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SENSITIVITY OF GUINEA-PIG HIPPOCAMPAL GRANULE CELL FIELD POTENTIALS TO HEXOSES *IN VITRO*: AN EFFECT ON CELL EXCITABILITY?

BY HERMAN S. BACHELARD*, DAVID W. G. COX AND JANET DROWER
From the Department of Biochemistry, St. Thomas's Hospital Medical School, London, SE1 7EH

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SUMMARY

1. Evoked granule cell field potentials, and levels of tissue metabolites, in superfused guinea-pig hippocampal slices have been studied in the presence of low glucose and an alternative glycolytic substrate (D-fructose). The effects of glucose analogues (5-thio-D-glucose, 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose) in the presence of glucose were also tested.

2. Concentrations of glucose or fructose in excess of 2 mM and 10 mM respectively were required to maintain normal evoked activity. 5-Thioglucose (15 mM) in the presence of 5 mM-glucose decreased the amplitude of the population spike by 60% with little effect on population excitatory post-synaptic potential (e.p.s.p.).

3. Tissue levels of phosphocreatine and ATP were essentially unchanged under all conditions tested, with the exception of 10 mM-fructose. The decrease in rates of lactate efflux from superfused tissue during and after superfusion with 3-*O*-methylglucose, 2-deoxyglucose or 5-thioglucose was found to be positively correlated with the extent of attenuation of field potentials.

4. Analysis of the relationship between population spike amplitude and rates of rise of e.p.s.p., under conditions where field potentials were attenuated, showed that the population spike was always more sensitive to metabolic perturbation than was the e.p.s.p., thus indicating an effect on cell excitability.

5. It is suggested that some aspect of non-oxidative glucose metabolism is important in maintaining this granule cell excitability.

INTRODUCTION

The results of numerous studies *in vivo* have indicated that moderate degrees of hypoglycaemia, associated with the appearance of neurological deficit, fail to produce detectable changes in the over-all cerebral energy state (Hinzen & Müller, 1971; Ferrendelli & Chang, 1973; Horton, Meldrum & Bachelard, 1973; Lewis, Ljunggren, Ratcheson & Siesjö, 1974), but the reasons for this remain obscure. Whereas the use of *in vivo* techniques may suffer from the disadvantage that the

* To whom correspondence should be addressed.

immediate environment of brain tissue is difficult to control and to manipulate, this is considerably less of a problem with slices of cerebral tissue superfused *in vitro*. Furthermore, it is possible to use such *in vitro* preparations to relate some measure of physiological activity recorded from the tissue to biochemical events occurring within the tissue. Using the hippocampal slice preparation it has been shown that extracellular field potentials, evoked from dentate granule cells by stimulation of the perforant path, are particularly sensitive to moderate decreases in the glucose available to the tissue. Furthermore, substituting the mitochondrial substrates, pyruvate plus malate, or 3-hydroxybutyrate, for glucose failed to support normal evoked activity (Cox & Bachelard, 1982; Bachelard & Cox, 1983) even though the concentrations of these alternative substrates used are known to maintain a normal cerebral energy state *in vitro* (Woodman & McIlwain, 1961; Bachelard & Cox, 1983). Thus it would seem that *in vitro* as well as in the whole brain *in vivo*, electrophysiological activity can be modified under conditions of decreased glucose availability which apparently do not affect the over-all energy state.

In view of the apparent inability of non-glycolytic substrates to maintain normal evoked activity we have compared the ability of glucose and fructose to maintain field potentials, and have investigated the sensitivity of the evoked activity to the glucose analogues, 5-thio-D-glucose, 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose. Use of these glucose analogues is of interest in view of their different metabolic fates. All three share the glucose uptake carrier to the brain (Bachelard, 1971; Betz, Drewes & Gilboe, 1975), but differ in metabolism thereafter as described in the Discussion. We present evidence that the sensitivity of the evoked activity to perturbations of glucose metabolism may be partly due to an elevation of the threshold for cell discharge and discuss the results in terms of the importance of glycolysis in maintaining normal evoked activities.

METHODS

Female Dunkin–Hartley guinea-pigs (220–500 g) were stunned by a blow to the cervical vertebrae, exsanguinated, and the cerebral hemispheres removed. A tangential slice, consisting of parts of the para- and pre-subiculum, dentate gyrus and CA3 division of the hippocampus proper, was prepared as previously described (Cox & Bachelard, 1982). Slices (0·4 mm thick) were superfused at about 37 °C at a rate of 2·5 ml/min with a Krebs–Ringer–bicarbonate medium (mM: NaCl, 124; KCl, 5; KH_2PO_4, 1·2; $MgSO_4$, 1·2; $CaCl_2$, 1·2; $NaHCO_3$, 26; D-Glucose, 10), equilibrated with 95% O_2/5% CO_2.

