***Dose-Dependent Effects of Cardiotonic on Myocardial Contractility: Insights from Isolated Heart Studies***

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**Abstract**

Cardiotonics are of pivotal importance in the treatment of heart failure through modulation of contractility of the myocardium. These drugs, such as cardiac glycosides (e.g., digoxin), β-adrenergic agonists (e.g., dobutamine), and phosphodiesterase inhibitors (e.g., milrinone), increase cardiac output by modulating intracellular calcium handling and myocardial energy metabolism. Appreciation of their dose-dependent effects is important in order to realize optimal therapeutic benefits with reduced side effects, such as arrhythmias and myocardial toxicity.(1)

This review deals with the pharmacodynamics of cardiotonics, including their mode of action, receptor interaction, and downstream signaling. It also deals with the methods in isolated heart preparations, i.e., Langendorff and working heart models, which are important to the understanding of drug-induced alterations in myocardial contractility. Further, it deals with the important observations regarding their dose-dependent effects on myocardial contractility, having in view the therapeutic benefit vs. toxicity ratio.(2)

Particular emphasis is placed on comparative investigation of various cardiotonic agents, the contribution of genetic heterogeneity to drug efficacy, and new principles to increase the safety and effectiveness of the drugs in the clinic. New directions for new cardiotonic agents and their therapeutic application in heart failure are also addressed.

**Keywords:**

**Introduction**

Cardiotonics, including cardiac glycosides, β-adrenergic agonists, and phosphodiesterase inhibitors, are widely used to enhance cardiac output in heart failure patients. Their effects on myocardial contractility depend significantly on dosage, as both subtherapeutic and supratherapeutic doses can lead to diminished efficacy or toxicity. This review aims to provide a comprehensive understanding of the dose-dependent effects of cardiotonics using evidence from isolated heart models.(3)

Cardiotonics are used to correct heart failure since they can increase the force of

contraction (positive inotropy) of a hypodynamic heart. Experimentally hyperdynamic heart might

was replicated using an isolated frog heart perfused with altered Frog Ringer Solution. This

modified Ringer contains 1/4 of the CaCl2 of the standard Frog Ringer solution. The standard

Ringer contains 0.12 g/lit of CaCl2 while the modified Ringer which was utilized to establish a hyperdynamic heart has 0.03 g/lit of CaCl2(4)

**Heart failure**

Heart failure is defined as a condition in which the cardiac output is decreased and the heart is unable to meet the body‘s oxygen and blood supply demands.

**Cardiotonic:**

These are the drugs that increase the force of contraction of the cardiac muscles in the failing heart (during heart failure). These drugs include Na+-K+-ATPase inhibitors, and positive inotropic agents (sympathomimetics, parasympatholytics, xanthines, etc.)

**Prepation**

**a. Frog heart isolation and mounting**

Holding the frog: Hold the frog so that the thumb of the left hand is against its back. The right front leg of the frog is placed between the left-hand index and middle finger and remaining two fingers are on its back. The frog's left front leg and hind legs are not held back.

**b. Pithing position:**

Pithing is performed at the intersection of the cranium and atlas vertebra (this is similar to the foramen magnum). The position of foramen magnum is determined by gliding pithing needle along the midline on the frog's head. Pithing must be performed at the site where first slight depression is experienced.(5)

**c. Pithing:** Put a sharp needle in the foramen magnum in the direction of the brain and kill part of it. Then pull out and put the needle inside the opened spinal canal and kill part of the spinal cord by putting the needle in the opposite direction. This can make the frog urinate and put its hind legs into convulsion.

**d. Testing the reflexes:**

 To determine if the frog has been properly pithed, touch eye cornea with needle and observe if corneal responses have totally ceased. Even 'touch and pain' reflexes can be tested by superficial pricking of hind leg of the frog to observe if jerking movement is elicited. A properly pithed frog does not exhibit corneal or pain reflexes.

**e. Place the pithed frog** back on its back. Make a small 'V' shaped cut in the abdominal skin along the pelvic girdle with fine scissors. Insert curved scissors inside this 'V' shaped cut and dissect the abdominal skin until the pectoral girdle.(6)

**f. The deeper muscular part** reveals the rectus abdominis muscle. Make a sharp cut on one side of the mid-vein. With this, cut by the blunt edge of scissors and continue up to the pelvic girdle without cutting the visceral organs.

