reducing (EEG) wakefulness, with relatively less locomotor activity impairment during waking than triazolam and zolpidem. In addition, the benzodiazepine receptor ligands showed distinct "rebound" wakefulness after the NREM sleep-promoting effect subsided, although the neuroactive steroids did not. In addition, in vitro binding studies and in vivo pharmacological data confirmed that CCD-3693 was orally active in standard tests of anxiety, anticonvulsant, loss-of-righting and passive avoidance.

The clinical efficacy of benzodiazepines as sedative/hypnotics is unquestioned. These compounds are limited, however, by negative effects on psychomotor performance, by interactions with alcohol and by dependence liability (Shader and Greenblatt, 1993). A recent advance in the hypnotic area has been the clinical introduction of zolpidem, a nonbenzodiazepine with an approximately 20-fold greater affinity for benzodiazepine receptors on GRCs containing α1 subunits than those containing α2 or α3 subunits and a >800-fold greater affinity for α1 over α5 containing GRCs (Pritchett and Seeberg, 1990). This increase in benzodiazepine receptor subtype specificity appears to result in an improved hypnotic profile over classical benzodiazepines; however, the 20-fold separation in receptor subtype affinities defines the narrow dosing range needed not to disrupt normal sleep architecture and maintain a superior clinical profile. Dosing above this range can result in disruptions of normal sleep architecture (Hoehns and Perry, 1993) and rebound insomnia on the next night’s sleep (Roehrs et al., 1986).

Barbiturates, which are also allosteric modulators of the GRC, also have been used extensively as hypnotics, with their clinical use limited by their abuse potential and low therapeutic indices (Mellinger et al., 1985). Neuroactive steroids are the first class of endogenous compounds (Hu et al., 1987) known to act as positive allosteric modulators of the GRC (Cottrell et al., 1987; Gee et al., 1988; Lan et al., 1990, 1991; Puia et al., 1990; Shingai et al., 1991; McNeil et al., 1992; Paul and Purdy, 1992; Woodward et al., 1992). Consistent with their site of action, they have also been shown to possess hypnotic and anesthetic actions (Atkinson et al., 1965; Gyermek, 1967; Mendelson et al., 1987; Mok and Krieger, 1990; Steiger et al., 1993). Although significant synthetic efforts have been applied to the design of neuroactive steroids with i.v. anesthetic activity (Phillips, 1975), the de...
sign of orally bioavailable hypnotics has not been fully assessed. We present the pharmacological profile of CCD-3693, a synthetic orally bioavailable analog of the endogenous neuroactive steroid, pregnanolone. The overall pharmacological profile of this compound is compared with that of pregnanolone and with the approved hypnotics, triazolam (Edgar et al., 1991a and b) and zolpidem (Depoortere et al., 1986).

Methods

Materials. TBPS (60–100 Ci/mmol) and [3H]flunitrazepam (74–84 Ci/mmol) were obtained from NEN (Boston, MA). HPβCD was obtained from American Maize Products (Indianapolis, IN). Triazolam and sterile 0.25% methylcellulose and zolpidem were the generous gifts from Upjohn (Kalamazoo, MI) and Synthelabo (Le Plessis-Robinson, France), respectively. All other reagents and drugs were obtained from Sigma Chemical Co. (St. Louis, MO).

In Vitro Pharmacology

Allosteric modulation of [35S]TBPS binding to rat brain cortex. The rat neocortical membrane preparation and subsequent binding assay were performed as described in detail previously (Hawkinson et al., 1994). All values are the means of at least three replicate experiments with less than 10% S.D. Briefly, fresh rat cortical P2 membrane preparations were washed three times and used for assay with 2 nM [35S]TBPS in the presence of 5 μM GABA and using 2 μM cold TBPS to define nonspecific binding. Incubations were 90 min at room temperature and were terminated by filtration.

