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Glucose intolerance in thiamine-deficient rats

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The mechanism of glucose intolerance in thiamine-deficient rats has been examined. Deficient rats showed marked glucose intolerance. However, the hypoglycaemic effect of insulin (1 i.u. kg^{-1}, i.p.) was similar in the deficient, pair-fed and normal groups, though somewhat weaker in the normal group than in the other two groups. After injection of tolbutamide (40 mg kg^{-1}, i.p.), the hypoglycaemic effects in the three groups were the same. Tyramine (10 mg kg^{-1}, s.c.) restored the impaired glucose tolerance of deficient rats to normal, but not that of alloxan diabetic rats. Furthermore, tyramine did not restore the intolerance of deficient rats pretreated with alloxan. These results suggest that the main factor causing glucose intolerance in the deficient rats may be suppressed insulin secretion.

Previously, we observed that thiamine-deficient rats showed alterations of adrenergic mechanisms (Iwata, Fujimoto & others, 1968; Iwata, Nishikawa & Fujimoto, 1969; Iwata, Watanabe & others, 1969; Iwata, Nishikawa & Watanabe, 1969; Iwata, Nishikawa & Baba, 1970; Iwata & Nishikawa, 1970; Iwata, 1972). In these reports, we suggested a relation between depressed adrenergic mechanisms and disturbance of nervous function in the deficient rats.

On the other hand, it has been reported that deficient rats show decreased tolerance to dextrose (Lepkovsky, Clarence & Evans, 1930; Pachman, 1941), and that the concentration of insulin-like substance in the serum of deficient mice is abnormally low (Machida, 1956). But the mechanism of these disturbances is not clear. Furthermore, it is well known that adrenergic mechanisms are involved in the regulation of insulin secretion from the pancreas. Accordingly, the mechanism of glucose intolerance in deficient rats was investigated.

MATERIALS AND METHODS

The animals and the methods used to obtain thiamine-deficient, pair-fed and control rats were reported previously (Iwata & others, 1968). Deficient rats were used when the heart rate was reduced to below 70% of the normal rate (about 350 beats min^{-1}). Alloxan diabetic rats were obtained by withholding food from rats, ~250 g, for 2 days, alloxan (160 mg kg^{-1}, i.p.) was given on the second day. The rats were used 3 days later when the blood glucose level was over 400 mg dl^{-1}. With deficient rats, food was removed when the heart rate was about 420 beats min^{-1}, these animals were treated with alloxan in the same way as normal rats. The heart rate of these animals before decapitation was about 300 beats min^{-1}. The acute mortality rates of alloxan diabetic and alloxan + thiamine-deficient rats were 11 and 21%, respectively. The concentration of blood glucose was measured by the anthrone method. Glucose tolerance was measured as the change in the blood glucose level after intraperitoneal

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administration of 2 g kg^{-1} of glucose. Statistical significance was calculated using Student's *t*-test. Tolbutamide was suspended in 0·5% carboxymethylcellulose. Insulin, tyramine and alloxan were dissolved in 0·9% NaCl.

RESULTS

Glucose tolerance in thiamine-deficient rats

The glucose tolerance test was performed on normal, control, pair-fed and thiamine-deficient rats. A specific change of the blood glucose curve was seen in deficient rats, as shown in Fig. 1. In these animals the maximum level was about 300 mg dl^{-1} and the level remained high for about 30 min and did not return to the initial level within 3 h. Other groups did not exhibit any significant increase of the blood glucose level except for controls which showed a slight increase 30 min after glucose injection.

