MECHANISMS UNDERLYING THE PROLONGED DURATION OF ACTION OF MUSCLE RELAXANTS CAUSED BY EXTRAHEPATIC CHOLESTASIS


SUMMARY

The neuromuscular blocking effects of Org 6368 and Org NC 45 were studied in rats with experimental cholestasis and in controls. The effect of Org NC 45 during infusion of taurocholate was investigated. In cholestatic rats we observed a three-fold increase of the duration of action of Org 6368 and Org NC 45. The same was observed for Org NC 45 with taurocholate. In the rat phrenic nerve-hemidiaphragm preparation taurocholate potentiated the neuromuscular blockade of Org 6368, pancuronium and gallamine, but not Org NC 45. Increased bile salt concentrations caused strong inhibition of hepatic uptake and biliary excretion of Org 6368 and tubocurarine in isolated perfused livers. Taurocholate and glycocholate were more potent than cholate and chenodeoxycholate. Cholestatic livers exhibited a clearance of Org 6368 which was 50% of control. We conclude that the prolonged duration of action of certain muscle relaxants because of cholestasis results from both inhibition of hepatic uptake by the accumulated bile salts and a general deterioration of liver transport function.

For the muscle relaxants pancuronium bromide, Org 6368 (2, 16-dipiperidino-5-androstan-3-ol acetate dimethobromide), Org NC 45 (N-methyl-N-(3,17-diacetoxy-2-piperidino-5-androstan-16-yl)piperidinium bromide) a new monoquaternary analogue of pancuronium bromide, and tubocurarine, the liver plays a central role in the pharmacokinetics in various species (Agoston, Kersten and Meijer, 1973; Agoston et al., 1973; Agoston et al., 1977; Ham et al., 1978; Durant, Houwertjes and Agoston, 1979; Meijer et al., 1979). Somogyi, Shanks and Triggs (1977) reported a prolongation of neuromuscular blockade by pancuronium bromide in patients with extrahepatic cholestasis as a result of a decrease in systemic clearance. Livers from patients and animals with extrahepatic cholestasis show only minor changes in ultrastructure by light-microscopy (Schaffner et al., 1971; Greim et al., 1972; Cooper et al., 1974). These findings make it clear that altered kinetics of drugs during extrahepatic cholestasis are probably not only a result of degeneration of hepatocytes or cirrhosis. Decrease of microsomal biotransformation of drugs in the hepatocytes during extrahepatic cholestasis is an explanation for altered kinetics of drugs that are largely metabolized in the liver (McLuen and Fouts, 1961; Hutterer et al., 1970; Schaffner et al., 1971). For drugs not or only slightly metabolized, pharmacokinetic and pharmacodynamic alterations during extrahepatic cholestasis may occur, although the precise mechanisms remain unclear (Somogyi, Shanks and Triggs, 1977; Vonk, Westra et al., 1979; Westra et al., 1980).

The aim of the present investigation was to clarify the possible reasons for such alterations. We studied:
(a) The neuromuscular blocking effect of the steroid muscle relaxants Org 6368 and Org NC 45 in rats in vivo during experimental cholestasis compared with animals without biliary obstruction.
(b) The effect of the bile salt taurocholate on the neuromuscular blocking effect of Org NC 45 in rats with no cholestasis.
(c) The influence of the bile salt taurocholate on the neuromuscular blocking effect of Org 6368, pancuronium bromide, Org NC 45 and gallamine in the rat phrenic nerve–hemidiaphragm preparation.
(d) The influence of a number of naturally occurr-
ing bile salts (cholate, taurocholate, glycocholate and chenodeoxycholate) on the hepatic elimination of the muscle relaxants Org 6368 and tubocurarine in more detail in vitro. Isolated perfused livers from rats without cholestasis were used to exclude possible interactions outside the liver. We included experiments with rat livers isolated from animals with cholestasis to establish whether an abnormal disposition of muscle relaxants would still be present in the absence of increased bile salt concentrations.