Allosteric modulation of [3H]flunitrazepam binding to rat brain cortex. The rat neocortical membrane preparation and subsequent binding assay were performed as previously described in detail (Hawkinson et al., 1994). All values are the means of at least three replicate experiments with less than 10% S.D. Briefly, washed P2 membrane preparations were incubated with 1 nM [3H]flunitrazepam, in the presence of 1 μM GABA, for 90 min at room temperature and the incubations terminated by filtration. Nonspecific binding was determined with 1 μM cold clonazepam.

Behavioral Pharmacology

The behavioral testing of pregnanolone, CCD-3693 (fig. 1), triazolam and zolpidem was conducted in male mice and rats (Harlan Sprague-Dawley, Indianapolis, IN) according to procedures previously described in detail (Wieland et al., 1995). However, the in vivo evaluation of neuroactive steroids is complicated significantly by the difficulties associated with formulating these compounds. Initial studies were undertaken using 0.25% methylcellulose as a vehicle; however, subsequent studies used HPβCD (American Maize Products, Indianapolis, IN). For i.p. or s.c. dosing 50% HPβCD (w/v in 0.9% NaCl) solutions were used although oral dosing was with 10 to 20% HPβCD solutions. For these studies, initial drug solutions were made in 50% HPβCD (15 mg/ml of CCD-3693 and 40 mg/ml of pregnanolone), and subsequent dilutions made with 0.9% NaCl. In the rat i.p. and s.c. dosing was done at a volume of 1 ml/kg, although oral dosing was up to 10 ml/kg. With mice the corresponding numbers were 100 μl/20 g, i.p. and 400 μl/20 g, p.o.

Anxiolytic testing. The elevated plus-maze (Lister, 1987), was used as an ethological model in NIH Swiss-Webster mice and the Geller-Seifert conflict procedure was performed with Sprague-Dawley rats.

Anticonvulsant testing. Anti-PTZ activity was evaluated both in Sprague-Dawley rats and CF/1 mice. The PTZ dose used in mice was 85 mg/kg, s.c. and in rats was 70 mg/kg, s.c.

Motor function. Roto-rod deficits were evaluated both in the rat and mouse. LRR was also determined in mice. The ability of drugs to potentiate the motor deficits of ethanol (Frye and Breese, 1992) were evaluated in the rat (1.0 g ethanol/kg) roto-rod paradigm and in the hanging wire-mesh test for mice (1.5 g ethanol/kg).

Passive-avoidance paradigm. The acquisition test was conducted according to previously published methodologies (Holmes and Drugan, 1991). The CF/1 mice were placed in the “bright” side of a two-compartment shuttle box (GEMINI Avoidance System; San Diego Instruments, San Diego, CA) and allowed to explore the chamber for 120 sec. At the end of the exploration period, a guillotine door opened to allow access to a “dark” chamber. The mice had 120 sec to enter the dark chamber. If the mice failed to enter the dark chamber within 120 sec they were gently pushed across. Upon entering the dark chamber, the guillotine door closed and the mice received a footshock of 0.2 mA intensity and 2 sec. The mice were removed immediately and placed into their home cages. To monitor drug effects on acquisition, they were administered 10 min before behavioral testing.

On the testing day, 24 hr after training, the mice were tested for their ability to remember that they received a footshock within the dark chamber. The mice were placed in the bright chamber for a 120 sec exploratory period. After exploratory period, the guillotine door was raised and the latency to enter the dark chamber measured automatically. Upon entering the dark chamber, the guillotine door closed and the testing session was completed. The mice did not receive a footshock during testing.

Pharmacokinetics. The oral pharmacokinetic profile of pregnanolone was evaluated after a dose of 100 mg/kg. The time points evaluated were 0.5, 1, 2 and 4 hr. To evaluate the pharmacokinetic profile of CCD-3693 after acute and repeated dosing, rats were dosed orally with 15 mg/kg and plasma samples taken at 0.25, 0.5, 0.75, 1, 1.5 and 2 hr after drug administration. Animals that had been dosed daily for 3 days at 15 mg/kg, were evaluated identically on day 4. Plasma samples were extracted with hexane and the hexane extracts analyzed by GC-MS using a DB-17 column (J&W: 30 m × 0.32 mm i.d.; 0.1-μm film thickness; 180°C for 2.5 min followed by a 20°C/min gradient to 230°C), for chromatography and a Finnigan ITS 40 as the detector. Total ion current chromatograms were integrated for quantitation.

