SUMMARY

We have studied five pigs undergoing bilateral clamping of the renal pedicles, seven pigs undergoing orthotopic liver transplantation and three control animals without surgery in order to examine the roles of the kidney and liver in the plasma clearance of pipecuronium. An i.v. infusion of pipecuronium was controlled to maintain a constant 90-95% twitch depression throughout the investigation. The right sciatic nerve was stimulated continuously with supra-maximal stimuli at 0.1 Hz and the force of the corresponding evoked isometric muscle contraction was recorded continuously. Control pigs needed an infusion rate of pipecuronium 8-10.7 μg kg⁻¹ min⁻¹. In the renal group, it was necessary to reduce the infusion rate of pipecuronium by about 25% after clamping both renal vascular pedicles (P < 0.05 compared with controls); in pigs undergoing liver transplantation, it was necessary to reduce the rate by approximately 80% after clamping hepatic vessels (P < 0.05 compared with controls and from the period after clamping of renal vessels). After hepatic recirculation, the infusion rate of pipecuronium was increased progressively to a rate which corresponded to 50% of baseline values (P < 0.05 compared with the anhepatic phase and from controls). Plasma concentrations of pipecuronium were comparable in the three animal groups and did not change significantly during the study. These data suggest that the liver plays a more important role than the kidney in the plasma clearance of pipecuronium in pigs.

KEY WORDS

Methods

Fifteen pigs (Sus scrofa domesticus) weighing 22–25 kg were included in the study, after approval of the Ethics Committee on animal research of our institution. Animals were allocated to three groups: group A as control group (n = 3), group B undergoing ligation of both renal vascular pedicles (n = 5), and group C undergoing ortho-
After a 60-min period of stabilization during which both renal vascular pedicles were prepared, and operation.

Beginning of pipecuronium infusion to the end of measurement of pipecuronium concentrations were drawn every 15 min, from 30 min after the triggering of spontaneous ventilation, to 90 min after the beginning of pipecuronium infusion. Blood samples for measurement of plasma concentrations of pipecuronium were obtained by a new technique using iodine-labelled bengal rose. By comparison with an appropriate calibration curve, measurement of radioactivity of this complex permits measurement of pipecuronium. The use of $^{125}$I-bengal rose with a specific activity of 150 uCi mg$^{-1}$ permitted measurement of pipecuronium to a detection limit of 5 ng ml$^{-1}$. This assay is linear over the range 5–5000 ng ml$^{-1}$, with a correlation coefficient of 0.99 in pig plasma. Inter-assay coefficients of correlation (for 10 assays) were 61.1% at 10 ng ml$^{-1}$ and 5.4% at 100 ng ml$^{-1}$ of pipecuronium. Intra-assay coefficients of correlation (for 10 assays) were 60.5% at 10 ng ml$^{-1}$ and 3.2% at 100 ng ml$^{-1}$ of pipecuronium. Metabolites of pipecuronium and other anaesthetic agents do not interfere with measurement of pipecuronium concentrations.

Measurement of plasma concentrations of pipecuronium

One millilitre of whole blood was collected in a heparinized tube and centrifuged immediately at 5000 g for 30 s. 500 µl of plasma was transferred into an Eppendorf tube and frozen quickly to $-70$ °C in acetone and dry ice. Samples were stored at $-70$ °C until analysed. Plasma concentrations of pipecuronium were determined by a new technique using iodine-labelled bengal rose. This technique consists of producing a complex between pipecuronium and iodine-labelled bengal rose. By comparison with an appropriate calibration curve, measurement of radioactivity of this complex permits measurement of pipecuronium. The use of $^{125}$I-bengal rose with a specific activity of 150 µCi mg$^{-1}$ permitted measurement of pipecuronium to a detection limit of 5 ng ml$^{-1}$. This assay is linear over the range 5–5000 ng ml$^{-1}$, with a correlation coefficient of 0.99 in pig plasma. Inter-assay coefficients of correlation (for 10 assays) were 61.1% at 10 ng ml$^{-1}$ and 5.4% at 100 ng ml$^{-1}$ of pipecuronium. Intra-assay coefficients of correlation (for 10 assays) were 60.5% at 10 ng ml$^{-1}$ and 3.2% at 100 ng ml$^{-1}$ of pipecuronium. Metabolites of pipecuronium and other anaesthetic agents do not interfere with measurement of pipecuronium concentrations.

Statistical analysis

In each animal group, recorded variables over
time were compared by one-way analysis of variance for repeated measurements followed by Bonferroni's multiple comparisons test; comparisons between the three animal groups were made using a one-way analysis of variance followed by a Duncan's multiple comparisons test. For all statistical comparisons, differences were considered significant if $P < 0.05$.

RESULTS

Pipecuronium-induced neuromuscular block was stable at 90–95% twitch depression in each animal of the three groups during the entire investigation. In control animals, a mean infusion rate of pipecuronium of 8.3 μg kg$^{-1}$ min$^{-1}$ (range 8–10.7 μg kg$^{-1}$ min$^{-1}$) was necessary to maintain neuromuscular block at 90–95% twitch depression during the 240-min study period (fig. 1). To maintain a similar neuromuscular block in pigs with bilateral renal exclusion, a mean infusion rate of pipecuronium of 8.9 μg kg$^{-1}$ min$^{-1}$ (range 7–11 μg kg$^{-1}$ min$^{-1}$) was necessary before clamping both renal pedicles (ns at each time interval compared with control animals). After exclusion of both kidneys, the infusion rate had to be reduced significantly within 15 min to mean 6.9 (SEM 0.8) μg kg$^{-1}$ min$^{-1}$ ($P < 0.05$ compared with the period before clamping of renal vessels), and it remained at this lower rate for the 2 h to the end of the study. To maintain block at 90–95% twitch depression in pigs undergoing liver transplan-

\begin{figure}
\centering
\includegraphics[width=\linewidth]{fig1}
\caption{Mean infusion rate of pipecuronium adjusted to maintain a stable 90–95% twitch depression in three control pigs (△), in seven pigs undergoing orthotopic liver transplantation (■), and in five pigs undergoing bilateral renal exclusion (○). Data points represent mean (SEM) of values measured every 15 min. Clamp = clamping of renal and hepatic vessels; Unclamp = restoration of hepatic circulation. $P < 0.05$: *compared with control; †compared with pigs with bilateral renal exclusion.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\linewidth]{fig2}
\caption{Plasma concentrations of pipecuronium during a 240-min i.v. infusion of pipecuronium adjusted to maintain a stable 90–95% twitch depression in control pigs. Data points represent mean (SEM) of three animals measured every 15 min.}
\end{figure}
block (up to 100%), despite reduction in the
infusion rate. In order to restore 90–95% neuromuscular block rapidly, we stopped the infusion for a short time (10–15 min) and subsequently the infusion rate of pipecuronium was maintained at 1.2 (0.5) and 1.5 (0.7) µg kg⁻¹ min⁻¹ up to recirculation of the liver (P < 0.05 for each data point compared with the period before clamping of hepatic vessels, controls and the period after clamping of renal vessels) (fig. 1). To maintain a comparable neuromuscular block after restoration of the circulation to the liver, the mean infusion rate of pipecuronium was increased progressively over 45 min to 4.3 (0.4) µg kg⁻¹ min⁻¹ and maintained at these values during 60 min up to the end of the study. These infusion rates were significantly (P < 0.05) greater than those during the anhepatic phase, but significantly (P < 0.05) less than those during the period before exclusion of the liver.

Plasma concentrations of pipecuronium in the control group remained between 1416 (365) ng ml⁻¹ (smallest) and 2098 (299) ng ml⁻¹ (greatest) with a mean value of 1783 ng ml⁻¹ and did not change significantly during the 240-min study period (fig. 2).

Before and after bilateral renal exclusion, plasma concentrations of pipecuronium remained between 1241 (105) ng ml⁻¹ (smallest) and 1677 (212) ng ml⁻¹ (greatest) with a mean value of 1460 ng ml⁻¹ (ns compared with control group) and did not change significantly during the entire investigation, despite a 120-min duration of bilateral renal exclusion (fig. 3).

Plasma concentrations of pipecuronium during the three different phases of liver transplantation remained between 1332 (257) ng ml⁻¹ (lowest) and 2513 (620) ng ml⁻¹ (greatest) with a mean value of 1988 ng ml⁻¹ (as compared with control group) and did not change significantly during the entire investigation, despite a 90-min duration of liver exclusion (fig. 4).

Rectal temperature was maintained in the control group at 38.0 ± 0.1 °C, during the three phases of liver transplantation (before liver ex-
**TABLE I.** Time course, haemodynamic variables, $P_{aCO_2}$, arterial pH, and rectal temperature (mean (SEM)) in the three groups of animals. MAP = Mean arterial pressure; CVP = central venous pressure. $P < 0.05$: *compared with before crossclamping of liver vessels; †between control group and the period before crossclamping of liver vessels.

<table>
<thead>
<tr>
<th>Duration (min)</th>
<th>Control</th>
<th>Before clamping liver vessels</th>
<th>Anhepatic phase</th>
<th>After hepatic recirculation</th>
<th>Renal exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>240</td>
<td>128 (7)</td>
<td>95 (5)</td>
<td>122 (10)</td>
<td>90</td>
</tr>
<tr>
<td>Heart rate (beat min$^{-1}$)</td>
<td>118 (6)</td>
<td>112 (5)</td>
<td>113 (5)</td>
<td>151 (5)*</td>
<td>115 (6)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>69 (2)†</td>
<td>92 (3)</td>
<td>78 (3)</td>
<td>88 (3)</td>
<td>74 (6)</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>3 (1)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>$P_{aCO_2}$ (kPa)</td>
<td>4.7 (0.13)</td>
<td>4.5 (0.13)</td>
<td>4.7 (0.13)</td>
<td>4.8 (0.13)</td>
<td>4.8 (0.13)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.43 (0.01)</td>
<td>7.43 (0.01)</td>
<td>7.38 (0.01)*</td>
<td>7.35 (0.01)*</td>
<td>7.43 (0.01)</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.0 (0.1)</td>
<td>37.8 (0.1)</td>
<td>37.9 (0.1)</td>
<td>37.6 (0.1)</td>
<td>37.9 (0.1)</td>
</tr>
</tbody>
</table>

In the control group and in animals with bilateral renal exclusion, the cardiovascular variables remained within 20% of baseline during the entire investigation (Table I). In animals undergoing liver transplantation, mean systemic arterial pressure and central venous pressure did not change significantly during the three phases of liver transplantation. In contrast, these animals developed significant tachycardia after restoration of hepatic circulation (from 112 ± 5 to 151 ± 5 beat min$^{-1}$) ($P < 0.05$).

**DISCUSSION**

The results of the present study demonstrate that in pigs, bilateral renal exclusion and absence of the liver produced respectively 25% and 80% decreases in the dose requirement of pipecuronium necessary to maintain constant neuromuscular block without significant change in plasma concentrations of pipecuronium. This indicates that the liver plays a more important role than the kidney in plasma clearance of this agent in pigs.

To determine plasma concentrations of pipecuronium, we used a new method based on the production of a complex between pipecuronium and iodine-labelled bengal rose [6]. This assay is specific for the parent compound and does not measure metabolites of pipecuronium. It has detection limits less than those of the colorimetric assay described earlier [7, 8] and gives results comparable to those of the recently published capillary gas chromatographic assay for quaternary ammonium steroidal neuromuscular blocking agents [9].

In control animals, administration of a 150-μg kg$^{-1}$ bolus and of a 8.3-μg kg$^{-1}$ min$^{-1}$ constant rate i.v. infusion of pipecuronium allowed us to produce and maintain a stable 90–95% twitch depression, which was associated with stable plasma concentrations of pipecuronium during the entire study. Doses of pipecuronium used in this study in pigs were twice the dose necessary in humans [10]. This high requirement for myoneural blockers in pigs has been reported for other drugs, such as atracurium [11], vecuronium and gallamine [12].

To maintain a similar twitch depression in animals undergoing ligature of both renal vascular pedicles, before renal exclusion we needed an infusion rate of pipecuronium comparable to that required in control animals. Plasma concentrations of pipecuronium measured before clamping of renal vessels were also comparable to those determined in the control group. Similarly, infusion rate and plasma concentrations of pipecuronium measured before crossclamping of hepatic vessels were comparable to those measured in
control animals. These findings suggest that our control animals may be considered as true controls, although they did not undergo surgery. Isoflurane anaesthesia (1 MAC) alone does not modify hepatic blood flow [13], and isoflurane in a concentration similar to that used in the present study in association with surgical stress (laparotomy) produces only a small decrease in hepatic blood flow [14].

Exclusion of both kidneys produced a 25% reduction in dose requirement of pipecuronium. Despite significant reduction in infusion rate, plasma concentration of pipecuronium remained stable. Our data are comparable to the 34% reduction in plasma clearance of pipecuronium observed in patients undergoing cadaver renal transplantation [4] compared with clearance in patients with normal renal function. In contrast, in dogs studied 1 h after bilateral renal exclusion, an 85% reduction in plasma clearance of pipecuronium was observed in comparison with control animals [15]. These differences probably reflect species variation.

Exclusion of the liver produced an 80% reduction in dose requirements of pipecuronium necessary to maintain similar neuromuscular block as that before clamping of liver vessels without significant changes in plasma concentrations of this agent. Many factors may explain this change. We did not evaluate renal function of our pigs undergoing liver transplantation but urine output was maintained at values greater than 1 ml kg\(^{-1}\) h\(^{-1}\) during the entire investigation, and we found a maximal decrease in dose requirements of pipecuronium, after bilateral renal exclusion, of only 25%. Therefore, it is unlikely that changes in renal function alone could explain this decrease in dose requirement. Other important factors affecting pharmacodynamics, such as systemic haemodynamics, temperature or blood pH, remained within the physiological range during the period of liver exclusion. Increased receptor sensitivity and a decrease in volume of distribution of pipecuronium induced by exclusion of the liver could also be evoked. However, during the anhepatic phase, we used an external veno-venous bypass to allow drainage of the splanchnic circulation which represents an important part of the total blood volume in pigs, avoiding possible changes in volume of distribution of pipecuronium. In a previous study, we used the same animal model to investigate atracurium, a neuromuscular blocking drug known to be independent of the kidneys and liver for its elimination [11]. We have found that plasma concentrations and infusion rates of atracurium did not change significantly during the anhepatic phase compared with the period before clamping of the hepatic vessels. In contrast, plasma concentrations of its metabolite laudanosine, known to be metabolized in the liver, increased significantly after exclusion of the liver compared with the period before clamping of the hepatic vessels. Therefore, it is unlikely that increased sensitivity of the neuromuscular junction during the anhepatic phase could explain our findings and we conclude that there is significant hepatic uptake of this neuromuscular blocker in animals with normal liver function. The importance of the liver was further confirmed by the fact that, in all animals, it was necessary to increase the infusion rate of pipecuronium significantly during the first 1 h after reperfusion of the liver graft. However, dose requirement of pipecuronium was significantly less than that before crossclamping of hepatic vessels, probably because of decreased metabolic function of the hepatic graft immediately after transplantation [16]. The reperfusion of the liver graft was followed immediately by a transient decrease in arterial pH, which could also result in a larger dose requirement of pipecuronium. However, the change observed was probably not related to pH, as changes in the dose requirement of pipecuronium occurred after correcting the metabolic acidosis.

In contrast with our findings, Agoston and colleagues reported that, in cats, temporary hepatic exclusion did not alter significantly the intensity and the time-course of the neuromuscular blocking effects of pipecuronium [17]. However, these authors did not measure plasma concentrations of pipecuronium before and during a brief period of portocaval shunting (10 min). It is possible therefore, that changes in the pharmacokinetics of pipecuronium induced by the short exclusion of the liver are not reflected in neuromuscular effects. There may also be species-related differences in hepatic uptake and excretion of pipecuronium, as reported by the same research group for pigs and cats in hepatic uptake of other myoneural blockers [16, 18]. Previous studies have also suggested that there are differences in hepatic uptake and distribution of neuromuscular blocking drugs between the cat and humans [19, 20]. As pig and human liver have similar enzyme systems [21], the pig is probably more rep-
PIPECURONIUM, HEPATIC AND RENAL FUNCTIONS

representative than the cat for studying the influence of the liver on the neuromuscular effects of muscle relaxants.

REFERENCES


