Biochemical Pharmacology, Vol. 23, pp. 1545–1548. Pergamon Press, 1974. Printed in Great Britain.

SHORT COMMUNICATION

Effect of barbital on cerebral cortical respiration of barbital-tolerant rats*

(*Received* 22 *June* 1973; *accepted* 19 *October* 1973)

TOLERANCE to barbital has been shown to occur in rats by either shortened sleeping periods following a standard dose of barbital[1] or by higher barbital brain levels at awakening.[2] In contrast to other barbiturates, tolerance to barbital is not of metabolic origin, since barbital is metabolized only to a minor extent.[3] Although barbital tolerance appears to be central in origin, no specific biochemical change of brain tissue *in vitro* has been detected in the presence of tolerance to barbital.

Barbiturates have long been known to depress cerebral oxidative metabolism *in vitro*. Potassium-stimulated respiration of cerebral cortical slices seems to be most sensitive to depression by barbiturates[4] as well as other CNS depressant drugs. Furthermore, it has been shown that the sensitivity of this K^+-stimulated respiration to morphine progressively decreases as tolerance to morphine develops in the intact animal.[5] Thus, for morphine there is a good correlation between development of tolerance *in vivo* and cellular adaptation of brain tissue *in vitro*. No such correlation has been shown to exist for barbiturates, yet changes *in vitro* in the sensitivity to a barbiturate and the metabolic excitability of cortical slices from rats with certain subcortical lesions have been correlated with changes *in vivo* in the response to the same barbiturate.[6] The present study was undertaken to answer the following question: can barbiturate-induced cerebral tolerance *in vivo* be correlated with measurable biochemical changes of brain tissue *in vitro*?

We have therefore examined the effect of repeated barbital administration to rats on: (1) the duration of sleep induced by a test dose of the barbiturate, (2) the brain levels of barbital at awakening, and (3) the K^+-stimulated respiration of slices of cerebral cortex *in vitro* in both the presence and absence of the barbiturate.

Male albino Sprague–Dawley rats were used throughout. They were injected i.p. daily with 200 mg/kg of barbital sodium. Untreated animals and rats injected with saline (pH 9·5) served as controls. All animals were maintained at the same weight level by removing food during the daytime. Sleeping time was determined after 200 mg/kg of barbital sodium i.p. at various time points of chronic treatment. In determining sleeping times, control animals never received more than one injection of barbital, since delayed hypersensitivity to other barbiturates has been observed.[7]

Sleeping time was obtained by standard procedure[8] in a quiet room of 24° ± 3°. Onset of sleep was tested every 5 min after the injection and was determined as the animal's inability to right itself. Sleeping time was defined as the time elapsed between loss and recovery of the animal's righting reflex.

For the studies *in vitro*, animals were decapitated and the brains removed within 2 min after decapitation. Whole brain barbital levels were measured in some animals at the moment of waking according to the method of Mazel and Bush.[9] Cortical slices were prepared and incubated according to previously described methods.[10] Respiratory rates were expressed as μl of oxygen consumed/mg of dry weight/hr. Metabolic excitability was expressed as the change in oxygen consumption of the cortical slice after the addition of 100 mM KCl. Barbital sensitivity of the stimulated respiration was expressed as the change in oxygen consumption in the presence of 3·8 mM barbital sodium.

The results in Table 1 show that sleeping time in naive rats after 200 mg/kg of barbital-Na^+ is approximately 200 min. Due to the long half-life of barbital, sleeping time has usually been tested after 1 or 2 days of withdrawal so as to allow excretion of excess barbital.[1] After 4 days of barbital injection and 2 days of withdrawal, no significant change in sleeping time could be detected between barbital-treated, saline-injected and untreated control animals. After 8 days of barbital treatment followed by 2 days of withdrawal, sleeping time in the barbital-pretreated rats was significantly reduced. The same phenomenon was observed after 5 days of withdrawal. Animals have been tested for up to 34 days of daily barbital injections (data not included here) and reduced sleeping times have been reproduced in several groups of rats. It should be noted that at no time could we detect any difference in the onset of sleep between any of the experimental groups ($P > 0{\cdot}05$).

* This investigation was supported by Public Health Service Research Grant No. 5 PO1 GM13749-05 from the National Institute of General Medical Sciences.

TABLE 1. BARBITAL-INDUCED SLEEPING TIME IN RATS DURING CHRONIC BARBITAL ADMINISTRATION AND AFTER ITS WITHDRAWAL*

No. of days injected/withdrawn rats†		N	Weights‡ (g)	Sleeping time‡ (min)	P
1/0	U	13	186 ± 1·5	187 ± 14·4	
	S	15	190 ± 1·4	192 ± 19·1	NS
	B	13	187 ± 1·5	186 ± 13·5	NS
4/2	U	10	199 ± 3·1	145 ± 20·7	
	S	9	194 ± 3·0	163 ± 21·7	NS
	B	10	200 ± 2·0	149 ± 18·6	NS
8/2	U	10	218 ± 3·5	253 ± 14·8	
	S	10	203 ± 5·3	303 ± 32·9	NS
	B	10	210 ± 3·0	198 ± 17·6	<0·05
8/5	U	10	249 ± 1·0	236 ± 24·8	
	S	10	260 ± 2·3	200 ± 16·9	NS
	B	9	245 ± 2·6	166 ± 12·3	<0·02

* Sleeping time is defined as time (min) elapsed between loss and recovery of righting reflex after 200 mg/kg of barbital-Na^+ i.p.

† U = untreated control rats; S = saline (pH 9·5) injected; 2 ml/kg i.p. daily; B = barbital-Na^+ (pH 9·5) injected; 200 mg/kg i.p. daily.

