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Uptake and Excretion of Vecuronium Bromide and Pancuronium Bromide in the Isolated Perfused Rat Liver

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Using the isolated perfused rat liver preparation, the disappearance from the perfusate and the excretion in the bile of vecuronium bromide and pancuronium bromide and their metabolites were followed for 2 h after the addition of 1 mg of either drug to the perfusate. In addition, the rate of change of the hepatic content of these two compounds was calculated by serially subtracting the amount of the compound and the metabolites in the bile and in the perfusate from the dose of drug added to the perfusate. It was found that, whereas the concentration of pancuronium in the perfusate declined slowly and monoexponentially, vercuronium concentration in the perfusate declined rapidly in a biexponential manner. No metabolites of either drug were detected in the perfusate. Approximately 40% of the injected dose of vecuronium was excreted in the bile as unchanged vecuronium and another 30% as the 3-hydroxy metabolite. No other metabolites of vecuronium were found in the bile. In total only about 7% of pancuronium (unchanged) was collected in the bile by the end of the experiment. It is concluded that, in comparison to pancuronium, the rat liver takes up large amounts of vecuronium rapidly, half of which is eliminated as unchanged vecuronium and half as the 3-hydroxy derivative. A small amount of vecuronium or its 3-hydroxy metabolite is returned to the perfusate from the liver. Some possible mechanisms underlying these differences are discussed. (Key words: Liver: biotransformation; neuromuscular relaxants. Neuromuscular relaxants: pancuronium, vecuronium.)

VECURONIUM BROMIDE (Org NC 45) is an intermediate-acting, monoquaternary analog of pancuronium with minimal cardiovascular side effects.[1–3] In humans, the shorter duration of action of vecuronium can be explained by a rapid disappearance from the plasma, the plasma clearance rate being approximately twice that of pancuronium.[4,5] It is likely that renal excretion accounts for only a small fraction of this sizable plasma clearance because studies in humans with renal failure[6,7] have shown that there is little change in the pharmacokinetic parameters of vecuronium after intravenous injection. In contrast, the liver seems to be an organ of major importance for the elimination of vecuronium. Thus, Upton *et al.*[8] have shown that as much as 46% of a dose of vecuronium is found in the bile of rats within 7 h after injection. Furthermore, Bencini *et al.*[9] have calculated that as much as 75% of an injected dose of vecuronium may be excreted in the bile in humans within 24 h. Moreover, these authors found large concentrations of vecuronium in a number of liver biopsies from patients as early as 30 min after injection and estimated that as much as 80% of the dose of vecuronium might be present in the liver of patients half an hour after injection. This pointed to the possibility that, as in the case of hexafluorenium,[10] uptake of vecuronium by the liver may proceed at a faster rate than excretion into the bile, and this may result in accumulation of vecuronium in the liver, thus accounting for the high concentrations of vecuronium in this organ. To examine this hypothesis further we have turned to the isolated perfused liver preparation,[11,12] using in this case the rat as the experimental animal. This preparation has been shown to preserve the hepatic transport function for organic anions, cations, and uncharged compounds.[13] In this model the liver is the only organ of elimination for vecuronium so that the quantitative contribution of the liver to the removal of vecuronium from the medium as well as the pattern of uptake, elimination, and excretion of this compound by the liver can be accurately studied. The results were compared to those obtained after pancuronium was added to the system.

Materials and Methods

Isolated Perfused Rat Liver Preparation

Details of our modification of the original isolated perfusion technique as described by Miller *et al.*[11] are given in Meijer *et al.*[14] In brief, the preparation consisted of a completely isolated liver, from a male Wistar rat, which was perfused by a circulating medium run in at the portal vein and drained *via* the hepatic veins. The perfusing medium consisted of a Krebs-bicarbonate solution supplemented with 1% bovine albumin. This was circulated by a rotary pump in a closed system, which allowed addition of chemicals and drugs as well as sampling at two ports.

