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INFLUENCE OF EXTRA-CELLULAR CALCIUM ON THE ADDITIVE EFFECT OF CAFFEINE ON THE CARDIAC RESPONSE TO CATECHOLAMINE

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ABSTRACT

At different extra-cellular calcium concentrations, the positive inotropic effect of isoproterenol and isoproterenol in combination with caffeine, a phosphodiesterase inhibitor have been evaluated in the isolated frog heart and the isolated guinea pig left atria to investigate whether extra-cellular calcium produces any effect on the additive effect of the caffeine on the cardiac response to catecholamine.

Cumulative dose response study of isoproterenol and isoproterenol in presence of caffeine at different extra-cellular calcium concentration was performed. The study revealed an increase in additive effect of caffeine on the cardiac response to catecholamine with increase in extra-cellular calcium concentration, but increase in extra-cellular calcium concentration decreased the myocardial responsiveness to additive effect of caffeine and isoproterenol combination.

The mechanism of positive inotropic effect of isoproterenol and caffeine involves increase in intracellular cAMP by different pathways and extra-cellular calcium produces positive inotropic effect by initiating the interaction between the contractile proteins actin and myosin.
The study revealed that an increase in the concentration of extra-cellular calcium increased the additive effect of caffeine and isoproterenol combination, but a decrease in the myocardial responsiveness was observed.

Keywords: *Positive initopic effect, isoproterenol, caffeine, catechloamine, intracellular cAMP.*

INTRODUCTION

Isoproterenol produces positive inotropic effect[1]. It increases cAMP by stimulating adenyl cyclase, which is activated through a guanine nucleotide binding protein 'Gs' subsequent to occupation of the beta-adrenergic receptors by isoproterenol.[2] Caffeine produces positive inotropic effect by inhibiting the phosphodiesterase enzyme, which is required for the inactivation of cAMP intracellularly[3].Activation of cAMP dependent protein kinase causes phosphorylation of calcium channels of sarcolemma, which leads to enhanced calcium influx.[4] The elevated calcium in the cytosol triggers calcium induce calcium release from sarcoplasmic reticulum which further increases calcium concentration in the cytosol. This calcium causes actin myosin interaction through calcium binding to troponin-c and brings about contraction.[5]
Calcium is one of the essential factor for the excitation-contraction coupling in the cardiac muscle cell. Intracellular calcium stores can be separated in to four main compartments 1.

Intracellular free or activator calcium 2. Calcium troponin complex 3. Sarcoplasmic reticulum 4. Mitochondria[7]. Influx of extracellular calcium in to the cell triggers the release of calcium from intracellular stores such as the cystarnae of sarcoplasmic reticulum, mitochondria and various fixed and soluble intracellular proteins. Thus there is increase in the intracellular free calcium concentration from a resting level of 0.1um to an activating level of 10um[8].The increase in intracellular calcium, binds to troponin c resulting in a conformational change in the tropomysin protein and myosin interacts with the active site on the actin filament. Sliding of the actin filaments over the myosin filaments produces the myocardial contraction[5].

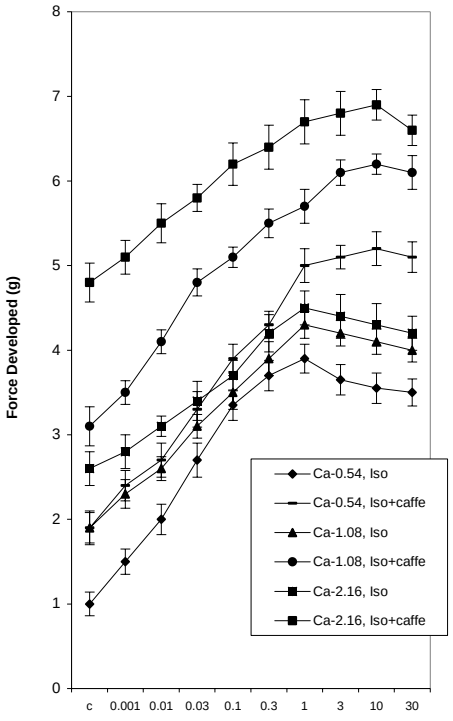

Fig.1: Dose response curve for the positive inotropic effect of isoproterenol (Iso) alone and with Caffeine (Caffe) on isolated frog heart at different calcium concentrations

Fig.1: Dose response curve for the positive inotropic effect of isoproterenol and isoproterenol in combination with caffeine on isolated frog heart at different calcium concentrations.

