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HOW WE TEACH | *Classroom and Laboratory Research Projects*

Classical and novel pharmacological insights offered by the simple chick cardiomyocyte cell culture model: a valuable teaching aid and a primer for "real" research

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Freestone NS, Sam CL. Classical and novel pharmacological insights offered by the simple chick cardiomyocyte cell culture model: a valuable teaching aid and a primer for "real" research. *Adv Physiol Educ* 41: 163–169, 2017; doi:10.1152/advan.00178.2015.—The chick embryo cardiomyocyte model of cell culture is a staple technique in many physiology and pharmacology laboratories. Despite the relative simplicity, robustness, and reproducibility inherent in this model, it can be used in a variety of ways to yield important new insights that help facilitate student understanding of underlying physiological and pharmacological concepts as well as, more generally, the scientific method. Using this model, this paper will show real data obtained by undergraduate students in the authors' laboratories. It will first demonstrate classical pharmacological concepts such as full and partial agonism, inverse agonism, and competitive reversible antagonism and then move on to more complex pharmacology involving the characterization of novel receptors in these cells.

chick cardiomyocytes; β-adrenoceptors; chronotropy

THE CHICK EMBRYO CARDIOMYOCYTE cell culture model has been a standard technique in teaching and research laboratories for a number of years across many countries. Such is its comparative straightforwardness that it may even be used as a teaching technique in high schools (16). The reason for its popularity is due to the relative ease with which students can obtain spontaneously beating sheets or separate colonies of electrically connected heart cells. This feature of the immature heart, the fact that isolated single cells will develop into beating groups of cells when cultured, lends itself greatly to studies of the different receptors that mediate changes in the beating rate of these cell cultures. Thus, chronotropic changes mediated via a variety of receptor populations can be easily studied (6, 9). Apart from these physiological and pharmacological observations, biochemical analyses can also be used to elucidate intracellular signaling pathways in these cells (11, 12).

β-Adrenoceptor Signaling Pathways

In terms of the research interests of the authors, the work undertaken by their own students has focused on pharmacologically characterizing the contribution of four different β-adrenoceptor subtypes to cardiac contractility. The β-adrenoceptor family comprises members of the superfamily of protein receptors called G protein-coupled receptors (GPCRs). The G_s version of this protein links to a receptor (the β_1-adrenoceptor and often the β_2-adrenoceptor) that mediates stimulation of the activity of cardiac cells in terms of positive force generation, rate of contraction, and speed of relaxation (positive inotropy, chronotropy, and lusitropy, respectively). It does this largely through activation of a membrane-bound enzyme, adenylate cyclase, which produces increased amounts of an intracellular signaling molecule called cAMP. cAMP goes on to stimulate the production of more PKA, a molecule that can phosphorylate a number of cellular protein targets to change their function by this change in structure. Targets include the L-type Ca^{2+} channel (causing the entry of more Ca^{2+} into the cell, thus facilitating the positive inotropic and chronotropic changes) and phospholamban, a protein that regulates Ca^{2+} reuptake into the intracellular store for Ca^{2+}, the sarcoplasmic reticulum (facilitating the positive lusitropic changes). There also exists a G_i version, which mediates negative inotropic and chronotropic effects (via the β_3-adrenoceptor physiologically and the β_2-adrenoceptor pathophysiologically in heart failure). In cardiac cells, this is done by the stimulation of a cytosolic enzyme, guanylate cyclase, which generates increased amounts of cGMP and consequently PKG. Phosphorylation events mediated by PKG include a reduction in the opening probability of the L-type Ca^{2+} channel (facilitating the negative inotropic and chronotropic changes).

Apart from the chronotropic analyses possible in spontaneously beating cardiac cell cultures, students have also undertaken biochemical studies looking at cAMP and PKA mobilization due to various β-adrenoceptor agonists. In addition, atomic absorbance analyses of media samples to look at Mg^{2+} efflux out of these cells in response to β-adrenoceptor stimulation has also been performed. Thus, it is evident that once students are routinely able to isolate spontaneously beating cells, there are a plethora of research avenues that could be pursued depending on their own interests and level of engagement.