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Experimental Procedure

A volume of 100 ml of perfusion medium was used in all experiments, and the flow of the perfusion medium was set at 35 ml/min. The medium was oxygenated by a membrane oxygenator and the temperature of the whole system was maintained at 38° C. The *p*H of the medium was maintained at 7.4 by titration with small amounts of sodium bicarbonate; 1 mg of either vecuronium bromide (n = 6) or pancuronium bromide (n = 6) was injected into the perfusion medium for each experiment. One-milliliter samples of the perfusion medium were collected at 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80, 100, and 120 min after injection. The *p*H of all the samples was brought to 5 by titration with 1 M disodium hydrogen phosphate to prevent degradation of vecuronium and the samples were then deep frozen to await further analysis. Bile was collected at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 min after injection, buffered, and then deep-frozen until further analysis as described above for the medium.

At the end of each experiment the liver was immediately perfused *via* the hepatic veins with 30–40 ml of ice-cold saline to remove medium and drugs therein. The livers were then homogenized (1:3 v:v) in ice-cold saline and the amount of drugs and metabolites determined as described below.

Chemical Analysis

A two-step procedure was used to measure the concentration of vecuronium and its metabolites in the perfusion medium, bile, and liver homogenate. This method is a modification of the method used to measure the concentration of pancuronium by Kersten *et al.*[15] In this method the amount of vecuronium and pancuronium and their metabolites are first determined fluorimetrically after the formation of ion-pair complexes of the muscle relaxants and their metabolites with the fluorescent dye Rose Bengal. This method has a sensitivity of 5 ng/ml but does not distinguish between the parent compounds and their metabolites. The relative quantities of vecuronium or pancuronium and their metabolites are then determined by separation with thin-layer chromatography. The size and density of the spots are compared visually with spot sizes and densities of known amounts of reference compounds on the same plate. This semiquantitative method has a coefficient of variation of ±10% and as little as 150 ng of a compound can be detected. The accuracy of this semiquantitative technique has been recently tested in the laboratory of one of the authors (D.K.F.M.); the quantities of vecuronium, pancuronium, and their metabolites in spots of the compounds on silicagel plates were measured both visually as described above and then fluorimetrically after scraping of the spots from the silicagel plate, followed by extraction after recomplexation of the compounds with Rose Bengal. The values thus obtained were reproducible and showed a coefficient of variation of ±15% in the concentration ranges of the compounds used in this study.††

Data Analysis

Curves of the rate of decline of the concentration of the compounds in the medium as well as the biliary excretion rate of vecuronium and pancuronium were fitted to the experimental data by the use of an iterative linear least square regression analysis program developed by one of the authors (A.H.J.S.). Fitting of the experimental data to equations with a varying number of exponential terms (maximum of five) was statistically compared at the 95% level of significance according to the criteria described by Boxenbaum *et al.*[16] The liver content of vecuronium or pancuronium was calculated by subtracting the amount of these drugs and their metabolites found in the medium and bile from the administered dose. The derived curve, showing the change of liver content of the muscle relaxant with time, was also analyzed by the curve-fitting program mentioned above.

Results

Disappearance from the Medium

The mean rate of the decline of the concentration of vecuronium (and its metabolites) is compared to that of pancuronium (together with its metabolites) in figure 1*A*. Vecuronium was rapidly removed from the medium by the liver in a biexponential manner in sharp contrast to pancuronium, which was very slowly removed from the medium by the liver in an apparently monoexponential process. None of the three possible metabolites of vecuronium or pancuronium could be detected in the medium with the available method of analysis.