Section the pelvic girdle by a bone cutter or bigger scissors to reveal the heart.

**g. Cut pericardium** with the aid of blunt forceps but not to cause injury to the heart. Use the left hand's thumb to push upward the heart's ventricle and identify the sinus venosus.

**h. Initiate a poor flow of P. S. S**. through the cannula. Pass the cannula into the central vein of the sinus venosus through the incision and secure it with the thread. Open the aorta to expel the perfusate.

**i. Grasp the cannula** with the left middle and left index finger and just raise it up. Cut the adherent tissues around the heart slowly with scissors and let go of the perfused heart.

**j. Restraint released heart** on the stand in the position depicted. Pass pin Attached to Starling's lever, superficially into ventricle wall tip. Position the lever so that it becomes horizontal.

**k. After placing the heart** with normal Ringer solution, introduce the flow of the modified Ringer and see if the force of contraction (amplitude of recording) of the heart decreases.

**l. After a decreased force** of contraction is seen, begin the dosing with Digoxin as 01. ml, 0.2 ml, 0.3 ml etc. and note the activity of the heart. Record the heart rage and see how the force of contraction varies(7)

**2. Pharmacodynamics of Cardiotonic**

**2.1 Cardiac Glycosides**

Cardiac glycosides like digoxin, ouabain, and digitoxin are potent inotropic drugs used mainly to treat heart failure and some arrhythmias. Their main mode of action is Na⁺/K⁺-ATPase inhibition, which is a vital membrane-bound enzyme that helps maintain ionic gradients across the cardiac myocyte membrane.(8)

By inhibiting Na⁺/K⁺-ATPase, cardiac glycosides lead to an increase in intracellular sodium concentration. This interferes with the sodium-calcium exchanger (NCX), leading to a decrease in the extrusion of calcium and an intracellular accumulation of calcium. The increased calcium enhances calcium-induced calcium release (CICR) from the sarcoplasmic reticulum, eventually enhancing myocardial contractility (positive inotropic effect).

But the dose-dependent actions of cardiac glycosides have to be carefully titrated. In therapeutic doses, digoxin enhances cardiac output and lowers ventricular filling pressures, causing symptomatic relief in heart failure patients. Its vagomimetic effect on the parasympathetic nervous system also prolongs AV conduction, and hence it is a good drug in the management of atrial fibrillation.(9)

At toxic levels, hyperaccumulation of calcium produces delayed afterdepolarizations (DADs), which cause ventricular arrhythmias, such as premature ventricular contractions (PVCs), ventricular tachycardia (VT), or fatal ventricular fibrillation (VF). Nausea, vomiting, blurred vision (yellow-green halos), and central nervous system impairment are also manifestations of digoxin toxicity.

**Clinical Considerations:**

- Therapeutic Window: Tapered; needs close regulation of plasma concentration (0.5–2.0 ng/mL).

- Electrolyte Sensitivity: Hypokalemia, hypomagnesemia, and hypercalcemia predispose to toxicity.

- Drug Interactions: Quinidine, amiodarone, and verapamil raise digoxin levels, with the need to adjust doses appropriately.

- Antidote: Digoxin-specific antibody fragments (Digibind® or DigiFab®) for severe toxicity.(10)

**2.2 β-Adrenergic Agonists**

β-Adrenergic agonists are a group of cardiotonic drugs that increase myocardial contractility through the stimulation of β1-adrenergic receptors in the heart. These drugs, such as dobutamine, dopamine, and isoproterenol, are commonly employed in acute heart failure, cardiogenic shock, and short-term inotropic support situations.(11)

**Mechanism of Action**

β-Adrenergic agonists work by stimulating β1-adrenergic receptors, which are associated with the Gs protein. This results in:

Activation of adenylyl cyclase, the enzyme that converts ATP to cyclic AMP (cAMP). Augmentation of levels of cAMP, leading to activation of protein kinase A (PKA). Phosphorylation of L-type calcium channels with increased entry of calcium into myocardial cells. Increased calcium-induced calcium release (CICR) from sarcoplasmic reticulum with increased myocardial contractions (positive inotropic effect).(12)

**Dose-Dependent Effects**

**Low to Moderate Doses:** Enhance cardiac output by enhancing stroke volume but having little effect on heart rate or peripheral resistance.

