

# The effect of $\beta$ 2-adrenoceptor agonists on leucocyte-endothelial adhesion in a rodent model of laparotomy and endotoxaemia

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10 **Microcirculation; Surgery**

## 11 **Abstract**

### 12 **Background**

13 The  $\beta$ 2-adrenoceptor agonist dopexamine may possess anti-inflammatory actions which could reduce  
14 organ injury during endotoxaemia and laparotomy. Related effects on leucocyte-endothelial adhesion  
15 remain unclear.

### 16 **Methods**

17 Thirty anaesthetised Wistar rats underwent laparotomy followed by induction of endotoxaemia with  
18 lipopolysaccharide and peptidoglycan (n=24) or sham (n=6). Animals received dopexamine at 0.5 or  
19 1  $\mu$ g kg<sup>-1</sup> min<sup>-1</sup> (D0.5 and D1), salbutamol at 0.1  $\mu$ g kg<sup>-1</sup> min<sup>-1</sup>, or saline vehicle (Sham and  
20 Control) for five hours. Intravital microscopy was performed in the ileum of the small intestine to  
21 assess leucocyte-endothelial adhesion, arteriolar diameter, and functional capillary density. Global  
22 haemodynamics and biochemical indices of renal and hepatic function were also measured.

### 23 **Results**

24 Endotoxaemia was associated with an increase in adherent leucocytes in post-capillary venules,  
25 intestinal arteriolar vasoconstriction as well reduced arterial pressure and relative cardiac index, but  
26 functional capillary density in the muscularis was not significantly altered. Dopexamine and  
27 salbutamol administration were associated with reduced leucocyte-endothelial adhesion in post-  
28 capillary venules compared to control animals. Arteriolar diameter, arterial pressure and relative  
29 cardiac index all remained similar between treated animals and controls. Functional capillary density  
30 was similar for all groups. Control group creatinine was significantly increased compared to sham  
31 and higher dose dopexamine.

### 32 **Conclusions**

33 In a rodent model of laparotomy and endotoxaemia, β2-agonists were associated with reduced  
34 leucocyte-endothelial adhesion in post-capillary venules. This effect may explain some of the anti-  
35 inflammatory actions of these agents.

## 36 **Introduction**

37 Complications following major gastrointestinal surgery have a significant impact on both short and  
38 long-term survival (1-3). Inotropic agents may have important effects on outcomes for this patient  
39 group (4). Dopexamine is a dopamine analogue with agonist activity at β2 and dopaminergic  
40 receptors. This spectrum of activity confers vasodilator actions in addition to chronotropic and mild  
41 inotropic effects (5). Dopexamine has been used to increase cardiac output and hence tissue oxygen  
42 delivery in several trials of peri-operative haemodynamic therapy (6, 7). Other cardiovascular effects  
43 of dopexamine may include improved tissue microvascular flow and oxygenation (8). Various groups  
44 have studied the effects of dopexamine in patients following major gastrointestinal surgery (6, 9),  
45 with promising results, although the findings of a recent large trial were inconclusive (7).

46 Investigators have previously demonstrated potent anti-inflammatory effects of β2-adrenoceptor  
47 agonists (10-18), in particular dopexamine (19-22). However, it is unclear that β2-adrenoceptor  
48 agonism is responsible for the dopexamine-dependent reduction of leucocyte-endothelial adhesion  
49 seen in several endotoxaemia studies (19-20). The findings of previous laboratory and clinical  
50 investigations suggest dopexamine may improve tissue microvascular flow and oxygenation (8, 19,  
51 20, 23-26), and it is thought that these effects may account for much of the potential benefit of  
52 inotropic agents in the critically ill (27). However, in a previous laboratory study from our group, the  
53 haemodynamic actions of dopexamine infusion appeared to be less important than anti-inflammatory  
54 effects, including decreased plasma cytokine levels, modulation of neutrophil CD11b surface  
55 expression, and decreased pulmonary neutrophil infiltration (28). We sought to further clarify how  
56 leucocyte-endothelial adhesion under endotoxaemia might relate to the β2-adrenoceptor agonist  
57 effects of dopexamine, and its effects on arterial pressure, cardiac output and the microcirculation.

58 We therefore investigated the effects of dopexamine on leucocyte-endothelial adhesion within the  
59 microcirculation. Our hypothesis was that, in a rodent model of laparotomy and endotoxaemia,  
60 dopexamine would decrease leucocyte-endothelial adhesion in intestinal post-capillary venules,  
61 through β2-adrenoceptor mediated actions. The relative contribution of β2-adrenoceptor agonism to  
62 these effects was assessed by using the β2 adrenergic agonist salbutamol as a comparator.