Statistics. ED50 and TD50 values were calculated for each experiment within each drug using the method of Litchfield and Wilcoxon (1949). Calculations were computed using a commercially available computer program included in the “Manual of Pharmacological Calculations with Computer Programs” (R. J. Tallarida and R. B. Murray, eds., Springer Verlag, 1987). Statistical inferences were made with the Neuman-Keuls post-hoc procedure, subsequent to an ANOVA.

Sleep-Wake Bioassay

Animal surgery. Adult, male Wistar rats (275–350 g at time of surgery, Charles River Laboratories, Wilmington, MA) were anesthetized (Nembutal, 60 mg/kg) and surgically prepared with a cranial implant that permitted chronic EEG and EMG recording. Body temperature and locomotor activity were monitored via a miniature transmitter (Minimitter) surgically placed in the abdomen. The cranial implant and telemetry surgical procedures have been described in detail elsewhere (Edgar et al., 1991a). In brief, EEG was continuously monitored using differential leads across one each of two frontal (+3.9 AP from bregma, ±2.0 ML) and two occipital (−6.4 AP, ±5.5 ML). Two Teflon-coated stainless steel wires positioned under

Pregnanolone

CCD 3693

Fig. 1. Structure of the endogenous neuroactive steroid, pregnanolone and the synthetic neuroactive steroid, CCD-3693.
the nuchal trapezoid muscles permitted continuous EMG recording. All implants were sterilized with ethylene oxide. A minimum of 3 wk was allowed for recovery from surgery.

**Recording environment.** Rats were housed individually within specially modified Nalgene microisolator cages equipped with a low-torque swivel commutator and filter-top riser. These cages were located within separate, ventilated compartments of a stainless steel recording chamber. Food and water were available *ad libitum*. A 24-hr (LD 12:12) light-dark cycle was maintained throughout the study (32–35 lux inside the cage during lights-on, <0.05 lux during lights-off). Animals were undisturbed for 3 days both before and after treatments.

**Automated data collection.** Sleep and wakefulness were determined using “SCORE”—a microcomputer-based sleep-wake and physiological monitoring system. A detailed description and functional validations of this system for rodents have been described elsewhere (Van Gelder *et al.*, 1991; Edgar *et al.*, 1991a; Seidel *et al.*, 1995). Briefly, the system monitors amplified EEG (bandpass 1–30 Hz; digitization rate 100 Hz), integrated EMG (bandpass 10–100 Hz), Tb, nonspecific LMA and drinking activity, from up to 64 rodents simultaneously. Arousal states were classified on-line as NREM sleep, REM sleep, wake, or θ-dominated wake every 10 sec based on SCORE EEG feature extraction and pattern-matching algorithms. The classification algorithm used individually-taught EEG-arousal-state templates and EMG criteria to differentiate REM sleep from θ-dominated wakefulness, plus behavior-dependent contextual rules (e.g., if the animal was drinking, it was awake). Drinking and LMA were recorded as discrete events every 10 sec. Body temperature was recorded each minute. Tb and LMA was detected by a telemetry receiver (Datasciences, Inc., St. Paul, MN) beneath the cage. Telemetry measures (LMA and Tb) were not part of the scoring algorithm; thus, sleep-scoring and telemetry data were independent measures. The quality of data was assured by frequent on-line inspection of the signal. Graphical and statistical summaries of the 3 days before and after each animal’s injection were also inspected to determine stability of the scoring. Data quality was further ensured by examining the raw EEG file (covering the first 5 hr posttreatment) for every individual treatment.

**Drug administration.** All treatments were administered to parallel groups under dim red illumination 6 hr after lights-out. This time-point is usually designated “CT-18” (CT = circadian time, CT-0 = lights-on). Pregnanolone, triazolam and zolpidem were suspended in sterile 0.25% methylcellulose and administered i.p. in a volume of 1 ml/kg although CCD-3693 was administered orally in 10% HPβCD in a volume of 10 ml/kg. All treatment groups had a
Variables and statistics. NREM and REM sleep were expressed as percent of time asleep per hour. The sleep-scores were derived from automated EEG/EMG scoring, described above. LMA was measured in counts per hour. This measure was independent of sleep-scoring. Tb was normalized for each animal by computing the hourly average °C change from the 24-hr base-line (pretreatment) mean. By inspection of the data, it was evident that for all treatments primary hypnotic effects (e.g., increased NREM sleep) occurred within the first 5 hr. Therefore, for each animal and for each variate, the average hourly response across the first 5 hr posttreatment was subtracted from the corresponding average of the 5-hr pretreatment baseline period taken 24 hr earlier. For each variate, this change-from-base-line score was then compared against the appropriate vehicle control using one-way ANOVA. In the presence of a significant main effect, Dunnett contrasts (α = 0.05) tested the difference between an active treatment group and controls. Comparisons of compensatory (“rebound”) wakefulness was performed in a similar manner, except that the interval of interest was 7 to 10 hr posttreatment.

Results

In vitro binding. The actions of CCD-3693 at the GABA_A receptor complex were monitored via the negative allosteric modulation of [35S]TBPS binding to the chloride channel and the positive allosteric modulation of [3H]flunitrazepam binding to associated benzodiazepine receptors. Pregnanolone inhibited [35S]TBPS binding to a high affinity site with an IC50 of 37 nM (64%) and a low affinity site with an IC50 of 8600 nM (36%); CCD-3693 possessed a slightly lower affinity (76 nM) but recognized only one apparent binding site (see fig. 2). In the case of [3H]flunitrazepam binding, pregnanolone also demonstrated biphasic actions with a 65 nM high affinity site (56% enhancement) and a 8.6 μM low affinity site (17% enhancement), with an overall enhancement of 72%. A 70% increase in [3H]flunitrazepam binding with an EC50 of 210 nM was seen with CCD-3693 (see fig. 3).

In Vivo Pharmacology

The general CNS pharmacology of CCD-3693 was evaluated both in rats and mice and comparisons made with the endogenous neuroactive steroid, pregnanolone and the hypnotics, triazolam and zolpidem.

Anxiolytic activity. All compounds, except zolpidem, demonstrated parenteral activity in the mouse elevated plus maze paradigm. Similarly, in the Geller-Seifter conflict paradigm, in rats, CCD-3693 demonstrated potent activity both after parenteral and oral administration. In contrast, pregnanolone was potent parenterally but not after oral administration (table 1).

Anticonvulsant activity. All compounds were potent in blocking PTZ-induced convulsions, both in mice and rats, after parenteral administration (see fig. 4; table 1). However,
as with anxiolytic testing, CCD-3693, but not pregnanolone, was active after oral dosing.

**Motor function.** In the mouse, all compounds elicited decreased performance on the rotorod, but with TD$_{50}$ values that were multiples of their anticonvulsant ED$_{50}$ values. Loss-of-righting reflex was also noted with all compounds except triazolam. In the rat, the oral TD$_{50}$ of CCD 3693 on the rotorod was 30 mg/kg, distinctly higher than doses needed for potent NREM-sleep-promoting effects on rat (10 mg/kg, p.o.; see below).

**Interactions with ethanol.** In the mouse, all compounds potentiated the actions of ethanol in the hanging wire mesh paradigm (table 1). Similarly, CCD-3693 was also active in potentiating ethanol disruption of rat performance on the rotorod after oral administration.

**Cognitive deficits.** In the mouse passive avoidance paradigm, all compounds decreased acquisition; however, in the case of pregnanolone and CCD-3693, these actions only occurred at ataxic doses, although triazolam and zolpidem disrupted performance at doses significantly below ataxic doses (see fig. 5; table 1).