Excretion in the Bile

Figure 1*C* compares the rate of excretion of vecuronium and pancuronium in the bile. Pancuronium and vecuronium appeared early in the bile, *i.e.*, with the first sample taken at 5 min after injection. However, pancuronium was excreted much more slowly so that pancuronium was still being excreted at the end of the experiment. By this time only 6.6% of the administered dose of pancuronium (table 1) had been excreted in the bile. In contrast, biliary excretion of vecuronium had virtually ended by 120 minutes, by which time about 70% of the administered dose had been excreted in the bile (table 1).

†† Mol W: Personal communication, 1987.

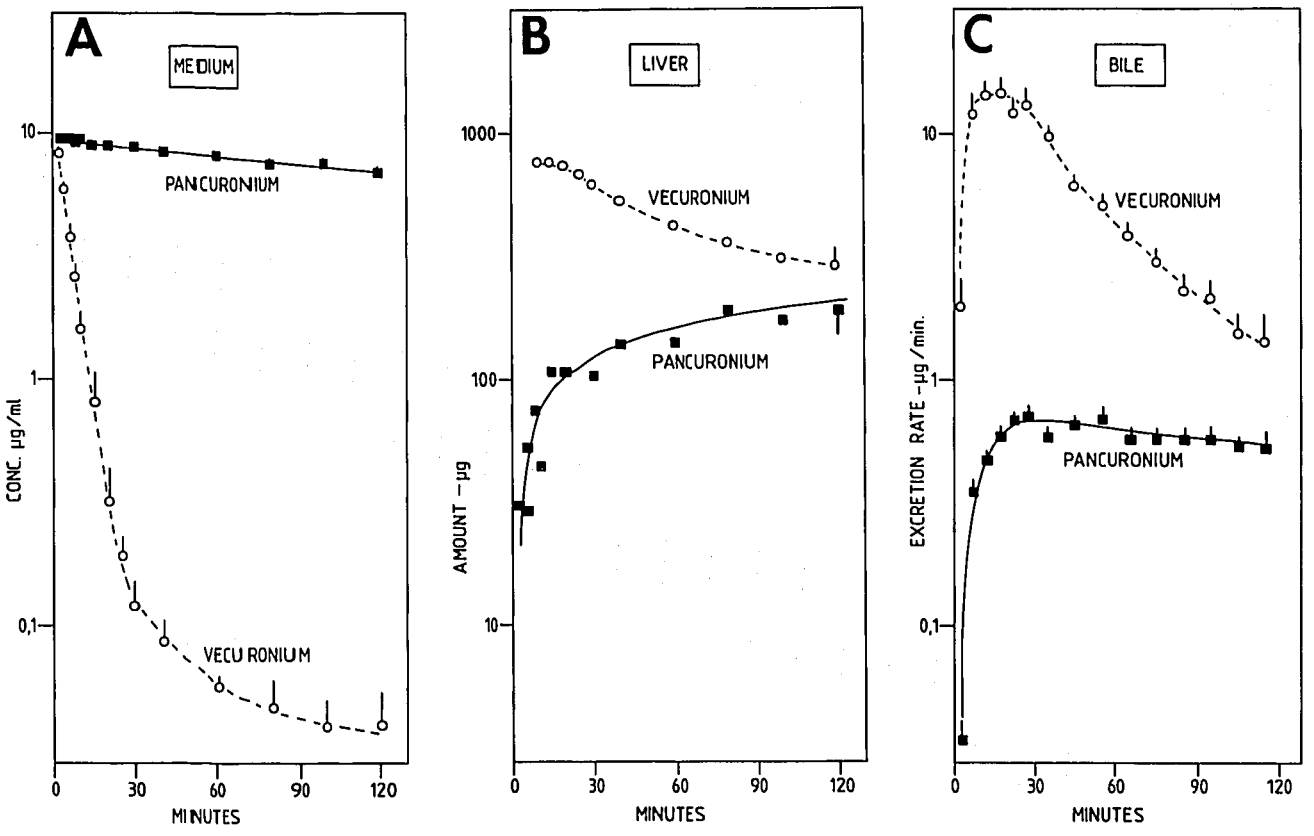

FIG. 1. Concentration in medium (*A*), amount in liver (*B*), and excretion in bile (*C*) of vecuronium and pancuronium. Values in *B* are mean only except for last points since values derived. Other values are mean ± SEM.