**High Doses:** Activate β1- and β2-adrenergic receptors, resulting in augmented heart rate (positive chronotropic effect), arrhythmias, and enhanced myocardial oxygen consumption. Under some circumstances, β2-mediated vasodilation will result in hypotension.(13)

**Clinical Considerations**

**Dobutamine**: Acts mainly on β1 receptors with potent inotropic activity and minimal chronotropic activity, thereby being a drug of choice in acute decompensated cardiac failure.

**Dopamine**: Exhibits dose-dependent effects—low doses stimulate dopamine receptors, moderate doses stimulate β1 receptors, and high doses stimulate α1 receptors with vasoconstriction.

**Isoproterenol**: A non-selective β-agonist with augmented contractility and heart rate but with a high risk of arrhythmias.(14)

**Adverse Effects & Drawbacks**

Tachycardia & Arrhythmias: Because of β1 overstimulation.

**Increased Myocardial Oxygen Demand**: May worsen ischemia, especially in patients with coronary artery disease.

**Development of Tolerance:** Long-term administration results in desensitization of β-receptors with loss of effect over time.

**2.3 Phosphodiesterase Inhibitors**

Milrinone and other PDE inhibitors prevent the degradation of cAMP, leading to increased calcium influx and contractility. While effective, their dose-dependent effects include risks of hypotension and arrhythmias at higher doses.(15)

**3. Methodologies in Isolated Heart Studies**

**3.1 Langendorff Perfusion Model**

Langendorff perfusion model is a commonly applied ex vivo model for cardiac pharmacology, physiology, and toxicology research on an isolated heart. The model offers a controlled model to investigate the direct effect of cardiotonic drugs on myocardial contractility without interference from systemic circulation and neurohumoral control effects.(16)

**Principle & Methodology**

In Langendorff preparation, the animal heart (usually a rat, guinea pig, or rabbit) is removed and perfused retrogradely from the aorta with oxygenated physiological buffer solution (e.g., Krebs-Henseleit solution). This allows for even delivery of drugs and nutrients with preservation of myocardial function.(17)

**There are two perfusion modes:**

Constant Pressure Mode: The perfusion pressure is kept constant and change in coronary flow mirrors vascular responses.

Constant Flow Mode: Perfusing flow rate is held constant, and perfusion pressure changes reflect coronary resistance changes.(18)

**Key Measured Parameters**

Langendorff model facilitates dose-dependent action of cardiotonics to be observed in real time by measuring:

**Left Ventricular Developed Pressure (LVDP): Reflects myocardial contractility.**

**Heart Rate (HR):** Evaluates the chronotropic effect of drugs.

**Coronary Flow (CF):** Evaluates the vasodilatory or vasoconstrictive effect.

**Peak Rate of Pressure Development (+dP/dtmax):** Represents systolic function.

**Peak Rate of Pressure Decay (−dP/dtmax):** Reflects systolic contraction.

**Peak Rate of Pressure Decay (−dP/dtmin):** Reflects diastolic relaxation.(19)

**Applications in Cardiotonics Research**

**Cardiac Glycosides:** Assist in evaluating their inotropic effect and arrhythmogenicity based on calcium overload.

**β-Adrenergic Agonists:** The Langendorff perfusion model offers a controlled environment for the study of the direct effects of β-adrenergic agonists on myocardial oxygen consumption and contractility uninfluenced by systemic factors. The method is especially advantageous in assessing their dose-dependent inotropic and chronotropic effects while observing for possible adverse effects like arrhythmias and augmented metabolic requirements.(20)

**Measurement of Contractility Effects**

β-Adrenergic agonists like dobutamine, isoproterenol, and dopamine increase myocardial contractility by stimulating β1-adrenergic receptors and resulting in:

✔ Augmented Left Ventricular Developed Pressure (LVDP): Direct contractility measure.

