HEMODYNAMIC AND METABOLIC EFFECTS OF 2-DEOXY-D-GLUCOSE IN CONSCIOUS WISTAR RATS

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Recently 2-deoxy-D-glucose (2DG) has been extensively used to create neuroglycopenia in a study of reactions induced by it. 2DG is a competitive inhibitor of glycolysis, for like glucose, it passes through the blood-brain barrier and competes with it for hexokinase. The 2DG-6-phosphate thus formed inhibits glucose-6-phosphate metabolism. This results in the development of intracellular glycopenia of the CNS [6]. Impulses from glucose-sensitive brain zones then pass to the medullary layer of the adrenals, and catecholamines, mainly adrenalin, are released [9, 11]. One result of this is activation of glycogenolysis in the liver, accompanied by an increase in the blood glucose and insulin concentrations [12]. Meanwhile changes in the hemodynamic parameters are observed: blood pressure falls and bradycardia develops [3].

However, there is no information in the literature on metabolic changes taking place in different organs during the long-term action of 2DG.

The aim of this investigation was to study the effect of 2DG as a stress-inducing factor on changes in hemodynamic parameters and metabolism of conscious animals.

EXPERIMENTAL METHOD

Under pentobarbital anesthesia (40 mg/kg) a polyethylene catheter was implanted into the abdominal aorta of male Wistar rats weighing 350-450 g through the femoral artery. The end of the catheter was exteriorized in the interscapular region. After 24-48 h the animals' blood pressure and heart rate were measured on an electromanometer ("Siemens Elema," Sweden) on a "Harvard Apparatus Biograf 2120" recorder before and after systemic injection of 2DG in a dose of 250 mg/kg. A blood sample was taken a definite time after injection of 2DG (from 15 min to 6 h), for determination of catecholamines, glucose, lactate, and immunoreactive insulin. The animals were then killed by intraarterial injection of air. The brain and heart were homogenized in liquid nitrogen and proteins were precipitated with perchloric acid [15]. Altogether six series of experiments were carried out: the first series corresponded to the initial state, and in the subsequent series biochemical parameters were determined 15 and 40 min and 2, 4, and 6 h after injection of 2DG. Catecholamine concentrations were determined by HPLC with electrochemical detection [1]. Insulin was investigated by RIA (using a kit from the "Radiopreparat" Uzbek Research Institute of Scientific and Technical Information). The samples were counted on a "Compugamma model 1282" gamma-counter (LKB-Wallac, Finland). Glucose and lactate levels in the blood plasma and extracts were determined by enzymic methods [5, 8]. The results were subjected to statistical analysis on the Labtam/3015 computer, using Student's t test. The results are given in the form $M \pm m$.

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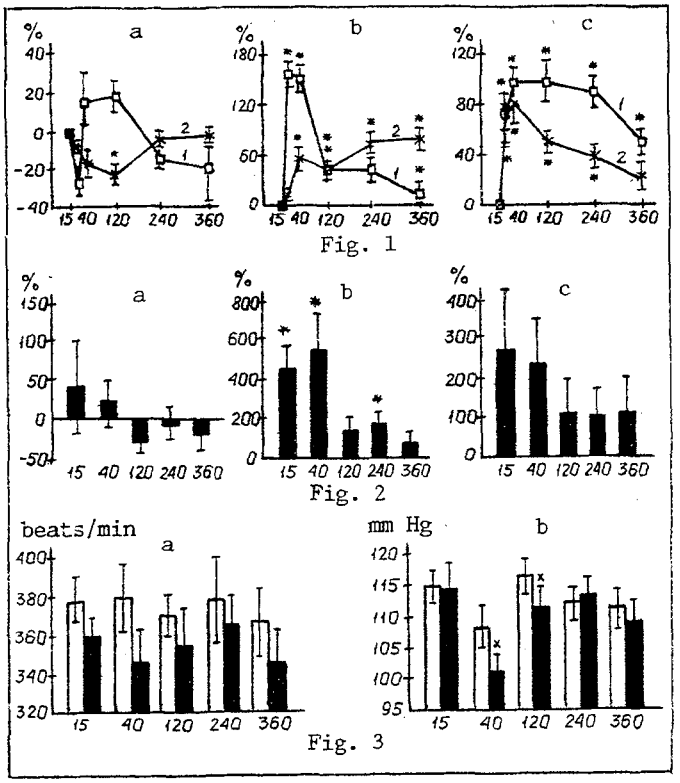

Fig. 1. Effect of 2-deoxy-D-glucose (250 mg/kg) on glucose and lactate levels in brain (a), heart (b), and blood plasma (c) in conscious Wistar rats (n = 6). Abscissa, time (in min); ordinate, percent relative to background; 1) time course of glucose level, 2) lactate; *p < 0.05 compared with background values.

Fig. 2. Changes in concentrations of noradrenalin (a), adrenalin (b), and insulin (c) in blood plasma (in percent of background level) in waking rats in response to injection of 2-deoxy-D-glucose (dose 250 mg/kg, n = 6). Abscissa, time (in min); ordinate, per cent relative to background; *p < 0.05 compared with background values.

Fig. 3. Changes in blood pressure (a) and heart rate (b) in response to injection of 2-deoxy-D-glucose (dose 250 mg/kg, n = 6). Abscissa, time (in min); ordinate: a) heart rate (in beats/min), b) blood pressure (in mm Hg). Unshaded columns – background values, black columns – after injection of 2DG; *p < 0.05 compared with background values.

