DEPENDENCE OF ACTIVITY OF CHOLINERGIC MECHANISMS OF THE HEART ON THE STATE OF ITS SYMPATHETIC INNERVATION AND NORADRENALIN CONTENT IN HYPOXIA

N. K. Khitrov, A. I. Svistukhin, and E. B. Tezikov

UDC 612.173-06: [612.178.2+612.273.2

Removal of the inferior cervical and stellate ganglia abolishes the property of moderate hypoxia (7-8% O_2 in N_2) to increase the reserves of acetylcholine (ACh) and acetylcholine-like substances in the heart and leads to exhaustion of these reserves during deep hypoxia (3-4% O_2 in N_2). Under hypoxic conditions reduction of the noradrenaline (NA) reserves in the heart by reserpine weakens, whereas perfusion of the heart with Krebs–Henseleit solution containing NA, potentiates the action of ACh on the isolated heart. In hypoxia, exogenous NA raises the potassium level in the myocardium. It is suggested that NA stimulates cholinergic mechanisms and modified potassium metabolism in the myocardium. Exhaustion of the NA reserves in hypoxia leads to functional isolation of the heart not only from sympathetic, but also from parasympathetic influences.

KEY WORDS: hypoxia; heart; sympathetic and parasympathetic regulation; interaction.

Acetylcholine (ACh) inhibits the transport of noradrenaline (NA) from the terminals into the synaptic space [10] and cyclic AMP formation in the myocardium [9], as a result of which the response of the heart to sympathetic stimuli is weakened. There are few data on the influence of the sympathetic system on the cholinergic mechanisms of the heart, and the role of this interaction in nervous regulation of the myocardium in hypoxia has not been studied. Dependence of the ACh content in the heart and its action on the myocardium on the state of the sympathetic regulation during hypoxia was studied in the investigation described below.

EXPERIMENTAL METHOD

Forty male rabbits weighing 2.5-3.0 kg and 45 male albino rats weighing 180-200 g were used. In the experiments of series I the ACh concentration was determined in the tissues of the heart after bilateral removal of the inferior cervical and stellate ganglia (10-12 days before the investigation) in rabbits exposed to moderate and deep hypoxia in a gas mixture containing 7-8 and 3-4% O_2 in N_2. The action of exogenous ACh in increasing concentrations ($1 \cdot 10^{-9}$, $5 \cdot 10^{-9}$, and $1 \cdot 10^{-8}$ g/ml) on the hearts of rats isolated by Langendorf's method was investigated during hypoxic perfusion (Krebs' solution saturated with a gas mixture containing 30% O_2, 65% N_2, and 5% CO_2). In the experiments of series II hearts were used after preliminary reduction of their NA reserves by administration of reserpine to the rats (4 and 2 mg/kg Rausedil, intraperitoneally, 24 and 12 h before isolation of the hearts). In the experiments of series III, with the same reduced oxygenation, the isolated hearts were perfused for 10 min with Krebs–Henseleit solution containing NA (200 ng/ml of NA hydrotartrate, calculated as base), and the action of ACh was tested 5 min later. The effect of ACh on isolated hearts of intact rats during normal (95% O_2, 5% CO_2) and hypoxic perfusion was studied in the control.

General hypoxia was produced in rabbits and hypoxic perfusion of isolated rats' hearts with recording of the actual systolic pressure in the left ventricle and the cardiac rhythm, and with calculation of an index of integral cardiac function (Opie's index) were carried out by methods described in [4, 7]. To assess the effectiveness of procedures directed against sympathetic regulation the NA content was investigated in the hearts of animals of the various groups [3]. The concentration of free ACh [1], the content of free and reserve

Department of Pathological Physiology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 6, pp. 523-525, June, 1979. Original article submitted June 6, 1978.

 0007-4888/79/8706-0542$07.50

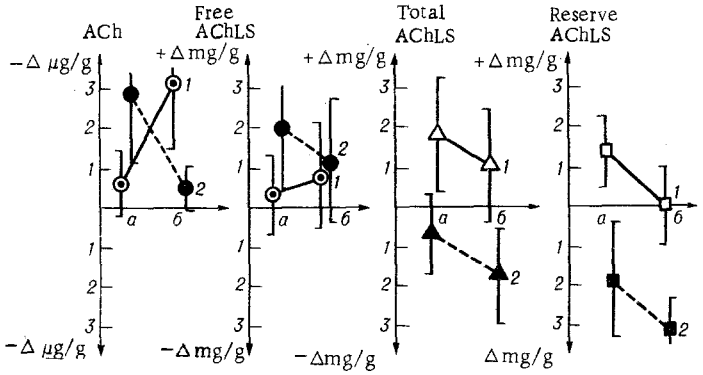

Fig. 1. Effect of sympathectomy on changes in content of free ACh and of various fractions of AChLS in heart tissue of rabbits during exogenous hypoxia: a) 7-8% O_2; b) 3-4% O_2. 1) Hypoxia (control); 2) sympathectomy + hypoxia. Ordinate, difference (Δ) between experimental and corresponding control indices.

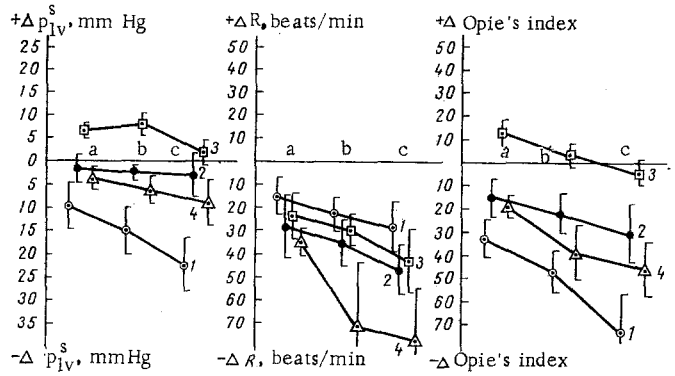

Fig. 2. Changes in actual systolic pressure in left ventricle (p^s_{lv}, cardiac frequency (S), and Opie's index for isolated rats' hearts under influence of different doses of exogenous ACh during hypoxia, depending on NA concentration in heart. ACh in doses of: a) $1 \cdot 10^{-9}$ g/ml, b) $5 \cdot 10^{-5}$ g/ml, ($1 \cdot 10^{-8}$) g/ml. 1) High oxygenation (95% O_2, 5% CO_2); 2) hypoxic perfusion (30% O_2, 65% N_2, 5% CO_2); 3) reserpine + hypoxic perfusion; 4) NA + hypoxic perfusion. Ordinate, difference (Δ) between experimental and corresponding control indices.

acetylcholine-like substances (AChLS) [8], and cholinesterase (ChE) activity were determined in the left ventricle of the rabbits' hearts.

EXPERIMENTAL RESULTS

The NA concentration in the rabbits' hearts 10-12 h after sympathectomy was reduced compared with the control (from 1.31 to 0.71 μg/g; $P < 0.05$). Moderate hypoxia (7-8% O_2) increased the concentrations of free ACh and AChLS in the control and sympathectomized rabbits, but the increase in their level in the heart after sympathectomy was considerable. In the sympathectomized animals moderate hypoxia caused a decrease in the AChLS reserves in the heart, and not an increase as in the control. The concentrations of free ACh and AChLS in the heart increased in the sympathectomized rabbits during deep hypoxia, but this increase, unlike in the control, was smaller than during moderate hypoxia. The content of stored AChLS in the heart of the sympathectomized animals was reduced by 80% during deep hypoxia, compared with only 30% in the control (Fig. 1). ChE activity in the myocardium of the control and sympathectomized animals at both levels of hypoxia fell to equal values. The increase in the content of free ACh and AChLS in the heart of the experimental rabbits was probably due to increased liberation of these substances from the depots rather than to inhibition of their hydrolysis. Exhaustion of the AChLS reserves stored in the heart of the sympathectomized animals, during deep hypoxia, was not associated with any increase in liberation of ACh and AChLS, evidence of their reduced formation.

