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Chronic Administration of S-(−)-Pentobarbital in Pigeons and Rats: Tolerance Development[1]

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WENGER, G. R. *Chronic administration of S-(−)-pentobarbital in pigeons and rats: Tolerance development.* PHARMACOL BIOCHEM BEHAV 31(2) 459–465, 1988.—The development of tolerance to pentobarbital and cross-tolerance to other barbiturates has been documented in both laboratory animals and man. This study was undertaken to determine the extent of tolerance development to S-(−)-pentobarbital in rats and pigeons receiving 10 mg/kg/day S-(−)-pentobarbital, PO. In addition, the extent of cross-tolerance was determined to R-(+)-pentobarbital and both isomers of secobarbital. Rats were trained to respond under a variable-interval 60-sec (VI60) schedule of food presentation while pigeons were trained to respond under a multiple fixed-ratio 30, fixed-interval 600-sec schedule of food presentation. After responding had stabilized, dose-response curves were determined for R-(+)-pentobarbital, S-(−)-pentobarbital, R-(+)-secobarbital, and S-(−)-secobarbital in both species. Upon the completion of the acute dose-response curves, both rats and pigeons were given 10 mg/kg/day S-(−)-pentobarbital, PO for 30 consecutive days prior to the redetermination of all four dose-response curves. Upon the completion of this second determination of each curve, the daily administration of the S-(−)-pentobarbital was discontinued, and the rats and pigeons remained drug free for 30 days. Following this 30-day drug free period, dose-response curves for the isomers of both pentobarbital and secobarbital were redetermined for a third time. In rats receiving chronic S-(−)-pentobarbital, a 1.5-fold shift to the right (tolerance) was observed in the dose-response curve for the R-(+)- and S(−)-isomers of pentobarbital, and a somewhat smaller shift was observed in the dose-response curves for the isomers of secobarbital. In pigeons receiving 10 mg/kg/day S-(−)-pentobarbital, no shift was observed in the dose-response curve for either isomer of pentobarbital or secobarbital. Upon the discontinuation of the chronic S-(−)-pentobarbital treatment no signs of withdrawal were observed in either species, and in rats the dose-response curves shifted back toward their original positions. Thus, in rats, 10 mg/kg/day of S-(−)-pentobarbital produced a tolerance to the effects of S-(−)-pentobarbital and a cross-tolerance to R-(+)-pentobarbital and both isomers of secobarbital. A similar tolerance was not observed in pigeons receiving 10 mg/kg/day of S-(−)-pentobarbital. No signs of physical dependence were observed in either species.

Pentobarbital Secobarbital Stereoisomers Tolerance Rats Pigeons Operant behavior

TOLERANCE to barbiturates has been studied in several species including man. In most species tolerance to short-to-intermediate acting barbiturates develops in 1 to 8 days (1–3, 8). For barbiturates such as pentobarbital, the tolerance which develops is thought to be a combination of a drug dispositional tolerance as well as functional tolerance, but the two components of the total tolerance development do not always have the same time course. Okamoto *et al.* (12) showed that in cats receiving maximally tolerable daily doses of pentobarbital drug dispositional tolerance developed rapidly to a peak within one week. However, functional tolerance developed much slower. In addition, it has been suggested that tolerance to barbiturates is not complete and a "ceiling effect" exists. This was suggested by Isbell and White (10) who noted that even in addicts who were fullly tolerant to a daily dose of barbiturate, an increase in the daily dose of as little as 100 mg produced a profound and prolonged state of intoxication. However, Boisse and Okamoto (4) and Okamoto *et al.* (12) suggested that a "ceiling effect" does not exist for barbiturates such as pentobarbital. They reasoned that because functional tolerance develops slowly and only after the subject has been challenged with higher doses, the dose can only be increased very slowly.

