Effect of conflict stress on adrenoreceptors and metabolic activity of the rat heart

M. N. E. Harri, R. Tirri[1] and A. Kärki[1]

Department of Physiology, University of Kuopio, SF-70101 Kuopio 10 (Finland); and Department of Biology, University of Turku, SF-20500 Turku 50 (Finland), 27 October 1976

Summary. Exposure of rats to a conflict situation (electric shocks associated with water supply) increased the activities of oxidative enzymes and of heksokinase in the myocardium. The stress also led to subsensitivity of atrial α-adrenoreceptors.

Increased activity of mainly oxidative enzymes in the myocardium has been found in rats following prolonged cold exposure or physical training[2]. These stresses also cause subsensitization of cardiac α-adrenoreceptors without concomitant changes in the sensitivity of β-receptors[3,4]. The aim of the present study was to elucidate whether a prolonged conflict situation, i.e. a stress of a more psychic nature, would induce changes in metabolic reactions and/or in the sensitivity of adrenoreceptors of the heart.

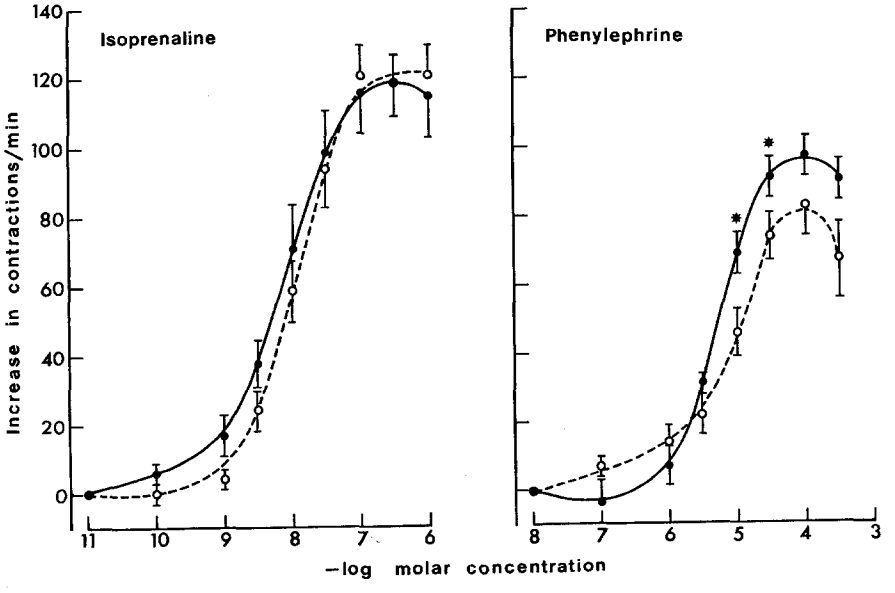
Material and methods. A total of 48 adult male Sprague-Dawley rats, 150–300 g, were used in these studies. 8 days before being sacrified, they were transferred to cages composed of two compartments. The floor of the first compartment (49 × 40 cm) was covered with pine chips while the floor of the second one (19.3 × 15.8 cm) was composed of wire screen. A drinking bottle with tap water was attached at the back wall of this second compartment. An AC electric current of 60 V was conducted to the wire screen, so that the animals had to tolerate electric shocks while drinking. In the course of the 1-week conflict period, the voltage was gradually raised to 90–95 V. Food was always freely available. Rats housed in similar cages without electric current served as controls.

The animals were killed after 7 days of the stress situation. The tips of the ventricles were homogenized in Tris-HCl buffer (0.1 M, pH 7.6) to a 2% homogenate, and centrifuged for 10 min at 1000 × g to remove unbroken cells and particulate debris. The supernatants were used for the determination of succinate dehydrogenase (SDH)[5], malate dehydrogenase (MDH)[6], citrate synthase (CS)[7], hexokinase (HK)[8], phosphofructokinase (PFK)[9] and lactate dehydrogenase (LDH, by the commercial kits of Biochemica Boehringer) activities. The protein content of homogenates were estimated by the phenol method[10]. The stressed and corresponding control groups were always assayed together.

The cumulative concentration-response curves for the positive chronotropic response to an α-adrenergic drug, phenylephrine (PHE), and to a β-adrenergic drug, isoprenaline (ISO), were determined on isolated atria at 37 °C in Tyrode's solution. The rate of spontaneous contractions was recorded on a Mingograph 24 B jet recorder by means of a suction electrode as described earlier[3].

Results. After the beginning of the stress period, in which electric shock was combined with water supply, the daily water consumption of the rats in this cage was reduced from the control level of 20–33 ml/animal (6 animals/cage) to 7.0–8.3 ml/animal. At the 3rd day, however, the

1 Department of Biology, University of Turku (Finland).
2 M. N. E. Harri and J. Valtola, Acta physiol. scand. *95*, 391 (1975).
3 M. N. E. Harri, L. Melender and R. Tirri, Experientia *30*, 1041 (1974).
4 R. Tirri, A. Siltovuori and M. Harri, Acta physiol. scand. (in press).
5 D. C. N. Earl and A. Korner, Biochem. J. *94*, 721 (1965).
6 S. Ochoa, in: Methods in Enzymology, vol. 1, p. 735. Ed. S. P. Colowick and N. O. Kaplan. Academic Press, New York and London 1955.
7 P. A. Srere, in: Methods in Enzymology, vol. 13, p. 3. Ed. I. M. Lowsstein. Academic Press, New York and London 1969.
8 A. Bass, D. Brdiczka, P. Eyer, S. Hofer and D. Pette, Eur. J. Biochem. *10*, 198 (1969).
9 S. Boström, M. Fahlén, Å. Hjalmarson and R. Johansson, Acta physiol. scand. *90*, 544 (1974).
10 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).



Log concentration-response curves for the chronotropic responses to isoprenaline and phenylephrine in isolated atria of control rats (●) and of animals subjected to a conflict situation for 7 days (○). The curves are the means ± SE of 6–8 rats. *, Significantly different from controls ($p < 0.05$).

Activities of enzymes and protein content in the myocardium from rats subjected to a conflict situation for 7 days

Enzyme	Control	Stressed	p
SDH	42.1 ± 2.64	52.1 ± 2.00	< 0.02
MDH	616 ± 10.5	844 ± 26.5	< 0.001
CS	159 ± 3.28	181 ± 3.52	< 0.001
HK	6.38 ± 0.12	6.97 ± 0.14	< 0.01
PFK	39.3 ± 1.12	41.6 ± 1.13	NS
LDH	916 ± 32.2	876 ± 16.9	NS
Protein	92.6 ± 0.80	95.8 ± 0.75	NS

Enzyme activities are expressed as μmoles of substrate utilized per min per g wet weight, and protein content as mg protein in cell-free homogenate from 1 g of muscle. Values are the means ± SE of 10 animals.

daily water consumption of stressed rats attained the level of control animals. The total water consumption during the 7-day observation period ranged from 122 to 154 ml/animal in the 'stress group' in comparison with 198–216 ml/animal in the control group.

The relative weight of the adrenals increased by 15% in response to the conflict situation, being 18.3 ± 0.30 mg/100 g of body weight in the controls and 21.1 ± 0.70 mg/100 g in the stressed animals ($p < 0.005$). The relative weight of the hearts was not changed (269 ± 7.7 mg/100 gr) as compared to the controls (267 ± 8.4 mg/100 g).

The results presented in the table show that exposure of rats to a conflict situation for 7 days significantly increased the activities of aerobic enzymes (SDH, MDH, CS) in the myocardium. Of the anaerobic enzymes studied, only HK showed significant activation in response to the stress. The protein concentration in the cell-free homogenate from stressed animals was not significantly different from that of the controls, an indication of increased activity rather than increased content of the enzymes.

The graphs in the figure show that the stress situation did not cause any significant changes in atrial response to ISO, while it lowered the sensitivity to PHE. This can be seen in the shifting to the right of the concentration-response curves. Because this treatment reduced the maximum response, the EC_{50}-values, however, did not differ from each other in control and experimental groups. The basic contraction frequency of isolated atria in the stressed rats (254 ± 13 beats/min) was not significantly different from that of the controls (226 ± 15 beats/min).

Discussion. Our present results show that exposure of rats to a conflict situation decreases the sensitivity of isolated atria to PHE, while it does not change the sensitivity to ISO. Our previous studies have shown that similar changes can be induced also by physical stresses, such as cold exposure or physical training[3,4]. Furthermore, subsensitization of α-adrenoreceptors can be produced by repeated injections of α- or β-adrenergic amines or of adrenocorticotropic hormone[4,11]. Thus the conclusion was drawn that prolonged stimulation of the heart by catecholamines, as released in the organism in stress situations or as produced by repeated injections of exogenous drugs, or a sensitization of adrenergic actions by corticosteroids (released by ACTH)[12], are responsible for subsensitization of α-adrenoreceptors[3,4,11]. In these situations, however, the sensitivity of β-receptors remains unchanged, which means a shift from α- to β-receptors. The 'oxygen wasting effect' of cardiac stimulation through β-receptors has been suggested[13,14]. Thus the increased relative sensitivity of β-receptors in stress situations increases the oxygen demand of the myocardium. This can be compensated for by increasing the capacity of oxidative metabolism and thereby the enzymes involved. Later this situation can result in compensatory cardiac enlargement, as observed earlier in cold-acclimatized and trained rats[2]. This was not seen in the present study of psychically stressed animals, due to the relative short duration of the stress period. The response of HK to a single short period of exercise has been shown[9,15]. Thus, the changed activity of this enzyme is more an indication of the acute nature of the stress situation than of prolonged alterations of metabolic pathways.

11 R. Tirri, A. Siltovuori and M. Harri, Experientia *32*, 1283 (1976).
12 A. J. Kaufmann, Naunyn-Schmiedebergs Arch. Pharmak. *273*, 134 (1972).
13 W. C. Govier, J. Pharmac. exp. Ther. *159*, 82 (1968).
14 P. J. Privitera and I. Rosenblum, J. Pharmac. exp. Ther. *156*, 325 (1967).
15 R. J. Barnard and J. B. Peter, J. appl. Physiol. *27*, 691 (1969).

Improved diffraction patterns from isolated heart muscle with infrared light[1]

J. W. Krueger[2]

Departments of Medicine and Physiology, Albert Einstein College of Medicine, Bronx (New York 10461, USA),
19 October 1976

Summary. Diffraction measurement of myofilament overlap can be made with greater resolution or extended to thicker heart muscle preparations by using light of a near IR wavelength.

Light diffraction methods have been widely used to observe sarcomere length changes in strips of isolated striated muscle[3–5]. Recently these techniques have been applied to study sarcomere motion and thus the role of myofilament sliding in cardiac contraction[6,7], but recognizable diffraction patterns have been obtainable only from very thin specimens. Thin (i.e., less than 200 μm), suitably shaped mammalian heart muscles are scarce, so that such studies have been limited to trabecular and right ventricular papillary muscles from rats. Diffraction spectra from thicker trabeculae of other species[8] tend to obscure upon contraction. In view of the ability of IR

1 Supported in part by National Heart and Lung Institute, grant 5 R01 HL 18668-02.
2 I wish to thank Dr E. H. Sonnenblick for support, Dr M. Kawai for advice, and Mr Andrew Hopkins, Jr, for technical assistance.
3 A. Sandow, J. cell. comp. Physiol. *9*, 55 (1936).
4 G. Goldspink, R. E. Larson and R. E. Davies, Experientia *26*, 16 (1970).
5 D. R. Cleworth and K. A. P. Edman, J. Physiol. *227*, 1 (1972)
6 R. Nassar, A. Manring and E. A. Johnson, in: The Physiological Basis of Starling's Law of the Heart, p. 57. Ciba Found. Symp. *24* (1974).
7 J. W. Krueger and G. H. Pollack, J. Physiol. *251*, 627 (1975).
8 I. Matsubara and B. M. Millman, J. molec. Biol. *82*, 527 (1974).