Furthermore, no pancuronium metabolites were found in the bile in contrast to vecuronium, which was excreted in the unchanged form and as the 3-hydroxy metabolite. None of the two other putative metabolites of vecuronium, *viz.*, the 17-hydroxy and the 3,17-dihydroxy metabolite, were found in the bile (fig. 2).

Unchanged vecuronium was found in the bile in the first sample 5 min after injection and seemed to cease being excreted at approximately 70 min after injection. On average 38% of the injected dose of vecuronium was recovered unchanged in the bile during this period. The 3-hydroxy metabolite appeared in the bile about 15 min after injection. Such a delay presumably denotes the time it takes to transform vecuronium to its 3-hydroxy derivative. At the end of the experiment, 2 h after injection, the 3-hydroxy vecuronium was still being excreted. The amount of the 3-hydroxy metabolite excreted by this time amounted to about 32% of the injected dose.

LIVER CONTENT

The amount of vecuronium (and metabolites) and pancuronium (and metabolites) contained in the liver during the period of the experiment is illustrated in figure 1*B*. The data shown here was derived as described in the section on data analysis. The two points at the end of the experiment show the average amount of drug actually found in the liver homogenate (table 1). These points adequately approximate the amounts calculated from the plots. Figure 2 and preliminary experiments in our laboratory†† indicate that the amount of vecuronium in the liver at the end of the experiment consists mainly of 3-hydroxy vecuronium.

As shown in figure 1*B*, the bulk of the vecuronium dose was found early in the liver after injection. In con-

TABLE 1. Isolated Rat Liver Perfusion

	Present in the Medium	Removed from the Medium by Sampling	Collected in Bile	Present in Liver	Total
Total vecuronium and metabolites	0.3	1.5	69.4	21.75*	92.95
Total pancuronium and metabolites	59.5	11.3	6.6	19.3	96.7

Values are given as mean total amounts (% of dose) of vecuronium and pancuronium found in the medium, bile, and liver at the end of the experiment; n = 6 except *n = 5.

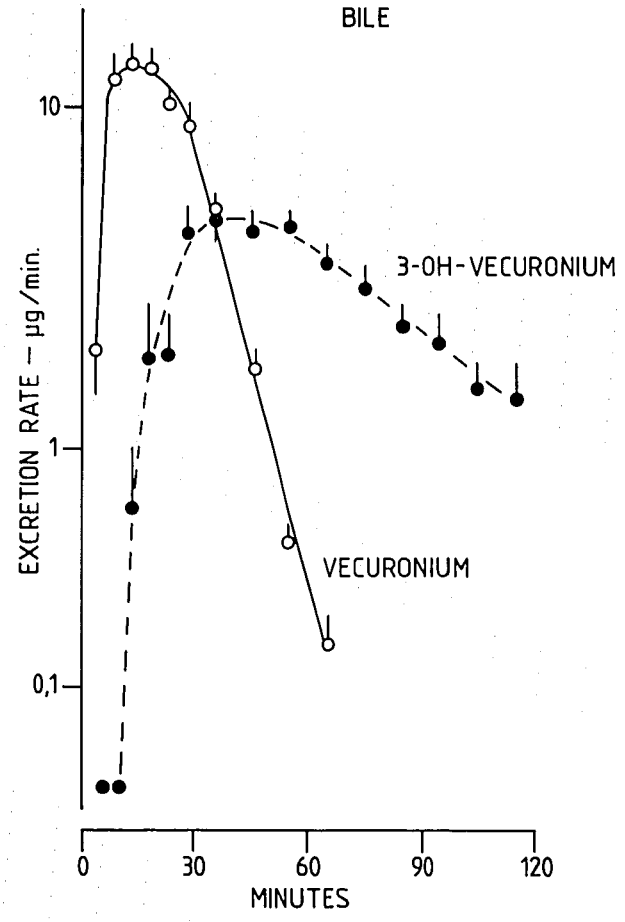

FIG. 2. Change of excretion rate of unchanged vecuronium and the 3-hydroxy metabolite (3-OH vecuronium) in the bile (mean ± SEM).

