Pharmacology Biochemistry & Behavior, Vol. 25, pp. 375–380, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Behavioral Effects of the Isomers of Pentobarbital and Secobarbital in Mice and Rats[1]

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Received 11 February 1986

WENGER, G. R. *Behavioral effects of the isomers of pentobarbital and secobarbital in mice and rats.* PHARMACOL BIOCHEM BEHAV 25(2) 375–380, 1986.—To determine what stereoselective differences there may be in the behavioral effects of the isomers of pentobarbital and secobarbital, the effect of each isomer was determined on the spontaneous motor activity (SMA) and multiple fixed-ratio 30, fixed-interval 600-sec (mult FR30 FI600) responding of mice, and on the variable-interval 60-sec (VI60) responding of rats. The S-(−) isomers of pentobarbital and secobarbital decreased SMA at lower doses than those required for the R-(+) isomers. At moderate to high doses of R-(+)-pentobarbital (30–42.5 mg/kg) and low to moderate doses of S-(−)-secobarbital (5.6–17.5 mg/kg) SMA was increased. An increase in SMA following R-(+)-secobarbital was only observed at 30 mg/kg, and no increases were observed with S-(−)-pentobarbital. No potency differences were observed between the isomers of pentobarbital and secobarbital on the responding of mice under the mult FR30 FI600 schedule over a dose range of 1–30 mg/kg. Increases in FI600 responding were only observed following moderate doses of the S-(−) isomer of pentobarbital (5.6–17.5 mg/kg). In rats responding under the VI60 schedule of food presentation, no qualitative stereoselective differences were observed in the behavioral effects of the isomers of pentobarbital (1–13 mg/kg) and secobarbital (1–13 mg/kg), but small differences in potency were observed. Thus, differences in the effects of the isomers were usually restricted to differences in potency, but in some cases differences in efficacy were observed.

Stereoisomers Pentobarbital Secobarbital Mice Rats Spontaneous motor activity Schedule-controlled behavior

SEVERAL of the commonly used barbiturates have asymmetrical carbon atoms which are optically active. Pentobarbital and secobarbital both have asymmetric carbons and the optical isomers can be synthesized and separated to a high degree of purity. Yet, most of our knowledge of the behavioral effects of these barbiturates has been obtained using the racemic mixtures. Pfeiffer [8] postulated that racemic mixtures with relatively high pharmacological potency would display significant differences in the activity of the antipodes when studied separately. This generalization has proven to be correct for many chemicals which affect the CNS, and for some classes, a large difference in activity can be demonstrated. For anesthetic and lethal effects, the S-(−) isomers of pentobarbital and secobarbital have been shown to be 2–4 fold more potent than the R-(+) isomers [2, 4, 11]. For other effects, the differences in potency have not always been as great, and in some cases, no differences could be demonstrated. For anticonvulsant effects, no difference could be demonstrated between the potency of the isomers of secobarbital in protecting against chemically-induced seizures [6]. In studies on the effects of the isomers of pentobarbital and secobarbital on the responding of pigeons under a multiple fixed-ratio 30, fixed-interval 600 sec (mult FR30 FI600) schedule of reinforcement and under a multiple fixed-ratio 30 variable-interval 60 sec (mult FR30 VI60) schedule in which responding was suppressed by the presentation of a mild electrical shock upon a response, the S-(−) isomer of both barbiturates was more potent than the R-(−) isomer. The difference was 2-fold or less, but it was consistent across schedule components and across suppressed and non-suppressed responding [14]. In rats responding under a mult FR30 FI300 schedule of food presentation, the S-(−) isomer of pentobarbital was more potent than the R-(+), but the difference in potency was less than 2-fold [9]. Using drug discrimination procedures, Herling and Winger [7] studied the isomers of pentobarbital in pigeons trained to discriminate racemic pentobarbital from saline. Both isomers showed generalization to the racemic pentobarbital stimulus, but the S-(−) isomer was more potent than the R-(+) isomer. In rats trained to discriminate saline from 3 mg/kg of diazepam, both the S-(−) and R-(+) isomers of pentobarbital generalized to the diazepam stimulus, but the S-(−) isomer was more potent [15]. Thus, depending on the effect being studied the S-(−)

[1]This work was supported by National Institute on Drug Abuse Research Grant DA-03393. A preliminary abstract of this study appears in *Fed Proc* **44**: 495, 1985.

isomers of pentobarbital and secobarbital are reported to be either more potent than the R-(+) isomers, or they are reported to be equipotent with the R-(+) isomers.

