ELEVATION OF CALCIUM-DEPENDENT PHOSPHOLIPASE C ACTIVITY IN THE MYOCARDIUM DURING ADAPTATION TO SHORT-TERM STRESS

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Adaptation to repeated stress regularly limits the disturbances of energy metabolism and contractile function of the heart in acute anoxia and subsequent reoxygenation [1], prevents disturbances of electrical stability of the heart and the onset of arrhythmias in acute experimental infarction and postinfarction cardiosclerosis, and also increases the resistance of the isolated heart of adapted animal to toxic concentrations of adrenalin and, finally, to an excess of Ca^{2+} [7]. This combination of changes has been called the adaptive stabilization of structures phenomenon (ASSP) [2]. A study of the nature of ASSP has shown that a significant role in it may be played by an increase of many times in the concentration of heat shock proteins (HSP) in the myocardium,which develops during adaptation to stress, and it has been suggested that an important role in the activation of synthesis and accumulation of HSP is probably played by activation of a certain intracellular regulatory mechanism, which leads to the formation of inositol triphosphate (ITP) and diacylglycerol (DAG) [9]. The initial stage of this cascade, phospholipase C (PL-C) is known to be activated both by stimulation of α_1-adrenoreceptors and by the direct influence of an increase in the intracellular Ca^{2+} concentration [5].

The aim of this investigation was to assess the effect of adaptation to repeated stress (a) on the number and affinity of α_1-adrenoreceptors in the rat heart and (b) on PL-C activity and its dependence on the Ca^{2+} concentration.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats. Adaptation to short-term stress was produced by repeated immobilization of the animals in the supine position on alternate days, with an increase in duration from 15 min the 1st time to 1 h the 4th time. The course of adaptation consisted of eight immobilization sessions. The animals were killed on the day after the end of the course of adaptation The plasma membranes of the heart were isolated by the method in [3] with certain modifications. Binding of [^{3}H]-prazocin with α_1-adrenoreceptors was measured by the method in [6] with some modifications. The reaction of ligand-receptor binding was stopped by rapid addition of 15 ml of cold buffer of the same composition and pH at 4°C, followed by filtration through GF/C filters (Whatman, England). The filters were transferred to flasks with dioxan scintillator and radioactivity was measured on a "RackBeta" scintillation counter (LKB, Sweden). The number of receptors (B_{max}) was counted and the dissociation constant of the ligand (K_d), the reciprocal of affinity of the receptors, was determined on a personal computer, using the EBDA/Ligand program for IBM-PC (McPherson, 1984). Activity of PL-C, specific for phosphoinositides, was determined in 50-μl medium containing 25 mM Tris-maleate buffer, pH 6.0, 1 mM Mg^{2+}, 1.5 mM Na cholate, 200 μM EGTA, and $CaCl_2$ from 0 to 300 μM. The incubation medium contained liposomes prepared from the coarse fraction of bovine brain phosphoinositides (Sigma, USA, 10 μg per sample), containing [^{3}H]-phosphatidylinositol-4,5-diphosphate (PIP_2) (Amersham International, England, 16,000-20,000 dpm per sample). To determine dependence of PIP on Ca^{2+}, measurements were made in the absence of exogenous calcium and with the

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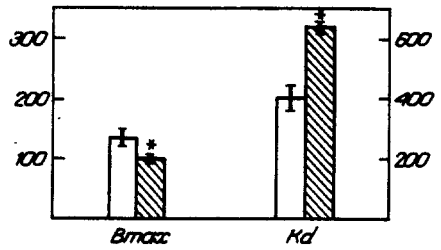

Fig. 1. Content of α_1-adrenoreceptors (B_{max}) in rat heart and their affinity (K_d) during adaptation to stress. Ordinate: on left – B_{max} (fmoles/mg protein); right – K_d (in nM). Unshaded columns – control, shaded columns – adaptation to stress.

