It can be concluded from these findings that changes in the electrical parameters of striated muscle fibers and, in particular, in the value of MP, in botulinus poisoning are evidently the result of weakening of neurotrophic influences on the skeletal muscle.

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RESISTANCE OF THE MYOCARDIUM TO ADRENALIN IN RATS ADAPTED TO HYPOXIA

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The intensity of the changes produced by adrenalin in the myocardium of rats adapted to hypoxia was studied after its administration to the intact animal and perfusion of the isolated heart. The changes were revealed by histochemical reactions for succinate dehydrogenase activity and staining for lipids. After intramuscular injection of a cardiotoxic dose of adrenalin (2.0 mg/kg) into adapted rats no damage to the myocardium was found, whereas perfusion of the isolated heart with adrenalin (20 µg/ml) caused the formation of micronecroses of the cardiocytes. However, their volume was statistically significantly smaller than in the isolated heart of intact rats under similar conditions. Differences in the sensitivity of the myocardium in vivo and in vitro indicate that the phenomenon of protection of the myocardium against the harmful effects of adrenalin in rats adapted to hypoxia is manifested at the level of the intact organism. The increase in the resistance of the myocardium itself is probably due to an increase in the power of the metabolic systems during adaptation.

KEY WORDS: *rat myocardium; adaptation to hypoxia; resistance to adrenalin; isolated heart.*

Adaptation to hypoxia, physical exertion, and other extremal environmental factors is accompanied by an increase in the nonspecific resistance of the organism to several pathogenic agents [2, 7]. In particular, preliminary adaptation to high-altitude hypoxia [7, 13]

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TABLE 1. Response of Isolated Hearts of Control and Adapted Rats to Anoxia

	Original indices: control (12)/ adaptation (10)	Anoxia (absolute values): control (7)/ adaptation (10)
P, mm Hg	60±8	—13±4
	68±7	—9±5
R, beats/min	295±13	—216±17
	293±13	—203±24
IFS	30,3±0,8	—23,9±1,3
	30,0±0,9	—22,0±1,4
Uptake of glucose, mm/g/min	5,4±0,5*	+5,1±0,7*
	3,4±0,6	+11,0±0,9
Excretion of lactate, mm/g/min	5,8±0,6*	16,0±1,2*
	3,7±0,5	+37,4±1,4

Legend. Number of hearts shown in parentheses. Statistically significant differences between experimental and control series marked by asterisk ($P < 0.05$).

or to physical exertion [6] has been shown to reduce the mortality of animals and the extent of necrotic damage to the heart and to prevent disturbances of cardiac activity following subsequent injection of large doses of sympathomimetics. These observations are interesting in the light of existing views on the role of hypoxia in the formation of catecholamine lesions in the myocardium [14]. However, it is not yet clear whether the factors responsible for tolerance of the myocardium to sympathomimetic drugs are to be found in the heart itself or whether they exist at the level of the intact organism. Hence the interest in the comparative study of the reaction of the myocardium in rats adapted to hypoxia to the harmful effect of adrenalin when injected in vivo and after perfusion of the isolated heart.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-220 g were exposed intermittently in a semi-airtight chamber for 5 h daily, six times a week for 3 weeks, to a gas mixture of O_2 and N_2 in which the oxygen concentration was lowered gradually from 15% on the first day of the experiment to a final working concentration of 8-9% on the sixth day of the experiment. The rats were investigated 18-20 h after the final training session. The criteria of adaptation were: 1) the resistance of the rats to deep hypoxia and, 2) the resistance of the isolated heart to anoxia. Resistance was determined in vivo from the periods of survival of the animals (eight rats) under the conditions of acute testing hypoxia (3-4% O_2). The response of the isolated heart to anoxia in vitro was assessed from indices of contractile activity: the systolic pressure in the left ventricle (P, mm Hg) and the heart rate (R, beats/min) and Meerson's index of the intensity of function of structures (IFS) (P•R/wet weight of the heart), and also from the activity of the glycolytic system: the uptake of glucose [5] and excretion of lactate [9]. Intact rats and their hearts served as the control.

Resistance to the harmful action of adrenalin was assessed as follows. Adrenalin was injected intramuscularly into the animals of group 1 (seven rats) in a dose of 2.0 mg/kg body weight. Seven intact rats receiving the same dose of adrenalin served as the control. The animals were decapitated 24 h later and the heart examined morphologically. In group 2 the degree of damage to the myocardium of the trained and intact rats (seven in each case) by adrenalin was compared after perfusion of their isolated hearts. In the control the hearts of experimental and intact rats were perfused without adrenalin. The hearts of all the animals in this group were studied morphologically.

The hearts were isolated and perfused by Langendorff's method with Krebs's solution in the modification of Neely et al. [11] under a pressure of 70 mm Hg, at a temperature of 37°C, and at pH 7.4. The solution was aerated with a mixture of 95% O_2 + 5% CO_2. To study the effect of anoxia, after perfusion for 10 min under normal conditions the heart was perfused

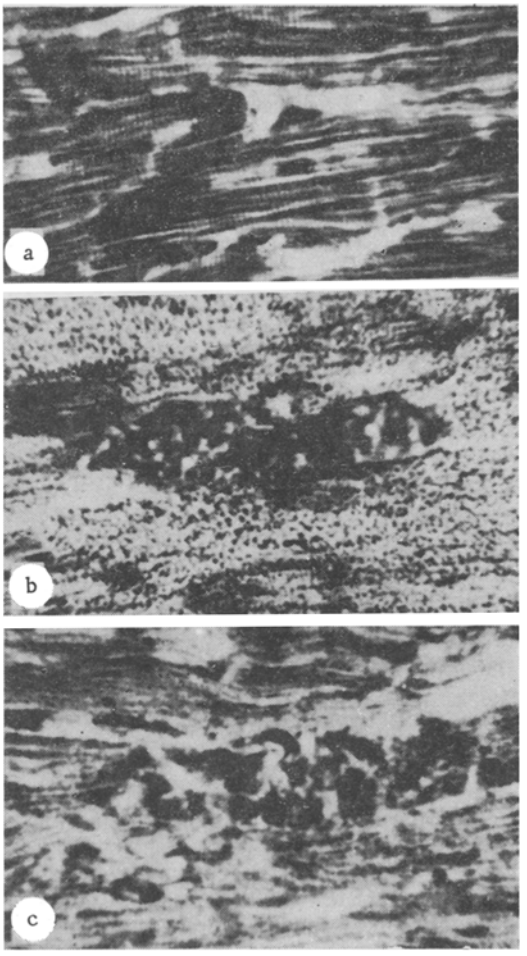

Fig. 1. Myocardium of rats after administration of adrenalin. Reaction for succinate dehydrogenase, 320 ×. a) Injection of adrenalin (2.0 mg/kg) into rats adapted to hypoxia: "linear" deposits of formazan predominate; b) injection of adrenalin (2.0 mg/kg) into intact rats: irreversibly damaged cell with variegated appearance in center, coarse-grained deposits of reaction products in adjacent myocytes; c) perfusion of isolated heart of rat adapted to hypoxia with adrenalin (20 μg/ml): injured cell with variegated appearance in center.

for 20 min under anoxic conditions (aeration of the Krebs's solution with a mixture of 95% N_2 + 5% CO_2). After perfusion for 5 min with the original solution (without recording the pressure in the left ventricle) the heart was perfused for 10 min with a solution containing adrenalin hydrochloride in a concentration of 20 μg/ml (calculated as the base), after which it was again perfused for 2 h with the original solution.

The heart was stopped in cold Krebs's solution, cut in half in the frontal plane, and frozen in liquid nitrogen; cryostat sections 10 μ thick were cut. To detect damage to the myocardium the reaction of Nachlas et al. [10] for succinate dehydrogenase (SD) activity was carried out with nitro-BT. Serial sections were stained for lipids with Oil red O. The degree of damage to the myocardium of the left ventricle was assessed by a quantitative stereometric method [1]. The numerical results were subjected to statistical analysis. The significance of differences between the mean values was determined by Fisher's criterion using the φ method.

EXPERIMENTAL RESULTS

Under conditions of acute testing hypoxia the experimental rats showed increased resistance to severe oxygen deficiency. Eight of the trained rats of this group survived 62 ± 9 min. The control rats died 36 ± 4 min after the beginning of exposure.

When the original indices of contractile activity of the isolated hearts of the experimental and control rats were compared no difference was found. Anoxia led to less marked inhibition, not statistically significant ($P > 0.05$), of the function of the isolated heart of the adapted rats than in the control. The original values of glucose uptake and lactate excretion by the hearts of the trained rats were significantly lower than in the control ($P < 0.05$), but under anoxic conditions the glucose uptake and lactate excretion by the hearts of the experimental animals rose much more, to reach values significantly higher than those in the control (Table 1).

Injection of adrenalin into the trained animals did not cause the appearance of lesions in their heart revealed by the reaction for SD (Fig. 1a). Staining for lipids was always negative. Conversely, injection of the same doses of adrenalin into intact rats produced marked changes in the pattern of formazan deposition in their myocardium. Foci of micronecrosis were formed, in which the myocytes acquired a distinctive "variegated" appearance because of the irregular distribution of formazan in their cytoplasm (Fig. 1b). More extensive changes also were observed, in the form of deposition of large polymorphic granules of diformazan (Fig. 1b), which was always combined with the presence of lipids in these cells.

Perfusion of the hearts of the trained animals with Krebs's solution caused no changes in the character of the histochemical reaction for the enzyme, whereas perfusion with adrenalin constantly led to the appearance of cells with the characteristic variegated appearance (Fig. 1c), similar to those described in necrotic foci in intact animals receiving adrenalin in vivo. However, neither coarse-grained deposits of formazan nor lipids could be observed in the myocardium of the isolated heart. The same result was observed after perfusion of the hearts of intact rats. However, on histostereometric assessment of the degree of injury, its volume in the heart of the trained rats was significantly less than in the intact ($6.6 \pm 0.02\%$ compared with $4.14 \pm 0.02\%$, $P < 0.001$).

The results are evidence of the development of a phenomenon of specific adaptation in the experimental animals, as is confirmed by the increase in the period of survival of the rats under conditions of acute testing hypoxia. At the level of the isolated heart the phenomenon of adaptation was characterized by relatively greater resistance to anoxia, and also by an increase in the functional reserves of the glycolytic system. Similar phenomena during adaptation of rats in a pressure chamber were also observed by other workers [3, 7, 12], who found a parallel increase in the power of systems of aerobic oxidation under these circumstances.

A fact of importance in principle is the absolute absence of any harmful effect of adrenalin when injected in vivo into the experimental animals, whereas in the isolated heart of rats adapted to hypoxia perfusion with adrenalin constantly caused the formation of micronecroses. This difference indicates that the phenomenon of protection of the myocardium against the damaging effect of adrenalin is exhibited at the level of the intact organism. Meanwhile the higher tolerance of the myocardium of the adapted rats to adrenalin than of the intact animals, revealed in the experiments with perfusion of the isolated heart, indicates that the prophylactic effect of training is manifested at the organ level also. The mechanisms of this protection are not clear. The possibility cannot be ruled out that one factor in protection at the organism level may be increased ability of the blood to bind catecholamines, as has been found in rats adapted to physical exertion [8]. The increase in the resistance of the myocardium itself was perhaps due to an increase in the power of its metabolic systems. On the one hand, an increase in the power of the glycolytic system, resulting in increased formation of lactate under anoxic conditions, correlates with the prophylactic effect of lactic acid against the cardiotoxic action of adrenalin described in [4]. On the other hand, the absence of fatty degeneration of the myocardium (24 h after injection of adrenalin into adapted rats), which is invariably found as a result of the action of increased concentrations of biogenic amines in the intact organism, could also be evidence of the more intensive catabolism of lipids mobilized by adrenalin.

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EFFECT OF PARASYMPATHETIC ACCELERATION ON BIOELECTRICAL ACTIVITY OF PACEMAKER CELLS OF THE DESYMPATHIZED AND RESERPINIZED FROG HEART

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The mechanisms of parasympathetic acceleration was studied in experiments on the heart of *Rana temporaria* after preliminary exhaustion of the catecholamine reserves by desympathization and reserpinization of the animals. Electrical activity of cells of the isolated pacemaker was recorded. During parasympathetic acceleration the rate of rise of slow diastolic depolarization was found to increase (evidence of the active mechanism of this acceleration), and this was accompanied by slight hyperpolarization and by shortening of the duration of the action potential. After treatment of the preparation with atropine the accelerating effect and the changes in shape of the action potential disappeared, confirming the cholinergic nature of the parasympathetic acceleration. It is suggested that acetylcholine, the mediator of the parasympathetic system, may reduce potassium or increase sodium permeability of the pacemaker cell membrane, thus increasing the rate of rise of slow diastolic depolarization and causing acceleration of discharges.

KEY WORDS: *desympathization; reserpinization; parasympathetic acceleration; atropine; action potential.*

Much experimental evidence has now been obtained of the ability of the parasympathetic system both to inhibit and to accelerate the heart beat [2, 3, 7, 8, 10]. It has been shown by pharmacological methods that both types of parasympathetic influences travel along cholinergic nerve pathways [1, 4].

In this investigation the mechanism of parasympathetic acceleration was studied on frogs' hearts after preliminary exhaustion of the catecholamine reserves by desympathization and treatment with reserpine.

EXPERIMENTAL METHOD

Experiments were carried out on a preparation of the isolated sinus of the heart of *Rana temporaria* with the extracardiac nerves running to it. The frogs were desympathized by bilateral extirpation of the sympathetic chain at the level of the second and third ganglia, after which the animals were kept at 11-14°C for 27-30 days. Reserpine (or Rausedil) was injected subcutaneously 2 days before the experiments in a dose of 50 μg/g. The catecholamine fluorescence of the heart is known to disappear almost completely under these circumstances [9]. The results of control experiments showed that stimulation of the sympathetic chain in frogs after reserpinization does not accelerate the cardiac rhythm.

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