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THE EFFECT OF THE CHRONIC ADMINISTRATION OF BARBITONE SODIUM ON LABILE COMPOUNDS IN THE RAT BRAIN

B. E. LEONARD

Department of Pharmacy, The University, Nottingham, England

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Abstract—Changes in labile nitrogen and phosphorus compounds in the brains of 2 substrains of albino rats were measured both during habituation to barbitone sodium and also for a short period after the animals were deprived of this drug. Differences were found in the response of these substrains to the period of habituation and withdrawal. One substrain showed a considerable rise in brain ammonia and lactate during habituation and these rises were maximal 2 days after withdrawal of the barbiturate. The other substrain showed only a slight rise in brain ammonia whilst the lactate level was unchanged. The changes in brain ammonia could not be entirely explained by changes in either blood ammonia or brain glutamine or brain free amino nitrogen. In both substrains there was a fall in ATP and creatine phosphate both during habituation and immediately after withdrawal. Most of the labile compounds returned to the control levels by 1 week after withdrawal of the barbiturate.

SEVERAL investigations have demonstrated that withdrawal symptoms occur in animals which have been deprived of a chronically administered central depressant drug.[1–3] It is well established that cats and dogs exhibit withdrawal symptoms when deprived of chronically administered barbiturates[4–6] and Bentley[7] has reported that rats show an increase in susceptibility to audiogenic seizures shortly after they have been withdrawn from narcotic drugs. In our laboratories it has been found that rats can readily be habituated to barbiturates by increasing the concentration of the drug in their drinking water over a period of 4–5 weeks and on withdrawal of the barbiturates the animals become hyperexcitable and susceptible to audiogenic seizures.[8] A preliminary communication of this investigation was presented at the Second International Pharmacological Meeting.[9]

The present investigation was undertaken to determine the effect of chronic barbiturate administration on labile compounds in the rat brain.

METHODS

Female albino rats (48–52 g) originally of the Wistar strain were used. These rats were obtained from two sources* and housed in single cages. Barbitone sodium was added to the drinking of the experimental rats in increasing doses over a period of 4 weeks, for the Boots' substrain, and 5 weeks, for the Tuck's substrain, as described elsewhere.[8, 8a]

*Boots Pure Drug Co. Ltd., Nottingham, England. A. Tuck Ltd., Rayleigh, Essex, England.

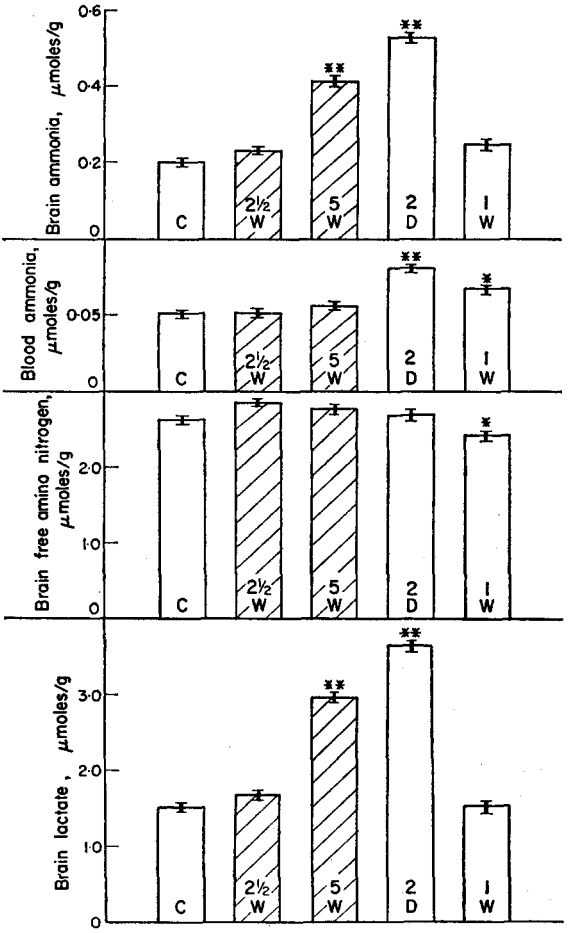

FIG. 1. Effect of the chronic administration of barbitone sodium on the levels of labile nitrogen compounds and on lactate in the rat brain (Tuck's substrain).

▨ rats killed 2½ and 5 weeks after habituation

□ rats killed 2 days and 1 week after withdrawal of barbiturate.

The first column, C, gives the control value. All results expressed as the mean ± standard error. Results significant at $*P < 0{\cdot}05$, $**P < 0{\cdot}01$. Free amino nitrogen levels given in terms of glutamic acid.

