3. M. D. Gaevyi, Fiziol. Zh. SSSR, No. 3, 417 (1967).
4. M. I. Gurevich, S. A. Bernshtein, D. A. Golov, et al., Fiziol. Zh. SSSR, No. 3, 350 (1967).
5. L. N. Didiani and L. S. Andreeva, Patol. Fiziol., No. 3, 91 (1972).
6. L. G. Erokhina and L. N. Naumov, Zh. Nevropatol. Psikhiat., No. 3, 378 (1974).
7. G. I. Kositskii, Afferent Systems of the Heart [in Russian], Moscow (1975).
8. V. G. Krasil'nikov, Patol. Fiziol., No. 4, 15 (1974).
9. R. S. Mirzoyan, "Pharmacology of adrenergic regulation of the cerebral circulation," Author's Abstract of Doctoral Dissertation, Moscow (1977).
10. B. I. Tkachenko, S. A. Polenov, and A. K. Agnaev, Cardiovascular Reflexes [in Russian], Leningrad (1975).
11. L. A. Usov and L. G. Miller, Patol. Fiziol., No. 2, 40 (1978).
12. M. Sale, N. Hussain, and W. Lixfeld, Cir. Res., 47, 166 (1980).
13. L. Weaver, J. Auton, Nerv. Syst., 3, 253 (1981).
14. A. Vogt, F. Vetterlein, H. Ri, et al., Arch. Int. Pharmacodyn., 239, 86 (1979).

CHOLINERGIC MECHANISM OF REGULATION OF CARDIAC FUNCTION IN ACUTE TRANSIENT CORONARY INSUFFICIENCY

P. F. Litvitskii

UDC 616.132:2-008.64-036.11-07:616.12-008.1-092:616.127-008.94:577.175.822

KEY WORDS: cholinergic control mechanism; transient local myocardial ischemia.

Cholinergic regulation of cardiac activity plays an essential role in the maintenance of homeostasis during hypoxia. It does so, in particular, by reducing the oxygen demand of the myocardium and its utilization of high-energy phosphates and glycogen and by reducing the degree of disturbance of the transmembrane ion distribution [10]. Collectively these effects increase the resistance of the heart to energy-deficient states of varied genesis. The writer previously found that disturbance of the contractile function and rhythm of the heart in local myocardial ischemia (MI) of coronary genesis and during subsequent reperfusion (RP), takes place in stages which are accompanied by phasic changes in the functional state of the adrenergic control mechanism of cardiac activity [4, 6]. Depression of the parameters of cardiac contractility in the early stages of local MI and during postischemic RP under these circumstances was combined with maintenance of a high functional reserve of the myocardium, reflected in the pressure difference in the left ventricle at rest and during isometric contraction. This fact suggests that the reduction in the contractile function of the heart may be the result not only of regional hypoxic changes, but also of activation of controlling, mainly parasympathetic, influences on the myocardium.

It was accordingly decided to study the state and the role of the cholinergic control mechanism of the heart in acute transient coronary insufficiency (ATCI) of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred male albino rats weighing 200 ± 10 g, kept under animal house conditions on an ordinary diet. ATCI was produced by the method described previously [4, 5] under urethane anesthesia (1200 mg/kg) and with artificial ventilation of the lungs with atmospheric air. The duration of MI was 10, 40, or 120 min, and of subsequent RP 40 min. In the zone of MI and in the same zone during subdequent RP of the myocardium, and also in remote parts of the heart the acetylcholine (ACh) concentration was determined by a biological method on the dorsal muscle of the leech [1]; total and bound acetylcholine-like substances (ACLS) [3] and total cholinesterase (ChE) activity [7] also were determined. The response of the heart to ACh was assessed by the time course of the chronotropic reaction,

Department of Pathological Physiology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR F. I. Komarov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 94, No. 12, pp. 12-15, December, 1982. Original article submitted November 6, 1981.