Several of the short-to-intermediate acting barbiturates used clinically (pentobarbital and secobarbital) are most frequently studied as racemic mixtures. When the individual

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isomers have been studied separately the S-(−) isomers of both pentobarbital and secobarbital have generally been shown to be more potent than the R(+) isomers. Using several different endpoints: lethality and anesthetic potency (5, 6, 15), responding under various schedules of reinforcement (13, 16, 17), discriminative stimulus properties (9, 18, 20) and spontaneous motor activity (16), the S-(−) isomers have been shown to be from 1.5 to 4 times more potent than the R-(+) isomers. However, the role of the individual isomers in the development of tolerance has never been explored. This study was undertaken to determine the degree of tolerance development to S-(−)-pentobarbital and the degree of cross-tolerance to R-(+)-pentobarbital, S-(−)-secobarbital and R-(+)-secobarbital. To examine the species generality of the effects observed, tolerance development was assessed in two species, pigeon and rat. While the schedules of reinforcement were not the same in both species, the schedules used were schedules for which a large data base exists in this laboratory and the published literature. Dose-response curves were determined for responding under both schedules of reinforcement for each isomer of pentobarbital and secobarbital in both species, before, during and after chronic daily oral administration of 10 mg/kg S-(−)-pentobarbital.

METHOD

Subjects

Six adult, male CD rats weighing between 395 and 475 g (Charles River Breeding Laboratories, Portage, MI) and five adult, male White Carneaux pigeons weighing between 510 and 650 g (Palmetto Pigeon Plant, Sumter, SC) when given free access to food and water, were used in the study. Both rats and pigeons were food deprived to a body weight equivalent to 80% of their free-feeding weight and maintained at this weight throughout the experiment by postsession feeding. Subjects were housed individually with water freely available in their home cages. A 12-hr light/dark cycle was maintained with lights on from 0700–1900. All testing was conducted between 0730 and 1730.

Variable Interval 60-Second (VI60) Schedule

Responding under the VI60 schedule was measured in a standard rat test chamber (Gerbrands Model G7322). The chamber was housed inside a sound and light attenuating enclosure (Gerbrands Model G7210). 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 delivered. 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. 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 pair of 28-V DC bulbs (No. 1819) was mounted on 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 sessions except during the 10-sec time-out periods. Schedule programming and data collection were accomplished on a TRS-80, Model III (Radio Shack) microcomputer.

The rats were trained to respond under a VI60 schedule of reinforcement. Under this schedule, in the presence of the illuminated stimulus lights mounted above the right-hand lever, the first response to occur upon the completion of a variable interval of time produced a 97 mg food pellet. Following the presentation of the food pellet, a 10-sec period was initiated during which all lights in the chamber were extinguished, and responses on the right-hand lever had no consequence and were not recorded (time-out). If no responses were made during the first 30 sec following the completion of the variable-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 each interval was determined by the computer controlling the experiment. A given interval could be as short as 10 sec or it could be several minutes long, but it averaged 60 sec in duration. Each test session consisted of 60 VI60 component presentations and lasted approximately 4200 sec including the time-out periods. Test sessions were conducted 5 days/week, Monday through Friday.

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

Pigeons were tested in a standard pigeon operant chamber (Gerbrands pigeon chamber, Model G7311). The chamber was housed in a sound and light attenuating chamber (Gerbrands Model G7211). A force of approximately 0.15 N (15 g) was required to open the key contacts. Opening of the key contacts defined the response and produced an audible feedback click inside the chamber. The chamber was illuminated with two 28-V DC bulbs (No. 1819) at all times except during the presentation of food (mixed grain). Water was not available in the experimental chambers. A TRS-80, Model III (Radio Shack) microcomputer controlled the experiment and recorded the data.

Pigeons were trained to respond under a mult FR30 FI600 schedule of reinforcement. Under this schedule, in the presence of a green key-light, every 30th response produced 5-sec access to a hopper of mixed grain (FR30). In the presence of a red key-light, the first response to occurr after a 600-sec interval of time had elapsed produced 5-sec access to the hopper of mixed grain. The key lights and their associated schedules alternated throughout the experiment. If 30 responses were not made during the first 60-sec period following the onset of the green key-light, the key-light was extinguished, and the red key-light was illuminated signalling the start of the FI600 component. If no response occurred during the first 60 sec after the 600-sec interval had elapsed, the key-light color was switched back to green signalling the start of a FR30 component. The session terminated after seven presentations of the FR30 component and seven presentations of the FI600 component (approximately 72 min under control conditions).

