REACTIONS OF THE SYSTEMIC AND REGIONAL HEMODYNAMICS TO METABOLIC STRESS INDUCED BY 2-DEOXYGLUCOSE

O. S. Medvedev, E. V. Oranovskaya, O. P. Ashirova, and A. N. Murashev

UDC 616.16-008.1-02:613.863]-07

KEY WORDS: stress, adrenalin, hemodynamics, adrenals

It is well known that one component of the response of the body to stress stimuli is activation of the adrenal glands followed by release of both corticosteroids and catecholamines into the blood stream [10]. Considering the important role of emotional stress in the pathogenesis of arterial hypertension, it is very interesting to study the role of a factor of humoral regulation of the cardiovascular system such as adrenalin in the reactions of the hemodynamics observed during stress.

Emotiogenic stimuli are incapable of inducing a sufficiently standardized release of adrenalin from the adrenals, for responses of animals to the same stimulus are highly individual. It is more promising to use preparations inducing sufficiently selective stimulation of the adrenal medulla in man and animals. The list of such substances includes insulin and 2-deoxy-D-glucose (2-DG) [2, 7].

In the investigation described below, adrenalin release from the adrenals was stimulated by 2-DG. The mechanism of action of 2-DG is based on competition with glucose for a place on the carbohydrate carrier through the blood-brain barrier (BBB) and through the neuron membrane, and on inhibition of the isomerase reaction predominantly in nerve tissue, causing the development of intracellular glycopenia in neurons of the CNS [14], and inhibiting glycolysis. Preferential accumulation of 2-DG in the brain is due to the fact that the rate of glucose consumption by the brain (about 780 nmoles/g tissue/min) is 10-15 times faster than the rate of its utilization in the skeletal muscles and most other organs [13]. Glucose-sensitive zones of the hypothalamus and medulla, descending influences from which potentiate activity in the adrenal nerve, are the most sensitive to the action of 2-DG [9, 12]. Neurogenic stimulation of the adrenal medulla leads to adrenalin release followed by an increase in the plasma glucose concentration [4].

Thus 2-DG stimulates adrenalin secretion from the adrenals through central activation of sympathetic efferent pathways, which is very close to the stimulation of the adrenals observed in emotional stress. Because of the absence of information in the literature on the hemodynamic consequences of injection of 2-DG, the aim of the investigation described below was to make a detailed study of responses of the cardiovascular system to acute administration of 2-DG to conscious animals.

EXPERIMENTAL METHOD

Experiments were carried out on conscious male Wistar rats weighing 280-350 g. Under pentobarbital anesthesia, 24 h before the experiment polyethylene catheters were introduced into the left ventricle and abdominal aorta of the rats. Responses of parameters of the systemic and regional hemodynamics were studied with the aid of isotope-labeled 15-μ microspheres (NEN, USA). Details of the method were described previously [1]. The suspension of microspheres was injected into the left ventricle, and arterial blood samples were taken from the abdominal aorta. The number of microspheres in samples of organs and tissues was determined on a "Compu-Gamma Model 1282" gamma-counter (LKB-Wallac, Finland).

Laboratory of Experimental Pharmacology, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Department of Biochemistry, Biophysics, and Physiology, N. G. Chernyshevskii Saratov University. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 132-135, February, 1991.

 0007-4888/91/0002-0148$12.50

TABLE 1. Changes in Parameters of Systemic Hemodynamics of Conscious Rats under the Influence of 2-DG (250 and 500 mg/kg, intraarterially)