Furthermore, despite the apparent simplicity and robustness of this preparation, novel data can still be generated (7). Therefore, this technique is an ideal tool to give students an initial insight into the research process from constructing hypotheses, elaborating methods, and obtaining data to critically analyzing and potentially reporting their findings in

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peer-reviewed journals. This inclusion of undergraduate students as partners in collaborative research endeavors has been much discussed in the United Kingdom (UK) context (for a review, see Ref. 15) and is an ever-increasing feature of the student experience at UK Higher Education Institutions.

A typical trajectory for undergraduate students learning this technique in the authors' own laboratories would first involve sessions on aseptic techniques and then the mechanics of enzymatically isolating viable populations of beating cardiac cells. Students usually observe the cell isolation procedure being conducted by their project supervisor on two or three separate occasions. They are told to write detailed notes of the procedure as it is being performed and then to come up with their own step-by-step guide to this method. Having produced their own guide, students undertake the cell isolation themselves with their project supervisor in close attendance offering verbal guidance and physical aid if necessary. This stage is repeated as often is necessary to convince the project supervisor that the students are competent enough to undertake the cell isolation procedure alone. In practice, this usually takes no longer than three supervised cell isolations. After this time, students are able to work unaided and independently of the project supervisor.

Once this has been successfully achieved and beating cells are routinely obtained, students are encouraged to design their own experiments around a suitable testable hypothesis. After data has been gathered, students analyze their data, subject it to statistical analysis, and present it graphically as plots and graphs. Finally, students are enjoined to discuss their own results with those already existing in the literature that might have relevance to the work they have completed. In the authors' settings, these projects are finally presented by the students in the form of oral or poster presentations to an audience of peers and academic assessors.

General Teaching Points

The first experiments in the authors' laboratories would involve adding the most potent sympathomimetic agonist, isoproterenol (ISO), to show the students the extent of the chronotropic changes this cell preparation can reveal. Addition of a nonselective β-adrenoceptor blocker, such as propranolol, would demonstrate the concept of competitive, reversible antagonism on the ISO response. These initial experiments are methodologically easy to undertake and conceptually simple for the students to understand. Indeed, such data are often displayed in graphical idealized terms in most introductory pharmacology textbooks. However, undertaking these experiments themselves gives students a real insight into fundamental concepts such as the efficacy and potency of drugs as well as a useful general introduction into primary cell culture techniques. Once mastery of these initial steps has been achieved, students can then progress to more complicated experiments either of their own design (in an ideal scenario) or with more guidance from their academic supervisor.

Under these circumstances, students have been able to obtain results that portray (extremely well) classical elements of receptor pharmacology such as full and partial agonism, competitive reversible antagonism, and inverse agonism. They have also been able to reveal some unusual aspects of β-adrenoceptor pharmacology by confirming the presence of a propranolol-insensitive low-affinity β_1-adrenoceptor as well as a cardioinhibitory β_3-adrenoceptor in these cells.

Specific Teaching Points

A number of areas of uncertainty in the successful completion of any set of experiments have been evident in the many students who have undertaken this type of work. The first question invariably asked concerns the addition of cardioactive drugs. Should this be done by adding a small aliquot of a more concentrated stock solution to the existing media bathing the cells in the culture dish or should all the media be replaced by new media already containing the final concentration of the desired drug? The pros and cons of each approach can be debated, and students make the final choice largely depending on their analysis of the amount of data they can obtain by each approach. If adopting the former approach (as most students do), then apart from having to calculate volumes of drug that should be added to obtain appropriate final concentrations from an initial stock concentration, students also have to realize that the volume of drug added to the cells should not be so large as to significantly change the final volume of solution bathing the cells and thus dilute the final desired drug concentration.

Second, in terms of experimental design and efficient use of their time, students often have to think about the plating density of their freshly isolated cells when culturing them. This is because if the cells are plated too densely, the culture dishes will subsequently develop into a monolayer of cells all beating in unison. This restricts students to one experimental observation per culture dish. If the cells are plated too sparsely, few connections are formed between isolated groups of cells and little, if any, spontaneous beating activity may be observed. Previous experience has shown that using a cell plating density of $0.7–0.9 \times 10^6$ cells per 35-mm culture dish results in the formation of numerous, electrically separate groups of cells that beat independently of each other. This results in the number of observations per culture dish dramatically increasing. In the experiments outlined here, students usually obtained 6–8 million cells from 12 embryos and thus had at least 6 culture dishes full of separate groups of independently beating cells to experiment on per cell isolation cycle.

The next area of uncertainty experienced by the students is counting the number of beats per minute of the spontaneously contracting cardiac cells in the culture dishes. Should they endeavor to try and find the exact same groups of cells they counted before the addition of a drug or after that addition? In the same vein, students often discuss whether they should use culture dishes with a grid etched into the culture plastic or not to aid in finding the location of specific cell groupings. This then leads onto a discussion of what kind of statistical analyses might be undertaken with the data gathered. At the simplest level, this is based on the fact that statistical significance is more easily reached if one could be confident that exactly the same groups of cells are counted after the addition of the drug as before, using a paired *t*-test. More realistically, however, students soon learn that finding the same individual groups of cells each time is very difficult, and thus an unpaired *t*-test is usually used.

MATERIALS AND METHODS

Egg Incubation

Every week, 12 fertilized chicken eggs (Dekalb White) were ordered from a source specializing in supplying eggs for scientific research (Henry Stewart, Lincolnshire, UK). Eggs were incubated for 7 days at 37–38°C in a humidified egg incubator. Use of chick embryos at this early stage of their development renders them outside the auspices of The Humane Killing of Animals under Schedule 1 to the Animals (Scientific Procedures) Act in the United Kingdom.

The temperature and humidity of the incubator were checked regularly throughout the day. The humidity was maintained by topping up the water reservoirs present inside the incubator, and the temperature was checked using a thermometer. The incubators used were the Brinsea Octagon 10 and Maino MPS24 A, but there are many alternative suppliers of suitable incubators.

Solutions for Cell Culture

Trypsin solution. A 0.05% trypsin solution was prepared with sterile Ca^{2+}- and Mg^{2+}-free Dulbecco's PBS (DPBS) containing (in mM) 137 NaCl, 2.7 KCl, 8.1 Na_2HPO_4, 1.5 KH_2PO_4, and 4.2 $NaHCO_3$ (Thermo Scientific Hyclone) by adding 0.01 g trypsin (Worthington) to 20 ml sterile DPBS in a sterile 50-ml tube (Falcon). DPBS was filter sterilized by passing the solution through a 0.22-μm filter. The solution was then incubated in a 37°C water bath.

Cell growth medium. Five milliliters of FBS and 50 μl of 0.1% antibiotic antimycotic solution [containing penicillin G (10,000 units), streptomycin (10 mg) and amphotericin B (25 μg/ ml)] were added to 45 ml of medium 199 (M199) into a 50-ml sterile tube (all reagents from Sigma-Aldrich). The solution was filter sterilized and transferred into another 50-ml sterile tube. This solution was also incubated inside a 37°C water bath. The antibiotic-antimycotic solution was used to protect against microbial contamination during the cell isolation process.

Materials Used for Chick Embryo Dissection

A dissecting microscope (CETI Steddy-T microscope), 15-ml sterile tubes (Falcon), forceps, razor blades, Pasteur pipettes, a bowl for waste, and tube racks were all sprayed with 70% alcohol and placed inside a laminar flow hood to ensure sterile conditions throughout the process. Eggs were taken out from the incubator, placed into a bowl, wiped with 70% alcohol, and then placed inside the laminar flow hood.

Heart Dissection and Ventricular Cell Isolation

Dissecting the hearts. The egg shell was cracked by tapping the blunt end of the egg using sterile scissors. The top of the egg shell was removed to reveal a circular opening at the top of the egg. The chick embryo was then removed from the egg using sterile forceps. The embryo was placed inside a sterile petri dish along with some of the amniotic fluid from the egg. The head was separated from the body using two forceps, one for holding the neck and the other to pull the head off the main body. The heart was located underneath the left or right wing, pinched out with a fine pair of forceps, and placed into a petri dish containing sterile DPBS. All isolated hearts were subsequently placed into this petri dish.

Isolating the ventricles from the atria. The ventricles were separated from the atria using the dissecting microscope. This was done by holding the aorta with sterile forceps and cutting off the atria using sterile microdissecting scissors. All separated ventricles were then transferred to a new petri dish containing fresh sterile DPBS. The ventricles were then sliced up using sterile razor blades to help increase the surface area for the dissociative enzyme, trypsin, to act on.

Separation and isolation of single ventricular myocytes. The minced ventricles were then transferred, using a sterile Pasteur pipette, into a 15-ml sterile tube, and 2 ml of warm 0.05% trypsin solution in DPBS were added to the ventricular fragments. This tube was then shaken gently for 7 min in a 37°C water bath to help separate the ventricles into single cells.

The resulting supernatant from this first digest was discarded. Fresh trypsin solution was added a second time to a sterile tube containing the ventricular fragments and was gently agitated again for 7 min at 37°C. The supernatant from the second digestion was transferred into a 15-ml sterile tube topped up with warm sterile growth media. More fresh enzyme was added to the remaining ventricular fragments once more, and these were again shaken for another 7 min in the water bath at 37°C followed by transfer of the supernatant into sterile growth media. This procedure was repeated for as long as it took for all ventricular tissue to be digested, forming a suspension of isolated, single ventricular cells.

Centrifugation and Resuspension

The tubes containing the supernatants were topped up with growth media to ensure they all contained an equal volume of solution. The tubes were then centrifuged at a low speed (<9 *g* if possible) for 3 min. The supernatant from the centrifuged tubes was discarded as it still contained traces of the dissociative enzyme. Fresh, warmed, sterile growth media was added to the pellets to resuspend the cells into a final homogenous cell suspension. This final tube was made up with warm sterile growth media to an appropriate final volume.

Cell Counting

One hundred microliters of the cells resuspended into a homogenous solution were withdrawn and placed in an Eppendorf tube. This was followed by addition of 100 μl trypan blue solution (Sigma-Aldrich), which was mixed with the cell suspension. A hemocytometer was used to count the number of viable ventricular cells in the homogenous cell suspension to calculate the number of cells to plate out in each petri dish.

Cell Plating and Culturing

The desired number of cells for plating was determined through trial and error and was found to be between 0.7 and 0.9×10^6 cells for obtaining spontaneously beating cardiac cells. Once the cells had been aliquoted out into the culture dishes, they were placed inside a CO_2 incubator (Heraeus HERAcell 150i Double Stacked CO_2 Incubator), which was set at 5% CO_2 and 37°C. Cells were cultured in the incubator for between 48 and 96 h to allow growth and proliferation of the cells into monolayers of cells or separated colonies (depending on the density of cells seeding the petri dish).

Addition of Drugs and Assessment of Chronotropic Changes

Stock concentrations of a variety of cardioactive agents were made, filter sterilized, and kept in a −20°C freezer, usually at a concentration of 1 mmol/l. On the day of experiments, these stock solutions were thawed when needed, and students either made more dilute stock solutions or calculated the volume of stock solution needed to be added to the cells to obtain the required final concentration. CGP-12177A, BRL-37344, CGP-20712A, and ICI-118551 were obtained from Tocris Bioscience; propranolol, ISO, epinephrine, and norepinephrine were obtained from Sigma-Aldrich.

For ISO and CGP-12177A, a range of concentrations was used to produce log concentration-response curves. The nonselective β-adrenoceptor blocker propranolol was used at a final concentration of 200 nmol/l, the β_1-adrenoceptor antagonist CGP-20712 was used at a final concentration of 300 nmol/l, and the β_2-adrenoceptor antagonist ICI-118551 was used at a final concentration of 50 nmol/l. To assess negative chronotropic effects revealed by stimulation of the β_3-

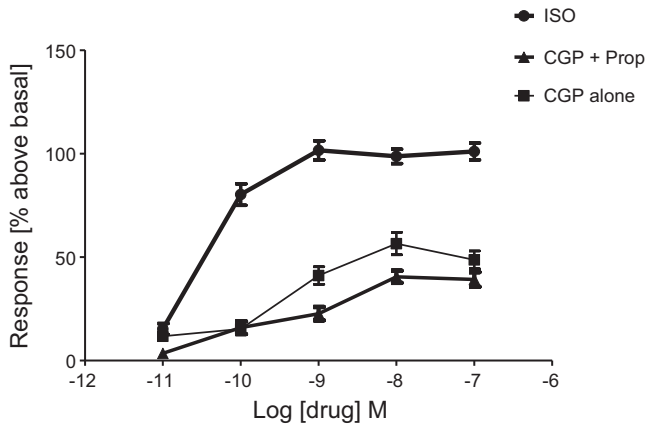

Fig. 1. Full and partial agonism mediated via β-adrenoceptors in chick cardiac myocytes. Data are presented as means ± SE of n = 12–20 beating groups of cells for each agonist condition. ISO, isoproterenol (a nonselective β_1- and β_2-adrenoceptor full agonist); Prop, propranolol (a nonselective β_1- and β_2-adrenoceptor antagonist, 200 nmol/l); CGP, CGP-12177A (a partial agonist at the low-affinity site of the β_1-adrenoceptor).

adrenoceptor, BRL-37344 was used at a final concentration of 600 nmol/l. The cell cultures in sterile petri dishes were removed from the CO_2 incubator and placed onto the heated plate of an inverted light microscope (Heatable Universal Mounting Frame M Series, Leica, Wetzlar, Germany), and the basal spontaneous contraction rate was counted. This counting procedure could either be performed through the eyepiece of the microscope (e.g., Leica DM series) or on a computer monitor (using, for example, Leica Application Suite microscope software) when a suitable camera was attached to the microscope (e.g., Leica DFC 420 C camera). Once the basal beating rate had been ascertained from the cells, they were placed into the laminar flow hood, and the required drug was aseptically added to the growth media bathing the cells.

Once the drug had been added, the cells were replaced into the CO_2 incubator to allow the drug to equilibrate with the specific receptors on the cells. Initial experiments have shown that equilibrium effects of the added drugs were achieved after 20 min. After this drug incubation period, the cells were once more remounted onto the heated stage of the light microscope, and any changes to the spontaneous cell beating rate were recorded. Cell beating rate counts were conducted as quickly as possible so as to minimise any changes arising from having the cells outside the CO_2 incubator.

Statistical Analysis

Invariably, students initially use a paired or unpaired *t*-test to test for the significance of any changes in the beating rate per minute observed, and the data are presented as means ± SE with n corresponding to the number of individual groups of cells responding to drug additions. However, students are counselled that when comparing the effect of multiple additions of drugs, an ANOVA test to test differences between two or more means should be applied. A suitable post hoc test (Tukey's test, for example) can then be performed to ascertain which of the pairs of the three or more means manifests any statistically significant difference.

RESULTS AND DISCUSSION

After observing the chick embryo ventricular myocyte cell isolation technique and subsequent culture of these cells to form spontaneously beating sheets or colonies on a number of occasions (typically two or three), students are soon able to undertake the mechanics of the process themselves. After this training period, students are able to relatively quickly obtain data, as shown below.

Figure 1 shows the effect that the nonselective β-adrenoceptor agonist ISO has on the spontaneous beating rate of chick embryo ventricular cardiac myocytes. The basal beating rates were 58 ± 13, 65 ± 19, and 66 ± 18 beats/min for ISO and CGP-12177 in the absence and presence of propranolol, respectively. This represents a standard pharmacological set of responses by these cells. Figure 1 also shows, however, something more advanced in nature. This is partial agonism mediated via a novel low-affinity β_1-adrenoceptor. The agonist for this receptor is a nonconventional partial agonist called CGP-12177A. At low concentrations, this agent acts as a β-adrenoceptor blocker but at higher concentrations as a partial agonist at the same receptors. Even more unusual is the fact that the β-adrenoceptor targeted by this agonist is relatively impervious to blockade by the nonselective β-adrenoceptor blocker propranolol.

To demonstrate that propranolol normally inhibits β-adrenoceptor-mediated positive chronotropic effects caused by ISO, the log dose-response curve for ISO can be done again but this time in the presence of propranolol (200 nmol/l). Figure 2 shows this effect very convincingly. The addition of propranolol shifts the log dose-response curve for ISO to the right. This demonstrates a classical shift in the potency of an agonist in the presence of an appropriate antagonist and would be very similar to idealized plots that students would see in their textbooks that illustrate the concept of competitive, reversible antagonism.

In this case, however, the above data also extend student understanding from this relatively straightforward concept to also encompass the novel nature of the data obtained in Fig. 1 for the effect of the nonconventional partial agonist CGP-12177A in the presence of propranolol. Here, the β-adrenoceptor blocker produces little, if any, rightward shift of the data.