Unlike in the control, during hypoxic perfusion of the heart the negative inotropic effect of ACh was not manifested over the whole range of its concentrations tested. On the contrary, the negative chronotropic effect of ACh on the heart was potentiated under these conditions. As a result of dissociation between the inotropic and chronotropic reactions of the heart, the index of integral myocardial function (Opie's index) fell under the influence of ACh by a lesser degree during hypoxic perfusion than under normal conditions. The decrease in the ACh reserves in the heart [6] and inhibition of the response of the heart to the parasympathetic mediator may lead to weakening of vagus nerve effects inhibiting myocardial activity. Similar changes take place in the sphere of sympathetic regulation during deep hypoxia [4, 7], suggesting functional isolation of the myocardium from regulatory nervous influences.

Reserpine caused a decrease (from 1.15 to 0.60 μg/g; $P < 0.05$), whereas perfusion of the isolated heart during hypoxia with Krebs–Henseleit solution containing NA caused an increase (from 0.66 to 0.97 μg/g; $P < 0.05$) in the concentration of sympathetic mediator in the heart. Reserpine weakened the negative inotropic action of ACh but did not change its rhythmic effect, so that Opie's index of cardiac function fell only slightly during hypoxia. Perfusion of the heart with NA under hypoxic conditions had little effect on the negative inotropic action of ACh, but its negative chronotropic action (especially in doses of $5 \cdot 10^{-9}$ and $1 \cdot 10^{-8}$ g/ml) was increased compared with the control. Correspondingly, under the influence of ACh Opie's index fell significantly (Fig. 2).

The ACh and AChLS content in the heart during hypoxia thus depends on the state of the extracardiac sympathetic innervation. Sympathectomy abolishes the ability of moderate hypoxia to increase the ACh and AChLS reserves in the heart and promotes their exhaustion in deep hypoxia. In hypoxia the intensity of the inhibitory effect of the parasympathetic mediator on cardiac activity is controlled by NA. The decrease in the NA reserves in the heart prevents, whereas an increase, on the contrary, facilitates manifestation of the inhibitory effect of ACh on the heart during hypoxic perfusion. ACh formation in cholinergic neurons depends on the potassium concentration in them [11]. An increase in the intracellular potassium concentration increases the resting potential of the postsynaptic membranes and potentiates the reaction of the myocardium to mediators [2]. During hypoxic perfusion of the heart, NA in a dose of 200 ng/ml increased the potassium concentration in the myocardium (from 7.5 to 9.5 mg/g dry tissue; $P < 0.05$), although during high oxygenation of the heart this effect of NA was not significant. The sympathetic innervation probably controls the state of the cholinergic mechanisms of the heart through the action of NA on potassium metabolism in the myocardium. Deep hypoxia causes functional isolation of the heart from nervous influences stimulating and inhibiting its activity. Functional isolation of the heart from parasympathetic influences may be connected with inhibition of activity of extracardiac sympathetic mechanisms.

LITERATURE CITED

1. K. B. Vinnitskaya, Lab. Delo, No. 2, 89 (1972).
2. B. Hoffman and P. Cranefield, Electrophysiology of the Heart [Russian translation], Moscow (1962).
3. V. V. Men'shikov, in: Investigation of the Functional State of the Adrenal Cortex and the Sympatho-Adrenal System under Clinical and Experimental Conditions [in Russian], Moscow (1963), p. 149.
4. V. P. Nuzhnyi, N. K. Khitrov, and A. M. Alaverdyan, Fiziol. Zh. SSSR, No. 4, 539 (1977).
5. V. A. Sveshnikov and E. Ya. Pekker, Lab. Delo, No. 6, 327 (1965).
6. N. K. Khitrov and A. A. Abinder, Probl. Éndokrinol., No. 6, 90 (1972).
7. N. K. Khitrov and A. I. Svistukhin, Patol. Fiziol., No. 2, 21 (1975).
8. S. Hestrin, J. Biol. Chem., 180, 249 (1949).
9. M. N. Levy, Circulat. Res., 29, 437 (1971).
10. K. Löffelholz and E. Muscholl, Arch. Pharmacol., 265, 1 (1969).
11. P. J. G. Mann, M. Tennenbaum, and J. H. Guastel, Biochem. J., 33, 822 (1939).