Abstract

Our educational module offers a new approach to studying cardiovascular function by utilizing a high-interest topic, effects of endotoxin-related septicemia (Lipopolysaccharides, LPS) from gram-negative bacteria. Research on LPS is abundant in the area of neuroscience and neuromuscular junction activity. However, little is known on its effects in cardiac tissue. We have developed and piloted several hands-on laboratory exercises including simple heartbeat counts (HR) to more advanced recording of diastolic and systolic periods with in-situ hearts. Furthermore, the laboratory module is highly flexible for virtual or in-person completion: we have adapted this module to both the frog and larval Drosophila melanogaster. The flexibility of this module also makes it adaptive to different classroom and laboratory settings, including CURiE courses. Utilization of comparative laboratory modules to combine topics with primary literature reviews can be used to foster a deeper understanding of the diversity of cardiovascular mechanisms possible among animals. Finally, this module contains pedagogical support for instructors interested in open inquiry and active learning approaches.

Methods

This module is divided into two main experimental procedures. One involves examining the effect of exposure to LPS on frog hearts to determine if there is an acute effect on the heart rate. The second and more challenging activity is to determine if there is any effect by LPS on the heart rate in larval Drosophila. The experimental exercises will be conducted following the protocol below. After the experimentation is completed, then the second lesson assignments can be completed and turned in along with the laboratory report.

Objectives

Students will learn:
1. How the frog heart is arranged anatomically compared to mammalian hearts.
2. The general anatomy of the cardiovascular system, relevant subsystems, and practical health-related terms associated with the cardiovascular system.
3. How the heart generates electrical activity and conducts electrical activity.
4. Types of septicaemia and how they arise.
5. The effects of bacterial septicaemia on the physiology of the body and general immune response.
6. The potential mechanism of action of LPS on heart rate.
7. Comparative cardiac physiology and immune response with insects and mammals.

Introduction

Traditional undergraduate anatomy and physiology courses follow standard protocols for laboratory experimentation, partly because of the commercialization of student equipment and pre-packaged software. As a result, much student laboratory coursework utilizes similar experiments across colleges and universities, such as measuring lung volumes as a human respiration lab or measuring properties of the frog gastrocnemius muscle. While such laboratory exercises have been shown to increase the students’ understanding in factual concepts, chronic reuse of the same pre-packaged lab exercises raises many concerns, including reduced academic diversity, loss of critical thinking opportunities, transfer of complex workflows directly among students of the same institution or indirectly from other institutions (e.g. downloading completed reports from internet websites), and lack of student interest and motivation to complete the work (Henige, K., 2011; Espaço et al., 2020).

To increase student interest and motivation, foster critical thinking skills, and promote academic diversity and independence of student work, there is strong interest in developing effective course-based undergraduate research experiences, or ACUREs (Bakshi et al., 2016; Linn et al., 2015). Effective ACUREs also have the potential to empower students to communicate novel scientific findings to the scientific community, thus helping the student develop scientific communication skills as well as a sense of scientific identity (Staub et al., 2016; Espaço et al., 2020). Furthermore, there are now increased numbers of journals which promote peer-reviewed undergraduate research (e.g. Malley et al., 2017). Such journals as IMPULSE (The Premier Undergraduate Neuroscience Journal; https://impulse.aaas.org/issues/2017) and American Journal of Undergraduate Research (AJUR) are overseen by faculty with the emphasis on publications by undergraduates.

In this paper, we describe the development and implementation of a flexible mini-ACUREs within a pre-existing upper division animal physiology course, that utilized pre-existing equipment, standardized lab procedures and readily available software packages. The novelty of the project is the use of experimental treatments with unknown effects within the context of a traditional laboratory exercise: the effects of lipopolysaccharides (LPS) from gram-negative bacteria on the contractile properties of frog and Drosophila hearts. Further, new findings are of interest to the scientific community, since LPS is known to trigger illness in humans such as septicaemia.

Summary

The frog preparation is amenable to student laboratories in physiology and for demonstrating pharmacological concepts to students. This preparation has been in use for over 100 years, and it still offers much as a model for investigating the generation and regulation of pacemaker rhythms and for describing the mechanisms underlying their modulation. This robust preparation is well suited to training students in physiology and pharmacology. The students will also learn to present data in graph form for statistical analysis. The frog heart is very easy to exposes with minimal dissection and the contractions are easy to record. A novel twist in standard physiology laboratory teaching exercises in the use of insects or invertebrates to address cardiac function. Crabfish and crabs are easy to use to monitoring heart rate in the intact functioning animal (insects-Bellenoit et al., 2010 and Bier and Bodmer, 2004; crab–crayfish Bierbower and Cooper, 2009; crabs-Wycoff et al., 2018). Injection of LPS (Saeltner et al., 2019) or other substances such as serotonin or dopamine (Listerman et al., 2000) can also be performed. Injecting compounds into the intact animal will likely have an effect on various physiological systems, such as ventilation rate (Schapker et al., 2002; Shuranova et al., 2003), and not just heart. Addressing how compounds might also have an effect on the autonomic nervous system in crustaceans can be addressed as a comparative exercise in the autonomic nervous system of different animals (Choma et al., 2011; Shuranova et al., 2006). Annelids (i.e., worms and leeches) are also able to be used with exposed hearts for direct application of compounds (Bohner 2008; Hallmann and Crisp, 2011; Sternt, et al., 1979).

If these experimental labs are to be performed each year or semester small variations can be implemented such as a different concentration of a substance or different strains of LPS. At some point in time, it would be nice to have multiple environments so it may add to the scientific literature. By tweaking the procedure with varied compounds, the lab reports will not likely be shared from student to student over the years as well.

Details are provided on web page: http://web.as.ksu.edu/Biology/Faculty/school/ABLE-2021/ABLE-2021_frog%20septicemia%20heart-LPS/Home.html.

References

Halfmann K, Wycoff M, Stoecklein K, Kojima K, 2009. A model for monitoring heart rate in the intact functioning animal (insects-Bellenoit et al., 2010 and Bier and Bodmer, 2004; crab–crayfish Bierbower and Cooper, 2009; crabs-Wycoff et al., 2018). Injection of LPS (Saeltner et al., 2019) or other substances such as serotonin or dopamine (Listerman et al., 2000) can also be performed. Injecting compounds into the intact animal will likely have an effect on various physiological systems, such as ventilation rate (Schapker et al., 2002; Shuranova et al., 2003), and not just heart. Addressing how compounds might also have an effect on the autonomic nervous system in crustaceans can be addressed as a comparative exercise in the autonomic nervous system of different animals (Choma et al., 2011; Shuranova et al., 2006). Annelids (i.e., worms and leeches) are also able to be used with exposed hearts for direct application of compounds (Bohner 2008; Hallmann and Crisp, 2011; Sternt, et al., 1979).

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