Figure 3 shows that the basal spontaneous beating rate of chick embryo ventricular cardiac myocytes can be altered by the addition of propranolol. This feature is known as inverse agonism and demonstrates the fact that an antagonist even in the absence of the requisite agonist(s) can mediate effects on the empty receptor. The more perceptive among the students

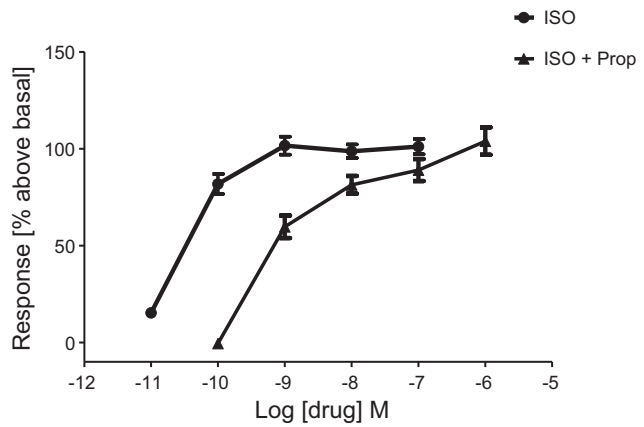

Fig. 2. Effect of Prop on the positive chronotropy caused by ISO. Data are presented as means ± SE of n = 12–15 beating groups of cells for each condition. The basal beating rates were 56 ± 19 and 60 ± 14 beats/min for ISO in the absence and presence of Prop (200 nmol/l), respectively.

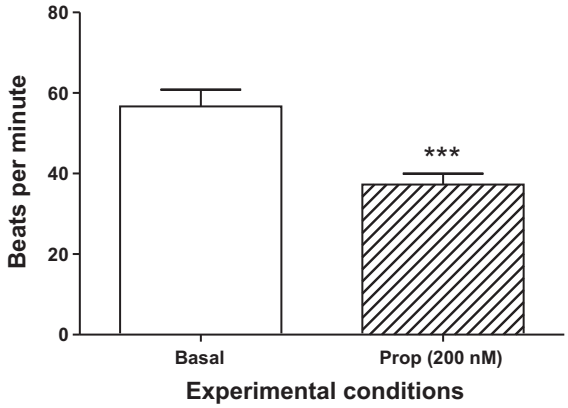

Fig. 3. Inverse agonism caused by Prop in chick embryo ventricular cardiac myocytes. Data are presented as means ± SE of $n = 38 – 45$ beating groups of cells for each condition. ***$P < 0.001$.

may notice this phenomenon in the course of their use of propranolol when generating data for the first two figures (Figs. 1 and 2). This observation then leads on to the theoretically more challenging areas of constitutive activity in empty receptors and the conventional two-state drug-receptor interaction model. Students may struggle to understand these concepts as portrayed in textbooks and other didactic forms of learning. Obtaining and interpreting data themselves that naturally leads them to the appropriate conclusions makes more real this particular concept, which is of increasing importance in the therapeutic application of a variety of drugs (see Ref. 10 for a review of inverse agonism and its therapeutic utility).

The inverse agonistic effect of propranolol on the basal spontaneous beating rate of the chick cardiac cells shown in Fig. 3 may initially be missed by students, as dealing with the sympathetic nervous system and β-adrenoceptor ligands they may only be expecting positive responses and an increase in the spontaneous beating rates of these cells. Another series of experiments may be performed that reveals negative chronotropic effects of a specific agonist at another novel cardiac receptor. Here again, a decrease in the initial basal spontaneous beating rate is revealed, as shown in Fig. 4. This time, the decrease in basal beating rate is attributed to an agonist initiating an intracellular cascade rather than an antagonist blocking the constitutive activity of an empty receptor. The β_3-adrenoceptor was first identified in cardiac tissue by Gauthier and colleagues in a number of mammalian species and subsequently in human hearts (8). Students will probably have come across this receptor in their studies in the context of lipolysis. Here, activation of the sympathetic nervous system and the "fight-or-flight" mechanism will cause the liberation of free fatty acids from fat cells to ensure an adequate energy supply to the fighting or fleeing organism. This is done by epinephrine/norepinephrine binding to fat cells via β_3-adrenoceptors.

While there have been a number of papers published on the cardiac effects mediated by the β_3-adrenoceptor, the literature still exhibits some ambiguity on the exact role of this receptor in health and disease (see debate between Refs. 2 and 14). As such, this presents a great opportunity to show students that a clear cut consensus on fundamental aspects of physiology and pharmacology is only reached after numerous viewpoints and opposing data sets have been published and analyzed. This uncertainty in the data is a very valuable learning point for students who may seek the comfort and simplicity of yes or no answers and incontestable facts.

