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Mechanisms contributing to barbiturate intolerance in rats

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Summary

1. Female rats, 3 weeks after pretreatment with 200 or 400 (mg/kg)/day barbitone for 2 or 30 days, exhibited a prolonged sleeping time and a reduced awakening barbiturate brain level when challenged with either barbitone or pentobarbitone. After 3 additional weeks, the latter responses had returned, or were returning to, control values.

2. Barbitone pretreatment schedules had no residual effect upon *in vitro* hepatic pentobarbitone-metabolizing activity measured 3 or 6 weeks later, except in one instance, when hepatic enzyme activity was significantly enhanced 3 weeks after 30 daily doses of 200 mg/kg barbitone. In this case, however, an enhanced barbiturate sleeping time, together with a reduced awakening barbiturate brain level, were observed.

3. It is concluded that barbitone administered intraperitoneally in doses of 200 to 400 (mg/kg)/day for 2 or 30 days induces a non-hepatogenic intolerance to barbiturates, related to an increased sensitivity of the central nervous system to these drugs. This central intolerance is seen 3 weeks, but not 6 weeks, after pretreatment. Furthermore, this central intolerance has been observed to co-exist with an hepatic tolerance, a situation which could result in a reduced LD_{50} coupled with an increase in ED_{50}.

Introduction

Both an acutely- and a chronically-induced barbiturate intolerance have been reported in the literature. In acutely-induced intolerance, two daily doses of a barbiturate induces a hypersusceptibiity which is maximal after 3 to 4 weeks and reversed after 6 weeks (Aston & Hibbeln, 1967; Stolman & Aston, 1970). This intolerance appears to be the result of an alteration in the sensitivity of the central nervous system to the drug. In the chronically-induced type of intolerance, administration of barbiturate for a period of 32 days results in an exaggerated barbiturate response, manifested maximally after 3 weeks of abstinence and lasting for up to 4 months (Stevenson & Turnbull, 1970). This intolerance is associated with a reduction in hepatic drug-metabolizing activity.

Since the induction of central and hepatic intolerance appear to depend upon pretreatment dosage schedule, the present study was designed to determine the effects of alterations in the dose level and duration of barbitone pretreatment upon the manifestation of intolerance, as well as to investigate the level of central barbiturate sensitivity and hepatic enzyme activity resulting from such pretreatment.

Methods

Female rats of the Charles River CD strain were subjected to varying barbitone pretreatment schedules and, after abstinence intervals of different durations, these animals were challenged, as were saline-pretreated controls, with either barbitone or pentobarbitone. In all cases the intraperitoneal route of administration was employed. Both sleeping times (the time between loss and regaining of the righting reflex) and awakening barbiturate brain levels were determined in these rats. *In vitro* hepatic pentobarbitone-metabolizing activity was measured in livers obtained from unchallenged animals from each pretreated group, as well as from control animals.

Pretreatment schedules included 50 or 200 (mg/kg)/day barbitone administered for a total of 2 or 30 days. An additional group of rats received 400 (mg/kg)/day barbitone for 2 days. Daily doses of 400 mg/kg barbitone beyond 2 days, however, resulted in a high mortality among the experimental animals. For this reason the following 30 day barbitone dosage schedule was adopted: 100 mg/kg every 12 h for 7 days, 150 mg/kg every 12 h for 7 days, and 200 mg/kg every 12 h for 16 days. Each of these 6 pretreated groups was divided into 2 subgroups subjected to abstinence intervals of 3 to 6 weeks. Adequate saline-pretreated controls were included. Pretreatment schedules were arranged so that terminal challenge doses of barbiturate fell on the same calendar date. To ensure that terminal experimental body weights were equivalent, rats on the 3 and 6 week abstinence intervals weighed 80 and 120 g respectively at the beginning of the study.

The above experimental schedule provided 12 groups of pretreated rats. The barbiturate response level of each of these groups was assessed by administering barbitone (200 mg/kg as a 3% aqueous solution) or pentobarbitone (30 mg/kg as a 1% solution adjusted to isotonicity) and recording sleeping times.

Awakening brain levels of barbiturate in challenged animals were determined as an index of central nervous system sensitivity to the drugs, by means of a spectrophotometric method (Stolman & Aston, 1970) based upon the technique of Williams & Zak (1959). In addition, hepatic pentobarbitone-metabolizing activity was determined in unchallenged rats from each of the 12 pretreated groups, by the method of Kato & Chiesara (1962), to determine possible changes in the metabolic disposition of barbiturates.

The significance of differences between mean values was estimated by Student's *t*-test, following the methods of Burn, Finney & Goodwin (1950). All statements of significance refer to the 5% level of probability.