Parameter	Initial value	Changes in % after injection of 2-DG	
		15 minute	40 minute
2-DG, 250 mg/kg (n = 15)			
Average blood pressure, mm Hg	110,2±6,7	4,3±3,7	5,7±4,3
Heart rate, beats/min	344±15	—6,8±3,2	—5,1±3,6
Cardiac index, ml/min/100 g body weight	36,7±2,1	14,9±11,0	7,73±8,2
Stroke volume, ml	39±03	20,5±5,0**	10,2±3,5
Total peripheral vascular resistance, mm Hg (ml/min/100 g)	3,15±0,31	—1,7±6,8	7,4±10,1
2-DG, 500 mg/kg (n = 10)			
Average blood pressure, mm Hg	97,5±4,8	—0,4±1,8	—2,3±1,0
Heart rate, beats/min	407±12	—14±2***	—6±3
Cardiac index, ml/min/100 g body weight	43,8±3,9	7,9±6,0	9,93±8,4
Stroke volume, ml	41±04	24,9±6,0**	17,4±8,5
Total peripheral vascular resistance, mm Hg (ml/min/100 g)	2,39±0,25	—5,0±5,3	—6,0±7,6

Legend. Here and in Tables 2 and 3: *$p < 0.05$, **$p < 0.01$ compared with initial level, taken as 100%.

TABLE 2. Changes in Parameters of Regional Hemodynamics in Conscious Rats Under the Influence of 2-DG (250 mg/kg, intraarterially, n = 15)

Parameter	Av. blood pressure, mm Hg	Changes in per cent after injection of 2-DG	
		15 minute	40 minute
Blood flow (in ml/min/g) in:			
skin	0,17±0,02	—6,8±16,3	—19,1±11,2
skeletal muscles	0,22±0,09	41,8±20,8	34,7±23,8
brain	1,43±0,10	59,0±17,6*	—3,3±4,3
small intestine	2,60±0,27	57,93±38,0	44,0±23,4
spleen	2,47±0,32	66,0±27,8*	120,6±52,8*
heart	7,20±0,71	54,3±17,9*	57,3±19,8*
kidneys	5,84±0,63	31,6±15,1	15,1±9,4
adrenals	11,18±3,55	42,8±14,2*	89,6±52,3

2-DG was injected intraarterially into 15 rats in a dose of 250 mg/kg and into 10 rats in a dose of 500 mg/kg. In each experiment the basic parameters of the hemodynamics were determined three times: before injection of 2-DG (basal level) and 15 and 40 min after injection of 2-DG. The 2-DG and pentobarbital (Nembutal) were obtained from "Serva," West Germany.

EXPERIMENTAL RESULTS

Parameters of the systemic hemodynamics (blood pressure, cardiac index) showed no significant change after injection of 2-DG in doses of 250 and 500 mg/kg (Table 1). Significant lowering of the heart rate from 407 ± 12 to 350 ± 13 beats/min ($p < 0.05$) and an increase in the stroke volume of blood from 410 ± 40 to 500 ± 50 μl ($p < 0.01$) were observed 15 min after injection of 2-DG in a dose of 500 mg/kg.

Relative changes in the velocity of the blood flow in several regions of the body were much more marked than changes in the parameters of the systemic hemodynamics (Tables 2 and 3). The group of organs in which the blood flow was increased included the brain, heart, adrenals, stomach, intestine, liver, and spleen. The blood flow was reduced in the skeletal muscles, skin, diaphragm, and testes of the animals. No significant changes in the blood flow were observed in the kidneys. The intensity of the changes in the blood flow in the organs and tissues in rats receiving 2-DG in a smaller dose (250 mg/kg) was less than the responses of the rats of the other group. Responses of the blood flow in the skeletal muscles were opposite in direction on the two groups of rats studied.

TABLE 3. Changes in Parameters of Regional Hemodynamics in Conscious Rats under the Influence of 2-DG (500 mg/kg, intraarterially, n = 10)

Parameter	Av. blood pressure, mm Hg	Changes in per cent after injection of 2-DG	
		15 minute	40 minute
Blood flow (in ml/min/g) in: skin	0,24±0,03	−13,2±9,3	−21,3±6,2*
skeletal muscles	0,23±0,05	−35,5±10,5	−38,8±14,5*
brain	1,98±0,18	105,3±16,2**	−11,8±8,3
small intestine	3,04±0,30	72,33±17,8*	84,8±18,6*
spleen	2,34±0,37	30,8±23,3	150,5±43,5*
heart	7,94±1,04	53,9±12,1*	92,5±21,8*
kidneys	6,74±0,62	6,0±7,5	23,9±13,8
adrenals	4,70±0,42	114,6±20,5**	131,0±18,6**

Taken as a whole the hemodynamic reactions in several regions in response to injection of 2-DG were very similar to those arising in animals and man in stress-inducing situations. For instance, an adrenalin-dependent increase in the cerebral blood flow takes place in immobilization stress [11]. Raised plasma adrenalin concentrations are one cause of dilatation of cerebral vessels [8], and an increase in the blood flow in different parts of the cortex, the globus pallidus, and ventral thalamus [17].

The combination of cardiovascular and endocrine responses to 2-DG arising in these experiments was very similar to those arising in response to insulin-induced hypoglycemia. In experiments on anesthetized [3] and conscious rats [5] lowering the plasma glucose concentration from 8.4-8.7 to 2.6-1.5 μmole/ml caused a two-threefold increase in the blood flow in the brain, whereas a lesser degree of hypoglycemia did not necessarily increase the cerebral blood flow [16].

Unlike the increase in the blood flow in the brain observed 30-90 min after injection of insulin [5], in the present experiments an increase in the blood flow was observed on the 15th minute after injection of 2-DG, whereas at the 40th minute the cerebral blood flow did not differ significantly from its initial value. The reasons for these differences are not yet clear. It can be postulated that the increased plasma glucose concentration found under the influence of high adrenalin concentrations [3, 7] can compete with 2-DG for the carrier through the BBB and neuron membranes, and can thereby reduce the glucose deficiency in the brain. The hypothermia developing after injection of 2-DG may be an additional factor reducing the brain's requirement of glucose [7].

The increase in blood flow in the adrenals by 2.1-2.3 times is in good agreement with data on stimulation of adrenalin secretion and increased activity in the adrenal nerve [2, 12]. It can be tentatively suggested that activation of the efferent fibers in the composition of the adrenal nerve does not cause vasoconstriction. This statement is confirmed also by the results of experiments [6] in which denervation of the adrenals led to reduction of the blood flow, i.e., to an increase in the vascular resistance.

Reduction of the blood flow in the skeletal muscles against the background of the action of a large dose of 2-DG was very unexpected, for it is widely believed that high concentrations of adrenalin can induce dilatation of muscular vessels through activation of postsynaptic $beta_2$-adrenoreceptors [15]. The increased vascular resistance in the skeletal muscles can be explained by the increase in activity in sympathetic vasoconstrictor nerves, demonstrated for injection of 2-DG in man [7] and in conscious rats [12]. In conclusion, it must be pointed out that responses of the systemic and regional hemodynamics induced by 2-DG (the dose-dependent increase in the stroke blood volume, the increase in the blood flow in the brain, heart, and adrenals) are very closely similar to emotiogenic changes in the hemodynamics. Taking into consideration previous data [12] on the ability of 2-DG to potentiate the flow of impulses in the adrenal nerve and to increase the adrenalin concentration in the plasma preferentially [2], there are good grounds for taking this substance to be a convenient pharmacological tool with which to simulate emotiogenic responses of the cardiovascular system.

LITERATURE CITED

1. O. S. Medvedev, A. N. Murashev, F. E. Meertsuk, and S. F. Dugin, Fiziol. Zh. SSR, **72**, No. 2, 253 (1986).
2. O. S. Medvedev, A. I. Kuz'min, G. Ya. Khulup, and O. B. Anosova, Byull. Éksp. Biol. Med., No. 2, 193 (1982).