The present study was designed to extend our knowledge about the relative properties of the isomers of pentobarbital and secobarbital. The effects of the isomers were determined on the spontaneous motor activity (SMA) and mult FR30 FI600 responding of mice, and on the variable-interval 60 sec (VI60) responding of rats.

METHOD

Subjects

Adult male C57BL/6N mice and adult male CD rats (Charles River Breeding Laboratories, Portage, MI) were used in the study. All animals were drug naive prior to this study. For experiments on SMA, mice were maintained on a free feeding schedule with food and water available at all times in their home cages. They were housed 4/cage except during testing. Mice used in experiments on mult FR30 FI600 responding were food deprived to a body weight equivalent to 80% of their free feeding weight and maintained at this weight throughout the experiment by post-session feeding. They were housed 3/cage with water available at all times in the home cages. Rats used in the experiments on VI60 responding were food deprived to a body weight equivalent to 80% of their free feeding weight and maintained at this weight throughout the experiment by post-session feeding. Rats were housed individually with water available at all times in the home cages. A 12-hr light/dark cycle was maintained with lights on from 0700–1900. All testing was conducted between 0730 and 1730.

Spontaneous Motor Activity (SMA)

A total of 15 mice were used in the experiments on SMA. Seven mice were used in the experiments with the isomers of pentobarbital and 8 mice were used for the determination of the effects of the secobarbital isomers. Each mouse was placed individually in a standard polyproplyene mouse cage (19×25 cm). Each cage was placed on top of an Automex activity monitor (Columbus Instruments, Columbus, OH). The sensitivity setting on each platform was adjusted to a level which produced a significant number of counts under control conditions, an increase in counts after a low dose of d-amphetamine, and a decrease in counts after a high dose of d-amphetamine. The activity was monitored for the duration of a 60-min test session on Monday, Wednesday and Friday. Typically, drugs were administered 5-min before the test session on Monday and Friday. Vehicle control injections were given 5-min before the test sessions on Wednesday.

Multiple Fixed-Ratio 30, Fixed-Interval 600-sec (Mult FR30 FI600)

Responding under the mult FR30 FI600 schedule was measured in a test chamber developed for mice, described previously [13]. Briefly, a light beam crosses the width of a blind corridor striking a photocell. Interruption of the light beam defined the response and produced an audible click inside the experimental chamber (feedback). The test chamber was illuminated with a 28-V DC bulb (No. 1819) at all times except during the presentation of the milk dipper. The test chamber was housed inside a sound and light attenuating chamber. Schedule programming and data collection were accomplished on a TRS-80, Model III (Radio Shack) microcomputer.

Six mice were trained to respond under the mult FR30 FI600 schedule of milk presentation. Under this schedule, in the presence of a green light, every 30th response produced 10-sec access to a 0.025 ml dipper of evaporated milk (FR30). In the presence of a second stimulus, audible white noise, the first response to occur upon the completion of the 600-sec interval produced 10-sec access to the dipper of evaporated milk (FI600). If 30 responses were not made within 60-sec following the onset of the green light, the green light was extinguished and the white noise turned on, signaling the start of the FI600 component. Likewise, if no response was made during the first 60-sec following the completion of the 600-sec interval, the white noise was turned off and the green light illuminated, signaling the return to the FR30 component. The two components alternated throughout the session. The session terminated after the presentation of 7 FR30 components and 7 FI600 components (approximately 72 min). Test sessions were conducted 5 days/week, Monday through Friday. Typically, drug was administered 5-min before the test session on Tuesdays and Fridays, and vehicle control injections were given 5-min before the test sessions on Thursdays.

Variable-Interval 60-Second (VI60)

Responding under the VI60 schedule was measured in a standard rat test chamber (Gerbrands Model G7322). In the center of the front panel, approximately 2 cm off the floor, a rectangular opening provided access to a feed tray into which 97-mg pellets were dropped. Two levers were mounted on either side of the rectangular opening. A downward force of approximately 0.15 N (15 g) was required to close the contacts and defined the response. Only responses on the right-hand lever resulted in food presentation, and responses on the left lever were not recorded and had no programmed consequence. Two 28-V DC bulbs (No. 1819) were located on the front panel immediately above the right-hand lever. An additional 28-V DC bulb was mounted in the ceiling of the chamber (houselight). The stimulus lights above the right-hand lever and the houselight were illuminated at all times during the test session except during the 10-sec time-out periods. Schedule programming and data collection were accomplished on a TRS-80, Model III (Radio Shack) microcomputer.

