

Effect of Hypothermia on the Hepatic Uptake and Biliary Excretion of Vecuronium in the Isolated Perfused Rat Liver

Ton M. Beaufort, M.D., Ph.D., Johannes H. Proost, Pharm.D., Ph.D.,*†‡ Jan-Gerard Maring, Pharm.D.,‡ Emiel R. Scheffer, Pharm.D.,‡ J. Mark K. H. Wierda, M.D., Ph.D.,§ Dirk K. F. Meijer, Pharm.D., Ph.D.‖*

Background: **Hypothermia prolongs the time course of action of nondepolarizing muscle relaxants. It is not known whether this prolongation is caused by a reduced rate of extrahepatic distribution or elimination, liver uptake, metabolic clearance, or biliary excretion. Therefore, the authors studied the effects of hypothermia on the net hepatic uptake, metabolism, and biliary excretion of vecuronium in isolated perfused rat liver.**

Methods: **Livers of Wistar rats were perfused with Krebs Ringer solution (1% albumin, 3.3% carbon dioxide in oxygen, pH 7.36–7.42, 38°C). Each perfusion experiment (recirculatory perfusion system) was divided into three phases. In phase 1, a bolus dose of vecuronium (950 μg) was followed by a continuous infusion of vecuronium (63 μg/min) throughout the perfusion experiment. In phase 2, the temperature was reduced to 28°C. In phase 3, temperature was restored. In controls, the temperature was kept constant throughout the perfusion. Concentrations of vecuronium and its metabolites were measured in perfusion medium, bile, and liver homogenate. Parameters of a multicompartmental liver model were fitted to the concentration patterns in perfusion medium and in bile.**

Results: **Hypothermia increased vecuronium concentrations in the perfusion medium from 4.0 μg/ml (range, 2.5–6.6) to 15.6 μg/ml (11.5–18.4 μg/ml; $P = 0.018$). Hypothermia reduced the biliary excretion rate of 3-desacetyl vecuronium from 18% (range, 6–37%) to 16% (range, 4–19%) of that of vecuronium ($P = 0.018$). Pharmacokinetic analysis confirmed that hypothermia reduced the rate constants of hepatic uptake and metabolism from 0.219 to 0.053 and from 0.059 to 0.030, respectively.**

Conclusions: **Hypothermia significantly and reversibly reduced the net hepatic uptake of vecuronium. Hypothermia reduced the metabolism of vecuronium and the biliary excretion rate of 3-desacetyl vecuronium.**

HYPOTHERMIA prolongs the time course of action of nondepolarizing muscle relaxants.[1–10] The mechanism underlying this prolongation may be pharmacodynamic, pharmacokinetic, or both. Buzello *et al.*[11] hypothesized that hypothermia mainly influences the pharmacodynamics of muscle relaxants. However, this raises the question why the effect of hypothermia on the time course of action of pancuronium is less pronounced than on that of vecuronium while their mechanism of action is identical.[6] Because, after bolus dosing, the time course of action of most muscle relaxants is, apart from the dose, mainly determined by redistribution processes, a pharmacokinetic mechanism should also be considered.[12,13] In addition, biliary excretion is an important route of elimination for *d*-tubocurarine and vecuronium.[14–16] Both in animals and humans, the duration of action of vecuronium is largely determined by the rate of uptake in the liver.[16–19] At present, it is unknown whether the hypothermia-induced prolongation of the time course of vecuronium results from a reduced rate of extrahepatic distribution or elimination, for instance caused by reduced cardiac output and muscle blood flow, or from a reduced rate of liver uptake, reduced metabolic clearance, or reduced biliary excretion.

To elucidate the mechanism of this hypothermia-induced prolongation, we used an isolated perfused rat liver model in which the liver perfusion rate is fairly constant. This particular model allows one to study the influence of hypothermia on just the liver, because other organs that are important for the distribution or elimination of vecuronium are absent.

Methods

After obtaining approval from the Ethical Committee on Animal Experiments of the Faculty of Medical Sciences, Groningen, The Netherlands, 22 male Wistar rats (median weight, 270 g; range, 239–315 g) were used in the study.

Rat Liver Perfusion Technique

Sixteen liver perfusions were performed as described by Meijer *et al.*[20] Rats were anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg), and cannulae were inserted into the portal vein and the common bile duct. The hepatic artery was ligated. After surgical removal, the liver was placed in the perfusion apparatus and perfused with recirculating perfusion medium, consisting of Krebs bicarbonate buffer supplemented with 1% bovine serum albumin and insufflated with a mixture of 3.3% carbon dioxide in oxygen.[19] A volume of 100 ml of perfusion medium was used in all

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

† Associate Professor, § Professor, * Research Group for Experimental Anesthesiology and Clinical Pharmacology, University of Groningen. † Associate Professor, ‖ Professor, ‡ Department of Pharmacokinetics and Drug Delivery, University Centre for Pharmacy, Groningen, The Netherlands.

Received from the Research Group for Experimental Anesthesiology and Clinical Pharmacology, University of Groningen, Groningen, The Netherlands. Submitted for publication April 26, 1999. Accepted for publication August 30, 2000. Supported by the Foundation for Experimental and Clinical Anesthesiology, Groningen, The Netherlands.

Address reprint requests to Dr. Beaufort: Department of Anesthesiology, University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands. Address electronic mail to: a.m.beaufort@anest.azg.nl.

experiments. The perfusion experiments were performed at a temperature of 38°C and with a pH of the perfusion medium between 7.36 and 7.42. Liver temperature was maintained by performing the experiments in a perfusion cabinet that was heated thermostatically. The temperature of both the air in the cabinet and of the perfusion medium directly entering the liver was measured. The pH of the perfusion medium leaving the main reservoir was measured online by means of a pH electrode connected to a WTW Microprocessor pH Meter (TüV, Bayern, Germany), pH 535 MultiCal. To replace bile salts removed from the liver during the experiment, constant infusion of sodium taurocholate (15 μmol/h) was given throughout the perfusion. During each experiment, the viability of the liver was assessed according to the following criteria: the color of the liver (uniformly pinkish-brown), a stable flow of the perfusion medium (35 ml · min^{-1} · 10 g^{-1} liver) at a hydrostatic pressure of 12 cm H_20 or less and a constant bile flow (approximately 1.2 μl · min^{-1} · g^{-1} liver).[20]

Experimental Design

After removal from the rat, the liver was perfused and allowed to stabilize for 30 min before the start of the experiment (t = 0). The experiment was divided into three phases, each lasting 30 min (fig. 1). In phase 1 (t = 0–30 min), which was similar in all experiments, a bolus dose of vecuronium bromide (950 μg in 950 μl) was given at t = 0. The bolus dose was followed immediately by a continuous infusion of vecuronium (63 μg/min, 4 mg/ml), which was maintained throughout the experiment, *i.e.*, 90 min. An infusion was chosen to establish a stabile medium concentration, and a bolus dose was given to rapidly achieve a steady state. Infusion rate and bolus dose were estimated by means of computer simulation based on data of Mol *et al.*[21] and Bencini *et al.*[22] 3-Desacetyl vecuronium was not administered. The syringes containing vecuronium solution were weighed before and after administration for calculation of the amount of vecuronium administered. In phase 2 (t = 30–60 min), the temperature (n = 7) was reduced and maintained at this unphysiologic level (*vide infra*). In phase 3, the temperature was restored to the value as present in phase 1 (physiologic level). In the control experiments, the conditions as mentioned in the rat liver perfusion technique were maintained throughout the perfusion experiment (n = 6).

