Use of an “Isolated Chicken Embryonic Heart” In Vitro System to Study Dobutamine’s® Effect on Heart Rate and Ventricular Contractility

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The Effects of Cardiovasuclar Drugs on Heart Development
Abstract

The chick embryo is a classic model system used to study the pharmacological effect(s) of drugs, both in vivo and in vitro, on vertebrate heart development and physiology (McLaughlin and McCain, 1998). Herein, the McLaughlin and McCain protocol is redesigned in order to research the effects of specific cardiovascular drugs on the embryonic vertebrate heart without innervation. Data to support the use of “isolated chicken hearts in CMRL media” as an improved in vitro model system is presented, as is data revealing how the drug DOBUTamine™ affects the embryonic heart rate when utilizing this system. Clinically, DOBUTamine™ is administered as a short term therapy used to treat patients undergoing heart failure. It’s distributed as a racemic mixture consisting of a (+) and (-) isomers. The (+) isomer is a β1-adrenergic agonist which acts on β1 receptors and stimulates cardiac output (CO) by augmenting stroke volume (SV) through enhanced left ventricular contractions (Ahonen et. al., 2008). The (-) isomer is a α1 agonist responsible for stimulating vasoconstriction in the systemic circuit. In vitro data from “isolated chicken embryonic hearts in CMRL media” suggests that DOBUTamine™ stimulates the embryonic HR in a dose dependent manner and induces strong ventricular contractions at the high concentrations. Additionally, cardiac arrest and specific arrhythmias are noticed, such as atrial flutter and tachycardia, at toxic levels. The use of “isolated chicken hearts in CMRL media” is recommended as an ideal in vitro research and/or teaching model system to study
drug induced effects on the vertebrate heart’s developing intrinsic circuitry and cardiac cycle.

McLaughlin, J. S. and McCain, E. R. (1998). Developmental and physiological aspects of the chicken embryonic heart. Tested Studies for Laboratory Teaching, Volume 20 (C.A. Goldman, Editor). Proceedings of the 20th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 20: 85-100. [This publication has recently been peer-reviewed and is now showcased as multi-media learning material for the SDB BioScience Education Network and also appears as a nationally-recognized learning corpus of material on chicken heart development for the Education website of the Professional Development and Curriculum Committee of the Society of Developmental Biology (SDB) URL: www.sdbonline.org/index.php?option=com_content&task=view&id=27&it.]

Introduction

Dobutamine was synthesized by Dr. Ronald Tuttle at Eli Lilly & Company. Clinically, DOBUTamine™ is administered as a short term therapy used to treat patients undergoing heart failure. It’s distributed as a racemic mixture consisting of a (+) and (-) isomers. The (+) isomer is a β1-adrenergic agonist. This agonist acts on β1 receptors and stimulates the cardiac output (CO) in a variety of ways (Ahonen et. al., 2008). Dobutamine causes an increase in CO by augmenting stroke volume (SV) through enhanced left ventricular contractions (Ahonen et. al., 2008). The (-) isomer is a α1 agonist. This isomer is responsible for stimulating vasoconstriction in the systemic circuit.

The chick embryo is the classic model system used to study the pharmacological effects of drugs on vertebrate heart development due to its similar developmental patterns and processes (Harvey et. al., 1999); (McLaughlin and McCain,1999). Herein, the McLaughlin and McCain protocol was redesigned using “isolated chicken hearts in CMRL media” to research dobutamine’s effect on the 5-day embryonic chick heart rate (HR) without innervation. It was hypothesized that dobutamine would increase heart rate (HR) and enhance left ventricular contractility in a dose dependent manner until reaching toxic levels. It was also reasoned that arrhythmias would be diagnosed at higher concentrations.
Materials and Methods

I. “Windowing” the Egg according to the methods of Cruz, 1993:

An egg was placed in a bed of cotton and Magic Scotch tape was used to cover its surface. A hole was punctured in the ovular side of the egg using stainless steal scissors and 4mls of albumen was removed from the egg. Next, a “window” was cut in the egg to expose the embryo itself. The in vivo HR was taken 3x’s at 15 second intervals each, using a stopwatch. Data was then multiplied by 4 to get beats per minute (bpm). Average HR in bpm was then calculated.

Figure 1. A) An egg lying in a Syracuse dish lined with cotton; B) Albumen being removed from the egg; C) Cutting a “window” D) A “windowed” egg showing the in vivo embryo

II. Explanting” the Embryo according to the methods of Cruz, 1993:

A filter paper doughnut was placed a top the embryo and allowed to adhere. Using surgical scissors and forceps, the extra-embryonic membranes were cut around the filter paper and attached embryo. Using an embryonic spoon the filter paper and attached embryo were scooped into a pre-warmed Syracuse dish containing CMRL media then placed under a dissecting microscope. Gooseneck lamps were focused on the stage of the microscope to ensure a warm environment. The in vitro HR was taken 3x’s at 15 second intervals each, using a stopwatch. Data was then multiplied by 4 to get bpm. Average HR in bpm was then calculated.

Figure 2: A) Explanted 5-day embryo in embryonic spoon; B) Explanted 5-day embryo in Syracuse dish containing CMRL media.
III. Isolating the heart according to the methods of McLaughlin and McCain, 1998:
The heart was “isolated” by cutting above the bulbus cordis (BC) and below the sinus venosus (SV) using surgical scissors. The isolated heart was then placed into a clean, warm Syracuse dish containing new CMRL media. The HR of the isolated heart was taken in CMRL solution 3x’s at 15 second intervals each, using a stopwatch. Data was then multiplied by 4 to get bpm. Average HR in bpm was then calculated.

