Fentanyl protects the heart against ischaemic injury via opioid receptors, adenosine A1 receptors and KATP channel linked mechanisms in rats

R. Kato*, S. Ross and P. Foëx

Nuffield Department of Anaesthetics, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, UK

*Corresponding author

We have investigated if fentanyl protects against myocardial ischaemic injury and if so, if the mechanism of this protection is mediated via opioid and adenosine A1 receptors, and KATP channels. Langendorff rat hearts were subjected to global ischaemia (30 min) and reperfusion (60 min). The drugs were administered before induction of ischaemia and maintained throughout the experiment. Treatment with fentanyl 740 nmol litre⁻¹ improved post-ischaemic mechanical function, assessed as developed pressure, +dP/dtmax and –dP/dtmin, compared with controls after 60 min of reperfusion. These effects were abolished by naloxone 1 µmol litre⁻¹, DPCPX 10 µmol litre⁻¹, a selective adenosine A1 antagonist and sodium 5-hydroxydecanoate 100 µmol litre⁻¹, a KATP channel blocker. We conclude that fentanyl protected the heart against post-ischaemic injury by a mechanism which was blocked by an opioid and an adenosine A1 receptor antagonist and also by a KATP channel antagonist.

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Transient myocardial ischaemia may occur during anaesthesia and surgery. Myocardial performance is compromised to a variable degree after ischaemia and subsequent reperfusion. This dysfunction reflects either irreversible (infarction) or reversible injury. The latter is often termed myocardial stunning where dysfunction persists despite return of full perfusion. Recovery of this dysfunction is facilitated by some drugs, including halogenated anaesthetics. In many models investigating this improvement, the protective drug is given before the ischaemic insult and, therefore, may act by preconditioning. This term was originally coined for the protection which brief periods of ischaemia confer on the heart against subsequent damage caused by prolonged ischaemia. This phenomenon, ‘ischaemic’ preconditioning, has been investigated extensively because of its clinical relevance and scientific interest. Several mediators are involved in myocardial preconditioning, including protein kinase C (PKC) and ATP-sensitive potassium channels (KATP channels). The existence of mediators has suggested the possibility for novel interventions which would represent ‘pharmacological’ preconditioning; protection could be induced by administering drugs which activate effectors intervening in ischaemic preconditioning before the ischaemic period, and serve the same purpose as brief ischaemic episodes.

Opioids are linked to PKC and are therefore putative mediators in preconditioning. Indeed, opioid receptor agonists have been shown to protect the heart from the insult of ischaemia. Administration of morphine before an ischaemic insult mimics ischaemic preconditioning and protects against infarction in anaesthetized rats and in isolated rat hearts. Furthermore, the opioid antagonist naloxone abolishes ischaemic preconditioning. However, there has been little work on the protective effects which activation of the opioid system may confer against contractile dysfunction which occurs after brief periods of ischaemia.

Adenosine is released endogenously from the myocardium during ischaemia and reperfusion, and alleviates ischaemic damage. Exogenous adenosine receptor agonists have been shown to protect against injury in various myocardial ischaemia models. Several mechanisms have been postulated for their protective action, including inhibition of norepinephrine release from sympathetic nerve endings and attenuation of production of free radicals and calcium influx into reperfused myocardium. There is convincing evidence to suggest involvement of adenosine receptors in ischaemic preconditioning. Adenosine A1 receptors share with opioid receptors a common cellular pathway linked to PKC, and an adenosine A1 blocker abolishes
the cardiac protection induced by ischaemic preconditioning. Furthermore, protective effects by activating α1 adrenergic receptors, equivalent to opioid receptors in the PKC activation pathway, have been blocked by an adenosine A1 antagonist, suggesting that adenosine may be a key effector in pharmacologically induced preconditioning.

KATP channels are activated as a result of a decrease in the level of intracellular ATP, and are well characterized in perfused retrogradely through the aorta using a Langendorff Concentrations of the drugs were fentanyl 740 nmol rapidly, arrested in ice-cooled Krebs–Henseleit perfusion solution (concentrated at 100 cm H2O and kept constant. of ventricular mechanical function were measured. The

Materials and methods
The study was performed in accordance with the UK Animal Act (scientific procedures) 1986 (Home Office licence number 30/00980).

Preparation of rat hearts
Adult male Wistar rats (325–400 g) were anaesthetized with pentobarbital 60 mg kg⁻¹ i.p. The hearts were excised rapidly, arrested in ice-cooled Krebs–Henseleit buffer, and perfused retrogradally through the aorta using a Langendorff apparatus. The Krebs–Henseleit perfusion solution (containing (mmol litre⁻¹) NaCl 118.7, KCl 4.7, MgSO4 1.2, KH2PO4 0.95, NaHCO3 28, CaCl2 1.3 and glucose 10) was filtered through a 5.0 μm filter, oxygenated with a mixture of 95% oxygen and 5% carbon dioxide, and maintained at 37°C throughout the procedure. pH was 7.4 when equilibrated with carbon dioxide at 37°C. Perfusion pressure was set at 100 cm H2O and kept constant.

The left and right ventricles were vented by apical puncture with a polyethylene drain and ventriculotomy, respectively. A latex balloon, slightly larger than the ventricle, was inserted through the left atrium into the left ventricle and connected by a polyethylene catheter to a saline-filled syringe and to a pressure transducer (Medex Medical Inc, Lancashire, UK). All hearts were paced with right ventricular epicardial electrodes at a fixed rate of 5 Hz (2 ms, 5 V square pulses). The volume of the balloon was adjusted to achieve an end-diastolic pressure (EDP) of 5–10 mm Hg which remained unchanged throughout the study.

After a stabilization period of 10 min, only those hearts that met the following criteria were studied: peak systolic pressure (PSP) >85 mm Hg; EDP <10 mm Hg; and coronary flow (CF) >12.0 ml min⁻¹.

Experimental design
Hearts were allocated to one of 18 groups.

Non-ischaemia study
There were six groups in this study. The hearts in these groups did not undergo ischaemia. Group NI-Fent was treated with fentanyl (n=5), group NI-Nal with the non-selective opioid receptor antagonist naloxone (n=5), group NI-HD with the KATP channel blocker sodium 5-hydroxy-decanote (5-HD) (n=5), group NI-DPC with the adenosine A1 antagonist 1,3-depropyl-8-cyclopentylxanthine (DPCPX) (n=5) and group NI-Cont with no drug.

Ischaemia study 1
There were 10 groups according to which drug(s) was administered. Group Fent was treated with fentanyl (n=7), group Nal with naloxone (n=6), group HD with 5-HD (n=5), group Veh with DMSO (n=6), group DPC with DPCPX (n=6), group Fent+Nal with fentanyl and naloxone (n=7), group Fent+HD with fentanyl and 5-HD (n=5), group Fent+Veh with fentanyl and DMSO (n=5) and group Fent+DPC with fentanyl and DPCPX (n=6). The control group (Cont) (n=8) received no drug treatment.

Ischaemia study 2
The hearts were allocated to one of two groups depending on the period of fentanyl incubation before ischaemia. Group Fent10 (n=7) was perfused with fentanyl for 10 min and group Fent15 (n=6) for 15 min.

Concentrations of the drugs were fentanyl 740 nmol litre⁻¹ (Janssen-Cilag Ltd, Buckinghamshire, UK), naloxone 1 μmol litre⁻¹ (Du Pont Pharmaceuticals Ltd, Hertfordshire, UK), 5-HD 100 μmol litre⁻¹ (Sigma-Aldrich, Dorset, UK) and DPCPX 10 μmol litre⁻¹ (Sigma-Aldrich) (in 0.04% DMSO (Sigma-Aldrich)).

The experimental procedure is summarized in Figure 1. After a period of stabilization (10 min), pre-drug levels of ventricular mechanical function were measured. The appropriate drug was then administered to the perfusate and maintained until the end of the study. In the non-ischaemia study, measurements were obtained at 20, 60, 70, 80, 90, 100 and 110 min after administration of 5-HD in group NI-HD, and at 15, 55, 65, 75, 85, 95 and 105 min after the pre-drug measurement in the remainder of the non-ischaemia
Non-ischaemia study
Groups Ni-Cont, Ni-Fent, Ni-Nal, Ni-HD, Ni-Veh, Ni-DPC
No drug (Ni-Cont)
Fentanyl (Ni-Fent)
Naloxone (Ni-Nal)
5-HD (Ni-HD)
Vehicle (Ni-Veh)
DPCPX (Ni-DPC)

Stabilization
10 115 [120] (min)

Ischaemia study 1
Groups Cont, Fent, Nal, Veh, DPC
No drug (Cont)
Fentanyl (Fent)
Naloxone (Nal)
Vehicle (Veh)
DPCPX (DPC)

Stabilization
10 15 30 60 (min)

Ischaemia
10 5 10 30 60 (min)

Reperfusion

Groups Fent + Nal, Fent + Veh, Fent + DPC
Naloxone (Fent + Nal)
Vehicle (Fent + Veh)
DPCPX (Fent + DPC)

Stabilization
10 10 10 30 60 (min)

Ischaemia
10 5 10 60 (min)

Reperfusion

Groups HD, Fent + HD

Stabilization
10 10 10 30 60 (min)

Ischaemia
10 5 10 60 (min)

Reperfusion

Ischaemia study 2
Groups Fent10, Fent15

Stabilization
10 10 10 30 60 (min)

Ischaemia
10 5 10 60 (min)

Reperfusion

Fig 1 Study design.

groups. 5-HD had a longer pre-treatment time so that the results from the non-ischaemia study were comparable with ischaemia study 1 where 5-HD needed an additional 5 min pre-incubation time (see below). In the ischaemia studies, measurements just before ischaemia were taken as pre-ischaemic values. Ischaemia was caused by stopping the flow of the perfusate to the heart, and pacing was stopped at the onset of ischaemia. After 30 min of ischaemia, aortic flow was reintroduced and continued for a further period of 60 min. Pacing was reintroduced 8 min into reperfusion and continued until the end of the study. Measurements were obtained at 10, 20, 30, 40, 50 and 60 min during reperfusion. 5-HD was pre-incubated for 10 min before administration of fentanyl, 5 min longer than the other antagonists. This was because preliminary experiments showed that the effect of fentanyl was not blocked by 5-HD with 5 min pre-incubation (data not shown).

Measurements included PSP, EDP, developed pressure (DP), maximum positive and minimum negative left ventricular pressure derivatives (+dP/dmax and −dP/dmin) and CF.

Data collection and analysis
Left ventricular pressure was monitored continuously throughout the experiment. The left ventricular pressure signal was digitized at 250 Hz (analogue–digital converter AT-MIO, National Instruments Corporation, TX, USA) and stored on the hard disk of a desktop computer. Data were
collected and processed using data acquisition and analysis software developed in our department.

PSP and EDP were averaged values computed respectively from 10–14 heart beats. DP was defined as the difference between PSP and EDP. \( +\frac{dP}{dt}\text{max} \) and \( -\frac{dP}{dt}\text{min} \) were obtained by differentiation of the ventricular pressure signal. These were also averaged values from 10–14 heart beats. EDP was defined as the pressure at the first positive deflection of the left ventricular \( \frac{dP}{dt} \) signal.

**Statistical analysis**

All values are expressed as mean (SEM). Differences in indices between groups at various times were compared using two-way analysis of variance (ANOVA) for treatment and time with repeated measures on a time factor. If ANOVA indicated significant differences between groups, further comparisons on specific times were performed using Fisher’s test. Statistical significance was assumed at \( P<0.05 \).

**Results**

**Non-ischaemia study**

The mechanical indices in the non-ischaemic groups are shown in Table 1. There were no significant differences between groups throughout the study.

**Ischaemia study 1**

Figures 2 and 3 show changes in DP and \( -\frac{dP}{dt}\text{min} \) in groups Cont, Fent, Nal and Fent+Nal. There were no significant differences between groups during the pre-ischaemic period. At the end of reperfusion (60 min), group Fent had a significantly higher DP (58 (3) mm Hg) than all other groups (group Cont 34 (5), group Nal 40 (4) and group Fent+Nal 37 (7) mm Hg) (\( P<0.01 \)). A similar result was obtained for \( -\frac{dP}{dt}\text{min} \), which was also greater in group Fent (943 (34) mm Hg s\(^{-1} \)) compared with the other groups (group Cont 578 (83), group Nal 659 (75) and group...
between groups for EDP or CF. antagonist or the K ATP channel blocker. Fentanyl reperfusion. Table 2 shows the changes in the other indices in the recovery of mechanical function and, therefore, the mechanical function in groups Cont, Fent, HD and an opioid has not been reported previously, studies showed –

\[ P/H11001 \]

Changes in minimum negative left ventricular pressure derivative strictly comparable with groups Fent

\[ P/H11001 \]

compared with group Fent

\[ P/H11001 \]

ischaemia in groups Cont, Fent, Nal and Fent

\[ P/H11001 \]

Fig 2

Changes in developed pressure (DP) before and after global There were no differences between groups during the pre-

\[ \mu \]

ences between groups before the ischaemic period. DP Although fentanyl is preferentially a

\[ P/H11021 \]

t

\[ \min \] (Fig. 5) showed similar recovery pro

\[ P/H11021 \]

Figures 6 and 7 and Table 3 show the changes in indices of mechanical function in groups Veh, Fent+Veh, DPC and Fent+DPC. Groups Veh, DPC and Fent+DPC showed similar recovery profiles for DP (Fig. 6), +dP/dmax (Table 3) and –dP/dmin (Fig. 7). DP in group Fent+Veh was significantly greater at the end of reperfusion than in all other groups (group Fent+Veh 59 (5), group Veh 37 (5), group DPC 33 (7) and group Fent+DPC 33 (5 mm Hg) \[ P<0.01 \]). +dP/dmax and –dP/dmin were also greater in group Fent+Veh compared with the other groups at the end of reperfusion \[ P<0.01 \]. There was no significant difference in EDP or CF between groups at any time. There were no differences between groups during the pre-

\[ \text{Ischaemia study 2} \]

No difference was found between groups Fent10 and Fent15 for any index at any time (Table 4). The recovery profiles of DP, +dP/dmax, –dP/dmin and EDP in groups Fent10 and Fent15 were similar to those in group Fent.

Discussion

The non-ischaemia study showed that none of the drugs had any effect on the mechanical function of the non-ischaemic heart. It is clear from ischaemia study 1 that fentanyl protected the ischaemic myocardium, enhancing both post-ischaemic systolic (DP and +dP/dmax) and diastolic (–dP/dmin) function. Furthermore, ischaemia study 1 suggests that this protection may be blocked by naloxone, 5-HD and DPCPX, as the recovery profiles of mechanical function after ischaemia were similar between groups Nal and Fent+Nal, groups HD and Fent+HD, and groups DPC and Fent+DPC. However, group Fent was not strictly comparable with groups Fent+Nal, Fent+HD and Fent+DPC, because the pre-incubation time of fentanyl was 10 min in groups Fent+Nal, Fent+HD and Fent+DPC and 5 min shorter in group Fent. Therefore, we performed ischaemic study 2 to show that the difference in the pre-incubation time with fentanyl did not cause any difference in the recovery of mechanical function and, therefore, the extent of protection. Our studies demonstrated that the protective effect of fentanyl was blocked by addition of an opioid receptor antagonist, an adenosine A1 receptor antagonist or the KATP channel blocker.

While protection against post-ischaemic dysfunction by an opioid has not been reported previously, studies showed that morphine protected against infarction in an in vivo and an in vitro rat model. This effect was blocked by naloxone and a KATP channel blocker glibenclamide.

Although fentanyl is preferentially a μ opioid receptor

\[ \text{Fent+Nal} 588 (117) \text{ mm Hg s}^{-1} \] \[ P<0.01 \] at 60 min reperfusion. Table 2 shows the changes in the other indices of mechanical function in groups Cont, Fent, Nal and Fent+Nal. +dP/dmax showed similar recovery to DP and –dP/dmin. There were no significant differences between groups for EDP or CF.

Figures 4 and 5 and Table 2 show the indices of mechanical function in groups Cont, Fent, HD and Fent+HD. DP (Fig. 4), +dP/dmax (Table 2) and –dP/dmin (Fig. 5) showed similar recovery profiles in groups Cont, HD and Fent+HD. There were no significant differences between groups before the ischaemic period. DP

\[ P<0.05 \], +dP/dmax \[ P<0.05 \] and –dP/dmin \[ P<0.01 \] in group Fent were significantly greater at the end of reperfusion than in all other groups. There were no significant differences in EDP and CF between groups at any time.

\[ \text{Kato et al.} \]

![Fig 2](image1)

**Fig 2** Changes in developed pressure (DP) before and after global ischaemia in groups Cont, Fent, Nal and Fent+Nal (ischaemia study 1). Cont=Control (no drug); Fent=fentanyl; and Nal=naloxone. \[ aP<0.05 \] compared with group Cont, \[ bP<0.05 \] compared with group Nal, \[ cP<0.05 \] compared with group Fent+Nal.

![Fig 3](image2)

**Fig 3** Changes in minimum negative left ventricular pressure derivative (–dP/dmin) before and after ischaemia in groups Cont, Fent, Nal and Fent+Nal (ischaemia study 1). Cont=Control (no drug); Fent=fentanyl; and Nal=naloxone. \[ aP<0.05 \] compared with group Cont, \[ bP<0.05 \] compared with group Nal, \[ cP<0.05 \] compared with group Fent+Nal.

\[ \text{Fent+Nal} 588 (117) \text{ mm Hg s}^{-1} \] \[ P<0.01 \] at 60 min reperfusion.
Cardioprotection against ischaemia by fentanyl

Table 2 Indices of mechanical function before and after global ischaemia in groups Cont, Fent, Nal, Fent+Nal, HD and Fent+HD (ischaemia study 1). DP=Developed pressure; EDP=end-diastolic pressure; +dP/dt max=maximum positive left ventricular pressure derivative; –dP/dt min=minimum negative left ventricular pressure derivative; CF=coronary flow. Cont=Control (no drug); Fent=fentanyl; Nal=naloxone; and HD=5-HD. Results are mean (SEM). *P<0.05 compared with group Cont, **P<0.05 compared with group Nal, ***P<0.05 compared with group Fent+Nal, ****P<0.05 compared with group HD, *****P<0.05 compared with group Fent+HD

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agonist, it is capable of binding to δ and κ receptors.33 Of the several known subtypes of opioid receptor agonists, δ opioid agonists have been shown to provide protection against ischaemic injury. Schultz and colleagues reported that a δ opioid receptor agonist decreased infarct size in an in vivo rat model after 30 min of regional ischaemia and that δ, but not μ or κ, opioid antagonists eliminated the protection conferred by morphine in anaesthetized rats.35 In contrast, κ agonists appeared to enhance cardiac ischaemic injury. Indeed, dynorphin, a κ agonist, decreased ventricular mechanical function in vivo in rats,36 and was arrhythmogenic in both in vitro and in vivo rat ischaemia models. δ and κ opioid receptors have been characterized in the rat heart,39 40 but the existence of μ opioid receptors is doubtful. It has been reported that μ opioid receptors are not expressed in the whole heart or in isolated myocytes of rats.

The cellular mechanisms by which fentanyl exerts its post-ischaemic protective action, and the mechanisms whereby an opioid and an adenosine A1 receptor antagonist block such protective effects are unknown. One hypothesis is based on the PKC model of pharmacologically induced preconditioning. Activation of PKC in the myocyte has been suggested to be the key event in the process of protection.6 7 Stimulation of PKC-linked receptors by α1 adrenergic agonists,41 42 endothelin-1 43 44 and bradykinin44 5 has been shown to induce protective effects via PKC activation. δ-Opioid receptor agonists also enhance PKC activity via G proteins in the myocyte.8 9 Furthermore, a PKC antagonist has been reported to inhibit morphine-induced preconditioning in the isolated heart preparation.13 Although involvement of either G proteins or PKC was not examined in our study, PKC activation is a possible explanation for the protective characteristics of fentanyl. In common with α1 adrenergic, bradykinin and δ opioid receptors, A1 adenosine receptors have been reported to activate PKC via G proteins in the myocyte.21 Pretreatment with A1 adenosine receptor agonists protected the heart against ischaemic injury.17 18 46
and this protection was eliminated by PKC block. Downey and Cohen have suggested that myocardial protection is obtained by the combined activities of several types of receptors, all acting via the PKC pathway. According to their model, protection is achieved only if the activity of PKC exceeds a threshold level. Our results are consistent with such a model, where there were at least two sources for stimulation of PKC: fentanyl acting at the δ opioid receptor which was added to the perfusate, and adenosine released during ischaemia. For protection to occur, the combined effects of these two agonists must increase the activity of PKC above threshold. Hence removing one by addition of either opioid or adenosine A1 antagonists reduces the activity of PKC below the threshold, abolishing its protective action.

An alternative hypothesis to explain the protection provided fentanyl and the ability of two different types of receptor antagonists to abolish it, is that of an indirect mechanism. For example, the interaction of fentanyl with opioid receptors could result in release of adenosine, which in turn acts on adenosine A1 receptors to induce a protective effect. There is some evidence for such an indirect action in the spinal cord, where μ and δ opioid receptors have been shown to mediate adenosine release from synapto-
Cardioprotection against ischaemia by fentanyl

Table 3 Indices of mechanical function before and after global ischaemia in groups Veh, Fent+Veh, DPC and Fent+DPC (ischaemia study 1). DP=Developed pressure; EDP=end-diastolic pressure; +dP/dtmax=maximum positive left ventricular pressure derivative; −dP/dtmin=minimum negative left ventricular pressure derivative; CF=coronary flow. Veh=Vehicle; Fent=fentanyl; and DPC=DPCPX. Results are mean (SEM). *P<0.05 compared with group Veh, †P<0.05 compared with group DPC.  ‡P<0.05 compared with group Fent+DPC.

<table>
<thead>
<tr>
<th>Reperfusion (min)</th>
<th>Pre-drug</th>
<th>Pre-ischaemia</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
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</tr>
<tr>
<td>Veh</td>
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<td>90 (6)</td>
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<td>9</td>
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<tr>
<td>Fent+Veh</td>
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<td>3</td>
<td>f</td>
<td>21</td>
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<td>f</td>
</tr>
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<td>DPC</td>
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<td>92 (7)</td>
<td>8</td>
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<td>11</td>
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<td>91 (3)</td>
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</tr>
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<td>5 (1)</td>
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<td>2540 (147)</td>
<td>136</td>
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<td>228</td>
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<td>172</td>
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<td>59 (12)</td>
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Table 4 Indices of mechanical function before and after ischaemia in groups Fent10 and Fent15 (ischaemia study 2). DP=Developed pressure; EDP=end-diastolic pressure; +dP/dtmax=maximum positive left ventricular pressure derivative; −dP/dtmin=minimum negative left ventricular pressure derivative; CF=coronary flow. The hearts were perfused with fentanyl in the pre-ischaemic period for 10 min in group Fent10 and for 15 min in group Fent15. No differences between groups.

<table>
<thead>
<tr>
<th>Reperfusion (min)</th>
<th>Pre-drug</th>
<th>Pre-ischaemia</th>
<th>10</th>
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<td>105 (2)</td>
<td>101 (2)</td>
<td>15</td>
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<td>Fent15</td>
<td>108 (3)</td>
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<tr>
<td>Fent10</td>
<td>6 (1)</td>
<td>5 (1)</td>
<td>64</td>
<td>4</td>
<td>53</td>
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<td>44</td>
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<td>Fent15</td>
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<td>71</td>
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<td>4</td>
<td>51</td>
<td>4</td>
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<tr>
<td>+dP/dtmax (mm Hg s⁻¹)</td>
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<tr>
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<td>2709 (64)</td>
<td>2573 (54)</td>
<td>328</td>
<td>53</td>
<td>540</td>
<td>88</td>
<td>836</td>
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<tr>
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<td>2655 (81)</td>
<td>296</td>
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<td>852</td>
<td>138</td>
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<td>−dP/dtmin (mm Hg s⁻¹)</td>
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<tr>
<td>Fent10</td>
<td>1858 (38)</td>
<td>1757 (46)</td>
<td>247</td>
<td>60</td>
<td>370</td>
<td>58</td>
<td>562</td>
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<td>1763 (73)</td>
<td>171</td>
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<td>CF (ml g⁻¹ min⁻¹)</td>
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<tr>
<td>Fent10</td>
<td>79 (3)</td>
<td>74 (2)</td>
<td>57</td>
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<td>Fent15</td>
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<td>71 (4)</td>
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By extrapolation from the central nervous system, such release could be thought to occur in the heart. Adenosine release can induce pharmacological preconditioning via PKC, as mentioned above, and may also offer protection via other mechanisms, such as attenuation of production of oxygen-derived free radicals and calcium influx into the myocardium on reperfusion. Indeed, in our study, the heart was perfused with fentanyl not only during the pre-ischaemic period but also during reperfusion. Therefore, fentanyl could have exerted its protective effect during reperfusion by a mechanism independent of preconditioning. There is much speculation that K_ATP channels may be the end-effector of myocardial protection. Addition of K_ATP
channel openers results in ischemic preconditioning of the heart. Furthermore, the protection produced by PKC-linked receptor agonists, such as adenosine 22 and endothelin-1, is blocked by KATP channel blockers. Hence it has been proposed that activation of PKC results in the opening of KATP channels which produce myocardial protection. There is little evidence of a direct link and KATP channels. We are currently undertaking further studies to investigate the cellular mechanisms by which fentanyl exerts this protective effect, in particular whether PKC is involved, and whether the protection affects the extent of infarction and/or the degree of stunning. Elucidation of the mechanisms of opioid-mediated protection could pave the way for the development of interventions reducing myocardial ischemic injury in clinical practice.

In our study, variables of mechanical function were used to examine the extent of ischemic damage resulting from 30 min of global ischemia. We cannot determine if improved recovery was caused by more limited irreversible injury (infarction), reduced degree of reversible damage (stunning) or a mixture of both. Isolated rat hearts reperfused after 20 min of total ischemia have been reported to exhibit no irreversible injuries. In contrast, ischemic periods of longer than 25 min have been shown to result in a mixture of reversible and irreversible histological features. Infarction was found after 30 min of global ischemia in recent preliminary experiments in our laboratory (unpublished data). Therefore, we assume that the mechanical dysfunction observed in our study reflects both reversible and irreversible myocardial damage. Further studies are needed to determine if fentanyl protects against infarction or stunning.

Regarded as an early diastolic index, \(-dP/d\text{dmin}\) represents the speed of relaxation. Adequate supply of ATP is required for the uptake of \(\text{Ca}^{2+}\) into the sarcoplasmic reticulum so that the myocardium can be relaxed during diastole. It is believed that the supply of ATP is impaired during reperfusion and that this is responsible for the decrease in \(-dP/d\text{dmin}\). EDP indicates compliance of the left ventricle. The increase in EDP is reported to be caused by a combination of intracellular \(\text{Ca}^{2+}\) overload and other altered myocardial properties, but the detailed mechanism is unknown. In this study, both \(-dP/d\text{dmin}\) and EDP were used to evaluate diastolic function. Fentanyl improved \(-dP/d\text{dmin}\) significantly but not EDP. The reason for this result remains to be elucidated.

The concentration of fentanyl which exhibited protection in our study was 740 nmol litre\(^{-1}\) (250 ng ml\(^{-1}\)). The plasma concentration of fentanyl required for anaesthesia in rats is unknown. The clinical plasma concentration of fentanyl in cardiac surgery is 10–30 ng ml\(^{-1}\) with a loading dose of 30 \(\mu\)g kg\(^{-1}\) followed by a continuous infusion of 0.3 \(\mu\)g kg\(^{-1}\) min\(^{-1}\). However, in dogs, a dose of more than 500 ng ml\(^{-1}\) was required to obtain anaesthesia when only fentanyl was administered. Pharmacodynamics are largely dependent on species and therefore it is difficult to infer whether or not the concentration used in this study was within the ‘therapeutic range’. However, the finding that fentanyl improved post-ischaemic mechanical function was important as fentanyl may offer some cardiac protection in patients with ischemic heart disease who may suffer from myocardial ischemia during anaesthesia and surgery.

In summary, the results of our study suggest that fentanyl, an opioid agonist, enhanced the recovery of cardiac mechanical function after ischemia via an unknown mechanism involving opioid and \(\alpha_1\) adenosine receptors, and KATP channels. We are currently undertaking further studies to investigate the cellular mechanisms by which fentanyl exerts this protective effect, in particular whether PKC is involved, and whether the protection affects the extent of infarction and/or the degree of stunning: Elucidation of the mechanisms of opioid-mediated protection could pave the way for the development of interventions reducing myocardial ischemic injury in clinical practice.

Acknowledgements
R. K. was in receipt of a Honjo International Scholarship. S. R. was in receipt of a Medical Research Council studentship and a bursary from the Medical Research Fund (University of Oxford). The study was supported in part by a grant from the Garfield Weston Trust for Medical Research into diseases of the heart.

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