Drugs

S-(−)-Pentobarbital, R-(+)-pentobarbital, S-(−)-secobarbital, and R-(+)-secobarbital were synthesized by Research Triangle Institute (Research Triangle Park, NC). Drug was dissolved in 0.05 M sodium bicarbonate buffer, pH 10.5. For both rats and pigeons, the volume of each injection was 1 ml/kg of body weight. For the determination of dose-response curves, drugs were administered IP to rats and IM to pigeons 5 min before the start of the test session. Typically, drug was administered on Tuesday and Friday with a vehicle control on Thursday. As a routine procedure, normal saline was administered as the vehicle control on

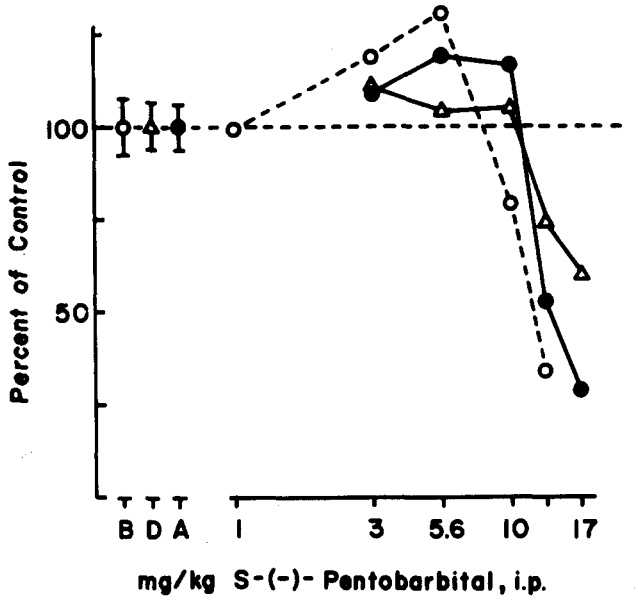

FIG. 1. The effects of S-(−)-pentobarbital on the rate of responding of rats under the VI60 schedule of reinforcement before (B, ○), during (D, △), and after (A, ●) chronic S-(−)-pentobarbital administration. Abscissa: dose in mg/kg on a log scale; ordinate: rate of responding expressed as a percentage of each animal's own control rate of responding. Each data point represents the mean of single determinations in each of 6 rats. Points and brackets above B, D and A represent the vehicle injection control mean±S.E.

Thursday. However, on numerous occasions the actual vehicle, 0.05 M sodium bicarbonate buffer, pH 10.5, was administered. This was not done routinely out of concern for the tissues at the injection site following the injection of an alkaline solution. No difference was observed following either saline or sodium bicarbonate buffer, and all vehicle control values have been pooled. Doses of the isomers were calculated as, and are expressed as, the free acid. Doses of the isomers were administered in a mixed sequence.

Experimental Protocol

This experiment was conducted in three phases. During phase 1 *(before)* of the experiment, dose response curves were determined for each isomer of pentobarbital and secobarbital in both species. Upon the completion of phase 1, phase 2 (*during*) was initiated. For the first 30 days of phase 2 the subjects were tested in their experimental sessions, Monday through Friday, and administered 10 mg/kg/day of S-(−)-pentobarbital at the end of the session. This dose was administered about noon on Saturday and Sunday. After 30 days of chronic dosing, dose-response curves for each of the isomers were determined as before with the chronic dose of S-(−)-pentobarbital administered after the daily session. Upon the completion of the dose-response curves, phase 3 (*after*) was initiated. For the first 30 days of phase 3, the animals were tested Monday through Friday as before, but no drugs were given during this period. Following this 30-day washout period, dose response curves for each isomer were determined a 3rd time.

Measurement of Drug Effects

Average rates of responding, responses/sec, were determined for rats responding under the VI60 schedule, and for pigeons responding under each component of the mult FR30 FI600 schedule. Due to differences in the control rate of responding in several rats, drug effects in the rats were expressed as a percentage of the vehicle control rate of responding. In pigeons which had a more homogenous control rate, the drug effects were expressed as responses/sec. All response rate data are expressed as a group mean±S.E. The S.E. for vehicle control sessions was determined by calculating the total standard deviation (n−1) for all vehicle control observations and dividing by the square root of the number of subjects in the study. Calculation of ED_{50} values was according to the method of Tallarida and Murray (14). For the rats, this was sometimes an extrapolated value since the highest dose tested did not decrease responding to less than 50% of control. However, in all cases, the data points used in the calculation were those obtained following 10, 13 and where tested, 17 mg/kg of the respective isomers. The nonparametric Wilcoxon-Mann-Whitney U-test was used for determination of significance ($p<0.05$) of differences in treatment results.

