Relaxation by glucagon of potassium contracture in cat ventricle

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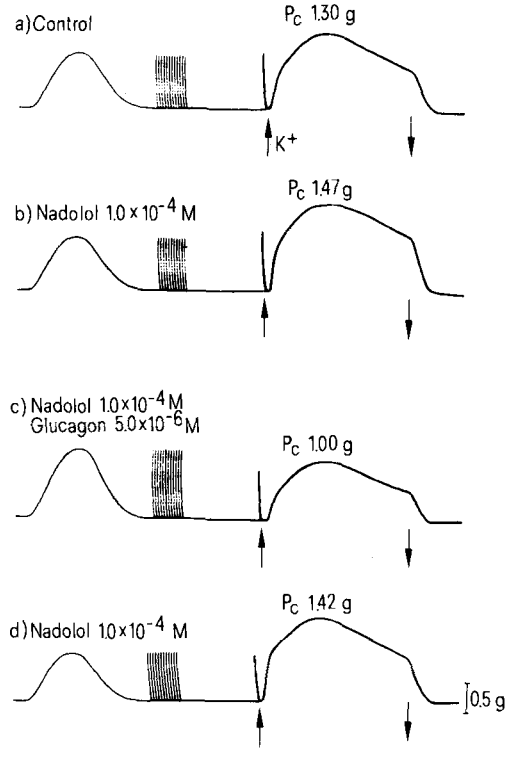
Summary. Glucagon reduces K^+-contracture, increases peak active force but does not alter time to peak force and relaxation of the isometric twitch of cat ventricle.

Catecholamines[3,4] and cyclic AMP derivatives[5] increase active force and rate of force development, shorten time to peak force and relaxation time of the cardiac isometric twitch. These agents also relax high K^+-induced contracture in mammalian myocardium[5,6]. The cardiac actions of catecholamines probably arise as a consequence of cyclic AMP-dependent reactions which occur following β-adrenergic receptor stimulation[7]. It has been suggested that the positive inotropic action of glucagon is mediated by cyclic AMP[8-10]. However, in contrast to catecholamines and cyclic AMP, glucagon does not alter time to peak force[9] or relaxation of the isometric twitch[4]. The differences in mechanical response suggest that cyclic AMP does not play a role in mediating the cardiotonic actions of glucagon, however, cellular ionic events which occur during phasic contraction may obscure glucagon's influence on relaxation. We used high K^+ medium to depolarize cat ventricular muscle and assessed the effects of glucagon on maintained contracture force developed by these preparations in the absence of repolarizing currents and changes in excitability[11,12].

Methods. The methods used in this study have been described in detail previously[11]. Force was monitored from small (< 1 mm diameter, in situ) cat right ventricular papillary muscles maintained in Tyrode's solution and stimulated at 0.5 Hz. Control Tyrode's solution contained (in mM): NaCl, 129; KCl, 4; $NaHCO_3$, 20; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; dextrose, 5.5; $CaCl_2$, 2.7; pH 7.4. Contracture solution (high K^+-Tyrode's) was prepared by replacing all the NaCl with KCl (final concentration, 133 mM; residual Na^+ salts, 21.8 mM), pH 7.4. Solutions were gassed with 95% O_2-5% CO_2. In selected experiments, β-adrenergic blockade was induced with nadolol (1.0×10^{-4} M); this concentration has no effect on isometric contraction of mammalian myocardium[11]. Once initiated, β-blockade was maintained. Crystalline glucagon (Sigma) was dissolved in 0.1 M Tris buffer at pH 9.2. The solvent for glucagon did not affect the results. Muscles were equilibrated 60 min prior to exposure to high K^+-Tyrode's solution and were reequilibrated in control Tyrode's solution (30–60 min) after each contracture.

Results and discussion. A representative experiment is shown in the figure. Exposure of 3 muscles to glucagon (5.0×10^{-6} M) during β-blockade with nadolol (1.0×10^{-4} M) increased peak active force $28\pm1.8\%$ and rate of force development $26\pm3.1\%$ ($p<0.05$); time to peak force and relaxation time of the isometric twitch were not significantly altered but glucagon significantly reduced K^+-contracture force $31\pm3.8\%$ ($p<0.05$). These effects were not significantly different from those obtained for 3 other muscles in the absence of nadolol. Thus, we confirm that the positive inotropic action of glucagon persists during β-adrenergic blockade[4,13] and also demonstrate that its action to reduce K^+-contracture is not due to β-receptor stimulation.

Recently, Blinks et al.[14] reported that both glucagon and isoproterenol increase the rate constant for decay of the light signal in ventricular muscle cells injected with aequorin. This was considered as evidence for enhanced Ca^{2+} sequestration by the sarcoplasmic reticulum (SR). Biochemical studies indicate that enhancement of myocardial relaxation by cyclic AMP probably arises from its stimulation of SR Ca^{2+} sequestration. It has been demonstrated, for example, that catecholamines[16] and cyclic AMP derivatives[17] stimulate Ca^{2+} transport into isolated cardiac microsomes and the relaxing effects of cyclic AMP in skinned



Effects of glucagon (5.0×10^{-6} M) on contractile and K^+-induced contracture force of cat ventricular muscle in the presence of the β-receptor antagonist, nadolol (1.0×10^{-4} M). *A* Control recordings of an isometric twitch followed by a high K^+-induced contracture. (The twitch was recorded at a paper speed of 100 mm/sec and the K^+-contracture at 25 mm/min). Electrical stimulation was discontinued 10 sec before exposure of the muscle to high K^+-Tyrode's solution. High K^+-Tyrode's is introduced at the upward arrow and after 2 min removed at the downward arrow. Exposure of the muscle to the high K^+-Tyrode's stimulates an initial phasic response followed by contracture. Control contractile and contracture force was 0.87 g and 1.30 g, respectively. Following release of the contracture, stimulation was resumed and the muscle reequilibrated for 30 min. *B* Response of the same muscle after exposure to nadolol for 45 min. Note that contractile force is unaltered but contracture force is increased 13%; β-adrenergic blockade eliminates the effects of endogenous catecholamines released by the high K^+ and the subsequent relaxation of contracture[6]. *C* After reequilibration in control Tyrode's solution, the muscle was exposed to glucagon for 30 min in the presence of maintained β-adrenergic blockade. Note that glucagon increased contractile force 31% while contracture force was reduced 32%. *D* Wash with control Tyrode's solution containing nadolol for 60 min. Contractile and contracture force return toward control values (B). Muscle length and diameter were 4.6 mm and 0.68 mm, respectively.

cardiac fibres have been attributed to modifications of SR Ca^{2+} uptake[17]. Glucagon has been reported to stimulate myocardial adenyl cyclase activity in broken cell preparations[8] and to increase tissue levels of cyclic AMP in intact heart cells[10] and it is possible that glucagon's relaxation of K^+-induced contracture occurs as a consequence of a cyclic AMP stimulated increase in SR Ca^{2+} uptake. This, in turn, would increase the quantity of Ca^{2+} available for release to the contractile elements upon subsequent depolarization and account for the augmentation of force.

Catecholamines and dibutyryl cyclic AMP increase the slow inward Ca^{2+} current in heart[3]. The resultant elevation in $[Ca_i^{2+}]$ reduces the electrochemical gradient for Ca^{2+} into the cell and/or effects a more rapid activation of K^+ repolarizing currents[18,19]; as a result the action potential shortens. Membrane potential modulates myocardial force[20] and catecholamine (cyclic AMP)-induced earlier repolarization might accelerate the initiation of relaxation and shorten the twitch. This would be independent of the enhancement of SR Ca^{2+} uptake by cyclic AMP. The lack of a significant effect of glucagon on twitch duration probably reflects its modest actions on Ca^{2+} influx[21], repolarization[4] and presumably triggered relaxation or alternatively, as Marcus et al.[9] suggest, glucagon does not shorten time to peak isometric force because of its limited inotropic potency.

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Prostaglandin E_2 increases mechanically evoked potentials in the peripheral nerve

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Summary. Subdermal injections of PGE_2 (5 µg) in the rat foot lead to increases in the potentials evoked in sensory nerve branches by the mechanical stimulation of the skin. This sensitization of both A and C fibres complements the previously described hyperalgesic effects of prostaglandins of the E series.

In addition to the well known potentiating effects of prostaglandins of the E series (PGE_1 and PGE_2) on the action of pain-producing substances[1,2], their direct intradermal injection or subdermal infusion cause erythema, oedema and, in some cases, long-lasting hyperalgesia[3,4]. The purpose of this research was to study the effects of algesic doses of PGE_2 on the electrical activity of a sensory nerve. To that end we injected 5 µg PGE_2 in the subdermal space of the rat foot and recorded the potential evoked in a branch of a sensory nerve by the innocuous stimulation of the skin. Additionally, the activity of single sensory units with A fibres was also studied.

Material and methods. Male Wistar rats (250–320 g) were anaesthetized (1.25 mg/kg urethane i.p.) and had their left leg tied down securely and the saphenous nerve exposed within a paraffin pool formed by the flaps of the incision. A platinum electrode was positioned under a branch of the nerve and another hooked onto nearby moist skin. The nerve was ligated proximally and records were made of its activity as the appropriate receptive area of the skin was stimulated mechanically.

Stimulation consisted of the electronically-controlled contact of a metal stylus with the skin at a distance of 40–52 mm from the point of recording (tip diameter of stylus: 0.5 mm; force adjustable to a maximum of 2.5 g). Contact time was set at between 10 and 500 msec in cycles of up to 32 deliveries and at rates of 0.2–1 delivery per sec. This stimualtion was designed to activate skin mechanoreceptors with A and C fibres but not high threshold 'mechanical' or 'polymodal' nociceptors[5]. Both the onset and the withdrawal of the stimulus evoked nerve potentials. Single units, responding to the mere bending of hairs, were isolated in specially dissected few fibre preparations. After calculations of conduction velocity these were assigned as rapidly adapting hair follicle receptors[6]. In all cases recordings took place both before and at various intervals after the injection of PGE_2 into the interdigital subdermal space of the foot.