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Physiological and drug-induced changes in the glycogen content of mouse brain

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Summary

1. The effect of the method of killing on the concentration of glycogen in mouse brain was determined. The cerebral glycogen content of mice killed by immersion in liquid nitrogen did not differ significantly from that of animals decapitated and the heads immediately frozen. A delay before freezing led to the rapid loss of brain glycogen, with a 17% fall at 10 s and an 82% loss after 5 min.

2. Hyperglycaemia, induced by the administration of D-glucose, resulted in an 8·3% loss of brain glycogen after 120 min. Insulin hypoglycaemia produced a 10·7% fall in glycogen at 60 min followed by an 11·2% increase at 120 min.

3. Exposure to either high (32° C) or low (10° C) ambient temperatures caused a depletion of brain glycogen.

4. A circadian rhythm of brain glycogen concentration was found, with a nadir which was coincident with the peak of locomotor activity and body temperature.

5. Drugs from several pharmacological classes were studied for their *in vivo* effect on the concentration of glycogen in mouse brain.

6. Brain glycogen was increased by all the depressant drugs tested, and by some drugs which had little effect on behaviour (diphenhydramine, phenytoin and propranolol), or which caused excitation (caffeine and nialamide).

7. Glycogen was depleted only by amphetamine-like compounds or by bemegride-induced convulsions.

8. The results are discussed with particular reference to the possible relation between catecholamines and glycogen metabolism in the brain.

Introduction

It has been known for many years that the rapid fixation of cerebral tissue *in situ* is essential for the estimation of labile intermediary products of carbohydrate metabolism such as glycogen and lactic acid (Kerr, 1936). The most satisfactory method for the fixation of cerebral tissue of small animals is by immersion in liquid nitrogen or liquid air (McIlwain & Rodnight, 1962). There have been several recent communications in the literature, however, in which the products of cerebral carbohydrate metabolism have been estimated without the use of rapid-freezing techniques and the maintenance of the deeply frozen state (Jacobowitz & Marks, 1964 ; Moore, Sawdy & Shaul, 1965 ; Pfeifer & Fodor, 1968 ; Chowdhury & Spector, 1969). It is therefore possible that the conclusions drawn from such experiments

may need to be modified by the likelihood of post-mortem biochemical changes. Experiments were made to confirm that the method of killing and tissue fixation is of prime importance in the estimation of cerebral glycogen.

Many physiological variables are known to demonstrate circadian rhythms, and the time of day at which an experiment is conducted may well influence its outcome. As liver glycogen concentration shows a striking circadian rhythm (Halberg, Barnum, Silber & Bittner, 1958), the possibility of a similar rhythm in brain glycogen content has been investigated. Some other physiological and environmental factors which may influence the concentration of brain glycogen have also been studied.

The influence of catecholamines on glycogen metabolism in peripheral tissues has been investigated extensively (Hess & Haugaard, 1958 ; Sutherland & Rall, 1960 ; Stetten & Stetten, 1960), particularly with reference to the adenyl cyclase system. The effect of drugs on this system has also been studied (Haugaard & Hess, 1965). In the brain the following enzymes and co-factors associated with glycogenolysis have been isolated: adenyl cyclase (Sutherland, Rall & Mennon, 1962), phosphodiesterase (Sutherland & Rall, 1958 ; Drummond & Perrot-Yee, 1961), glycogen phosphorylase (Cori & Cori, 1940 ; Breckenridge & Norman, 1962) and cyclic 3′,5′-adenosine monophosphate (cyclic AMP) (Rall & Sutherland, 1958 ; Kakiuchi & Rall, 1965 ; Klainer, Chi, Freidberg, Rall & Sutherland, 1962). The concentration of cyclic AMP in the brain is increased, *in vitro* by noradrenaline, and to a lesser degree by dopamine, whereas the concentration of this co-factor is not altered by 5-hydroxytryptamine (Kakiuchi & Rall, 1968a ; 1968b). Changes in the metabolism of catecholamines in the central nervous system (CNS) may be reflected, therefore, by changes in cerebral glycogen metabolism.

Noradrenaline and dopamine are believed to function as neurotransmitter substances at central synapses, and many centrally acting drugs are known to affect the synthesis, release and breakdown of cerebral catecholamines. It is possible, therefore, that these drugs may also affect cerebral glycogen metabolism.

In this report the effect of various centrally active drugs on the concentration of glycogen in mouse brain has been determined.

Methods

The experiments were performed on male albino mice weighing between 20 and 30 g. The animals were allowed free access to food and water before and during the experiments and were kept in groups of between four and six per cage. Unless otherwise stated, the environmental temperature was controlled at 20°–22° C.

Body temperature was measured by means of an electric thermometer and oesophageal probe (Light Laboratories, Brighton).

Spontaneous motor activity was recorded using an activity cage (Ugo Basile, Milan) in which movement of the mice was measured by completion of electronic circuits as the animals moved across the bars on the floor of the cage. Eighteen mice were used, in groups of three. The animals were placed in the activity cage for 24 h before recording.

Drugs

The drugs used were D-amphetamine sulphate, atropine sulphate, bemegride, caffeine (base), chlordiazepoxide hydrochloride, chlorpromazine hydrochloride,

cocaine hydrochloride, diphenhydramine hydrochloride, fencamfamin hydrochloride, haloperidol (base), imipramine hydrochloride, iproniazid phosphate, meprobamate (base), mepyramine maleate, mescaline hydrochloride, morphine hydrochloride, nialamide (base), pargyline hydrochloride, pentazocine (base), pethidine hydrochloride, phenoxybenzamine hydrochloride, phenobarbitone sodium, phentolamine mesylate, phenylbutazone sodium, phenytoin sodium, propranolol hydrochloride, physostigmine sulphate, pyrogallol, reserpine, tranylcypromine sulphate, tremorine hydrochloride. Doses are given in terms of the salts where these were used.