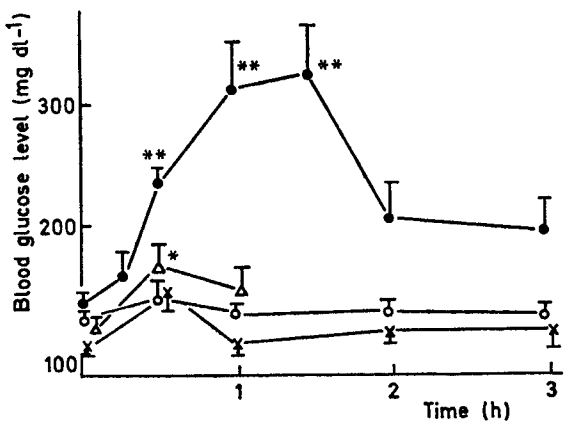

FIG. 1. Glucose tolerance of normal (○——○), pair-fed (×——×), control (△——△) and thiamine-deficient rats (●——●). Glucose (2 g kg^{-1}, i.p.) was loaded at 0 time. Each point represents the mean (± s.e.) of 6 to 10 observations. The statistical significance is calculated with respect to the corresponding value at 0 time. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$.

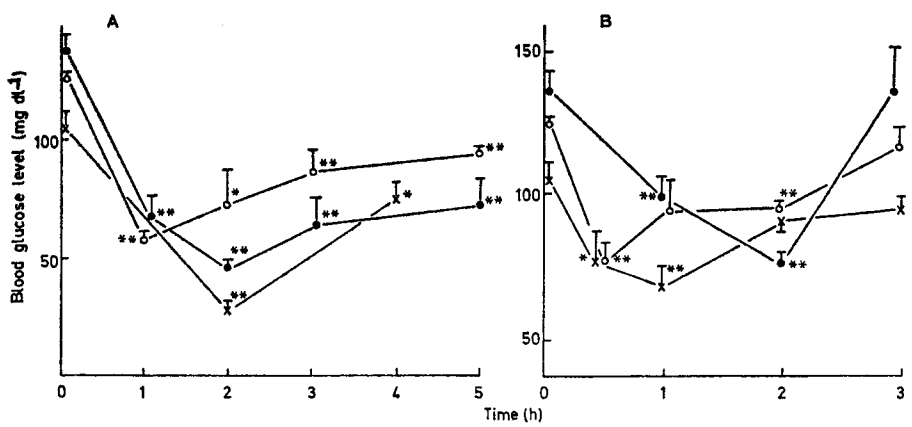

FIG. 2A. Effect of insulin (1 i.u. kg^{-1}, i.p.) and B. tolbutamide (40 mg kg^{-1}, i.p.) on the blood glucose level of normal (○——○), pair-fed (×——×) and thiamine-deficient rats (●——●). Each point represents the mean (± s.e.) of 4 to 6 observations. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$.

Hypoglycaemic effects of insulin and tolbutamide in thiamine-deficient rats

Next, to test the insulin response of deficient rats, the blood glucose level was examined after insulin injection (1 i.u. kg^{-1}, i.p.) (Fig. 2A). The action of insulin in deficient rats was similar to that in pair-fed rats, though it was somewhat less in normal rats than in the other two groups. To investigate the hypoglycaemic action of endogenous insulin in these animals, the effect of tolbutamide was examined (Fig. 2B). After tolbutamide injection (40 mg kg^{-1}, i.p.) the blood glucose level reached a minimum level in normal and pair-fed rats within 30 to 60 min, while in the deficient rats, the minimum level was only reached after 2 h, though it was the same as in the other two groups.

Effect of tyramine on glucose intolerance in thiamine-deficient and alloxan diabetic rats

Tyramine (10 mg kg^{-1}, s.c.) did not cause any change in the basal glucose level in deficient or normal rats after 3 h. Moreover, it did not cause any change in the glucose tolerance of normal rats but it restored the impaired glucose tolerance of deficient rats (Fig. 3).