Results

Fifty and 200 mg/kg dose levels

Pretreatment of rats with 50 mg/kg barbitone, regardless of the dosage schedule employed, was without significant effect upon any of the response parameters examined. Thus, 3 or 6 weeks after 2 or 30 such doses, hepatic enzyme activity, sleeping times, and awakening barbiturate brain levels (following both pentobarbitone and barbitone challenge) did not differ from control values.

Pretreatment of rats with 2 daily doses of 200 mg/kg barbitone resulted, 3 weeks later, in a 21% prolongation of sleeping time when the animals were challenged

TABLE 1. *Responses of female rats to a 30 mg/kg i.p. pentobarbitone challenge 3 weeks after barbitone pretreatment*

Barbitone pretreatment			Pentobarbitone challenge	
Dose (mg/kg)	No. daily doses	Hepatic enzyme activity*	Sleep time (min)	Awakening brain level (μg/g)
50	2	29·9±1·4 (7)	210·8±10·6 (5)	21·6±2·2 (5)
50	30	29·9±4·1 (8)	203·2±13·1 (5)	21·5±1·2 (5)
200	2	32·2±1·7 (8)	257·0±7·2 (10)**	15·9±1·0 (9)**
200	30	39·1±2·7 (8)**	241·5±12·0 (10)**	16·9±0·9 (10)**
400	2	32·0±1·2 (11)	261·4±12·3 (12)**	17·1±1·1 (12)**
400†	30	30·2±4·0 (5)	247·7±11·6 (10)**	15·9±1·3 (10)**
Naive controls		29·2±2·0 (22)	211·8±8·3 (20)	21·1±1·1 (14)

* Expressed as (μg pentobarbitone metabolized/g wet liver)/2 hours. ** Significantly different from corresponding control value at $P=0·05$. † Maximum incremental dose (see **Methods**). Number of animals is shown in parentheses.

TABLE 2. *Responses of female rats to a 200 mg/kg i.p. barbitone challenge 3 weeks after barbitone pretreatment*

Barbitone pretreatment		Barbitone challenge	
Dose (mg/kg)	No. daily doses	Sleep time (min)	Awakening brain level (μg/g)
50	2	228·4±9·5 (5)	138·4±1·6 (5)
50	30	212·4±13·4 (5)	135·9±2·4 (5)
200	2	273·0±11·5 (5)*	124·4±2·6 (5)*
200	30	277·8±11·0 (9)*	120·1±2·0 (9)*
400	2	265·0±11·8 (12)*	125·0±2·1 (11)*
400**	30	263·8±15·4 (9)	122·6±2·0 (9)*
Naive controls		230·8±9·7 (16)	141·4±3·0 (16)

* Significantly different from corresponding control value at $P=0·05$. ** Maximum incremental dose (see **Methods**). Number of animals is shown in parentheses.

with pentobarbitone, and a 25% reduction in awakening barbiturate brain level, compared to control (Table 1). Parallel changes were observed with barbitone challenge (Table 2). Sleeping time was increased by 18%, and awakening barbitone brain level was reduced by 12%, compared to control values. Hepatic enzyme activity was unaltered by this pretreatment schedule. Six weeks after barbitone pretreatment all measured parameters had returned to control values (Tables 3 and 4).

Pretreatment of rats with 200 mg/kg barbitone daily for 30 days caused, after 3 weeks, a 34% increase in hepatic pentobarbitone metabolizing activity (Table 1), indicating the development of hepatic tolerance. At the same time, however, pentobarbitone and barbitone sleeping times were significantly prolonged by 14 and 26% respectively (Tables 1 and 2). In the case of barbitone this was associated with a 15% decline in the barbitone brain level upon awakening. A 20% decline was observed in the awakening pentobarbitone brain level. Six weeks after the 30 daily doses of barbitone, all measured parameters were approaching control levels (Tables 3 and 4).

Four hundred mg/kg dose level

Two daily doses of 400 mg/kg barbitone resulted in essentially the same response patterns at 3 and 6 weeks, as did 2 daily doses of 200 mg/kg barbitone. The

TABLE 3. *Responses of female rats to a 30 mg/kg i.p. pentobarbitone challenge 6 weeks after barbitone pretreatment*

Barbitone pretreatment			Pentobarbitone challenge	
Dose (mg/kg)	No. daily doses	Hepatic enzyme activity*	Sleep time (min)	Awakening brain level (μg/g)
50	2	29·7±1·4 (8)	215·2±13·6 (5)	21·0±2·2 (4)
50	30	33·7±3·4 (8)	220·7±38·9 (8)	18·1±2·5 (8)
200	2	32·1±2·0 (8)	201·7±11·4 (5)	21·4±2·3 (5)
200	30	35·9±3·6 (8)	238·7±14·2 (10)	19·4±1·7 (10)
400	2	29·8±0·7 (9)	214·1±10·7 (8)	24·0±0·9 (8)
400**	30	24·7±1·1 (10)	184·6±15·2 (9)	18·0±1·2 (9)
Naive controls		29·2±2·0 (22)	211·8±8·3 (20)	21·1±1·1 (14)

* Expressed as (μg pentobarbitone metabolized/g wet liver)/2 hours. ** Maximum incremental dose (see Methods). Number of animals is shown in parentheses.