63

## 64 **Materials & methods**

65 Thirty male Wistar rats (240-340 g) received a standard diet and water ad libitum before the  
66 experiments. All procedures were performed with institutional approval and in accordance with the  
67 United Kingdom Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act  
68 1986 under the project license PPL 70/6526. Anaesthesia was induced by intra-peritoneal injection of  
69 thiopental (120 mg kg<sup>-1</sup>) and maintained with supplementary injections administered according to  
70 regular testing for limb withdrawal to a standard stimulus. Animals were placed on a warming mat to  
71 maintain a core temperature of 37 ± 0.5 °C. A tracheostomy was performed, following which a short  
72 section of polyethylene tubing (internal diameter, 1.67 mm) was inserted to maintain airway patency  
73 and to facilitate spontaneous respiration. The right carotid artery was cannulated to allow blood  
74 sampling and continuous monitoring of heart rate (HR) and mean arterial pressure (MAP). The left  
75 jugular vein was cannulated for drug and fluid administration.

76 A 2 cm midline incision was then made through the abdominal wall to expose the peritoneum.  
77 Following laparotomy, bowel was evacuated into a moist cotton receptacle. Blunt dissection was then  
78 performed to access the abdominal vasculature. After isolation from the vena cava, a 1.5 mm  
79 ultrasonic aortic transit time flow probe (MA1.5PRB; Transonic Systems Inc., Ithaca, USA) was  
80 placed on the infra-renal aorta to measure aortic blood flow allowing calculation of relative stroke  
81 volume and relative cardiac index. The bowel was then replaced in the abdominal cavity, except for a  
82 loop of ileum just proximal to the caecum. The exposed bowel was kept moist by the application of  
83 0.9% saline drops through a pipette. The laparotomy incision above and below the exit of the  
84 terminal ileal loop from the abdomen was then closed with 5.0 vicryl to prevent excessive insensible  
85 fluid losses. The animal was maintained on a warming mat on an intravital microscopy platform and  
86 placed in the right lateral position so the ileal loop fell on to a raised section of the platform at the  
87 level of the laparotomy incision. The temperature of the raised section was thermostatically  
88 controlled at 37.5 °C to ensure the temperature of the exposed bowel was similar to the core  
89 temperature. This position did not interfere with the ability of the ultrasonic probes to measure aortic  
90 blood flow. Subsequently the bowel was covered with Saran wrap to prevent evaporative losses from  
91 its surface and maintain bowel microvascular integrity (29). This was followed by a 5 ml kg<sup>-1</sup> bolus  
92 of normal saline to replace insensible fluid losses and a 15 minute stabilisation period to allow  
93 microvascular flow to stabilise. A first set of arterial blood samples was then taken (see below), the  
94 volume being replaced with an equal volume of normal saline. Animals were allowed to stabilise for  
95 15 min before being allocated randomly to one of five groups (sham, control, D0.5, D1, S).

96 Endotoxaemia was induced over a 10 minute period in four of five groups by administering 1ml kg<sup>-1</sup>  
97 of a solution containing Escherichia coli lipopolysaccharide 0111:B4 (LPS, 1 mg ml<sup>-1</sup>) and  
98 peptidoglycan (PepG, 0.3 mg ml<sup>-1</sup>) intravenously, the sham group received 0.9% saline vehicle alone.  
99 In all groups this was followed by an infusion of 0.9% saline at 4.3 ml kg<sup>-1</sup> h<sup>-1</sup> though different doses  
100 of dopexamine or salbutamol were added to the D0.5, D1 and S groups' infusion fluid. This resulted  
101 in dopexamine infusion rates of 0.5 and 1 µg kg<sup>-1</sup> min<sup>-1</sup> for groups D0.5 and D1, respectively, and a  
102 salbutamol infusion rate of 0.1 µg kg<sup>-1</sup> min<sup>-1</sup> in group S. This dose of salbutamol was selected as  
103 previous studies conducted in isolated guinea-pig tracheal preparations showed a ten-fold greater  
104 potency of salbutamol at the β2-adrenoceptor when compared with dopexamine (30). Intravital  
105 microscopy in the intestinal ileum was performed after 150 minutes, midway through resuscitation. It  
106 was not possible to measure global haemodynamics during this procedure. The experiment ended  
107 after five hours of resuscitation when the heart and lungs were excised.

#### 108 **Analysis of plasma lactate, base deficit and renal and hepatic function**

109 200 µl of blood was taken at baseline and at the end of the experiment for measurement of plasma  
110 lactate concentration (Accutrend Lactate; Roche Diagnostics, Basel, Switzerland) and base deficit  
111 (Radiometer ABL77, Copenhagen, Denmark). A 1ml blood sample was also taken at the end of the  
112 experiment for measurements of urea, creatinine, alanine aminotransferase and aspartate  
113 aminotransferase by a commercial veterinary laboratory (IDEXX Laboratories Ltd, Sussex, UK).

