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THE EFFECT OF EXPERIMENTALLY MODIFIED SLEEPING PERIODS ON BRAIN LEVELS OF BARBITAL*

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Abstract—Whole brain levels of barbital were determined in 158 albino rats. The drug levels in the brain were related to experimentally induced alterations in the latent and sleeping periods following barbital injection. The brain barbital levels were related to observed alterations in latent and sleeping periods. Significant changes in latent times and in periods of sedation occurred in the absence of substantial differences in the brain levels of barbital from those measured in control animals at equivalent times following the injection of barbital.

ALTERATIONS in the duration of barbiturate hypnosis have been induced experimentally by the use of a wide variety of pharmacological agents. As pointed out by Richards in his excellent review of the subject,[1] several mechanisms may be operative in the modification of barbiturate effects. The chemical makeup and metabolic fate of the barbiturates are factors, as well as the dosage and species employed. Many of the previous investigations concerned with the duration of the sedative effects of the barbiturates have not included data on brain levels of the drug, so that accurate criteria for analysis of possible modes of action have frequently not been available.

The purpose of this investigation was to study the effect of experimentally produced alterations in the duration of barbiturate-induced sedation on the level of the drug in brain tissue at the beginning and end of sedation. In order to obviate variability that might occur due to changes in barbiturate biotransformation, barbital, which is not metabolized and is excreted unchanged, was employed for the induction of sedation.

METHODS

Adult albino rats of the Denver strain were employed as experimental animals. Sedation was induced by the intraperitoneal injection of 200 mg of sodium barbital per kg. A latent period was measured from the time of injection to loss of the righting reflex. Sleeping periods were measured from the time of loss of righting reflex to the return of the same reflex. All animals were killed by decapitation and the entire brain was removed, weighed and immediately homogenized. Total brain barbital levels were determined for each animal by the method of Brodie,[2] in which the tissue is extracted with petroleum ether in acid solution. The drug is then transferred from

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the ether to an aqueous alkaline phosphate buffer and quantitated spectrophotometrically. Brain levels of barbital were expressed as micrograms per gram of brain tissue. Brain levels of barbital determined in a series of control groups of male rats at the onset of sleep, the end of the sleeping period, 80 min, 210 min and 300 min respectively, after injection with sodium barbital. The drug was administered intraperitoneally as a saline solution in a dose of 200 mg kg. A group of male animals were treated with reserpine, 100 μg per kg, injected subcutaneously. Twenty hours following the administration of reserpine, the animals were administered sodium barbital. In another group of male rats, 19·5 hr following treatment with the same dose of reserpine, lysergic acid diethylamide (LSD) was injected subcutaneously in a dose of 1 mg per kg, followed in 30 min by the injection of the barbital. One group of male rats was injected with sodium barbital dissolved in distilled water. Two groups of female animals were utilized. A control group was injected with sodium barbital in distilled water via the intraperitoneal route. A second group of females was subjected to bilateral ovariectomy and administered the same dose of barbital 9 days after operation. Brain levels of barbital were determined in all of the experimental groups at the end of the latent period and at the end of the sleeping period. The dose of barbital given intraperitoneally, was 200 mg per kg in all animals.

RESULTS

Latent periods and sleeping times observed for the various groups of animals are summarized in Table 1. Significant differences from control values were observed in the latent periods in animals treated with reserpine plus LSD, and, in the groups in which distilled water was employed as the vehicle for barbital. Administration of reserpine prior to barbital sedation resulted in greatly prolonged sleeping times. Injection of LSD after treatment with reserpine prior to induction of barbital hypnosis was followed by sleeping times greatly reduced from those seen in the reserpine-treated animals. These observations on the effect of reserpine, and of reserpine plus LSD, on sleeping times are in accord with results reported by Taeschler and Cerletti[3] for thiopental and Brodie *et al.*[4] for hexobarbital. The sleeping times of castrate females were generally longer than those for female controls, but due to considerable variability from animal to animal the difference was not statistically significant. No significant differences were noted between the latent periods or sleeping periods in male and female animals of the control groups, with distilled water as the vehicle for the barbital.

Brain levels of barbital at the beginning and at the end of sleep are presented in Table 2, and are related graphically to latent and sleeping periods in Fig. 1. Included in the data are brain levels of the drug in control animals at various intervals of time following barbital injection. In the group treated with reserpine prior to barbital injection, no significant difference occurred in the brain levels of barbital between these animals and the brain levels in control animals, at equivalent times following injection of barbital. From Fig. 1 it may be seen that the brain level of the drug in the animals treated with reserpine and LSD, as they awoke, was not greatly lower than the level in reserpine-injected, sleeping animals at the same point in time. Very small differences in brain levels of barbital were recorded between female control and castrate groups, and the male group given barbital in distilled water, although most of the castrate females slept for much longer periods. In the presence of significant

TABLE 1. LATENT PERIODS AND SLEEPING PERIODS AFTER SODIUM BARBITAL INJECTION

	N	Dose (mg/kg)	Latent period (min)	s.e.	*N*	Sleeping period (min)	s.e.	*P*	
Controls, male barbital, saline	26	200	46·7	±1·86	30	122·4	±6·17		
Males, reserpine + barbital, saline	20	0·1 200	41·0	±1·60	10	227·0	±25·30	LP vs. controls SP idem	>0·01 <0·001
Males, reserpine + LSD + barbital, saline	21	0·1 1·0 200	37·0	±1·64	11	98·0	±11·10	LP vs. controls SP idem LP vs. reserpine SP idem	<0·001 >0·01 idem <0·001
Males, barbital in dist. H_2O	25	200	68·0	±3·10	14	149·0	±11·17	LP vs. controls SP idem	<0·001 >0·01
Controls, female barbital in dist. H_2O	19	200	73·0	±3·60	10	118·0	±13·00	LP vs. males, dist. H_2O SP idem	>0·01 idem
Females, ov'cty barbital in dist. H_2O	18	200	55·6	±2·59	9	160·0	±23·50	LP vs. female controls SP idem	<0·001 >0·01

LP latent period.
SP sleeping period.