Electrophysiological experiments

Following a minimum pre-incubation period of 1·5 h at about 36·5 °C, stimulation of the perforant path and recording from the layer of granule cells was performed as before (Cox & Bachelard, 1982). Paired supramaximal stimuli of 0·1 ms duration, separated by 25 ms (Lømo, 1971) were provided, from a glass micropipette containing 2% NaCl, at a frequency of 0·5 Hz and intensity of 1·5 V. The recording glass micropipette contained 0·9% NaCl. Every 60 s four paired responses were averaged using a Neurolog NL750 Signal Averager (Digitimer, Welwyn Garden City, U.K.) and were displayed on a Rikadenki R104 Pen Recorder (Centronic, New Addington, U.K.). The field potentials were partially analysed by an HP85 microcomputer (Hewlett Packard, Redhill, U.K.), and recorded as a time series of 256 digital values (bins) on magnetic tape or floppy disk for subsequent detailed analysis.

The rate of rise of population excitatory post-synaptic potential (e.p.s.p.) was determined for a series of averaged field potentials by finding the latest bin at which the positive e.p.s.p. wave

began and the earliest bin at which this wave was interrupted by the negative-going population spike. A least-squares regression analysis was then made for the bin values between these two points. Amplitude of the population spike was determined as previously described (Cox & Bachelard, 1982). Electrophysiological results are expressed as percentages of the means of those recorded under control conditions (10 mM-glucose; except in the case of the glucose analogues: 5 mM-glucose) during a 5 min period prior to introduction of test superfusion medium. Slices were exposed to test media containing lowered glucose concentrations and/or added compounds for periods of 10 min or more, after which control superfusion conditions were resumed for a minimum of 15 min, and usually for at least 20 min before re-introduction of test media. Curves showing the relationship beween the rate of rise of the conditioning e.p.s.p. and the amplitude of the corresponding population spike were set up using two different methods.

Method 1. The perforant path was stimulated every 2 s in the following cycle, repeated every 3 min. Responses were collected for averaging over an 8 s period every min. For the first 1 min 50 s of each cycle, stimuli were supramaximal (1·5 V) and from then to 2 min 25 s (i.e. a period of 35 s) submaximal stimuli were applied. The intensities of the submaximal stimuli varied, from cycle to cycle, from 0·95 to 1·3 V, and were applied 10 s before collection of responses for averaging. Thus, each averaged response obtained with submaximal stimuli was separated by two averaged sets of responses evoked by supramaximal stimulation. This method was used to obtain e.p.s.p./population spike relationships in the control medium, except in one experiment (Fig. 3) where the variable submaximal stimuli were not separated by two supramaximal stimuli. The results obtained were the same.

Method 2. The field potentials were attenuated as a result of superfusion with test media (e.g. low glucose). Because the field potentials were continuously attenuating in the test media, it was not possible to plot a routine (variable recruitment) input–output curve. However, an index of the relationship between e.p.s.p. and population spike amplitudes could be obtained by plotting attenuating population spike as a function of attenuating e.p.s.p., both expressed as a percentage of values observed in control medium prior to exposure to test medium. Thus, the basic differences between Methods 1 and 2 is that whereas in Method 1, the field potentials were attenuated briefly in response to the intermittent submaximal stimulation, in Method 2 the evoked activity gradually decreased in amplitude over a period of some minutes during supramaximal stimulation. In both methods the data plotted were restricted to those obtained during periods when the population spike was clearly attenuated. The data were tested for statistical significance using the appropriate *t* test.