RESULTS

Under control conditions, both the VI60 and mult FR30 FI600 schedules maintained rates and patterns of responding characteristic of the schedules (7). The VI60 schedule maintained responding in rats at a nearly constant rate, approximately 1 response/sec, throughout the experimental session. The responding of pigeons under the FR30 component of the mult FR30 FI600 schedule was characterized by a brief pause followed by a high continuous rate of responding, approximately 2 responses/sec, which was maintained up to the presentation of the mixed grain. Responding under the FI600 component was characterized by an initial period of a very low rate of responding. The rate of responding gradually increased as the interval progressed resulting in an overall rate of responding for the interval of 0.4–0.5 responses/sec. Typically 50–60% of the interval elapsed before 25% of the responses were made in the interval.

Prior to chronic administration, both isomers of pentobarbital produced increases in the VI60 responding of rats. Increases were observed following doses of 3 and 5.6 mg/kg of the S-(−) isomer and after 5.6 mg/kg of the R-(+) isomer (Figs. 1 and 2). As with the doses which increased rate, a lower dose of the S-(−) isomer (10 mg/kg) was required to decrease responding than was observed with the R-(+) isomer (13 mg/kg). However, the shift in the ED_{50} was not significant (Table 1). When the dose-response curves were redetermined during the period of chronic postsession administration of 10 mg/kg S-(−)-pentobarbital, P.O., the rate increases observed earlier with both isomers were blocked (Figs. 1 and 2). In addition, the descending leg of the dose-effect curve was shifted to the right resulting in higher calculated ED_{50} values (Table 1). The chronic administration of the S-(−) isomer of pentobarbital resulted in a 1.6-fold shift in the ED_{50} value for S-(−)-pentobarbital and a 1.9-fold shift in the ED_{50} value for R-(+)-pentobarbital. Upon the completion of the dose-response curves during the period of chronic dosing, the chronic dosing was discontinued, and the rats remained totally drug free for 30 days prior to a third determination of the curves. As can be seen in Figs. 1 and 2, the curves which were determined after the period of chronic dosing showed a return towards the original curves. Once again, rate increases were observed following low doses of

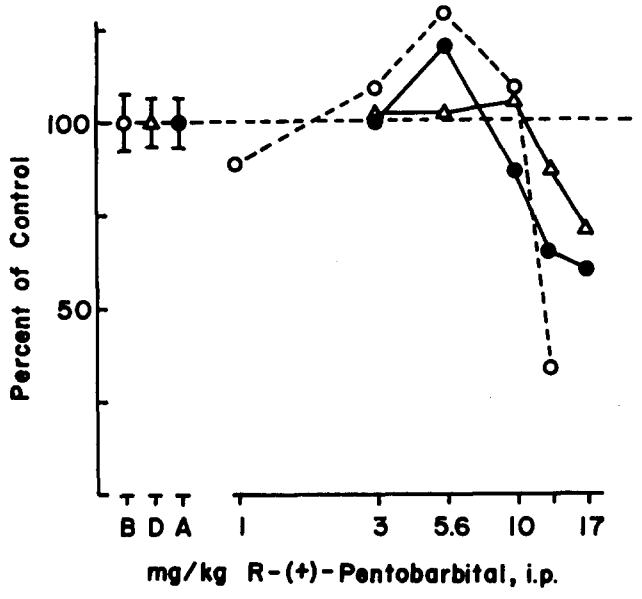

FIG. 2. The effects of R-(+)-pentobarbital on the rate of responding of rats under the VI60 schedule of reinforcement before (B, ○), during (D, △) and after (A, ●) chronic S-(−)-pentobarbital administration. Data expressed as in Fig. 1.