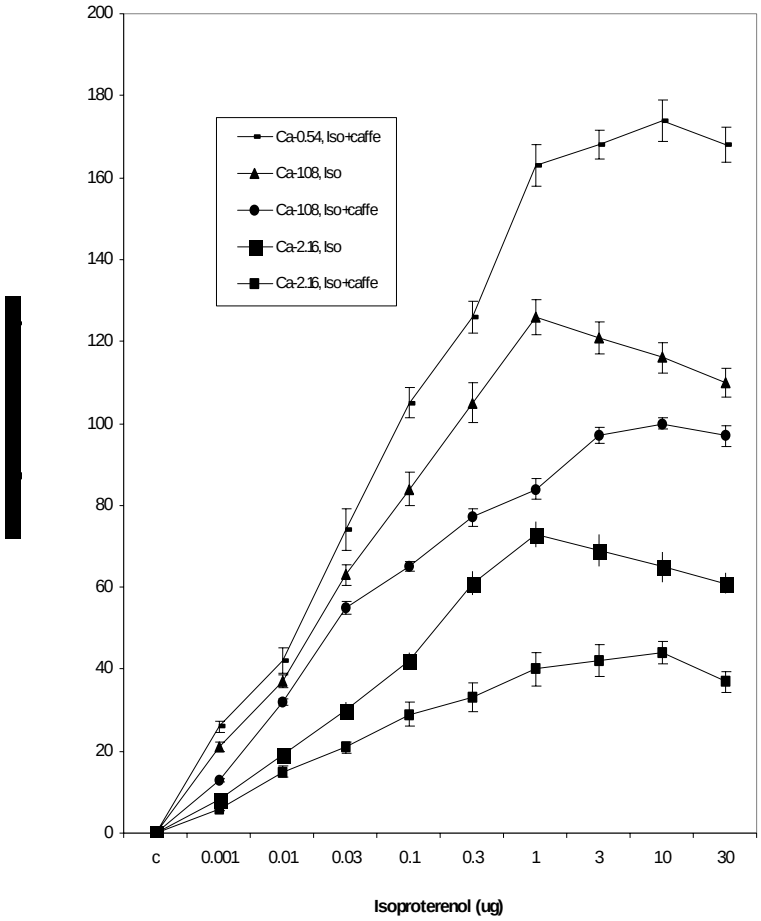

Fig. 2 Dose response curve for the positive inotropic effect of isoproterenol (Iso) alone and with Caffeine (Caffe) on isolated frog heart at different calcium concentrations

Fig.2 :Dose response curve for the positive inotropic effect of isoproterenol and isoproterenol in combination with caffeine on isolated frog heart at different calcium concentrations

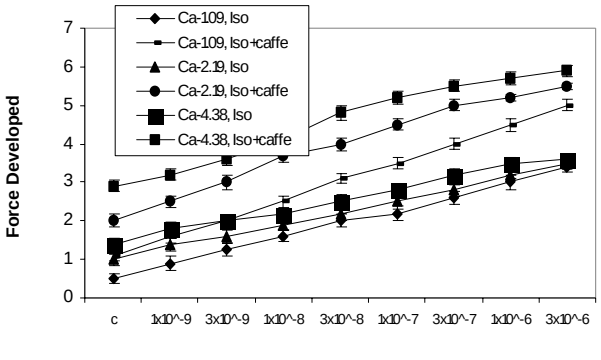

Fig. 3: Dose response curve for the positive inotropic effect of isoproterenol (Iso) alone and with Caffeine (caffe) on isolated guinea pig left atria at different calcium concentrations

Fig.3 :Dose response curve for the positive inotropic effect of isoproterenol and isoproterenol in combination with caffeine on isolated guinea pig left atria at different calcium concentrations

EXPERIMENTAL

Isoproterenol (Sigma, USA), caffeine (E-Merck India Limited, Mumbai.), calcium chloride (Loba-Chemie and S. D. Fine chemicals, Mumbai), and all other chemicals and reagents were of analytical grade and were used as received.

