Differential Activation of Sympathetic Nervous System and Release of Catecholamines during Neuroglycopenia in Awake Rats

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Neuroglycopenia induced by administration of 2-deoxy-D-glucose (125-375 mg/kg) is characterized by bradycardia and increased sympathetic activity, overall release of norepinephrine, plasma epinephrine, and norepinephrine at stable arterial pressure and norepinephrine plasmatic clearance. Sympathoneural activity is markedly enhanced in the adrenal-demedullated rats, while the levels of norepinephrine and its overall spill into circulation are the same as in the intact animals. It is concluded that neuroglycopenia is accompanied by differential activation of the sympathetic system subdivisions and selective release of norepinephrine by sympathetic terminals, but not by the adrenal medullae.

Key Words: *sympathetic nervous activity; catecholamines; labeled norepinephrine; hemodynamics; 2-deoxy-D-glucose*

The leading role in urgent mobilization of physiological functions and energy resources belongs to sympathoadrenal system, although reactions of neural and adrenomedullar parts on different kind of external stress or on variations of the internal medium are rather specific. Determinations of plasma catecholamines [14], which is often performed in clinical practice, does not obligatorily reflect the degree of activation of sympathoadrenal system, because plasma epinephrine and norepinephrine (NE) depend on the rate of their release by sympathetic terminals and adrenal medulla, as well as on the rate of their removal from plasma, i.e., clearance [6]. In this study activation of the neurohumoral component of stress reaction was simulated by administration of 2-deoxy-D-glucose (2-DG), which led to intracellular glycopenia mostly in the glucosensitive areas of hypothalamus and medulla oblongata [13]. It is known to be accompanied by activation of centrifugal influences to adrenal medullae [11] and, consequently, by increased epinephrine release with subsequent development of hyperglycemia [8], which also accompanies stress of different origins.

Our aim was to study the interactions between neural and humoral components of the sympatheticoadrenal system in experimental neuroglycopenia.

MATERIALS AND METHODS

The study was carried out on awake intact and adrenalectomized male Wistar rats. The rats were operated 2-3 weeks before experiments. In all experiments the first dose of 2-DG (Fluka) was 125 mg/kg intravenously, which was followed by additional 375 mg/kg after 15 min. The animals were preliminary implanted with an electrode to record sympathetic activity in the renal nerve, a catheter to directly measure arterial pressure (AP) and heart rate (HR) via abdominal aorta as well as to take blood samples, and the triple luminal catheter of original design to administer substances into jugular vein. The method of data recording was described elsewhere [2].

The overall activity of sympathetic nervous system was assessed with the help of dilution of tracer NE

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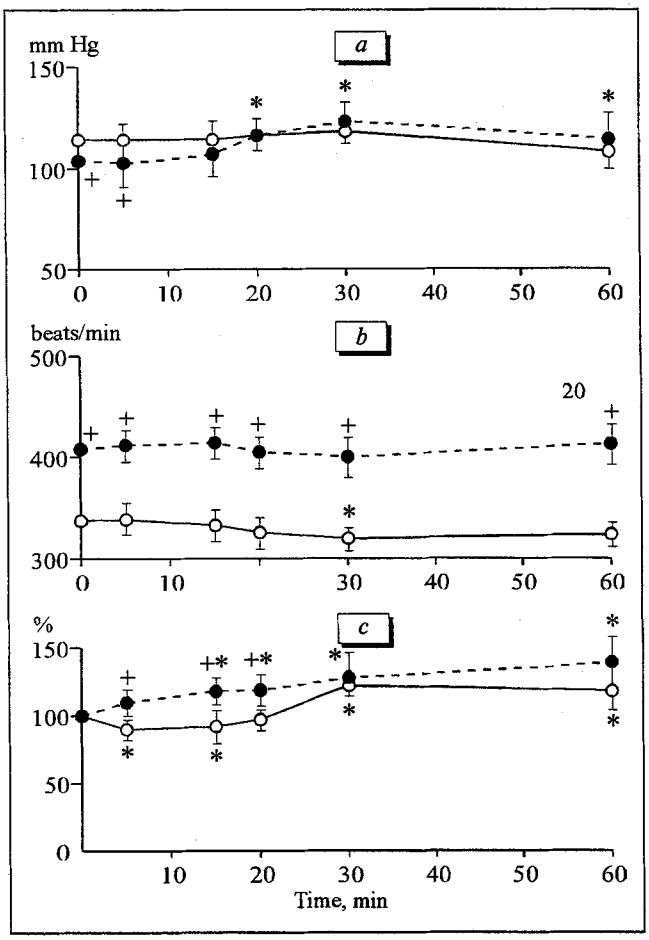