Metabolic experiments

Tangential slices of the hippocampal formation were each mounted in quick transfer holders (McIlwain, 1975) in superfusion chambers containing 4·0 ml. Following a 40 min pre-incubation, four test slices were superfused with test medium usually for 15 min, while another four continued to be superfused with control medium. A chamber volume of 4·0 ml superfused at a rate of 2·4 ml/min requires approximately 4 min after changing the medium for the constituents to reach 90% of their final concentrations. In contrast the chamber volume in the electrophysiological experiments was only *ca.* 0·5 ml with a corresponding mixing time of *ca.* 0·5 min. For this reason, test superfusions in the metabolic experiments were extended by 5 min to allow for this difference. At the end of the 15 min incubation period (except in some experiments in the presence of 2-deoxyglucose) slices were floated off the quick transfer holder into control medium, and were immediately homogenized in 1 ml ice-cold 0·6 M-perchloric acid containing 1 mM-EDTA. A 50 μl aliquot was removed for protein determination using the method of Lowry, Rosebrough, Farr & Randall (1951), and the remainder was centrifuged at 10000 *g* for 10 min at 4 °C. The supernatant was divided into two parts. Total tissue K^+ was determined in a 200 μl aliquot using a Corning 400 Flame Photometer (Halstead, U.K.). An equivalent of ice-cold 1 M-$KHCO_3$ was added to the remainder of the perchloric acid extract and stored on ice. After 5 min of occasional mixing, the samples were centrifuged at 2000 *g* for 5 min at 4 °C. The decanted neutralized supernatants were stored at −20 °C until required for metabolite assay. In some experiments the superfusate was collected in flasks on ice and stored at −20 °C prior to analysis for lactate. Phosphocreatine, ATP and lactate levels were measured independently using fluorometric enzyme assays (Lowry & Passonneau, 1972).

RESULTS

Glucose and fructose as substrates

We reported previously that slight decreases in glucose in the superfusing medium caused an attenuation of the evoked activity when the energy state appeared to be unchanged (Bachelard & Cox, 1981; Cox & Bachelard, 1982). In subsequent metabolic studies we have found that the concentration of glucose can be decreased below 0·5 mM without any detectable change in the tissue levels of phosphocreatine, ATP or total K^+ (Table 1). This is consistent with ^{31}P nuclear magnetic resonance spectroscopic data obtained from superfused brain slices (Cox, Morris, Feeney & Bachelard, 1983). It seems clear that the evoked field potentials are far more sensitive than is the energy state to decreases in the glucose concentration of the superfusing medium.

Fructose at 10 mM in the absence of glucose resulted in a 40% decrease in phosphocreatine and ATP, with a 20% decrease in K^+; these were normal when fructose was present at 20 mM (Table 1). In strict parallel to the results on the energy state, whereas 10 mM-fructose caused a substantial decrease in evoked activities, these were normal in three out of five experiments when fructose was present at 20 mM (Table 2). In only one slice was the evoked activity strongly depressed within 9 min, although in one test where superfusion with 20 mM-fructose was prolonged for a further 5 min there was a significant decrease in field potentials.

Glucose analogues

Where these analogues were tested, it was decided to use them at a concentration of 15 mM in the presence of 5 mM-glucose, in view of the findings of Meldrum & Horton (1973) where the production of hypoglycaemic symptoms *in vivo* required plasma concentrations of 2-deoxyglucose some 3 times that of glucose. In order to avoid excessively hyperosmotic conditions the tissues were presented with a total hexose concentration of 20 mM. Glucose at a concentration of 5 mM is as effective as the normal control level of 10 mM in maintaining evoked activity (Table 2), while increasing the glucose concentration to 20 mM had no effect on the responses.

When the hippocampal slices were superfused for 9 min with a 5 mM-glucose medium containing 15 mM-5-thioglucose, the amplitudes of both conditioning and conditioned population spikes were decreased by about 60% (Table 2). In four out of five cases changes in the conditioning e.p.s.p. were very small, and over-all the synaptic potential remained insignificantly different from control. Recovery of field potentials following a return to 5-thioglucose-free medium tended to be intermittent and was considerably slower than occurred following superfusion with low glucose medium (Fig. 1).

As reported previously (Bachelard & Cox, 1983), 15 mM-2-deoxyglucose in the presence of 5 mM-glucose had no significant effect on evoked activity until the analogue was withdrawn, when there was profound attenuation. 3-*O*-methylglucose (in the presence of glucose) had no effect. Tissue levels of phosphocreatine and ATP were not significantly decreased under the conditions where evoked activity was strongly attentuated.