Guinea pigs were a generous gift from Hindustan Lever Research Centre Mumbai. Industrial Oxygen Company Ltd., Patalganga, supplied Carbogen gas.

Preparation of Isoproterenol stock solution: Isoproterenol was dissolved in 0.05 N HCl and serially diluted with distilled water to obtain concentrations in the range of 1ng to 100µg / 0.1 ml and administered alone and in presence of caffeine (1×10^{-7} M) in the isolated frog heart. In case of guinea pig left atrial experiments a stock of 1×10^{-2} M isoproterenol was prepared in 0.05 M. HCl. Appropriate volumes of the stock of the isoproterenol was then administered to the bathing solution to obtain the required final concentration ranging from 1×10^{-9} M to 3×10^{-6} M alone and in the presence of caffeine (1×10^{-7} M).

Preparation of Caffeine stock solution: The stock solution of caffeine (1×10^{-3} M) was prepared in distilled water. Appropriate volume of this solution was added to the frog ringer solution to obtain the concentration of 1×10^{-7} M. and administered as a continuous infusion to the isolated frog heart. In the isolated guinea pig left atria appropriate volume of the stock solution was added to Chenoweth-Koelle solution to obtain the concentration of 1×10^{-7} M.

Measurement of inotropic effect in isolated frog heart: Frogs (Rana tigriana) of either sex were used throughout the study. Frogs were stunned by a sharp blow on the head and then pithed. The heart was dissected out from the animal and mounted on a modified heart perfusion apparatus.[5] The frog's ringer solution was used as perfusate having the composition (mM): NaCl: 11.11, KCl- 1.88, $CaCl_2$- 1.08, $NaHCO_3$- 2.38, NaH_2PO_4- 0.07 and glucose 11.1 (pH 7.8). The solution was constantly bubbled with air at room temperature. The contractile force developed by the heart was recorded on a student's physiograph (Bio-Devices, Ambala- 134003) through force displacement transducer (Inco Model- T305).

Measurement of inotropic effect in isolated guinea pig left atria: Experiments were performed on the guinea pigs of either sex weighing between 300 to 400 g. Guinea pigs were stunned by a sharp blow on the head and cervical dislocation was quickly performed. The left atria was rapidly isolated from the heart of animal and placed in 20 ml organ bath containing Chenoweth-Koelle solution of composition (mM): NaCl: 135, KCl: 5, $CaCl_2$: 2.18, $MgCl_2$: 2, $NaHCO_3$: 19 and glucose: 9.9 (pH 7.4). A resting tension of 1g was applied to tissue. The atrium was electrically stimulated to 1Hz with square wave pulses of 1.5 m/sec duration, at twice threshold voltage by bipolar platinum electrode connected to a stimulator (Medicare student stimulator). Left atrial contractions was recorded on a student's physiograph (Bio-Device, Ambala- 134003) through force displacement transducer (Inco Model T- 305) and the tension developed was expressed in gram.

In case of frog heart experiments, 0.1 ml isoproterenol was administered as a bolus injection. Caffeine was administered as a continuous infusion at the rate of 1 ml/min and calcium of different concentrations were administered as a part of ringer solution. In the guinea pig left atrial experiments isoproterenol was administered via bathing solution containing calcium and caffeine. Force measurements were made after peak response was developed.

DATA ANALYSIS

All the experiments were repeated six times. The values are expressed as mean ± s.e.m. Statistical evaluation were performed by using the student's 't' test for paired values. A 'P' value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Positive inotropic effect of isoproterenol under the influence of different extracellular calcium concentrations: In the isolated frog heart isoproterenol produced dose dependent positive inotropic effect at different extracellular calcium concentrations (fig.1). At low (0.54 mM), Normal (1.08 mM) and high (2.16 mM) buffer calcium level, isoproterenol produced dose dependent positive inotropic effect in the dose range of 1ng to 1 µg with increase in magnitude of inotropic effect with increase in calcium concentration. At all calcium concentrations maximum inotropic response reached at the dose of 1µg. When the effect of isoproterenol is expressed as a percent change in basal force development, 1 µg of isoproterenol at 0.54 mM, 1.08 mM and 2.16 mM calcium resulted in 290%, 126% and 73% increase in contractile force respectively (fig 2).