The drugs were dissolved in distilled water except for haloperidol (1% w/v tartaric acid), nialamide (distilled water adjusted to pH 5 with N HCl), meprobamate (25% v/v propylene glycol) and reserpine (supplied as the manufacturers' solution).

Each drug was administered in a volume of 1·0 ml/100 g body weight. The intraperitoneal route of injection was used for all drugs except bemegride, which was administered subcutaneously. Control animals received either distilled water or the appropriate drug solvent.

Experimental animals were killed within one hour of the control mice in order to minimize the effect of the circadian rhythm found to occur in brain glycogen concentration. Experiments were performed in the late evening when the mice were most active.

Estimation of brain glycogen

Unless otherwise stated, the mice were killed by complete immersion in liquid nitrogen, and the brains were chiselled out of the skull whilst in the deeply frozen state. Each brain was rapidly weighed before crushing in a stainless steel anvil cooled with liquid nitrogen (Stone, 1938). One mouse brain was used for each determination.

Cerebral glycogen was estimated by a modification of the method of Le Baron (1955). The crushed, frozen brain tissue was digested in 10 ml of alcoholic potassium hydroxide (20% w/v potassium hydroxide in 60% ethanol) at 80° C for 20 min. The tissue solution obtained was cooled to room temperature when the impure glycogen precipitated out. After centrifuging at 3,000 rev/min at room temperature for 30 min, the supernatant was decanted and the precipitate dispersed in 10 ml of a methanol:chloroform mixture (4:1 v/v) which was briefly heated in a hot water bath. After cooling, the suspension was centrifuged as before. The residue was again washed with a further 10 ml volume of methanol:chloroform.

The purified glycogen was hydrolysed by heating with 5 ml 1N sulphuric acid for 3 h, and the residual solution was neutralized and assayed for glucose in 1 ml aliquots using a glucose oxidase enzyme method based on that of Huggett and Nixon (1957) with reagents available in kit form (Boehringer, Mannheim).

The results are expressed as mg glycogen per 100 g brain tissue, wet weight (mg%).

Estimation of blood glucose

Blood was obtained from mice by decapitation. Portions of 0·2 ml were deproteinized with 2 ml uranyl acetate solution (0·16% w/v) and were then assayed for glucose using glucose oxidase enzyme (Boehringer, Mannheim).

In the analysis of results, statistical significance was calculated using Student's *t* test.

Results

Effect of method of killing on brain glycogen concentration

The mice were decapitated and the heads were immersed and frozen in liquid nitrogen, either immediately or after a delay of between 10 s and 20 min, during which time they were kept at room temperature. Decapitation followed by immediate freezing of the heads resulted in no significant change in glycogen concentration from those values obtained when the mice were killed by total body immersion in liquid nitrogen (Fig. 1). If there was a delay before freezing, however, glycogen was lost rapidly from the brain, with a 46% reduction in the first minute. After 5 min, only 18% of the glycogen remained. This residual concentration was maintained for at least 20 min.

Although there was no loss of glycogen from the brain tissue when the mice were killed by decapitation directly into liquid nitrogen, the head frequently fractured while cooling and the brain became brittle and difficult to chisel out *in toto*. For this reason, the technique commonly used in this laboratory involves total immersion in liquid nitrogen to kill and freeze the animals.

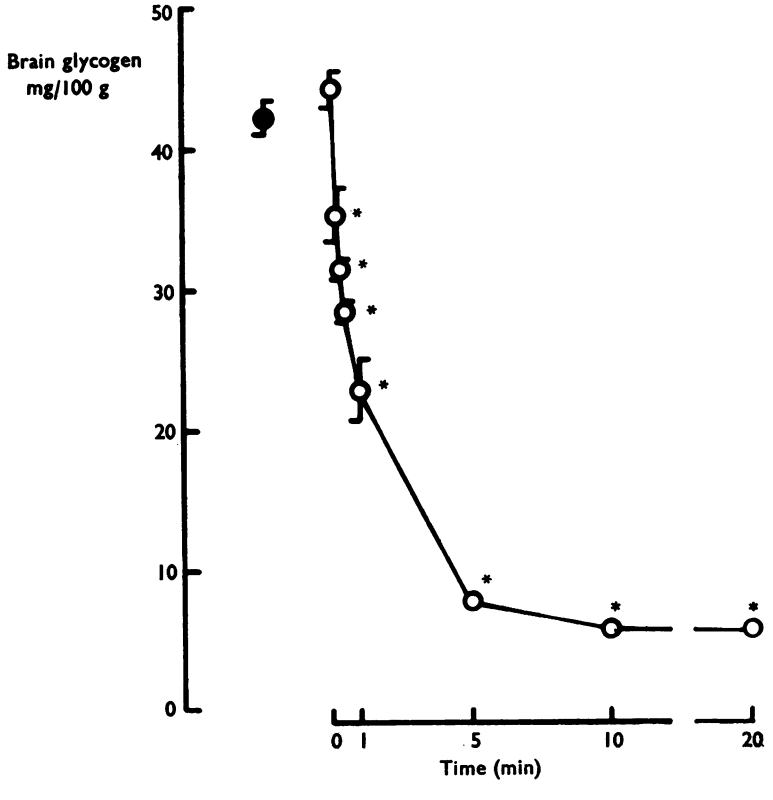

FIG. 1. Effect of method of killing on the concentration of glycogen in mouse brain. Mice were killed by immersion in liquid nitrogen (●) or the heads were frozen in liquid nitrogen at different times after decapitation (○——○). Each point is the mean of five animals. The S.E.M. is indicated by vertical lines through each point. All points marked with an asterisk are significantly different from the mean value obtained from brains frozen immediately after decapitation ($P<0{\cdot}05$).

