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USE OF 2-DEOXYGLUCOSE TO ANALYZE THE HUMORAL COMPONENT OF THE CARDIOVASCULAR RESPONSE TO STRESS

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Various types of stressors, inducing marked responses of the cardiovascular system, are widely used as models of neurogenic experimental hypertension [2]. One typical response of the endocrine system to stress is the release to catecholamines (mainly adrenalin) from the adrenals [7], which is accompanied by elevation of the blood glucose level [3]. The role of increased adrenalin concentrations in the realization of stress-induced circulatory reactions is not yet clear. The discovery of β-adrenoreceptors on sympathetic nerve endings provided a basis for the hypothesis that release of noradrenalin (NA) from endings can take place, and the hypertensive responses can thereby be increased, leading to the development of hypertension [8]. A gap in the experimental proof of the hypothesis is that elevation of the blood pressure (BP) takes place only after prolonged administration of exogenous adrenalin in large doses [13].

The aim of this investigation was to analyze the role of raised concentrations of endogenous adrenalin in the regulation of the cardiovascular system in waking animals. For this purpose a model of "metabolic stress," induced by 2-deoxyglucose (2-DG) was used. 2-DG and glucose utilized the same transport system during passage through the blood–brain barrier and nerve cell membranes [14]. Unlike glucose, 2-DG inhibits the isomerase reaction and induces intracellular glucopenia in the CNS, which is accompanied by activation of hypothalamic structures, by potentiation of the flow of impulses in the sympathetic nerve of the adrenals, and by adrenalin release from the adrenals [6, 11]. Thus under the influence of

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TABLE 1. Plasma Adrenalin and NA Concentrations (in mg/ml) in Rats before and after Intravenous Injection of 2-DG and Glucose in a Dose of 500 mg/kg

Substance determined	Initial level	Time after injection		
		15 min	40–50 min	24 h
		2-DG		
NA	0,483±0,068 ($n = 17$)	0,938±0,159* ($n = 7$)	0,713±0,116 ($n = 6$)	0,680±0,186 ($n = 4$)
Adrenalin	0,170±0,036	2,680±0,310**	2,360±0,270**	0,263±0,174
		Glucose		
NA	0,527±0,185 ($n = 5$)	—	0,373±0,086 ($n = 5$)	0,644±0,140 ($n = 4$)
Adrenalin	0,160±0,049	—	0,185±0,069	0,131±0,045

Legend. *p < 0.05, **p < 0.001 compared with initial level.

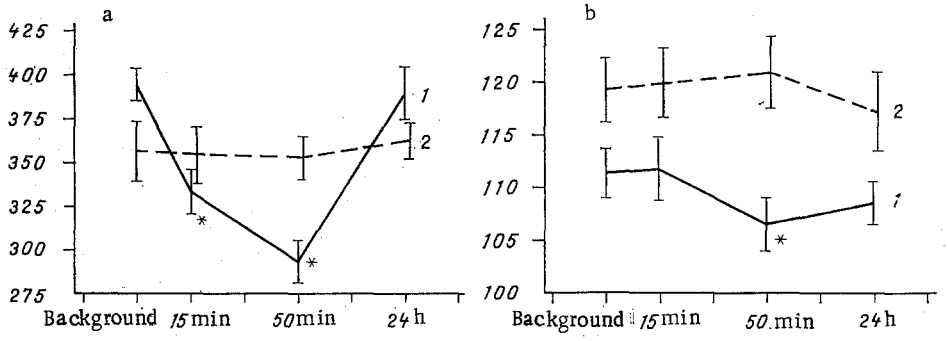

Fig. 1. Changes in BP and HR in conscious Wistar rats under the influence of intravenous injection of 500 mg/kg of 2-DG (1) or glucose (2). a) HR, beats/min; b) BP (mm Hg). *p < 0.05 compared with background.

2-DG, as in most types of stress, central activation of the adrenal medulla takes place, and it is this property of 2-DG which makes it a convenient tool with which to analyze the humoral mechanisms of realization of autonomic responses.

EXPERIMENTAL METHOD

Experiments were carried out on 21 conscious male Wistar rats weighing 300-350 g. Under pentobarbital anesthesia aortic (through the femoral artery) and venous (through the jugular vein) polyethylene catheters were implanted in the rats 2 days before the experiment. During the experiment, the BP of the unrestrained rats was measured by means of a Statham 23FD transducer and the heart rate (HR) was measured by a cardiotachometer, triggered by the pulse wave of BP. In the experiments of series I 1.5 ml of arterial blood was withdrawn before and 40-50 min after intravenous injection of 500 mg/kg of 2-DG (Merck, West Germany). Although taking even 3 ml of blood does not affect the blood catecholamine concentration of conscious rats [7], the volume of blood withdrawn was replaced by injection of an equal volume of blood from a conscious donor animal of the same line. Instead of 2-DG, the control animals were given an injection of the same dose of glucose. BP and HR were recorded continuously during the first 6 h, after which they were measured every 2 h for 24 h. In the experiments of series II the same volumes of blood were withdrawn before and 15 min after injection of 2-DG. After 40-50 min the animals were given an intravenous injection of atropine sulfate in a dose of 1 mg/kg to block vagal influences on the heart. In the course of the experiments BP and HR were recorded on a Grass 7D automatic writer (USA). To determine the adrenalin and NA concentrations, blood samples were centrifuged, 40 µl of a 5% solution of sodium metabisulfite was added to 0.7 ml of plasma and the samples were kept at −20°C. The plasma catecholamine levels were determined by high-performance liquid chromatography, using an electrochemical detector [1]. The results were subjected to statistical analysis by Student's t test (the results are given in the form M ± m).