TABLE 2. BRAIN LEVELS OF BARBITAL DURING AND AFTER SODIUM BARBITAL SEDATION

	N	Dose (mg/kg)	Brain levels μg barbital/gm brain At end of latent period	s.e.	N	At end of sleeping period	s.e.	P	
Controls, male barbital, saline	10	200	79·1 (a)	±2·54	14	83·1 (b)	±2·29		
Controls, male barbital, saline	11	200	Killed 80 min after injection			82·9 (c)	±2·74		
Controls, male barbital, saline	12	200	Killed 210 min after injection			82·0 (d)	±2·15		
Controls, male barbital, saline	9	200	Killed 300 min after injection			67·9 (e)	±1.18		
Males, reserpine +barbital, saline	10	0·1 200	70·8 (f)	±3·20	10	67·5	±2·70	LP vs. a SP vs. e	>0·01 idem
Males, reserpine +LSD +barbital, saline	10	0·1 1·0 200	71·3	±1·80	11	74·2	±2·24	LP vs. f SP vs. b	>0·01 <0·01
Males, barbital in dist. H_2O	10	200	83·2 (g)	±2·84	14	73·7 (h)	±2·19	LP vs. c SP vs. d	>0·01 <0·01
Controls, female barbital, dist. H_2O	9	200	75·2	±1·75	10	73·5	±1·09	LP vs. g SP vs. h	>0·01 idem
Female, ov'cty barbital, dist. H_2O	9	200	76·1	±1·40	9	70·8	±2·11	LP vs. female controls SP idem	>0·01 idem

LP brain level at end of latent period.
SP brain level at end of sleeping period.

differences in latent periods which occurred in the various experimental groups, no equivalent differences were noted in the brain levels of barbital at the onset of sleep. The animals injected with reserpine awakened on an average of 260 min after the injection of barbital, with brain levels of barbital which were essentially the same as those of the control animals which had been awake on the average 100 min. A greater

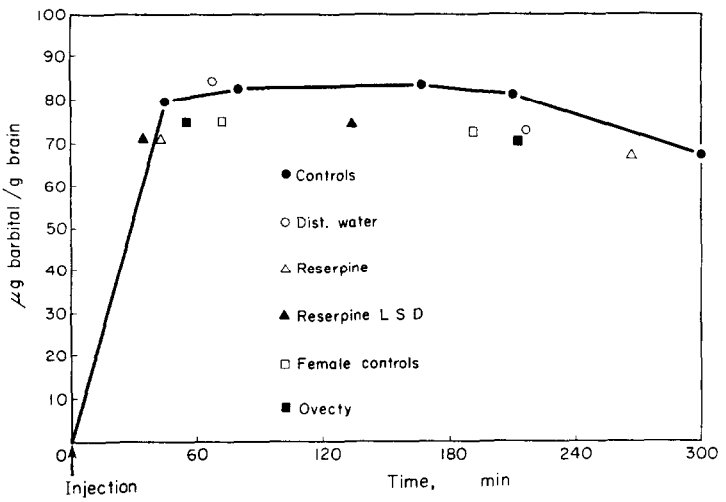

FIG. 1. Brain levels of barbital at the beginning and at the end of barbital sedation. Symbols between 0 time and 80 min on the time scale represent brain levels of barbital at the induction of sleep. Symbols to the right of the 120 min mark show the brain levels of barbital at the end of sleep. Solid line and circles indicate male control animal brain levels.

than 50 per cent reduction of the reserpine-induced prolonged sleeping period was achieved by LSD administration without the occurrence of any large difference in the brain level of barbital.

DISCUSSION

It is of interest to note that these data are in agreement with those of Giarman *et al.*[5] for thiopental, in that a critical level of barbital must be attained prior to induction of sleep. Those workers clearly showed that below a similarly critical level of thiopental, consciousness invariably returned. In the case of barbital, awakening can occur at any point along the gradually descending curve of brain barbital content. It should be noted that, in our experiments, a single standard dose of barbiturate was employed, and that no bio-transformation is involved with barbital.

The results of these experiments suggest that the brain tissue content of barbital is not the primary determinant of the duration of sedation, once sleep is induced. Since alterations in metabolic rate and degradation are not important factors with this particular drug, other mechanisms must be considered. Changes in the rate of excretion could affect the duration of sedation, but elevated brain levels of the drug would be expected. It is possible that the brain cells may be conditioned to react quantitatively in a different manner to equivalent amounts of barbital by virtue of

relative estrogen lack, or of altered quantities of brain amines, initiated by the neuropharmacological actions of drugs such as reserpine or LSD.

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