In the isolated guinea pig left atria isoproterenol produced dose dependent positive inotropic effect at different extracellular calcium level (fig 3). At low (1.09 mM), normal (2.19 mM) and high (4.38 mM) buffer calcium level isoproterenol produced dose dependent positive inotropic effects in the dose range of 1×10^{-9} to 3×10^{-6} M. with increase in magnitude of inotropic effect with increase in calcium concentration. At all calcium concentrations maximum inotropic response reached at the dose of 3×10^{-6} M. When the effect of isoproterenol is expressed as a

percent change in basal force development, then at 1.09 mM, 2.19 mM and 4.38 mM calcium, 3 x 10^{-6} M isoproterenol resulted in 580%, 250% and 157% increase in contractile force respectively (fig 4).

Positive inotropic effect of isoproterenol in combination with caffeine (1 x 10^{-7} M) under the influence of different extracellular calcium concentrations: In the isolated frog heart isoproterenol in combination with caffeine (1 x 10^{-7} M) produced dose dependent positive inotropic effect at different extracellular calcium levels (fig 1). At low (0.54 mM), Normal (1.08 mM) and high (2.16 mM) buffer calcium level, isoproterenol in combination with caffeine produced dose dependent positive inotropic effect in the dose range of 1ng to 1 µg with increase in magnitude of inotropic effect with increase in calcium concentration. At all calcium concentrations maximum inotropic response reached at the dose of 1ug. When the effect of isoproterenol in combination with caffeine is expressed as a percent change in basal force development, 1 µg of isoproterenol at 0.54 mM, 1.08 mM and 2.16 mM calcium resulted in 174%, 100% and 44% increase in contractile force respectively (fig 2).

In the isolated guinea pig left atria isoproterenol in combination with caffeine (1 x 10^{-7} M) produce dose dependent positive inotropic effect at different extracellular calcium level (fig 3). At 1.09 mM, 2.19 mM and 4.38 mM buffer calcium level isoproterenol in combination with caffeine produced dose dependent positive inotropic effects in the dose range of 1 x 10^{-9} M to 3 x 10^{-6} M. with increase in magnitude of inotropic effect with increase in calcium concentration. At all calcium concentrations maximum inotropic response reached at the dose of 3 x10^{-6} M. When the effect of isoproterenol in combination with caffeine is expressed as a percent change in basal force development, then at 1.09 mM, 2.19 mM and 4.38 mM calcium, 3 x 10^{-6} M isoproterenol resulted in 354%, 175% and 103% increase in contractile force respectively (fig 4).

Isoproterenol is a beta-adrenergic agonist. It selectively acts on the beta-receptors of the heart and produces positive inotropic and chronotropic effect. The beta-adrenergic receptors belong to the super family of membrane receptor and its signal transduction mechanism involves coupling to 'G' protein. Beta-receptors are coupled to adenyl cyclase which catalyses the conversion of ATP to cAMP via guanine nucleotide protein Gs[10].In the absence of agonist, guanine diphosphate (GDP) is bound reversibly to the Gs protein. Interaction of the agonist with the receptor brings about a conformational change in protein receptors which causes reduction in the affinity of Gs protein for GDP and a concomitant increase in affinity for guanosine triphosphate (GTP). The alpha 3 subunit of Gs protein and GTP bound to it, dissociates from the receptor G protein ternary complex and binds to adenyl cyclase and activates the enzyme. The bound GTP then undergoes hydrolysis to GDP and the receptor Gs protein complex returns to basal state[11].

The intracellular function of the second messenger cAMP activates the protein kinases, which phosphorylates the specific proteins and alter their function, producing inotropic effect.[16] The action of cAMP is terminated by a class of enzyme phosphodiesterase, which catalyses the hydrolysis of cAMP to AMP.