Fig. 1. Effect of adrenal demedullation on metabolic stress-evoked changes in arterial pressure (*a*), heart rate (*b*), and sympathetic neural activity in renal nerve (*c*) of awake normotensive rats. Solid line: intact rats (n=16), broken line: adrenalectomized rats (n=8). Here and in Fig. 2 and 3: $p<0.05$: *in comparison with initial level; +in comparison with intact animals.

in plasma [6] as the total spillover of NE into the bloodstream. The volume of plasma, which was cleared from NE per unit time, i.e., NE plasma clearance was determined by infusing labeled NE (^{3}H-NE, 56.9 Ci/mmol, Du Pont, New England Nuclear) in tracking amount (1.8×10^{-4} mmol/min). When ^{3}H-NE reached a plateau, arterial blood was taken, and the concentrations of endogenous NE and ^{3}H-NE were determined. The following values were calculated from the given formulas: clearance of NE = (^{3}H-NE rate of infusion)/[^{3}H-NE]; overall spillover of NE into the bloodstream = (^{3}H-NE rate of infusion)× [NE]/[^{3}H-NE]. Blood samples (0.75 ml) were taken on minutes 40 and 75 (before administration of the second dose of 2-DG), as well as on minutes 90 and 120 after onset of infusion. The volume of taken blood was substituted in each time by an equal volume of blood from awake intact or adrenalectomized donor rat. The concentrations of endogenous epinephrine and NE in plasma samples (0.5 ml) were determined by high-performance liquid chromatography with electrochemical detection [1]. In the moment of NE peak arising during chromatographic separation, the eluent was collected into a container with an OptiPhase MP scintillating cocktail (LKB). Specific activity of ^{3}H-NE (dpm) was measured in a 1215 Rack-beta II scintillation counter (LKB).

The data were statistically analyzed using ANOVA package for repeated measurements, with subsequent application of either Fischer test or nonparametric analog of variation analysis (Mann—Whitney test).

RESULTS

Neuroglycopenia was accompanied by a decrease in HR, while AP was stable in intact rats and enhanced in the adrenal-demedullated rats (Fig. 1). In contrast to intact rats, in which a small dose of 2-DG induced a significant decrease in the renal nerve sympathetic activity, this activity increased by 19% in adrenalectomized rats and persistently grew during 30 min, exceeding the initial level by 39% after administration of the second dose. In 15 min after administration of the first 2-DG dose, epinephrine increased by about 12 times, its maximum (41-fold) increase was observed 15 min after administration of the second dose; thereafter it did not vary throughout the entire experiment (Fig. 2). Neuroglycopenia did not affect NE clearance, so NE concentration changed in parallel to overall NE spillover into the bloodstream; both these parameters increased after administration of the first dose by 1.4 times, and after the second dose by 2.5-2.7 times.

As expected, plasma epinephrine in adrenalectomized rats did not exceed the limit of its chromatographic detection (0.02 ng/ml). Extirpation of adrenal medullae did not affect NE clearance. As in the control series, this parameter was not changed by 2-DG. Basal plasma NE and NE spillover in adrenalectomized rats were much higher than in rats with intact adrenal glands. These differences were maintained at any period after administration of 2-DG (Fig. 3).