Six rats were trained to respond under a VI60 schedule of reinforcement. Under this schedule, in the presence of a stimulus light mounted above the response lever, the first response to occur upon the completion of a variable-interval of time produced a 97-mg food pellet. A 10-sec period during which all lights in the chamber were extinguished and responses on the lever were not recorded followed the presentation of the food pellet (time-out). If no response occurred during the first 30-sec after the completion of the interval, all lights were extinguished, and the 10-sec time-out period was initiated. The length of each interval was constantly varied, and the exact length of any given interval was determined by the computer program controlling the experiment. Briefly, an internal clock generated 10-sec intervals. At the end of each 10-sec interval, a random number generator was sampled, and on the average one of every six times the generator was sampled the program determined that the interval had elapsed. Thus, a given interval could be as short as 10-sec. or a given interval could be several minutes long, but on the

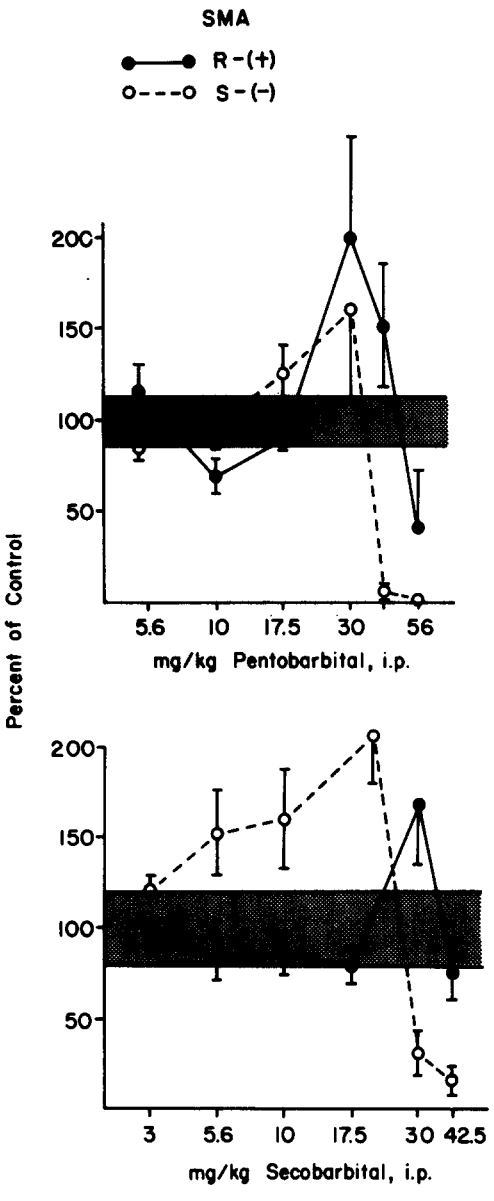

FIG. 1. The effect of the isomers of pentobarbital and secobarbital on the SMA of mice. Abscissa: dose in mg/kg of body weight on a log scale; ordinate: drug effect expressed as a percentage of the vehicle control level of activity. Points and brackets represent the mean±S.E. for single determinations in each of 7 mice for pentobarbital and in each of 8 mice for secobarbital. Shaded area represents the mean±S.E. for vehicle control injections (see the Method section for calculation of control S.E.). Mean vehicle control activity=944 counts per 60 min.

average the interval was 60-sec long. Each test session consisted of 60 VI60 component presentations, and each session lasted approximately 4200 sec including the 10-sec time-out periods. Test sessions were conducted 5 days/week, Monday through Friday. Typically, drug was administered 5-min before the test sessions on Tuesdays and Fridays with vehicle control injections made 5-min before the test sessions on Thursdays.

Drugs

R-(+)-pentobarbital, S-(−)-pentobarbital, R-(+)-secobarbital and S-(−)-secobarbital were custom synthesized by Research Triangle Institute, Research Triangle Park, NC. Drug was dissolved in 0.05 M sodium bicarbonate buffer and the pH adjusted to 10.5 with NaOH. All injections were given by the intraperitoneal (IP) route in a volume of 1 ml/100 g body weight for mice, and a volume of 1 ml/1000 g body weight for rats. All doses were calculated and are expressed as the free acid. Doses were administered in a mixed sequence.

Measurement of Drug Effects

In the experiments on SMA, total activity counts for the 60-min test session were recorded following vehicle control and drug administration. The effect of the drug is expressed as a percentage of the vehicle control activity level ±S.E. In the experiments on responding of mice under the mult FR30 FI600 schedule and of rats under the VI60 schedule, average rates of responding in each component were determined for the session as responses/second. The effect of the drug was expressed as a percent±S.E. of the vehicle control injection rate of responding. The S.E. for vehicle control injections in both the SMA and the schedule-controlled experiments was determined by determining the total standard deviation (n-1) for all control days in all animals and dividing by the square root of the number of animals in the group being studied.

RESULTS

Spontaneous Motor Activity (SMA)

The effects of the isomers of pentobarbital and secobarbital on the SMA of mice is seen in Fig. 1. The S-(−) isomers of pentobarbital and secobarbital were more potent than the R-(+) isomers with respect to activity decreasing effects. At lower doses, both isomers of pentobarbital tended to increase SMA, but the increases in SMA were of greater magnitude following the R-(+) isomer. However, the peak in the mean values for activity occurred at the same dose, 30 mg/kg, of both isomers of pentobarbital. Both isomers of secobarbital produced increases in SMA, but the increases observed with the S-(−) isomer were of greater magnitude, occurred at lower doses, and were observed over a wider dose range than those produced by the R-(+) isomer of secobarbital.

Multiple Fixed-Ratio 30, Fixed-Interval 600 sec (Mult FR30 FI600)

The mult FR30 FI600 schedule of reinforcement maintained responding of mice similar to what has been reported previously for this schedule in other species [5]. Responding under the FR30 component, following saline administration, occurred at a high, continuous rate of approximately 1.8 responses/sec. Responding under the FI600 component, following saline, was characterized by an initial period of very low response rates followed by a gradually increasing rate until the presentation of the dipper of evaporated milk. Average rate of responding for the FI600 component was 0.34 responses/sec, and typically 50–60% of each interval had elapsed before 25% of the total responses/fixed-interval had been made (FI 1/4-life).

Responding under the FR30 component did not show stereoselective effects following the isomers of pentobarbital (Fig. 2). Both isomers of pentobarbital produced dose-related decreases in response rates, and no differences in