‡ Values are given ± S.E.M.

Since in this study we were interested in the minimal time required to produce barbital tolerance, observations were performed *in vitro* after 8 days of daily barbital injections. Brain waking levels of barbital of some animals are summarized in Table 2. The limited number of animals involved in each group did not allow meaningful statistics, yet it seems apparent that barbital-pretreated rats awoke at higher brain levels of barbital than did animals of either control group.

In the studies *in vitro*, slices from untreated or saline-injected control animals did not differ from each other in either metabolic excitability or their sensitivity to barbital. Therefore, these two control groups were used interchangeably. Respiratory rates prior to the addition of potassium chloride ranged from 10·1 to 12·0 with a mean of 11·2 μl of oxygen/mg dry weight of tissue/hr. There was no statistically significant difference in the unstimulated respiratory rates between any of the groups nor between different days of withdrawal. Metabolic excitability of cortical slices is shown in Fig. 1 as a function of the time of withdrawal after eight consecutive injections of barbital. As can be seen, at a time when tolerance to barbital can be shown *in vivo*, metabolic excitability of cortical slices is not altered to any significant degree.

The sensitivity of cortical slices to barbital, however, did decrease progressively during the period of withdrawal (Fig. 2). At days 5 and 6 after withdrawal, cortical slices from animals pretreated with barbital reached complete insensitivity to the depressant action of barbital *in vitro*. It should be pointed out that cortical slices taken from rats at the eighth day of injection responded equally to the depressant effect of barbital *in vitro* with 45 ± 5 per cent and 51 ± 4 per cent ($P < 0·05$) for control and barbital-treated animals respectively.

TABLE 2. BRAIN WAKING LEVELS OF BARBITAL DURING BARBITAL WITHDRAWAL IN RATS*

No. of days injected/withdrawn	Untreated Controls (μg/g brain)	Saline (pH 9·5) (2 mg/kg i.p. daily) (μg/g brain)	Barbital-Na^+ (200 mg/kg i.p. daily) (μg/g brain)
8/2	156·4 ± 1·74 (3)	149·5 ± 10·3 (4)	170·4 ± 5·6 (3)
8/5	151·6 ± 6·40 (4)	137·2 ± 4·00 (4)	172 ± 13·04 (4)

* Waking levels determined at waking from 200 mg/kg of barbital i.p. The numbers in parentheses refer to number of animals. Values are given ± S.E.M.

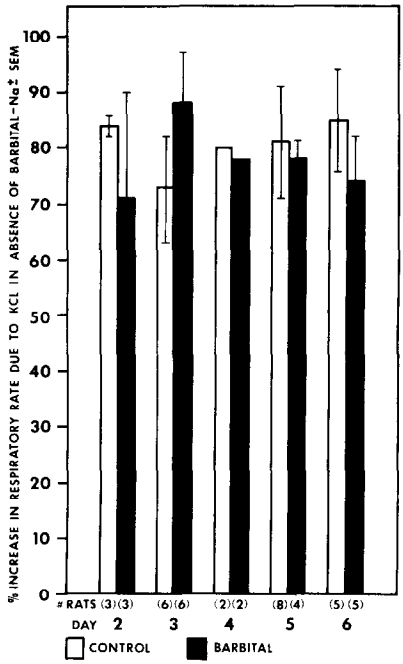

FIG. 1. Effect of withdrawal from barbital on metabolic excitability of cortical slices *in vitro*. Days shown refer to number of days after withdrawal from eight daily injections of 200 mg/kg i.p.

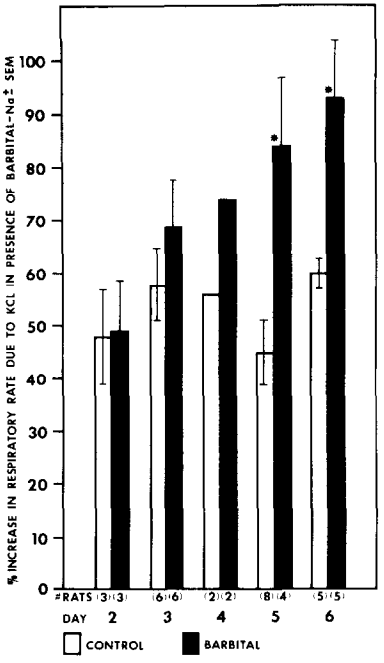

FIG. 2. Effect of withdrawal from barbital on barbital sensitivity of cortical slices *in vitro*. Days shown refer to number of days after withdrawal from eight daily injections of 200 mg/kg i.p. The asterisks indicate values that are significantly different from control levels ($P < 0.05$).

The results obtained indicate that in rats tolerance to barbital can be produced by a minimum of eight daily injections of 200 mg/kg. This tolerance can be demonstrated by a shortened sleeping time together with higher brain barbital waking levels in the barbital-pretreated rats. Tolerance could best be demonstrated after several days of withdrawal. This confirms previous observations by investigators[1] and has been interpreted as due to the long half-life of barbital. Although that is probably a contributing factor, the results reported in this communication suggest that some cellular adaptive processes did occur during the presence of high levels of barbital. During withdrawal of the drug, these processes were unmasked and manifested themselves as decreased sensitivity of brain tissue *in vitro* to the depressant action of barbital. Although the cellular adaptation observed here is different in onset and occurrence from that observed with morphine, the original question asked in this study can be answered in a limited way. The presence, rather than onset, severity and duration, of central tolerance to barbital *in vivo* coincides with measurable subsensitivity of brain tissue to barbital *in vitro*.

Department of Pharmacology,
The George Washington University,
School of Medicine,
Washington, D.C., U.S.A.

BARBARA F. ROTH-SCHECHTER

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