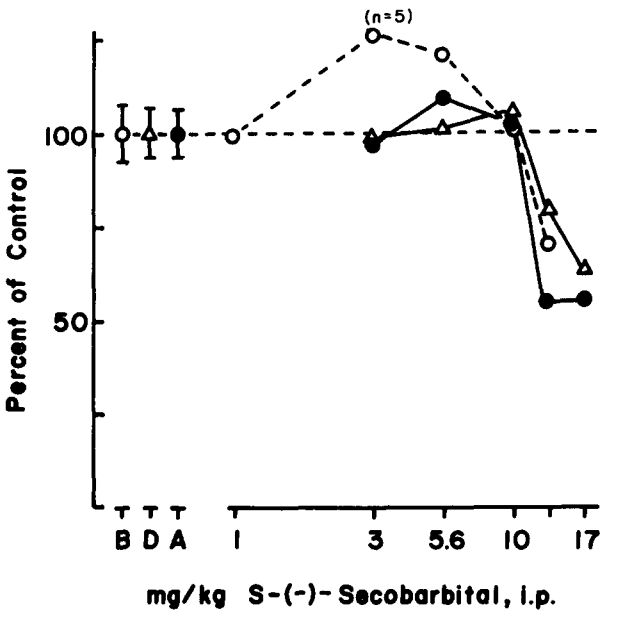

FIG. 3. The effects of S-(−)-pentobarbital on the rate of responding of rats under the VI60 schedule of reinforcement before (B, ○), during (D, △), and after A(A, ●) chronic S-(−)-pentobarbital administration. Data expressed as in Fig. 1.

TABLE 1

THE CALCULATED ED_{50} VALUES (95% CONFIDENCE LIMITS) IN mg/kg FOR THE RATE-DECREASING EFFECTS OF EACH ISOMER ON THE RESPONDING OF RATS UNDER THE VI60 SCHEDULE OF REINFORCEMENT

	Before	During	After
S-(−)-Pento-barbital	11.8 (10.9–12.7)	18.8* (18.0–19.6)	14.4 (13.4–15.3)
R-(+)-Pento-barbital	12.3 (11.4–13.2)	23.1* (22.5–23.7)	20.3* (19.5–21.1)
S-(−)-Seco-barbital	15.4* (14.8–16.0)	19.4* (18.6–20.2)	16.6 (15.9–17.3)
R-(+)-Seco-barbital	16.4* (15.9–16.9)	20.8* (20.0–21.6)	23.5* (23.1–23.9)

*Value represents an extrapolated ED_{50} value.

both isomers, and the descending leg was partially shifted back to its original position. During the 30-day drug-free period following the discontinuation of chronic dosing, no changes were observed in the rate or pattern of responding and no changes were observed in the physical appearance of the rats.

As was seen with the isomers of pentobarbital, before the period of chronic dosing, the isomers of secobarbital produced increases in the VI60 responding of rats at low doses and decreases at higher doses (Figs. 3 and 4). At doses up to 17 mg/kg the decreases in response rate were not as great as those observed following 17 mg/kg of the isomers of pentobarbital. As was seen with the isomers of pentobarbital, the S-(−) isomer of secobarbital was more potent than the R-(+) isomer with respect to the rate increasing effects.

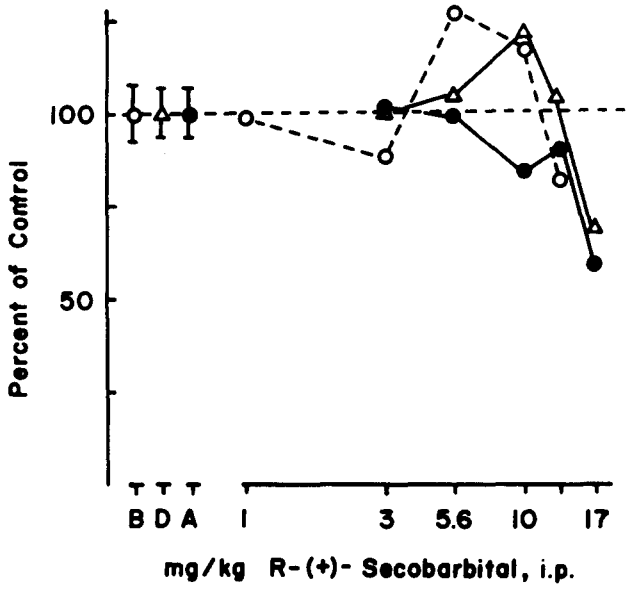

FIG. 4. The effects of R-(+)-secobarbital on the rate of responding of rats under the VI60 schedule of reinforcement before (B, ○), during (D, △), and after (A, ●) chronic S-(−)-pentobarbital administration. Data expressed as in Fig. 1.