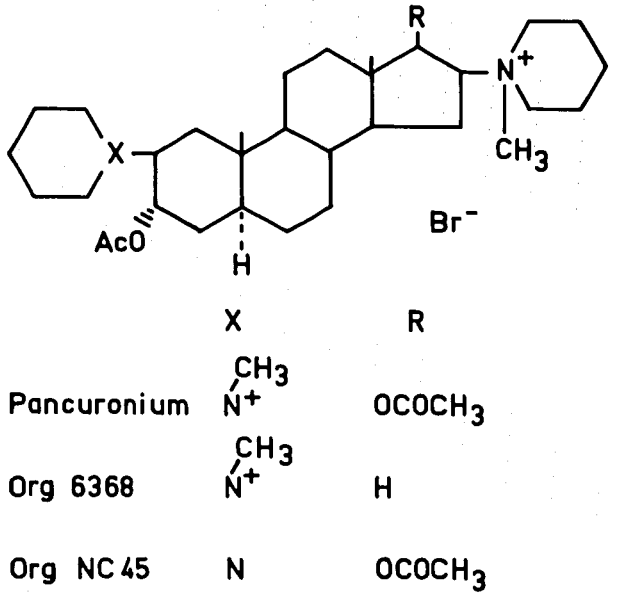

FIG. 3. Chemical differences between pancuronium, vecuronium (Org NC45), and Org 6368.

trast, the amount of pancuronium in the liver probably never reached more than 20% of the injected dose in this study.

Discussion

Figure 1 shows that vecuronium is removed in a biexponential manner from the perfusion medium in contrast to pancuronium, which was apparently removed monoexponentially. The word *apparently* deserves emphasis here because pancuronium is probably also removed biexponentially from the perfusion medium by the liver. This fact is deducible from the biexponential manner of appearance of pancuronium in the liver (fig. 1*B*). Therefore, the monoexponential decline of pancuronium concentrations in the perfusion medium seen in figure 1*A* possibly conceals a small initial phase of more rapid decline of pancuronium concentration, so short-lasting that it is unidentified by the sampling protocol used in these experiments.

The results of these experiments support the currently available body of evidence that the liver is a major determinant of the sizable plasma clearance of vecuronium (relative to other nondepolarizing muscle relaxants). Assuming an average size of a rat liver of 10 g and using this value to convert the amount of vecuronium or pancuronium found in the liver (fig. 1*B*) to concentration, it is deduced that, apart for the first 10 min when vecuronium is rapidly taken up by the liver from the medium, the concentration of vecuronium in the liver greatly exceeds that in medium or bile. Vecuronium is then partly excreted unchanged and partly metabolized before excretion into the bile. Together these two pathways of elimination account for 70% of the administered dose in this study (table 1).

These events agree with the conclusion reached in a previous clinical study that the liver acts as an organ of elimination, not only by metabolizing and excreting vecuronium, but also by primarily accumulating large amounts of this compound before excreting it into the bile.[9] Little or no vecuronium was considered in that study to be returned to the plasma. However, the biexponential decay of vecuronium concentration in the perfusing medium supports the contention that a little of the vecuronium transfers back from the liver into the perfusing medium.

