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A NEW METHOD FOR STUDYING THE RELATIONSHIP BETWEEN HEPATIC UPTAKE OF DRUGS AND THEIR PHARMACODYNAMIC EFFECTS IN ANAESTHETIZED CATS

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1 A new *in vivo* experimental method is described whereby the liver can be temporarily excluded from the general circulation by means of a portocaval shunt operation. The influence of this manoeuvre upon the effects of pancuronium and Org 6368 was investigated using the tibialis muscle preparation of anaesthetized cats.

2 The procedure also allowed intraportal injections of the drugs to be made so that the effect of first-passage uptake by the liver could be compared with hepatic exclusion in the same animal.

3 Hepatic exclusion greatly increased the duration of action of both drugs. Whereas intraportal injection did not significantly alter the effect of pancuronium on the tibialis muscle, the effect of Org 6368 was greatly diminished when given by this route.

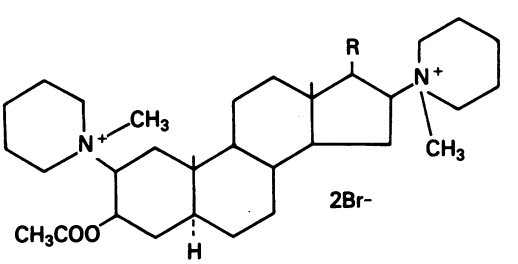
4 The liver appears to tolerate short periods of hepatic exclusion and it is concluded that this technique may become a useful tool for studying the handling of drugs by this organ.

Introduction

Liver uptake and metabolism is a major factor in determining the duration of action of many non-depolarizing muscle relaxants. Thus, the plasma disappearance of (+)-tubocurarine (Cohen, Brewer & Smith, 1967; Meijer & Scaf, 1968), pancuronium (Agoston, Vermeer, Kersten & Meijer, 1973b; Buzello, 1975) and hexafluorenium (Meijer, Vermeer & Kwant, 1971) appears to be dependent to a significant extent upon liver uptake. It also seems probable that, for instance, the longer duration of action of pancuronium compared to that of its closely related analogue, Org 6368, (Sugrue, Duff & McIndewar, 1975) may be related to the more rapid and complete hepatic uptake of the latter steroid (Agoston, Kersten & Meijer, 1973a; Agoston, Crul, Kersten, Houwertjes & Scaf, 1977).

Apart from *in vitro* studies of the isolated perfused liver (Meijer & Scaf, 1968) and hepatocytes (Berry & Friend, 1969), the techniques available for examining the specific role of the liver in drug metabolism in the intact animal are relatively few. Disappearance of drugs from the plasma following intravenous (Agoston, Vermeer, Kersten & Scaf, 1978) or intraportal (Hughes, 1972) injection, combined with estimation of the level of the drug in bile and in the liver itself (Agoston *et al.*, 1973a; 1977) have so far provided the bulk of the information concerning liver uptake of neuromuscular blocking agents.

For this reason we have developed a new operative technique for use in anaesthetized cats which allows the liver to be temporarily excluded from the systemic circulation by a procedure for shunting blood from the hepatic portal vein into the inferior vena cava combined with temporary closure of the hepatic artery and the portal vein proximal to the shunt. The technique also allows drugs to be injected intraportally when the normal hepatic circulation is restored.



Org 6368 R = H
Pancuronium R = OOCCH3

Figure 1 Formulae of pancuronium bromide and Org 6368.

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Using this new technique, we have investigated the role of the liver in the inactivation of pancuronium and Org 6368 (2β,16β-dipiperidino-5α-androstan-3α-ol acetate dimethobromide), drugs which are known to be treated in a quantitatively different manner by the liver of cats. The formulae of these two drugs is given in Figure 1. The neuromuscular blocking activity of the drugs has been monitored during hepatic exclusion and after intraportal injection.

Methods

General

Adult cats (2 to 4 kg body weight) were given 40 mg/kg pentobarbitone sodium (Nembutal, Abbott) intraperitoneally. After intubation, using a cuffed Magill tube of 5.5 mm external diameter, ventilation was maintained with a Braun type 1905 respirator pump at a frequency of 30 per min and a tidal volume of air of 18 ml/kg. Blood pressure was monitored by a Statham P23Db pressure transducer connected to a polythene cannula inserted into the carotid artery. The pressure transducer and cannula were filled with heparinized saline. The mean arterial pressure was seen to be maintained above 100 mmHg throughout the investigation. Arterial blood pH was also regularly monitored and was maintained throughout between 7.41 and 7.43. A further polythene cannula was placed in the external jugular vein and this was used for injection of pancuronium, Org 6368, pentobarbitone (maintenance doses of 3 mg/kg) and heparin. Unless otherwise stated, drugs were injected via this route during the experiment. In order to replace fluid loss during the proceedings, an infusion of glucose 2.5% and saline 0.45% was maintained at a rate of 8 ml kg^{-1} h^{-1}. Rectal temperature was continuously measured and maintained at 37.5 ± 0.5°C by means of a heating blanket placed under the cat.

Peroneal nerve—tibialis anterior muscle preparation

The twitch tension of the left tibialis anterior muscle, elicited by supramaximal square wave stimuli of 0.3 ms duration applied to the common peroneal nerve at 0.3 Hz, was recorded by means of a Hottinger-Baldwin force displacement transducer and an MFE recorder.

Portocaval shunt preparation

A skin incision was made from the xiphisternum to the umbilicus. Bleeding points were diathermised. After loosening the skin from the underlying muscle, the rectus sheath was divided longitudinally along the junction of the rectus abdominus muscle bodies. The peritoneum was opened and the viscera were reflected to expose the hepatic artery and the hepatic portal vein. The portal vein was cleared of fat and connective tissue, starting at the intestinal end and a length of vein, 2 cm long, was exposed; at the same time any small tributaries were tied off. This procedure was repeated in an adjacent section of the inferior vena cava. The cat was then heparinized with 500 iu heparin (Heparin, Leo Products, Denmark) given intravenously. The vena cava was clamped at either end of the freed segment, i.e. 2 cm apart. An incision was made in its wall, 0.5 to 1 cm long, and the vena cava T-piece (Figure 2) was inserted, one end at a time, both ends being secured with thread. Air was removed from the T-piece by releasing momentarily each clamp in turn. The balloon in the side arm was then inflated to occlude the side arm while the shunt was being prepared and the clamps could then be removed to allow normal blood flow along the vena cava to return. A 10 cm length of rat jejunum, freshly prepared by washing repeatedly in saline, was connected and tied to the side arm of the T-piece. The balloon in the T-piece was momentarily deflated to allow the jejunum segment to fill with venous blood and thereby exclude air.

The injection cannula of the portal vein T-piece (Figure 3) was filled with normal saline via a syringe and needle attached to its distal end. The T-piece was then inserted into the freed length of portal vein by a method similar to that for the vena cava. After filling the T-piece with blood (by releasing the clamps) the free end of the jejunum was tied to the T-piece and this connecting vessel was positioned so that no kinks could obstruct the free flow of blood through it (Figures 4 and 5).

To operate the shunt, a large clamp was placed across the portal vein proximal to the T-piece and across the hepatic artery, thereby preventing blood flow to or from the liver. Deflation of the balloon in each T-piece then diverted blood from the portal vein into the inferior vena cava along the pressure gradient.

Experimental protocol

The intensity and duration of the neuromuscular blocking effects of either pancuronium, 20 μg/kg, or Org 6368, 75 μg/kg, were compared under the different experimental conditions described below. Each cat received only one of the two neuromuscular blockers tested. The intensity of the effect was defined as the percentage depression of twitch height compared to the control twitches. The duration of action was defined as the time elapsing from the end of the drug injection until 90% recovery of twitch height.

In each cat, the same dose of either pancuronium or Org 6368, dissolved in 0.2 ml water, was injected

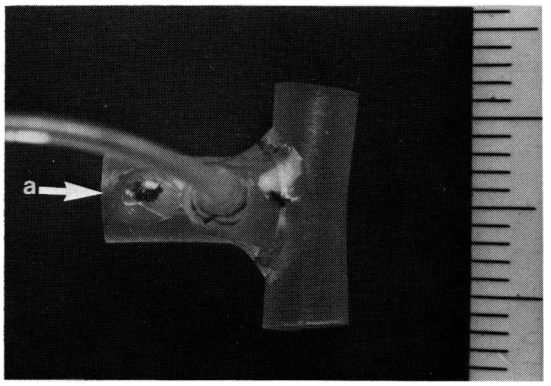

Figure 2 Vena cava T-piece used in portocaval shunt preparation. Scale: 1 mm per small division. (a) Shunt outlet.

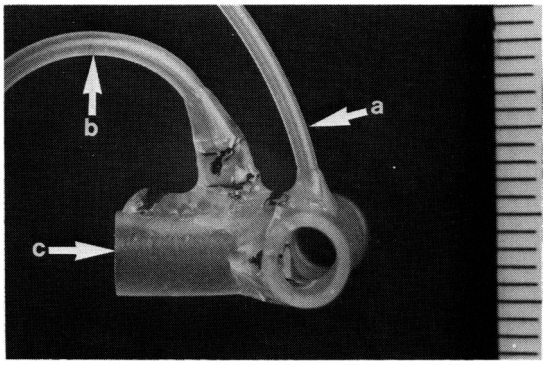

Figure 3 Portal vein T-piece used in portocaval shunt preparation. Scale: 1 mm per small division. (a) Portal injection route; (b) connection to balloon; balloon deflated; (c) shunt outlet.

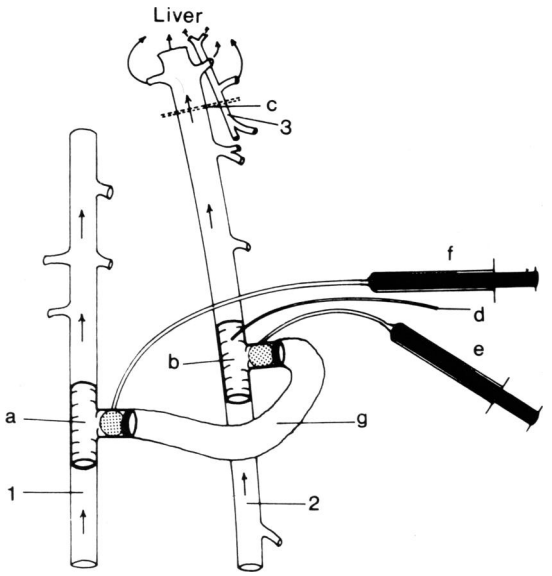

Figure 4 Diagram of the portocaval shunt with balloons inflated and clamp (c) off. Thus, blood flows normally to the liver through both hepatic artery and hepatic portal vein. The shunt is operated by deflating both balloons and clamping the hepatic portal vein, so diverting hepatic portal venous blood to the inferior vena cava. Clamp (c) also occludes the hepatic artery thereby preventing blood flow to or from the liver. (1) Caudal vena cava; (2) portal vein; (3) common hepatic artery; (a) T drain; (b) T drain; (d) catheter for intraportal injection; (e) syringe; (f) syringe; (g) connecting vessel.

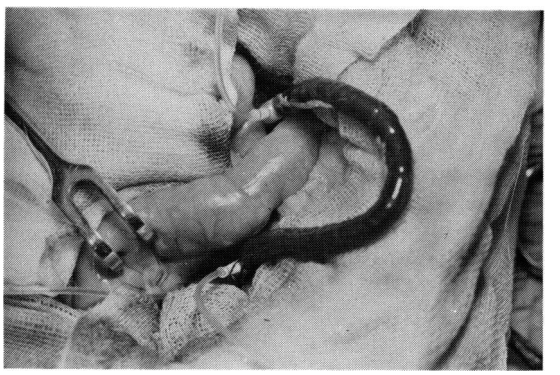

Figure 5 Portocaval shunt in operation.

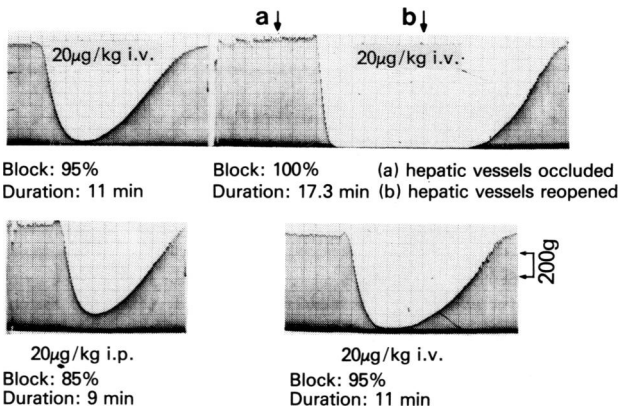

Figure 6 Typical records of contractions of cat tibialis anterior in response to stimulation of the peroneal nerve and the effect of pancuronium 20 μg/kg under various conditions. Trace reads from left to right as follows: control i.v. injection, hepatic exclusion plus i.v. injection, intraportal injection, control i.v. injection.

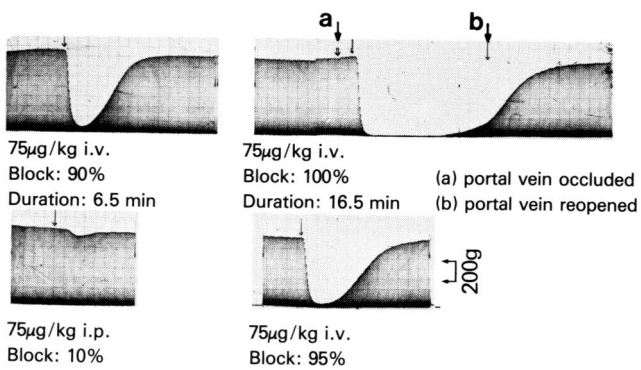

Figure 7 Typical records of contractions of cat tibialis anterior in response to stimulation of the peroneal nerve and the effect of Org 6368 75 μg/kg under various conditions. Trace reads from left to right as follows: control i.v. injection, hepatic exclusion plus i.v. injection, intraportal injection, control i.v. injection.

over a 10 period on four separate occasions, at 1 h intervals. The first administration was a control injection given intravenously with normal hepatic blood flow. Three min before the second (intravenous) injection the shunt was opened and the hepatic vessels and proximal portal vein clamped, as described above. This period of hepatic exclusion was maintained for 10 min, when the balloons were re-inflated and the clamps removed thereby closing the shunt. The third injection was intraportal via the catheter entering the portal T-piece, and during this part of the experiment the shunt was still closed. The final injection was a further control, identical to the first, in an attempt to assess any alterations in hepatic handling of the drug and the effects of accumulation of the neuromuscular blockers.

Blood samples were taken when the muscle twitch response had returned to 25% and 75% of the control height, after each of the four drug administrations. The levels of pancuronium or Org 6368 were assayed in these samples by a fluorimetric determination method, described by Kersten, Meijer & Agoston (1973) and Agoston *et al.* (1977).

Statistical comparisons were made by Student's paired *t* test with a 95% significance level. The values given are mean values ± s.e. mean.

Results

A typical sequence of experimental results using pancuronium and Org 6368 are shown in Figures 6 and 7, respectively. Hepatic exclusion for 10 min resulted in a marked prolongation of the action of the two drugs on the contractions of the cat tibialis muscle. Intraportal injection of pancuronium under normal conditions was associated with a slight decrease in intensity of block in the illustration shown (Figure 6). However, data from all the cats studied with these drugs showed that there was no significant difference from the control effect (see Table 1). On the other hand there was significant hepatic first-passage uptake of Org 6368, as shown by the resultant diminution of intensity and of duration in the action of this drug on the tibialis muscle after its intraportal injection (Figure 7).

Although the final control injections of both pancuronium and Org 6368 appeared to produce a slight increase in both intensity and duration of block compared to the first control injection (Table 1) the increases were not significant. This suggests that the liver is able to withstand a 10 min period of hepatic exclusion without measurable damage to its ability to inactivate the neuromuscular blocking drugs tested here. Also it shows that significant accumulation of drugs did not occur during the course of the experiment. This point is further borne out by the fact that no significant difference in the concentrations of either pancuronium or Org 6368 could be detected in the plasma during the recovery phase of each part of the experiment. Thus, after the first intravenous injection of pancuronium, plasma levels of 0.04 ± 0.01 and 0.03 ± 0.01 μg/ml were measured at the times of 25% and 75% recovery from neuromuscular blockade respectively. No statistically significant differences from these levels were apparent when samples were estimated at the corresponding times after the second, third, fourth injections. Similarly, the corresponding plasma levels of Org 6368 (initially 0.19 ± 0.03 and 0.10 ± 0.02 respectively) were not significantly different from the values obtained after subsequent injections.

The Rose Bengal method used here for determining pancuronium and Org 6368 also measures their metabolites and so the figures given above represent the total amount of unchanged and metabolized drug. However, the pharmacodynamic response is the sum of the effects of all these metabolites plus parent com-

Table 1 Magnitude (% twitch depression) and duration (min) of the neuromuscular blockade following equipotent doses of pancuronium and Org 6368

Experimental conditions and routes of administration	*Max. block (%)* (*mean ± s.e. mean*) ($n = 3$)		*Duration (min)* (*mean ± s.e. mean*) ($n = 3$)	
	Pancuronium	*Org 6368*	*Pancuronium*	*Org 6368*
Intravenous	81.7 ± 8.7	80.0 ± 3.1	10.5 ± 0.4	4.6 ± 0.6
Intravenous	100	100	17.1 ± 0.7*	16.3 ± 0.3*
(hepatic vessels occluded)				
Intraportal	89.0 ± 3.5	11.3 ± 4.1*	10.0 ± 0.9	1.83 ± 0.8*
Intravenous	93.7 ± 0.6	87.3 ± 3.8	11.7 ± 0.7	6.4 ± 1.0

* Significantly different from initial control intravenous injection.

pound since these metabolites are themselves pharmacologically active (Miller, Agoston, Booij, Kersten, Crul & Ham, 1978). Moreover it is not likely that metabolites will be formed to any considerable extent within 10 to 15 min (Agoston *et al.*, 1973a), the period during which neuromuscular transmission is fully restored in the present experiments.

Discussion

This technique for producing a temporary complete exclusion of the liver from the general circulation in anaesthetized cats could prove to be an extremely useful tool for elucidating the role of the liver in the handling of a wide variety of drugs in the intact animal. Isolation of the liver by the procedure described allows an examination of drug pharmacodynamics after removal of one of the major variables affecting the action of drugs, namely hepatic uptake.

The technique is simple to perform. It also allows some measure of first-passage uptake of drugs by the liver because their intraportal injection can also be compared with a normal control injection in the same animal.

In our experiments we have monitored the neuromuscular blocking potency of pancuronium and Org 6368 during the various manoeuvres affecting liver function (i.e. hepatic exclusion and biliary injection) by noting the simultaneous contractions of the tibialis muscle. Obviously, other classes of compound could be investigated in this preparation for liver exclusion combined with, say, measurement of plasma levels or biological responses of other organs (e.g. heart rate, contraction of the nictitating membrane) depending upon the type of drug being tested.

The viability of the preparation also appears to be good. No evidence was found for any change in ability of the liver to inactivate the neuromuscular blockers tested during the second control period which followed a 10 min period of hepatic exclusion.

In our hands, the technique provided confirmation of findings previously obtained in this laboratory in which the actual hepatic uptake of pancuronium and Org 6368 was measured after intravenous injections in normal cats. In those studies significant hepatic uptake of both drugs was found (Agoston *et al.*, 1973a; 1977) and this correlates well with the enhancement of duration of action of both drugs by hepatic exclusion. In the present preliminary studies a 90% blocking dose of both drugs was used, so that it is not possible to comment upon the potentiation of the intensity of the block produced by hepatic exclusion. A quantitative analysis of the enhancement of neuromuscular blocking effects of these drugs by hepatic exclusion is now in progress. In the earlier studies it was found that whereas only 24% of an injected dose of pancuronium was present in the liver after 8 h the measured hepatic uptake of Org 6368 occurred at double this rate (51%) (Agoston *et al.*, 1977). The avidity of the liver for Org 6368 compared to pancuronium is shown in the present experiments by the marked decrease in potency of Org 6368 following its intraportal injection. This is compared to the lack of effect of hepatic first-passage inactivation of pancuronium, and confirms our previous results on the relative affinity of the liver for pancuronium and Org 6368.

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