Upon the completion of this initial determination of the dose-response curves, the period of chronic postsession dosing with 10 mg/kg S-(−)-pentobarbital PO was initiated. A redetermination of the dose-response curves during the period of chronic dosing revealed an absence of the rate increasing effects observed earlier with the S-(−) isomer of secobarbital, and a shift to the right in the dose which

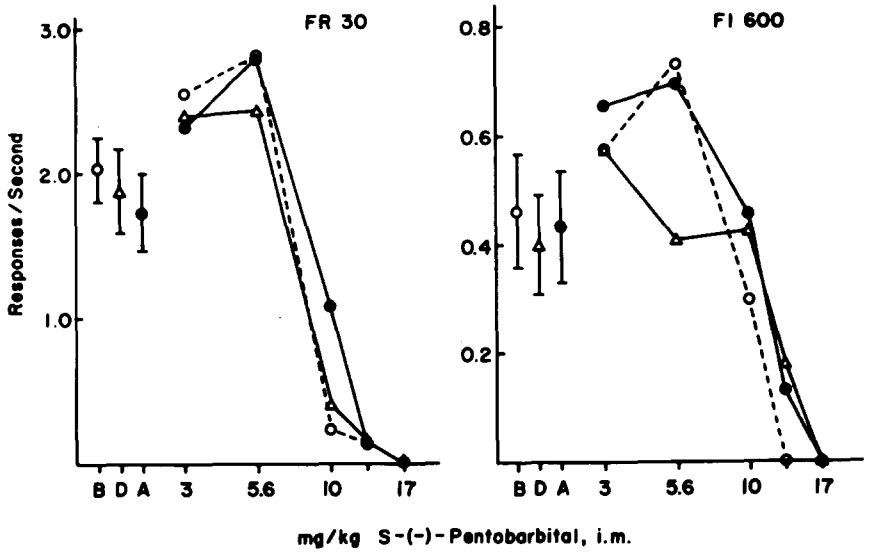

FIG. 5. The effects of S-(−)-pentobarbital on the rate of responding of pigeons under the mult FR30 FI600 schedule of reinforcement (B, ○), during (D, △) and after (A, ●) chronic administration of S-(−)-pentobarbital administration. Abscissa: dose in mg/kg on a log scale; ordinate: rate of responding in responses/second. Each point represents the mean of duplicate (B) or single (D and A) determinations in each of 5 pigeons. Points and brackets above B, D, and A represent the vehicle injection control mean±S.E.

produced the peak rate increases following R-(+)-secobarbital. In addition, the descending leg of the dose-response curve was shifted to the right (Fig. 3 and 4). However, the shift of the descending leg to the right observed with both isomers of secobarbital was not as great as the shift observed with the isomers of pentobarbital, and only a 1.3-fold shift of the ED_{50} values (Table 1) was observed.

When the curves were determined for a third time, 30 days after the discontinuation of chronic S-(−)-pentobarbital, the dose-response curve for S-(−)-secobarbital partially returned to its initial position and shape. However, the rate increases initially observed following doses of 3 and 5.6 mg/kg were not seen in this third determination of the curve. The third determination of the R-(+)-secobarbital curve also failed to show any rate increases at low doses, and no return was observed in the ED_{50} value for rate-decreasing effects.

In pigeons responding under the mult FR FI schedule, S-(−)-pentobarbital increased responding under both schedule components at doses of 3–5 mg/kg (Fig. 5). At higher doses, the rate of responding decreased in both components. Although the rate increases observed at low doses were not always as large, similar effects to those observed for S-(−)-pentobarbital were seen with R-(+)-pentobarbital and both isomers of secobarbital (data not shown). For both pentobarbital and secobarbital, the S-(−) isomers were more potent than the R-(+) isomers. Under both schedule components the ED_{50} values for the rate-decreasing effects were lower following the S-(−) isomers than that observed with the R-(+) isomer (Table 2).