Effect of changes in blood glucose on brain glycogen

Hyperglycaemia was induced in mice by the repeated administration of D-glucose solution by mouth (50% w/v, 1·0 ml/100 g body weight) and hypoglycaemia was induced by insulin injected subcutaneously (1·0 u./kg body weight). Each substance was administered at 0, 30 and 60 min (Fig. 2). The hyperglycaemia resulted in a small but significant fall in concentration of brain glycogen at 120 min. Insulin hypoglycaemia produced a small but significant fall at 60 min and an equally small but significant rise at 120 min.

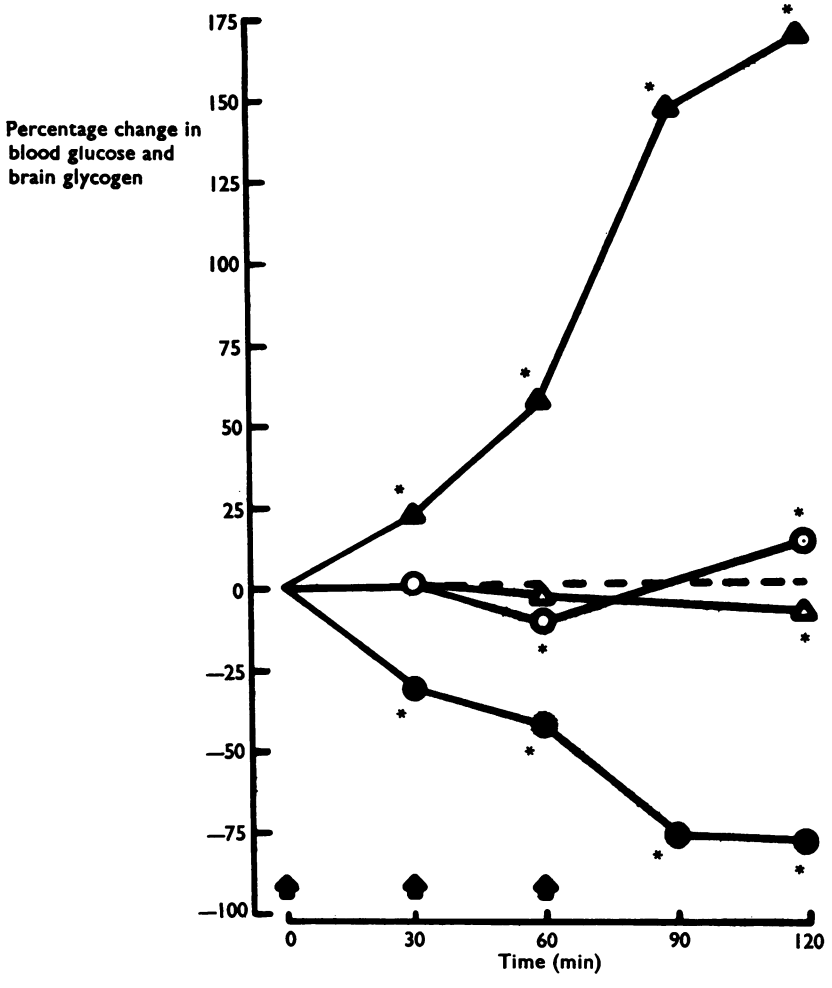

FIG. 2. Effect of hyperglycaemia and hypoglycaemia on the concentration of glycogen in mouse brain. The figure shows the levels of blood glucose (▲——▲) and brain glycogen (△——△) following administration of D-glucose and the levels of blood glucose (●——●) and brain glycogen (○——○) following administration of insulin. D-glucose was given by mouth (50% w/w solution, 1·0 ml/100 g body weight). Insulin was injected subcutaneously (1·0 u./kg). Each substance was administered at 0, 30 and 60 min as shown by the arrows. Each point is the mean of five animals. In the control groups the mean concentration ± S.E.M. for brain glycogen was 40·9 ± 0·9 mg/100 g and for blood glucose 119·9 ± 3·2 mg/100 ml. All points marked with asterisks are significantly different from control values ($P<0·05$).

Effect of ambient temperature on brain glycogen

Mice were placed in a ventilated oven at 30°–32° C in groups of five, or in a refrigerator at 8°–10° singly to avoid thermal insulation by grouping. Brain glycogen was estimated after periods of from 1 to 8 h. There was a significant fall in the glycogen concentration after 4 h at 10° C and after 8 h at 32° C (Table 1).

TABLE 1. *Effect of ambient temperature on the glycogen concentration of mouse brain*

Temperature (°C)	Time (h)	Brain glycogen concentration (mg/100 g)
8–10	0·0	41·2±1·4
	1·0	42·4±2·7
	2·0	41·5±2·8
	4·0	33·7±2·0*
30–32	0·0	41·9±1·1
	1·0	40·6±1·1
	4·0	37·7±2·6
	8·0	35·6±1·7†

Mice were maintained at 30°–32° C in groups of five, or at 8°–10° C singly to avoid thermal insulation by grouping.
Significance of difference from control values: * $P<0{\cdot}02$; † $P<0{\cdot}01$.

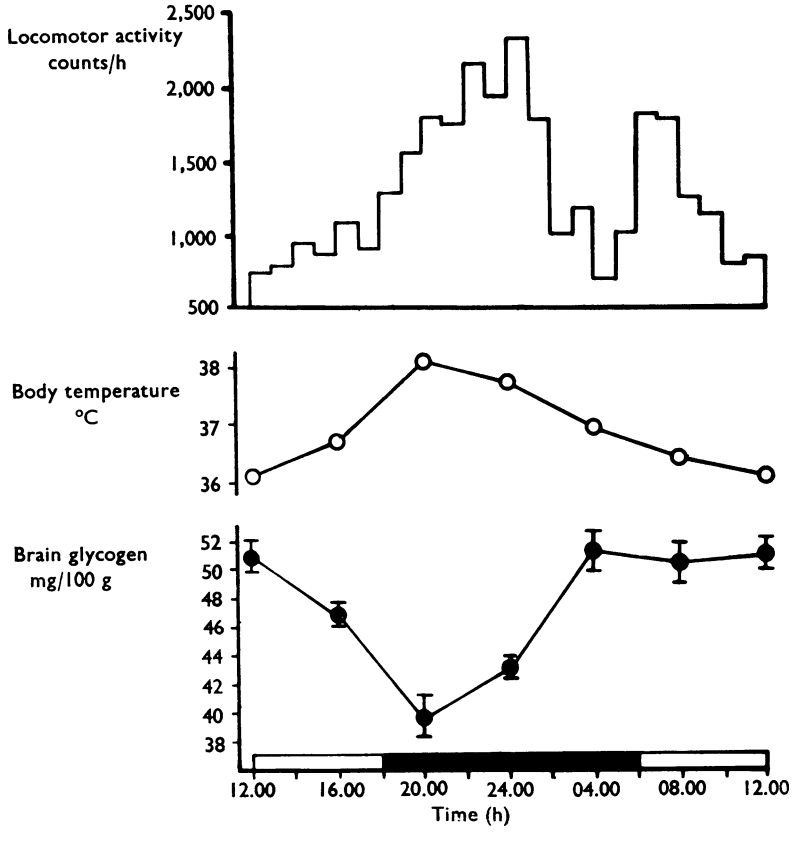

FIG. 3. Circadian rhythms for locomotor activity, body temperature and brain glycogen in the mouse. The clear bar indicates the light period and the black bar indicates the dark period of the illumination cycle. Vertical lines show the S.E.M. of the glycogen values.

Circadian changes in locomotor activity body temperature and brain glycogen

The mice used in this study were maintained from birth in a room in which the light cycle was controlled, the light period lasting from 06.00–18.00 h daily.

Locomotor activity increased from a low level in the early afternoon to a peak value at 20.00–01.00 h, then declined rapidly until 05.00 h (Fig. 3). A second but less pronounced increase in activity occurred lasting about 2 h (06.00–08.00 h).

Brain glycogen maintained a constant concentration of 50–51 mg/100 g from 04.00–12.00 h. The concentration then decreased by approximately 20% to a minimal value of 39·6 mg/100 g at 20.00 h coincident with the period of maximal motor activity and body temperature. The glycogen concentration then increased to the previous level of 51 mg/100 g by 04.00 h. The results shown in Fig. 3 were obtained over one period of 24 h. Similar results have been obtained on two further occasions. This study of circadian rhythm was made in June ; the effect of seasonal variation is under investigation.

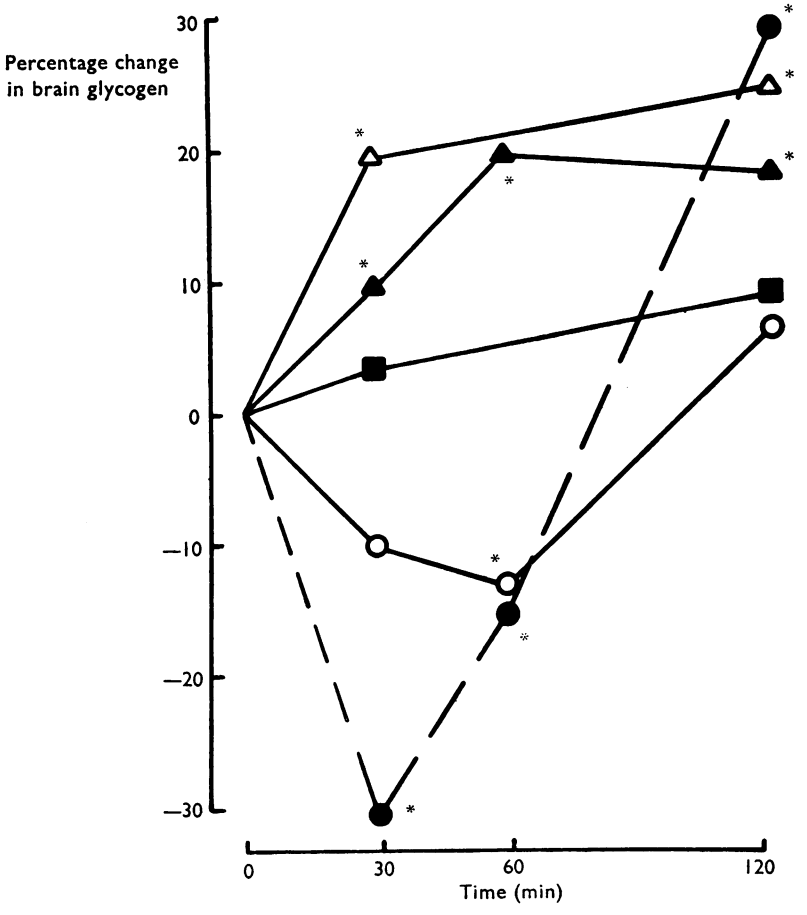

FIG. 4. Effect of the central stimulants D-amphetamine sulphate (5 mg/kg, ●– – –●), caffeine (10 mg/kg, ▲——▲), caffeine (200 mg/kg, △——△), cocaine hydrochloride (30 mg/kg, ■——■), and fencamfamin hydrochloride (20 mg/kg, ○——○) on the glycogen content of mouse brain. Each point is the mean of four animals. The mean concentration ±S.E.M. for the control group was 41·1±0·8 mg/100 g. All points marked with an asterisk are significantly different from control values ($P<0·05$).

Effect of drugs on brain glycogen

Central stimulants

The effect of central stimulant drugs on the concentration of glycogen in the mouse brain is shown in Fig. 4. A decrease of 32% was observed 30 min after amphetamine injection. This depletion was followed by an equally pronounced increase at 120 min. Fencamfamin also lowered brain glycogen, but the effect was less marked than that of amphetamine.