The difference in the period of barbiturate administration were due to the observation, in a preliminary experiment, that the time of administration was 5 weeks and 4 weeks respectively for these substrains to show an increased susceptibility to audiogenic seizures 2 days after withdrawal. Furthermore after these periods of administration the daily intake and the brain levels of barbiturate were approximately the same for the 2 substrains.[8]

Groups of at least 5 rats were killed by immersion in liquid oxygen at intervals during the period of barbiturate administration and at 2 days and 1 week after withdrawal. The brains were chipped out while still frozen, homogenized in cold 10% trichloracetic acid (T.C.A.) and centrifuged at approximately 2,500 rev/min for 5 min. Aliquots of the clear supernatant were then taken for the estimation of ammonia[10] total free amino nitrogen,[11] glutamine,[12] lactate,[13] adenosine triphosphate (ATP),[14] creatine phosphate,[15] inorganic phosphate[14] and "alkaline soluble" phosphates[16] (mainly hexose phosphates). In a separate experiment, groups of rats were killed by

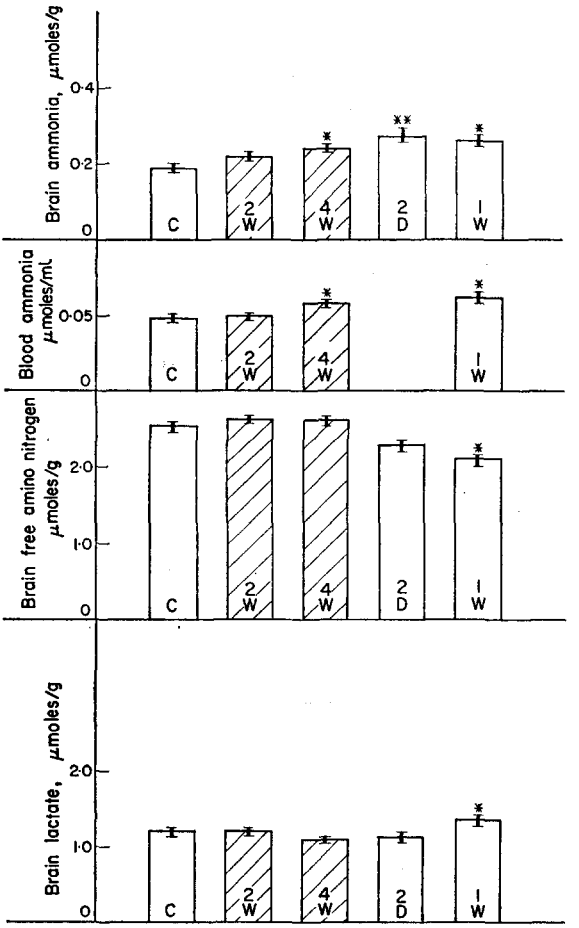

FIG. 2. Effect of the chronic administration of barbitone sodium on the levels of labile nitrogen compounds and on lactate in the rat brain (Boots' substrain).

Details of the histograms are the same as those for Fig. 1.

decapitation at the same time intervals, the blood collected in cooled centrifuge tubes containing 0·1 ml heparin (5000 IU/ml) and the ammonia[10] estimated. All estimations were done whenever possible on the day the animals were killed. However it was sometimes necessary to keep the T.C.A. extracts in a refrigerator (4°) for up to 2 days before making the estimations.

RESULTS

The brain ammonia level rose during habituation and just before withdrawal was 106 per cent above the control value in the Tuck's substrain (Fig. 1) but the increase was slightly less in the Boots' substrain (20 per cent above control; Fig. 2). Two days after withdrawal of the barbiturate there was a rise in brain ammonia in the Tuck's substrain (164 per cent above control value) and in the Boots' substrain (75 per cent above control value). This corresponded to the period when all the rats were hyperexcitable and susceptible to audiogenic seizures.[8] One week after withdrawal, the Tuck's but not the Boots' rats were no longer susceptible to audiogenic seizures. In the Tuck's but not in the Boots' rats the brain ammonia level returned approximately to the control value 1 week after withdrawal. The rise in brain ammonia was not reflected in the changes in blood ammonia (Figs. 1 and 2). The only significant rise in blood ammonia (60 per cent above control value) occurred 2 days after withdrawal in the Tuck's substrain. There was little change in the blood ammonia level

