Intravenous anaesthesia and the rat microcirculation: the Dorsal Microcirculatory Chamber†

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The use of the dorsal microcirculatory chamber in male Wistar rats (n=7) to study the effects of induction and maintenance of anaesthesia on the microcirculation is described. Different patterns of responses were observed. At induction, arteriolar dilation was found following propofol and thiopental but ketamine produced constriction. During maintenance, constriction of arterioles was seen with ketamine and thiopental but dilation persisted with propofol. The dorsal microcirculatory chamber appears to be a useful tool for the study of microcirculatory changes related to anaesthesia.

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Previous studies investigating the effect of anaesthesia on the microcirculation have been limited by the absence of a conscious control and the inability to measure the initial effects of agents in the absence of background anaesthesia. The major problem has been that it is not possible to observe these effects in microcirculatory beds that require surgical preparation without previously providing anaesthesia and analgesia.

We are describing the use of the Dorsal Microcirculatory Chamber (DMC) which allows serial study of the rat striated muscle microcirculation in the conscious state and during induction and maintenance of anaesthesia with different agents. The DMC is essentially a lightweight polycarbonate chamber enclosing a single layer of cutaneous muscle which is chronically implanted in the dorsal skin fold. This allows longitudinal studies using a number of agents in the same animal. The aim of this initial study was to evaluate the use of this technique by comparing the effects of induction and maintenance of anaesthesia with propofol, thiopental and ketamine with a conscious control on the striated muscle microcirculation.

Methods and Results

Seven male Wistar rats were entered into the study protocol which was conducted over a 10-week period. The studies were conducted under Home Office Licence PPL.50/1252. During the first 2 weeks the rats were trained to sit at rest in the restrainer required for observation of the microcirculation. This familiarized the animals with the study environment thus minimizing any physiological effects of stress on the observations. At the start of the third week the DMC was implanted under fentanyl/fluanisone/diazepam (0.1 ml 100 g⁻¹) anaesthesia using the technique described by Smith et al.¹ and Hutchins et al.² Training in use of the restrainer was recommenced in weeks 4 and 5. The studies investigating responses to the different anaesthetic agents were conducted between weeks 6 and 10.

Each animal was studied on four occasions 1 week apart and received, in randomized order, either propofol, thiopental, ketamine or saline (control). The study protocol lasted 90 min. Animals were placed in the restrainer positioned on a horizontally mounted Nikon Optiphot 2 microscope (magnification 590×) equipped with transmitted light for observation of the microcirculation. An area of muscle containing A1–A4 arterioles and V1–V4 venules was identified before starting the 90 min study period. Vessels are classified by their order of branching, A1 being the largest (60–80 μm) with subsequent branching down to A4 (8–12 μm). Following a 30 min baseline period, anaesthesia was induced over 5 min and then maintained for a further 55 min. The area for observation was recorded on videotape, during transmitted light exposure, every 10 min for up to 30 s. The video images were subsequently analysed using a computerised image analysis system (Image Pro Plus, Media Ciberkinetics, USA) which allowed measurement (in μm) of change in vessel diameter.

¹This study was presented, in part, at the British Microcirculation Society meeting in April 1999.
Measured changes in vessel diameter with each agent at the end of induction and during maintenance were compared with baseline (t=30), using Kruskal-Wallis and Wilcoxon Rank Sum tests (P<0.05 taken as significant). Kruskal-Wallis and Mann-Whitney U-test were used to compare changes produced by each agent with control study period.

Results are summarized in Figure 1 where the changes following induction (t=35) and during maintenance (t=60) are illustrated using A1 and A4 arterioles and V1 and V4 venules.

**Control**

Following 30 min baseline period, saline (2 ml kg⁻¹) was administered over 5 min via the tail vein followed by a continuous infusion at 5 ml kg⁻¹ h⁻¹ for the remaining study period. There were no significant changes in vessel diameter observed in any of the vessel groups throughout the 90 min study. There were no observed effects following either bolus or continuous infusion of saline. There were no significant changes from baseline during the control study period.

