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ORIGINAL ARTICLE

Revisiting the slow force response: The role of the PKG signaling pathway in the normal and the ischemic heart☆

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Abstract
Introduction: The myocardial response to acute stretch consists of a two-phase increase in contractility: an acute increase by the Frank-Starling mechanism and a gradual and time-dependent increase in force generated known as the slow force response (SFR). The SFR is actively modulated by different signaling pathways, but the role of protein kinase G (PKG) signaling is unknown. In this study we aim to characterize the role of the PKG signaling pathway in the SFR under normal and ischemic conditions.
Methods: Rabbit papillary muscles were stretched from 92 to 100% of maximum length (L_{max}) under basal conditions, in the absence (1) or presence of: a PKG agonist (2) and a PKG inhibitor (3); under ischemic conditions in the absence (4) or presence of: a PKG agonist (5); a nitric oxide (NO) donor (6) and a phosphodiesterase 5 (PDE5) inhibitor (7).
Results: Under normoxia, the SFR was significantly attenuated by inhibition of PKG and remained unaltered with PKG activation. Ischemia induced a progressive decrease in myocardial contractility after stretch. Neither the PKG agonist nor the NO donor altered the myocardial response to stretch under ischemic conditions. However, the use of a PDE5 inhibitor in ischemia partially reversed the progressive deterioration in contractility.
Conclusions: PKG activity is essential for the SFR. During ischemia, a progressive decline in the force is observed in response to acute myocardial stretch. This dysfunctional response can be partially reversed by the use of PDE5 inhibitors.

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PALAVRAS-CHAVE
Proteína cínase dependente do GMP cíclico tipo 1;
Óxido nítrico;
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Resposta inotrópica tardia: o papel da via de sinalização da PKG no miocárdio normal e na isquemia)

Resumo
Introdução: A resposta ao estiramento agudo do miocárdio consiste num aumento bifásico da contractilidade: um aumento agudo pelo mecanismo de Frank-Starling e um aumento gradual denominado resposta inotrópica tardia (SFR). A SFR é modulável por diferentes vias de sinalização, no entanto, o papel da via da Proteína Cínase G (PKG) permanece desconhecido. Assim, no presente estudo, pretendemos caracterizar o papel da via de sinalização da PKG na SFR em condições normais e em condições isquémicas.
Métodos: Músculos papilares de coelho foram estirados de 92 para 100% de L_{max} em condições basais, na ausência (1) ou na presença de: um agonista da PKG (2) e um inibidor da PKG (3); em condições isquémicas, na ausência (4) ou na presença de: um agonista da PKG (5), um dador de óxido nítrico (NO) (6) e um inibidor da fosfodiesterase 5 (PDE5) (7).
Resultados: Em condições basais, a SFR foi significativamente atenuada pela inibição da PKG, não tendo sido alterada pela ativação da PKG. A isquemia induziu uma diminuição progressiva da contratilidade após o estiramento agudo. Esta resposta não foi alterada pela adição de agonista da PKG nem pelo uso de um dador de NO. No entanto, o uso de um inibidor da PDE5 durante a isquemia foi capaz de reverter parcialmente a deterioração progressiva da contractilidade.
Conclusões: A atividade da PKG é essencial para a SFR. Durante a isquemia observa-se uma diminuição progressiva da contratilidade em resposta ao estiramento agudo. Esta resposta disfuncional pode ser revertida pelo uso de inibidores da PDE5.

List of abbreviations

AT	active tension
cGMP	cyclic guanosine monophosphate
EDV	end-diastolic volume
FSM	Frank-Starling mechanism
NO	nitric oxide
PDE	phosphodiesterase
PKG	protein kinase G
SFR	slow force response
TnI	troponin I

Introduction

The heart is able to adapt continuously to ever-changing hemodynamic conditions. An acute hemodynamic overload, such as the beginning of aerobic exercise, increases venous return, eliciting myocardial stretch and an increase in end-diastolic volume (EDV) and pressure. The systolic arm of the response to myocardial stretch was first described over a century ago by Ernest Starling and Otto Frank,[1–5] who discovered that increased EDV promotes an immediate increase in contractility and stroke volume, the Frank-Starling mechanism (FSM). After the initial response, there is a progressive and time-dependent increase in contractility, first described in 1912 by von Anrep[6] and later explored by Parmley and Chuck in 1973.[7] In vivo, this mechanism allows the return of EDV to its initial value and is known as the von Anrep effect. Its in-vitro counterpart is known as the slow force response (SFR).

The SFR is a consequence of an increase in magnitude of calcium transients, due to activation of the Na^+/H^+ antiporter. This mechanism favors the reverse operating mode of the Na^+/Ca^{2+} antiporter, leading to an increase in intracellular calcium.[8–10] This phase of the myocardial response to stretch is known to be dependent on various signaling pathways and acutely modulated by various neurohumoral mediators.[8,9,11] Despite the importance of the PKG signaling pathway in cardiovascular homeostasis, its role in the SFR still mostly unexplored. Pharmacological modulation of this pathway, mostly by drugs that activate NO pathways (nitrates), is extremely common in ischemic heart disease and myocardial infarction. Recently, there has been considerable interest in the beneficial effects of phosphodiesterase 5 (PDE5) inhibitors in the treatment of acute myocardial ischemia.[12,13] Myocardial ischemia promotes contractile dysfunction and acute hemodynamic overload, thereby leading to myocardial stretch. Therefore, discerning the influence of the PKG pathway on the contractile response to stretch under ischemic conditions and, particularly, its pharmacological modulation (by NO donors and PDE5 inhibitors) is central to more accurate treatment decisions.

Methods

Modulation of the PKG signaling pathway during acute myocardial stretch

The effects of acute muscle stretch from 92 to 100% of maximum length (L_{max}) on contractile function of rabbit papillary muscles were assessed under basal conditions, in the absence (control group, n=8) or the presence of a