**Pharmacokinetics.** In the rat, oral dosing with 100 mg/kg of pregnanolone results in peak plasma levels of 40 ng/ml with rapid subsequent clearance (see fig. 6). In contrast, CCD-3693, after oral dosing with 15 mg/kg, resulted in peak blood levels of about 200 ng/ml. These data support the pharmacological data (table 1) that demonstrated improved oral activity with CCD-3693, as compared to pregnanolone. Daily dosing of rats for 3 days also did not alter the pharmacokinetic profile of CCD-3693 on day 4, suggesting that no induction of acute drug metabolism occurred. Long-term studies are still needed, however, to fully evaluate induction of drug metabolism.

**Sleep-Wake Bioassay**

Figures 7, left and right depict the timecourse of the effects of the higher dose of CCD-3693 (30 mg/kg p.o.) and pregnanolone (30 mg/kg i.p.) on NREM and REM sleep, LMA and T$_b$. The first 24 hr of each panel reveal the normal (undisturbed) circadian cycle for each variable, allowing the magnitude of the treatment effects to be appreciated relative to normal circadian variation. Active and vehicle group pre-treatment baselines were closely similar.

**Sleep.** Pregnanolone and CCD-3693 potently promoted NREM sleep in a dose-related manner, with a rapid onset of action (fig. 8). The route of administration and vehicles for these two compounds differed (CCD-3693 p.o. vs. pregnanolone i.p.), and therefore may account for the more rapid
onset of action observed for pregnanolone. Table 2 shows that 30 mg/kg of pregnanolone or CCD-3693 exerted markedly stronger NREM-promoting effects than the highest doses tested of triazolam and zolpidem, even though with respect to LMA reduction, these highest-dose treatments were roughly comparable.

The neuroactive steroids did not measurably interfere with REM sleep when administered at CT-18, whereas the highest doses of triazolam and zolpidem produced statistically significant reductions of REM sleep. For CCD-3693, a significant main effect ($P < .05$, ANOVA) for REM was due to differences between active doses; however, contrasts with vehicle were not significant.

Although a robust increase in NREM sleep was observed after treatment with 30 mg/kg CCD-3693, no compensatory (“rebound”) increase in wakefulness was observed in the subsequent rest phase (lights-off) of the circadian cycle (fig. 9). Therefore, in the rat, CCD-3693 generated a “surplus” of NREM sleep relative to the same time-period 24 hr earlier. In figure 9, the accumulating surplus after CCD-3693 is contrasted with the benzodiazepine receptor ligands. In the rat, both triazolam and zolpidem showed a distinct “rebound” wakefulness that counterbalanced the surplus NREM accumulated during the interval of hypnotic efficacy.

**Locomotor activity.** All active treatments showed significant dose-related reductions of LMA (table 2). Compared to the highest doses tested of triazolam and zolpidem, pregnanolone or CCD-3693 (30 mg/kg) promoted NREM more but reduced LMA less. Because these treatments reduced both EEG-wakefulness and LMA, it is desirable to know which variable was more strongly affected by a given treatment. Toward these ends, we defined **locomotor activity intensity** as the number of counts of LMA per minute of EEG-wakefulness. Plotting the high-dose treatments (fig. 10) shows that the benzodiazepine ligands reduced LMA more than wake, although the neuroactive steroids did the opposite. The main

---

**Fig. 7.** Time series plots comparing the effects of CCD-3693 (30 mg/kg; left) and pregnanolone (30 mg/kg; right) on NREM sleep, REM sleep, LMA and $T_b$. Light-dark cycle is indicated along the abscissa for each variable. Time of treatment (CT-18; 6 hr after lights-off) is indicated by a vertical dotted line. Data are plotted as hourly means ± S.E. (%NREM/hr; LMA counts/hr; hourly $T_b$ difference from circadian mean). Note that both compounds markedly increase NREM for several hours posttreatment, but showed no evidence of subsequent rebound insomnia or REM sleep abnormality. Locomotor reduction was commensurate with increased sleep time, yet had little effect on the body temperature circadian rhythm.
effects computed as the change from baseline for 5 hr posttreatment for CCD-3693 (F[2,23] = 4.14, P < .05), for pregnanolone (F[2,33] = 6.98, P < .005) and for triazolam and zolpidem (F[7,93] = 22.60, P < .0001) and the Dunnett contrasts for all of the high-dose treatments were statistically significant. Relative to vehicle controls, figure 10 suggests that the neuroactive steroids more specifically affect sleep-wakefulness than LMA when compared to the benzodiazepine receptor ligands.