addition of increasing concentrations of $CaCl_2$ (50, 100, 200, 300 μM) against the background of 200 μM EGTA. In this way the free Ca^{2+} concentration in the incubation medium could be varied from below 10^{-7} M to levels of the order of 100-200 μM, between which limits Ca^{2+}-dependence of PL-C is observed [12]. The reaction was started by the addition of a suspension of plasma membranes (50 μg protein per sample). After exactly 5 min of incubation at 37°C the reaction was stopped by the addition of 188 μl of a chloroform:methanol:HCl (100:200:3) mixture, the sample thoroughly mixed, and after 10 min 50 μl of chloroform and 50 μl of 1.8 M KCl were added, which was followed by further thorough mixing. After separation of the phases, a 50-μl sample was taken from the uppermost phase, added to flasks with Triton-toluene scintillator, after which radioactivity was counted on a "RackBeta" scintillation counter (LKB, Sweden). Activity of PL-C was expressed in per cent of [^{3}H]-PIP_2 per 50 μg protein hydrolyzed in the course of 5 min. All experiments were carried out in three parallel tests. Protein was determined as in [11]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The data in Fig. 1 show that a considerable shift took place in the α_1-adrenoreceptor system under the influence of adaptation to repeated stress: a phenomenon of deep sensitization took place. Desensitization of α_1-receptors was expressed as a simultaneous decrease of 25% in the number of receptors and an increase in their K_d, i.e., their affinity was reduced by more than 1.5 times.

The data in Table 1 show that activity of PL-C in the plasma membranes of the heart of the control animals exhibits marked dependence on Ca^{2+} concentration. For instance, with a $CaCl_2$ concentration in the medium of 100 μM, PL-C activity increased more than fourfold compared with that without addition of exogenous calcium and at 200 μM or more, activity was increased by more than an order of magnitude. Significantly stronger activation of PL-C with an increase in the $CaCl_2$ concentration was observed in membranes of animals adapted to short-term stress. For instance, whereas at a concentration of 50 μM only a tendency was observed for the value to exceed that in the control animals, if the medium contained 100 μM $CaCl_2$ in the presence of 200 μM EGTA, corresponding to the intracellular Ca^{2+} concentration under physiological conditions [5], PL-C activity was twice as high as in the control animals ($p < 0.01$). With concentrations of 200 and 300 μM, PL-C activity in animals adapted to stress was about 1.5 times higher than that in the control animals ($p < 0.01$).

Thus during adaptation to repeated short-term stress desensitization of α_1-adrenoreceptors takes place, with a simultaneous increase in Ca^{2+}-dependent PL-C activity.

The results demonstrate that an increase in activity of the initial component of phosphoinositide metabolism, namely phospholipase C, which hydrolyzes phosphoinositides with the formation of ITP and DAG, takes place under these conditions, not in any way as the result of increased α_1-adrenoreceptor activity, but as a result of an increase in sensitivity of PL-C to Ca^{2+}. This shift can be brought about during adaptation to stress as a result of a repeated increase in the intracellular Ca^{2+} concentration during repeated exposure to stress. This view is in agreement with the well-known fact that an increased inflow of Ca^{2+} into cardiomyocytes and an increased release of Ca^{2+} from the sarcoplasmic reticulum are an essential component of the adrenergic cardiotropic effect during stress [8]. In the process of adaptation to stress the

TABLE 1. Phospholipase C Activity in Plasma Membranes of Heart of Rats Adapted to Short-Term Stress, on a Change in Calcium Concentration in Incubation Medium

$CaCl_2$ concentration in incubation medium at 200 μM EGTA, μM	Phospholipase C activity, % of [^{3}H]-PIP_2 hydrolyzed per 50 μg protein in 5 min	
	control, n = 9	adaptation to stress, n = 10
0	1,18±0,34	1,41±0,49
50	2,26±0,57	3,07±0,78
100	4,79±0,44	9,45±1,88*
200	11,38±0,38	16,01±0,62*
300	16,01±0,95	22,91±1,13*

Legend. Asterisk indicates differences significant at $p < 0.01$.

repeated Ca^{2+}-determined activation of PL-C becomes the initial stage triggering the regulatory cascade, in which ITP and DAG are formed. Since, besides hydrolysis of PIP_2, Ca^{2+} activated PL-C can also degrade PIP and PI, this leads to the formation of a larger quantity of DAG than of ITP [5]. This also takes place because DAG is formed not only by hydrolysis of inositol phospholipids, but also by hydrolysis of phosphatidylcholine and, perhaps, of other phospholipids, and that DAG accumulation in this system is at least one order of magnitude higher than that from myoinositol phosphates and myoinositol [4]. The importance of this state of affairs for an understanding of the mechanism of long-term adaptation and, in particular, of ASSP formation, is due to the fact that DAG is an activator of protein kinase C, which activates the genetic apparatus and protein synthesis in the cell [10]. It can be tentatively suggested that the increase in synthesis and accumulation of HSP found in the myocardium in our experiments [9] takes place on account of this mechanism. If this suggestion is correct, the increase in activity of phospholipase C and subsequent stages of the regulatory cascade, namely ITP and DAG, may play an important role in HSP accumulation and in adaptive protection of the heart.

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