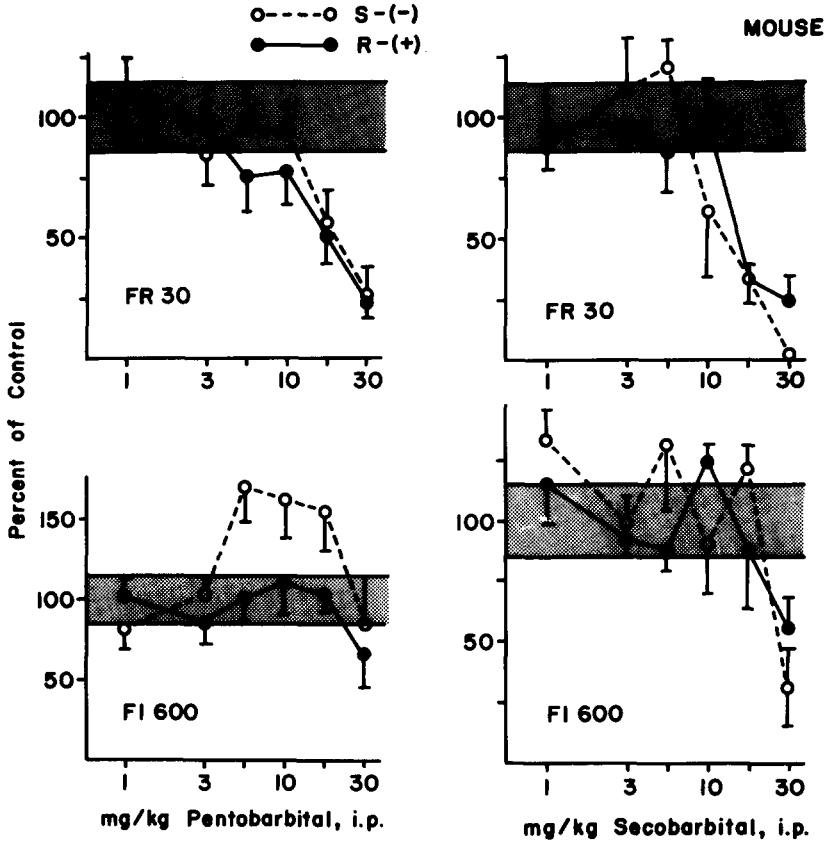

FIG. 2. The effect of the isomers of pentobarbital and secobarbital on the responding of mice under a mult FR30 FI600 schedule of milk presentation. Abscissa: dose in mg/kg of body weight on a log scale: ordinate; drug effect expressed as a percentage of the mean vehicle control rate of responding. Points and brackets represent the mean±S.E. for single determinations in each of 6 mice. Shaded area represents the vehicle control mean±S.E. (see the Method section for calculation of control S.E.). Mean vehicle control rates of responding were 1.81 and 0.34 responses/sec for the FR30 and FI600 components, respectively.

potency were observed. Under the FI600 component, responding was increased following doses of 5.6–17 mg/kg of the S-(−) isomer of pentobarbital. No increases in FI responding were observed following any dose of the R-(+) isomer of pentobarbital. Following 30 mg/kg of both isomers of pentobarbital, responding early in the session was nearly totally suppressed. Later in the session, responding returned at a rate greater than that observed following vehicle injections. Thus, with respect to the rate-decreasing effects, no estimate can be made on the relative potency of the two isomers.

The effect of both isomers of secobarbital were also determined on responding under the mult FR30 FI600 schedule of reinforcement (Fig. 2). No stereoselective differences were observed under either component of the schedule, and no differences in potency were observed. Only response rate decreases were observed with increasing doses of both isomers.

Variable-Interval 60 sec (VI60)

The VI60 schedule of reinforcement maintained responding of rats in a manner similar to what has been reported previously for this schedule in other species [5]. Rats responded at a consistent rate of responding throughout the VI at a rate of approximately 1 response/sec. The effects of the isomers of pentobarbital on VI60 responding in rats are seen in Fig. 3. Both isomers increased response rates at moderate doses, but the S-(−) isomer increased responding at a lower dose (3 compared to 5.6 mg/kg), and decreased responding at a lower dose (10 compared to 13 mg/kg) than the R-(+) isomer. However, the maximum rate increase occurred at the same dose of both isomers (5.6 mg/kg), and the magnitude of the rate decrease produced by 13 mg/kg of both isomers was identical. The effects of the isomers of secobarbital on VI60 responding (Fig. 3) were similar to that observed for the isomers of pentobarbital. There were no qualitative differences observed between the isomers of secobarbital on VI responding, but the dose required to increase responding and the dose required to decrease responding was lower for the S-(−) isomer than for the R-(+) isomer of secobarbital.

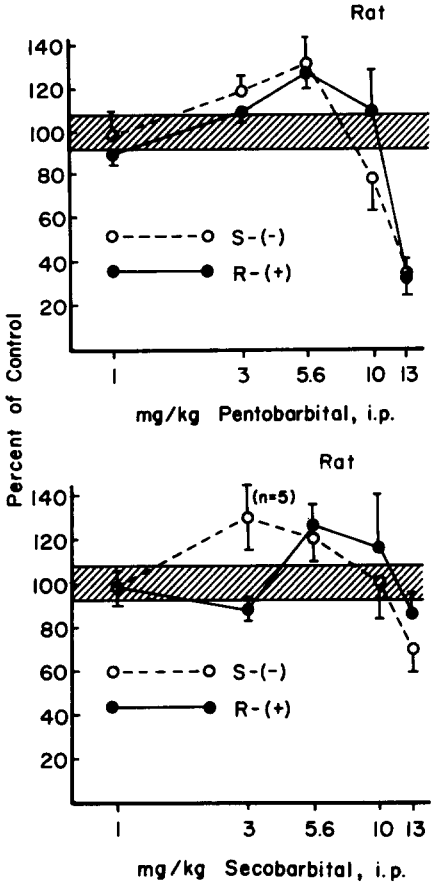

FIG. 3. The effect of the isomers of pentobarbital and secobarbital on the responding of rats under a VI60 schedule of food presentation. Data expressed as in Fig. 2. Points and brackets represent the mean±S.E. for single determinations in each of 6 rats. Mean vehicle control rate of responding was 1.09 responses/sec.