Cocaine in a dose that increased the motor activity of the mice did not affect the glycogen level. The effect of caffeine on activity was found to be dose dependent. The animals became excited and showed an increased motor activity after a dose of 10 mg/kg, although this effect was less than that produced by the same dose of amphetamine. Following a dose of 200 mg/kg of caffeine the activity of the mice was depressed for a period of more than 2 h. The content of cerebral glycogen was significantly increased by both the stimulant and depressant doses of caffeine.

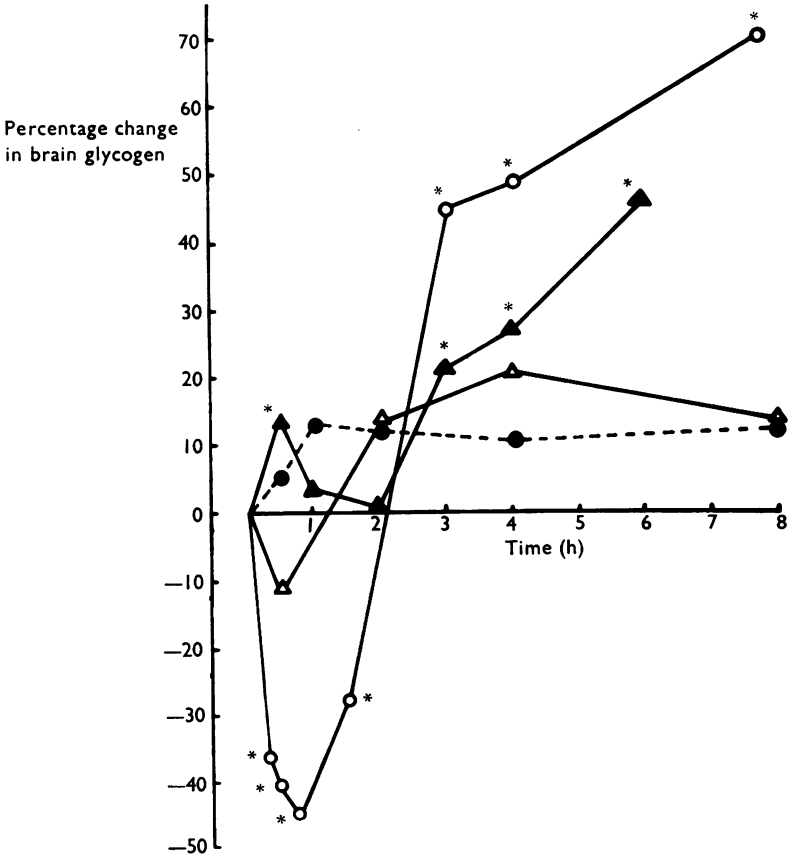

FIG. 5. Effect of the monoamine oxidase inhibitors iproniazid phosphate (500 mg/kg, △——△), nialamide (500 mg/kg, ▲——▲), pargyline hydrochloride (100 mg/kg, ●- - -●), and tranylcypromine sulphate (15 mg/kg, ○——○) on the glycogen content of mouse brain. Each point is the mean of five animals. The mean value ± S.E.M. for the control group was 39·3 ± 1·0 mg/100 g. All points marked with an asterisk are significantly different from the control value ($P<0.05$). All animals were killed at approximately the same time of day (22.00 h) to mitigate the influence of circadian changes in brain glycogen concentration.

Monoamine oxidase inhibitors

Tranylcypromine and nialamide were the only drugs in this group to affect behaviour or produce a significant change in brain glycogen (Fig. 5). Tranylcypromine induced an immobile hyperexcited response which lasted for 2–3 h, after which time the mice showed increased motor activity. The changes in glycogen concentration produced by this drug followed the pattern and time course of the changes produced by amphetamine.

Nialamide caused initial depression of activity followed by excitement, with head twitching and increased motor activity after 3–4 h. From 2–6 h after injection the brain glycogen showed a constant rate of increase.

Pargyline and iproniazid produced a small, constant, but non-significant increase in the glycogen concentration for a period of up to 8 h.

Bemegride convulsions

The effect of the analeptic agent bemegride on brain glycogen was determined in mice weighing 25–30 g using a dose of 40 mg/kg. Only animals within this weight range sustained a regular pattern of convulsive behaviour. This consisted of clonic convulsions at approximately 4·5 min after the injection with a quiescent period before further convulsions at 12 min after the injection. Each convulsive period lasted for approximately 15 s.

The glycogen concentration increased in the initial preconvulsive depression period, but was then depleted at a constant rate after the initiation of the convulsions (Fig. 6).

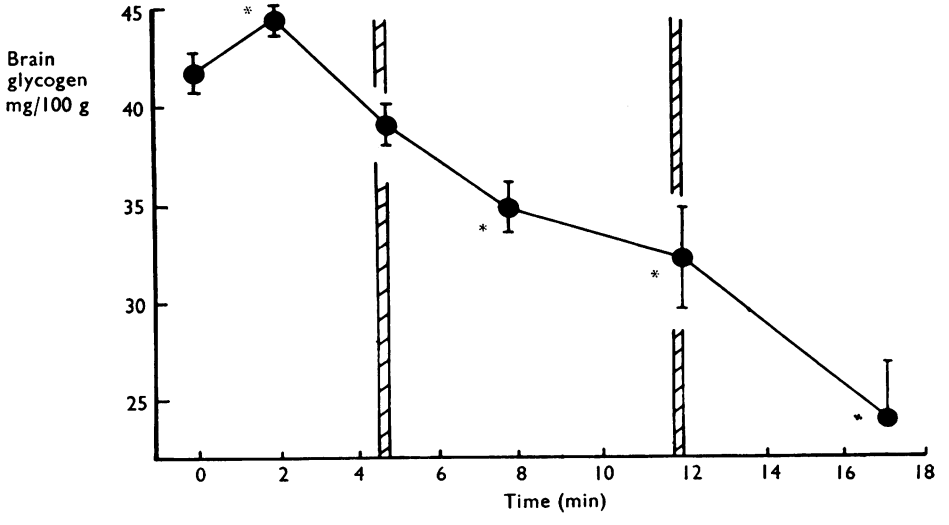

FIG. 6. Effect of bemegride convulsions on the glycogen content of mouse brain. Mice were killed: (i) 2 min after injection of bemegride (40 mg/kg), (ii) immediately after the first convulsion, (iii) 3 min after the first convulsion, (iv) immediately after the second convulsion, (v) 5 min after the second convulsion. The hatched areas show the mean periods of convulsive activity. Vertical bars indicate the S.E.M. Each point is the mean of six animals. All points marked with an asterisk are significantly different from the control value ($P<0·05$).