Recording of AP and HR together with determination of plasma epinephrine showed that in normotensive rats a 40-fold increase in plasma epinephrine did not elevate these parameters. Moreover, bradycardia was observed after administration of 2-DG. The absence of AP and HR changes was described previously in the studies, where a drastic increase in plasma epinephrine (2.1 ng/ml, which is close to the value of 2.9 ng/ml in our experiments) was created in awake rats by a 30-min infusion [15].

Development of bradycardia was the most pronounced reaction to metabolic stress. As we previously demonstrated with metoprolol [3], 2-DG-induced bradycardia was mostly related to central activation of

vagal traffic to the heart rather than to a decrease in adrenergic cardiac stimulation. This may be a factor that prevents revealing the positive chronotropic affect of adrenaline. 2-DG elevates the mean filling pressure, which may cause an increase in cardiac output in normotensive rats due to enhanced venous return [12].

In our experiments, a decrease in sympathetic activity in the renal nerve after the first dose of 2-DG was accompanied by a small increase in plasma epinephrine, which can evoke vasodilation [9]. This is confirmed by the finding that 2-DG (125 mg/kg) produces a small decrease in venous tone [12]. The results of direct recording of the sympathetic nerve discharge agree qualitatively with biochemical measurements of the overall sympathetic activity. However, while the maximum elevation of renal nerve activity in response to metabolic stress was 23% against the initial level, the overall sympathetic activity increased by 170% according to the NE spillover measurements. Such a difference can be explained by regional and organ-specific differentiating of sympathetic activity. Indeed, infusion of sodium nitroprusside enhanced neural activity in the renal nerve of narcotized rats only by 90%, while the renal and total NE release into the bloodstream increased by 155 and 268%, respectively, and NE concentration in arterial blood increased by 326% [5].

The 40-fold increase in plasma epinephrine indicates almost maximum activation of the adrenal glands in this study, which exceeds that evoked by 2.5-hour immobilization [10]. The intensity of stress evoked by administration of 500 or 250 mg/kg 2-DG also exceeded by many times the reactions to emotional stress [4]. Only the reaction to 125 mg/kg 2-DG was comparable to that evoked by the known stressors.

The 2-DG-evoked elevation of plasma NE and epinephrine can be caused not only by activation of sympatheticoadrenal system, but also by a possible decrease in plasma clearance of catecholamines. Experiments with labeled NE used in this work demonstrated that administration of 2-DG to the awake rats did not affect NE clearance; therefore, 2-DG-evoked increase in plasma NE results only from its release by the nerve terminals and probably by chromaffin cells of the adrenal glands.

Extirpation of adrenal medullae resulted in a persistent elevation of plasma NE. Stability of NE clearance indicates that this effect reflects a compensatory enhancement of the overall activity of sympathetic system in response to disappearance of plasma epinephrine. Investigating the effect of general anesthesia on the plasma catecholamines in the adrenalectomized rats, the authors [7] have concluded that there is an extensive reciprocal interaction between neural and adrenomedullar subdivisions of the sympatheticoadrenal system. The absolute value of reaction of the sympathetic system to neuroglycopenia was larger in adrenalectomized rats than in the

