

# Physiological Research Pre-Press Article

**Phosphodiesterases inhibition enhance the effect of glucagon on cardiac automaticity in the isolated right ventricle of the rat.**

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**Short title:** Effects of glucagon and phosphodiesterases on ventricular automaticity

**Summary:**

We evaluated the effect of glucagon on cardiac automaticity as well as the possible role of cyclic nucleotide phosphodiesterases (PDE) in regulating this effect. Concentration response curves for glucagon in the absence and in the presence of the non selective PDE inhibitor IBMX were performed in the isolated right ventricle of the rat. We did find that glucagon produces only a minor increase of ventricular automaticity ( $11.0 \pm 4.1$ ,  $n=5$ ) when compared to the full agonist of  $\beta$ -adrenoceptor, isoproterenol ( $182.2 \pm 25.3$ ,  $n=7$ ). However, IBMX enhances the  $E_{max}$  of glucagon on cardiac automaticity ( $11.0 \pm 4.1$ , in the absence and  $45.3 \pm 3.2$  in the presence of IBMX,  $n=5$ ,  $P < 0.05$ ). These results indicate that PDE blunts proarrhythmic effects of glucagon in rat myocardium.

**Key words:** glucagon, cyclic nucleotide phosphodiesterases (PDE), cardiac automaticity, isoproterenol.

Glucagon is a polypeptide hormone produced and secreted by the alpha cells of the pancreatic islets of Langerhans which increases heart rate and contractility (White 1999). Consequently, it is used for the management of poisoning caused by cardiodepressant drugs such as  $\beta$ -adrenoceptors ( $\beta$ -AR) blockers or calcium channel blockers (DeWitt and Waksman 2004). Cardiac effects of glucagon are considered to be resultant from stimulation of glucagon receptors associated with Gs protein, which causes adenylyl cyclase (AC) activation and the consequent increase of 3',5',-cyclic adenosine monophosphate (cAMP) production (White 1999).

Cell cAMP levels are regulated by the activity of cyclic nucleotide phosphodiesterases (PDE) enzymes which break down cAMP into its chemically inactive product 5'AMP (Bender and Beavo 2006). In contrast to the positive inotropic and chronotropic effects of glucagon which are well established, there is controversy about its effect on cardiac arrhythmias since no proarrhythmic and arrhythmogenic effects have been reported for glucagon (Farah 1983). Abnormal automaticity is an important mechanism underlying cardiac arrhythmias (Shu et al 2009) and cAMP increases the spontaneous firing rate of pacemaker cells by inducing a local  $Ca^{+2}$  release resulting from a protein kinase A-dependent phosphorylation of sarcolemmal ryanodine receptors (Vinogradova and Lakatta 2009). The isolated right ventricle of the rat contains pacemaker cells, in the His-Purkinje fibers, which develop spontaneous activity resembling an idioventricular rhythm (Mangoni and Nargeot 2008) and it is a useful experimental model for assessing the effect of drugs on ventricular automaticity (Hernandez et al. 1994, Hernandez and Ribeiro 1996). The present work was aimed to evaluate the effect of glucagon in this experimental model as well as the possible role of PDEs in regulating this effect. For comparison, we have studied the effect of the full  $\beta$ -AR agonist isoproterenol which also produces its cardiac effects by activating the Gs/AC/cAMP pathway (Brodde and Michel 1999). To

our knowledge, this is the first report showing that PDE inhibition increases the effect of glucagon on cardiac automaticity in this tissue.