When the dose-response curve for S-(−)-pentobarbital was redetermined during the period in which the pigeons were receiving daily doses of 10 mg/kg/day S-(−)-pentobarbital, PO, the rate increases initially observed following low doses were blocked, but the rate-decreasing effects of higher doses were not greatly affected. The ED_{50} value for the rate decreasing effects under the FI600 component shifted only 1.26-fold, and the ED_{50} did not shift for the rate-decreasing effects under the FR30 component (Fig. 5 and Table 2). When the dose-response curve was determined for R-(+)-pentobarbital the rate increases initially observed at low to moderate doses were blocked. However, either no shift (FI600) or only a very small shift (FR30) was observed in the ED_{50} value for the rate-decreasing effects at higher doses. With the isomers of secobarbital the rate increases initially observed were not affected by the chronic treatment with S-(−)-pentobarbital, and, in general, a similar lack of an impressive shift was observed in the ED_{50} values for the rate decreases at higher doses (Table 2). Clearly, the degree of tolerance development observed was not as large or as consistant as that observed in rats responding under the VI60 schedule. Table 2 shows that the ED_{50} values for the rate decreasing effects of the isomers in pigeons were shifted only 1.1–1.36-fold. This compares to shifts of 1.3–1.9-fold in rats (Table 1). In addition, it can be seen that under the FR30 component the shift was never larger than 1.15-fold, and in many cases under both the FR30 and the FI600 schedules the 95% confidence limits overlap.

Upon the completion of the second determination of the dose-response curves in the pigeons, the daily administration of 10 mg/kg/day of pentobarbital was discontinued, and the birds remained drug free for 30 days. When the curves were redetermined a third time following the 30-day drug-free period, the ED_{50} values for the most part remained unchanged. The only exception being the ED_{50} values for R-(+)-secobarbital which for some unexplained reason shifted to the right (Table 2).

For both rats and pigeons, no changes in the rate and/or the pattern of responding were observed during the 30-day drug-free period following chronic dosing. Similarly, no

TABLE 2

THE CALCULATED ED_{50} VALUES (95% CONFIDENCE LIMITS) IN mg/kg FOR THE RATE-DECREASING EFFECTS OF EACH ISOMER ON THE RESPONDING OF PIGEONS UNDER EACH COMPONENT OF THE MULT FR30 FI600 SCHEDULE OF REINFORCEMENT

	Before	During	After
		FR30	
S-(−)-Pentobarbital	9.06 (8.16– 9.96)	9.71 (8.9–10.5)	11.1 (10.2–12.0)
R-(+)-Pentobarbital	13.2 (12.3 –14.1)	15.0 (14.2–15.8)	14.5 (13.7–15.3)
S-(−)-Secobarbital	9.9 (9.0 –10.8)	11.3 (10.5–12.1)	11.0 (10.1–11.9)
R-(+)-Secobarbital	14.7 (14.0 –15.4)	16.5 (15.8–17.2)	18.1* (17.5–18.7)
		FI 600	
S-(−)-Pentobarbital	10.4 (9.56–11.2)	13.1 (12.2–14.0)	12.5 (11.6–13.4)
R-(+)-Pentobarbital	14.7 (14.0 –15.4)	15.2 (14.5–15.9)	15.6 (14.8–16.4)
S-(−)-Secobarbital	16.1 (15.3 –16.9)	18.1* (17.4–18.8)	17.2 (16.6–17.8)
R-(+)-Secobarbital	16.9 (16.3 –17.5)	23.0* (22.5–23.5)	28.0* (27.7–28.3)

*Value represents an extrapolated ED_{50} value.

differences were observed in the animals reaction to daily handling, or in their outward appearance.

DISCUSSION

Prior to this study relatively little, if any, data existed on the stereospecificity of tolerance development to a single isomer of a barbiturate with optically active centers. This study shows that with repeated administration in the rat, tolerance develops to the effects of S-(−)-pentobarbital on responding under a VI60 schedule of reinforcement. Furthermore, a cross-tolerance develops to R-(+)-pentobarbital and to both isomers of secobarbital. The shift to the right in the ED_{50} value for the R-(+)-isomer of pentobarbital was slightly larger than that observed for S-(−)-pentobarbital, but no difference was observed in the degree of shift for the ED_{50} values for the isomers of secobarbital. Thus, in the rat it would appear that if a stereospecificity does not exist for the degree of tolerance development, it does not apply in a general way for all barbiturates.

