

## **Biopta Ltd Technical Summary**

### **Preservation and Transport of *Ex Vivo* Human Cardiac Tissue for *In Vitro* Pharmacology Studies**



**Human Tissue Contract Research Services**



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## Biopta Technical Summary - Validation of *Ex Vivo* Human Atrial Trabeculae

### Aim:

To verify that *ex vivo* human atrial trabeculae received at Biopta following hypothermic transportation and storage in RS-I physiological solution (Aqix Ltd., UK) retain their expected functional characteristics during normothermic perfusion using RS-I physiological solution and thereby represent a valuable model to study human cardiac muscle responses.

### Methods:

Fresh human atrial appendages were obtained from donors who had undergone cardiopulmonary bypass. All appropriate ethical approvals, informed patient consent and tissue transfer agreements were arranged prior to receipt of any tissues. Following removal, the atrial appendage was immediately placed in an ice cold washing solution (RS-I physiological solution (RS-I solution, table 1), and then placed in cardioplegic solution (RS-I physiological solution and  $MgSO_4$  25mM) designed to reduce the activity of the heart tissues during transportation and storage. The tissue was transferred to Biopta via a specialist courier, arriving at Biopta within 4-6 hours. Upon arrival the tissue was removed from the cardioplegic solution, placed in ice cold RS-I solution and gassed continuously with 95%  $O_2$ /5%  $CO_2$ . The atrial appendage was then cut open to expose the trabeculae inside and suitably sized trabeculae were removed and mounted on parallel platinum electrical stimulating heads in 5 ml wire myography baths. The trabeculae were then stretched isometrically to approximately 7.5 mN tension in RS-I solution heated to approximately 37°C and gassed continuously with 95%  $O_2$ /5%  $CO_2$ . Electrical field stimulation ('EFS') was then continuously applied to the tissue (1 Hz, 0.5 msec pulse width, 5 V) and the changes in force produced following the EFS stimulations were recorded.

### Protocols:

Experiments were carried out to examine the typical responses to three agonists with well characterized effects on heart function.

Agonists:
Isoprenaline ( $\beta$ adrenergic receptor agonist)
Milrinone (phosphodiesterase III inhibitor)
Digoxin (cardiac glycoside)

### Results:

To the best of our knowledge the stability of the tissue during transport and dissection is far in excess of that previously achieved with other physiological solutions. The novel cardioplegic solution (AQIX RS-C; Muryama & Chambers, 2008), together with careful collection, dissection and handling processes, has contributed to the robust responses of the tissue as shown below.

#### Isoprenaline

A cumulative concentration response curve (CCRC) to isoprenaline was performed. In Figure 1, the positive inotropic activity of isoprenaline can clearly be seen over the concentration range  $1 \times 10^{-8}M$  to  $1 \times 10^{-4}M$  ( $EC_{50}$  value 36 nM). The response shown is expressed as a percentage of the response to  $1 \times 10^{-5}M$  norepinephrine, which was applied to the trabeculae after washing out the isoprenaline at the end of the experiment.

Figure 1a:

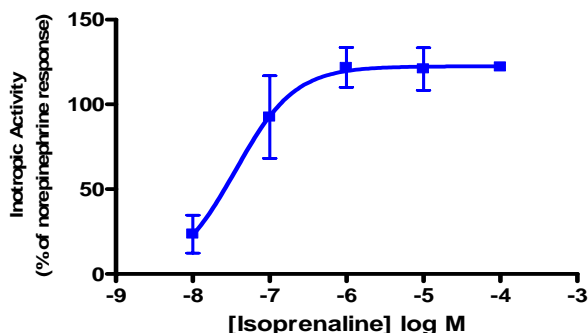


Figure 1b:

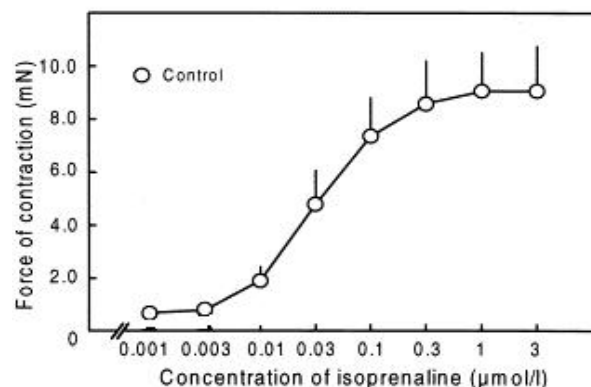


Figure 1: Figure 1a shows the positive inotropic effects of cumulative concentrations of isoprenaline in human atrial muscle strips from experiments conducted in-house, over the concentration range  $1 \times 10^{-8}M$  to  $1 \times 10^{-4}M$ . The response to isoprenaline is expressed as a percentage of the response to norepinephrine. For comparison, figure

1b shows the inotropic effect of isoprenaline (Flesch *et al.*, 1999 (edited)) with a reported EC<sub>50</sub> value of approximately 30nM, which is very similar to the findings in Biopta's laboratory.

**Milrinone**

Milrinone, as shown in figures 2a and 2b, increased cardiac muscle contractility in a concentration-dependent manner, over the range 1 x 10<sup>-8</sup>M to 1 x 10<sup>-5</sup>M. Figure 2a demonstrates that the positive inotropic effect of milrinone was reversed by the addition of one single high concentration (1 x 10<sup>-5</sup>M) of the muscarinic receptor agonist, acetylcholine (ACh).

Figure 2a:

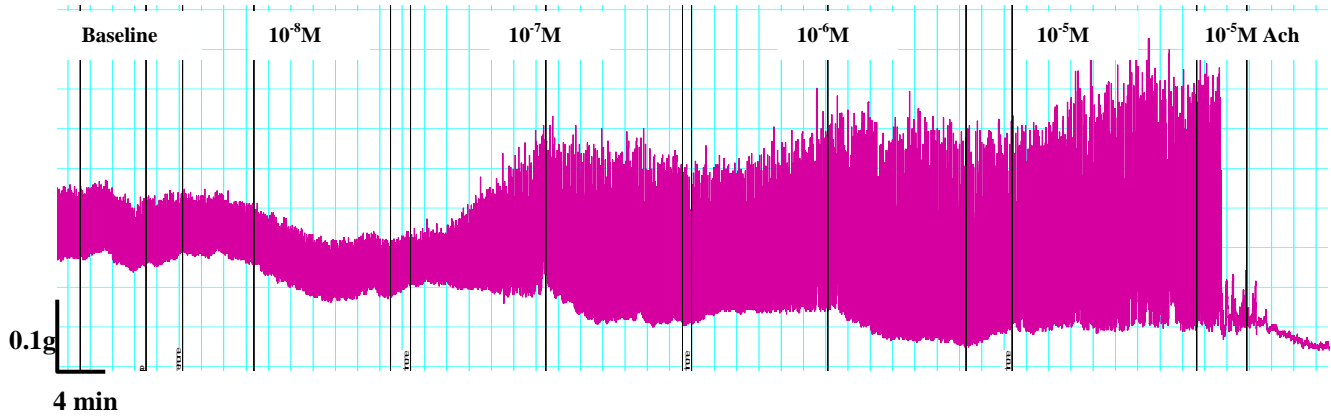


Figure 2b:

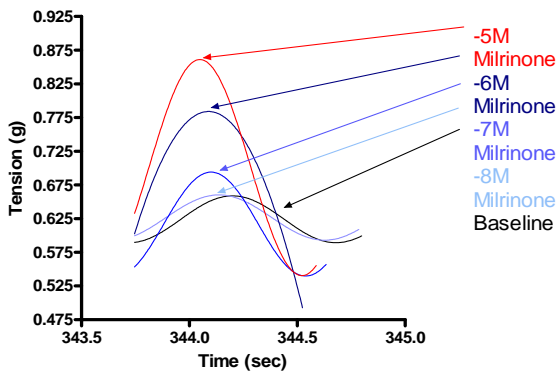


Figure 2c:

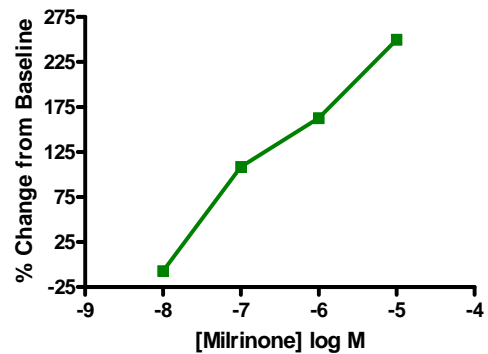


Figure 2: The raw data trace in Figure 2a shows the effect of cumulative concentrations (1 x 10<sup>-8</sup>M to 1 x 10<sup>-5</sup>M) of milrinone and the reversal of this effect by acetylcholine. The graph in figure 2b shows this effect more clearly from one contraction cycle taken from the baseline response and each concentration of milrinone tested (1 x 10<sup>-8</sup>M to 1 x 10<sup>-5</sup>M). Figure 2c shows the effect of milrinone expressed as the percentage change from the baseline EFS response.

## Digoxin

Digoxin was once widely used as the standard treatment for heart failure but, due to its narrow therapeutic index, is now used in patients who have congestive heart failure and are still symptomatic despite proper diuretic and ACE inhibitor treatment. The inotropic effects of digoxin on atrial heart muscle can clearly be seen in Figure 3, below, where one high dose of digoxin ( $1 \times 10^{-6}\text{M}$ ) was added.

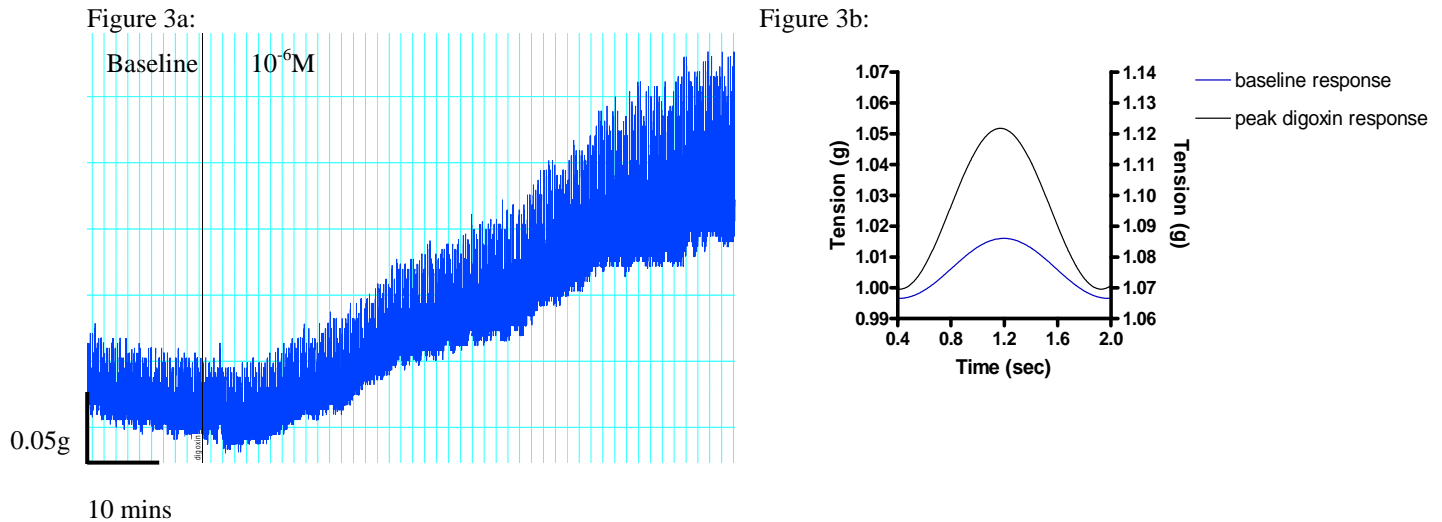


Figure 3: Figure 3a shows raw data trace showing the inotropic effects of digoxin on human atrial heart muscle. Figure 3b shows this effect more clearly from one contraction cycle taken from the baseline response and the peak digoxin response.

## Conclusion:

*Ex vivo* human atrial trabeculae received via Biopta's UK-wide tissue network retain their expected functional characteristics and therefore represent a valuable model for prediction of human cardiac muscle efficacy and safety pharmacology.  $EC_{50}$  values for isoprenaline compared favourably to published results. All compounds tested, isoprenaline, milrinone and digoxin, showed positive inotropic effects in line with published data and their expected pharmacological activity.

The use of RS-I avoided the need for continuous perfusion of the tissues during the experiment, which helped to minimize the required volume of test drugs. To the best of our knowledge the stability of the tissue during transport and dissection is far in excess of that previously achieved. The novel cardioplegic solution, together with careful dissection and handling processes, have contributed to the creation of a robust human heart test system for the testing of potential new drugs.

## References:

- Milrinone: Floreani *et al.* (2003) *Naunyn-Schmiedeberg's Arch Pharmacol* 367 : 109–118; Jaski *et al.* (1985) *J Clin Invest.* 1985 February; 75(2): 643–649
- Isoprenaline: Ledda *et al.* (1975) *Br J Pharmacol* 54, 83-90; Flesch *et al.* (1999) *Journal of the American College of Cardiology* 33 ( 4) , :1062-1070
- Digoxin: Hauptman *et al.* (1999) *Circulation* 99, 1265-1270
- Muryama, Y and Chambers, D.J (2008): Myocardial protection: Efficacy of RS-C (AQIX®), a novel cardioplegic solution, compared to St Thomas' Hospital solution. *J Mol Cell Cardiol.*, 44; 711-825: abst. No. 117

Table 1                      **Patented Formulation of AQIX® RS-I**  
**Perfusion & Preservation Solution**

<i>Component</i>	<i>Conc.(mmol/L)</i>	
NaCl	110.00	
KCl	5.00	
CaCl <sub>2</sub>	1.25	(Salts)
MgCl <sub>2</sub>	0.45	
NaHCO <sub>3</sub>	25.0}	(pH Buffer)
BES }	5.00}	
D-glucose	10.00	
Glycerol	0.11	
L - Glutamate	0.30	
L - Glutamine	0.40	(Substrates)
L - Aspartate	0.02	
L - Carnitine	0.05	
Choline Chloride	0.01	
TPP (cocarboxylase)	40.00 <i>nmol</i>	
Human (recom.) Insulin	28.00 <i>mIU</i>	

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[\* aerate with *carbogen*, ie, 95%O<sub>2</sub> / 5%CO<sub>2</sub> ]

(**pH** = 7.13 - 7.41 ± 0.5 @ 10-37 °C )

The concentration of the naturally occurring components in this solution, with the exception of the taurine-based Good's buffer, BES, equate to the levels found in human serum. The zwitteronic characteristics (pK<sub>a</sub>) of BES ensures pH stability over the temperature range 10-38 °C and allows optimal, serum levels of hydrogen carbonate to be utilised.