EXPERIMENTAL RESULTS

Injection of 2-DG into conscious rats induced maximal responses of the cardiovascular system after 50 min. BP was lowered by 5 mm Hg and HR by 101 beats/min. The most likely cause of development of bradycardia was strengthening of vagal influences, for injection of atropine into animals with maximal bradycardia restored the normal HR.

Adrenalin and NA concentrations in the arterial blood plasma are given in Table 1. Background catecholamine concentrations in the experiments of series I and II were similar, and they are therefore pooled in Table 1. The data show that 15 min after injection of 2-DG the plasma adrenalin concentration rose by 15.8 times, whereas the NA level rose by only 1.9 times. In previous experiments [11] on anesthetized rats, release predominantly of adrenalin also was observed from the adrenals after systemic injection of the same dose of 2-DG. A similar response of the adrenals has been observed in various types of stress, especially immobilization stress [7]. Proof of the central genesis of the response of the adrenals to 2-DG is given by the fact that it could arise to central injection of such smaller doses of 2-DG (1-2 mg) [6, 10], and also by its disappearance after denervation of the adrenals [4].

The 13-16-fold increase in the plasma adrenalin concentration observed in the present experiments after injection of 2-DG is evidence of near-maximal activation of the adrenals. It was equal to or greater than that observed in the response of the adrenals to factors such as immobilization for 15 min [15] or immobilization in the supine position for 2.5 h [7]. It has been shown that 3 h after injection of 500 mg/kg of 2-DG the adrenalin concentration in the adrenals falls by two-thirds [3].

Continuous recording of BP and HR showed that the 13-16-fold increase in the plasma adrenalin concentration did not lead to any increase in these parameters. On the contrary, BP was significantly lower 40-60 min after injection of 2-DG, and HR fell by 101 beats/min. No change in BP and HR likewise was observed in other investigations [15] during a 30-min infusion of adrenalin into conscious rats at the rate of 400 mg/kg/ml, which created a blood concentration of 2110 pg/ml, i.e., close to that observed in the present experiments. Thus even a very considerable rise of the plasma adrenalin concentration in rats causes no change in HR. Man is evidently more sensitive to the action of adrenalin, for HR in man begins to rise in response to a two-five-fold increase to the adrenalin concentration [9]. The hypotensive response which coincides in time with a 15-16-fold increase in the plasma adrenalin concentration is evidently associated with the ability of adrenalin to activate vascular β-adrenoreceptors. An increase in the plasma adrenalin concentration in spontaneously hypertensive rats during immobilization in the supine position also leads to a fall of BP [7]. The increase in the adrenalin concentration during stress may perhaps participate in the response of increased cardiac output observed in Wistar rats during immobilization [2].

The most marked autonomic response to injection of 2-DG was the development of severe bradycardia (Fig. 1). The experiments of series II on seven rats showed that 40 min after injection of 2-DG HR fell from 391 ± 15.6 to 303 ± 19 beats/min (difference 88 ± 10; $p < 0.001$). After injection of atropine in a dose of 1 mg/kg HR rose to 397 ± 18 beats/min. The bradycardia was thus most probably attributable to central activation of vagal influences on the heart. Since glucose is the only substrate which can provide energy for CNS neurons [14], the hypoglycemia induced by 2-DG can induce the same responses of the autonomic nervous system as anoxia. In fact, anoxia induces bradycardia through activation of the cardiac fibers of the vagus nerve [12]. Activation of vagal influences on the heart through the action of 2-DG may be a factor preventing manifestation of the positive chronotropic action of adrenalin on the heart.

The results of this investigation are evidence against the participation of endogenous adrenalin in the triggering on enhancement of pressor responses to stress. We consider that in physiological concentrations adrenalin has a predomonantly hyperglycemic and cardiac-stimulating action (leading to increased cardiac output).

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ELEVATION OF BRAIN AND ADRENAL IMMUNOREACTIVE OPIOID PEPTIDE LEVELS IN RATS DURING ADAPTATION TO PHYSICAL EXERCISE

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It was shown previously that preliminary adaptation of animals to physical exercise prevents stress-induced disturbances of the contractile function of the heart [5, 8] and other stress-induced injuries [5]. It can be tentatively suggested that one step in the protective effect of this adaptation to stress is activation of the opioid peptide system during adaptation; these peptides constitute a regulatory system which can modulate the function of several neuroendocrine systems [1] and, in particular, it can limit activation of the stress-realizing adrenergic system [10, 14]. This hypothesis is supported by data showing elevation of the blood β-endorophin level in trained humans and animals [12], which is accompanied by increased resistance to pain.

The content of opioid peptides in different parts of the brain and in the adrenals was studied in the present investigation in rats adapted to physical exercise by swimming.

EXPERIMENTAL METHOD

Male Wistar rats weighing 300-350 g were used. Two groups of animals were investigated: 1) intact rats (control), 2) rats adapted to exercise. Adaptation was produced by making the animals swim unloaded 5 times a week for 7 weeks in water at a temperature of 32°C in a bath; the area of the water surface per animal was 400-440 cm^2. The rats swam for 15 min on the 1st day, 30 min on the 2nd day, 45 min on the 3rd day, and 1 h on the 4th and all subsequent days.

Endorphin and enkephalin concentrations were determined by radioimmunoassay in the cerebral cortex, cerebellum, corpus striatum, hypothalamus, and pituitary and adrenal glands.

The rats were killed by decapitation and the above-mentioned tissues were quickly removed and washed with physiological saline to remove blood, weighed, frozen without dealy in liquid nitrogen, and kept, until required for determination, at -70°C. Tissue extracts were obtained by the method of Rossier et al. [13] and their peptide content was determined by radioimmunoassay (RIA) by the method described previously [2, 9]. The specificity of the

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