This study was performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethical Committee of the University of Murcia. 17 Sprague-Dawley rats of either sex, 250-300 g were stunned and exsanguinated, hearts were removed and placed in warm Tyrode solution containing (mM): NaCl 136.9, KCl 5, MgCl<sub>2</sub> 1.05, CaCl<sub>2</sub> 1.8, NaH<sub>2</sub>PO<sub>4</sub> 0.4, NaHCO<sub>3</sub> 11.9 and dextrose 5. The right ventricle was freed, the atrial end fixed to a metallic support and the apical end was attached to a Grass FT 03 force-displacement transducer. A 30 ml organ bath was used and the bathing Tyrode solution was maintained at 37°C, pH 7.4 and bubbled with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Contractions were displayed on the screen of a computer by using a Stemtech Inc., Houston, U.S.A.) and the AT-CODAS computer programme (Dataq Instruments, Ohio, U.S.A.). The resting tension was set at 1 g and the preparations were allowed to stabilize for at least 30 min. After the stabilization period, drugs were applied in volumes less than or equal to 0.1 ml and each concentration left 5 min; after, higher concentration of the test drug was applied until a cumulative concentration-response curve had been constructed. To ascertain the role of PDEs in regulating glucagon responses, we also determined concentration response curves for glucagon in the presence of the nonselective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX) (Bender and Beavo 2006). The concentration of IBMX used was 10 µM which effectively inhibits PDEs activity in the rat heart (Bian et al., 2000). IBMX was left in contact with the tissue for 15 min before construction of the concentration response curves for glucagon. Recorded data were then processed and the spontaneous ventricular frequency (beats min<sup>-1</sup>) was calculated as the average rate of the preparation recorded during the incubation period for each drug concentration. The change of frequency, compared with the control rate, was considered to be the effect of a given drug concentration.

Glucagon was generously supplied by Novo Nordisk Pharma S.A. (Madrid, Spain). Isoproterenol and IBMX were obtained from Sigma Chemicals Co. (Madrid, Spain) and dimethyl sulphoxide (DMSO) from Probus (Barcelona, Spain). Glucagon and IBMX were dissolved in DMSO and Tyrode solution (20% DMSO in Tyrode) and isoproterenol was dissolved in Tyrode solution. The drugs were added to the organ bath at an appropriate concentration so that the concentration of DMSO in the test solution was less than 0.3%, which produced no effect on these preparations. Results are expressed as mean values  $\pm$  S.E.M. Student's t test or one way analysis of variance followed by Scheffe' method for multiple comparisons was used. The criterion for significance was that P values should be less than 0.05.

We performed a total of 17 experiments in which a spontaneous ventricular rate was obtained by using the technique above described. The mean frequency before addition of any drug was  $12 \pm 5$  beats  $\text{min}^{-1}$ . Typical effects of glucagon (Fig. 1a) and isoproterenol (Fig. 1c) on ventricular automaticity are shown. As it can be seen, glucagon produces a small increase in ventricular frequency when compared to isoproterenol. For instance, a concentration 1  $\mu\text{M}$  of either glucagon and isoproterenol increases ventricular frequency by  $10.1 \pm 3.5$  (n=5) and  $182.2 \pm 25.3$  (n=7) beats  $\text{min}^{-1}$  respectively ( $P < 0.05$ , Figure 2). This agrees with results obtained in the dog heart showing that glucagon is devoid of effect (Shanks and Zaidi 1972, Wilkerson et al. 1977), or only produces a minor increase in ventricular automaticity in contrast to the huge enhancement of it induced by isoproterenol (Wilkerson et al. 1971). Cardiac effects of both, glucagon and isoproterenol are considered to be resultant of the stimulation of specific receptors (glucagon receptors and  $\beta$ -adrenergic receptors, respectively) associated with Gs protein, which causes AC activation and the consequent increase of cAMP production in myocardium (Brodde and Michel 1999, White 1999). However, glucagon seems to be weaker than isoproterenol in activating AC in rodents (Ostrom et al. 2000) as well as in human hearts (Kilts et al. 2000). This agrees with the lower efficacy of glucagon when compared to isoproterenol

for increasing automaticity (present results) as well as contractility in the rat ventricular myocardium (Gonzalez-Muñoz et al. 2008).

