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AVOIDANCE LEARNING UNDER HYPO AND HYPERGLYCEMIA IN RATS

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Abstract: Learning behaviour under different glycaemic conditions were studied in albino rats using an avoidance box. When insulin and glucose levels were low after fasting, animals showed delay in avoidance learning. But there was no change in acquisition of learning after hypoglacaemia induced by insulin. This difference in behaviour under hypoglycaemia of almost similar severity is possibly due to difference in its rate of induction and activation of counter regulatory neuro-endocrine mechanisms.

Diabetic (alloxan) rats failed to improve learning. Besides, hyperglycemia, other factors like metabolic disturbances, cytotoxic effects of alloxan may have inhibited learning in this group.

Hypo or hyperglycemia disturb the function of neuronal substrates responsible for learning and memory.

Key words: hypoglycemia avoidance learning hyperglycemia experimental diabetes

INTRODUCTION

Variations in blood or extracellular glucose level is reported to influence cortical functions (1, 2). Most of the cerebral structures do not require insulin for utilization of glucose from surrounding extracellular fluid. In conditions like fasting when insulin and glucose levels in the blood are low, glucose present is mostly utilized by the brain because other tissues use less glucose in the absence or at low levels of insulin. Similarly hypoglycemia following insulin administration compromises availability of glucose as a fuel for brain cells.

There is evidence to indicate that hyperglycemia of diabetes mellitus can also adversely affect brain function (3). A number of reports (4, 5, 6) provide evidence of impairment of cortical functions after acute fluctuations in blood glucose levels in insulin dependent diabetes (IDDM). These studies in IDDM, assessed performance of a learned behaviour or speed of learning a new task.

Taking such reports into account, it is pertinent to consider the effects of repeated glycaemic changes on behaviour. Such glycaemic changes commonly occur in IDDM patients receiving insulin regularly.

In view of its clinical significance, the present study investigates avoidance behaviour in rats under hypoglycemic and hyperglycemic conditions.

METHODS

Adult male albino rats (n = 36) weighing 140-230 gms were assigned randomly to following 4 groups.

Group I: (n = 9) Rats were allowed food and water ad lib and served as normoglycemic controls.

Group II: (n = 9) In these animals, hypoglycemia was produced by food deprivation for 16 hrs (water *ad lib*).

Group III: (n = 9) In this group Hypoglycemia was induced by injecting insulin (4 units/100 gm sc) 30 min before the behavioural study. The dose of insulin was adjusted by a pilot study to achieve

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a hypoglycemia of the same severity as in group II.

Group IV: (n = 9) The rats were made diabetic by administration of alloxan monohydrate (12 mg/100 g in 0.9% saline ip) and diabetes was confirmed by glycosuria and hyperglycaemia exceeding 3 to 3.5 times that of controls.

Avoidance behaviour: The animals were familiarised to the avoidance box for 15 min in the morning for 2 days prior to the experiments. An avoidance box (Takaki & Co, Japan) consisting of two compartments each measuring 40 cm × 20 cm × 12 cm was used for this study. After placing the animal in the left compartment, the trial commenced with presentation of a light signal (C. S) of 0.5 sec provided by low tungsten filament bulb, followed by a foot shock (UCS) 1 mA for 0.5 sec delivered through the floor made up of stainless steel bars spaced 1 cm apart. Interstimulus interval was kept at 5 sec. Movement of the rat to the other chamber immediately after CS to avoid UCS indicated the learning of avoidance behaviour and was designated as a successful trial. Shifting of rat to the chamber after UCS or random movements were not taken into consideration.

One session for each animal consisting of five trials was completed between 10-12 hrs and was repeated on consecutive days. The intertrial interval was approximately 1-2 min.

The experiment was terminated when the animal reached a 80% criterion (4 successive trials out of five) or after the 8th day of the study.

Blood glucose estimation: Three animals from each of the above groups were exclusively used for blood glucose estimation. Tail blood samples were collected at 9.30 a.m. for Group I, II, IV.

For Group III, blood sample was collected at 10 a.m. after 30 min of insulin injection. Blood glucose was estimated with the help of Glucometer (Ames). Glycosuria was confirmed by Benedict's method.

RESULTS

As depicted in Fig. 1, insulin-induced hypoglycemia did not change the acquisition of avoidance learning as compared to the normoglycemic group. However, the animals with fasting hypoglycemia showed a significant delay in attaining the criterion of learning, indicating that the learning was slowed down.

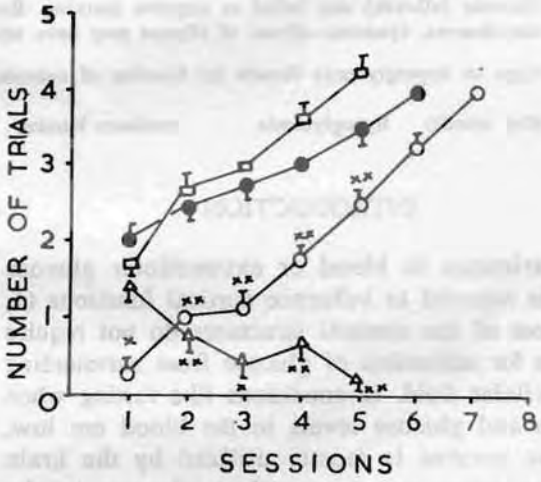

Fig. 1: Showing the acquisition of avoidance behaviour in normoglycemic n = 6, (□ - □); fasting hypoglycemic n = 6, (O-O) ; insulin induced hypoglycemic n = 6, (●-●) ;diabetic hyperglycemic n = 6, (Δ-Δ) rats.

Each value is mean (± SEM) of successful trials in each group per session conducted on consecutive days. For diabetic group, trials were started on 4th day after alloxan administration. X $P<0.005$ and XX <0.001 level of significance compared to corresponding values of normoglycemic rats. Other values are not significant.

In the alloxan group, there was no increment in the number of successful avoidance trials even after 9th day of administration of alloxan.

As shown in Table I the hypoglycemia of the fasting and the insulin groups was almost of the same severity. Alloxan treated animals exhibited hyperglycemia and glycosuria on 4th day of

alloxan administration. They showed 40% mortality. There were no death following insulin administration.

TABLE I : Blood glucose levels in mg/dl, mean ± SD.

	Normoglycemic (*n* = 3)	*Fasting hypoglycemic* (*n* = 3)	*Insulin hypoglycemic* (*n* = 3)	*Diabetic* (*n* = 3)
Blood Glucose	62 ± 2	37.7 ± 2*	35 ± 0.5*	242 ± 5*

* P<0.001 compared with normoglycemia.

DISCUSSION

Restriction of the number of trials to five and limiting each session to about 15 min were essential to prevent further aggravation of hypoglycaemia, especially in the insulin group.

Previous reports (4, 5, 6) in IDDM subjects, where glycaemia was controlled by glucose-insulin infusions, suggest that hypoglycaemia may affect cortical functions and delay learning. In our study, insulin induced hypoglycaemia did not change the avoidance behaviour.

It is not clear whether counter-regulatory endocrine mechanisms (7) decrease the adverse effect of hypoglycaemia on cortical functions. After prolonged fasting these mechanisms may not mobilise energy substrates, since the energy stores are low, but rapid induction of hypoglycaemia by insulin may be effective in mobilising energy substrates and raising peripheral blood glucose level (7). It is however suggested that neuronal functions under hypoglycaemia may be independent of the peripheral changes triggered by counter-regulatory hormones. It is also reported that counterregulatory mechanisms decline after prolonged hypoglycaemia (7). Hyperinsulinaemia may facilitate glucose transport across blood-brain barrier (8, 9, 10) and this increase in brain glucose may be beneficial in rats receiving insulin. Adaptation to hypoglycaemia, demonstrated in earlier studies (7), may be favoured by high insulin levels. In these experiments, precautions were taken to complete the behavioural study before the animals developed a severe fall in blood glucose during which the behaviour may have been entirely different. It was observed that blood glucose level declined to 10-15 mg/dL at the end of 45-60 min. after insulin injection.

Neurones with functional differences may vary in their sensitivity to the duration or rate of induction of hypoglycaemia (4, 11). Such functional and regional variations may determine the speed of learning. Memory processes may be inhibited severely in the face of non-availability of a metabolic substrate like glucose after fasting. This can be one of the reasons for prolongation of learning activity.

Peripheral blood glucose levels are considered to reflect the glucose concentration in neuronal environment. Therefore, whether prolonged fasting alone permits sufficient time to achieve equilibrium in glucose concentration at the centre and periphery can not be overlooked in the analysis of these results.

Drastic effects on behaviour in alloxan diabetes can be explained on the basis of diabetic disturbances. These rats were responding to UCS\ but showed progressive deterioration in learning with duration of diabetes. Therefore, untoward effects on behaviour in this group may be due to metabolic distur- bances (12), prolongation of reaction time (13) and cytotoxic effects of alloxan (12). In mice, passive avoidance was affected following experimental diabetes (14).

Alloxan and fasting group showed similarity in respect of lower levels of insulin during behavioural study. In addition to the glucose availability, whether insulin or insulin dependant neuro-endocrine process facilitate learning needs further exploration.

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