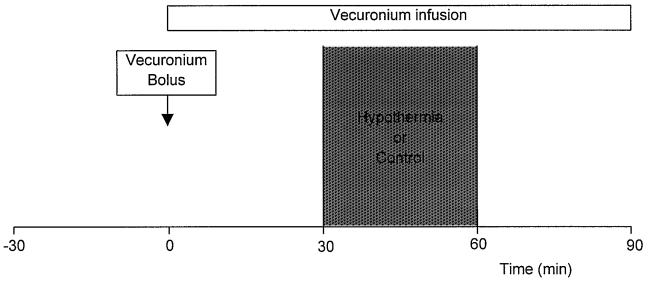

Fig. 1. Experimental design: after a stabilization period of 30 min, a bolus dose of vecuronium bromide (950 μg) was given at t = 0, followed immediately by a continuous infusion (63 μg/min) throughout the liver perfusion. Between t = 30 and t = 60 min, the temperature in the gassing mixture was reduced to 28°C or not reduced (control). At t = 60, the temperature was normalized.

Samples (800 μl) of the perfusion medium were taken before entering the liver at 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, and 90 min and were immediately mixed with 1 M NaH_2PO_4 (200 μl) to prevent hydrolysis of vecuronium. Bile was collected from the start of the perfusion at intervals of 10 min and from the start of phase 1 (t = 0) until the end of the experiment at intervals of 5 min. To collect bile, an Eppendorf tube containing 1 M NaH_2PO_4 (100 μl) was placed under the liver, and the collected bile was vortexed after sampling. The amount of bile collected in an interval was determined by weighing each Eppendorf tube before and after collecting. At the end of the experiment, the liver was weighed and homogenized in 0.2 M NaH_2PO_4 (40 ml). Perfusion medium, bile, and liver homogenate were stored at −18°C until analysis.

In hypothermia experiments, between t = 30 and t = 60 min, the temperature was reduced to 28°C within 3–5 min by opening the doors of the cabinet, turning off the heating fan, resetting the thermostat to 28°C, and exposing the main reservoir of perfusion medium to melting ice. At t = 60 min, the temperature in the cabinet was restored to 38°C within 2–3 min by removing the ice, turning on the heating fan, resetting the thermostat to 38°C, and closing the doors of the cabinet. The medium temperature was normalized within 3 min.

Influence of Temperature on Vecuronium Hydrolysis in Bile

The aim of these experiments was to assess whether metabolism, chemical decomposition of vecuronium in bile, or both occur and, if so, whether hypothermia influences these processes. The liver was removed and perfused from three rats anesthetized with intraperitoneal pentobarbital (*vide supra*). Bile was collected during 90 min. The pH of the bile was measured and 200 μl bile was added to each of four collecting tubes. Bile was incubated in duplicate at 37°C and 28°C.

Simultaneously with each bile tube, a blank solution (phosphate buffer, pH 7.4) was included to measure the extent of vecuronium decomposition in the absence of bile. When bile and the blank solution had reached a temperature of 37°C or 28°C, 100 μg vecuronium was added at t = 0. Samples of 20 μl were taken from the bile and blanks at t = 1, t = 30, t = 60, and t = 90 min. NaH_2PO_4 80 μl 0.2 M (pH 4.0) was added to each bile sample, which was subsequently vortexed. Samples were stored at −18°C until analysis. Total body weight was used for calculation of the biliary excretion rate.

Influence of Temperature on Vecuronium Hydrolysis in Liver Homogenate

The aim of these experiments was to investigate whether metabolic conversion of vecuronium in liver homogenate occurs and, if so, whether hypothermia reduces the rate of metabolism. In three rats anesthetized with pentobarbital, the liver was removed and then rinsed with an ice-cold sucrose solution (250 mM sucrose, 1 mM EDTA, pH 7.0, 4°C). Subsequent steps were performed at 4°C. The liver was added to a glass beaker containing 35 ml sucrose solution. The beaker was weighed with and without the liver to obtain the exact liver weight. The liver was then cut into pieces, and the suspension was put in a Potter-Elvehjem tube (1,000 rpm, 12 strokes) to make a homogenate. Each homogenate was split in two. One half was centrifuged at 6,800*g* for 8.5 min to remove nuclei and mitochondria. Consequently, a "rough" homogenate containing intact liver cells and the whole cell particulate and a "centrifuged" homogenate containing the particulate minus nuclei and mitochondria were obtained. One-milliliter samples of these homogenates were incubated in duplicate with vecuronium at 37°C and at 28°C. Simultaneous with incubation, a blank solution (phosphate buffer, pH 7.4) was included to measure the chemical decomposition of vecuronium in the absence of liver tissue. Incubations were performed at a temperature of 37°C or 28°C, and 50 μg of vecuronium was added at t = 0. Samples of 100 μl were taken from the homogenates and blank solutions at t = 1, t = 15, t = 30, t = 60, and at t = 90 min. NaH_2PO_4 900 μl 0.2 M (pH 4.0) was then added to each sample, and the mixture was then vortexed. Samples were stored at −18°C until analysis.

Isolation of Rat Mitochondria

Mitochondria were isolated from rat liver by a modified method of Rickwood *et al.*[23] Briefly, three rats, weighing 240–270 g each, were killed by decapitation. Their livers were homogenized in isolation medium containing 75 mM sucrose, 225 mM mannitol, 5 mM HEPES, and 1 mM EGTA, adjusted to pH 7.4. The homogenate was centrifuged for 10 min at 1,000*g* at 4°C. A mitochondrial fraction was obtained by centrifugation of the supernatant for 10 min at 10,000*g*. The resulting pellet was washed twice. The protein concentration of the mitochondrial fraction was determined according to the procedure of Lowry *et al.*[24]

Influence of Temperature on the Uptake of Radioactive-Labeled Vecuronium in Isolated Mitochondria

Mitochondria suspension (20 μl, 0.5–1.0 mg protein) and incubation medium (80 μl) were incubated together for 5 min either at 28°C or 38°C. Final concentrations were 75 mM mannitol, 25 mM sucrose, 1 mM EGTA, 95 mM KCl, 5 mM KH_2PO_4, 20 mM Tris, 10 mM succinate, 5 mM malate, and 1 mM ATP, at pH 7.4. The uptake of muscle relaxant in mitochondria could start after addition of 20 μl solution of the muscle relaxant, at a final concentration of 1 μM. Ten minutes after addition of the muscle relaxant, the uptake was rapidly stopped by addition of ice-cold 0.2 M NaH_2PO_4 (0.5 ml). Each incubation was performed in triplicate. To allow calculation of the uptake in and total recovery from the mitochondrial fraction, the following incubations were performed. At 0 min, the muscle relaxant was added to the mitochondria suspension and incubation medium. At 10 min, either 0.5 ml of 0.2 M NaH_2PO_4 was added (mixture 1) or muscle relaxant, mitochondria suspension, and incubation medium were centrifuged over 30 s at 10,000*g* to separate supernatant and mitochondria, immediately followed by addition of 0.5 ml of 0.2 M NaH_2PO_4 both to the supernatant (mixture 2) and to the mitochondrial pellet (mixture 3). Mixture 1 (mitochondria suspension + incubation medium + muscle relaxant + NaH_2PO_4) and mixture 4 (muscle relaxant + NaH_2PO_4) served as controls.