In view of the apparent importance of glycolysis in maintaining normal evoked activity (Cox & Bachelard, 1982; Bachelard & Cox, 1983) it was decided to assess any effect on lactate output from the superfused tissues. Under conditions where the

TABLE 1. Effects of substrates or glucose analogues on tissue metabolites. The results are means ± S.E. of mean of data expressed as percentage of control values. Concentrations (mM) of substrate or analogue are shown in parentheses. Results significantly different from control: $P < 0.05$ (*) and $P < 0.005$ (†). (a) 5 min after return to analogue-free medium. 2-DOG, 2-deoxy-D-glucose; 5-TG, 5-thio-D-glucose. Control levels of metabolites were (μmol/100 mg protein): phosphocreatine, 2·73 ± 0·08; ATP, 0·97 ± 0·09; K^+, 66 ± 4. Results previously reported (Bachelard & Cox, 1983) are included for completeness (‡)

Substrate	Addition	Phosphocreatine (percentage of control)	ATP (percentage of control)	K^+ (percentage of control)
Glucose (1)	—	91 ± 12 (3)	94 ± 16 (3)	92 ± 9 (3)
Glucose (0·1)	—	100 ± 10 (4)	86 ± 10 (4)	104 ± 11 (4)
Fructose (20)	—	91 ± 20 (4)	100 ± 12 (4)	—
Fructose (10)	—	59 ± 6 (4)†	56 ± 6 (4)†	80 ± 6 (4)*
Glucose (5)	2-DOG (15)	105 ± 15 (4)‡	107 ± 10 (4)	102 ± 9 (4)‡
		[a]83 ± 10 (4)‡	101 ± 8 (4)	100 ± 8 (4)‡
Glucose (5)	5-TG (15)	96 ± 13 (8)	108 ± 17 (8)	—

TABLE 2. Effects of substrates or glucose analogues on components of evoked potentials. The results are means ± S.E. of mean of the amplitude of each component 9 min after beginning the test superfusion, expressed as percentage of control values obtained during a 5 min period prior to introduction of test medium. Concentrations (mM) of substrate or analogue are shown in parentheses. Results significantly different from control: $P < 0.05$ (*) and $P < 0.005$ (†). (a) 5 min after return to inhibitor-free medium. 3-OMG, 3-*O*-methyl-D-glucose; 2-DOG, 2-deoxy-D-glucose; 5-TG, 5-thio-D-glucose. (‡) Results previously reported (Bachelard & Cox, 1983) are included for completeness

		Conditioning response		Conditioned response
Substrate	Addition	E.p.s.p. (percentage of control)	Population spike (percentage of control)	Population spike (percentage of control)
Glucose (5)	—	103 ± 5 (5)	104 ± 9 (5)	103 ± 3 (6)
Glucose (2)	—	96 ± 4 (4)	66 ± 15 (7)*	65 ± 13 (7)*
Glucose (1)	—	97 ± 4 (8)	57 ± 9 (7)†	74 ± 12 (8)*
Fructose (20)	—	99 ± 15 (5)	72 ± 19 (5)	83 ± 22 (5)
Fructose (10)	—	88, 96 (2)	16 ± 8 (3)†	43 ± 4 (4)†
Glucose (5)	3-OMG (15)	107 ± 6 (3)	92 ± 5 (4)	96 ± 3 (6)
		[a]109 ± 9 (3)	91 ± 6 (4)	96 ± 6 (6)
Glucose (5)	2-DOG (15)	106 ± 1 (8)‡	97 ± 12 (6)‡	86 ± 8 (8)
		[a]99 ± 5 (8)‡	28 ± 11 (6)‡†	60 ± 12 (8)*
Glucose (5)	5-TG (15)	97 ± 7 (5)	38 ± 15 (5)*	42 ± 13 (5)†

presence of 5-thioglucose with glucose had no effect on tissue levels of phosphocreatine or ATP the rate of lactate efflux was significantly decreased to 33% of control (Table 3). In the presence of 2-deoxyglucose with glucose, the rate of lactate efflux was also decreased (to 68% of control) but this only became significant after with-

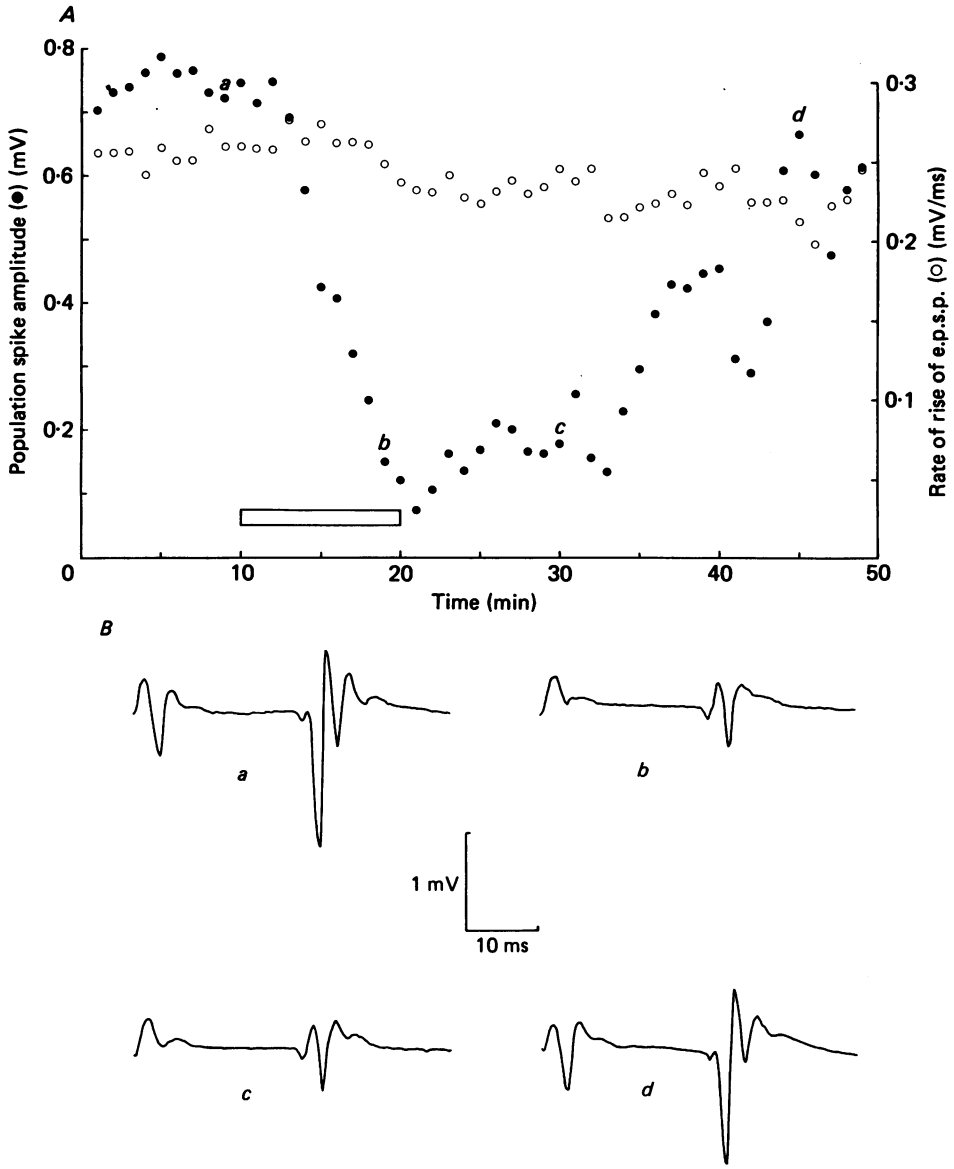

Fig. 1. *A*, time course of the amplitude of conditioning population spike (●) and rate of rise of conditioning e.p.s.p. (○) before, during and after superfusion in the presence of 15 mM-5-thio-D-glucose; 5 mM-glucose was present throughout. The bar shows the duration of superfusion with the analogue present. *B*, responses obtained at the times indicated in *A*.

drawal of the analogue (Table 3). Here too there was no detectable effect on the energy state. 3-*O*-methylglucose was without significant effect either during superfusion with the analogue or after withdrawal.

Granule cell excitability

During analysis of the results, it became apparent that the population spike appeared to be attenuated with little if any effect on the corresponding rate of rise of e.p.s.p. It therefore seemed possible that these metabolic perturbations might have some specific effect on granule cell discharge of which the population spike is a representation. It is known, however, that small changes in e.p.s.p. amplitude are associated with large effects on the size of the population spike (Richards & White, 1975). Thus the apparently specific attenuation of the population spike seen here might merely be secondary to some small undetected decrease in e.p.s.p. amplitude.

TABLE 3. Effects of glucose analogues on efflux of lactate from superfused tissue. The results are means ± S.E. of mean of data expressed as percentage of control values. Concentrations (mM) of substrate or analogue are shown in parentheses. Results significantly different from control: $P < 0.05$ (*) and $P < 0.005$ (†). (a) Superfusate collected during last 5 min of 15 min superfusion in the presence of analogue; (b) superfusate collected 5–10 min after return to analogue-free medium. Mean control rates of lactate efflux were in the range 502–862 nmol/100 mg protein.min. Abbreviations as in Table 2

Substrate	Addition	Lactate efflux (percentage of control)
Glucose (5)	3-OMG (15)	[a]89 ± 8 (3)
		[b]97 ± 19 (3)
Glucose (5)	2-DOG (15)	[a]68 ± 15 (4)
		[b]43 ± 11 (4)*
Glucose (5)	5-TG (15)	[a]33 ± 5 (4)†

To test this possibility, relationships between the conditioning population spikes and their corresponding e.p.s.p.s were set up as described in the Methods. Linear regression analyses of data obtained from the use of media containing 2 mM-glucose, 1 mM-glucose, or 10 mM-fructose gave positive correlations significant at $P < 0.005$, < 0.005 and < 0.05 respectively. Threshold e.p.s.p.s for granule cell discharge were 68·8 ± 3·7, 84·9 ± 2·7 and 92·5 ± 1·9 (percentage of control e.p.s.p.; mean + S.D.; $n \geqslant 8$) respectively compared with a value of 47·3 ± 10·9 for four separate input–output determinations in control medium. Results for 1 mM-glucose and 10 mM-fructose are illustrated together with control data in Fig. 2. The greater scatter of the points obtained for 1 mM-glucose compared to those observed for 10 mM-glucose or fructose is attributed to the results for low glucose being derived from the pooled data of four separate experiments, whereas those illustrated for 10 mM-glucose and fructose are each derived from a single experiment.

The values of threshold e.p.s.p. obtained in low glucose, or with fructose, were significantly different from those obtained with control media ($P < 0.001$).

Similar results were obtained using 5-thioglucose. In this case the population spike disappeared at an e.p.s.p. value of 86·5 ± 2·2% of control (Fig. 3). In all these situations, therefore, the submaximal population spikes had lower amplitudes for a given e.p.s.p. value than was observed in control media suggesting that cellular excitability may be particularly sensitive to some disturbance of glucose metabolism.

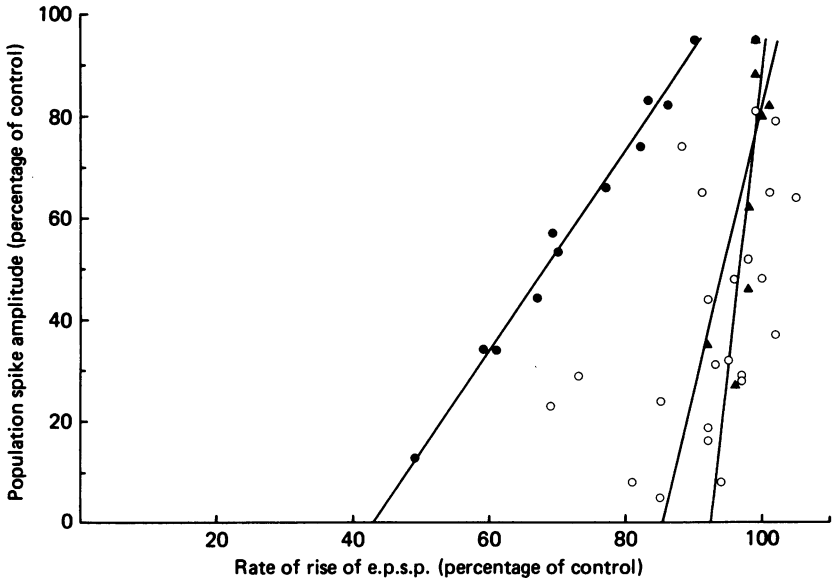

Fig. 2. Relationships of population spikes to rate of rise of e.p.s.p.s for tissue superfused in control medium (10 mM-glucose, ●; obtained by Method 1, $n = 11$), medium containing 1 mM-glucose (○; Method 2, $n = 24$), or medium containing 10 mM-fructose (▲; Method 2, $n = 8$).

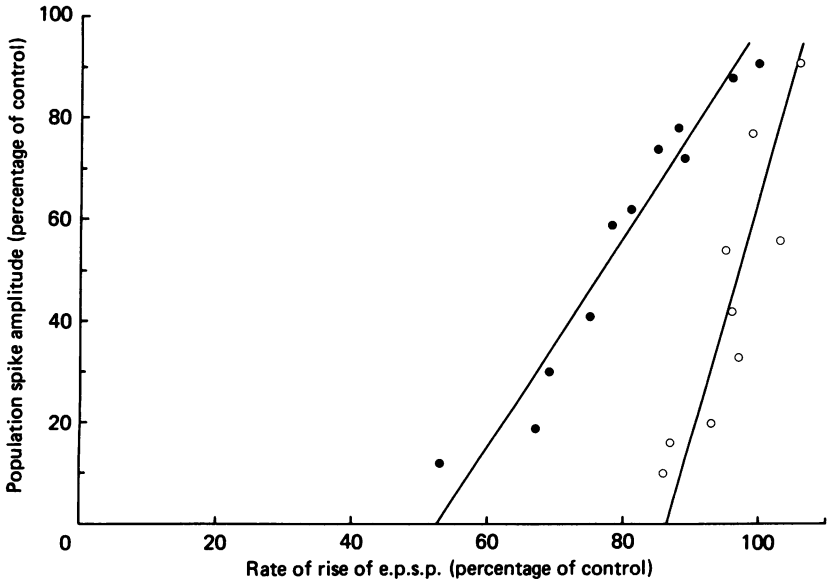

Fig. 3. Relationships of population spikes to rates of rise of e.p.s.p.s for tissue superfused in control medium (10 mM-glucose, ●; Method 1, $n = 11$), or medium containing 15 mM-5-thioglucose plus 5 mM-glucose (○; Method 2, $n = 9$).

DISCUSSION

Effect of lowered glucose

It has been shown elsewhere using the non-invasive technique of ^{31}P nuclear magnetic resonance that the tissue energy state is remarkably resistant *in vitro* to low glucose availability (Cox *et al.* 1983). In the present experiments, 0·1 mM-glucose was able to support normal levels of ATP, phosphocreatine and K^+ in the hippocampal slice yet glucose concentrations in excess of 2 mM were required for the maintenance of normal evoked dentate granule cell activity. This *in vitro* observation is consistent with data obtained *in vivo* from moderately hypoglycaemic animals where the cerebral energy state is unaffected at a time when the animals are exhibiting abnormal behaviour and a modified electroencephalogram (Hinzen & Müller, 1971; Ferrendelli & Chang, 1973; Horton *et al.* 1973; Lewis *et al.* 1974).

In the work presented here, the threshold concentration of glucose below which attentuation of field potentials occurred was between 2 and 5 mM, which is somewhat lower than previously reported (Cox & Bachelard, 1982). This may be due to differences in the temperature of *pre*-incubation (36·5 °C in the present experiments and 30 °C in the previous work) since the evoked activity is known to be temperature sensitive (Fujii & Yoshizaki, 1976).

Fructose as alternative substrate

Though fructose cannot replace glucose as a cerebral substrate *in vivo* due to its limited access to the brain from the bloodstream, it is known to be able to replace glucose *in vitro*. McIlwain (1953) showed that considerably higher concentrations of fructose than glucose were required to maintain normal rates of oxygen uptake *in vitro*. Fructose is phosphorylated in the brain, with a K_m value some 200 times higher than that for glucose (Sols & Crane, 1954) and thus if present at a sufficient concentration can act as a satisfactory precursor for cerebral glycolysis. In our hands, 20 mM-fructose maintained a normal energy state and, to a considerable extent, normal evoked activity. This sugar is the only substrate other than glucose we have tested so far that has been able to support normal or nearly normal evoked activity. Neither pyruvate plus malate nor 3-hydroxybutyrate was able to maintain field potentials in the dentate gyrus, although the energy state of the tissue was apparently normal (Bachelard & Cox, 1981, 1983). It is perhaps significant that whereas pyruvate plus malate and 3-hydroxybutyrate are mitochondrial substrates, and therefore do not produce ATP by substrate level phosphorylation in the cytoplasm, fructose can enter glycolysis before the stage of glycolytic production of ATP. Thus, glycolytically produced ATP may be important in maintaining evoked activity in the dentate gyrus, perhaps through replenishing a cytoplasmic pool of ATP close to the plasma membrane ATPases involved in restoring ion gradients following excitation (Scott & Nicholls, 1980; Nicholls & Åkerman, 1981).

Glucose analogues

Both 3-*O*-methylglucose and 2-deoxyglucose compete *in vitro* with glucose for transport across the membranes of cerebral tissues (Bachelard, 1971). 3-*O*-methylglucose is not metabolized further (Csáky & Wilson, 1956), but 2-deoxyglucose

is phosphorylated by hexokinase at the same maximum rate and with a higher K_m than that of glucose (Sols & Crane, 1954; Bachelard, Clark & Thompson, 1971). Unlike glucose-6-phosphate which inhibits its own formation by hexokinase, 2-deoxyglucose-6-phosphate does not, but will inhibit hexosephosphate isomerase at high concentration. Deoxyglucose-6-phosphate is not significantly metabolized further under these conditions (Horton *et al.* 1973; Bachelard, 1976). 5-Thioglucose enters the brain, but its further metabolism is uncertain. In other tissues it is converted to the 1-phosphate which strongly inhibits phosphoglucomutase (Chen & Whistler, 1975; Betz *et al.* 1975). Since the metabolism of other glucose analogues (3-*O*-methylglucose and 2-deoxyglucose) is similar in the various tissues studied, it is reasonable to assume that the cerebral metabolism of 5-thioglucose will also be similar to that in the systems (yeast and muscle) where it has been studied. If so, its action would be expected to be on glycogen metabolism.

In simple terms, the efflux of lactate from superfused tissues reflects the excess of glycolytic flux over pyruvate oxidation. Thus, under conditions where glucose availability is limited, or glycolytic flux is inhibited, lactate efflux would be expected to decrease. 5-Thioglucose strongly inhibited lactate efflux (Table 3) at a time when evoked activity was considerably attenuated. 2-Deoxyglucose was less effective than thioglucose at decreasing lactate efflux, and it was only during re-superfusion with deoxyglucose-free medium that the degree of inhibition of lactate efflux approached that observed *in the presence* of thioglucose (Table 3). Perhaps significantly, it was during the period of greatest decrease of lactate efflux *following* deoxyglucose superfusion that evoked activity was attenuated. Indeed, 3-*O*-methylglucose which is not metabolized and had no effect on evoked activity, also had no apparent effect on lactate efflux. These observations are consistent with some special role for glycolysis in maintaining normal evoked activity.

The relative lethargy of recovery of evoked activity following 5-thioglucose and 2-deoxyglucose superfusions may reflect some long-term effect on glycolytic flux. This same mechanism might underlie the delayed effect of 2-deoxyglucose on evoked activity. From the discussion above it would appear that the phosphorylated intermediates of 2-deoxyglucose and 5-thioglucose are mainly responsible for inhibition of glucose metabolism. While the unesterified sugar analogues presumably freely enter and leave cerebral tissue on the glucose carrier, the phosphorylated intermediates would be unable to leave the tissue, a characteristic utilized in the 2-deoxyglucose method of measuring regional cerebral metabolism (Sokoloff, Reivich, Kennedy, Des Rosiers, Patlak, Pettigrew, Sakwada & Shinohara, 1977).

Sensitivity of granule cell discharge

In every condition investigated here: low glucose, fructose, 5-thioglucose with glucose, or *subsequent* to superfusion with 2-deoxyglucose plus glucose, the granule cell population spike was considerably more sensitive to low glucose than was the population e.p.s.p. For *varying* stimulus strengths in control media, the population spike threshold occurred at an e.p.s.p. value less than 50% of its maximal value. In contrast, in slices superfused with media containing fructose, low glucose, or glucose plus 5-thioglucose and subjected to *constant* stimulus strengths, the population spike threshold occurred at an e.p.s.p. which was 69–92% of control. In the slices superfused

with analogue-free medium *after* exposure to 2-deoxyglucose, the population spike appeared to decrease without any attenuation of the e.p.s.p.

The steep relationship between e.p.s.p. and population spike, such that small decreases in the former are associated with large attenuations of the latter, has been noted previously (Richards & White, 1975). The threshold e.p.s.p. values reported here are consistent with equivalent figures derived from the data of Richards & White (1975), which suggest that in normal media the population spike threshold occurs at an e.p.s.p. of about 50% of its maximal value. The discharge of granule cells, represented by the population spike, is a post-synaptic process. Thus there is evidence that this post-synaptic component of the evoked activity is particularly sensitive to the metabolic perturbations of these experiments. The e.p.s.p. represents the near simultaneous depolarization of a population of granule cell dendrites, and in that sense is a post-synaptic process. However, the size of e.p.s.p. is dependent, at least in part, on the amount of transmitter released from presynaptic nerve endings. Thus the e.p.s.p. is a representation of both pre- and post-synaptic processes. While the data presented here indicate at least one specific post-synaptic effect in deficient media, where the e.p.s.p. is attenuated as did occur in some of the experiments, a presynaptic effect cannot be ruled out.

Taken together the data reported here would seem to suggest that some early intermediate in, or product of, non-oxidative carbohydrate metabolism is important in maintaining granule cell excitability.

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