PDE hydrolyzes cAMP produced by glucagon and limits its contractile effect in rat myocardium since inhibition of PDE enhance both cAMP levels and inotropic responses to glucagon in this tissue (Juan-Fita et al. 2004, Juan-Fita et al. 2005), but whether or not PDE blunts its effect on ventricular automaticity was unknown and was investigated in the present work. To this purpose, we studied the effects of glucagon in the presence of the non selective PDE inhibitor IBMX. IBMX (10  $\mu$ M), on its own, increased ventricular frequency by  $9.5 \pm 4.3$  beats  $\text{min}^{-1}$ , (n=5). This is consistent with other results showing enhancement of cardiac automaticity after PDE inhibition and suggest a basal PDE activity which reduces ventricular automaticity by hydrolyzing cAMP (Vinogradova and Lakatta 2009). Our results reveal that IBMX increases the effect of glucagon on ventricular automaticity (Fig. 1b, Fig. 2). Indeed, the maximal efficacy of glucagon increased from  $11.0 \pm 4.1$  beats  $\text{min}^{-1}$  in the absence to  $45.3 \pm 3.2$  beats  $\text{min}^{-1}$  in the presence of IBMX (n=5,  $p < 0.05$ ); thus, indicating that PDE also blunts proarrhythmic effects of glucagon in this tissue. Consequently, basal PDE activity seems to limit ventricular automaticity and protects myocardium against arrhythmias resulting from latent pacemaker overactivity induced either spontaneously or by cAMP producing agents such as glucagon. Therefore, PDE inhibition might be deleterious due to a potential proarrhythmic effects resulting from enhancement of cardiac automaticity. Indeed, a higher incidence of sudden death, attributed to cardiac arrhythmias has been reported in patients taken PDE inhibitors (Crickshank 1993).

In summary, the present study provides the first evidence that PDE limits proarrhythmic effects of glucagon. This might have some practical interest since both glucagon and PDE inhibitors are considered to be useful agents for treating toxicity induced by cardiodepressant agents such as  $\beta$ -adrenoceptor blockers or calcium channels blockers (DeWitt and Waksman 2004). Although combining

PDE inhibitors and glucagon produce a synergist inotropic effect (Gonzalez-Muñoz 2008), which should be beneficial in this case, it might also enhance automaticity thus increasing the risk of cardiac arrhythmias. However, the clinical relevance of these finding remains to be determined.

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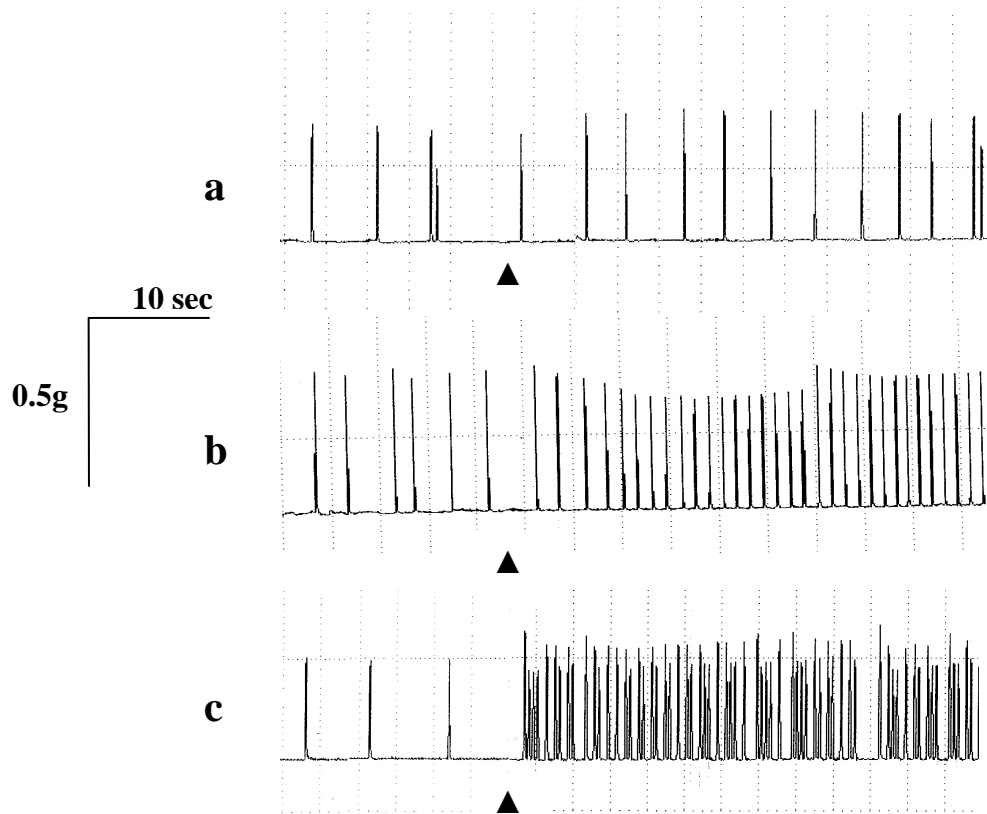
**Figures Legend:**