METHODS

Isolated liver perfusion experiments

The equipment and method used in the perfusion experiments were those reported by Meijer and Weitering (1970) with slight modifications. In the present experiments the perfusion fluid was Krebs–bicarbonate buffer solution (pH 7.4) with 3% bovine serum albumin. Glucose and ampicillin were added at concentrations of 2 mg ml⁻¹ and 25 µg ml⁻¹ respectively. About 100 ml of the medium was used in the experiments and perfused through the liver via the portal vein and recirculated. To replace the bile salts, physiologically originating from the enterohepatic circulation of bile salts, in the control experiments an infusion of taurocholate 15 µmol h⁻¹ was given.

The viability of the isolated perfused livers was tested by measuring perfusion flow through the liver, pH of the perfusion medium and the bile flow. Perfusate flow was approximately 36 ml min⁻¹ and the bile flow 12 µlitre min⁻¹ at the start of the experiments in the controls. A priming dose (75 µmol) was given of the primary bile salts taurocholate (n = 2), glycocholate (n = 2) and cholate (n = 2) and chenodeoxycholate (n = 2) and a constant infusion of 150 µmol h⁻¹ of these bile salts was started (0.95 ml h⁻¹; Perfusor V, Salm & Kipp) and stopped after 30 min. In the case of taurocholate the infusion was continued after 30 min in four experiments. After 30 min Org 6368 1000 µg (n = 18) or tubocurarine 1000 µg (n = 4) was added to the perfusion medium. In addition four livers removed from rats after 3 days of common bile duct ligation were perfused without bile salts after relief of the ligation of the common bile duct; Org 6368 1000 µg was studied in these perfusion experiments. Medium samples and bile samples were collected at fixed intervals. After 2.5 h of perfusion, the liver was weighed after removing excess of moisture.

Male Wistar rats weighing 290–310 g were used as liver donors.

In vitro rat phrenic nerve–hemidiaphragm preparation

Male Wistar rats of approximately 300 g were decapitated after ether anaesthesia and two phrenic nerve–hemidiaphragm preparations (Bülbring, 1946) were removed and mounted in 45-ml organ baths containing mammalian Krebs' solution. The Krebs' solution was bubbled with 5% carbon dioxide in oxygen and maintained at 37 °C. The nerve was stimulated supramaximally with square-wave pulses of 0.2 ms duration at 0.1 Hz. The contractions were measured isometrically. After a sufficient equilibration period in which the contractions became stable the concentration of the bile salt taurocholate in the medium was gradually increased to 1 mmol litre⁻¹ in 0.25-mmol litre⁻¹ steps at 5-min intervals and effects were observed. Subsequently the muscle relaxants were added. The other side of the rat diaphragm, with no added taurocholate, served as control. The muscle relaxants pancuronium bromide, Org 6368, Org NC 45 and gallamine were added in partially blocking doses to give approximately 50% neuromuscular blockade.

Pharmacokinetics

According to one-compartment kinetics, the clearances (Cl) were calculated from the semi-logarithmic plots of the plasma concentration–time profiles using the equations:

\[ Cl = 0.693 \times VD/T_a \]

and

\[ VD = D/C_0 \]

where Cl is the clearance, VD is the volume of distribution, D is the dose and \( C_0 \) the concentration at \( t = 0 \) found by extrapolation of the log concentration–time curves.

Radiochemical analysis

\(^3\)H-d-tubocurarine chloride, \(^3\)H-taurocholate, \(^1\)C-chenodeoxycholate, \(^1\)C-glycocholate and \(^3\)H-cholate (New England Nuclear Corp., Boston, Mass.) were estimated by liquid scintillation counting with external standardization.
Operative procedures for experimental cholestasis

After a mid-line abdominal incision under ether anaesthesia, the common bile duct was ligated with Perma-Hand Seide 4-0 (Ethicon GmbH, Norderstedt, Germany). The incision was closed with Perma-Hand Seide 2-0. Sham-operated (all manipulations without ligation) rats served as control subjects. The obstruction of the common bile duct lasted 3 days. The entire operation took less than 15 min.

Animal in vivo experiments

Experiments were performed in male Wistar rats (320-420 g) under pentobarbitone anaesthesia (Nembutal, Abbott; 60 mg kg$^{-1}$ i.p.; maintenance doses of 12 mg i.p.). After tracheotomy, artificial ventilation with air was maintained with a Braun respirator type 74072, set at a rate of 70 b.p.m. and a tidal volume of 10 ml kg$^{-1}$. In order to check the general condition of the rat, mean arterial pressure (Statham P23 D6-transducer; KWS 3085 HSE Elektro-Manometer) was monitored. Body temperature was kept constant at 36-38 °C with a heating lamp. Neuromuscular studies (isometric twitch tension) were performed on the tibialis anterior muscle which was stimulated through the common peroneal nerve with supramaximal square-wave pulses of 0.2 ms duration at a frequency of 0.1 Hz. The stimulus was supplied by a Grass S88 stimulator and an SIU5 stimulus isolation unit. The tendon of the tibialis anterior muscle was connected to a load cell (Uh4-5, Statham), attached to a force-displacement transducer (UC3 Gold Cell, Statham), mounted securely on a myograph. Muscle contractions were recorded continuously on an MFE two-channel polygraph (d.c. to 40 Hz, full scale). Org 6368 10 mg kg$^{-1}$ (Organon Ltd) and Org NC 45 0.8 mg kg$^{-1}$ (Organon Ltd) were injected i.v. over 5 s. A constant infusion of 0.9% saline 0.5 ml h$^{-1}$ per 100 g was given and the primary bile salt taurocholate was infused at a rate of 50 µmol h$^{-1}$ per 100 g starting 10 min before injection of the muscle relaxants.

A short pilot study with Org 6368 0.83 mg kg$^{-1}$ was carried out in two male Wistar rats, weight 300 g, with ligated renal pedicles in order to determine the importance of hepatic elimination for Org 6368. The time to the onset of maximum block was defined as the time which elapsed from the injection until the twitch had reached a minimum. For the duration of action, two indices were used:

(a) the time from administration of the drug until the return of the twitch tension to 50% of the control value (duration 50).
(b) the time from administration of the drug until reversal to 90% of control tension (duration 90).

The recovery rate was the time from 25 to 75% recovery of control twitch tension (recovery rate 25-75).

Analysis of Org 6368 and of some physiological plasma constituents

The determinations of total bilirubin, direct bilirubin, total cholesterol, glutamic-pyruvic transaminase, alkaline phosphatase, urea and creatinine in the plasma of the rats were performed with an Automatic Chemical Analyzer of E. I. du Pont de Nemours & Co. (Inc.), Wilmington, DE 19898. Plasma cholinesterase activity was measured at pH 7.4 and 37 °C with butyrylthiocholine as substrate. Enzyme activities were expressed in international units per litre (iu litre$^{-1}$). Total bile salt concentrations in the plasma were measured by means of an enzymatic kit for spectrofluorimetric end-point determinations (Sterognost-Flu, Nyegaard & Co. AS, Oslo, Norway) with an Aminco Bowman Spectrofluorimeter (Am. Instr. Comp.). Excitation wavelength was 565 nm and emission wavelength 580 nm. Spectrofluorimetric determination of Org 6368 and thin-layer chromatography have been described in detail elsewhere (Kersten, Meijer and Agoston, 1973). The fluorimetric method for bis-quaternary ammonium compounds does not discriminate between Org 6368 and its metabolite(s).

Histology and enzyme histochemistry

The localization of alkaline phosphatase, 5'-nucleotidase, amino-peptidase, NADH-tetrazoliumreductase and ATP-ase in the cholestatic rat livers was studied to define the extrahepatic cholestasis on a morphological base (Hardonk et al., 1977). Histological sections of the cholestatic rat livers were stained with haematoxylin and eosin.

Statistics

All values are given as mean ± SEM. Statistical significance was tested using either the paired or the unpaired Student's $t$ test with a 95% significance level.
RESULTS

In the rats with extrahepatic cholestasis a normal glomerular filtration was found, as shown by normal plasma concentrations for creatinine and urea. The enzymes alkaline phosphatase (AF) and glutamic–pyruvic transaminase (GPT) were increased significantly in the jaundiced rats. Also the plasma concentrations of total cholesterol and total bilirubin were significantly increased compared with the sham-operated rats. Approximately 50% of the total bilirubin in the plasma was present as a conjugate with glucuronic acid. Total plasma bile salt concentrations, after 3 days of experimental cholestasis, were increased more than 200 times in comparison with the sham-operated rats. The plasma concentrations of pseudocholinesterase (butyrylthiocholinesterase) did not show a significant difference between the cholestatic rats and the sham-operated rats. The data are listed in table I.

Pharmacodynamics

The neuromuscular blocking parameters of Org 6368 and Org NC 45 in rats with experimental cholestasis clearly differed from those of the sham-operated rats, except for the onset of action (table II). In the rats with cholestasis the duration to 50% and 90% recovery of control twitch tension showed approximately three-fold increases compared with the sham-operated rats. The same pattern was observed for Org NC45 in sham-operated rats during infusion of taurocholate (table II).

Compared with controls, the plasma taurocholate concentrations were increased more than 200 times just before administration of the muscle relaxants in the cholestatic rats or during infusion of taurocholate in sham-operated rats.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Block (%)</th>
<th>Onset (min)</th>
<th>Duration 50</th>
<th>Duration 90</th>
<th>Recovery rate 25–75 (min)</th>
<th>Total bile salt &quot;0&quot; min (µmol litre⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Org 6368 10.0</td>
<td>4</td>
<td>100</td>
<td>0.92 ± 0.20</td>
<td>14.95 ± 1.91</td>
<td>18.05 ± 1.41</td>
<td>2.96 ± 0.32</td>
</tr>
<tr>
<td>Org NC 45 0.8</td>
<td>4</td>
<td>100</td>
<td>0.88 ± 0.14</td>
<td>49.36 ± 17.34</td>
<td>59.88 ± 17.34</td>
<td>12.27 ± 3.43</td>
</tr>
<tr>
<td>Org NC 45 0.8</td>
<td>4</td>
<td>100</td>
<td>1.08 ± 0.16</td>
<td>5.62 ± 0.64</td>
<td>7.58 ± 0.96</td>
<td>1.33 ± 0.21</td>
</tr>
<tr>
<td>Org NC 45 0.8</td>
<td>4</td>
<td>100</td>
<td>1.13 ± 0.18</td>
<td>16.11 ± 2.60</td>
<td>20.15 ± 3.84</td>
<td>3.71 ± 0.75</td>
</tr>
<tr>
<td>Org NC 45 0.8</td>
<td>4</td>
<td>100</td>
<td>0.80 ± 0.13</td>
<td>16.33 ± 3.31</td>
<td>21.73 ± 5.39</td>
<td>4.54 ± 1.29</td>
</tr>
</tbody>
</table>

*Significantly different from sham-operated rats.

Figures in parentheses indicate the observed range.
**Pharmacokinetics of Org 6368**

The cumulative biliary excretion of Org 6368 after 30 min in rats with ligated renal pedicles \((n = 2)\) was 39% and 47% of the administered dose and the liver content was then respectively 57% and 56% in the two rats. The plasma disappearance in the perfused liver experiments could be described by a one-compartment model (fig. 1).

A comparable pattern was seen when using the bile salts cholate, glycocholate and chenodeoxycholate (table III). The medium concentrations of the bile salts taurocholate, cholate, glycocholate and chenodeoxycholate at which the normal disappearance rate of Org 6368 started were \(0.14 \pm 0.02\), \(0.43\), \(0.13\) and \(0.54\) mmol litre\(^{-1}\) respectively (fig. 1). Bile salt concentrations of the perfusion medium of the various bile salts at \(t = 0\) and \(t = 120\) min are listed in table III.

Perfusion experiments with livers removed from rats after 3 days of common bile duct ligation showed \(T_\lambda\) three times greater than in the control experiments and \(Cl\) of approximately 50% of the control value (table III). The liver content of Org 6368 after 120 min was significantly decreased during infusion of taurocholate 150 \(\mu\)mol h\(^{-1}\) compared with the control perfusions and a comparable pattern was shown for the cumulative biliary excretion (table IV).

The cumulative biliary excretion and liver content of Org 6368 after infusion of taurocholate, cholate, glycocholate and chenodeoxycholate to \(t = 0\) min showed similar results, except for the low cumulative biliary excretion of Org 6368 in the case of glycocholate infusion (table IV). The livers of the rats with cholestasis in the perfusion experiments showed a strongly reduced cumulative biliary excretion of Org 6368 (<1%). The liver content after 120 min was 75.0 ± 6.1% (table IV), but at this time plasma concentration of the drug was increased at least 20 times compared with controls.

**Pharmacokinetics of tubocurarine**

The semilogarithmic plot of the plasma disappearance of tubocurarine in the perfused liver experiments was linear, as reported earlier by Vonk, Scholtens and others (1978) (fig. 2). The taurocholate infusion with a priming dose of 75 \(\mu\)mol and a constant infusion of 150 \(\mu\)mol h\(^{-1}\) to \(t = 0\) min \((n = 4)\) resulted in an effect similar to that observed with Org 6368. Two phases, a slow one with \(T_\lambda\) of 554.1 ± 154.4 min and a rapid one with \(T_\lambda\) of 131.3 ± 20.2 min, were found. \(Cl\) calculated from the slow phase was 0.20 ± 0.06 ml min\(^{-1}\) and was 0.70 ± 0.11 ml min\(^{-1}\) for the rapid phase. The volume of distribution was 123 ± 2 ml, the cumulative biliary excretion 20.5 ± 3.5%, the total recovery 92.5 ± 1.3% and the liver content after 120 min 26.7 ± 2.2% of the
Table III. Pharmacokinetic parameters of Org 6368 and concentrations of bile salts in the perfusion medium in isolated perfused rat liver experiments. $\tau = 0$ indicates the time of addition of the muscle relaxant. Studies were performed during infusion of taurocholate in a dose of 15 mmol h$^{-1}$, $n = 4$ (controls) or 150 mmol h$^{-1}$ during the whole experiment with a priming dose of 75 mmol ($n = 4$) and 150 mmol h$^{-1}$ until $\tau = 0$ min with a priming dose of 75 mmol h$^{-1}$ ($n = 4$). Cholate, glycocholate and chenodeoxycholate were given in a dose of 150 mmol h$^{-1}$ to $\tau = 0$ min with a priming dose of 75 mmol (for each $n = 2$) and cholestatic livers were studied without addition of bile salts ($n = 4$). Mean values $\pm$ SEM. *Significantly different from taurocholate in a dose of 15 mmol h$^{-1}$ (controls). $S =$ slow elimination phase; $R =$ rapid elimination phase.

<table>
<thead>
<tr>
<th>Experiment (mmol h$^{-1}$)</th>
<th>$V_p$ (ml)</th>
<th>$V_d$ (ml)</th>
<th>$T_1$ (min)</th>
<th>$Cl$ (ml min$^{-1}$)</th>
<th>Bile salt ($\tau = 0$ min) (mmol litre$^{-1}$)</th>
<th>Bile salt ($\tau = 120$ min) (mmol litre$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate 15 ($n = 4$)</td>
<td>100</td>
<td>101 $\pm$ 4</td>
<td>14.2 $\pm$ 0.7</td>
<td>4.95 $\pm$ 0.38</td>
<td>0.009 $\pm$ 0.001</td>
<td>0.011 $\pm$ 0.001</td>
</tr>
<tr>
<td>Taurocholate (75) + 150 ($n = 4$)</td>
<td>100</td>
<td>107 $\pm$ 1</td>
<td>229.1 $\pm$ 81.1*</td>
<td>0.72 $\pm$ 0.42*</td>
<td>0.349 $\pm$ 0.054*</td>
<td>1.990 $\pm$ 537*</td>
</tr>
<tr>
<td>Taurocholate (75) + 150 ($\tau = 0$) ($n = 4$)</td>
<td>100</td>
<td>122 $\pm$ 12</td>
<td>S72.7 $\pm$ 15.1*</td>
<td>S1.33 $\pm$ 0.30*</td>
<td>S0.33 $\pm$ 0.056*</td>
<td>S0.04 $\pm$ 0.006*</td>
</tr>
<tr>
<td>Cholate (75) + 150 ($n = 2$)</td>
<td>100</td>
<td>116 (108-123)</td>
<td>S77.5 (61.0-94.0)</td>
<td>S1.10 (0.80-1.40)</td>
<td>S0.710-0.725</td>
<td>S0.169-0.192</td>
</tr>
<tr>
<td>Glycocholate (75) + 150 ($\tau = 0$) ($n = 2$)</td>
<td>100</td>
<td>110 (109-111)</td>
<td>S29.0 (28.0-30.0)</td>
<td>S2.60 (2.50-2.70)</td>
<td>S0.239-0.200</td>
<td>S0.045-0.056</td>
</tr>
<tr>
<td>Chenodeoxycholate (75) + 150 ($\tau = 0$) ($n = 2$)</td>
<td>100</td>
<td>122 (119-125)</td>
<td>S53.0 (29.0-77.0)</td>
<td>S1.95 (1.10-2.80)</td>
<td>S0.823-0.901</td>
<td>S0.039-0.029</td>
</tr>
<tr>
<td>Cholestatic livers ($n = 4$)</td>
<td>100</td>
<td>107 $\pm$ 9</td>
<td>47.1 $\pm$ 13.7*</td>
<td>2.50 $\pm$ 1.10*</td>
<td>0.030 $\pm$ 0.007*</td>
<td>0.108 $\pm$ 0.051*</td>
</tr>
</tbody>
</table>

dose (fig. 2). The concentration of taurocholate in the perfusion medium at $\tau = 0$ min was 0.40 $\pm$ 0.01 mmol litre$^{-1}$.

**Biotransformation of Org 6368**

Thin-layer chromatography of bile samples and perfused livers containing Org 6368 was performed to determine if increased bile salt concentrations or extrahepatic cholestasis, or both, did change the metabolism of Org 6368. In both cases no deviations from the biotransformation pattern observed in isolated perfused control livers could be detected (table IV).

**Neuromuscular blockade in the hemidiaphragm preparation**

In the in vitro phrenic nerve–hemidiaphragm preparation taurocholate 1 mmol litre$^{-1}$ caused no increase of the neuromuscular blockade of Org NC45 3.3 $\mu$g ml$^{-1}$ compared with the controls (without addition of taurocholate). In the case of Org 6368 55.6 $\mu$g ml$^{-1}$, pancuronium bromide 2.0 $\mu$g ml$^{-1}$ and gallamine 100.4 $\mu$g ml$^{-1}$ incubation with taurocholate 1 mmol litre$^{-1}$ caused a 55% $\pm$ 17, 58% $\pm$ 21 and 28% $\pm$ 6 increase of the neuromuscular blocking activity respectively. These alterations were statistically significant. Taurocholate alone in a concentration of 1 mmol litre$^{-1}$ did not cause an alteration of muscle contraction in the isolated hemidiaphragm.

**Histology and enzyme histochemistry**

The perfused livers of rats with cholestasis were compared with directly removed cholestatic livers, with a cholestasis period of 3 days. Haematoxylin and eosin staining revealed a marked increase in the prominence of the portal triads and bile duct proliferation both in the directly removed and in
TABLE IV. Mean percentage of the dose of Org 6368 in the liver and bile, the total recovery and the approximate percentage of the compound which is present in liver and bile in the form of metabolite in isolated perfused rat liver experiments after 2 h perfusion. See for further details legend table III. Mean values ± SEM. *Significantly different from taurocholate in a dose of 15 µmol h⁻¹ (controls). n.d. = not detectable

<table>
<thead>
<tr>
<th>Experiment (µmol h⁻¹)</th>
<th>Bile (% of dose)</th>
<th>Liver (% of dose)</th>
<th>Tot. rec. (% of dose)</th>
<th>% Metabolite (liver)</th>
<th>% Metabolite (bile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurocholate 15 (n = 4)</td>
<td>22.7 ±2.6</td>
<td>70.0 ±5.3</td>
<td>95.0 ±5.3</td>
<td>trace-5</td>
<td>trace-5</td>
</tr>
<tr>
<td>Taurocholate (75)+150 (n = 4)</td>
<td>3.8±2.5*</td>
<td>28.4±10.0*</td>
<td>85.0±2.2</td>
<td>5-10</td>
<td>none</td>
</tr>
<tr>
<td>Taurocholate (75)+150 (t = 0) (n = 4)</td>
<td>14.2±1.5*</td>
<td>75.7±2.3</td>
<td>93.0±4.2</td>
<td>5</td>
<td>trace</td>
</tr>
<tr>
<td>Cholate (75)+150 (n = 2) (t = 0)</td>
<td>20.9</td>
<td>60.3</td>
<td>85.0</td>
<td>trace-5</td>
<td>trace-10</td>
</tr>
<tr>
<td>Glycocholate (75)+150 (n = 2) (t = 0)</td>
<td>4.0</td>
<td>76.5</td>
<td>83.5</td>
<td>trace-5</td>
<td>trace</td>
</tr>
<tr>
<td>Chenodeoxycholate (75)+150 (n = 2) (t = 0)</td>
<td>18.0</td>
<td>58.9</td>
<td>80.0</td>
<td>trace</td>
<td>trace-5</td>
</tr>
<tr>
<td>Cholestatic livers (n = 4)</td>
<td>0.3±0.1*</td>
<td>75.0±6.1</td>
<td>92.5±4.4</td>
<td>trace</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Fig. 2. Plasma disappearance after addition of 1000 µg to the perfusion medium, liver content at t = 120 min and biliary excretion of ³H-d-tubocurarine in isolated perfused rat liver experiments during infusion of taurocholate 150 µmol h⁻¹ to t = 0 min with a priming dose of 75 µmol (n = 4). The perfused livers. In the removed cholestatic livers the haematoxylin and eosin staining showed many mitotic nuclei. The number of these nuclei in the perfused livers was markedly less. A slight degeneration of hepatocytes in the periportal zone was sometimes observed in the NADH-tetrazolium staining. Dilatation of the sinusoids in the centrolobular zone occurred in both groups of livers. After 2.5 h of perfusion the livers of rats with cholestasis showed large, less intensively coloured areas in the centrolobular zone. The histochemical stainings of bile canalicular activities showed pictures typical of extrahepatic cholestasis. The activity of alkaline phosphatase was increased mainly in the periportal zone; the perfused group of livers showed less increase. ATPase activity in the periportal zone and aminopeptidase activity in the centrolobular zone were decreased, but the most in the perfused group. 5' Nucleotidase showed a slight decrease in bile canalicular activity; the sinusoidal plasma membrane of the hepatocytes showed an increase of activity in both groups of livers.
DISCUSSION

Prolongation of action of the non-depolarizing muscle relaxants Org 6368 and Org NC 45 in rats with cholestasis and in rats during infusion of the primary bile salt taurocholate may have the following causes.

Decreased renal clearance

There is evidence that renal elimination of quaternary ammonium type muscle relaxants occurs mainly by glomerular filtration (Cohen, Brewer and Smith, 1967; Raaffaub and Frey, 1972) and the process may be altered following cholestasis. However, creatinine and urea concentrations in the plasma of the rats with cholestasis, after 3 days of common bile duct ligation, were within the normal range indicating an unchanged glomerular filtration rate in extrahepatic cholestasis. These findings suggest that renal failure is unlikely. This idea is supported by the finding of normal kinetics of gallamine in cholestatic cats and in patients with extrahepatic cholestasis (Westra et al., 1980; Westra, Vermeer et al., 1981). Gallamine is almost completely eliminated by the renal route in these and other species (Mushin et al., 1949; Feldman, Cohen and Golling, 1969; Agoston et al., 1978).