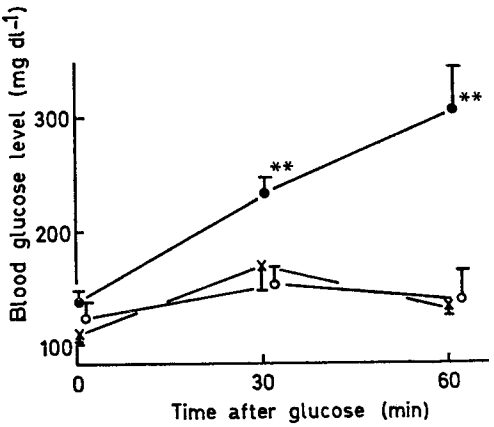

FIG. 3. Effect of tyramine on the glucose tolerance of thiamine-deficient rats. Thiamine-deficient (●——●), thiamine-deficient + tyramine (○——○), normal + tyramine (×——×). Tyramine (10 mg kg^{-1}) was administered 3 h before glucose. Each point represents the mean of 5 observations. $*P < 0.05$.

Table 1. *Effect of tyramine on glucose tolerance of alloxan diabetic rats.*

Time after glucose	Blood glucose level (mg dl^{-1})	
	0 min	30 min
Normal		
Untreated	125 ± 2	141 ± 16
Alloxan	455 ± 41	750 ± 58**
Alloxan + tyramine	432 ± 39	605 ± 37**
Thiamine-deficient		
Untreated	137 ± 7	235 ± 14**
Alloxan	479 ± 41	622 ± 52*
Alloxan + tyramine	410 ± 47	667 ± 87*

Tyramine (10 mg kg^{-1}) was administered 3 h before glucose. Alloxan diabetic rats were prepared as described in the methods. Each value represents the mean (±s.e.) of 4 observations. Statistically significant differences between values at 0 and 30 min at levels of $P < 0.05$ and $P < 0.01$ are indicated by * and **, respectively.

Alloxan diabetic rats exhibited a high level of blood glucose and marked glucose intolerance, which was not restored by tyramine (Table 1). As shown in Fig. 3, tyramine restored the impaired glucose tolerance of deficient rats but this restoration was no longer observed when the deficient rats had been treated with alloxan.

DISCUSSION

Thiamine-deficient rats showed a characteristic blood glucose curve in the glucose tolerance test, with a high peak level, delay in the time of the peak and slow recovery to the initial level. This indicates that they have remarkably impaired glucose tolerance. This observation agrees with those of others (Lepkovsky & others, 1930; Pachman, 1941). These workers reported that the poor glucose tolerance of deficient rats was observed irrespective of whether the sugar was given orally, intravenously or intraperitoneally. This eliminates the possibility of impaired intestinal absorption of sugar in these animals.

Our result showing that hypoglycaemic actions of insulin in deficient rats were similar to those in other groups suggested that in deficient rats the sensitivity of target organs to insulin is the same as in the other two groups but that insulin release from the pancreas is disturbed. This postulation was supported by the experiment with tolbutamide, which is known to cause insulin secretion (Coore & Randle, 1964). Furthermore, the fact that thiamine deficiency had no further effect in impaired glucose tolerance in diabetic rats may also support this postulation. In deficient rats which had been treated with alloxan, tyramine did not restore the impaired glucose tolerance. However, as thiamine deficiency had no further action in impaired glucose tolerance in alloxan diabetic rats, the implication of this result is not clear.

Previously we have shown sympathetic tone is to be depressed in thiamine-deficient rats (Iwata & others, 1970). Tyramine was found to improve the bradycardia in such rats and this action may be mediated through release of catecholamines (Iwata, Watanabe & others, 1969). In the present work we have found tyramine to improve the glucose tolerance of deficient rats. It is possible that this effect of tyramine is due to some action in improving insulin secretion or increasing the effectiveness of endogenous insulin. These may be direct actions of tyramine or secondary to the effect of the drug in correcting the impairment of sympathetic nerve function. It is possible that improvement of the efficiency of an impaired blood circulation, for example, due to correction of bradycardia, may explain the beneficial effects of tyramine in thiamine-deficient rats.

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