TABLE 4. *Responses of female rats to a 200 mg/kg i.p. barbitone challenge 6 weeks after barbitone pretreatment.*

Barbitone pretreatment		Barbitone challenge	
Dose (mg/kg)	No. daily doses	Sleep time (min)	Awakening brain level (μg/g)
50	2	231·0±15·7 (5)	136·5±3·2 (4)
50	30	221·5±12·5 (4)	134·6±3·8 (4)
200	2	236·7±19·5 (5)	142·1±2·0 (5)
200	30	252·2±14·0 (10)	134·3±2·3 (10)
400	2	255·4±10·7 (9)	140·6±2·9 (9)
400**	30	259·7±13·3 (11)	126·1±2·6 (11)*
Naive controls		230·8± 9·7 (16)	141·4±3·0 (16)

Number of animals in parentheses. *Significantly different from corresponding control value at $P=0·05$. **Maximum incremental dose (see Methods).

increased barbitone pretreatment dose did not augment the magnitude of induced changes beyond those observed following the 200 mg/kg dosage.

Three weeks after increasing barbitone doses to 400 (mg/kg)/day over 30 days, response parameters followed the same pattern as after 2 doses of 400 mg/kg barbitone, i.e. prolonged sleeping times coupled with reduced brain barbiturate levels upon awakening (Tables 1 and 2). No change in hepatic enzyme activity was observed. Six weeks after pretreatment with incremental barbitone doses to 400 mg/kg over 30 days, the response to a barbitone or pentobarbitone challenge was the same as after a 3 week abstinence (Tables 3 and 4). Hepatic pentobarbitone metabolizing activity was reduced below control levels, though not significantly. This was the only experimental group to show this response, all others exhibiting no change, or else a tendency to an increase, in enzyme activity.

Discussion

Effect of variation in pretreatment dose level

Neither 2, nor 30, daily doses of 50 mg/kg barbitone resulted in any changes in measured responses to barbiturate challenge 3 or 6 weeks later. Since the 200 mg/kg pretreatment dose level produced a significant enhancement of barbiturate sleeping

times following 3 weeks of abstinence, it is apparent that the appearance of delayed intolerance is a dose-related phenomenon, probably reflecting a critical threshold of central depression required for the development of hyper-susceptibility. This response may be quantal, rather than graded, in nature since an increase in the pretreatment dose level from 200 to 400 (mg/kg)/day barbitone caused no corresponding increase in the degree of induced intolerance. However, it is possible that a graded dose-response relationship between intolerance and pretreatment dose exists between the 50 and 200 mg/kg levels, reaching a plateau response at doses beyond this range. Barbiturate sleeping times, obtained 6 weeks after 2 daily doses of either 200 or 400 mg/kg barbitone, had returned to control levels, indicating that the size of the pretreatment dose had little or no effect upon the duration of the intolerance phase.

Effect of variation in duration of pretreatment

It might be anticipated that the magnitude of induced intolerance would be proportional to the duration of barbitone pretreatment. However, increasing the number of daily doses of 50 mg/kg barbitone from 2 to 30 in no way altered the responses of rats to later barbiturate challenge, when compared to controls. After 2 or 30 doses of 200 (mg/kg)/day barbitone, barbiturate challenge sleeping times at 3 weeks, were enhanced to similar extents. After 6 weeks abstinence, however, rats challenged with either barbitone or pentobarbitone still displayed a non-significant prolongation of sleeping time if pretreated for 30 days, while in rats pretreated for 2 days sleeping times had returned to control levels. These data indicate that the duration, but not the intensity, of induced intolerance is proportional to the length of the pretreatment schedule. This relationship was not evident when daily pretreatment doses of 400 mg/kg were employed.

Mechanism of intolerance

Three lines of evidence, in the present study, indicate a central nervous system, rather than hepatic, site of induced intolerance to barbiturates.