#### 114 **Measurement of aortic blood flow**

115 A 1.5 mm perivascular probe was applied with water-soluble sonicating gel and sited as described  
116 earlier. The probe was connected to a TS420 monitor (Transonic Systems Inc., Ithaca, USA), which  
117 was connected to a Powerlab/8SP monitoring system (AD Instruments). This allowed continuous  
118 recording of aortic blood flow and HR, and calculation of relative stroke volume and relative cardiac  
119 output (relative as infra-renal aortic blood flow is not equivalent to cardiac output). Aortic blood flow

120 was indexed to body weight to provide a measure of changes in relative stroke volume index (SVI)  
121 and relative cardiac index (CI). Probe calibration was performed daily according to the  
122 manufacturer's instructions before experiments.

### 123 **Intravital microscopy (IVM)**

124 15 minutes before the midpoint of fluid resuscitation, 0.2mls of 0.17 g L-1 rhodamine 6G (Sigma-  
125 Aldrich, Gillingham, UK) was administered intravenously to enhance the visibility of leucocytes.  
126 The animal platform was then transferred to the stage of an intravital microscope. Fluorescence  
127 microscopy was carried out using an Olympus BX61W1 microscope (Carl Zeiss Ltd.) connected to  
128 an Olympus BXUCB lamp, Uniblitz VCMD1 shutter driver and DG4-700 shutter instrument.  
129 Recordings were captured using Slidebook 5.0 software (Intelligent Imaging TTL) and saved for later  
130 offline analysis. All images were taken at x40 magnification. Leucocyte rolling and adhesion (>30 s  
131 stationary) was quantified in ileal post-capillary venules: the course of microvessels of the ileal  
132 submucosal layer was followed from collecting venules (V1) to postcapillary venules (V3), the latter  
133 being selected for analysis. Vessel length and diameter was measured and recorded. Images were  
134 recorded for a minimum of 40s. A further 0.2mls of 250 mg kg<sup>-1</sup> ml<sup>-1</sup> of FITC labelled bovine  
135 albumin (Sigma-Aldrich) was then administered intravenously in order to measure functional  
136 capillary density (FCD) and arteriolar diameters: the course of microvessels of the ileal submucosal  
137 layer was followed from supply arterioles (A1) to pre-capillary arterioles (A3), the latter being  
138 selected for analysis. Vessel diameter was measured and recorded. Capillaries were identified in the  
139 circular and longitudinal layers of the ileum and images were recorded for a minimum of 40s. These  
140 images were later analysed offline. The platform was then removed from the microscope stage and  
141 observations continued as before.

142 Recordings of intravital videos were stored electronically. These files were later analysed offline  
143 using Slidebook 5.0 Reader (Intelligent Imaging Innovations (3i)) by an observer blinded to the  
144 experiment groups. Leucocyte adhesion was quantified and indexed to endothelial surface area  
145 (mm<sup>2</sup>), calculated from the diameters and lengths of the vessel segments studied and assuming  
146 cylindrical vessel geometry. Firmly adherent leucocytes were defined as those that did not move or  
147 detach from the endothelial lining within an observation period of 30s. FCD was calculated as the  
148 total length of perfused capillaries indexed to the visualised area (mm<sup>-1</sup>).

### 149 **Statistical analysis**

150 Data were presented as Mean (SEM) unless expressed otherwise and specifically. Kolmogorov-  
151 Smirnov normality testing was performed for all groups. Normally distributed data were tested using  
152 one-way analysis of variance (ANOVA) for comparison across all groups at a given time point. Post-  
153 testing was performed with Bonferroni's tests. Occasionally when ANOVA revealed significant  
154 results but post-tests did not indicate which group was responsible, t-tests (with or without Welch's  
155 correction depending on the variance of data) were performed to gain additional insight to the data.  
156 When data were not normally distributed in at least one group for any measurement, data were  
157 expressed as median (IQR) and the Kruskal-Wallis test was used in place of one-way ANOVA with  
158 Mann Whitney post-tests (and a Bonferroni correction). Two-tailed paired t-tests were used to  
159 compare haemodynamics at baseline with those at other time points for animals within the same  
160 group. Data were analysed with PrismGraph 4.0 (GraphPad Software, San Diego, USA).  
161 Significance was set at p<0.05.

162

**163 Results**

164 Baseline characteristics and fluid management are described in Table 1 and Supplementary Table 1.  
165 There were no significant differences between groups regarding weight or volume of fluid received.  
166 Animals in the sham group required a slightly greater dose of thiopental to maintain anaesthesia  
167 (Table, Supplementary Digital Content 1). There were no baseline differences in haemodynamics,  
168 base deficit, lactate or haematocrit. In the sham group, MAP and HR did not change significantly but  
169 CI and SVI increased progressively (Table 1, Figure 1. Table, Supplementary Digital Content 1.  
170 Figure, Supplementary Digital Content 2). Compared with the sham group and baseline, controls had  
171 a significantly higher HR ( $p<0.05$ ) and a significantly lower SVI and CI at 5 hours (Table 1, Figure  
172 1. Figure, Supplementary Digital Content 2). At this point control group plasma base deficit and  
173 lactate were increased compared with sham animals, the latter significantly ( $p<0.05$ ) (Table 1.  
174 Figure, Supplementary Digital Content 3). Compared to shams, there were more firmly adherent  
175 leucocytes (control:  $703 \pm 86\text{mm}^{-2}$  vs. sham:  $186 \pm 68\text{mm}^{-2}$ ;  $p<0.001$ ), and fewer rolling leucocytes  
176 in the post-capillary venules of control animals (Figure 2. Figure, Supplementary Digital Content 4).  
177 Intestinal arteriolar diameters were reduced in control animals (control:  $21 \pm 2\mu\text{m}$  vs sham:  $39 \pm$   
178  $3\mu\text{m}$ ;  $p<0.01$ . Figure 3) although FCD in the muscularis and its component circular and longitudinal  
179 layers did not differ significantly from shams (Figure 4. Figure, Supplementary Digital Content 5).  
180 Endotoxaemia was associated with acute kidney injury but not liver dysfunction (Figure 5).

181 Dopexamine infusion had no significant effect on any haemodynamic parameters when compared to  
182 controls, except for an increase in heart rate (Table 1, Figure 1. Table, Supplementary Digital Content  
183 1. Figure, Supplementary Digital Content 2). Similarly, dopexamine was not associated with any  
184 improvement in plasma lactate or base deficit in endotoxaemic animals (Table 1. Figure,  
185 Supplementary Digital Content 3). The major finding of this study was that at the mid-point of  
186 resuscitation dopexamine significantly reduced leucocyte adhesion in post-capillary venules when  
187 compared to controls (D0.5:  $409 \pm 65\text{mm}^{-2}$ ,  $p<0.05$  vs. control; D1:  $361 \pm 66\text{mm}^{-2}$ ,  $p<0.01$  vs.  
188 control) (Figure 2). Furthermore dopexamine prevented the reduction in arteriolar diameter observed  
189 in control animals (Figure 3). The effects of dopexamine on FCD were complex. There were  
190 significant differences between groups in longitudinal muscle FCD (one-way ANOVA  $p=0.024$ ),  
191 (Figure 4), but post-tests did not show which group was responsible for this difference. However  
192 isolated t-tests comparing each group against controls reveal that only the D1 group had a  
193 significantly reduced longitudinal FCD compared to controls (unpaired t-test with Welch's  
194 correction,  $p=0.0034$ ). Dopexamine had no effect on FCD in the circular layer of the muscularis at  
195 any dose. When comparing total muscularis FCD for all groups, differences fell outside of the limits  
196 of statistical significance (one way ANOVA  $p=0.058$ ), (Figure, Supplementary Digital Content 4).  
197 Regarding organ function, renal function was improved in the D1 group compared to controls, but  
198 not the D0.5 animals (Figure 5).

199 The infusion of salbutamol was associated with a similar pattern of haemodynamics to those  
200 observed in dopexamine treated animals (Table 1, Figure 1. Table, Supplementary Digital Content 1.  
201 Figure, Supplementary Digital Content 2), and there was also no improvement in indices of tissue  
202 perfusion (Figure, Supplementary Digital Content 3). Compared to controls, salbutamol significantly  
203 reduced leucocyte-endothelial adhesion (S:  $365 \pm 49\text{mm}^{-2}$ ,  $p<0.01$  vs. control) (Figure 2).  
204 Salbutamol also appeared to prevent arteriolar vasoconstriction (Figure 3), but unlike dopexamine  
205 was not associated with any change in FCD in any layer of the muscularis (Figures 3 and 4. Figure,  
206 Supplementary Digital Content 5) and had no effect on organ injury (Figure 5).

207

**208 Discussion**

209 The principal findings of this experiment were that in a rodent model of laparotomy and  
210 endotoxaemia, clinically relevant doses of dopexamine were associated with decreased leucocyte-  
211 endothelial adhesion and reduced arteriolar constriction in the intestinal microvasculature. However,  
212 with the exception of an increase in heart rate, dopexamine infusion was not associated with any  
213 systemic cardiovascular effects and in particular, relative cardiac index was not improved. In the  
214 higher dose dopexamine group an amelioration of renal dysfunction as assessed by plasma creatinine  
215 was observed. Almost all these findings were replicated by the β2-adrenoceptor agonist salbutamol  
216 though salbutamol failed to improve renal function (28).

217  
218 This study provides evidence that dopexamine modulates the inflammatory response by reducing  
219 leucocyte-endothelial adhesion. We have previously demonstrated that in addition to reducing the  
220 pro-inflammatory cytokine response, dopexamine may also reduce the expression of leucocyte  
221 surface integrins following endotoxaemia. We have also previously demonstrated a reduction in  
222 neutrophil infiltration in the lung of dopexamine treated endotoxaemic rats (28). It is possible that  
223 these observations are linked, such that during endotoxaemia, a dopexamine mediated reduction in  
224 surface integrin expression results in reduced leucocyte-endothelial adhesion and consequential  
225 neutrophil transmigration into the tissues. Although this experiment does not elucidate the cellular  
226 events that result in reduced leucocyte-endothelial adhesion, the similar effects of salbutamol suggest  
227 a β2-adrenoceptor mediated mechanism. In this regard it has been shown that β2- and non-β2-  
228 adrenoceptor mediated elevations of cAMP reduce leucocyte adhesion (31, 32), whilst tonic activity  
229 of Protein Kinase A prevents β2-integrin activation (33). Other factors including, but not limited to,  
230 an amelioration of pro-inflammatory cytokines or drug effects on vascular endothelium may be of  
231 equal or greater relevance to these observed changes. Furthermore the relevance of reduced  
232 leucocyte-endothelial adhesion to the effect of dopexamine on organ function is also unclear because  
233 although salbutamol reduced leucocyte adhesion in post-capillary venules it did not ameliorate renal  
234 injury.

235  
236 The reduction in arteriolar vasoconstriction by dopexamine is consistent with the preservation of  
237 arteriolar diameters in villus arterioles and hepatic sinusoids observed in previous endotoxaemia  
238 studies with dopexamine. In those studies, dopexamine treated endotoxaemic animals had an  
239 associated preservation of total organ and microvascular blood flow compared to untreated groups  
240 (23, 26). However in our previous study we observed a reduction in ileal red cell flux during  
241 endotoxaemia (28). Assuming the same effect occurred in this study, then the similar muscularis  
242 FCD in control and sham animals suggests that any perfusion defect (assessed by FCD) must occur in  
243 the mucosa, as has been shown in other studies using intravital microscopy (34). The non-significant  
244 trend to reduction in longitudinal FCD in dopexamine treated animals may then indicate a  
245 dopexamine mediated re-distribution of blood from the outer layer of the ileum to the hypoxia prone  
246 mucosa. In this regard it is interesting to note that although salbutamol and dopexamine had similar  
247 effects on arteriolar diameters, salbutamol did not show any tendency to produce this effect on  
248 longitudinal layer FCD, did not improve renal function and showed no tendency to a reduced plasma  
249 lactate either. These differences, despite the similarity of effects otherwise, are notable and warrant  
250 further study.

251  
252 Previous clinical studies in major surgery including those by our own group have emphasised the role  
253 of cardiovascular optimisation in enhancing tissue oxygen delivery to reduce peri-operative  
254 morbidity (7, 8). Early studies drove clinicians to suggest this approach was beneficial as it reduced  
255 potentially harmful tissue ischaemia (35). The importance of maintaining tissue perfusion is still

256 supported by modern studies from peri-operative medicine where MAP is clamped at higher levels  
257 and shown to reduce the incidence of post-operative morbidity (36). This may also relate to  
258 improvements in microvascular perfusion (37). However we previously showed in surgical patients  
259 kept to a narrow MAP range that peri-operative stroke-volume guided fluid management protocols  
260 with continuous  $0.5 \mu\text{g kg}^{-1} \text{min}^{-1}$  dopexamine infusion produced improvements in tissue oxygenation  
261 but without beneficial effects on markers of systemic inflammation or organ dysfunction (8). We also  
262 showed in a rodent model that dopexamine  $1-2 \mu\text{g kg}^{-1} \text{min}^{-1}$  brings about potent immunomodulatory  
263 effects that are associated with improved organ function despite MAP and microvascular flow being  
264 similar to controls (28). This suggests that under surgical conditions therapeutic benefit is achievable  
265 through modulation of the host response to tissue injury and that further increases in tissue  
266 oxygenation or blood flow when perfusion is already guaranteed are redundant. In support of this, an  
267 analysis of surgical trials shows that patients with higher levels of baseline systemic inflammation are  
268 more likely to develop surgical complications (38). Similarly patients with impaired pre-operative  
269 microvascular function (who are known to have higher levels of inflammatory markers) are also  
270 more likely to suffer later complications (37, 39). Considering that previous studies have shown that  
271 surgical stress is associated with an upregulation of chemokines in the peritoneum and lungs,  
272 modulation of the host response to surgery becomes a potential explanation for the beneficial effects  
273 of dopexamine (40). However, the failure of salbutamol to improve renal function while producing a  
274 very similar spectrum of immune effects to dopexamine suggests that the mechanisms of renal  
275 protection with dopexamine are not necessarily only related to β2-adrenoceptor mediated effects on  
276 leucocyte-endothelial adhesion.

277  
278 Differential abilities of dopexamine and salbutamol to increase cellular cAMP may explain divergent  
279 effects on renal function (41). This might be the case if dopaminergic receptor activation by  
280 dopexamine further increased cAMP levels above that provided by β2-adrenoceptor activation (5).  
281 The importance of increasing cAMP is that regulated cell death that causes tissue injury in acute  
282 kidney injury is inhibited by cAMP mediated-pathways, as is mitochondrial biogenesis which is  
283 required for enhanced recovery from cell stress (42, 43). On the other hand β2-adrenoceptor  
284 activation has also been shown to alter systemic metabolism to increase tissue tolerance of injury  
285 (44). Therefore unexpected differential effects of the drugs at β2-adrenoceptors or in cAMP  
286 generation provide two mechanisms through which tissue damage can be minimised during the hours  
287 during and after emergency laparotomy and elective major abdominal surgery. This might also  
288 provide some explanation for the opposite findings in trials of β2-adrenoceptor agonism in acute  
289 respiratory distress syndrome (45). In those trials a week long infusion of higher doses of salbutamol  
290 were used to try and improve outcomes through a reduction in extravascular lung water but resulted  
291 in increased mortality. On the other hand in peri-operative medicine shorter-term infusions of similar  
292 agents are used to try and minimise tissue damage and organ dysfunction that can result from major  
293 surgery – trials in this setting with dopexamine have not shown any signal to increased mortality (7).

294  
295 Several findings of this study are consistent with previous investigations. The haemodynamic effects  
296 of endotoxaemia with or without dopexamine were replicated here and are in keeping with other  
297 studies (20, 28). Findings of intestinal arteriolar constriction are in keeping with the intense  
298 splanchnic vasoconstriction and rapid reduction of blood flow seen following endotoxaemia and  
299 shock in rodents (20, 28, 46). This study, in keeping with other studies, found arteriolar constriction  
300 could be ameliorated by dopexamine (23). Similar studies have found an increase in adherent  
301 leucocyte numbers in intestinal or mesenteric post-capillary venules that could be ameliorated by  
302 dopexamine (19, 20). A significant increase in adherent leucocytes (reduced by dopexamine) and a  
303 decrease in rolling leucocyte numbers in post-capillary venules at two and a half hours would likely  
304 have resulted in leukopenia, as found in other studies (19-23). However, some findings of the

305 experiment reported here, such as the failure of endotoxaemia to decrease longitudinal and circular  
306 muscularis functional capillary density are not consistent with previous studies (20). These  
307 inconsistencies are likely to be the result of differences in the endotoxin serotype, dose, method of  
308 administration and fluid loading conditions of each experiment. Our study also appears to contradict  
309 the findings of Schmidt et al regarding the role of β2-adrenoceptor agonism in leucocyte-endothelial  
310 adhesion (19). Importantly, our study design avoided the ablation of both endogenous and exogenous  
311 β2-adrenoceptor agonism that may account for differences in findings between the two studies.

312  
313 Our study has several strengths. The use of IVM gave qualitative and quantitative data that is  
314 unobtainable from laser Doppler flowmetry studies (20). The nature of endotoxaemia was modified,  
315 using peptidoglycan, which increases the generalizability of these findings outside of Gram negative  
316 septicaemia alone. Although the duration and nature of endotoxaemia differed from our previous  
317 study, the model behaved in a similar fashion to our previous study with respect to haemodynamics,  
318 markers of perfusion and resultant organ dysfunction. With respect to biochemical markers of tissue  
319 perfusion, it is possible that the lack of statistical significance in the D1 group where lactic acidosis  
320 and base deficit were less severe (as in our previous study) is the result of smaller sample sizes. If  
321 correct, it is notable that salbutamol neither showed any signal to an amelioration of plasma lactate  
322 nor resulted in any amelioration of renal dysfunction. This could suggest there are additional  
323 important mechanisms of action of reducing organ injury that dopexamine possesses (as discussed  
324 above).

325  
326 There are also limitations to the study performed. Although there were many similarities with our  
327 previous experiments, fundamental differences in design mean that it is not possible to be certain that  
328 the models behaved in an identical fashion. Secondly, although the use of IVM permitted direct  
329 visualisation of the intestinal microcirculation, expected differences in FCD were not seen between  
330 shams and controls. This may have related to the mild severity of the model (note no significant  
331 hepatic dysfunction was observed in any group), to visualising the intestinal microcirculation too  
332 early in the course of the experiment or even to the volume of fluid administered. In this regard the  
333 inability to observe changes in the microvascular bed over the entire course of the experiment was a  
334 weakness of this study. Furthermore although arteriolar diameters and muscularis FCD were  
335 observed, the inability to measure centre-line red cell velocity, mucosal FCD and mucosal inflow  
336 arteriolar diameters prevents a complete picture of the distribution of intestinal blood flow being  
337 made. Although reductions in leucocyte-endothelial adhesion were observed, it is not possible to  
338 ascertain the relative importance of this phenomenon to the reduction in organ injury seen in this or  
339 previous experiments. Derivation of the surrogates relative stroke volume and relative cardiac index  
340 from infra-renal blood flow is recognised in the literature. Nevertheless it should be noted that  
341 cardiac index may have differed between groups and the effects of different doses of vasoactive  
342 drugs may have led to differing organ blood flows above the level of measurement, none of which  
343 could have been detected by an infra-renal flow probe. Regarding the use of dopexamine and  
344 salbutamol, dose equivalence was based on previous studies in isolated tracheal preparations (30).  
345 Although haemodynamics were similar between D1 and S groups suggesting the dose selection was  
346 probably correct, it is still not possible to be certain that the effect at the β2-adrenoceptor was  
347 identical for both drugs. This may be compounded by the fact that salbutamol is a mix of two  
348 enantiomers, adding further complexity to the comparison. In this regard the use of a selective β2-  
349 adrenoceptor antagonist to further disentangle the role of this drug effects will be useful in future  
350 studies. Finally, although the use of peptidoglycan increases the generalizability of these findings  
351 outside of Gram negative septicaemia, the choice of an endotoxin based model may still be criticised  
352 for lack of a true clinical correlate.

353



354 In summary, we present experimental evidence confirming that clinically relevant doses of  
355 dopexamine reduce leucocyte-endothelial adhesion in the intestinal microvasculature and are  
356 associated with improved renal function at clinically relevant doses. As a consequence of our  
357 experiments some avenues warrant further research. The relationship between β2-adrenoceptor  
358 signalling and downstream effects on leucocyte CD11b expression, tissue tolerance mechanisms and  
359 inhibition of regulated cell death deserve further attention. The effect of dopexamine on  
360 microvascular recruitment and its relationship to cardiac index under differing fluid regimes and also  
361 the effect of dopexamine on the distribution of microvascular blood flows are two areas that also  
362 warrant further study given the disparity seen in results of our studies and others (8, 20, 23).  
363 Although peri-operative dopexamine use has been shown to be safe in randomised controlled trials  
364 and a Bayesian analysis of the OPTIMISE trial suggested a high probability of superiority of  
365 treatment efficacy, a new randomised controlled trial of peri-operative optimisation using β2-  
366 adrenoceptor agonists including dopexamine is underway and will inform clinicians definitively  
367 regarding the role of peri-operative dopexamine and haemodynamic optimisation (7, 47, 48).  
368

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### 378 **Author contributions statement**

379 MB carried out the in vivo studies and statistical analysis of all data. NP provided advice during in  
380 vivo studies and on study design. TA took offline measurements from intravital microscopy videos.  
381 CT participated in the design and coordination of the study and provided guidance  
382 throughout. RP conceived of the study, participated in its design and co-ordination and helped with  
383 statistical analysis. RP, CH, CT & MB together drafted the manuscript. All authors read and  
384 approved the final manuscript.

### 385 **Conflict of interest statement**

386 RP has received equipment loans from LiDCO Ltd and has performed consultancy work for Edwards  
387 Lifesciences and Massimo Inc. RP is a member of the associate editorial board of the British Journal  
388 of Anaesthesia. CT is CEO of William Harvey Research Limited, which is a CRO and has conducted  
389 contracted research in the area of critical care. CT is also Senior Associate Editor for the journal  
390 Shock. All other authors have no interests to declare.

### 391 **Contribution to the Field Statement**

392 Best practice in the haemodynamic optimisation of the patient at risk of peri-operative organ  
393 dysfunction is a clinically controversial and poorly understood area. Clinicians can select from a  
394 wide variety of strategies combining different intravenous fluids and vasoactive drugs in an attempt  
395 to prevent organ dysfunction. Exactly how and even whether these drugs and strategies can produce  
396 their purported clinical outcomes is not known and only adds to the confusion regarding best

397 practice. While ever-larger and costly human clinical trials are underway to try and address the  
398 question, mechanistic insight is still lacking. We here provide a second basic science study of  
399 immune, microvascular and organ preserving effects of dopexamine, the catecholamine with the best  
400 evidence for efficacy in the peri-operative setting. Through this study we further contribute to the  
401 understanding of how this drug might affect peri-operative organ dysfunction and clinical outcomes,  
402 focusing on leucocyte-endothelial interaction. This study (together with its preceding one) will add to  
403 mechanistic insight of the upcoming OPTIMISE II trial.

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585  
586 Tables:

587  
588 Table 1. Fluid administered, temporal changes in blood gas and haemodynamic parameters for  
589 each group (n=6 all groups)  
590 There were no significant differences between groups in baseline haemodynamics or volumes of  
591 fluid administered. Over the course of the experiment in shams SVI significantly increased and CI

592 also tended to increase, though HR and MAP did not change. In contrast HR significantly increased,  
 593 while SVI and CI significantly decreased in control and dopexamine groups. MAP was significantly  
 594 decreased, though this was not a consistent finding. HR also significantly increased in group S and  
 595 although CI and SVI remained relatively stable MAP decreased significantly. A significantly lower  
 596 lactate was seen in shams compared to controls. Data presented as mean (SEM) when all groups  
 597 normally distributed, otherwise median (IQR) if ≥ 1 group not normally distributed. One-way  
 598 ANOVA (post hoc Bonferroni's test, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. controls). Paired t-tests of  
 599 baseline vs end experiment for mean changes (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001)  
 600

	<b>Sham</b>	<b>Control</b>	<b>D 0.5</b>	<b>D1</b>	<b>S</b>
<i>Administered fluid (ml kg<sup>-1</sup>)</i>	29.9 (29.2 – 30.5)	29.4 (29.3 – 29.5)	29.8 (29.4 – 30.5)	30.1 (29.4 – 30.5)	29.8 (29.4 – 30.4)
<i>Baseline HR (bpm)</i>	378 (357 – 421)	399 (379 – 417)	415 (390 – 420)	379 (356 – 441)	392 (370 – 423)
<i>Baseline MAP (mmHg)</i>	120 (7)	111 (3)	114 (5)	108 (3)	110 (6)
<i>End experiment HR (bpm)</i>	371 (8)***	447 (12)	465 (15)	478 (9)	445 (5)
<i>End experiment MAP (mmHg)</i>	114 (95 – 133)	93 (69 – 101)	81 (76 – 106)	94 (79 – 106)	78 (70 - 106)
<i>End experiment lactate (mmol L<sup>-1</sup>)</i>	1.7 (0.2)*	3.4 (0.5)	3.1 (0.3)	2.6 (0.4)	3.8 (0.3)
<i>End experiment base deficit (mmol L<sup>-1</sup>)</i>	- 0.6 (1.0)	4.5 (1.6)	2.9 (1.5)	3.3 (1.5)	6.0 (1.0)
<i>Mean change in SVI during experiment (ml kg<sup>-1</sup>)</i>	0.044 (0.014)*	-0.036 (0.008)**	-0.050 (0.009)**	-0.054 (0.003)***	-0.020 (0.011)
<i>Mean change in CI during experiment (ml min<sup>-1</sup> kg<sup>-1</sup>)</i>	15.3 (6.2)	-10.5 (3.2)*	-16.1 (4.2)*	-14.4 (2.0)***	3.0 (4.9)

601

602  
603 Figure legends

604  
605 Figure 1  
606 Relative Stroke Volume Index during 5 hours of laparotomy and endotoxaemia recorded every 30  
607 minutes. Values not plotted for t180 – t210 (animals were undergoing IVM at this time and aortic  
608 flow and HR could not be measured (therefore relative SVI could not be calculated)).  
609 Relative stroke volume index in controls differed significantly for most of the experiment and until  
610 its end when compared to shams. However no significant differences in relative stroke volume index  
611 were observed between controls and groups treated with dopexamine or salbutamol at any time. The  
612 mean change in relative stroke volume index from baseline to end experiment was also significant in  
613 all groups except salbutamol treated animals (also see Table 1). Data presented as mean (SEM). One-  
614 way ANOVA at each time point (Bonferroni's post-tests, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs.  
615 controls).

616  
617 Figure 2  
618 (Left) Numbers of adherent leucocytes per square mm of endothelium in ileal post-capillary venules  
619 at 2.5 hours of laparotomy and endotoxaemia (n=6 all groups). Numbers of vessels observed per  
620 group ranged from 8-18.  
621 Sham, dopexamine and salbutamol treated groups demonstrated significantly less adhesion than  
622 controls. Data presented as mean (SEM). One-way ANOVA (Bonferroni's post-tests, \*p<0.05,  
623 \*\*p<0.01, \*\*\*p<0.001 vs. controls)

624  
625 (Mid) Intestinal arteriolar diameters of the ileum at 2.5 hours of laparotomy and endotoxaemia (n=6  
626 all groups). Numbers of vessels measured per group ranged from 8-19.  
627 When compared to shams and unlike controls, ileal arteriolar diameters were not significantly  
628 reduced in dopexamine and salbutamol treated groups. Data presented as mean (SEM). One-way  
629 ANOVA (Bonferroni's post-tests, \*\*p<0.01 vs. shams).

630  
631 (Right) Intestinal functional capillary density in longitudinal layers of the ileal muscularis at 2.5  
632 hours of laparotomy and endotoxaemia (n=6 all groups). Number of images per group ranged from  
633 16-25. Groups were significantly different with respect to longitudinal FCD at 2.5 hours. Data  
634 presented as mean (SEM). One-way ANOVA p=0.024 (no groups positive in post-tests, although  
635 p<0.01 when comparing control and D1 group with unpaired t-test with Welch's correction).

636  
637 Figure 3  
638 Plasma urea, creatinine, ALT and AST sampled 5 hours after laparotomy and endotoxaemia (n=6 all  
639 groups).  
640 5 hours of laparotomy and endotoxaemia caused significant acute kidney injury in controls. The  
641 degree of injury was significantly less in the D1 group. All data presented as mean (SEM). Plasma  
642 urea and creatinine: One-way ANOVA (Bonferroni's post-tests, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs.  
643 controls). ALT: One-way ANOVA, p = 0.0246 (no groups positive in post-tests).

644