Uptake of labeled muscle relaxant in mitochondria was determined by a 10-min incubation followed by rapid filtration over Whatman GF/C filters under a constant vacuum of 600 mbar. To determine the mitochondrial uptake of labeled muscle relaxant, the radioactivity associated with the filters was counted. Therefore, the filters were transferred into scintillation vials, and 3.0 ml of Safe Fluor (Packard, Groningen, The Netherlands) was added. Vials were counted for 5 min in a scintillation counter (Beckman LS 1800 Liquid).

Bioanalysis of Vecuronium and Its 3-Desacetyl Metabolite

The concentrations of vecuronium and its metabolite 3-desacetyl vecuronium, were determined by the high-performance liquid chromatography (HPLC) method previously described for rocuronium and modified and validated for vecuronium.[25] After introduction to the HPLC system, the compounds were separated followed by postcolumn ion-pair extraction and fluorimetric detection. Aliquots of 5–25 μl of the perfusion medium or 5 (10 times diluted) to 10 μl (undiluted) bile were injected directly onto the HPLC column. The liver homogenates, (10–100 μl) or the mitochondria suspension (5–25 μl) were subjected to a liquid–liquid ion-pair extraction before the introduction onto the HPLC system. For both compounds, the method showed a linear relation between the logarithm of the response ratio (peak height muscle relaxant divided by peak height internal standard) and the logarithm of the amount of the compounds. 3,17-Desacetyl rapacuronium was used as internal standard.

The linearity ranged from 1 to 100 ng in the injected perfusion medium and from 1 to 200 ng in the injected bile, with an intraday precision in the perfusion medium

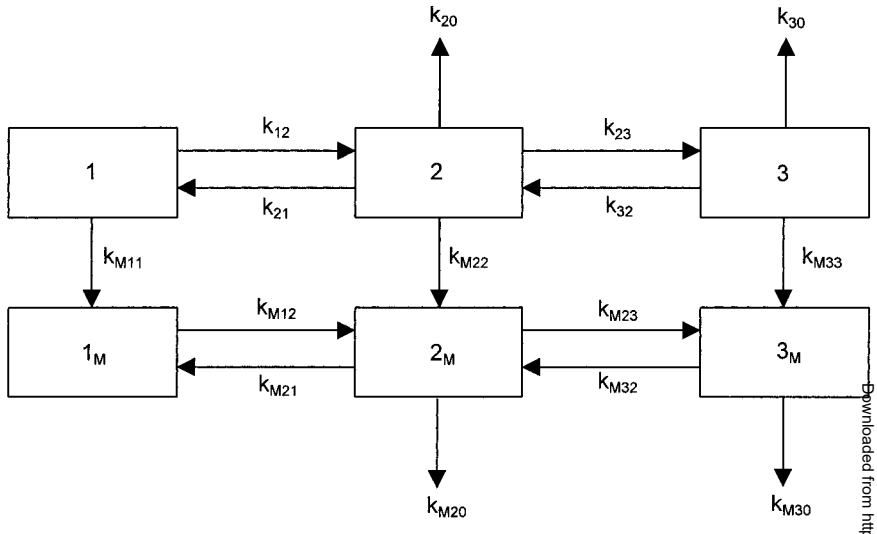

Fig. 2. Pharmacokinetic model used for simulations of the hepatic uptake, sequestration, metabolism, and biliary excretion of vecuronium by means of the computer program SIMULFIT. (1) Perfusion medium, central compartment; (2) part of the liver that is immediately accessible to the muscle relaxant; (3) part of the liver that is not immediately accessible to the muscle relaxant ("deep" compartment). k = rate constant; M = metabolite (3-desacetyl vecuronium); k_{12}/k_{21} = transport in and out of the liver; k_{23}/k_{32} = transport to and from the "deep" compartment; k_{20} = excretion into bile; k_{30} = excretion from "deep" compartment into bile; k_{M11}, k_{M22}, k_{M33} = metabolism of vecuronium in perfusion medium, liver, and "deep" compartment, respectively.

of 11.2% to 3.4% for vecuronium and 10.8% to 4.2% for 3-desacetyl vecuronium, and in the bile of 8.5% to 7.6% for vecuronium and 9.8% to 7.1% for 3-desacetyl vecuronium.

The mean absolute deviation of blindly assayed samples, covering amounts found in the injected perfusion medium and in bile, was found to be 4.8% and 9.8% for vecuronium and 3-desacetyl vecuronium, respectively. For the liver homogenate, the linearity ranged from 10 to 1,000 ng in the prepared sample, with an intraday precision of 12.5% to 1.5% for vecuronium and 14.2% to 2.7% for 3-desacetyl vecuronium.

In the prepared mitochondria samples, the linearity ranged from 10 to 250 ng, and the intraday precision was found to be 12.5% to 7.0% for vecuronium and 14.2% to 4.3% for 3-desacetyl vecuronium. In both matrices, the mean absolute deviation in the recovery samples was 5.5% for vecuronium and 10.3% for 3-desacetyl vecuronium.

The lower limit of quantification, defined as the lowest amount detected with a precision better than 15%, was 1.0 ng for vecuronium and 3-desacetyl vecuronium in the injected volume of perfusion medium and bile, while the lower limit of quantitation for both compounds appeared to be 10.0 ng in the prepared liver homogenate and mitochondria sample. The other potential metabolites, 17-desacetyl vecuronium and 3,17-desacetyl vecuronium, were also detectable in this system but were never found in samples taken during experimentation.

Pharmacokinetic Modeling

To study the influence of hypothermia on the hepatic uptake rate, metabolism, and biliary excretion of vecuronium, computer-aided simulations of the time course of the medium concentration and the biliary excretion rate of vecuronium and 3-desacetyl vecuronium were made by means of the computer program SIMULFIT (developed by J. H. P.).[21,26] SIMULFIT can simulate concentration profiles of vecuronium and of its quantitatively most important metabolite, 3-desacetyl vecuronium, simultaneously. The multicompartmental pharmacokinetic model used by SIMULFIT is shown in figure 2. SIMULFIT allows a change of model parameters such as the rate constants for hepatic uptake, metabolism, and biliary excretion over a specific time period, and therefore allows one to study the influence of external factors such as hypothermia on the model parameters. Fitting of model parameters was performed with the average values of all experiments carried out under the same external influence. The parameters to be fitted first were roughly estimated based on pilot experiments. Additional fitting was performed to reduce the residual variance. The F ratio test was used to test whether a change in the value of a parameter resulted in a significant difference with the initial value (5% level of significance).

Calculations and Statistical Analysis

The liver content was calculated by subtracting the amounts of vecuronium and 3-desacetyl vecuronium present in the perfusion medium, excreted into the bile and that removed by sampling, from the amount of vecuronium administered thus far.

The concentration of 3-desacetyl vecuronium was multiplied by 1.0705 to correct for the difference in molecular weight from the parent compound. The uptake of muscle relaxant in mitochondria was determined by dividing the mean concentrations of vecuronium in mixture 3 in each rat by that in mixture 4. The recovery of muscle relaxant in the mitochondria experiments was determined by dividing the mean of the concentrations of vecuronium in mixture 1 and in the sum of mixture 2 and 3 by that in mixture 4.

Concentrations in the perfusion medium, liver content, and biliary excretion rates of vecuronium and 3-desacetyl vecuronium at 30 min in the control and hypothermia experiments were compared with those at 60 min by means of a Wilcoxon test for paired data. Likewise, variables at 60 min were compared with those at 90 min. A Bonferroni correction was applied for multiple (2) comparisons. The concentrations of both com-

pounds in bile and liver homogenate experiments were compared with their initial values by means of a Wilcoxon test for paired data. Comparison of mitochondrial uptake between temperatures was performed by means of a Wilcoxon test for unpaired data. Differences were considered to be significant if the *P* value was less than 0.05. All values are expressed as median and range unless stated otherwise.

Results

In all perfusion experiments, the combination of a bolus administration and a continuous infusion of vecuronium resulted in a similar time course of the concentration of vecuronium in the perfusion medium, *i.e.*, an initial decrease in concentration until an apparent steady state was reached at approximately t = 20 min (fig. 3A). The liver content of vecuronium initially increased rapidly in the first 20 min and more slowly thereafter (fig. 3B). In all perfusions, the biliary excretion rate of vecuronium increased in phase 1 until a maximum excretion was reached at approximately t = 20 min (fig. 3C).

In the control experiments, the concentration of vecuronium in the perfusion medium remained stable also in phases 2 and 3 (fig. 3A) and were 4.3 μg/ml (range, 2.9–7.2 μg/ml) and 4.3 μg/ml (range, 3.5–8.1 μg/ml) at t = 30 min and t = 60 min, respectively ($P = 0.17$). The concentration of 3-desacetyl vecuronium in the perfusion medium increased gradually to 50% of that of vecuronium at t = 90 min (fig. 4A). The calculated liver content of vecuronium and 3-desacetyl vecuronium increased significantly (fig. 3B). Whereas the biliary excretion rate of vecuronium did not change significantly (fig. 3C), that of 3-desacetyl vecuronium increased to 74% of that of vecuronium at t = 90 min (fig. 4B). The bile flow remained within the specified range throughout the experiments.

In all hypothermia experiments, the reduction in temperature resulted immediately in an increase in the concentration of vecuronium in the perfusion medium in phase 2 from 4.0 μg/ml (range, 2.5–6.6 μg/ml) at t = 30 min to 15.6 μg/ml (range, 11.5–18.4 μg/ml) at t = 60 min ($P = 0.018$; fig. 3A). Conversely, return of the temperature to 38°C in phase 3 resulted in a decrease in the concentration of vecuronium in the perfusion medium to 10.3 μg/ml(range, 5.8–16.2 μg/ml) at t = 90 min ($P = 0.028$; fig. 3A). Comparison of the medium concentrations of vecuronium between the hypothermia group and the control group showed that at 30 min, the medium concentrations were not significantly different ($P = 0.67$), whereas at 60 min, the medium concentrations in the hypothermia group were significantly higher ($P = 0.003$). Figure 5 shows the medium concentrations of vecuronium of all hypothermia and control perfusion experiments. The concentration of 3-desacetyl vecuronium in the perfusion medium increased from zero at the start of the experiments to 10% (range, 7–26%) at t =

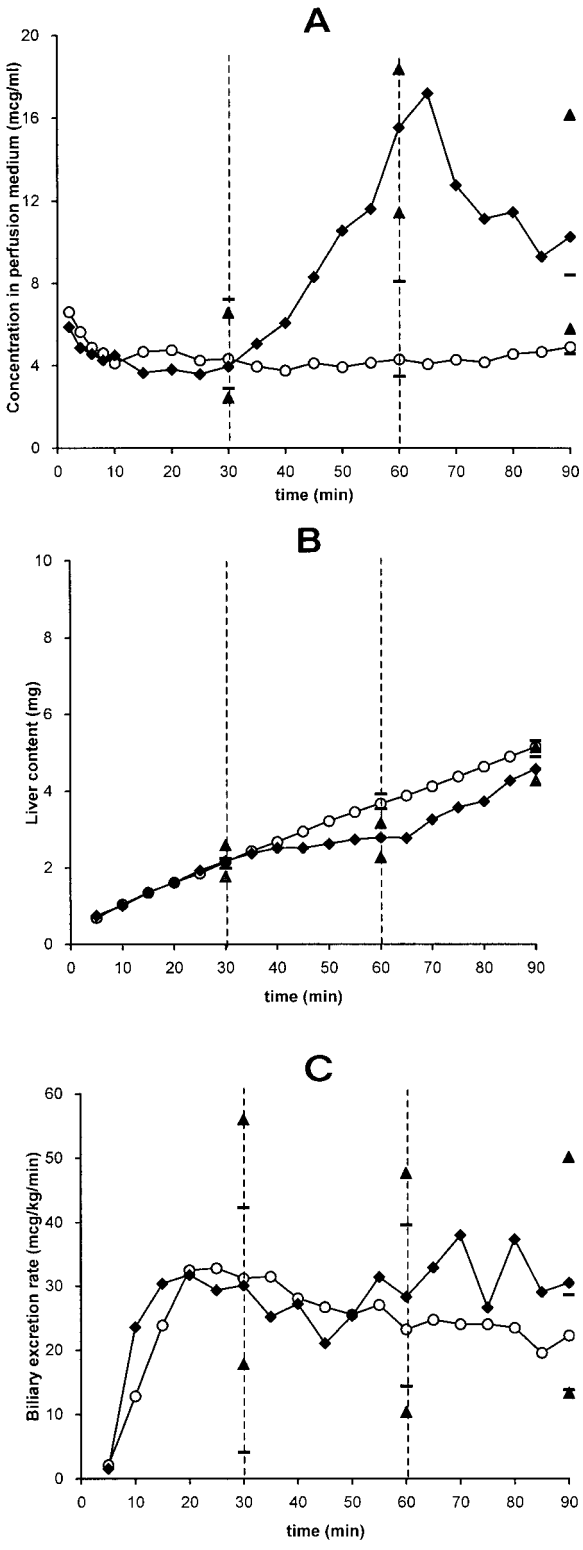

Fig. 3. Concentrations of vecuronium in perfusion medium (*A*), liver content (calculated; *B*), and biliary excretion rate (*C*) during liver perfusions. Between t = 30 and 60 min, the temperature was either reduced to 28°C (hypothermia, diamonds; n = 7) or not reduced (control, circles; n = 6). Ranges are given at 30, 60, and 90 min (hypothermia, triangles; control, dashes).

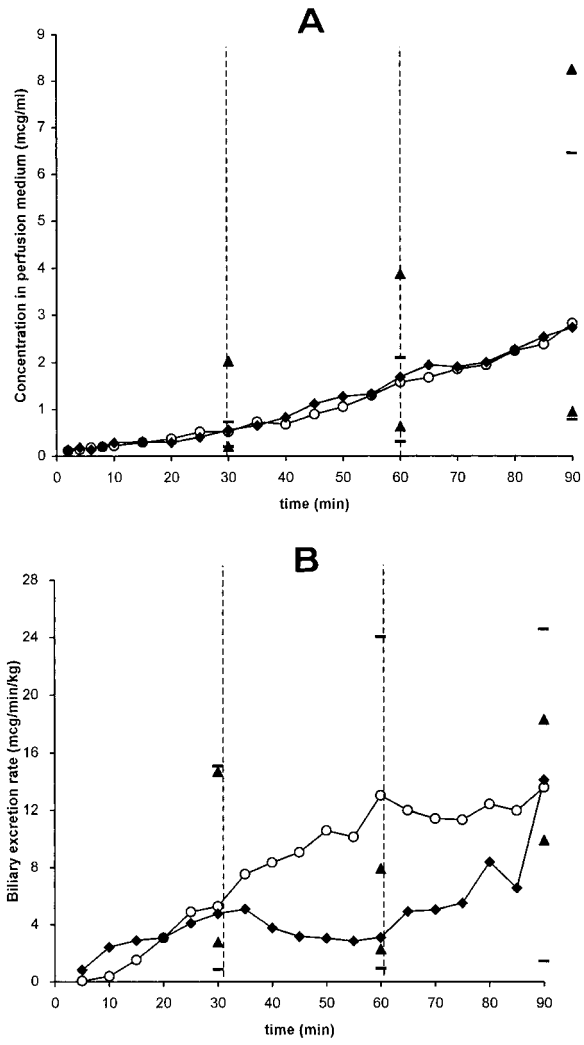

Fig. 4. Concentrations of 3-desacetyl vecuronium in perfusion medium (*A*) and biliary excretion rate (*B*) during liver perfusions. Between t = 30 and 60 min, the temperature was either reduced to 28°C (hypothermia, diamonds; n = 7) or not reduced (control, circles; n = 6). Ranges are given at 30, 60, and 90 min (hypothermia, triangles; control, dashes).

30 min, decreased during hypothermia to 8% (range, 4–22%; $P = 0.018$) at t = 60 min, and increased during normothermia to 30% (range, 13–80%) of that of vecuronium at t = 90 min (fig. 4A). In phase 2, the calculated liver content increased, although this increase was much smaller than that in phase 2 in the control experiments (0.5 mg [range, 0.4–0.9 mg] *vs.* 1.6 mg [range, 1.4–1.8 mg]; $P = 0.003$; fig. 3B). In phase 3, the liver content rapidly increased toward the value observed in the control experiments (fig. 3B). The biliary excretion of vecuronium was unaffected by hypothermia (fig. 3C). In contrast, the biliary excretion rate of 3-desacetyl vecuronium significantly and reversibly decreased by hypothermia (fig. 4B).

In phase 2, the median bile flow decreased to 54% of its initial (phase 1) value (from 1.15 to 0.62 $\mu l \cdot min^{-1} \cdot g^{-1}$ liver) and returned to its initial value in phase 3. During hypothermia, the pH slightly decreased to a lowest level of 7.29 at the lowest temperature of 28°C. Bicarbonate was not administered.

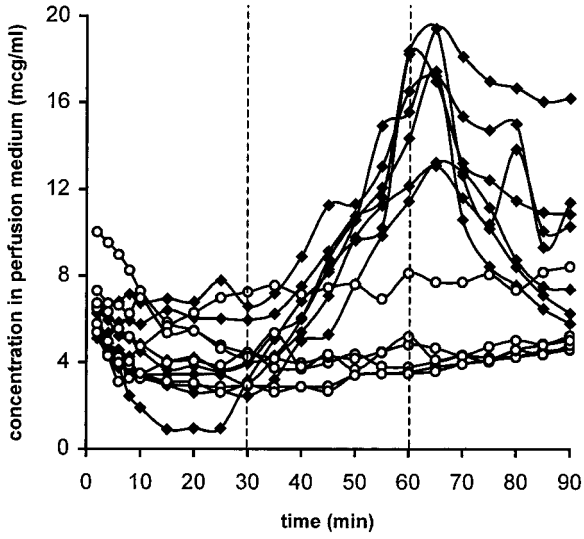

Fig. 5. Individual medium concentrations of vecuronium in all hypothermia and control perfusion experiments. Between t = 30 and 60 min, the temperature was either reduced to 28°C (hypothermia, diamonds; n = 7) or not reduced (control, circles; n = 6).

Influence of Temperature on Vecuronium Hydrolysis in Bile

Significant changes in the mean concentrations of vecuronium in bile incubated at 37°C and 28°C were not found. The mean concentration of 3-desacetyl vecuronium in bile at 37°C increased in time, whereas in bile at 28°C and in the absence of bile at 37°C and 28°C, the 3-desacetyl concentrations remained stable. We estimated that at 37°C, approximately 10% of the vecuronium in bile was hydrolysed in 90 min. At 28°C, virtually no hydrolysis of vecuronium occurred in bile.

Influence of Temperature on Vecuronium Hydrolysis in Liver Homogenate

In two rat liver homogenates, metabolism of vecuronium did not occur at all, and the effect of temperature lowering on vecuronium metabolism could not be investigated. In one liver homogenate, hypothermia reduced 3-desacetyl vecuronium formation by a factor of approximately 2. Differences in the influence of temperature on vecuronium hydrolysis between the total homogenate and the centrifuged homogenate (from which whole cells, nuclei, and mitochondria were removed) were not found.

Influence of Temperature on the Uptake of Radioactive-Labeled Vecuronium in Isolated Mitochondria

In the uptake studies of unlabeled and labeled vecuronium bromide in mitochondria, the protein concentration in the suspensions obtained from three rats was 30.6, 29.3, and 31.6 mg/ml, respectively. The uptake of

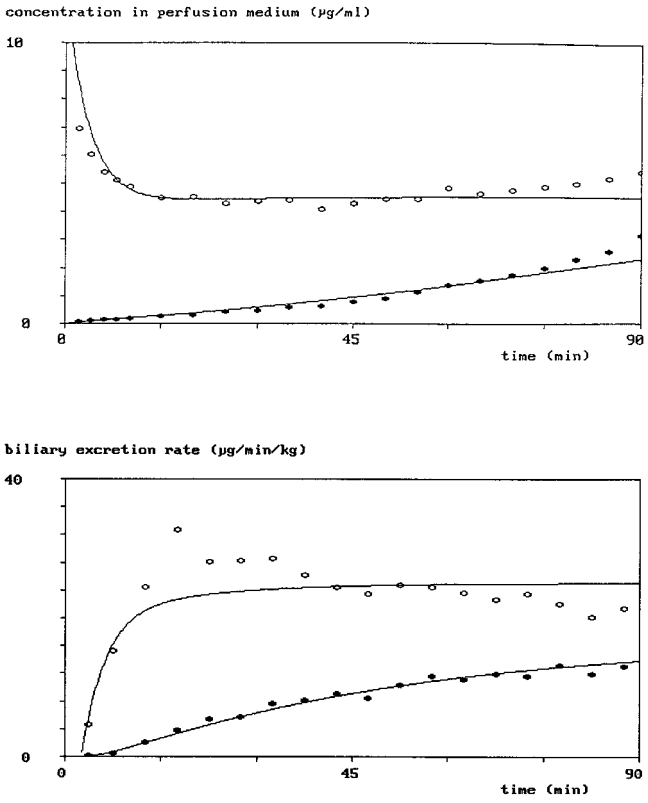

Fig. 6. Fitted curves of the concentrations in perfusion medium (*top*) and biliary excretion rates (*bottom*) of vecuronium (open circles) and 3-desacetyl vecuronium (closed circles) in the control experiments. Drawn lines indicate the fitted curves, and dots indicate the means of measured concentrations or excretion rates (n = 6).

unlabeled vecuronium in mitochondria was 33 ± 9% (mean ± SD) at 37°C and 29 ± 8% (mean ± SD) at 28°C ($P = 0.62$). The uptake of labeled vecuronium in mitochondria was 32 ± 8% (mean ± SD) at 38°C and virtually identical at 28°C (34 ± 8%, mean ± SD).

Pharmacokinetic Analysis

Pharmacokinetic analysis of the concentrations of vecuronium and its 3-desacetyl metabolite in perfusion medium and in bile in the control experiments resulted in reasonably good fits (fig. 6). Following the same fitting procedure as in the control experiments, the fits in the hypothermia experiments were poor with a large residual variance and large standard errors. In particular, the large increase in the concentrations of vecuronium in perfusion medium and the decrease in biliary excretion rate of 3-desacetyl vecuronium in phase 2 could not be approximated by the fitted curve. Variation of only one of the rate constants during phase 2 did not improve the fit substantially (not shown), whereas variation of the rate constants k_{12} and k_{M22} during phase 2 improved the goodness of fit significantly. During hypothermia, k_{12} and k_{M22} were calculated to be markedly reduced. The fit in phase 3 was improved by adapting k_{12} also in this phase. In this way, reasonably good fits were obtained (fig. 7). The pharmacokinetic parameters are listed in table 1.

Discussion

Hypothermia reversibly reduced the net uptake of vecuronium by the isolated perfused rat liver. At the same time, hypothermia substantially reduced the metabolism of vecuronium in the liver. Nondepolarizing muscle relaxants belong to the class of organic cations. On the basis of (mutual) inhibition studies of organic cations, cardiac glycosides and bile salts, at least three different carrier-mediated uptake systems have been identified in rat liver, the so-called type 1, type 2, and "multispecific" carrier systems.[27–32] This carrier-mediated uptake can be described as facilitated diffusion, *i.e.*, driven only by the electrochemical gradient of the cation.[33] However, the liver can accumulate organic cations much more extensively than can be calculated on the basis of passive distribution and equilibration according to the membrane potential. This accumulation process is caused by sequestration by mitochondria as energized by their highly negative membrane potential.[33,34] Hepatobiliary transport of organic cations is a highly concentrative "uphill" process; for vecuronium, the concentration ratio of unbound drug between bile and cytosol was calculated to be approximately 30.[33] This indicates that an energy-dependent transport system is involved in the excretion of such exogenous cations.[33] This is even more impressive realizing that transport to bile occurs against the electrochemical membrane gradient (*i.e.* negative inside).[35]

Manipulations of the membrane potential[36] or the absence of a Na^+ or K^+ gradient[36] do not influence the uptake rate of vecuronium, whereas some metabolic inhibitors do.[36] The influence of hypothermia, acidosis or hypoxia on the uptake of vecuronium is not extensively studied in intact liver. Steen *et al.*[37] showed that lowering of the temperature from 40 to 19°C significantly reduced the rate of uptake of the model cation methyl [^{3}H]tri-n-butylmethylammonium in isolated rat hepatocytes. Mol *et al.*[36] found that lowering of the temperature (42–17°C) reduced the initial rate of uptake of vecuronium in isolated rat hepatocytes, which is in accordance with a carrier-mediated transport mechanism. The decrease in the rate constant for liver uptake (k_{12}) in our study, corresponding with a Q_{10} value of 4.0, is in reasonable agreement with the Q_{10} value of 3.0 as found by Mol *et al.*[36] in isolated rat hepatocytes. Q_{10} is a measure of changes in the rate of reaction, *e.g.*, metabolic rate of oxygen consumption, for a change in temperature of 10°C.

In the present study, we used the isolated perfused rat liver technique to elucidate the influence of hypothermia on the uptake of vecuronium in the intact organ. The hypothermia-induced increase in the concentration

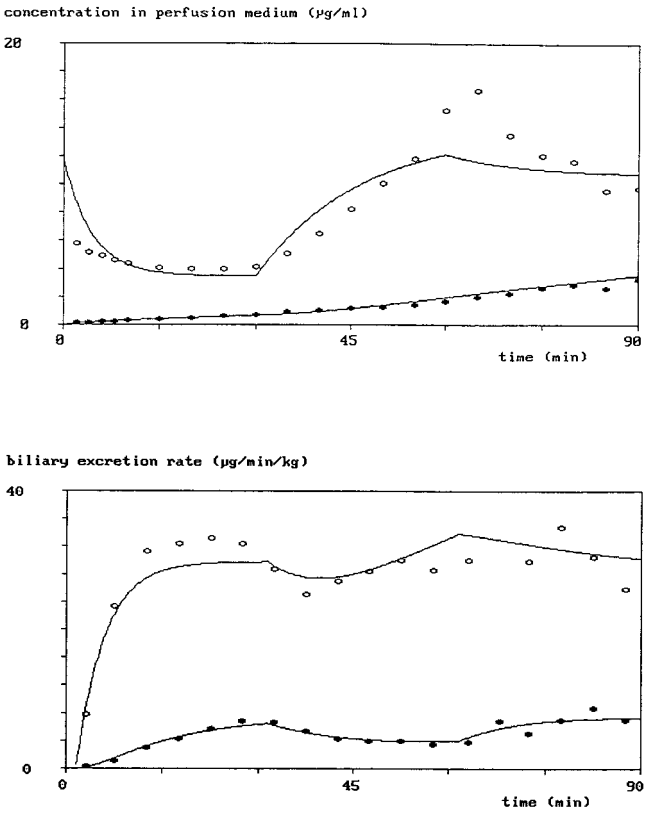

Fig. 7. Fitted curves of the concentrations in perfusion medium (*top*) and biliary excretion rates (*bottom*) of vecuronium (open circles) and 3-desacetyl vecuronium (closed circles) in the hypothermia experiments. Drawn lines indicate the fitted curves, and dots indicate the means of measured concentrations or excretion rates (n = 7).

of vecuronium in the perfusion medium and the secondary decrease when temperature restored during constant-rate infusion of vecuronium show that hypothermia reversibly reduces the net uptake of vecuronium in the liver. Release of vecuronium from the liver during hypothermia is very unlikely because pharmacokinetic modeling showed that variation of k_{21} did not improve the fit (see also table 1). Interestingly, whereas hypothermia reduced the net uptake of vecuronium in the liver extensively, its biliary excretion virtually remained intact. Consequently, we calculated that the increase in liver content during hypothermia was much smaller than that during normothermia (fig. 3B).