The question raised here is whether this back flow consists of vecuronium or the 3-hydroxy metabolite of vecuronium. Refering to figures 1 and 2, the slope of the second phase of the disappearance curve of vecuronium from the medium runs parallel to that of the second phase of the excretion of the 3-hydroxy metabolite. This implies that it is probably the 3-hydroxy vecuronium that is transferred back to the medium—a fact supported by the ap-

pearance of the 3-hydroxy metabolite in the urine in clinical studies.[7,9] Assuming that the human kidney itself does not metabolize vecuronium, the 3-hydroxy metabolite of vecuronium most probably was carried to the kidney from the liver *via* the blood in these clinical studies.

In contrast, the largest fraction of pancuronium is (throughout the duration of this experiment) found in the perfusion medium where it still makes up approximately 60% of the injected dose at the end of the experiment (table 1). The amount in the liver does not apparently even exceed 20% of the injected dose and the amount collected in the bile amounted to only 6.5%. Pancuronium is therefore poorly taken up by the rat liver.

Because vecuronium is a monoquaternary homologue of pancuronium (fig. 3), it is logical to assume that this difference in chemical structure determines the dissimilar handling of these two closely related drugs by the rat liver. Similar differences have been observed in the hepatic disposition of *d*-tubocurarine (monoquaternary, more rapidly taken up by the liver) and metocurine (bisquaternary, less rapidly taken up by the liver) under the same experimental conditions.[17] These two compounds, like vecuronium and pancuronium, also differ structurally only in the number of quaternary nitrogen groups they contain. In this context, Schanker noted that most of the strongly cholephilic quaternary ammonium compounds possess one positively charged group at one end of the molecule and a more lipophilic ring structure at the other end.[18] This would be the structure of about 10% of the vecuronium molecules at normal body *p*H of 7.4 because vecuronium, a weak base, has a *p*Ka value of 8.97.‡‡ Although small, this proportion of vecuronium molecules in the monoquaternary form is probably sufficient for the effective hepatic removal of vecuronium.

An additional factor that might explain the rapid hepatic uptake of vecuronium in contrast to pancuronium can be related to the differing lipid solubility of the two compounds. Experimental evidence suggests that there is a reasonable correlation between the octanol/water partition coefficient of monoquaternary compounds,[19] as well as bisquaternary compounds[17] and biliary excretion. Pancuronium has a very low lipid solubility (partition coefficient in an octanol/Krebs-Ringer system[10] is <0.01). In contrast, vecuronium's partition coefficient in an octanol/Krebs-Ringer system has been found in our laboratory to be 2.57. However, lipid solubility cannot be the only factor because other studies have shown that the bisquaternary homologue of pancuronium, Org 6368 (fig. 3), is also rapidly taken up by the liver[20] in spite of a small partition coefficient in an octanol/Krebs system of 0.001.§§

‡‡ Sleigh T: Personal communication, 1986.

§§ Kersten UW: Personal communication, 1986.

Finally, the observed differences between the biliary excretion of vecuronium and pancuronium might be due to the fact that several monoquaternary compounds have been shown to bind to bile acids or to be associated with biliary micelles in the liver, which might facilitate their net transport from the liver into the bile even though this process occurs against an electrochemical gradient.[21]

We therefore conclude that, in contrast to pancuronium, vecuronium bromide is rapidly taken up by the perfused isolated liver which, in turn, rapidly eliminates it into the bile. Almost 50% of the injected dose is metabolized to the 3-hydroxy derivative. The other two possible metabolites of vecuronium, the 17-hydroxy and the 3,17-hydroxy derivatives, were not detected. Small amounts of this 3-hydroxy derivative, or possibly vecuronium itself, are transferred back into the plasma. The striking differences in the handling of pancuronium and vecuronium by the liver could in part be due to the monoquaternary nature of vecuronium as opposed to the bisquaternary structure of pancuronium.

The results of this study substantiate the conclusion of Upton *et al.*[8] that the liver is a major organ of elimination of vecuronium bromide in the rat. Furthermore, they support the growing body of evidence that the liver is probably a major organ of elimination for vecuronium in humans.[9]

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