**Body temperature.** Benzodiazepine ligands reduced T_b, zolpidem being remarkably potent in this respect even at the lowest dose, whereas, of the neuroactive steroids, only the highest dose CCD-3693 reduced T_b significantly. None of these treatments reduced T_b below the normal circadian nadir, so physiologically, these effects could be concomitants of decreased motor activity.

### Discussion

The soporific efficacy of neuroactive steroids has long been known, but only recently considered within the framework of modern drug discovery and development. Selvey (1942) first demonstrated that progesterone, and the 5a-pregnane-3,20-dione precursor to the 3a-hydroxylated, 5-reduced pregnanes produced anesthesia in rodents (Figdor et al., 1957; Atkinson et al., 1965). Subsequent studies established that water-soluble salts of the progesterone metabolite pregnanolone, first identified in human pregnancy urine (Marker and Kamm, 1937), could produce sedation or anesthesia in mice (Figdor et al., 1957). In addition, the 3a-hydroxyl group appears essential for hypnotic activity (Atkinson et al., 1965). Our study shows that an orally bioavailable synthetic derivative of pregnanolone, CCD-3693, also has potent and dose-dependent soporific efficacy.

Neuroactive steroids are believed to affect neuron excitability at the level of the neuronal membrane by allosterically enhancing the action of GABA on chloride conductance via a unique site on the GABAA receptor complex (Lan et al., 1990). The pharmacological profile of pregnanolone and CCD-3693 are consistent with other positive modulators of GABA action (e.g., benzodiazepines and barbiturates), exhibiting anxiolytic, anticonvulsant and sedative hypnotic properties.

Early studies of neuroactive steroid efficacy relied on loss-of-righting reflex in rodents—a crude index of sedation and central nervous system depression that is inadequate for assessment of sleep parameters normally measured by electroencephalography. In contrast, this study focused on EEG sleep-stage assessments and parallel physiological and behavioral measures necessary to identify an ideal sedative hypnotic. This approach indicates the superior sensitivity of the EEG measures in the case of CCD-3693, where full hypnotic activity in the EEG assay was seen at 10 to 30 mg/kg, p.o. although loss-of-righting reflex Td50 for CCD-3693 in the rat was 45.1 mg/kg, p.o. Indeed, the combination of standardized physiological and behavioral measures in our study offered useful preclinical measures of drug onset of action (based on EEG sleep), rebound wakefulness after drug-induced sleep and locomotor activity inhibition. In addition, continuous body temperature measures were obtained that can provide an early indication of potential cardiovascular side-effects (because rat body temperature is highly sensitive to change in vasomotor tone).

**Timing of drug administration in the circadian cycle.** An important consideration in the design of this preclinical sleep-wake assay was the timing of drug treatment. Although one could postulate that the most relevant time to administer a novel soporific agent is at the beginning of the rat’s circadian rest phase (akin to bedtime drug administration in humans), we have found that, with the exception of REM sleep inhibition measures (see below), preclinical sleep-wake assessments of sedative hypnotics in nocturnal rodents are both sensitive and reliable (e.g., offer predictive use) when treatments are performed in the middle of the rat’s activity phase (e.g., CT-18). There are specific features of rodent sleep that serve to validate this otherwise empirically derived study design as well. In addition to existing reports that rats are sensitive to benzodiazepines and other soporific agents at CT-18 (Edgar et al., 1991a; Seidel et al., 1995), treatment at this time of day offers the advantage of a 5-hr window posttreatment in which sleep and wake levels are fairly stable (and reproducible) under control conditions. The day-to-day variability in NREM sleep levels at the daily cusp of the circadian activity to rest transition, confounded further by the polyphasic nature of sleep-wake in rodents, and potential NREM ceiling effects during the circadian rest phase (Mistlberger et al., 1983) can undermine the sensitivity of sleep-wake assessments in rodents when treated too close to CT-0. This is a particularly important consideration when drug effects are calculated as a function of base-line measures 24 hr earlier. Finally, treatment at CT-18 facilitates...
the detection of rebound wakefulness during the animals rest phase as a drug’s soporific effects subsides. As noted in our study, soporific drug effects were not detected beyond 5 hr posttreatment for both neuroactive steroids and the benzodiazepine receptor ligands. Any immediate compensatory posttreatment for both neuroactive steroids and the benzodiazepine receptor ligands is “gated” by the underlying physiological (homeostatic) need for sleep, whereas other classes of compounds may directly invoke NREM sleep (Edgar et al., 1991a and b, Treachsel et al., 1992). For example, α2-adrenergic agonists such as clonidine and dexmedetomidine invoke NREM sleep to high levels at any time in the circadian cycle (Seidel et al., 1995). Given their efficacy, it seems likely that this could also be true for the neuroactive steroids in our study, although additional treatments at different circadian times are needed to confirm this. All of the active compounds in this study appeared to have a relatively short duration of NREM-promoting action which can be estimated from figure 8. The detailed time course of reduced LMA (not presented here) closely coincided with the automated EEG-scoring data in figures 7 and 8.

