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THE GLYCOGEN, GLUCOSE AND LACTIC ACID CONTENT OF THE BRAIN IN EXPERIMENTAL CATATONIA

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Abstract—Rats were rendered catatonic by bulbocapnine and by audiogenic seizures. The glycogen content of the brain fell by approximately 14 per cent in both experimental situations. All parts of the brain lost glycogen, the 'free' glycogen fraction being predominantly affected. Brain glucose content rose in both forms of catatonia. In that due to audiogenic seizures, an early rise in the lactic acid content of the brain also occurred but lactic acid was unaffected in the early stages of bulbocapnine catatonia. Adrenal demedullation abolished the hyperglycaemic action of bulbocapnine. It reduced, but did not abolish, the effect of the drug on the glucose content of brain.

IT IS well known that many psychotropic drugs affect the extrapyramidal system, producing Parkinsonian-like symptoms in human beings. In experimental animals, extrapyramidal involvement more often manifests itself as catatonia, a condition characterized by immobility, increased muscle tone, the maintenance of abnormal postures (catalepsy), negativism and signs of autonomic stimulation.

Bulbocapnine is the drug classically associated with the production of catatonia in animals[1] but the condition also occurs in the course of audiogenic convulsions.[2] The catatonia due to bulbocapnine is not preceded by any overt signs of central excitation, whereas that arising during audiogenic seizures follows a period of violent convulsive activity. It was hoped that a study of the neurochemical changes occurring during catatonia associated with these two apparently different states of nervous activity might reveal changes fundamental to the catatonic condition.

The present paper reports the effects of experimental catatonia on the glycogen, glucose and lactic acid contents of rat brain.

A preliminary account of this work was presented to the British Pharmacological Society in January, 1967.

METHODS

Drug-induced catatonia

Female Wistar rats weighing between 140 and 160 g were used. Catatonia was produced by the i.p. injection of bulbocapnine at a dose level of 50 mg/kg. Control animals received i.p. injections of a comparable volume of 0·9% saline solution.

Post-convulsive catatonia

Over a period of four weeks some members of a group of female Wistar rats received barbitone sodium in their drinking water. The concentration was adjusted regularly

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so as to cause a progressive increase in the daily intake of barbitone from 100 mg/kg to 400 mg/kg. Forty eight hr after replacement of the drug solution by tap water the animals were exposed to the sound of an electric bell which induced convulsions followed by a well-marked catatonia.[3] The remaining members of the group were not given barbiturate but they were subjected to auditory stimulation at the same time as the barbiturate-treated rats when all the animals were between 140 and 160 g in weight.

Adrenal demedullation was performed under ether anaesthesia according to the operative procedure originally described by Evans.[4]

Biochemical determinations

In order to minimise changes which might occur in the concentration of labile metabolites during decapitation and after death, the rats were killed by immersion in liquid oxygen. When it was necessary to obtain samples of blood for glucose determination, only the head and the thoracic region of the animal was immersed in the liquid oxygen. After 30 sec the head was removed from the semi-frozen body and immediately returned to the liquid oxygen. Since the body had not been frozen and the heart was still beating, blood was readily obtainable from the severed vessels in the neck.

The brains were chipped out of the frozen heads and crushed in a stainless steel crusher of the type described by Stone,[5] taking care to prevent thawing of the frozen tissue until it was in contact with the extraction medium. In some experiments the frozen brains were separated into four portions corresponding to the medulla and pons, the cerebellum, the upper brain stem and the cerebral hemispheres. Because of the difficulty of accurately delineating these regions in the frozen brain, a series of unfrozen brains was dissected and the relative weights of these four portions determined. Comparison of these values with those obtained from each frozen brain served as a partial check on the accuracy with which the latter had been divided.

Glycogen

The crushed brain tissue was ground with ice cold 10% (w/v) trichloroacetic acid (2 ml acid/g of brain). The suspension was centrifuged and the supernatant solution containing the 'free' glycogen was separated from the residual brain tissue in which the 'bound' glycogen was attached to the precipitated protein. Four vol. of 95% ethanol were added to the separated supernatant solution. The glycogen precipitate was allowed to settle out overnight at 0–4° and was collected by centrifugation.

The 'bound' glycogen was extracted by a method essentially the same as that described by Le Baron.[6] The brain residue was digested for 15 min at 80° in alcoholic potassium hydroxide (10 ml solution/g of original brain tissue) and the glycogen which precipitated on cooling was separated and washed three times with hot methanolic chloroform.