![Figure 3](image1.png)

**Figure 3:** A) The process of surgically “isolating” the heart of a 5-day chicken embryo; B) An *in vitro* photomicrograph of isolated heart

IV. Preparation of serial dilutions.
Serial 10-fold dilutions were created using a 12.5mg/ml DOBUTamine™ stock solution (Bedford Laboratories™ and CMRL media to yield $1.25 \times 10^{-4}$ mg/ml, $1.25 \times 10^{-3}$ mg/ml, $1.25 \times 10^{-2}$ mg/ml, $1.25 \times 10^{-1}$ mg/ml and 1.25mg/ml. All dilutions were stored at 10° C and then heated to approximately 33° C in order to replicate the environment of the chick embryo.

![Figure 4](image2.png)

**Figure 4:** Serial dilutions of DOBUTamine.
V. Application of Dobutamine

All research on DOBUTamine™ was performed on “isolated” 5-day chicken hearts in CMRL media with or without DOBUTamine™. CMRL media was removed from the Syracuse dish containing an “isolated” heart using a pipette after control HR’s were determined. The lowest dilution $1.25 \times 10^{-4} \text{ mg/ml}$ was added to the Syracuse dish, and after 60 seconds, the \textit{in vitro} HR was recorded as above. Using a plastic pipette this concentration was removed and the next highest concentration was added. This process was repeated until the highest concentration was used or until cardiac arrest (CR) occurred.

\textbf{Figure 5. Application of DOBUTamine}
Results

After all the HR’s were taken, histograms were plotted for each concentration of dobutamine and the average HR was calculated (Figures 6-10). As the concentration of dobutamine increased, the HR increased until reaching $1.25 \times 10^{-1}$ mg/ml where the HR began to decline. The highest concentration, $1.25$ mg/ml, led to cardiac arrest in nine out of the ten isolated hearts. Figure 11 shows the average HR of all 10 “isolated” hearts in each concentration of dobutamine. Strong ventricular contractions were noted $1.25 \times 10^{-3}$ mg/ml and $1.25 \times 10^{-2}$ mg/ml. It was also noted that there was strong ventricular contractility in $1.25 \times 10^{-1}$ mg/ml along with arrhythmias such as bouts of tachycardia, atrial flutter and fibrillation.

Figure 12 plots the average HR in the four different dobutamine concentrations tested. The average in vivo HR of the ten isolated hearts equaled 147.4bpm. Once the embryos were explanted and put into phosphate buffered saline (PBS), the average HR dropped to 75.4bpm. When the hearts were isolated in PBS solution the HR increased to 104.3bpm. Finally, when the isolated hearts were placed in CMRL media the average HR increased significantly to 135bpm, close to the in vivo heart rate.
Discussion and Conclusion

The data gathered herein gives evidence for the use of the “isolated chicken hearts in CMRL media” as an improved in vitro model system. The HR of the explanted embryo decreased significantly after being removed from its natural environment. Once the heart was “isolated” and placed in PBS, the HR decreased even more. This could be due to two reasons. First, when the heart was removed from the body, hormones such as adrenaline are could no longer influence the HR. Secondly, the PBS media is merely a salt solution that helps regulate ion concentrations; there are no amino acids or essential nutrients to sustain the heart for longer periods of time. Once the “isolated” heart was placed in CMRL media the HR drastically increased. The “isolated” heart in CMRL media had an average HR of 135 bpm. This data is significant because it comes extremely close to mimicking the HR of the in vivo system (147bpm). Consequently, the use of “isolated chicken hearts in CMRL media” is recommended as an ideal in vitro research and/or teaching model system to study drug induced effects on the vertebrate heart’s developing intrinsic circuitry and cardiac cycle.

The improved in vitro model system was then used to examine the effects of dobutamine on the 5-day isolated chicken heart. The data suggests that dobutamine stimulates the HR in a dose dependent manner until reaching toxic levels (figure 11). An optimal concentration was found to be $1.25 \times 10^{-2} \text{mg/ml}$. After this concentration, arrhythmias such as tachycardia, atrial flutter and fibrillation were noticed. Eventually the concentration levels became so toxic that cardiac arrest was induced. It was also noted that dobutamine stimulated
ventricular contractility in a dose dependent manner until toxic levels and cardiac arrest were induced.

**Figure 6**: Heart rate (bpm) of individual, “isolated” 5-day chicken hearts (#1-10) in CMRL media. Average heart rate equaled 135 bpm.

**Figure 7**: Heart Rate (bpm) of individual isolated hearts (1-10) in $1.25 \times 10^{-4}$ mg/ml dobutamine.
**Figure 8:** Heart Rate (bpm) of individual isolated hearts (1-10) in $1.25 \times 10^{-3}$ mg/ml dobutamine. Strong ventricular contractility noted in this concentration.

**Figure 9:** Heart Rate (bpm) of individual isolated hearts (1-10) in $1.25 \times 10^{-2}$ mg/ml dobutamine. Strong ventricular contractility noted in this concentration.
Figure 10: Heart Rate (bpm) of individual isolated hearts (1-10) in $1.25 \times 10^{-1}$ mg/ml dobutamine. Arrhythmias such as bouts of tachycardia, atrial flutter and fibrillation were observed in this concentration.

Figure 11: Average HR’s (bpm) of 5-day “isolated” chicken hearts (#1-10) in increasing concentration of dobutamine.
**Figure 12:** Average HR (bpm) 5-day chicken heart in the four different environments tested.
References:


