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Original article

Optimising conditions for studying the acute effects of drugs on indices of cardiac contractility and on haemodynamics in anaesthetized guinea pigs

Laura Mooney [a], Louise Marks [b], Karen L. Philp [b], Matthew Skinner [b], Susan J. Coker [a,*], Susan Currie [a]

[a] Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow, G4 0RE, UK
[b] Safety Assessment UK, AstraZeneca R&D, Alderley Park, Macclesfield, SK10 4TG, UK

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ABSTRACT

Introduction: Detecting adverse effects of drugs on cardiac contractility is becoming a priority in pre-clinical safety pharmacology. The aim of this work was to optimise conditions and explore the potential of using the anaesthetized guinea pig as an in vivo model. **Methods:** Guinea pigs were anaesthetized with Hypnorm/Hypnovel, isoflurane, pentobarbital or fentanyl/pentobarbital. The electrocardiogram (ECG), heart rate, arterial blood pressure and indices of cardiac contractility were recorded. In further experiments in fentanyl/pentobarbital anaesthetized guinea pigs the influence of bilateral versus unilateral carotid artery occlusion on haemodynamic responses was investigated and the effects of inotropic drugs on left ventricular (LV) dP/dt_{max} and the QA interval were determined. **Results:** Pentobarbital, given alone or after fentanyl, provided suitable anaesthesia for these experiments. Bilateral carotid artery occlusion did not alter heart rate or arterial blood pressure responses to isoprenaline or angiotensin II. Isoprenaline and ouabain increased $LVdP/dt_{max}$ and decreased the QA interval whereas verapamil had opposite effects and strong inverse correlations between $LVdP/dt_{max}$ and the QA interval were found. **Discussion:** Conditions can be optimised to allow the pentobarbital-anaesthetized guinea pig to be used for simultaneous measurement of the effects of drugs on the ECG, haemodynamics and indices of cardiac contractility. The use of this small animal model in early pre-clinical safety pharmacology should contribute to improvements in detecting unwanted actions on the heart during the drug development process.

1. Introduction

In recent years there has been increasing awareness that drugs can have serious adverse effects on cardiac contractile function (Force & Kerkelä, 2008). Traditionally, cardiac safety pharmacology studies have focused on exploring whether drugs in development may have proarrhythmic activity but there is now a need to also consider the potential for cardiac contractile dysfunction in pre-clinical testing. A relatively simple, inexpensive, small animal model is therefore required. Rats are often used for haemodynamic measurements and cardiac contractility can be measured in this species. However, as repolarization in the rat heart is mainly dependent on the transient outward potassium current (I_{to}) rather than the rapid delayed rectifier potassium current (I_{Kr}) it is not suitable for screening for proarrhythmia. The anaesthetized guinea pig is a model which has been used to investigate the proarrhythmic potential of various drugs by assessing their ability to prolong the QT interval (Batey, Lightbown, Lambert, Edwards, & Coker, 1997; Hamlin, Kijtawornrat, Keene, & Hamlin, 2003; Hauser, Stade, Schmidt, & Hanauer, 2005; Testai et al., 2004; Yao et al., 2008) or induce torsade de pointes (Michael, Kane, & Coker, 2008). As far as we are aware only one study (Hauser et al., 2005) has examined the effects of drugs on both QT prolongation and cardiac contractility (via measurement of the maximum rate of rise of left ventricular pressure ($LVdP/dt_{max}$)) in anaesthetized guinea pigs.

In order to measure arterial blood pressure and left ventricular (LV) pressure simultaneously, Hauser et al. (2005) cannulated both common carotid arteries thus eliminating any input from the carotid sinus baroreceptors. This could substantially impair reflexes and affect responses to drugs that alter arterial blood pressure. As drugs that have inotropic activity may also affect arterial pressure, it is important to determine whether or not bilateral carotid occlusion (by cannulating both carotid arteries) alters the effects of drugs on heart rate and arterial blood pressure in guinea pigs. This is particularly important when planning to use $LVdP/dt_{max}$ as an index of cardiac contractility because this parameter can be altered by changes in heart rate, preload or afterload (Wallace, Skinner, & Mitchell, 1963).

Abbreviations: ANOVA, analysis of variance; BP, blood pressure; ECG, electrocardiogram; i.v., intravenous; LV, left ventricular; $LVdP/dt_{max}$, the maximum rate of rise of left ventricular pressure; I_{Kr}, rapid delayed rectifier potassium current; I_{to}, transient outward potassium current.

* Corresponding author. Tel.: +44 141 548 2405, +44 7866 869219 (mobile).
E-mail address: susan.coker@strath.ac.uk (S.J. Coker).

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Some time ago the QA interval was proposed as an alternative index of cardiac contractility which was not affected by changes in cardiac loading (Cambridge & Whiting, 1986) and more recently there has been a revival of interest in the potential of this parameter. Comparisons of the QA interval and LVdP/dt_{max} as indices of cardiac contractility in the dog (Norton, Iacono, & Vezina, 2009) and the rat (Adeyemi et al., 2009) have been published but no studies have been performed in guinea pigs.

Although some studies comparing different anaesthetics in guinea pigs have been published (Brown, Thorne, & Nuttall, 1989; Buchanan, Burge, & Ruble, 1998; Schwenke & Cragg, 2004) none of these has measured cardiac contractility or ECG intervals. The previous ECG studies in guinea pigs cited above employed a variety of anaesthetic regimes: pentobarbital (Batey et al., 1997; Testai et al., 2004), ketamine/xylazine (Hamlin et al., 2003), thiobutabarbital/urethane (Yao et al., 2008) and urethane (Hauser et al., 2005). It is therefore not clear which anaesthetic may be best for these types of experiment.

To test the hypothesis that the anaesthetized guinea pig is a suitable model for the simultaneous assessment of the effects of drugs on the ECG, haemodynamics and cardiac contractility, the aims of the present work were: to choose an anaesthetic regime suitable for the nature and duration of the experiments; to determine if bilateral carotid cannulation compromises drug effects on heart rate and blood pressure; and to compare the usefulness of the QA interval and LVdP/dt_{max} as indices of cardiac contractility.

2. Methods

2.1. Animals

All animal experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986, approved by institutional ethical review committees and conducted under the authority of Project Licences held at the University of Strathclyde or at AstraZeneca. Fifty-five male Dunkin Hartley guinea pigs (450–720 g) were purchased from Harlan (Bicester, UK) and allowed a minimum of 1 week acclimatisation before use. They were housed in small groups, on aspen chip bedding and sizzle nest (Datesand), or hay, in rooms held at temperatures between 16 and 23 °C, with 40 to 70% relative humidity and a 12 hour light/dark cycle. Food (Special Diet Services FD1 guinea pig diet or Teklad global higher fibre guinea pig diet 2041, plus fresh fruit and vegetables) and water were available ad libitum.

2.2. Anaesthesia

Preliminary experiments focused on determining suitable anaesthetic regimes. The anaesthetics tested were isoflurane (n = 3), a combination of Hypnorm® and Hypnovel® (n = 4), and sodium pentobarbital either given alone (n = 4) or after premedication with fentanyl (n = 4). Experiments with Hypnorm/Hypnovel and pentobarbital alone were carried out at AstraZeneca whereas those with isoflurane and fentanyl/pentobarbital were performed concurrently at the University of Strathclyde. Subsequently a different isoflurane protocol was assessed at AstraZeneca (n = 4). Animals anaesthetized with isoflurane were breathing 100% oxygen, the first 3 breathing spontaneously, the latter 4 artificially ventilated. All others were artificially ventilated with room air. Details of the doses and routes of administration for the induction and maintenance of anaesthesia with all agents are given in Table 1. All subsequent experiments at the University of Strathclyde were performed in guinea pigs anaesthetized with the fentanyl/pentobarbital combination. Body temperature was measured via a rectal thermometer and maintained at ~37 °C with a heating lamp.

2.3. Surgical preparation

Immediately after induction of an adequate level of anaesthesia, as determined by the absence of corneal and/or pedal withdrawal reflexes, the trachea was cannulated and artificial ventilation with room air was commenced. At the University of Strathclyde a Bioscience pump (Harvard Apparatus, Edenbridge, Kent, UK) was used, set at a rate of 60 breaths min^{-1} and a stroke volume of 7–8 mL kg^{-1}. Oxygen saturation and expired CO_2 were measured continuously using a Medair Lifesense™ Vet pulse oximeter/capnograph (Kruuse UK Ltd., Sherburn in Elmet, UK). If necessary, during the preparation and stabilisation phases, the stroke volume of the pump was adjusted to keep the expired CO_2 value between 35 and 45 mm Hg and oxygen saturation above 95%. At AstraZeneca a TOPO™ dual mode ventilator (Kent Scientific, West Malling, UK) was used with the rate set at 20–40 breaths per minute, and the peak inspiration pressure set at 19.5 cm H_2O. If necessary these ranges were adjusted to achieve arterial blood gas values within a set range: $PO_2 > 70$ and <110 mm Hg, $PCO_2 > 25$ and <45 mm Hg (Gem Premier Blood Gas analyser, Instrumentation Laboratory, Warrington, UK). Oxygen saturation was also assessed continuously using a pulse oximeter (Medair PulseSense™ Vet, Kruuse UK Ltd., Sherburn in Elmet, UK), where a desirable reading was >90%.

Needle electrodes were inserted subcutaneously to record limb lead ECGs. Both jugular veins were isolated and cannulated, one for infusion of maintenance doses of anaesthetic and the other for test drugs. A fluid filled cannula was placed in the right carotid artery for measurement of arterial blood pressure and/or blood sampling. All cannulae were filled with normal saline (0.9% w/v NaCl) containing 10 units mL^{-1} heparin. The left carotid artery was isolated and a 2F or 3F Millar Mikro-tip® catheter pressure transducer (Linton Instrumentation, Diss, UK) was advanced through the artery so that its tip lay in the lumen of the left ventricle. A stabilisation period of

Table 1
Doses and routes of administration of agents used for the induction and maintenance of anaesthesia in guinea pigs.

Anaesthetic	Induction		Maintenance	
	Dose	Route	Dose	Route
Isoflurane[a]	5%	Inhalation	2.0–2.5%	Inhalation
Isoflurane[a]	2–4%	Inhalation	1.5–2.5%	Inhalation
Hypnorm/Hypnovel[b]	8 mL kg^{-1}	i.p.	1.25 mL kg^{-1} h^{-1}	i.v.
Sodium pentobarbital[c]	60 mg kg^{-1}	i.p.	6 mg kg^{-1} h^{-1}	i.v.
Fentanyl[d] *plus*	50 μg kg^{-1}	s.c.		
Sodium pentobarbital[c]	50–60 mg kg^{-1}	i.p.	6 mg kg^{-1} h^{-1}	i.v.

[a] Isoflurane was given by inhalation in 100% oxygen delivered at 1 L min^{-1} for induction and at 0.5 L min^{-1} for maintenance of anaesthesia.
[b] A solution containing 1 part Hypnorm (a solution containing fentanyl citrate 0.315 mg mL^{-1} and fluanisone 10 mg mL^{-1}); 1 part Hypnovel (midazolam HCl 5 mg mL^{-1}) and 2 parts water made up freshly each day.
[c] Sodium pentobarbital was dissolved at 60 mg mL^{-1} in distilled water daily for induction of anaesthesia and diluted to 6 or 12 mg mL^{-1} in normal saline (0.9% w/v NaCl in water) for maintenance, just before the start of each experiment.
[d] Fentanyl (Sublimaze®) was supplied as a solution of 50 μg mL^{-1} and was given 5 min before sodium pentobarbital.