Caffeine, a phosphodiesterase inhibitor produces positive inotropic effect by inhibiting the phosphodiesterase enzyme, which is required for the inactivation of cAMP intracellularly[13] .

Isoproterenol and caffeine produces the positive inotropic effect by increasing myocardial cAMP concentration through beta-adrenergic receptors and through inhibition of phosphodiesterase enzymes respectively[7, 14]. Thus, isoproterenol and caffeine produces positive inotropic effect by increasing myocardial cAMP through different pathways.

Calcium is essential for the excitation-contraction coupling in cardiac muscle as well as for the conduction of electrical impulses in certain regions of the heart particularly through the AV node[6]. Depolarization of myocardial fibers open the voltage regulated calcium channels and causes slow inward calcium current that occur during the action potential plateau. The current allows permeation of calcium, which is sufficient to trigger the release of additional calcium from the sarcoplasmic reticulum thereby causing contraction[5].

In both the amphibian and mammalian myocardia, the cumulative-dose response study of isoproterenol alone and in combination with caffeine at different extra-cellular calcium concentration produced increase in the additive effect of caffeine on the cardiac response to catecholamine with increase in extra-cellular calcium concentration. The positive inotropic effect of isoproterenol and caffeine is produced by increase in intracellular cAMP by different pathways[1, 3] while extracellular calcium produces positive inotropic effect by initiating the interaction between the contractile proteins actin and myosin[5]

The responsiveness of myocardia to the effect of isoproterenol alone and in combination with caffeine decreases with the increase in extra-cellular calcium concentration because increase in the extra-cellular calcium concentration increases the basal developed force and it is possible that the maximum force generated by a cardiac tissue has its limit. Isoproterenol alone and in combination with caffeine at maximum concentration are expected to increase the contractile force up to the maximum limit of the tissue and not beyond it. As extracellular-calcium concentration increases, isoproterenol alone and in combination with caffeine produces a slight increase in the contractile force because at higher extra-cellular calcium level the basal developed force is already high. The increase in contractile force is very small as compared to the high basal force developed. **T**his could possibly explain reduction in the percent increase of the contractile force over the basal developed force when the extra-cellular calcium level was increased.

The study revealed that in the amphibian and mammalian heart increase in extra-cellular calcium concentration increased the additive effect of caffeine with isoproterenol, but decreased the sensitivity of isolated frog heart as well as isolated guinea pig left atria to the additive effect of caffeine with isoproterenol.

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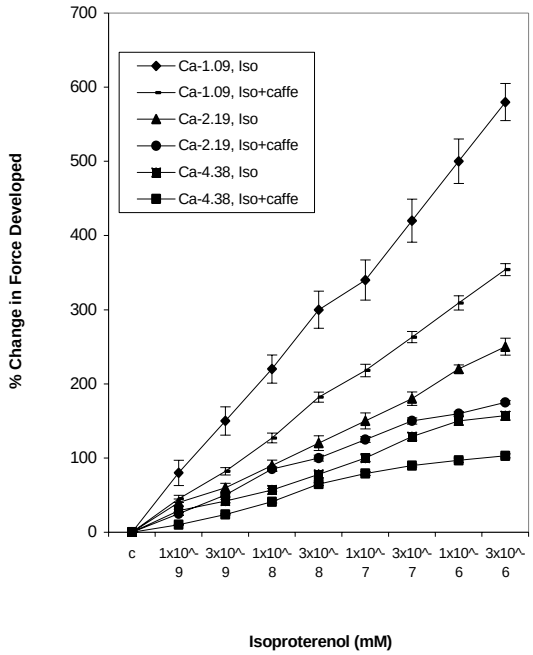

Fig. 4: Dose response curve for the positive inotropic effect of isoproterenol (Iso) alone and
with Caffeine (Caffe) on isolated guinea pig left atria at different calcium concentrations

Fig.4: Dose response curve for the positive inotropic effect of isoproterenol and isoproterenol in combination with caffeine on isolated guinea pig left atria at different calcium concentrations