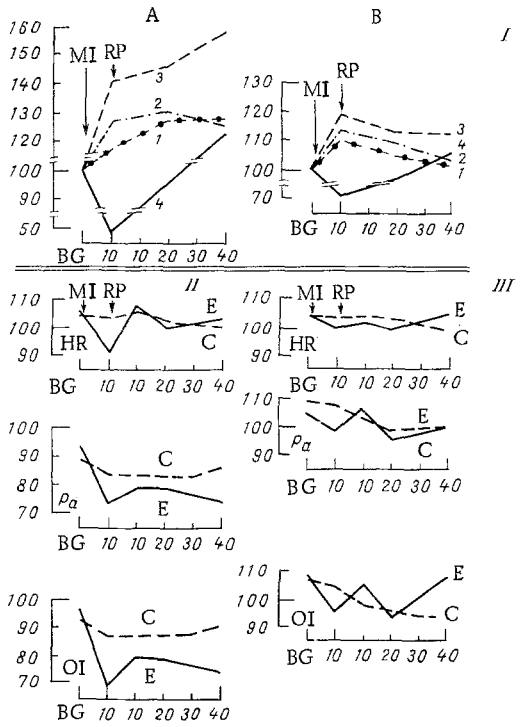

Fig. 1. Time course of concentrations of free ACh, free and bound ACLS, and ChE activity in myocardium (I) and response of heart to a standard dose of ACh (II and III) during ATCI with MI lasting 10 min. Abscissa, duration of periods of MI and RP (in min); ordinate: I) (in % of background) concentration of free ACh (1), of free ACLS (2), of bound ACLS (3), ChE activity (4); A) zone of MI and subsequent RP; B) parts of the heart remote from zone of MI and RP; II) response of heart to exogenous ACh 20 sec after its injection; III) 180 sec after injection. HR) Heart rate; P_a) pressure in left ventricle; OI) Opie's index; E) experiment; C) control; BG) Background. At each point n = 8.

changes in left intraventricular pressure, and Opie's index — an integral parameter of cardiac activity (the product of the heart rate and the pressure developed in the left ventricle) in response to injection of a standard dose of ACh (0.5 μg/kg) into the left ventricle at intervals of 10 min. The responses were recorded during the first 20 sec after injection of the neurotransmitter (the mean time of development of the maximal chronotropic response of the heart) and 180 sec after injection (the mean time of extinction of the response). The animals were divided into two groups: experimental (with the production of ATCI) and control — in which a mock operation only was performed (anesthesia, thoracotomy, artificial ventilation of the lungs, pericardiotomy, application of a ligature to the coronary artery without tying it).

EXPERIMENTAL RESULTS

The experiments showed that changes in the functional state of the cholinergic mechanism for the regulation of cardiac activity, at both the presynaptic and the postsynaptic level, operate in a series of regular stages.

The content of free ACh and ACLS in the myocardium in ATCI as a rule both changed in the same direction (Figs. 1-3, I). In the MI zone their concentration rose rapidly until the 10th minute, then fell a little but still remained above the background value until the 40th minute; later, until the 120th minute, MI fell progressively below the background value.

Restoration of the coronary blood flow after MI lasting 10 and 40 min was accompanied by a tendency for the content of free ACh and ACLS to return to normal in the previously ischemized zone of the heart, but by the 40th minute of RP it still remained above the background value. After 120 min of MI, however, RP was accompanied by a further decline in the

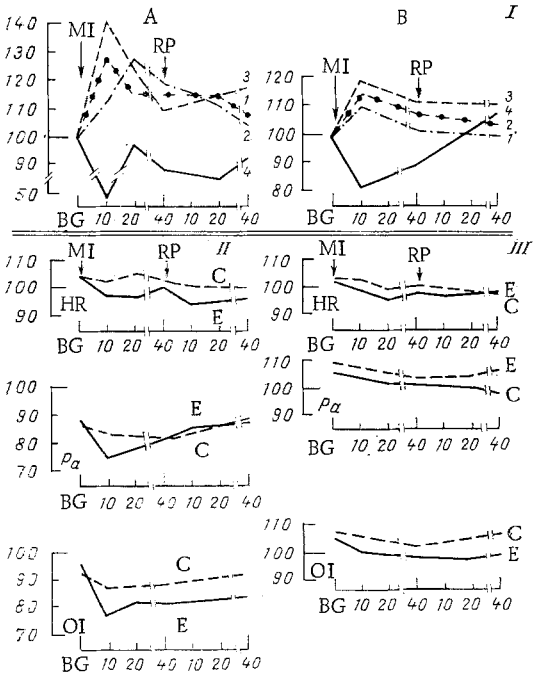

Fig. 2. Time course of concentration of free ACh, free and bound ACLS, ChE activity in myocardium (I), and response of the heart to a standard dose of ACh (II and III) during ATCI with MI lasting 40 min. Notation as in Fig. 1.

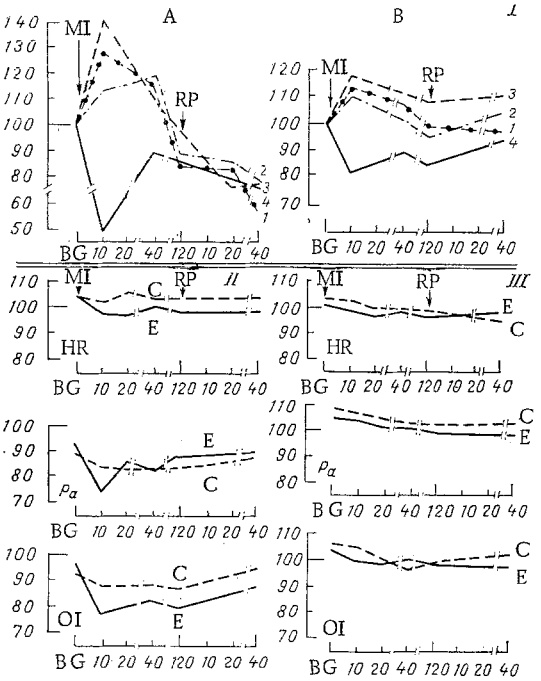

Fig. 3. Time course of concentration of free ACh and of free and bound ACLS, ChE activity in the myocardium (I), and response of the heart to a standard dose of ACh (II and III) during ATCI with MI lasting 120 min. Notation as in Fig. 1.

neurotransmitter level despite resumption of the blood flow in the coronary arteries. Changes in the concentration of free ACLS, reflecting to a certain extent the level of ACh synthesis, were of basically the same character (although with some quantitative differences).

In areas of the heart remote from the MI and RP zone the concentration of free ACh and ACLS during the first 10 min of MI increased, then decreased until the 40th minute, although still remaining above the background level, which is reached after 120 min. Just as in the zone of MI and subsequent RP the concentration of free ACh and ACLS changed in the same direction.

During the development of ATCI, AChE activity underwent considerable changes. These changes, it will be noted, during the 40 min of MI and during RP after MI lasting 12 and 40 min respectively, both in the zone of injury and in distant parts of the heart, were opposite in character to changes in the concentration of free ACh (Figs. 1-3, I).

The facts described above demand an answer to at least the two following questions: 1) which factors are responsible for the changes in ChE activity in the heart during ATCI (especially its fall during MI and RP after MI lasting 40 and 120 min); 2) what lies at the basis of the change in concentration of free ACh and ACLS and of bound ACLS in the myocardium during ATCI (especially the increase in their concentration during 40 min of MI).

The fall in ChE activity in the zone of MI and its subsequent RP, and in remote parts of the heart both during MI and during RP after ischemia for 40 and 120 min may be due, *inter alia*, to three factors: first, a biphasic (during MI and RP) and considerable increase in the intensity of free-radical oxidation of lipids in the cardiomyocytes, which the writers discovered previously [5]. Periods of maximal rise in the intensity of lipid oxidation coincide in time with periods of greatest decrease in ChE activity. At the same time, lipid peroxidation products are known to significantly depress ChE activity [2]; second, the energy deficiency arising during MI and continuing during RP after ischemia lasting 40 and 120 min [11]; third, the increase in the adrenalin concentration in the myocardium (during both MI and RP) [4]. Catecholamines and their oxidation products have been shown to inhibit ChE activity significantly [8].

The increase in the concentration of free ACh and ACLS in the zone of MI and RP and also in remote areas of the heart during the first 40 min of MI may be the result of the action of three main factors: 1) a decrease in hydrolysis of ACh as a result of depression of ChE activity, and also perhaps due to its uptake and fixation by nerve, muscle, and other cells of the heart [16]; 2) an increase in liberation of ACh by vagus nerve endings as a result of increased neuronal activity of the vagus nerves during MI and RP [9], an increase in the extracellular K^+ ion concentration characteristic of MI (it has been shown [12] that an increase in the extracellular K^+ concentration from 2.5 to 10 mM increases the frequency of liberation of ACh quanta tenfold, and an increase to 20 mM increases it 30-fold), and an increase in the concentration of catecholamines in the myocardium [4], activating receptors which facilitate ACh liberation [14]; 3) activation of ACh synthesis in connection with an increase in its liberation, and also under the influence of increased effector vagal impulsation [16]. This was seen to be a real possibility even during MI, for we know that the blood flow in the territory of distribution of the ligated coronary artery may amount to 2-10% of the total myocardial perfusion at rest [13] and that it can supply the necessary substrates to a certain extent for ACh synthesis.

The progressive decline in the ACh concentration in the previously ischemized zone of the heart during RP after prolonged (120 min) MI was evidently due to ischemic destruction of the majority of subcellular and cellular structures and to the substantial damage, increasing during RP, of structures which were still preserved [11]. Loss of ACh by the reperfused zone of the heart may also contribute to an increase in its liberation from neurons as a result of their destruction under the influence of Ca^{++} ions accumulating in the previously ischemized cells [11] and the "flushing out" of the neurotransmitter by the inflowing blood.