✔ Augmented dP/dtmax: Is the maximum ventricular pressure rise rate during systole.

✔ Elevated heart rate (HR): Because of positive chronotropic effects.

✔ Elevated coronary flow (CF): Secondary to β2 receptor stimulation in coronary arteries.(21)

**Oxygen Consumption Measurement (MVO₂)**

Langendorff preparation provides for online myocardial oxygen consumption (MVO₂) measurement of oxygenated buffer solutions and coronary effluent sampling. Observations are as follows:

MVO₂ increases proportionally to higher doses of β-agonist, which corresponds to increased ATP demand.

Ischemic states may be provoked by excessive β1 stimulation, particularly in energy-starved myocardium.(22)

β2-mediated vasodilation prevents ischemia but can potentially cause hypotension in vivo.(23)

**Clinical and Research Implications**

**Risk of Cardiotoxicity:** Dosage increase may enhance arrhythmogenicity, necessitating establishment of a safe therapeutic window.

**Development of Tolerance:** Cumulative exposure may result in desensitization of β-receptors with a consequent decrease in effectiveness with time.

**Drug Optimization:** Comparative research on selective and non-selective β-agonists assists in their clinical optimization.**Phosphodiesterase Inhibitors:** Observe their influences on myocardial contractility and relaxation.(23)

**3.2 Working Heart Model**

Working Heart Model is a very sophisticated ex vivo apparatus for simultaneous measurement of afterload and preload and is therefore superior to the Langendorff model for physiological cardiac function studies. This configuration is also most convenient for establishing dose-response relationships of cardiotonics in near-physiological conditions.(24)

**Principle & Methodology**

In contrast to the Langendorff model, in which the heart is perfused passively, the Working Heart Model re-establishes a more physiological blood flow by simulating ventricular filling (preload) and ejection (afterload).

**Left atrial filling (Preload):** Oxygenated buffer solution (e.g., Krebs-Henseleit buffer) is filled into the left atrium, a simulation of venous return.

**Left ventricular contraction:** The heart ejects the perfusate into an afterload system producing resistance, a simulation of systemic circulation.

**Measurement of cardiac output (CO**): The model records ventricular pressure, flow rate, and stroke volume, which are important indicators of cardiac function.(25)

**Most Significant Measured Parameters**

Cardiac Output (CO): Volume of fluid pumped per minute.

Stroke Volume (SV): Volume of fluid ejected per beat.

**Left Ventricular Pressure (LVP):** Records changes in contractility with cardiotonics.

Preload and Afterload Dependency: Allows for proper assessment of the influence of cardiotonics on myocardial function in varying circulatory states.(26)

**3.3 Key Parameters Measured**

* **Contractile force** (e.g., left ventricular pressure)
* **Heart rate variations**
* **Intracellular calcium transients**
* **Oxygen consumption and metabolic responses**

**4. Dose-Dependent Effects on Myocardial Contractility**

**4.1 Low-Dose Effects**

At low concentrations, cardiotonics enhance contractility with minimal adverse effects, improving cardiac efficiency without significant increases in myocardial oxygen demand.(27)

**4.2 Therapeutic Dose Range**

In the therapeutic range, cardiotonics optimize cardiac output by balancing enhanced contractility and controlled heart rate, reducing symptoms of heart failure.

**4.3 High-Dose and Toxic Effects**

Excessive doses can lead to calcium overload, arrhythmias, and myocardial ischemia. Studies in isolated hearts demonstrate that beyond a critical concentration, the risk of adverse cardiac events increases significantly.(28)

**5. Clinical Implications**

Understanding the dose-dependent effects of cardiotonics is essential for individualized therapy in heart failure management. Therapeutic monitoring, especially for drugs like digoxin, is crucial to avoid toxicity while ensuring efficacy. Insights from isolated heart studies contribute to safer and more effective clinical dosing strategies.(29)

**6. Conclusion**

Isolated heart studies provide valuable data on the dose-dependent effects of cardiotonics, revealing the fine balance between therapeutic benefits and toxicity. Future research should focus on developing novel cardiotonics with improved safety profiles and investigating personalized dosing strategies.(30)

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