EXPERIMENTAL RESULTS

Systemic administration of 2DG (250 mg/kg) into conscious rats caused the glucose level in the brain to fall at the 15th minute of the experiment, evidence of the development of intracellular glycopenia of the brain. At the 40th and 120th minutes of the experiment the brain glucose concentration was higher than initially, but later it fell again. The increase in the brain glucose concentration can evidently be explained by intensification of the cerebral blood flow [4] and by a sharp rise in the blood glucose concentration (Fig. 1c) after intracellular glycopenia of the brain. The subsequent fall of the glucose concentration can be attributed to washing out of the 2DG-6-phosphate from the brain cells and deinhibition of glycolysis.

Under the influence of 2DG the brain lactate concentration (Fig. 1a) fell, and reached a minimum of 25.2 ± 6.1% at the 120th minute. The lactate concentration then gradually was restored and by 6 h after injection of the glycolysis inhibitor it had almost completely regained the initial value. This character of the change in lactate levels may be connected with inhibition of glycolysis and subsequent restoration of the glycolytic flow in response to a fall in the intracellular 2DG-6-phosphate concentration [10, 13, 14].

The adrenalin concentration rose sharply (453.0 ± 130.7%, Fig. 2b) in the blood plasma in glycopenia of the CNS as early as 15 min after the beginning of the experiment. After 40 min the adrenalin concentration reached its maximal value (556.7 ± 214.9%). after which it fell to the initial level. The noradrenalin concentration was increased only at the beginning of the experiment.

These data are in agreement with an investigation [3] into the effect of 2DG on conscious animals in a dose of 500 μg/kg. Under the influence of this dose of 2DG the plasma adrenalin concentration was increased by 13-16 times, whereas the noradrenalin concentration was increased by only 1.9 times. The smaller change in the adrenalin and noradrenalin concentrations in the blood plasma in our experiments was probably connected with differences of dosage.

A raised adrenalin level causes intensification of glycogenolysis in the liver, and as a result, elevation of the blood glucose level [12]. In fact, it will be clear from Fig. 1c that the plasma glucose concentration was significantly raised at the 15th minute of the experiment (60.9 ± 14.3%). Maximal glucose concentrations were determined in the blood plasma at the 40th and 120th minutes – 98.5 ± 13.0 and 98.9 ± 16.6% respectively. After 4 h a fall of the glucose concentration was observed (91.2 ± 13.7%), although its level in the plasma remained higher than initially even 6 h after injection of 2DG (48.1 ± 9.6%).

The plasma lactate concentration increased significantly at the 15th minute (72.5 ± 19.6%). Its highest concentration occurred at the 40th minute of the experiment (81.1 ± 20.1%). Some decrease of the lactate concentration was observed 2 h later (49.8 ± 7.9%), but subsequently its level fell almost completely to its original value, possibly due to intensification of anaerobic glycolysis in the liver and muscles.

In response to the sharp rise of the blood glucose concentration the immunoreactive insulin level rose (Fig. 2c), and as a result the permeability of the cells of organs affected by glucose insufficiency was increased.

In the heart the glucose concentration rose sharply 15 min after injection of 2DG to 155.2 ± 17.6%. At the 40th minute a small decrease in its concentration was observed (149.4 ± 22.1%). Glucose accumulation in the heart in the early stages of the experiment could be associated: 1) with the increased intensity of transport into the cardiomyocytes as a result of an increase in its concentration and that of insulin in the blood; 2) with inhibition of glycolysis by 2DG-6-phosphate; and 3) with an increase in the blood flow in the heart, as the writers showed previously [4]. A sharp decrease in the glucose concentration in the heart was observed (41.3 ± 12.1%) 2 h after injection of 2DG. However, even 6 h after intracellular cerebral glycopenia the glucose concentration in heart muscle remained significantly higher than initially (17.5 ± 5.9%).

Under the influence of 2DG the lactate concentration in the heart rose steadily, and by 6 h it had reached its peak value (83.2 ± 15.7%). This may be caused by elevation of the adrenalin level in the heart [7] and the development of hypoxia as a result of inhibition of aerobic metabolism [2] and/or an increase in the work of the heart under the influence of 2DG [4].

Systemic injection of 2DG into conscious rats caused a significant fall of blood pressure (Fig. 3b) at the 40th minute of the experiment (−7.66 ± 1.99). Identical changes also were observed at the 120th minute (−5.13 ± 1.6). The heart rate under these circumstances showed only a tendency to diminish in all series of the experiment. When higher doses of 2DG (500 mg/kg) were used, the blood pressure fell and bradycardia developed [3].

Correlation between physiological processes and metabolic responses in our view is interesting. According to the results of our previous studies [4], in response to injection of the same dose of 2DG (250 mg/kg) there was a significant increase in regional blood flows in the brain, heart, liver, and adrenals; there was a tendency also for an increase in the pancreas. Thus the whole chain of 2DG – brain – adrenals – liver – pancreas – heart is involved to some degree or other in the response of the body to 2DG, in good agreement with the metabolic shifts we found.

A single injection of 2DG in a dose of 250 mg/kg causes prolonged changes in the systemic hemodynamics and metabolism and leads to considerable changes in the hormonal and metabolic status of the organism as a whole. These changes, being adaptive reactions of the body, are similar to reactions to stress.

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