In the determination of total glycogen, the brain tissue was digested in alcoholic potassium hydroxide without the preliminary treatement with trichloroacetic acid.

The glycogen precipitates obtained by these procedures were heated for $2\frac{1}{2}$ hr at 100° with N sulphuric acid (5 ml acid/g of original brain tissue). Glucose was determined in 1 ml aliquots of the neutralized hydrolysate by the Folin-Wu method.

Brain and blood glucose were estimated by the enzymatic method of Hugget and Nixon,[7] using reagents available commercially in kit form.

Perchloric acid is a satisfactory protein precipitant for blood but it was found that the enzymatic method could not be applied to perchloric acid extracts of brain tissue. A barium hydroxide-zinc sulphate mixture[8] provided a satisfactory alternative.

Other metabolites

Aliquots of trichloroacetic acid extracts of brain were taken for estimation of their contained lactic acid (method of Scholz, Schmitz and Lampen[9]), phosphorus compounds (Le Page[10]) and ammonia (Konitzer and Voigt[11]).

RESULTS

Comparison of the Folin-Wu and glucose oxidase methods for the estimation of brain glycogen

In preliminary experiments, the glucose obtained from hydrolysis of 'free' and 'bound' glycogen in brain extracts was determined by both the Folin-Wu and the glucose oxidase methods. The results of these experiments are presented in Table 1.

TABLE 1. COMPARISON OF THE FOLIN-WU AND THE GLUCOSE OXIDASE METHODS IN THE DETERMINATION OF FREE AND BOUND GLYCOGEN

	Glycogen concentration of rat brain (as glucose)		
	Folin-Wu method (μM/g)	Glucose oxidase method (μM/g)	Folin-Wu / Glucose oxidase (%)
Free glycogen			
cerebral hemisphere	1·38	1·28	108
cerebral hemisphere	1·34	1·15	116
mid brain	1·15	1·16	99
cerebellum	1·85	1·93	96
medulla-pons	1·77	1·51	117
			Mean 107
Bound glycogen			
cerebral hemisphere	2·58	2·31	112
cerebral hemisphere	2·53	2·28	111
mid brain	2·58	2·48	104
cerebellum	2·64	2·40	110
medulla-pons	3·01	2·83	106
			Mean 109

Since the Folin-Wu procedure only overestimated the glycogen content of brain by an average of 8 per cent it was used throughout in the glycogen estimations, and no correction is made to the figures obtained by this method. Glucose in blood and brain, however, was always determined by the enzymatic method.

Effect of catatonia on the glycogen, glucose and lactic acid content of brain

With the doses used in the present experiment, bulbocapnine produced catatonia which persisted for up to 20 min and which was accompanied by a fall in the concentration of glycogen in the brain and an increase in the concentration of glucose and

lactic acid (Table 2). The extent of the glycogen change was independent of the duration of the catatonia, but the effects on glucose and lactic acid were greater in the later stages of the condition. Indeed, during the first few minutes of catatonia no detectable change occurred in the lactic acid content of the brain.

TABLE 2. BRAIN GLYCOGEN, GLUCOSE AND LACTIC ACID DURING EXPERIMENTAL CATATONIA

	Glycogen (as glucose) μM/g ± S.E.	Glucose μM/g ± S.E.	Lactic acid μM/g ± S.E.
Bulbocapnine Early catatonia			
(1–2 min)			
Control	4·20 ± 0·12 (6)	0·65 ± 0·06 (4)	2·28 ± 0·08 (6)
Catatonic	3·58 ± 0·09 (6)	1·22 ± 0·09 (4)	2·22 ± 0·17 (6)
% change	— 14·7	+ 87·7	— 3·1
P	< 0·01	< 0·01	n.s.
Late catatonia			
(10–11 min)			
Control	3·92 ± 0·12 (6)	0·65 ± 0·06 (4)	2·30 ± 0·05 (6)
Catatonic	3·31 ± 0·16 (6)	1·66 ± 0·15 (3)	2·63 ± 0·07 (6)
% change	— 15·6	+ 155·4	+ 14·3
P	< 0·02	< 0·01	< 0·01
Post-convulsive catatonia			
1–2 minutes of seizures			
Control	4·50 ± 0·14 (5)	0·45 ± 0·09 (5)	1·88 ± 0·11 (5)
Catatonic	3·88 ± 0·10 (5)	0·93 ± 0·11 (3)	2·60 ± 0·15 (4)
% change	— 13·8	+ 106·8	+ 38·3
P	< 0·01	< 0·02	< 0·01

The catatonia which followed audiogenic seizures was of short duration, never exceeding 5 min, but it was associated with a fall in the glycogen content of brain which was virtually the same as that observed during the early stages of bulbocapnine catatonia. The lactic acid content of the brain, on the other hand, underwent a significant increase. It seemed likely that this was a consequence of the increased neuronal activity during the preceding convulsions. This view is supported by the results presented in Table 3 which show that seizures of longer duration involved

TABLE 3. BRAIN GLUCOSE AND LACTIC ACID FOLLOWING 4–6 MIN OF AUDIOGENIC CONVULSIONS

	Glucose μM/g ± S.E.	Lactic acid μM/g ± S.E.
Control	0·45 ± 0·09 (4)	1·51 ± 0·03 (4)
Post seizure	1·87 ± 0·09 (4)	3·86 ± 0·07 (3)
% change	+ 315	+ 155·6
P	< 0·02	< 0·01

much greater changes in glucose and lactic acid. Catatonia does not occur as a sequel to these longer-lasting seizures, and measurements of brain glycogen in these animals were therefore not undertaken.

TABLE 4. 'FREE' AND 'BOUND' GLYCOGEN IN DIFFERENT PORTIONS OF THE RAT BRAIN DURING BULBOCAPNINE CATATONIA

(μM/g brain weight as glucose $\pm$ S.E.)

		Free	Bound	Total (free and bound)
Cerebral hemispheres	Control	2·04 $\pm$ 0·19 (5)	3·14 $\pm$ 0·11 (5)	5·18 $\pm$ 0·13 (5)
	Catatonic	1·51 $\pm$ 0·18 (5)	2·83 $\pm$ 0·07 (5)	4·34 $\pm$ 0·21 (5)
	% change	— 26	— 10	— 16*
Mid brain	Control	2·02 $\pm$ 0·05 (3)	2·94 $\pm$ 0·17 (4)	4·91 $\pm$ 0·26 (3)
	Catatonic	1·54 $\pm$ 0·17 (4)	2·91 $\pm$ 0·14 (4)	4·37 $\pm$ 0·25 (3)
	% change	— 24	— 1	— 11
Cerebellum	Control	3·30 $\pm$ 0·24 (3)	3·96 $\pm$ 0·14 (4)	7·30 $\pm$ 0·17 (3)
	Catatonic	2·84 $\pm$ 0·16 (3)	4·11 $\pm$ 0·40 (4)	6·66 $\pm$ 0·22 (3)
	% change	— 14	+ 4	— 9
Medulla and pons	Control	2·39 (mean of 2)	3·84 (2)	6·23 (2)
	Catatonic	2·13 (2)	3·43 (2)	5·56 (2)
	% change	— 11	— 11	— 11

Figures in parenthesis refer to number of animals used. Significance of difference from controls: * $P < 0·02$.

Topographical distribution of 'free' and 'bound' glycogen

In order to investigate the possibility that the decrease in glycogen content might be localized to one or more areas of the brain, the topographical distribution of cerebral glycogen during experimental catatonia was examined. At the same time the experiment was extended to include a study of the different tissue glycogen fractions. The 'free' and 'bound' glycogen concentrations were estimated in four different areas of the rat brain during the two types of experimental catatonia. The results are presented in Tables 4 and 5. Because of the small numbers of animals used and the limited amounts of tissue available for analysis, apparently significant falls in the content of either fraction were, with one exception, not found. However, it is a reasonable conclusion from the figures presented that the glycogen was lost fairly uniformly from all areas of the brain and that the loss occurred predominantly from the 'free' fraction.

Origin of the brain glucose in animals treated with bulbocapnine

Klein, Hurwitz and Olsen[12] showed that glucose injection resulted in the appearance of increased amounts of glucose in the brain. Since it has also been reported that bulbocapnine causes hyperglycaemia,[13] it was necessary to determine the extent to which the glucose changes observed in the present experiments were a simple consequence of an increased supply in the blood.

The results set out in Table 6 show that bulbocapnine certainly caused hyperglycaemia in the intact rats. It had, however, no hyperglycaemic action in adrenal demedullated animals, though it still caused a small, but highly significant, increase in brain glucose content. The reduction in brain glycogen was greater in demedullated than in intact animals.

TABLE 5. 'FREE' AND 'BOUND' GLYCOGEN IN DIFFERENT PORTIONS OF RAT BRAIN DURING CATATONIA FOLLOWING AUDIO-SEIZURES

(μM/g brain weight as glucose ± S.E.)

		Free	Bound	Total (free and bound)
Cerebral hemispheres	Control	1·70 ± 0·1 (4)	2·82 ± 0·16 (4)	4·52 ± 0·19 (4)
	Catatonic	1·46 ± 0·13 (4)	2·69 ± 0·08 (4)	4·15 ± 0·21 (4)
	% change	— 14	— 5	— 8
Mid brain	Control	2·24 ± 0·40 (3)	2·70 ± 0·26 (4)	5·08 ± 0·69 (4)
	Catatonic	1·82 ± 0·21 (4)	2·72 ± 0·12 (4)	4·54 ± 0·24 (3)
	% change	— 19	+ 1	— 11
Cerebellum	Control	3·23 ± 0·17 (4)	3·24 ± 0·18 (4)	6·47 ± 0·22 (4)
	Catatonic	2·46 ± 0·16 (3)	3·14 ± 0·12 (4)	5·60 ± 0·31 (3)
	% change	— 24*	— 3	— 13
Medulla and pons	Control	3·10 ± 0·47 (4)	3·78 ± 0·67 (4)	6·89 ± 0·75 (4)
	Catatonic	2·66 ± 0·40 (3)	4·23 ± 0·38 (4)	7·20 ± 0·37 (3)
	% change	— 14	+ 12	+ 4

Figures in parenthesis refer to number of animals used. Significance of difference from controls: * $P < 0·05$.

TABLE 6. EFFECT OF ADRENAL DEMEDULLATION UPON BRAIN GLYCOGEN AND ON GLUCOSE IN THE BLOOD AND BRAIN OF THE RAT DURING BULBOCAPNINE CATATONIA

	Brain glycogen (as glucose) μM/g ± S.E.	Brain glucose μM/g ± S.E.	Blood glucose μM/ml ± S.E.
Intact animals			
(sham-operated)			
Control	3·87 ± 0·16 (4)	0·63 ± 0·04 (4)	5·09 ± 0·23 (4)
Late catatonia	3·28 ± 0·13 (4)	1·70 ± 0·12 (4)	7·93 ± 0·45 (4)
% change	— 15·3	+ 171	+ 55·7
P	< 0·05	< 0·001	< 0·002
Demedullated animals			
Control	4·04 ± 0·09 (4)	0·69 ± 0·03 (8)	5·17 ± 0·12 (7)
Late catatonia	3·00 ± 0·08 (5)	0·90 ± 0·07 (10)	4·89 ± 0·18 (9)
% change	— 25·8	+ 30·4	— 5·4
P	< 0·001	= 0·02	n.s.

Figures in parenthesis refer to number of animals used.

TABLE 7. EFFECT OF BULBOCAPNINE CATATONIA ON THE LEVELS OF HIGH ENERGY PHOSPHATES, INORGANIC PHOSPHATE AND FREE AMMONIA IN RAT BRAIN

(μM/g ± S.E.)

	Control (saline)	Bulbocapnine (50 mg/kg)
ATP	2·4 ± 0·2 (7)	2·3 ± 0·1 (8)
Creatine phosphate	1·9 ± 0·1 (7)	1·8 ± 0·1 (8)
Inorganic phosphate	3·8 ± 0·1 (7)	3·5 ± 0·1 (8)
Ammonia	0·085 ± 0·003 (8)	0·082 ± 0·007 (8)

Figures in parenthesis refer to number of animals used.

The results presented in Table 7 show that no significant changes occurred in the concentration of energy-rich phosphates, inorganic phosphate and ammonia in the brains of rats given bulbocapnine.