While this study clearly showed a tolerance development in rats following repeated administration of S-(−)-pentobarbital, a similar protocol in pigeons failed to show a clear tolerance development. The ED_{50} for the effects of S-(−)-pentobarbital on responding under the FR30 component failed to shift to the same degree as was seen in rats responding under the VI60 schedule. Under the FR30 component the ED_{50} value for the effects of S-(−)-pentobarbital shifted only 1.07-fold. This compares to a 1.59-fold for the same isomer in rats. A similarly unimpressive shift in ED_{50} values was observed for the effects of R-(+)-pentobarbital and the isomers of secobarbital on responding of pigeons under the FR30 schedule. Under the FI600 schedule, the degree of shift of the ED_{50} for S-(−)-pentobarbital, R-(+)-pentobarbital and S-(−)-secobarbital all tended to be smaller in magnitude than that observed in the rat. Only with R-(+)-secobarbital was the degree of shift in the ED_{50} for pigeons responding under the FI600 component of the multiple schedule equal to or larger in magnitude than that observed in the rat.

Tolerance development to the sedative/hypnotic effects to pentobarbital in several laboratory species is well documented: rat (1, 2, 8), cat (12). In addition, tolerance development to the effects of pentobarbital in rats responding under schedules of reinforcement has also previously been reported (11). However, in all of these studies the tolerance was produced by the repeated administration of racemic pentobarbital, and no information existed on what, if any, stereospecificity might exist in the development of tolerance. Based on the present study, it would appear that in the rat there is little evidence for stereospecificity in tolerance development beyond the documented difference in potency between the isomers seen in this study and earlier work (16). If a strong stereospecificity did exist for tolerance development, one would not expect to see the ED_{50} values for R-(+)-pentobarbital to be shifted approximately the same degree as that observed for the S-(−) isomer of pentobarbital. Nor would one expect to see a similar degree of cross-tolerance develop to the isomers of secobarbital. Thus, although tolerance development was only studied following repeated administrations of S-(−)-pentobarbital, and the effects of repeated administrations of the R-(+) isomer were not studied, it would appear that there is little evidence for the stereospecificity of tolerance development in the rat.

The failure to demonstrate a significant tolerance development in the pigeon was disappointing. This was especially true in light of the results obtained in the rat. The reason for the difference between the rat and the pigeon are not clear. It may, however, relate to the route of administration for the chronic dosing. Since the isomers were administered as the free base and dissolved in a buffer with a pH of 10.5, it did not seem wise to administer a solution with such a high pH into the breast muscle of a pigeon on a daily basis for an extended period of time. Thus, a decision was made to administer the daily dose of 10 mg/kg/day S-(−)-pentobarbital orally in both species. This decision may have been prudent based on pH considerations and IM injections, but it also may have resulted in lower blood levels in the pigeon compared to those obtained after IM administration. Drug administered by the oral route is absorbed into the portal system, and the drug is subjected to the metabolic processes of the liver before entering the general circulation. Interestingly, the same is usually true following IP administration. Thus, the blood levels obtained following IP and oral administration in the rat may not have differed as much as those obtained in the pigeon after IM and oral administration. These pharmacokinetic factors cannot be ruled out as a possible explanation for the difference in tolerance development between rat and pigeon. It should be noted, however, that Barrett and Witkin (19) failed to observe a development of tolerance in pigeons receiving 10 mg/kg/day of racemic Na. pentobarbital. The present study and the study by Barrett and Witkin (19) are the only studies that this author is aware of that have examined tolerance development to barbiturates in pigeons. The failure to observe any tolerance development may be a function of the chronic dose. However, the dose of the S-(−) isomer used in this study was sufficient to totally suppress responding for a significant portion of the

experimental session. Nevertheless, until tolerance in pigeons is demonstrated, the possibility that no tolerance develops in pigeons must remain a possible explanation for the results of this experiment.

Finally, it should be noted that the demonstration of a cross-tolerance from S-(−)-pentobarbital to R-(+)-pentobarbital and to both isomers of secobarbital, make it unlikely that the reported "ceiling effect" [Isbell and White (10)] for barbiturate tolerance is due to stereospecific differences in tolerance development.

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