“Rebound” wakefulness. After all but the lowest-dose treatments, rats slept more than usual for that time of day. This drug-induced “surplus” of NREM sleep was quantified by calculating the posttreatment amount minus the usual amount for that time of day (e.g., baseline 24 hr earlier), and then viewed as a running-sum in figure 9. After the NREM-promoting effect of benzodiazepine receptor ligands had subsided, a distinct compensatory decrease in the abundance of NREM was observed, which persisted until the total accumulated minutes of NREM approached values normal for a 24-hr period. This compensatory decrease in NREM may be related to “rebound insomnia” after benzodiazepine receptor ligands that has been reported in humans (Mitter et al., 1984; Roehrs et al., 1986, 1990), and, as such, can have considerable impact on the clinical use of hypnotics. As with the human response, it remains unclear whether this compensation in rats is a function of drug withdrawal (e.g., secondary to drug-induced receptor-sensitivity changes), is driven by the normal sleep-homeostatic process or both. Remarkably, the neuroactive steroids showed no evidence of compensatory wakefulness after the initial NREM-promoting effect had subsided, and therefore allows the possibility that “rebound insomnia” may be significantly less of a problem for neuroactive steroids than has been the case for some benzodiazepine receptor ligands.

---

**TABLE 2**

Dose-response comparisons, change from baseline during first 5 hr posttreatment

<table>
<thead>
<tr>
<th>Group</th>
<th>NREM (min/hr)</th>
<th>REM (min/hr)</th>
<th>LMA (cnt/hr)</th>
<th>T4 (°C/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>S.E.</td>
<td>N</td>
</tr>
<tr>
<td>CCD-3693</td>
<td>10 mg/kg</td>
<td>0.8</td>
<td>0.8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>6.5</td>
<td>1.3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>18.5</td>
<td>2.2</td>
<td>8</td>
</tr>
<tr>
<td>PREG</td>
<td>10 mg/kg</td>
<td>2.9</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>12.6</td>
<td>1.1</td>
<td>13</td>
</tr>
<tr>
<td>TRZ</td>
<td>0.1 mg/kg</td>
<td>4.0</td>
<td>0.8</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.2 mg/kg</td>
<td>5.9</td>
<td>1.0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>0.4 mg/kg</td>
<td>8.5</td>
<td>0.8</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>1.6 mg/kg</td>
<td>7.1</td>
<td>2.0</td>
<td>10</td>
</tr>
<tr>
<td>VEH</td>
<td>2.5 mg/kg</td>
<td>19</td>
<td>0.6</td>
<td>20</td>
</tr>
<tr>
<td>ZOLP</td>
<td>5 mg/kg</td>
<td>12</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg</td>
<td>20</td>
<td>0.8</td>
<td>20</td>
</tr>
</tbody>
</table>