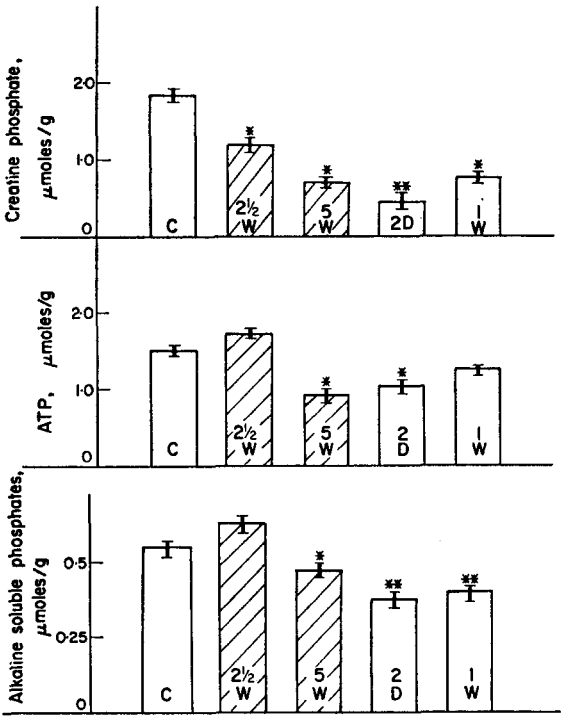

FIG. 3. Effect of the chronic administration of barbitone sodium on the levels of labile phosphate compounds in the rat brain (Tuck's substrain).

Details of the histograms are the same as those for Fig. 1.

'Alkaline soluble' phosphate levels given in terms of hexose phosphate.

in the Boots' substrain or in the total free amino nitrogen level in either substrain during the course of the experiment (Figs. 1 and 2). The free amino nitrogen level decreased immediately following withdrawal and in the Boots substrain was slightly lower than the control level 1 week after withdrawal. Brain glutamine was unchanged in both substrains throughout the experiment.

Changes in brain lactate levels only occurred to any extent in the Tuck's substrain. During habituation the lactate level rose (96 per cent above control) and reached a maximum 2 days after withdrawal (141 per cent above control) but returned to the control level 1 week after withdrawal (Fig. 1). In the Boots' substrain the only significant changes in brain lactate occurred 1 week after withdrawal (14 per cent above control; Fig. 2).

The changes in high energy phosphate compounds seem to reflect the changes in brain ammonia and the difference observed between the substrains was not so great for the high energy phosphate compounds as for brain ammonia and lactate. There was a significant fall in ATP (approximately 45 per cent control value) and creatine phosphate (approximately 70 per cent control value) during habituation and this decreased level was maintained 2 days after withdrawal (Figs. 3 and 4). In both substrains there was a tendency for the level of inorganic phosphate to rise during

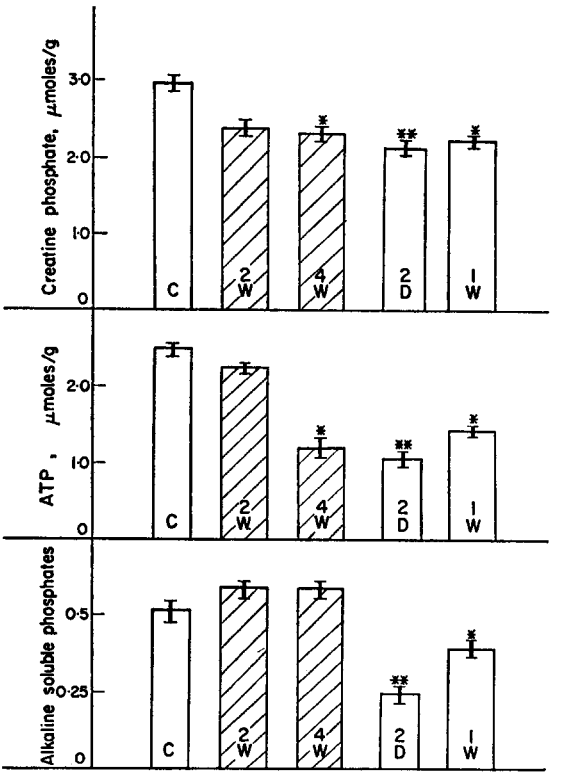

FIG. 4. Effect of the chronic administration of barbitone sodium on the levels of labile phosphate compounds in the rat brain (Boots' substrain).

Details of histograms are the same as those for Fig. 1.

habituation and reach a maximum level 2 days after withdrawal, although the changes were not significantly different from the control values. The level of 'alkaline soluble' phosphates also fell during habituation and reached a minimum (approximately 50 per cent control) 2 days after withdrawal.

Although the degree of change in the labile phosphorus compounds was similar in both substrains, there were differences between the control values for these groups. It is however unlikely that these are a reflection of *in vivo* differences between the strains and they probably reflect a greater breakdown of the labile compounds *in vitro* before the estimations were made.