3. A. Abdul-Rahman, C. D. Agardh, and B. K. Siesjo, Acta Physiol. Scand., **109**, 307 (1980).
4. R. Amann and F. Lembeck, Naunyn-Schmiederbergs Arch. Pharmakol., **339**, 71 (1986).
5. R. M. Bryan, B. R. Hollinger, K. A. Keefer, and R. B. Page, J. Cerebr. Blood Flow Metab., **7**, 96 (1987).
6. W. C. Engeland, M. P. Lilly, and D. S. Gann, Endocrinology, **117**, 1000 (1985).
7. J. Fagius and C. Berne, Am. J. Physiol., **256**, E714 (1989).
8. C. Iadecola, P. Lacombe, M. Underwood, et al., Acta Physiol. Scand., **127**, Suppl. 552, 74 (1986).
9. T. Katafuchi, Y. Oomura, A. Niijima, and H. Yoshimatsu, J. Autonom. Nerv. Syst., **13**, 81 (1985).
10. R. Kvetnansky, R. McCarty, N. B. Thoa, C. R. Lake, and I. J. Kopin, Am. J. Physiol., **236**, H457 (1979).
11. P. Lacombe, A. M. Reynier-Rebuffel, and J. Seylaz, Cerebral Blood Flow: Effects of Nerves and Neurotransmitters, Ed. by D. Heistad and M. Marcus, New York (1982), pp. 343-348.
12. O. S. Medvedev, M. Delle, and P. Thoren, Clin. Exp. Hypertens., **A10**, Suppl. 1, 375 (1988).
13. K. Meszaros, C. H. Lang, D. M. Hargrave, and J. J. Spitzer, J. Appl. Physiol., **67**, 1770 (1989).
14. L. P. Miller, J. B. Villeneuve, L. D. Braun, and W. H. Oldendorf, Stroke, **17**, 957 (1986).
15. M. P. Russel and N. C. Moran, Circulat. Res., **46**, 344 (1980).
16. F. E. Sieber, S. A. Derrer, C. D. Saudek, and R. J. Traystman, Am. J. Physiol., **256**, H697 (1989).
17. T. E. O. Sokrab and B. B. Johansson, Acta Physiol. Scand, **137**, 101 (1989).

EFFECT OF DIABETOGENIC AGENTS ON ZINC AND CALCIUM CONCENTRATIONS IN RABBIT PANCREATIC ISLET CELLS

E. D. Gol'dberg, V. A. Eshchenko, and V. D. Bovt

UDC 616.329-008.69

KEY WORDS: zinc, calcium, diabetes, pancreatic islets

Considering the important role of zinc and calcium in maintenance of the integral structure and function of cell membranes [4, 7], cytochemical studies of these metals in the pancreatic islets of animals receiving diabetogenic agents are interesting from the point of view of an explanation of the mechanism of the damaging action of these substances on insulin-producing cells. The present investigation was carried out by highly sensitive methods of determination of zinc and calcium in cells, developed by the writers [3].

EXPERIMENTAL METHOD

Experiments were carried out on 185 rabbits (intact and receiving single intravenous injections of the substances). Substances injected into the animals are widely used for the production of experimental diabetes [1, 2, 5]: dithisone, 8-(p-toluenesulfonylamino)-quinoline (8-TSQ), 8-(benzenesulfonylamino)-quinoline (8-BSQ) in doses of 40-50 mg/kg, and alloxan, in a dose of 100-200 mg/kg. In a separate group of investigations, rabbits received an injection of oxin in a dose of 50 mg/kg. This is an analog of 8-TSQ and 8-BSQ and, according to some workers [8, 10], it also exhibits diabetogenic properties.

The blood sugar level of the rabbits was determined by the Hagedorn Jensen method before and after injection of the substances. The animals were killed 5 days after the injection. Pieces of pancreas were used for fixation in Bouin's fluid, cold acetone, and 70 degrees alcohol, saturated with H_2S, and also to prepare frozen sections.

Research Institute of Pharmacology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR, Tomsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 135-137, February, 1991. Original article submitted June 20, 1990.

0007-4888/91/0002-0151$12.50