Hypothermia led to a drastic reduction in the biliary excretion rate of 3-desacetyl vecuronium in contrast to that of the parent compound (figs. 3C and 4B). This implies that hypothermia also reduced vecuronium conversion to its 3-desacetyl metabolite. Apparently, the reduction in the rate of metabolism results in keeping up the concentration of the parent compound in the liver despite the decreased uptake rate, thus maintaining the driving force for biliary excretion. In the single liver homogenate that showed metabolism of vecuronium, hypothermia reduced the rate of conversion by a factor of 2. No difference in metabolism was found between the homogenates with and without nuclei and mitochondria, suggesting that metabolism of vecuronium is not dependent on the presence of these organelles.

Extensive hydrolysis of vecuronium after excretion into bile could be excluded because at incubation of vecuronium with bile, only 10% is converted in 90 min at 37°C. Therefore, the measured biliary excretion rates of vecuronium and 3-desacetyl vecuronium could not be artefacts because of hydrolysis in bile. Because vecuronium metabolism in bile is insignificant, the hypothermia-induced reduction of metabolite excretion into bile is likely to be related to a temperature-dependent deacetylation process.

The mitochondrial uptake of both unlabeled and labeled vecuronium at 28°C did not differ from that at 38°C. This is in agreement with the results of Steen *et al.*,[3] who found that efficient uptake of the model cation [^{3}H]tri-n-butylmethylammonium occurred at 28°C.

Because the pharmacokinetic analysis of the data from the control experiments resulted in reasonably good fits, the hepatic uptake and biliary excretion of vecuronium could be adequately described by the compartmental model used. Reasonable fits in the hypothermia experiments could only be obtained when the rate constants for hepatic uptake and metabolism of vecuronium were allowed to decrease during the period of hypothermia. Although the calculated time profiles follow the trends in the concentration in perfusion medium and in the biliary excretion of vecuronium and 3-desacetyl vecuronium reasonably well, some systematic deviations can be observed (figs. 6 and 7). Therefore, the figures in table 1 should be interpreted carefully, *e.g.*, the measured concentration of vecuronium in the perfusion medium after return to the normal temperature decreases much faster than the calculated concentration profile, suggesting that the value of k_{12} during this period is underestimated. It may be expected that the true rate of liver

Table 1. Rate Constants (min^{-1}) Obtained by Simultaneous Pharmacokinetic Analysis of the Concentration in Perfusion Medium and of the Biliary Excretion Rate of Vecuronium and 3-Desacetyl Vecuronium Using the Model Shown in Figure 2

		Hypothermia		
	Controls	Phase 1 (0–30 min)	Phase 2 (30–60 min)	Phase 3 (60–90 min)
k_{12}	0.236	0.219	0.053	0.068
k_{21}	0.026	0	*	*
k_{20}	0.0070	0.0088	*	*
k_{M21}	0.0006	0	*	*
k_{M20}	0.0018	0.0033	*	*
k_{M23}	0.020	0.088	*	*
k_{M11}	0.0030	0.0048	*	*
k_{M22}	0.058	0.059	0.030	*

The parameters not listed became 0 during the fitting procedure.

* Same value as in phase 1, *i.e.*, 0–30 min (variation of this parameter did not result in a significantly better fit).

uptake is more close to that during the first period of normal temperature.

During hypothermia, the pH slightly decreased to a lowest value of 7.29, occurring at the lowest temperature of 28°C. It could be argued that the results obtained during hypothermia might also be the result of the slight acidosis. However, we also performed liver perfusion experiments (n = 4) according to the same protocol, reducing the pH to 7.10 in phase 2 (normothermia) by means of increasing the amount of carbon dioxide in the "inspiratory" gas mixture (respiratory acidosis). At such a low pH, the vecuronium concentrations in the medium increased nonsignificantly from 3.2 μg/ml (range, 2.4–3.9 μg/ml) to 6.0 μg/ml (range, 4.0–6.9 μg/ml; $P = 0.07$). The biliary excretion rates of vecuronium and 3-desacetyl vecuronium did not differ significantly from those in the control experiments. Because the net hepatic uptake of vecuronium at a pH of 7.10 was much less reduced than that during hypothermia, we consider the reduced hepatic uptake of vecuronium obtained during hypothermia mainly as a result of the reduction in temperature.

We also performed liver perfusion experiments (n = 7) according to the same protocol, establishing hypoxia in phase 2 (normothermia, normal pH) by means of reducing the concentration of oxygen in the "inspiratory" gas mixture to 70%. Hypoxia slightly but significantly increased the vecuronium concentrations in the medium from 4.9 μg/ml (range, 0.1–8.3 μg/ml) to 5.7 μg/ml (range, 0.6–9.4 μg/ml; $P = 0.018$). After restoration of oxygenation, the concentration in the perfusion medium did not change significantly. The biliary excretion rate of vecuronium decreased abruptly at reduction of the concentration of oxygen at 30 min (phase 2; $P = 0.018$) and did not recover at restoration of oxygenation. Most likely, irreversible liver damage occurred, specifically reflected in perturbation of the biliary excretion step. In contrast, the net liver uptake of vecuronium decreased only slightly, although irreversibly. This major detrimental effect of hypoxia on biliary output may be related to the involvement of adenosine triphosphate–dependent transport of vecuronium mediated by P-glycoprotein.[38,39]

In conclusion, hypothermia reversibly reduced the net uptake of vecuronium by the isolated perfused rat liver. At the same time, hypothermia substantially reduced the metabolism of vecuronium in the liver. Pharmacokinetic analysis showed that the hypothermia-induced changes in the pharmacokinetics of vecuronium could only be described adequately by the combination of a reduced hepatic net uptake and a reduced metabolism. The results of our study show that hypothermia extensively influences the pharmacokinetics of vecuronium and emphasize the need for effect measurement of muscle relaxants during hypothermia in patients to prevent overdosing.

The authors thank U. W. Kleef, B.Sc., and J. Roggeveld (Research Group for Experimental Anesthesiology and Clinical Pharmacology, University of Groningen, The Netherlands) for determining the concentrations of vecuronium and its metabolites in samples of perfusion medium, bile, liver homogenate, and mitochondria; and R. Oosting (Department of Pharmacokinetics and Drug Delivery, University Centre for Pharmacy, Groningen, The Netherlands) for assistance in the liver perfusions.