(a) Rats pretreated at dose levels of 200 and 400 (mg/kg)/day barbitone exhibited prolonged sleeping times whether challenged with pentobarbitone or barbitone. Since less than 4% of a dose of barbitone undergoes biotransformation in the rat (Ebert, Yim & Miya, 1964) it is unlikely that alterations in hepatic metabolizing activity can account for the observed hyper-susceptibility to this drug. In addition, it has been shown that the rate of decline of blood and brain levels of both unlabelled and of [^{14}C]-barbitone is the same in intolerant and in control rats up to 360 min after a challenge dose of the drug (Stolman & Aston, 1970).

(b) In all cases of significant intolerance observed in the present investigation, a corresponding significant decline in the awakening brain barbiturate concentration was observed. This provides direct evidence of an increase in the sensitivity of the central nervous system to pentobarbitone and barbitone. In these instances, reversion of sleeping times to control levels (observed 6 weeks after 2 daily doses of 200 or 400 mg/kg barbitone) was accompanied by a corresponding return of awakening barbitone brain concentrations to control values. A similar relationship between barbitone sleeping times and awakening barbitone blood levels has been previously reported from this laboratory (Stolman & Aston, 1970), corroborating the concept of intolerance as a central phenomenon.

(c) *In vitro* liver pentobarbitone-metabolizing activity was measured at 3 and 6 weeks following each of the 6 barbitone pretreatment schedules employed in this study. With one exception, no significant differences between hepatic barbiturate biotransformation rates in pretreated and control animals was observed, indicating a lack of correlation of liver enzyme activity and observed sleeping times. The exception among these data occurred in the case of rats examined 3 weeks after 30 daily doses of 200 mg/kg barbitone. In this instance, a significant increase of 34% in liver enzyme activity was seen, which was still present, though non-significant, after 6 weeks. Such enzyme induction should result in an objective hepatic tolerance. Apparently, however, in this group of animals, induced central intolerance was of such magnitude as to overshadow hepatic hypo-susceptibility, with the result that a significant prolongation of sleep time was observed. Conceivably, hepatic tolerance and central intolerance might coexist in such balance as to result in a significant enhancement of acute lethality to barbiturates with no change, or even a reduction in, the hypnotic or anaesthetic effectiveness of the drugs. Presumptive evidence of a similar interaction at hepatic and central levels has been recently obtained (Fredericks, Larson & Aston, 1973), in which central intolerance to pentobarbitone in rats was masked by hepatic enzyme induction caused by chronic dietary administration of chlorophenothane (DDT). The present study, however, appears to be the first report of such opposing mechanisms induced by a single drug.

The results reported here of a central intolerance induced by 30 doses of 400 (mg/kg)/day barbitone appear to be inconsistent with results of Stevenson & Turnbull (1968) who found a hepatogenic intolerance 3 weeks after 32 days of barbitone administration on a similar dosage schedule. The intolerance reported by the latter authors was characterized by a reduced hepatic hexobarbitone-metabolizing activity lasting up to 4 months, prolongation of sleeping time upon intraperitoneal pentobarbitone challenge, and a lack of evident intolerance following challenge with intraperitoneal barbitone or intraventricular pentobarbitone (Stevenson & Turnbull, 1970). In addition, awakening barbitone brain levels of intolerant animals did not differ from control values. Such hepatogenic intolerance, as opposed to the central intolerance observed in the present investigation, appears to be explicable on the basis of differences in route of administration. Stevenson & Turnbull observed hepatic intolerance following chronic barbitone administration, in incremental doses up to 400 (mg/kg)/day, in drinking water, while central intolerance has developed following intraperitoneal administration of similar doses of barbitone. It would appear, therefore, that central neuronal intolerance is achieved only after exposure of the central nervous system to high peak brain levels obtainable with parenteral administration of barbitone. As has already been pointed out, in reference to the relationship between central intolerance, induction and dose level, a critical central barbiturate threshold appears to exist which must be exceeded if an increase in neuronal sensitivity to the drugs is to be manifested. Such brain levels are unlikely to be achieved by allowing *ad libitum* access to barbitone in drinking water, as in the method of Stevenson & Turnbull.

On the other hand, no significantly reduced liver enzyme activity was obtained in the present study following long-term barbitone pretreatment, such as was observed by the former authors. Inhibition of hepatic enzyme activity may result from the exposure of liver to a continuous, though low, blood level of barbitone by the oral

route. Intermittent parenteral administration of the drug, though achieving high peak body levels of barbitone, may allow for significant periods during which barbitone blood levels are below those required to elicit the degree of enzyme induction necessary for subsequent exhaustion of drug metabolizing activity. It is of interest to note, in this regard, that a non-significant reduction of 15% in liver enzyme activity was observed, in the present study, following 30 daily doses of up to 200 mg/kg barbitone administered on a twice daily basis. This particular pretreatment schedule was the one most likely to result in the continuous barbitone blood levels achieved by Stevenson & Turnbull in their *ad libitum* oral pretreatment method.

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