Cholinergic and anticholinergic drugs

Atropine, physostigmine or tremorine failed to produce any significant changes in the content of cerebral glycogen (Table 2). Any behavioural effects produced by atropine and physostigmine were not obvious, whereas tremorine induced a pronounced tremor which lasted for 2 h.

Neuroleptic drugs and minor tranquillizers

Chlorpromazine, haloperidol, reserpine, meprobamate and chlordiazepoxide all increased the brain glycogen concentration, although the change produced by reserpine was not significant until 1 h after injection. Each of the five drugs tested reduced the activity of the mice.

Adrenoceptor blocking agents

Of the α-adrenoceptor blocking agents tested, phentolamine did not alter activity or glycogen concentration whereas phenoxybenzamine reduced activity and raised levels of cerebral glycogen. The β-adrenoceptor blocking agent propranolol produced mild sedation and an increase in glycogen which was slightly greater than that produced by phenoxybenzamine.

TABLE 2. *Effect of drugs on the concentration of glycogen in mouse brain*

Drug	No. of Animals	Dose (mg/kg)	Glycogen (mg/100 g) 30 min	60 min	120 min
Cholinomimetic agents					
Control	9	—	41·2±1·3	41·4±1·8	40·1±1·1
Atropine sulphate	5	25	43·5±1·2	38·6±3·9	—
Physostigmine sulphate	5	0·25	38·5±2·4	41·4±3·2	—
Tremorine hydrochloride	8	20	41·4±0·2	—	38·5±1·4
Analgesics					
Control	16	—	41·7±0·8	40·2±0·2	43·4±1·5
Morphine hydrochloride	16	50	39·5±0·9	39·7±1·3	—
Pethidine hydrochloride	4	50	39·1±1·9	38·5±0·8	—
Pentazocine	4	50	44·4±1·7	41·0±1·8	—
Phenylbutazone sodium	4	150	42·7±0·6	45·2±1·7*	42·6±0·7
Tranquillizers					
Control	16	—	40·7±0·9	—	40·7±1·1
Chlorpromazine hydrochloride	4	2	42·2±2·6	—	50·0±0·9†
Chlorpromazine hydrochloride	4	5	45·7±0·9†	—	48·0±1·2†
Haloperidol	4	5	48·8±1·5†	—	55·0±1·0†
Reserpine	5	10	39·2±1·2	45·2±1·0*	57·0±2·0†
Chlordiazepoxide hydrochloride	4	25	50·6±1·2†	—	49·8±2·5*
Meprobamate	8	150	45·7±1·1*	—	53·7±2·1†
Adrenoceptor blocking agents					
Control	5	—	42·1±1·9	37·8±1·0	45·5±1·8
Propranolol hydrochloride	4	20	47·9±2·1	47·3±1·2†	54·9±2·0*
Phentolamine mesylate	4	50	42·4±2·1	—	43·4±3·1
Phenoxybenzamine hydrochloride	4	50	45·2±2·4	—	52·3±1·9*
Other drugs					
Control	16	—	40·3±1·1	—	40·9±1·3
Mescaline hydrochloride	4	30	38·9±1·7	—	42·5±2·0
Mepyramine maleate	4	50	37·9±1·3	—	46·0±2·1
Diphenhydramine hydrochloride	5	10	38·5±1·7	44·8±0·8*	54·6±1·7†
Phenobarbitone sodium	4	250	38·1±0·6	—	47·0±0·8†
Phenytoin sodium	4	20	44·2±1·7	—	44·7±1·0*
Pyrogallol	4	300	35·4±2·0	37·1±2·0	—
Imipramine hydrochloride	4	20	39·7±2·1	—	40·7±1·4

Drugs were injected by the intraperitoneal route. Values are means and standard errors. Significantly different from control: * $P<0·05$; † $P<0·001$.

Analgesics

Mice injected with morphine or pethidine showed increased locomotor activity, whereas pentazocine was without effect on behaviour. None of these potent analgesics altered the brain glycogen concentrations. Phenylbutazone caused a reduction in activity which was accompanied by a small but significant increase in glycogen at 60 min.

Other drugs

Of the remaining drugs tested, only diphenhydramine, phenytoin and phenobarbitone caused significant changes in brain glycogen (increases). Phenytoin and diphenhydramine, like mepyramine, did not affect the behaviour of the mice ; phenobarbitone produced anaesthesia lasting at least 2 h. The catechol-O-methyltransferase inhibitor pyrogallol caused mild sedation. Mescaline increased sensitivity to auditory stimulation.

A summary of results is presented in Table 3.

Discussion

When mice are decapitated and the heads are dropped directly into liquid nitrogen, the concentration of brain glycogen does not differ significantly from that

TABLE 3. *Summary of drug effects on the glycogen content of mouse brain*

Drug	Activity response
Depletion of glycogen	
Amphetamine	Increased activity
Fencamfamin	
Tranylcypromine	
Bemegride	
No change in glycogen	
Cocaine	Increased activity
Morphine	
Pethidine	
Mescaline	
Tremorine	
Phentolamine	No effect
Imipramine	
Iproniazid	
Pyrogallol	
Atropine	
Physostigmine	
Mepyramine	
Pentazocine	
Glycogen increase	
Caffeine (10 mg/kg)	Increased activity
Nialamide (3–6 h)	
Diphenhydramine	No effect
Phenytoin	
Propranolol	
Chlorpromazine	Decreased activity
Phenobarbitone	
Haloperidol	
Meprobamate	
Chlordiazepoxide	
Reserpine	
Phenylbutazone	
Phenoxybenzamine	
Caffeine (200 mg/kg)	
Nialamide (30 min)	

of animals killed by direct immersion (Fig. 1). However, a delay of only 10 s before freezing results in a significant loss of brain glycogen. These results confirm the necessity for the immediate fixation of the brain tissue *in situ* if an accurate estimation of this labile metabolite is to be obtained.

It is of interest that a residual concentration of 18% of the total brain glycogen remained after 20 min. Lowry, Passonneau, Hasselberger & Schulz (1964) reported an almost total loss of glycogen from the brains of mice after 10 min of ischaemia, and suggested that the apparent remaining concentration was derived from non-glycogen sources such as gangliosides. In the present study it is unlikely that the values obtained include any contribution from such sources, as these complexes are removed at an early stage in the extraction procedure. The residual glycogen may be retained in a bound state, unavailable for glycolytic enzyme action. Evidence for the existence of " labile " and " bound " forms of glycogen has previously been reviewed by Stetten & Stetten (1960).

Although the glycogen concentration does not change when mice are decapitated and the heads are frozen immediately, these animals are subjected to greater stress than those mice which are dropped directly into liquid nitrogen. The possibility cannot be excluded, therefore, that the stress induced by the decapitation technique could affect results of experiments in which the environment of the animals is modified (for example by drug administration).

In order to establish that any drug-induced changes in cerebral glycogen were not mediated through changes in blood glucose, the effects on brain glycogen of moderate fluctuations in blood glucose concentrations were investigated. Acute changes in blood glucose concentrations were found to produce only minor effects on cerebral glycogen (Fig. 2). These results are not in accord with earlier reports that insulin hypoglycaemia depletes glycogen in the brains of dogs (Chesler & Himwich, 1944 ; Tews, Carter & Stone, 1965) ; cats (Kerr, Hampel & Ghantus, 1937 ; Olsen & Klein, 1947) and mice (Carter & Stone, 1961). The results obtained in the earlier experiments, however, were achieved after prolonged hypoglycaemia (3–14 h) induced by convulsant doses of insulin (1–130 u./kg) and often following surgical procedures in anaesthetized animals. Thus, the severity and duration of the hypoglycaemia may well account for the difference. Lowry *et al.* (1964) have shown that when the glucose supply to the brain is restricted, ATP and glycogen are the last sources of cerebral energy reserve to be utilized.

The hyperglycaemia which occurs following repeated doses of glucose leads to a small fall in the glycogen concentration after 2 h. Kerr & Ghantus (1936) have previously shown that the infusion of glucose does not alter the levels of glycogen in brains of dogs and rabbits, although prolonged exposure to hyperglycaemia has been reported to cause an increase in mouse brain glycogen (Nelson, Schulz, Passonneau & Lowry, 1968). Thus, moderate variation in the blood glucose concentration does not exert a large influence on brain glycogen concentrations in acute experiments, and it is likely that any changes in blood sugar caused by stress or drugs will also have little effect on brain glycogen.

Exposure to high or low environmental temperatures leads to metabolic and physiological responses necessary for the maintenance of thermal homeostasis. Cold exposure raises the metabolic rate, whereas prolonged exposure to heat leads to a gradual fall in metabolism. Brain glycogen was found to be depleted after

exposure to both high and low ambient temperatures (Table 1). This suggests that the changes in glycogen are not related to an alteration in the metabolic activity of the animal, but may be part of a response to the stress of heat or cold. Other forms of stress have been shown to cause a loss of brain glycogen (Jakoubek & Svorad, 1959 ; Jacobowitz & Marks, 1964 ; Karadzic & Mrsulja, 1969). Stress has also been shown to increase the turnover of cerebral noradrenaline (Barchas & Freedman, 1963 ; Maynert & Levi, 1964 ; Moore & Lariviere, 1964 ; Gordon, Spector, Sjoerdsma & Udenfriend, 1966 ; Corrodi, Fuxe & Hökfelt, 1968 ; Welch & Welch, 1968a ; Welch & Welch, 1968b ; De Shaepdryver, Preziosi & Scapagnini, 1969). Similarly, an increase in turnover of cerebral noradrenaline has been reported after exposure to both cold (Gordon *et al.*, 1966 ; Duce, Crabai, Vargiu, Piras, Adamo & Gessa, 1968 ; Reid, Volicer, Smookler, Beaven & Brodie, 1968 ; Simmonds, 1969) and heat (Corrodi, Fuxe & Hökfelt, 1967 ; Reid *et al.*, 1968 ; Simmonds, 1969). It is possible, therefore, that the release of catecholamines within the brain may be responsible for the loss of cerebral glycogen during exposure to heat or cold.

It is generally recognized that most metabolic systems exhibit rhythmic activities, and that some of these metabolic rhythms may interact with other circadian components. Brain glycogen shows a circadian rhythm, with a nadir at 20.00 h (Fig. 3) which coincides with the peaks of both body temperature and locomotor activity. Using lighting conditions identical to those described in the present experiment, Halberg *et al.* (1958) have observed that the liver glycogen of mice also exhibits a circadian rhythm with a trough at 20.00–22.00 h The magnitude of the changes in the liver glycogen is approximately 10 times as great as in the brain. The mechanisms responsible for the daily fluctuations in liver glycogen are not clear, but may be dependent on adrenocortical activity (Mills, 1966). Similar mechanisms are unlikely to obtain in the brain, because the chronic administration of hydrocortisone to mice causes an increase in the content of liver glycogen without altering the level of glycogen in the brain (Thurston & Pierce, 1969). The circadian depletion of brain glycogen may occur as a result of an increase in turnover of cerebral noradrenaline. Preliminary experiments in this laboratory using α-methyl-*p*-tyrosine, an inhibitor of catecholamine synthesis, indicate that the noradrenaline turnover in mouse brain shows a circadian increase at the time when glycogen levels are decreasing.

In the investigation of drug effects on brain glycogen, only amphetamine and fencamfamin, of those agents classified as CNS stimulants, depleted the glycogen concentration. Cocaine did not alter the glycogen level and has been shown also to have no effect on the catecholamine concentration in the CNS (Smith, 1965 ; this laboratory, unpublished observations). Although cocaine is known to inhibit neuronal re-uptake of noradrenaline in peripheral tissues, it does not influence the re-uptake process in the brain (Glowinski & Axelrod, 1965). Brain glycogen concentration was found to increase after doses of caffeine which stimulate activity and after doses which result in a depression of activity. Sutherland & Rall (1958) showed that methylxanthines inhibit phosphodiesterase *in vitro,* with a consequent increase in cyclic AMP. If these compounds exert similar biochemical activity *in vivo* caffeine might be expected to stimulate glycogenolysis. However, caffeine also stimulates the *in vitro* activity of phosphorylase inactivating enzyme (Wosilait & Sutherland, 1956) and therefore the effect that this drug may have on the phosphorylase system *in vivo* is not clear.

The glycogen changes observed after the monoamine oxidase inhibitor tranylcypromine followed a pattern very similar to that due to amphetamine. Tranylcypromine, like amphetamine, is a phenylethylamine derivative and is known to have many pharmacological effects similar to amphetamine. The chemical structures of nialamide, iproniazid and pargyline show no similarity to that of amphetamine, and these monoamine oxidase inhibitors did not deplete brain glycogen.

Bemegride has proved to be a useful tool in the determination of the pattern of glycogen change after convulsions, because repeated convulsions were obtained at fairly regular intervals. Depletion of brain glycogen in the mouse after convulsants has been reported by Carter & Stone (1961). The results presented here confirm their observations and show that the fall of glycogen occurs at a rapid but constant rate, and is not related to the overt signs of convulsive activity.

Atropine, physostigmine and tremorine are all known to affect the content of acetylcholine in the CNS. None of these drugs altered the glycogen content of mouse brain. Mrsulja, Terzic & Varagic (1968) reported that the intravenous injection of physostigmine induces a fall of glycogen concentration in the brains of rats. The reason for the difference in these results is not clear.

All of the tranquillizers tested reduced locomotor activity and increased the cerebral glycogen content. Similar changes of glycogen to those reported in this paper have been observed in mice after chlorpromazine and meprobamate (Estler, 1961). Chlorpromazine and haloperidol are believed to block central adrenoceptors (Carlsson & Lindqvist, 1963) and it is well known that reserpine depletes the brain of catecholamines.

The influence of the β-adrenoceptor blocking agent propranolol on the content and metabolism of glycogen in mouse brain has been discussed by Estler & Ammon (1966), who demonstrated an increase of glycogen of approximately half of that determined in this report. The pharmacological actions of the α-adrenoceptor blocking agents phentolamine and phenoxybenzamine on the brain are not clear. Phenoxybenzamine, unlike phentolamine, caused a reduction in activity which was associated with a significant increase in glycogen.

Cerebral catecholamine levels are not influenced by imipramine (Sulser, Watts & Brodie, 1962), although this drug is thought to potentiate the actions of catecholamines at adrenoceptors in the brain (see Schildkraut & Kety, 1967). No change in mouse brain glycogen was observed after the administration of imipramine.

The results summarized in Table 3 show that decreased locomotor activity is always accompanied by an increase in brain glycogen content, whereas there is clearly no correlation between an increase in locomotor activity and the glycogen changes. Except for bemegride-induced convulsions, only amphetamine-like compounds cause depletion of brain glycogen.

Amphetamine has been reported to release catecholamines, to inhibit their re-uptake and to exert a direct action on central adrenoceptors (see Schildkraut & Kety, 1967). Release of catecholamines and inhibition of their re-uptake results in an increase in the " free ", extracellular neurohumor which might then stimulate the adenyl cyclase system, raising the levels of cyclic AMP and increasing the rate of glycogenolysis. The monoamine oxidase inhibitors iproniazid, nialamide or pargyline increase the total concentration of brain catecholamines, but these drugs, alone, do not deplete brain glycogen. Following a single dose of these inhibitor

drugs the increase in monoamines occurs intracellularly (Carlsson, Dahlstrom, Fuxe & Lindqvist, 1965). However, when a monoamine oxidase inhibitor is administered in conjunction with dihydroxyphenylalanine, a treatment likely to result in an increased concentration of extracellular catecholamines, brain glycogen is depleted (Kakimoto, Nakajima, Takesada & Sano, 1964).

The fall in glycogen which accompanies convulsions has been suggested to occur as the result of an increase in cerebral activity (McIlwain, 1966). However, the role of glycogen as an energy reserve in the brain is not clear. Under normal physiological conditions blood glucose is known to provide an adequate supply of energy for the central nervous system. During hypoglycaemia only minor fluctuations in brain glycogen occur, suggesting that the glycogen is not readily available as a reserve of energy. On the other hand, brain glycogen shows circadian fluctuations, and is also depleted in situations of mild stress, indicating that cerebral glycogen metabolism is not essentially related to an overall demand for energy. Thus, during convulsions, the glycogen response may be mediated, at least in part, by a release of noradrenaline. Convulsive activity has been shown to cause loss of brain noradrenaline (Breitner, Picchioni & Chin, 1963). Furthermore, central stimulation *per se* does not always result in glycogen loss since caffeine and cocaine, drugs which evoke an increased locomotor activity, do not deplete brain glycogen, and there is no evidence that these drugs affect central adrenergic nerves in a similar manner to amphetamine.

Further aspects of the relation between glycogen and catecholamines in the CNS are under investigation.

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