References

1. Miller RD, Van Nyhuis LS, Eger EI: The effect of temperature on a d-tubocurarine neuromuscular blockade and its antagonism by neostigmine. J Pharmacol Exp Ther 1975; 195:237–41
2. Miller RD, Roderick LL: Pancuronium-induced neuromuscular blockade, and its antagonism by neostigmine at 29, 37 and 41°C. ANESTHESIOLOGY 1977; 46:333–5
3. Miller RD, Agoston S, van der Pol F, Booy LHDJ, Crul JF, Ham J: Hypothermia and the pharmacokinetics and pharmacodynamics of pancuronium in the cat. J Pharmacol Exp Ther 1978; 207:532–8
4. Ham J, Miller RD, Benet LZ, Matteo RS, Roderick LL: Pharmacokinetics and pharmacodynamics of d-tubocurarine during hypothermia in the cat. ANESTHESIOLOGY 1978; 49:324–9
5. Ham J, Stanski DR, Newfield P, Miller RD: Pharmacokinetics and pharmacodynamics of d-tubocurarine during hypothermia in humans. ANESTHESIOLOGY 1981; 55:631–5
6. Buzello W, Schluermann D, Schindler M, Spillner G: Hypothermic cardiopulmonary bypass and neuromuscular blockade by pancuronium and vecuronium. ANESTHESIOLOGY 1985; 62:201–4
7. Denny NM, Kneeshaw JD: Vecuronium and atracurium infusions during hypothermic cardiopulmonary bypass. Anaesthesia 1986; 41:919–22
8. Eriksson LI, Viby Mogensen J, Lennmarken C: The effect of peripheral hypothermia on a vecuronium-induced neuromuscular block. Acta Anaesthesiol Scand 1991; 35:387–92
9. Heier T, Caldwell JE, Sessler DI, Miller RD: Mild intra-operative hypothermia increases duration of action and spontaneous recovery of vecuronium blockade during nitrous oxide-isoflurane anesthesia in humans. ANESTHESIOLOGY 1991; 74:815–9
10. Diefenbach C, Abel M, Buzello W: Greater neuromuscular blocking potency of atracurium during hypothermic than during normothermic cardiopulmonary bypass. Anesth Analg 1992; 75:675–8
11. Buzello W, Schluermann D, Pollmaecher T, Spillner G: Unequal effects of cardiopulmonary bypass-induced hypothermia on neuromuscular blockade from constant infusion of alcuronium, d-tubocurarine, pancuronium, and vecuronium. ANESTHESIOLOGY 1987; 66:842–6
12. Law Min JC, Bekavac I, Glavinovic MI, Donati F, Bevan DR: Iontophoretic study of speed of action of various muscle relaxants. ANESTHESIOLOGY 1992; 77:351–6
13. Beaufort AM, Nigrovic V, Proost JH, Houwertjes MC, Wierda JMKH: Inhibition of the enzymatic degradation of suxamethonium and mivacurium increases the onset time of submaximal neurmomuscular block. ANESTHESIOLOGY 1998; 89:707–14
14. Cohen EN, Brewer HW, Smith D: The metabolism and elimination of *d*-tubocurarine-^{3}H. ANESTHESIOLOGY 1967; 28:309–17
15. Meijer DKF, Weitering JG, Vermeer GA, Scaf AHJ: Comparative pharmacokinetics of *d*-tubocurarine and metocurine in man. ANESTHESIOLOGY 1979; 51:402–7
16. Bencini AF, Houwertjes MC, Agoston S: Effects of hepatic uptake of vecuronium bromide and its putative metabolites on their neuromuscular blocking actions in the cat. Br J Anaesth 1985; 57:789–95
17. Bencini AF, Scaf AHJ, Agoston S, Houwertjes MC, Kersten UW: Disposition of vecuronium bromide in the cat. Br J Anaesth 1985; 57:782–8
18. Bencini AF, Scaf AHJ, Sohn YJ, Kersten-Kleef UW, Agoston S: Hepatobiliary disposition of vecuronium bromide in man. Br J Anaesth 1986; 58:988–95
19. Bencini AF, Scaf AHJ, Sohn YJ, Meistelman C, Lienhart A, Kersten UW, Schwartz S, Agoston S: Disposition and urinary excretion of vecuronium bromide in anesthetized patients with normal renal function or renal failure. Anesth Analg 1986; 65:245–51
20. Meijer DKF, Keulemans K, Mulder GJ: Isolated perfused liver technique, Methods in Enzymology, volume 77. Edited by Jacoby WB. New York, Academic Press, 1981, pp 81–94
21. Mol WEM, Rombout F, Paanakker JE, Oosting R, Scaf AHJ, Meijer DKF: Pharmacokinetics of steroidal muscle relaxants in isolated perfused rat liver. Biochem Pharmacol 1992; 44:1453–9
22. Bencini AF, Mol WEM, Scaf AHJ, Kersten UW, Wolters KT, Agoston S, Meijer DKF: Uptake and excretion of vecuronium bromide and pancuronium bromide in the isolated perfused rat liver. ANESTHESIOLOGY 1988; 69:487–92
23. Rickwood D, Darley-Usmar VM, Wilson MT: Mitochondria, A Practical Approach. Washington, DC, IRL Press, 1987, pp 1–16
24. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–75
25. Kleef UW, Proost JH, Roggeveld J, Wierda JMKH: Determination of rocuronium and its putative metabolites in body fluids and tissue homogenates. J Chromatogr 1993; 621:65–76

26. Proost JH, Roggeveld J, Wierda JMKH, Meijer DKF: Relationship between chemical structure and physicochemical properties of series of bulky organic cations and their hepatic uptake and biliary excretion rates. J Pharmacol Exp Ther 1997; 282:715–26

27. Schanker LS: Transport of drugs, Metabolic Pathways, volume Vl. Edited by Hokin LE. London, Academic Press, 1972, pp 543–79

28. Meijer DKF: The mechanism for hepatic uptake and biliary excretion of organic cations, Intestinal Permeation. Edited by Kramer M, Lauterbach F. Amsterdam, Amsterdam–Excerpta Med., 1976, pp 196–207

29. Meijer DKF: Current concepts on hepatic transport of drugs. J Hepatol 1987; 4:259–68

30. Meijer DKF: Transport and metabolism in the hepatobiliary system, Handbook of Physiology, The Gastro-Intestinal System III. Edited by Schulz SG, Forte JG, Rauner BB. Bethesda, American Physiology Society, 1989, pp 717–58

31. Steen H, Meijer DKF: Transport of endogenous and exogenous organic cations, Biliary Excretion of Drugs and Other Chemicals. Edited by Siegers CP, Watkins JBG. Stuttgart-New York, Fischer Verlag, 1991, pp 239–72

32. Steen H, Merema M, Meijer DKF: A multispecific uptake system for taurocholate, cardiac glycosides and cationic drugs in the liver. Biochem Pharmacol 1992; 44:2323–31

33. Mol WEM, Meijer DKF: Hepatic transport mechanism for bivalent organic cations: Subcellular distribution and hepato-biliary concentration gradients of some steroidal muscle relaxants. Biochem Pharmacol 1990; 39:383–90

34. Steen H, Maring JG, Meijer DKF: Differential effects of metabolic inhibitors on cellular and mitochondrial uptake of organic cations in the rat liver. Biochem Pharmacol 1993; 45:809–18

35. Meijer DKF, Mol WEM, Müller M, Kurz G: Carrier-mediated transport in the hepatic distribution and elimination of drugs, with special reference to the category of organic cations. J Pharmacokinet Biopharm 1990; 18:35–70

36. Mol WEM, Fokkema GN, Weert B, Meijer DKF: Mechanisms for the hepatic uptake of organic cations: Studies with the muscle relaxant vecuronium in isolated rat hepatocytes. J Pharmacol Exp Ther 1988; 244:268–75

37. Steen H, Oosting R, Meijer DKF: Mechanisms for the uptake of cationic drugs by the liver: A study with tributylmethylammonium (TBuMA). J Pharmacol Exp Ther 1991; 258:537–43

38. Meijer DKF, Smit JW, Müller M: Hepatobiliary elimination of cationic drugs: The role of P-glycoproteins and other ATP-dependent transporters. Adv Drug Del Rev 1997; 25:159–200

39. Smit JW, Duin E, Steen H, Oosting R, Roggeveld J, Meijer DKF: Interaction between P-glycoprotein substrates and cationic drugs at the hepatic excretory level. Br J Pharmacol 1998; 123:361–70