ATCI is thus accompanied by a regular series of stages of changes in the functional state of the presynaptic level of cholinergic mediation—activation of extracardial parasympathetic influences on the heart during the first 40 min of MI, during RP after ischemia for 10 and 40 min, and also the progressive decline of these influences toward the 120th minute of MI and during subsequent RP.

In the course of ATCI substantial reorganization also took place in the postsynaptic level of the cholinergic mechanisms regulating cardiac activity (Figs. 1-3, II-III). They

were characterized by an increase in the degree of the depressor cardiac response (judging by the pressure in the left ventricle and Opie's index) during the first 20 sec after injection of a standard dose of ACh, most marked at the 10th minute of MI. Later, toward the 120th minute and also during the period of RP this response decreased in magnitude although it still remained stronger than the background value when estimated by means of Opie's index, an integrative parameter of cardiac function. Similar changes affected the chronotropic response of the heart to exogenous neurotransmitter starting with the 10th minute of MI. However, there was one significant difference: the positive chronotropic response recorded parallel with the fall of pressure in the left ventricle in animals before reproduction of ATCI, was replaced by a negative response starting with the 10th minute of MI. This last fact is evidence of an increase in the cardiodepressor action of ACh in the initial stage of MI and a decrease in its action during prolonged (40-120 min) ischemia and also during subsequent RP. The cardiac response to exogenous ACh at the 180th second after its injection was characterized by a decrease in the disturbances of the parameters of cardiac function with an increase in the duration of MI (Figs. 1-3, III).

The results of this investigation thus indicate activation of parasympathetic influences on the heart in the early stage of MI and also during subsequent RP and a decrease in these influences during prolonged MI and the subsequent RP.

These facts, together with previous observations [6], constitute the basis for a conclusion that substantial changes arise in cholinergic mediation during ATCI. At the level of extracardial neuroeffector regulation of cardiac function, parasympathetic influences evidently "restrain" the cardiotonic effect and reduce the cardiotoxic effect of endogenous catecholamines (especially adrenalin), whose concentration in the myocardium is considerably increased, especially in the initial stage of MI [4]. During prolonged ischemia, accompanied by a decrease in the noradrenalin reserves in the myocardium [4], ACh may promote neuronal synthesis of the neurotransmitter of the sympathetic nervous system [15]. Parasympathetic influences may help to reduce an excessive hyperkinetic cardiac response, such as regularly develops in the initial stage of MI on account of an increase in the catecholamine concentration in the myocardium. At the metabolic level ACh may evidently prevent excessive consumption of oxygen by the myocardium, the development of a considerable energy deficit, and a disturbance of the transmembrane ion distribution in it, i.e., it may reduce the harmful action of the leading pathogenetic factors of coronary insufficiency.

LITERATURE CITED

1. K. B. Vinnitskaya, Lab. Delo, No. 2, 89 (1972).
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972).
3. K. E. Kalantaevskaya, I. V. Shutskii, N. K. Khitrov, et al., Lab. Delo, No. 2, 76 (1973).
4. P. F. Litvitskii, Kardiologiya, No. 10, 105 (1979).
5. P. F. Litvitskii, A. Kh. Kogan, A. N. Kudrin, et al., Byull. Éksp. Biol. Med., No. 3, 271 (1981).
6. P. F. Litvitskii, Byull. Éksp. Biol. Med., No. 8, 17 (1981).
7. M. E. Raiskina, Biochemistry of Nervous Regulation of the Heart [in Russian], Moscow (1962).
8. V. A. Sveshnikov and G. Ya. Pekker, Lab. Delo, No. 6, 327 (1965).
9. F. G. Sitdikov, in: Neuromediators under Normal and Pathological Conditions [in Russian], Kazan' (1979), pp. 318-320.
10. S. G. Skuratovskaya, Tr. Vtor. Mosk. Med. Inst., 129, 115 (1979).
11. R. Jennings, H. Hawkins, J. Love, et al., in: Metabolism of the Myocardium [Russian translation], Moscow (1981), pp. 321-335.
12. J. Hubbard, Physiol. Rev., 53, 674 (1973).
13. J. Lekwen and K. Andersen, Cardiovasc. Res., 14, 280 (1980).
14. W. Riker and M. Okamoto, Annu. Rev. Pharmacol., 9, 173 (1969).
15. H. Thoenen, A. Saner, P. Angeletti, et al., Nature, 236, 26 (1972).
16. S. Tucec, Acetylcholine Synthesis in Neurons, New York (1978).