**Fig. 1:**

Recorded data from representative experiments showing the effects of 1  $\mu\text{M}$  concentration (applied at the arrow) of glucagon in the absence (**a**) and in the presence (**b**) of the non selective phosphodiesterase inhibitor IBMX (10  $\mu\text{M}$ ) and isoproterenol (**c**) on the spontaneously beating right ventricle of the rat heart.

**Fig. 2:**

Effects of isoproterenol and glucagon on ventricular automaticity. Concentration-response curves to isoproterenol ( $\square$ ) and glucagon in the absence ( $\bullet$ ) and in the presence ( $\circ$ ) of IBMX (10  $\mu\text{M}$ ). The frequency (contraction rate per min) was calculated as the average rate of the preparation recorded during the incubation period for each drug concentration. Each point represents the mean value  $\pm$  SEM (vertical bars) of 5-7 experiments. \*P<0.05 versus glucagon alone.



**Fig. 1:**

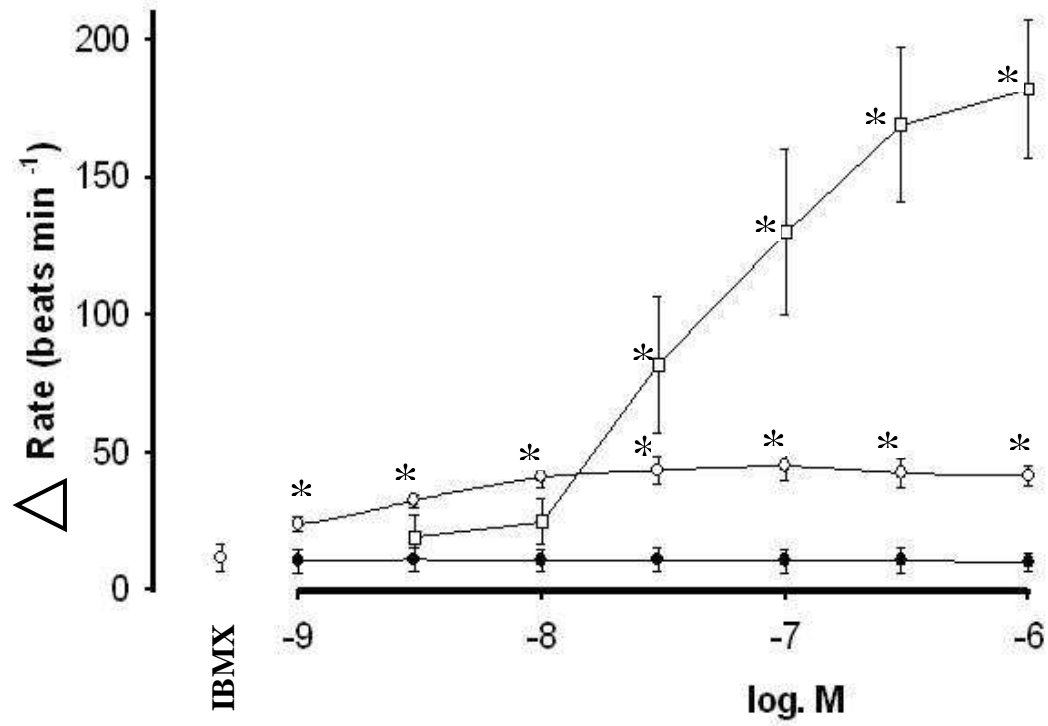


Fig. 2: