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Effect of Chronic Administration and Withdrawal of Sodium Barbitone on Protein Synthesis of Rat Brain

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LEITE, J. R. AND G. R. LORES ARNAIZ. *Effect of chronic administration and withdrawal of sodium barbitone on protein synthesis of rat brain.* PHARMAC. BIOCHEM. BEHAV. 8(4) 323–326, 1978. – After chronic administration of sodium barbitone to rats, a marked increase incorporation of [^{14}C]-Leucine into isolated nerve endings was observed. Withdrawal of the drug resulted in a decreased incorporation with respect to values obtained with chronic ingesting animals. On the other hand chronic administration of the barbiturate produced a decreased incorporation in mitochondrial and microsomal fractions. These results are discussed in relation to the development of tolerance and abstinence syndrome to this drug.

Chronic administration Sodium barbitone Protein synthesis Tolerance Abstinence syndrome

BARBITURATES have been widely used as hypnotics and anticonvulsivants. Tolerance appears after chronic administration and the withdrawal of the drug generally induces an abstinence syndrome ranging from paraoxysmal EEG abnormalities to severe convulsions. Although tolerance and abstinence syndrome to barbiturates have been behaviourally and pharmacologically well described, there is a lack of reports concerning the neurochemical correlates of these phenomena. Since barbiturates are generally transformed in the liver to inactive metabolites, the ability of this class of drugs to induce hepatic enzymes responsible for metabolizing drugs was accepted to be responsible for development of tolerance. However, other mechanisms should be involved because barbital which is not transformed in the liver also induces tolerance. Thus an adaptation of nervous tissue to the presence of the drug might possibly be involved [10].

Previous work reported that intraventricular injection of cycloheximide could block development of pentobarbital tolerance. It has also reported that chronic pellet implantation of the barbiturate stimulates protein synthesis in subcortical subcellular fractions, and this effect was postulated to be causually related to the tolerance [11]. On the other hand accute or in vitro barbiturates were able to inhibit brain protein synthesis [4, 9, 19] and a decreased incorporation of labelled aminoacid was also observed in brain after 3 doses of injected phenobarbitone [12].

The present study was undertaken to determine possible changes in protein synthesis in cerebral cortex of rats chronically exposed to sodium barbitone solution or in abstinence syndrome after drug withdrawal.

METHOD

Animals and Drug Administration

Wistar female rats, 8 weeks old, were placed in individual cages with food ad lib and sodium barbitone in water as the only drinking solution. The initial drug concentration was 1 mg/ml; then it was raised weekly 1 mg/ml up to 4 mg/ml. This last concentration was maintained for 1 month. The rats drank an average of 19 ml of solution every day and it was estimated that the amount of drug ingested was 450 mg/kg/day in the last week. A similarly treated lot of animals was deprived of the drug for 2 days before sacrifice and constituted the withdrawal group. An additional group receiving no drug was used as control.

Preparation and Incubation of Subcellular Fractions

The synaptosomes were separated from the crude mitochondrial fraction by a ficcoll gradient as previously described [2,3]. The isolated nerve endings were resuspended at a concentration of 0.8–1.0 mg protein/ml in a medium containing 100 mM NaCl, 10 mM KCl, 100 mM

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sucrose and 33 mM Tris/HCl buffer, pH 7.4 [5]. For the preparation of the microsomal and pH 5 fraction, the cortices were homogenized at 10% w/v in 0.32 M sucrose containing 2 mM $MgCl_2$, 25 mM KCl, 10 mM Tris/HCl pH 7.4 and submitted to differential centrifugation [20]. The incubation of the microsomal fractions was done in a medium containing 36 mM KCl, 7.1 mM $MgCl_2$, 0.71 mM ATP, 0.18 mM GTP, 3.4 mM 2-phosphoenolpyruvic acid, 13.6 mM reduced glutathion, 0.09 μg pyruvate kinase/ml and 36 mM Tris/HCl buffer, pH 7.4 [20]; the protein added was 0.4–0.6 mg of microsomes and 5–8 mg of pH 5 fraction/ml. Mitochondria were isolated by differential centrifugation according to Lovtrup and Zelander [13]. The mitochondrial pellets were resuspended in a solution containing 45 mM KH_2PO_4, 75 mM KCl, 1 mM EDTA, 7.5 mM $MgSO_4$ and 75 mM Tris/HCl buffer pH 7.4 [6] at a concentration of 0.6–0.9 mg protein/ml.

In each experiment 3 samples of 0.5–1.0 ml of the resuspended fractions were preincubated at 30°C for 10 min, then 0.5 μCi of [^{14}C]-leucine (330 mCi/mmole) was added to start the reaction; the incubation continued for another 30 min in a Dubnoff shaker bath with an air atmosphere. The reaction was stopped by plunging the tubes in an ice bath. The samples were filtered through paper discs and extracted as described by Mans and Novelli [15]: in all cases, blank at zero time were prepared. All steps, during the preparation of the samples and incubation were carried out under sterile conditions, at 4°C. Protein was determined according to Lowry *et al.* [14] using bovine serum albumin as standard.

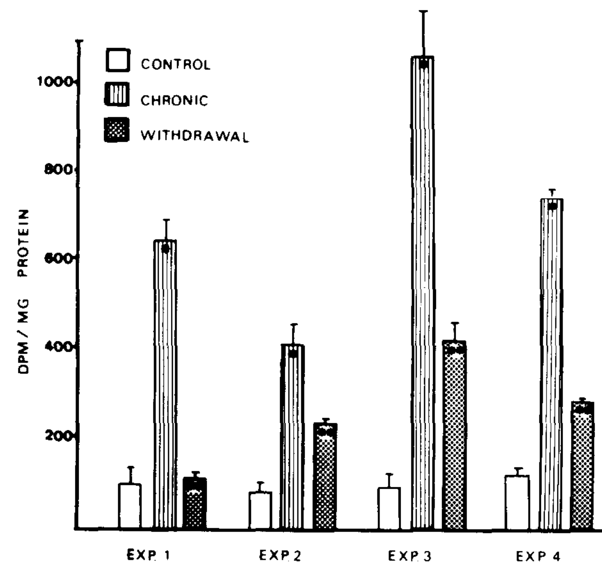

FIG. 1. Effect of chronic administration and withdrawal of sodium barbitone on protein synthesis in isolated nerve-endings. The following conditions were analysed: animals receiving no drug (control), animals chronically treated with sodium barbitone (chronic) and animals deprived of the drug 2 days prior to the sacrifice (withdrawal). Results are expressed in dpm per mg protein and represent the mean of three aliquots incubated and extrated separately (± SD). *denotes $p<0.05$ with respect to control. **denotes $p<0.05$ with respect to chronic.

RESULTS

No difference in liquid intake nor in weight was observed when controls and treated animals were compared. The chronic administration of sodium barbitone produced a marked increase in [^{14}C]-leucine incorporation in the isolated nerve endings (Fig. 1). Two days of withdrawal of the drug was enough to bring the values to nearly control levels, remaining in some instances higher than in the untreated group.

Table 1 shows the various experimental conditions in which the [^{14}C]-leucine incorporation to microsomes was determined. Control or treated microsomes could be mixed with control or treated pH 5 fraction. Chronic administration of barbitone reduced considerably protein synthesis in microsomes. This effect was also observed by adding the treated pH 5 fraction to the control microsomes or by adding pH 5 fraction from controls to treated microsomes (Table 1).

Table 2 shows the results for [^{14}C]-leucine incorporation in the mitochondrial fraction. Chronic animals presented a significant reduction in the incorporation of the labelled aminoacid. This reduction is even higher in those animals deprived of the sodium barbitone. It should be stressed that the protein content of the various subcellular fractions remained the same after chronic treatment with the barbitone.

TABLE 1

EFFECT OF CHRONIC ADMINISTRATION OF SODIUM BARBITONE ON PROTEIN SYNTHESIS IN MICROSOMAL FRACTIONS OF RAT CEREBRAL CORTEX

Experiment	Microsomal fraction	pH 5 fraction	dpm/mg protein
	control	control	1487±374
1	control	treated	726± 87*
	treated	control	458±114*
	treated	treated	536±106*
	control	control	1376±327
2	control	treated	531± 56*
	treated	control	576± 62*
	treated	treated	529± 82*
	control	control	1521±167
3	control	treated	1090± 54*
	treated	control	1317± 52
	treated	treated	1227±257*

In each experiment microsomal and pH 5 fractions were isolated from the brain cortex of 2 rats. The different conditions tested in each experiment are indicated. Results are expressed in dpm/mg protein; the figures are the mean of three samples incubated and extracted separately (± SD).

*Differ to values obtained with control microsomes plus control pH 5 fraction ($p<0.05$, Student t-Test).

DISCUSSION

The results of this study showed that chronic ingestion of sodium barbitone produced an altered [^{14}C]-leucine in corporation by subcellular fractions. A pronounced increase in aminoacid incorporation by synaptosomes obtained from

TABLE 2

PROTEIN SYNTHESIS IN ISOLATED BRAIN MITOCHONDRIA FROM RATS AFTER CHRONIC ADMINISTRATION AND WITHDRAWAL OF SODIUM BARBITONE

Experiment No.	Control	Chronic	Withdrawal
1	867±32	703±71*	590±32†
2	535±99	396±39*	299±38†
3	309±25	187±17*	160±36*
4	1044±45	614±39*	446±15†

Mitochondria were isolated from the cerebral cortex of chronically treated rats with sodium barbitone (chronic) or deprived of the drug two days before thg sacrifice (withdrawal). Results are expressed in dpm/mg protein; each value represents the mean of three samples ± SD.

*$p<0.05$ with respect to controls.

†$p<0.05$ with respect to the chronic values.

those animals chronically treated with the drug was observed. The withdrawal of the drug for 2 days was enough to bring the values back to nearly control levels. Results are in agreement with previous data which demonstrated a stimulation of protein synthesis in synaptosomal fraction after chronic pentobarbitone administration. This effect was proposed by the authors as being related to the appearance of tolerance to the drug, since protein synthesis inhibitors also prevent the development of tolerance [11]. On the other hand a decreased incorporation of aminoacid was observed in mitochondrial and microsomal fractions, isolated from brain of chronic ingesting animals. This decrease was still greater in mitochondria of treated animals, during the period of abstinence. Since an elevated excitability of the nervous system is observed during this period, leading the animals to convulsions, this effect could bear correlation to a similar effect on protein synthesis observed after electrical or chemical stimulation of the brain [2, 16, 17, 18], which has been interpreted as due to a change in the energetic metabolism giving rise to an impaired protein synthesis [16,17]. On the other hand chronic sodium barbitone administration also altered the pH 5 fraction reducing leucine incorporation (Table 1). Similar results were observed with animals chronically treated with ethanol [22]. In addition to decreased incorporation of labelled leucine and enzymatic activity of the pH 5 fraction, a strong inhibition in the aminoacylation [7] and an alteration in the transcription of RNA [21] were observed. As our findings with leucine incorporation correlated with those observed on alcohol treated rats it is possible that similar molecular mechanisms are involved. Therefore a study of aminoacylation and RNA synthesis could complement our findings. The possibility that chronic treatment with barbitone to affect the aminoacid pools modifying the specific activity of the precursor must be considered. Thus the interpretation that protein synthesis was directly altered by the drug treatment could be doubtful. However the differential effect of chronic barbitone on the three fractions (synaptosomal, microsomal and mitochondrial) speaks against the possibility that alterations in the aminoacid pools are affecting incorporation. Finally it could be important to point out possible hazardous effects of chronic ingestion of barbiturates on memory consolidation, since previous works have demonstrated the essential role of protein synthesis on this process [1,8]. Results on this subject showed that chronically treated animals or 2 days drug deprived animals presented an impaired performance on both active and passive avoidance, and no impairment on the acquisition of appetitive learning tasks.

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