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.

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

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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

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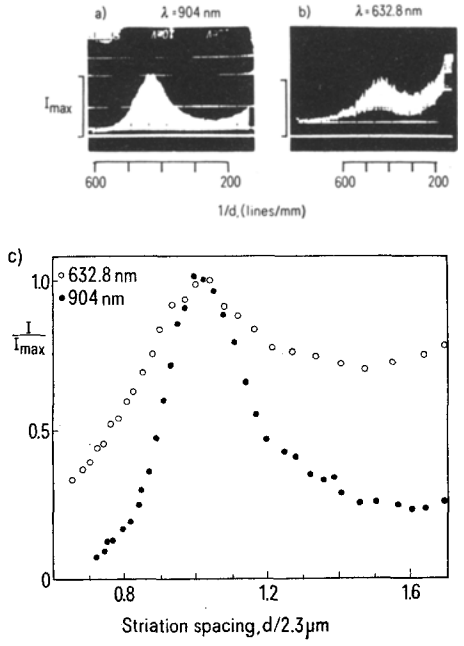

Fig. 1. Light intensity distribution in the first order diffraction pattern with *a* IR and *b* visible light. Diffraction patterns were sampled during the interval between contractions from the same area of a rat papillary muscle 350 μm thick. The position of the diffracted light in the display is directly proportional to the wavelength, λ, and the muscle's Fourier spatial frequency content, 1/d, where d is the striation spacing (10). *c* Direct comparison of the light intensity dispersion in *a* and *b* as a function of the striation spacing, d. The spatial coordinates have been normalized by the striation spacing occurring at the muscle length for maximum active force development, 2.3μm[7]. Similarly, light intensity, I, has been normalized to the first order maximum, I_{max}, to compensate for the wavelength dependent sensitivity of the silicon optical sensor.

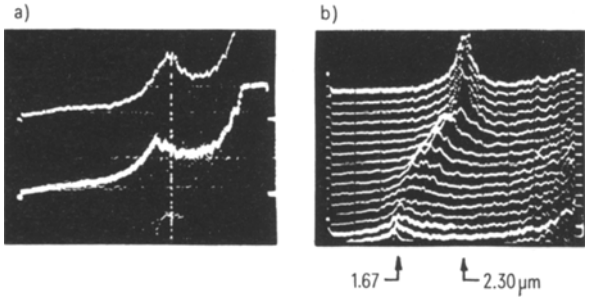

Fig. 2. IR spectra from rabbit papillary muscles. *a* First order intensity distribution at rest and at the peak of contraction (top and bottom traces, respectively) are shown from a specimen 750 μm thick. The ends of the muscle were held fixed. The vertical row of dots denotes the initial position, the difference at peak contraction corresponding to a sarcomere length change of 8%. The initial sarcomere length was 2.27 μm at a muscle length producing 95% maximal active force. *b* Appearance of the diffraction pattern during maximal shortening. The intensity distribution is displayed every 40 msec during a contraction in which a 300 μm thick muscle was shortened to keep tension near the preload level. The arrows denote an initial sarcomere length of 2.30 μm and that at peak shortening of 1.67 μm.

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light to penetrate cardiac tissue[9] it has been used in the present report to form the diffraction patterns. The resulting spectra has been shown to resolve myocardial sarcomere length populations more sharply than the visible spectra. This permits direct measurement of sarcomere length dynamics in relatively thicker papillary muscles from the rabbit and cat, where myocardial mechanics have been studied by more classical but potentially less precise means.

Materials and methods. Right ventricular papillary muscles were isolated from rats and rabbits and mounted horizontally between 2 clips in an experimental chamber seated on the stage of a microscope. One clip was attached to a force transducer, the other to the movable core of a small, servo-controlled galvanometer. The preparation was submerged in oxygenated (95% O_2, 5% CO_2) physiological salt solution (composition in mM: NaCl 117, $NaHCO_3$ 27, KCl 4.2, $CaCl_2 \cdot 2H_2O$ 1.9, $MgSO_4 \cdot 7H_2O$ 1.2, NaH_2PO_4 1.2, glucose 5.6) whose temperature was 25.2 ± 0.2 °C. The muscles were electrically stimulated to contract at rates of 25/min (rat) and 6/min (rabbit). The thickness of the preparation was measured directly using a mirror alongside the preparation angled 45° to the microscope's optical axis. A helium neon CW laser (632.8 nm wavelength, 5 mW power; Hughes Electrodynamics) and a gallium arsenide pulsed laser diode transmitter (904 nm peak emission, 3.5 nm spectral width, 2.9 mW average power, 40 ns pulsewidth; Laser Diode Labs, Inc.) were used as visible and IR light sources, respectively. Diffracted light was collected with either a 40X, N.A. 0.75 (Zeiss) or a 50X, N.A. 1.0 (Leitz) water immersion objective lens. The diffraction pattern at the objective's rear focal plane was focused simultaneously onto 2 optical sensors. The first, a silicon-vidicon television tube (Cohu 4400), was used to align and focus the IR diffraction pattern onto the second, a self scanned linear array of 256 photodiodes (Reticon Corp.). The light intensity distribution within the first order diffraction spectra was visualized by displaying the output voltages of the photodiode array on a cathode ray oscilloscope.

Results and discussion. The effect of wavelength upon the sharpness of the diffraction pattern from a rat papillary muscle is shown in figure 1. The muscle's thickness was chosen to give a relatively poor visible diffraction spectra. The first order spectral intensity of the IR pattern from the same area of the muscle appears noticeably sharper than that formed by visible light (figures 1a and 1b, respectively). When normalized and expressed directly as a function of spatial coordinates (i.e., striation spacing), the dispersion of the diffracted light is clearly narrower

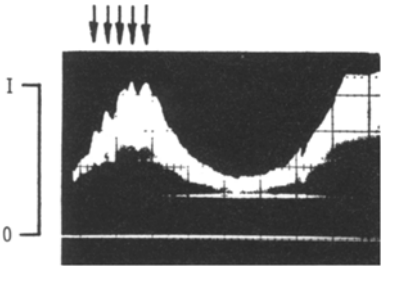

Fig. 3. Discrete light intensity distribution within first order spectra. The IR diffraction pattern from a rat papillary muscle 300 μm thick was sampled late in the interval between contractions. The arrows denote peaks corresponding to sarcomere lengths (from left to right) of 2.00, 2.07, 2.16, 2.24 and 2.36 μm. The peaks move slowly in a random fashion. There was no detectable change in resting tension during the process.

and sharper with the IR light (figure 1c). The relative improvement with IR illumination was more pronounced with thicker muscles.

IR diffraction spectra were often obtained from thicker muscles which showed no visible diffraction spectra. Figure 2a illustrates the IR diffraction spectra from such a case, a rabbit papillary muscle whose thickness (750 μm) was 3 times that of specimens giving comparable spectral clarity with helium neon laser illumination[7]. The use of IR light thereby permits direct comparison of the effects of sarcomere motion upon contractile behavior in mammalian heart muscles which have different intracellular pathways of contractile activation and control. For example, the maximum sarcomere shortening in another rabbit papillary muscle (2.30–1.65 μm, figure 2b) is comparable to that seen in rats[11], although the onset after stimulation is appreciably delayed (i.e., 40 msec vs 15 msec). No deleterious effect of IR illumination upon contractile force was noticed over the 3 h of observation. Improved spectral clarity with IR illumination suggests that the dispersion of first order light is affected more by low angle scattering than by the actual sarcomere length distribution within the muscle. When a uniform grating was illuminated by light passing first through the muscle, the dispersion of first order spectra was equivalent to the intensity distribution of figure 1c. Direct illumination of the grating produced equivalent, narrow dispersion for both light sources. Thus, the zero order light collimation is compromised at each diffracting layer of the muscle tissue, and the actual distribution of sarcomere lengths must be narrower than the light intensity dispersion shown in figure 1c. This speculation is supported by direct measurements by others which show little sarcomere length dispersion in living papillary muscles at rest[12].

Despite uncertainties about inference of sarcomere length distribution by diffractometry, it is often possible to detect discrete sarcomere lengths in these preparations. Since the light emitted by the gallium arsenide laser diode has a finite spectral bandwidth (3.5 nm), the IR diffraction patterns are not as speckled[13] as those obtained from the helium neon laser (refer to lack of small intensity fluctuations on figure 1a as compared to 1b). In the absence of speckle noise it is often possible to resolve small peaks which occur only on that part of the diffraction pattern where a component due to sarcomere diffraction would be predicted (figure 3). The position of these peaks is not stationary and may be related to the presence of small, nonpropagated foci of spontaneous contractile activity seen late during the interval between contractions in intact[7,14] and skinned[15] muscle preparations.

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GABA in the caudate nucleus: A possible synaptic transmitter of interneurons[1]

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Summary. Microiontophoretic application of GABA and its antagonist, picrotoxin, altered focal potentials evoked in the caudate nucleus by stimulation near the recording site to a much greater extent than potentials elicited by stimulation of afferent pathways, suggesting that GABA is a transmitter of interneurons in this nucleus.

The possibility that gamma-aminobutyric acid (GABA) acts as a neurotransmitter in the caudate nucleus (CN) is suggested by the fairly high concentration of GABA and its synthetizing enzyme, glutamic acid decarboxylase (GAD) in this nucleus[3–5]. GABA and GAD are reduced in the CN in Huntington's chorea[6,7], which is characterized by a loss of interneurons in the CN, and in Parkinson's disease[8,9], suggesting that striatal GABA plays a role in the pathophysiology of human movement disorders. When applied microiontophoretically to caudate neurons, GABA reduces their firing rate[10,11] and mimics the effect of synaptically induced inhibition[12]. Possibly, some GABA-containing terminals in the CN are recurrent collateral fibers from striato-nigral connections; these connections were first considered GABAergic because the GABA antagonist, picrotoxin, blocks focal evoked potentials and the depression of neuronal firing produced in the substantia nigra by stimulation of the CN[13,14]. However, it is unlikely that recurrent collaterals account for the high concentration and widespread effects of GABA in the CN, since less than 5% of the caudate neurons give rise to efferent fibers[15]. Moreover, because lesions of major connections to and from the CN do not reduce caudate GABA or GAD levels, it has been sug-

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