**Thiopental**

Anaesthesia was induced with a bolus of 30 mg kg⁻¹ given over 5 min and maintained with a continuous infusion at 40–90 mg kg⁻¹ h⁻¹. The diameters of all orders of vessels were stable during the baseline period. Induction of anaesthesia initially produced dilation of all arterioles (A1–A4) resulting in a 10–25% increase in diameter at the end of induction (t=35). The change was greatest in the A1 vessels. A similar magnitude of change was seen in the venules but V4 vessels dilated most.

During maintenance there was a change in all vessels (A1–A4, V1–V4) from dilation to constriction which occurred by t=50 and was maximal by t=60.

**Ketamine**

Anaesthesia was induced with a bolus dose of 30 mg kg⁻¹ given over 5 min and maintained with a continuous infusion at 60–90 mg kg⁻¹ h⁻¹. The diameters of all orders of vessels were stable during the baseline period. Induction of anaesthesia produced constriction of all arterioles and this was sustained throughout maintenance. This effect was evident at the end of induction (approximately 25% constriction). A different pattern of response was seen in venules where induction produced dilation and this changed to constriction during maintenance.

**Propofol**

Anaesthesia was induced with a bolus dose of 20–30 mg kg⁻¹ given over 5 min and maintained with a continuous infusion at 20–60 mg kg⁻¹ h⁻¹. The diameters of all orders of vessels were stable during the baseline period. Induction of anaesthesia produced a marked dilation of all arterioles (t=35, 20–40% change). Dilation continued throughout the maintenance period and tended to be greater in the smaller vessels (A3, A4) (NS). Venules dilated during induction, to a lesser extent than arterioles, and this dilation increased during maintenance.

With all agents the changes following induction and those during maintenance were significantly different from baseline (P<0.05). The changes during anaesthesia were all significantly different from control (P<0.05).
Comment
We have observed differences in the pattern of vessel diameter responses produced by the three anaesthetic agents used both at induction and during maintenance. The changes on induction are consistent with the known clinical effects of the drugs. Propofol and thiopental produced an initial dilation of vessels with the effect being greater following propofol, an effect that is in keeping with the decrease in arterial pressure that occurs at induction with these agents. There were differences in the pattern of changes with thiopental apparently having more effect on the larger arterioles (A1) and small venules and propofol having more effect on smaller arterioles and relatively less on venules. This is in agreement with the findings of our previous work on haemorrhage using a cremaster muscle preparation where dilation of small arterioles was more likely to occur along with lower arterial pressure. However, the use of the DMC model will allow the mechanism of the anaesthetic effects to be explored more fully. The constrictor effect of ketamine is consistent with its known sympathomimetic effect. However, it is interesting to note an effect was observed in the small arterioles which are thought to receive less sympathetic innervation.

The findings of this study are in broad agreement with the limited number of studies of the effects of these agents on microcirculation with a conscious control. In a hamster skin fold model, propofol at an infusion rate of 78 mg kg\(^{-1}\) h\(^{-1}\) produced arteriolar and venular dilation.\(^4\) In a bat wing model ketamine\(^5\) caused dilation of arterioles but thiopental\(^6\) had no effect on either type of vessel. In vitro studies have demonstrated vasodilation with all three agents.\(^7\) The differences we have demonstrated within arteriolar and venular responses and between induction and maintenance may indicate a dose-dependent effect as shown in the studies with ketamine in a bat wing model.\(^5\) We have previously shown that the doses used in this study produce a consistent light plane of anaesthesia.\(^3\)\(^8\) However, the DMC model is capable of differentiating the responses to both induction and maintenance with a single agent as background anaesthesia is not required.

The DMC model provides a useful method for defining the microvascular effects of anaesthetic agents. It can be used to explore the relationship between microcirculatory changes and arterial pressure at induction of anaesthesia and the effect of different agents on the response to events such as haemorrhage or sepsis.

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References