Having demonstrated the properties of agonism and antagonism by the use of nonselective β-adrenoceptor agonists and antagonists, it is possible with this model to elucidate the chronotropic contributions of the separate β_1- and β_2-adrenoceptors. Furthermore, the level of complexity inherent in this set of experiments can be further enhanced by looking at how β_1- and β_2-adrenoceptor-blocking drugs affect the constitutive activity of these two β-adrenoceptor subtypes in the absence of specific agonists.

Figure 5*A* shows that norepinephrine caused positive chronotropic changes in the presence of the β_2-adrenoceptor antagonist ICI-118551 (50 nmol/l), thus revealing β_1-adrenoceptor-specific stimulatory effects. In addition, inverse agonism was also seen when ICI-118551 was added alone. In this instance, any positive chronotropic effects can be attributed to stimulation of β_1-adrenoceptors by norepinephrine, because the expected K_D of ICI-118551 at β_2-adrenoceptors is 0.5–1.5 nM/l but is 50-300 times higher at the β_1-adrenoceptor (4).

On the other hand, Fig. 5B shows that the β_1-adrenoceptor blocker CGP-20712A had no effect on the basal spontaneous beating rate of chick embryo ventricular cardiac cells. Thus, no inverse agonism was observed at the β_1-adrenoceptor. However, the addition of epinephrine in the presence of CGP-20712A resulted in a small increase in the spontaneous beating rate of these cells, which, however, did not reach statistical significance. CGP-20712A (300 nmol/l) was chosen to block β_1-adrenoceptors since the expected K_D for this antagonist is 0.3-1 nmol/l at the β_1-adrenoceptor but 10,000 times higher at the β_2-adrenoceptor. Any positive chronotropic effect can thus reasonably be attributed to stimulation of β_2-adrenoceptors by epinephrine (4).

It was only while in the process of researching the literature that underpins much of the data supplied here in this article that the authors discovered that the data shown in Fig. 5 conform almost exactly with the literature on this topic. For example, an overexpression system of human cardiac β-adrenoceptors in COS 7 cells was used to show, in these artificially enhanced conditions, that constitutive activity was much lower in β_1-

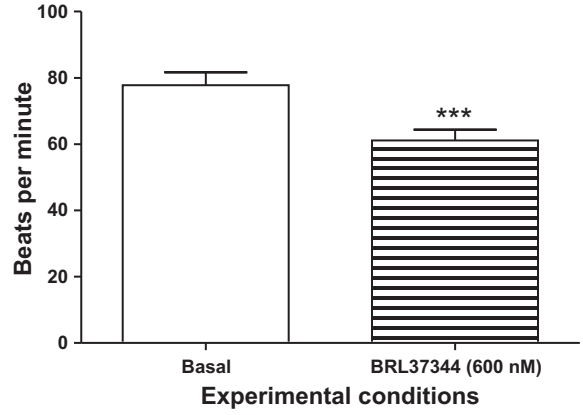

Fig. 4. Negative chronotropic effect of BRL-37344 in chick embryo ventricular cardiac myocytes. BRL-37344 is an agonist for the β_3-adrenoceptor. Data are presented as means ± SE of $n = 14–17$ beating groups of cells for each condition. ***$P < 0.001$.