DISCUSSION

The changes in some labile compounds in the brains of two substrains of Wistar rats are similar although there are several quantitative differences which ought to be mentioned. The rise in brain ammonia and lactate is considerably greater in the Tuck's substrain than in the Boots' substrain. This finding is not altogether surprising since the Tuck's rats were more excitable than the Boots' rats after withdrawal from the barbiturate. Furthermore, in other experiments in these laboratories, it has been found that the audiogenic seizures which occur in rats of the Tuck substrain 2 days after withdrawal of the barbiturate are more severe than those induced in the Boots' substrain which suggests that there are genetic differences in their responses to barbiturate administration. In neither substrain was there a significant change in brain glutamine during the experiment and the free amino nitrogen level only decreased during the withdrawal period which would suggest that these compounds are not the primary source of the increase in endogenous ammonia in the brain. The changes in the levels of high energy phosphate compounds and hexose phosphates reflect to some extent the changes in brain ammonia in both substrains. Thus the rise in brain ammonia during habituation and its even greater rise 2 days after withdrawal is coincident with the fall in high energy phosphate compounds and hexose phosphates during this period.

Some Russian investigators[17] independently found that when mice were injected with an anaesthetic dose of amylobarbitone or barbitone sodium twice daily for 7–15 days the brain ammonia level was approximately doubled; brain glutamine also increased during this period. The brain ammonia and glutamine levels returned to the control values 3 days after cessation of the anaesthesia. These authors cite an early experiment of Pavlov to suggest that the changes in brain ammonia were only secondary to a rise in blood ammonia. However it is evident from the present results and also from those of other investigators[18–20] that blood ammonia must be raised many times above its normal level before there is a significant increase in brain ammonia. Furthermore Navazio and co-workers[18] have shown in rats that neurological symptoms only occur when the brain ammonia level was raised approximately 12 times above the normal level. It therefore seems likely that changes in brain ammonia, and presumably the other labile compounds which have also been determined, are the result and not the cause of the chronic effects of the barbiturate within the central nervous system when given in non-anaesthetic doses.

Although the measurement of brain ammonia levels has been used as an index of brain excitability[21] Takahashi and co-workers[22] have clearly shown that in the rat

brain there is no such correlation. Many factors, apart from chemically or electrically induced convulsions, are now known to cause a rise in brain ammonia. These include anoxia[23, 24] hyperoxia,[25] inhibition of glutamine synthesis,[26, 27] increased functional activity of the central nervous system[28] and inhibition of the citric acid cycle.[29] Nevertheless despite the considerable amount of data available on conditions which lead to changes in brain ammonia, the sources of brain ammonia are far from certain. Glutamic acid and glutamine have long been implicated not only as a possible source of brain ammonia but also as the primary means whereby excess ammonia can be removed.[30] As the synthesis of glutamine requires 1 mole ATP per 1 mole of ammonia[31] several investigators have emphasized the importance of oxidative processes, and the concurrent synthesis of ATP.[27, 32, 33] The finding that brain glutamine is unaltered during the chronic administration of barbitone sodium despite the considerable rise in brain ammonia suggests that the glutamic acid-glutamine detoxification process is probably of little importance under these experimental conditions. Furthermore there was only a slight change in the level of free amino nitrogen following withdrawal of the barbiturate suggesting that any changes in individual amino acids which could act as precursors of brain ammonia (e.g. aspartic acid[34]) were too small to be detected.

The rise in brain lactate during habituation and immediately following withdrawal suggests that the activity of the glycolytic pathway is increased possibly as a consequence of the rise in brain ammonia which inhibits the citric acid cycle.[18, 35, 36] The increased glycolysis may be a direct result of the reduction of the rate of the citric acid cycle due to the barbiturate inhibiting mitochondrial NADH-oxidase[37] rather than a consequence of the rise in ammonia. Whatever may be the primary cause for the slowing down of the citric acid cycle the final effect would be a reduction in oxidative phosphorylation and a fall in high energy phosphate compounds, a situation which occurs after the chronic administration of barbitone sodium. There is evidence however suggesting that a rise in brain ammonia is not always accompanied by a fall in high energy phosphate compounds. Thus Schenker and Mendelson[38] found that there was no change in ATP levels in rats in a coma induced by ammonium acetate. Furthermore Rosado and co-workers[39] also concluded, from their investigation of ammonia intoxication in rats, that the reduction in oxygen uptake was not due to a reduced ATP formation.

It is difficult to visualize how the changes in the labile compounds found can explain the increased excitability of the central nervous system following the withdrawal of the barbiturate. The possibility remains however that ammonia, besides having a possible effect on oxidative metabolism, may also have direct effect on nervous transmission.[33]

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