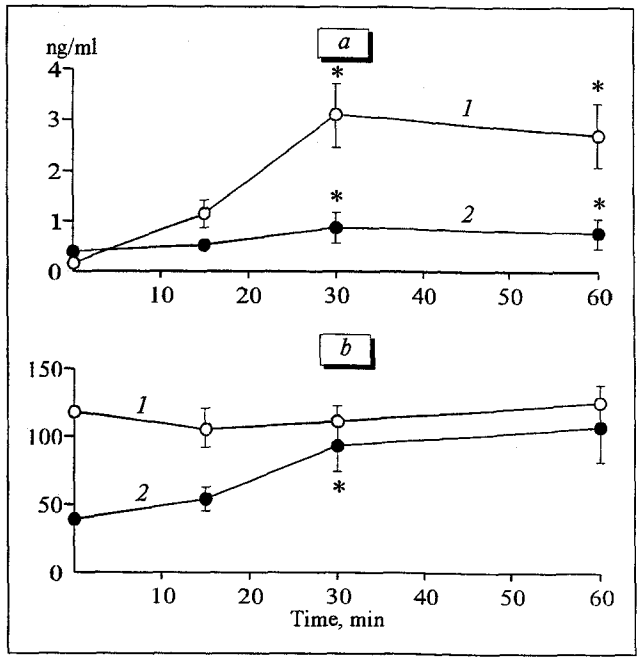

Fig. 2. Changes in concentrations of epinephrine (*a*, *1*) and norepinephrine (*a*, *2*), norepinephrine clearance (*b*, *1*, ml/min×kg), and overall norepinephrine spillover into the bloodstream (*b*, *2*, ng/min×kg) in response to administration of 2-deoxy-D-glucose to awake normotensive rats (n=11).

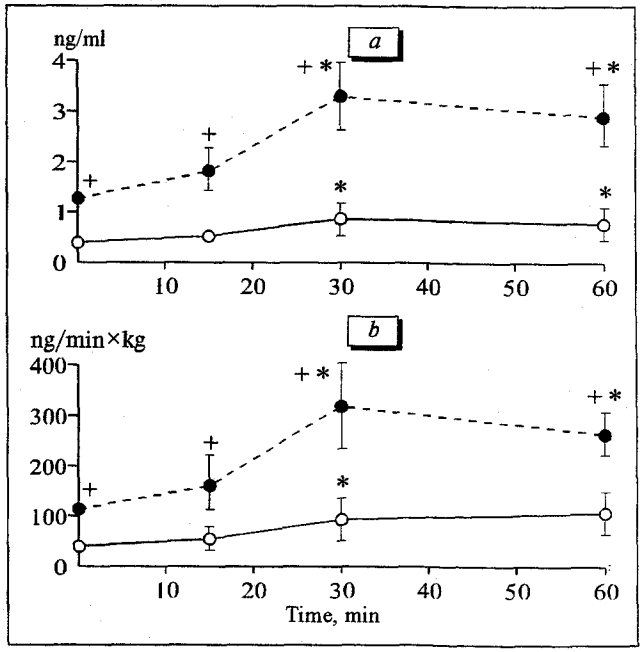

Fig. 3. The effect of adrenal demedullation on the metabolic stress-evoked changes in concentration of endogenous norepinephrine (*a*) and overall norepinephrine spillover into the bloodstream (*b*) in awake normotensive rats. Solid line: intact rats (n=11), broken line: adrenalectomized rats (n=8).

intact ones, but this difference virtually disappeared if the activity increase is assessed against the basal level. This reflects selective activation of NE release in response to the given stimulus by the sympathetic terminals, and not by the adrenal medullae.

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Nonopioid Nature of the Pressor Effect of FMRF-Amide

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The effect of the peptide FMRFa on arterial pressure, heart rate, and respiratory rate is examined in anesthetized rats. It is demonstrated that the effect of FMRFa is similar to that of epinephrine and is characterized by transient hypertension against the background of bradycardia and decreased respiratory rate followed by hypotensive phase. Opiate antagonists and agonists do not modify the effect of FMRFa. Pressor effect of FMRFa is inhibited by Aminazine and is abolished by dihydroergotamine, while clopheline, reserpine, propranolol, Dimedrol, and adrenalectomy have no appreciable effect on it. It is suggested that the effects of FMRFa are realized via vascular adrenoreceptors.

Key Words: *FMRFa; opioids; hypertension; adrenoreceptors*

The tetrapeptide Phe-Met-Arg-Phe-NH_2 (FMRFa) is a paraopioid [1] exhibiting pronounced therapeutic activity under conditions of clinical death and hypobaric hypoxia [2,7]. FMRFa produces hypertensive effects in intact rats [4,9]. The physiological mechanisms of these effects are obscure. In the present study we analyzed the possible mechanisms underlying cardiohemodynamic effects of FMRFa.

MATERIALS AND METHODS

Experiments were performed on 49 albino rats of both sexes (body weight 180-220 g) under Nembutal anesthesia. Before experiment, tracheotomy was per-

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