DISCUSSION

The majority of the work reported to date on the effects of pentobarbital and secobarbital in mice and rats has used racemic mixtures. Racemic pentobarbital was reported in mice to produce a small increase in SMA at low doses with decreases in SMA becoming evident as the dose was increased [10,12]. In mice responding under a mult FR30 FI300 schedule of reinforcement, pentobarbital produced a small increase in both FR and FI responding at 3 mg/kg. At 30 mg/kg FR responding was decreased, and at a dose of 56 mg/kg both FR and FI responding were almost totally suppressed [13]. In rats, as in other species, the effect of low doses of racemic pentobarbital on behavior maintained by schedules of food presentation is dependent on the control rate of responding. Typically, low doses will increase low rates of responding while having no effect or decreasing high rates of responding. At higher doses, only rate decreases are reported as a general finding in most species. There are relatively few studies on the effects of racemic pentobarbital and/or secobarbital in rats responding under a single component schedule in which responding was maintained by a VI schedule of food presentation. However, in rats, responding under a two component schedule in which responding in one of the components was maintained under a VI schedule of food presentation, increasing doses of racemic pentobarbital (3.75–30 mg/kg) produced decreases in VI responding [1].

This present study shows that in selected behavioral situations clear differences exist in the behavioral effects of the isomers of pentobarbital and secobarbital. The isomers showed clear differences in potency and magnitude of effect on the SMA of mice. Under a mult FR30 FI600 schedule of reinforcement the differences in the effects of the isomers in mice were not as clear. Under the FR30 component, no significant differences were observed for the isomers of pentobarbital or secobarbital, and under the FI600 component, no differences were observed for the isomers of secobarbital. FI600 responding was increased by S-(−)-pentobarbital while no rate increases were observed at any dose of the R-(+) isomer of pentobarbital. However, no potency differences were observed for the rate decreasing effects of either pentobarbital or secobarbital under the FI600 component of the schedule. In rats responding under the VI60 schedule, only small differences in potency were observed for the isomers of pentobarbital and secobarbital. In all behavioral tests and in both species where differences in potency were observed, the S-(−) isomers of pentobarbital and secobarbital were more potent than the R-(+) isomers.

In previous reports on the effects of the isomers of pentobarbital and secobarbital similar effects have been reported. With respect to anesthetic effects, the S-(−) isomers of pentobarbital and secobarbital have been reported to be 2–4 fold more potent than the R-(+) isomers in mice and rats [2, 4, 11]. Yet with respect to anticonvulsant activity in mice, the isomers of secobarbital are reported to be equipotent in a pentylenetretrazol model [6]. With another barbiturate, hexobarbital, the S-(−) isomer is reported to be less potent than the R-(+) isomer with respect to anesthetic activity in rats [3]. Thus, for the barbiturates with optical isomers which have been studied, with respect to anesthetic and anticonvulsant activity, the S-(−) isomer is either more potent, equipotent, or less potent than the R-(+) isomer.

With respect to the behavioral effects produced by lower doses of the isomers of pentobarbital and secobarbital, there are a very limited number of studies, but generally the S-(−) isomer has proven to be more pharmacologically active than the R-(+) isomer. In two previous reports from this laboratory, Rastogi *et al.* [9] reported that in rats responding under a mult FR30 FI300 schedule of reinforcement, the S-(−) isomer of pentobarbital was slightly less than 2 fold more potent than the R-(+) isomer, but the pharmacological effect of the isomers was the same. Wenger *et al.* [14] reported that in pigeons responding under a mult FR30 FI600 schedule of reinforcement and in pigeons responding under a schedule of suppressed responding (punishment), the S-(−) isomers of both pentobarbital and secobarbital were more potent than the R-(+) isomers. The S-(−) isomer of pentobarbital has also been shown to be more potent in a drug discrimination model than the R-(+) isomer in animals trained to discriminate racemic pentobarbital from saline [7]. Thus, the present data is somewhat in contrast to the general findings in that stereoselective differences were not consistently observed. However, where stereoselective differences were observed they were in agreement with the concensus that the S-(−) isomer is more potent than the R-(+) isomer. However, it must be pointed out that this work has used different species and different behaviors, and there may be behaviors for which the effects of the drugs will show stereoselective differences and other behaviors for which no differences can be demonstrated.

ACKNOWLEDGEMENTS

The author would like to thank Drs. D. E. McMillan and W. D. Wessinger for helpful comments during the preparation of the manuscript, Sherry Tate for technical assistance, Brenda Selby for final preparation of the manuscript and William Hardwick for preparation of the figures.

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