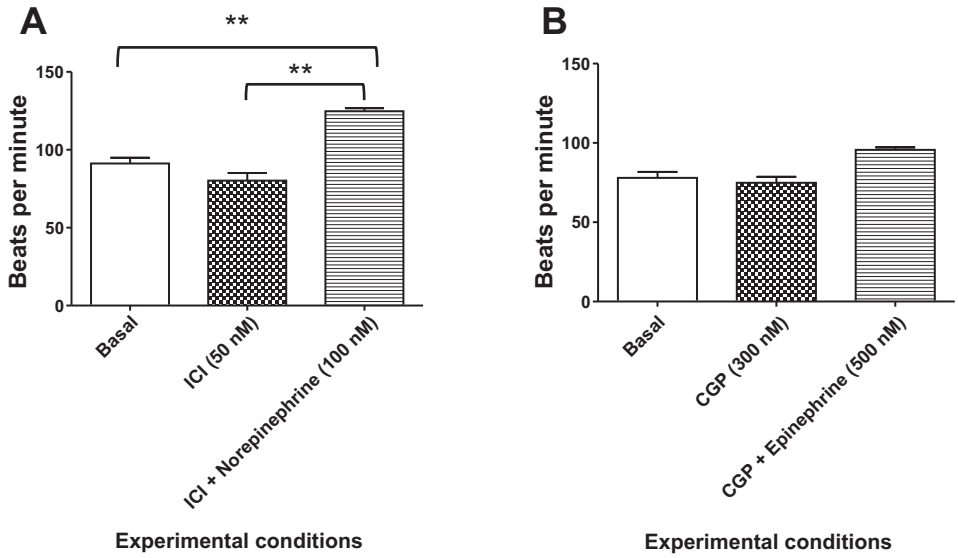

Fig. 5. Chronotropic changes mediated via specific β-adrenoceptors. *A*: β_2-adrenoceptor blockade with ICI-118551 (ICI) and β_1-adrenoceptor-specific effects revealed by norepinephrine. *B*: β_1-adrenoceptor blockade with CGP-20712A (CGP) and β_2-adrenoceptor-specific effects revealed by epinephrine. Data are presented as means ± SE of $n = 12–36$ beating groups of cells for each condition. An ANOVA test showed that there was a significant variation between the conditions in *A*. A post hoc Tukey's test revealed that ICI-118551 (ICI) in the presence of Prop differed significantly from both other conditions at $^{**}P < 0.01$.

compared with β_2-adrenoceptors (5). This underlines the robustness and usefulness of this technique as a general teaching tool.

Conclusions

This report has sought to demonstrate the utility, as a teaching tool, of the embryonic chick ventricular cardiac myocyte cell culture. First, it is a good general introduction to the essential physiological laboratory technique of cell culture. Students can see progress in developing their expertise in this technique by the successful culturing of uncontaminated, beating cell layers and colonies. Second, this model is extremely flexible, lending itself to routine experiments in cardiac cell anatomy (with appropriate fluorescent or histological staining techniques), physiology (effect of different media, temperatures, plating densities, well volumes, etc.), pharmacology (experiments on chronotropic changes exerted by different drugs), and biochemistry (assays are routinely done on cAMP in these cells, for instance, but there are kits available for literally hundreds of relevant assays).

The stability and consistency of the spontaneously beating cardiac cell model gives students confidence in their practical abilities and allows them the space to navigate their own learning. This deceptively simple technique scaffolds students' understanding of a number of important concepts and can, in the best cases, lead them to make their own novel contributions to the body of knowledge.

A possible criticism of this approach, at least as it pertains to its use in the authors' laboratories, is the limited number of students that can be exposed to this research experience on an annual basis. However, this limitation could be circumvented by providing larger groups of students with a similar experience by simply adding cardioactive agents to chick embryos remaining in their eggs or being explanted whole into petri dishes and then adding exogenous agents (1). This would remove the requirement for expensive cell culture infrastructure and equipment while still covering many of the relevant learning outcomes. Indeed, the crustacean *Daphnia magna* has also been commonly used for similar purposes (3).

Evaluation

Projects following the template described in this report have had an institutional, national, and even international impact. At the institutional level, projects of this kind are heavily oversubscribed, such is the perceived desirability of gaining this kind of experience. Furthermore, students undertaking these types of projects have often been recipients of prizes for best undergraduate research projects in their home school (up to 150 students in the year cohort) and, in one case, across the whole faculty (up to 1,000 students in the year cohort). At a national level, a high school-age student undertaking a Nuffield Summer Research project won a British Pharmacological Society Prize to become the first student at that level to present a poster at the annual British Pharmacological Society Winter Meeting in 2011 (13). Another of these undergraduate projects was presented at the South West Academic Network conference (involving a local consortium of four universities in southwest London), which was intended to highlight research being done by full-time research academics at these institutions. Finally, and at an international level, data using this experimental model were chosen from numerous other submissions to be given as a talk and subsequently published as a conference proceedings paper at the 6th European Congress on Pharmacology in Granada, Spain, in 2012 (7).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

N.S.F. conception and design of research; N.S.F. and C.L.S.S. performed experiments; N.S.F. analyzed data; N.F. and C.L.S.S. interpreted results of experiments; N.S.F. drafted manuscript; N.S.F. and C.L.S.S. edited and revised manuscript; N.S.F. and C.L.S.S. approved final version of manuscript; C.L.S.S. prepared figures.

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