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Convulsant Versus Typical Barbiturates: Effects on Conflict Behavior in the Rat

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COMMISSARIS, R L , R J VASAS AND T C McCLOSKEY *Convulsant versus typical barbiturates Effects on conflict behavior in the rat* PHARMACOL BIOCHEM BEHAV **29**(3) 631–634, 1988 —Typical barbiturates produce a spectrum of behavioral effects, including anti-convulsant, muscle relaxant, sedative hypnotic and anti-anxiety actions In contrast to these typical barbiturates, there exists a group of barbiturates which are *pro-*, rather than anti-convulsant The effects of these convulsant barbiturates on anxiety-related behaviors have not been examined Therefore, the present studies were designed to compare the effects of the convulsant barbiturate CHEB to those of a number of typical barbiturates in the Conditioned Suppression of Drinking (CSD) paradigm, an "animal model" for the study of anxiety and anti-anxiety agents In daily 10-minute sessions, water-deprived rats were trained to drink from a tube which was occasionally electrified (0 5 mA), electrification being signalled by a tone Within 3–4 weeks control responding had stabilized (10–15 shocks and 10–15 ml water/session), drug tests were then conducted at weekly intervals Consistent with previous reports, typical barbiturates (pentobarbital, secobarbital, phenobarbital) produced dose-dependent increases in the number of shocks received at doses which did not depress background responding (water intake) In contrast, sub-convulsant doses of CHEB (0 3–2 5 mg/kg) produced a dose-dependent depression of both punished responding and background responding Finally, it was found that pre-treatment with 1 25 mg/kg CHEB did not alter the anti-conflict effects of pentobarbital These results suggest that (1) convulsant and typical barbiturates have markedly different effects on conflict behavior in the rat and (2) CHEB appears not to possess any "barbiturate antagonist" qualities

Barbiturates Convulsants CHEB Conflict behavior Anxiety

BARBITURATES have a wide spectrum of actions including sedative hypnotic, anxiolytic, muscle relaxant and, for many barbiturates, anti-convulsant effects Prior to the introduction of the benzodiazepines, barbiturates were used extensively for these effects. Over the past fifteen years, however, the barbiturates have largely been replaced by the benzodiazepines, primarily because of the barbiturates' narrow therapeutic index [15,18].

It has been proposed that barbiturates exert actions on the chloride ionophore within the GABA-benzodiazepine receptor complex ([22,24], see also reviews by [23,26]). The increase in chloride permeability and the resultant membrane hyper-polarization have been suggested to account for many of the behavioral actions of barbiturates. However, since a barbiturate antagonist does not exist, critical data addressing this hypothesis are lacking.

Although a "barbiturate antagonist" does not currently exist, there do exist agents which are categorized as "atypical" or convulsant barbiturates [1, 2, 7–10, 16, 17, 19] Perhaps the most notable of these is cyclohexylideneethyl-5-barbituric acid (CHEB). This agent has been shown to be convulsant, rather than anti-convulsant [1,2] and to possess muscle stimulant, rather than relaxant, effects [7–10, 17, 19] Somewhat surprising, Darnell *et al* [6] have reported that CHEB decreases, rather than increases, spontaneous locomotor activity in rats. This effect of CHEB was qualitatively similar to the effects of the typical barbiturate secobarbital. There are as yet no reports on CHEB's possible "anxiolytic" or "anxiogenic" effects

One animal procedure which has been used extensively in the study of anxiety and/or anti-anxiety agents is the Conditioned Suppression of Drinking (CSD, [3–5, 11, 20, 21]), a modification of the Geller-Seifter conditioned conflict test [12–14] and the Vogel acute conflict test [27] Although the CSD procedure has been used in numerous studies examining benzodiazepines and typical barbiturates, there are no reports on the effects of CHEB or any other convulsant barbiturates in the CSD paradigm

Therefore, the present studies were designed to determine the effects of typical barbiturates (i e., phenobarbital, secobarbital and pentobarbital) and the convulsant barbiturate CHEB, alone and in combination, on the behavior of rats in the CSD paradigm.

METHOD

Animals

Female rats, purchased from Charles River Farms, Inc (Cambridge, MA), were housed in groups of five in a climate-controlled room with a 12 hour light 12 hour dark cycle (lights on 0700–1900 hours) Animals were given ad lib access to food with restricted water; details of the water restriction schedule are provided below under "Procedure "

Apparatus

Conditioned Suppression testing was conducted in an apparatus similar to that described by Commissaris *et al* [3] The testing chamber was a rectangular box with Plexiglas® sides and a metal floor and top Protruding from one wall was a metal drinking tube, to which a calibrated (±0 5 ml units) length of polyethylene tubing was attached for measuring the volume of water consumed Programming for the test session was controlled by solid state modular programming equipment (Coulbourn Instruments Co , Lehigh Valley, PA)

Procedure

Subjects were tested singly in 10-minute sessions at the same time of day Monday through Friday, and were allowed free access to water from Friday post-test until Sunday a m This schedule of five day/week testing was continued throughout the experiment For the first few sessions, water-restricted (24 hours water-deprived) subjects were placed in the experimental chamber and allowed to consume fluid freely without the shock contingency After one week of non-shock sessions, the tone/shock contingency was initiated The 7-second tone periods were presented at regular (22 second ISI) intervals to the subjects During the later 5 seconds of these tone periods, contact between the floor and the metal drinking tube completed a circuit which resulted in the delivery of a 0 5 mA shock to the rat Shocks were delivered by a Coulbourn Instruments Shocker (Model No E13-02)

Initially, the shock inhibited all fluid consumption in the test chamber After several days, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and very brief contacts with the tube during the tone, receiving a consistent number of shocks from day to day Drug testing was begun after 3 weeks of baseline CSD sessions

Drug Testing

Drug tests were conducted on Thursdays and Fridays each week Initially, the dose-response effects on CSD behavior of the typical barbiturates pentobarbital, phenobarbital and secobarbital and the convulsant barbiturate CHEB were determined using the "cross-over" procedure described by McCloskey *et al* [21] On the Thursday test days, half the subjects received the drug under examination and half received the appropriate vehicle These treatments were reversed on the Friday test days Thus, each animal served as its own control for the effects of a given drug dose All doses of the drug under investigation were evaluated in same group (n=20) of subjects The order of drugs and doses examined was randomized

Subsequent to the determination of the dose-response curves for these agents administered singly, the effects of various doses of pentobarbital were determined in CHEB (1 25 mg/kg) or vehicle pre-treated subjects In these studies, subjects were pre-treated (15 minutes prior to testing) with either CHEB or its vehicle on both the Thursday and Friday tests days while pentobarbital and its vehicle (saline) were administered on alternate days Thus, the pre-treatment was held constant for a given test week, but varied from week to week

Drugs

CHEB was received as a gift from Dr Hal Downes at the University of Oregon (Portland, OR) Pentobarbital HCl, phenobarbital HCl and secobarbital HCl were obtained through NIDA Pentobarbital, phenobarbital and secobarbital were dissolved in saline, CHEB was administered in slightly basic (pH=9 0) solution in distilled water All drugs were administered intraperitoneally in a volume of 1 ml/kg body weight

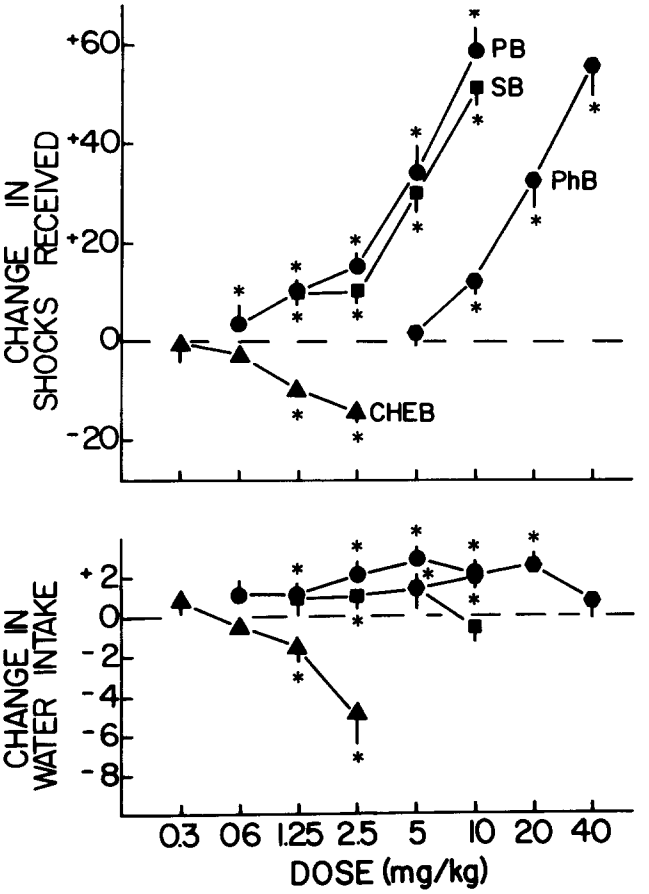

FIG 1 The effects of the typical barbiturates pentobarbital (PB, circles), secobarbital (SB, squares) and phenobarbital (PhB, hexagons) and the convulsant barbiturate CHEB (triangles) on behavior in the CSD paradigm Upper Panel The change in the number of shocks received following PB, SB, PhB or CHEB administration are plotted Each symbol and vertical bar represents the mean±SEM change in shocks received (Drug-Vehicle) obtained from 20 subjects PB, SB and PhB administration increased the number of shocks received in a dose-dependent manner In contrast, CHEB (triangles) administration resulted in a dose-dependent decrease in punished responding *$p<0$ 05, t-test for paired values Lower Panel The change in water intake (unpunished responding) following PB, SB, PhB or CHEB administration Again, each symbol and vertical bar represents the mean±SEM change in water intake obtained from 20 subjects *$p<0$ 05, Student's t-test for paired values

Statistical Analyses

The effects of single doses of various drugs on CSD performance were compared to drug vehicle using t-tests for paired values Dose-response curves for each drug were compared using one-way ANOVA with repeated measures The effect of CHEB (1 25 mg/kg) versus vehicle pre-treatment on the response to pentobarbital was analyzed

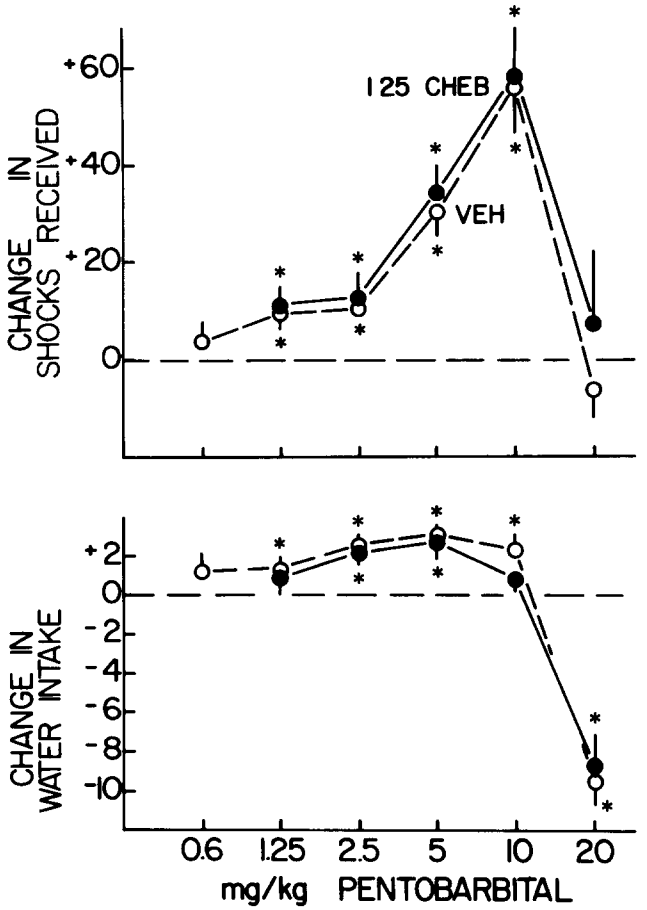

FIG 2 The effects of pentobarbital on CSD behavior in CHEB- and vehicle-pre-treated subjects Upper Panel The change in shocks received as a function of pentobarbital dose is plotted for both vehicle-pre-treated (open symbols) subjects and for subjects pre-treated with 1 25 mg/kg CHEB (filled symbols) See Fig 1 legend for further details As can be seen, CHEB pre-treatment did not influence the actions of pentobarbital on punished responding in the CSD Lower Panel The change in water intake as a function of pentobarbital dose in vehicle-pre-treated or CHEB-pre-treated subjects Again, CHEB pre-treatment did not alter the effects of pentobarbital on water intake

using a 2×5 Factorial ANOVA with repeated measures (MAIN EFFECTS ±CHEB, Pentobarbital Doses (5)) In all statistical comparisons, $p<0$ 05 was used as the criterion for statistical significance [25]

RESULTS

Baseline (i e., non-drug) responding in the CSD paradigm, 15±1 (Mean±SEM) shocks/session and 10 5±0.5 (Mean±SEM) ml water/session, was quite stable for each rat throughout the course of this study It should be noted that nearly all water intake occurred during the silent (i e , unpunished) periods

Figure 1 illustrates the change from these baseline values for various doses of the typical barbiturates pentobarbital, secobarbital and phenobarbital and the convulsant barbiturate CHEB As can be seen, administration of the typical barbiturates resulted in a dose-dependent increase in punished responding, these agents also produced small, yet statistically significant, increases in water intake at many doses tested In contrast to the effects of these typical barbiturates, CHEB administration resulted in a dose-dependent depression of both shocks received and water intake It should be noted that the highest dose of CHEB tested was slightly below the threshold for the production of convulsions (approximately 5 mg/kg IP, as determined in a separate group of subjects)

The upper panel of Fig 2 illustrates the effects of pentobarbital on punished responding in subjects pre-treated with either vehicle or 1 25 mg/kg CHEB Again, pentobarbital administration resulted in a dose-dependent increase in punished responding This was statistically supported by the significant MAIN EFFECT for Pentobarbital Dose, $F(4,76)=17$ 04, $p<0$ 01, for this measure The effect of pentobarbital on punished responding was not altered by CHEB pre-treatment, as indicated by the lack of a MAIN EFFECT for CHEB, $F(1,19)=2$ 24, n s , and the lack of a CHEB × Pentobarbital Dose Interaction, $F(4,76)<1$ 0, n s

The lower panel of Fig 2 illustrates the effects of pentobarbital administration on water intake in vehicle and CHEB-pre-treated subjects There was a significant MAIN EFFECT for Pentobarbital Dose on this measure, $F(4,76)=82$ 75, $p<0$ 01, with low to moderate doses increasing water intake and the highest dose (20 mg/kg) markedly decreasing water intake As with the effects of pentobarbital on punished responding, there was no MAIN EFFECT of CHEB on this measure, $F(1,19)<1$ 0, n s , nor was there a significant CHEB × Pentobarbital Dose Interaction, $F(4,76)<1$ 0, n s

DISCUSSION

As expected, typical barbiturates (phenobarbital, secobarbital and pentobarbital) produced dose-dependent increases in punished responding at doses which did not depress background responding These data are consistent with earlier data in the CSD [4, 20, 21] and other conflict procedures [14,27]

In contrast to the typical barbiturates, the convulsant barbiturate CHEB produced dose-dependent decreases in both punished responding and background behavior At no dose of CHEB was there observed a selective *pro*-conflict effect, i e , a decrease in punished responding without a comparable decrease in unpunished responding (water intake) Thus, although it's effects on CSD behavior are markedly different from those of the typical barbiturates, CHEB does not appear to exert an "anxiogenic" action on CSD behavior

Based on the observation that pretreatment with CHEB did not alter the effects of pentobarbital on shocks received or water intake, CHEB appears not to be an effective "barbiturate antagonist" for the effects of pentobarbital in the CSD procedure This observation is in agreement with the findings of Darnell *et al* [6], in which CHEB was found to be ineffective in blocking the locomotor depressant effects of secobarbital

In summary, the typical barbiturates pentobarbital, secobarbital and phenobarbital exerted relatively selective anti-conflict effects on CSD behavior, while the convulsant barbiturate CHEB did not selectively affect punished responding in the CSD Moreover, CHEB did not antagonize either the anti-conflict or the sedative effects of pentobarbital in the CSD These data further delineate the behavioral differences between CHEB and typical barbiturates and lend further support to the argument that CHEB is unlikely to be effective as a "barbiturate antagonist "

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