*veh, HP/pCD, p.o.
*veh, 0.25% methylcellulose, i.p. for pregnanolone (PREG) treatments.
*veh, 0.25% methylcellulose, i.p. for triazolam (TRZ) and zolpidem (ZOLP) treatments.
*P < .05, Dunnett’s t test, relative to corresponding vehicle treatment group.
floor effect. Nonetheless, we found that the neuroactive steroids did not measurably interfere with REM sleep at this time of day, whereas the highest doses of triazolam and zolpidem resulted in statistically significant reductions of REM sleep. Although these data are consistent with the action of neuroactive steroids in cats (Heuser, 1967), a more accurate assessment of the relative REM-interfering properties of these compounds in rats may be obtained by drug administration at a time-of-day when REM sleep was more abundant (e.g., CT-5).

**Behavioral and physiological specificity of actions.** Relative to vehicle controls, figure 10 suggests that neuroactive steroids more specifically affected sleep-wakefulness than LMA. In contrast, triazolam and zolpidem disproportionately reduced locomotor activity during waking episodes in the first 3 hr posttreatment, suggesting the benzodiazepine receptor ligands may not be as specific for sleep induction as the neuroactive steroids. Benzodiazepine receptor ligands generally show "myorelaxant" effects. Within a clinical context, this effect may be related to (or the same as) motor impairment, which could constrain the usefulness of benzodiazepine receptor ligands as hypnotics in some segments of the general population. For example, nocturnal motor impairment and disorientation could contribute to falls and hip fractures in the geriatric population. The relative specificity for the neuroactive steroids in promoting NREM sleep rather than reducing LMA could, therefore, indicate that motor impairment may be significantly less of a problem for neuroactive steroids than for benzodiazepine receptor ligands. Consistent with the foregoing, decreased acquisition of a passive avoidance paradigm in mice only occurred at ataxic doses of pregnanolone and CCD-3693, although triazolam and zolpidem disrupted performance at doses significantly below ataxic doses.

Taken together, these data suggest that the new orally bioavailable neuroactive steroid CCD-3693 has NREM-promoting potency comparable to the endogenous neuroactive steroid, pregnanolone. Although CCD-3693 potentiates alcohol comparable to benzodiazepine receptor ligands, some notable potential advantages over triazolam and zolpidem were observed: CCD-3693 appeared to be more intrinsically potent in promot-
ing NREM sleep. The neuroactive steroids did not interfere significantly with REM sleep and selectively reduced EEG wakefulness without disproportionate locomotor activity inhibition. In addition, the benzodiazepine ligands showed distinct “rebound” wakefulness after the NREM-promoting effect subsided, although the neuroactive steroids did not.

Because neuroactive steroids are naturally synthesized in the brain by enzymes in situ (Baulieu, 1981) and potently facilitate GABA-dependent chloride flux (Gee et al., 1988), it is plausible that these compounds are endogenous physiological regulators of brain excitability with diffuse action throughout the CNS. Neurotransmitters such as adenosine have similar diffuse inhibitory action and, on that basis, are postulated to be involved in normal sleep regulation (Benington, 1995).

The authors thank Drs. James D. Belluzzi and Larry Stein (UCI) for the rat Geller Seifter data and Humberto Garcia, Michael Halaas and Laura Alexandre for their expert technical assistance in sleep-wake studies performed at Stanford University.

Acknowledgments
The authors thank Drs. James D. Belluzzi and Larry Stein (UCI) for the rat Geller Seifter data and Humberto Garcia, Michael Halaas and Laura Alexandre for their expert technical assistance in sleep-wake studies performed at Stanford University.

References
DEPOORTERE, H., ZIVKOVIC, B., LLOYD, K. G., SANGER, D. J., PERRAULT, G., LANGER, M., HEDNOUR, G. D., CENTRE, DEPARTMENT OF PSYCHIATRY AND BEHAVIORAL SCIENCES, STANFORD UNIVERSITY, Send reprint requests to:}

Send reprint requests to: Dr. Dale M. Edgar, Sleep Disorders Research Center, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 701 Welch Rd. Suite 327, Palo Alto, CA 94304.