DISCUSSION

Despite assertions by Chance and his colleagues[14–17] that glycogen accumulates in the brain during convulsions, most of the available evidence indicates that convulsive activity is associated with a loss of glycogen from the brain.[18–24] It is generally accepted that the lactic acid content of brain increases during increased central activity.[5, 18, 19, 22] It seems not unreasonable, therefore, to assume that in the catatonic state which followed audiogenic seizures, the observed changes in the glycogen and lactic acid (Table 2) arose simply as a result of the accelerated carbohydrate metabolism accompanying the seizures. Support for this explanation comes from the observation that the lactic acid concentration was increased markedly in the brains of animals which had experienced the more prolonged convulsions (Table 3). An increased cerebral activity cannot, however, explain the fall in glycogen content which occurred in rats rendered catatonic by bulbocapnine. In these animals, catatonia was preceded by no overt sign of central excitation and it was accompanied by only small changes in the amount of lactic acid in the brain. It is true that a significant increase in brain lactic acid occurred after several minutes of catatonia (Table 2) but the extent of this increase was much less than that induced by the convulsions, and it was accompanied by no change in the concentration of other brain metabolites (energy-rich phosphates, inorganic phosphate and ammonia; Table 7). Moreover, in the early stages of bulbocapnine catatonia, when glycogen depletion was as well marked as in the later stages, no change at all was observed in the brain lactic acid concentration.

Both method of producing catatonia caused similar losses of glycogen from all areas of the brain. This finding is in conflict with those described by Svorad[23, 24] in the only other reported investigations into the relationship between catatonia and brain glycogen. Svorad reported that during bulbocapnine catatonia, significant decreases of glycogen content occurred only in the cerebellum and medulla of rat brain[23] while during post-convulsive catatonia, glycogen was lost from the diencephalon mesencephalon and medulla, was gained by the cerebellum and did not alter in the cerebral cortex.[24] The cause of the discrepancies between Svorad's results and those presented in this paper is not known, but since the values for cerebral glycogen quoted by Svorad are considerably higher than those generally reported in the literature, it is possible that reducing substances other than glucose may have prevented an accurate assessment of the glycogen changes.

Inspection of Tables 4 and 5 shows that during catatonia the glycogen loss in all parts of the brain affects predominantly the 'free' fraction. This observation supports the findings of several workers,[25–28] who have suggested that the separation of glycogen into its 'free' and 'bound' fractions is justified on the grounds that the 'free' form is more readily metabolised while the 'bound' fraction is relatively stable. The alternative suggestion[29, 30] that the different glycogen fractions are extraction artifacts is not supported.

In both forms of catatonia, the glucose content of the brain is increased. This is not surprising, in view of the fact that brain glucose is derived from that in the blood[12]

and that both convulsive activity[31] and bulbocapnine administration[13] cause hyperglycaemia. As might be expected, the brain glucose content increased more markedly in the animals which had had convulsions (Table 2).

Bulbocapnine did not cause hyperglycaemia in rats which had been subjected to adrenal medullation (Table 6), an observation which confirms that of Feldman, Cortell and Gellhorn.[13] In demedullated animals, however, a small but statistically significant increase in brain glucose content still occurred following bulbocapnine administration, while the loss of glycogen was even more pronounced than in intact rats. The cause of this increase in brain glucose level in animals in which no hyperglycaemia occurred is still unknown. It is unlikely to have come from glycogen since the activity of glucose-6-phosphatase in the brain is very low.

Although the glycogen depletion in animals subjected to audiogenic seizures can be adequately explained as a response to increased nervous activity, the quantitatively similar changes occurring after bulbocapnine administration are not susceptible to this explanation since they are not accompanied by any significant change in the other labile metabolites. An alternative explanation is that the glycogen-depleting action of bulbocapnine is due to interference with the enzymatic processes involved in the early stages of glycogen degradation. The principal enzyme specifically related to the breakdown of glycogen in mammalian tissue is glycogen phosphorylase. This enzyme has been shown to exist in the brain in an active 'a' form and an inactive 'b' form.[32, 33] Although it is known that the estimation of cerebral glycogen phosphorylase is complicated by difficulties of obtaining suitable samples of brain tissue,[32] attempts were made during the course of the work described in this paper to determine the enzyme activity in the brains of catatonic rats. It was found that the phosphorylase could only be extracted from the cerebral tissue in the active form. Similar difficulties have been described by Kakimoto, Nakajima, Takesada and Sano,[34] and for the present the question whether bulbocapnine affects brain phosphorylase must be left unanswered. In view of the rapid turnover of glycogen in the brain,[35] the possibility may also have to be considered that bulbocapnine inhibits glycogen synthetase activity.

The simultaneous occurrence of catatonia and glycogen depletion after bulbocapnine administration and after audiogenic seizures naturally raises the question whether these events are causally related, whether they are both due to the operation of a common third factor or whether they are quite unrelated to one another. This problem is now being investigated.

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