Decreased liver perfusion

A decrease of the blood flow through the liver can be expected to result in a decreased hepatic clearance and thus plasma disappearance of the muscle relaxants, especially if clearance is relatively high proportional to the flow. We did not specifically measure organ flow in the rat in vivo. Mean arterial pressure in the rats with cholestasis, however, did not differ significantly from that in the sham-operated animals. The flow of perfusate in the isolated rat liver perfusion experiments was adjusted to approximately 36 ml min⁻¹ in all studies and did not change during the experiments. Nevertheless a decreased plasma clearance of Org 6368 was clearly observed both in cholestatic livers and in livers exposed to high bile salt concentrations. Therefore, it is unlikely that a decreased liver perfusion in rats is a major cause for the prolonged actions of Org 6368 and Org NC 45.

Decreased metabolism

Impairment of biotransformation of drugs during extrahepatic cholestasis, caused by high concentrations of bile salts has been reported by several authors (McLuen and Fouts, 1961; Hutterer et al., 1970; Schaffner et al., 1971). Plasma cholinesterase may play a role in the breakdown of the acetylated steroid compounds such as pancuronium bromide and Org 6368 (F. F. Folds, personal communications) and this enzyme is synthesized by the liver (Kalow, 1959; Schuh, 1977). Plasma butyrylthiocholinesterase activity in our rats was low and did not decrease during extrahepatic cholestasis in rats. Furthermore, the biotransformation patterns of Org 6368 in the isolated rat liver perfusion experiments with increased bile salt concentrations in the perfusion medium and in the cholestatic livers did not differ from the control experiments, and the total amount of the metabolite excreted in bile was less than 15%. This is in line with the observation that biotransformation patterns of Org 6368 and pancuronium bromide in cats and patients with extrahepatic cholestasis were similar to the appropriate control group without hepatobiliary disease (Somogyi, Shanks and Triggs, 1977; Westra et al., 1980; Westra, Vermeer et al., 1981).

Decreased biliary excretion

Increased bile salt concentrations in the perfusion medium of the isolated perfused rat liver resulted in a decrease of the cumulative biliary excretion of Org 6368 (the present study) and of tubocurarine (Vonk, Scholtens et al., 1978). Bile production in the isolated perfused liver of rats with cholestasis on average was only 50% of the control initially; subsequently there was a further decrease. This was comparable to the bile flow pattern in the isolated normal livers with high bile salt infusion in the last part of the experiments. Even with normal bile flow, the biliary output of Org 6368 by these livers was very small. The same was found for pancuronium bromide in cats with extrahepatic cholestasis (Westra et al., 1980). Thus biliary excretion is considerably affected by experimental cholestasis.

Decreased hepatic uptake

Inhibition of the biliary excretion step alone with unaltered hepatic uptake would result in an increased or at least equal hepatic content, if biliary excretion is the rate-limiting step in the overall elimination process. We observed a de-
creased biliary excretion of Org 6368 combined with a decreased liver content, especially in the presence of accumulated bile salts (table IV, taurocholate experiments with 75 μmol as priming dose and 150 μmol h⁻¹ throughout the experiment). We consider, therefore, that the hepatic uptake process is also impaired. Output into bile of Org 6368 was almost absent in the isolated cholestatic livers whereas hepatic uptake was only reduced by about 50%. This implies that the bile canalicular excretion of the muscle relaxant Org 6368 is strongly reduced as a consequence of the preceding cholestasis and also that hepatic uptake and biliary excretion processes are unequally influenced in this condition.

Although the inhibition of the biliary excretion of Org 6368 in the isolated perfused cholestatic rat livers, without addition of bile salts, was stronger than the inhibition of the hepatic uptake, quantitatively the latter process is the more important. Thus, inhibition of hepatic uptake in the distribution phase of the plasma disappearance might largely explain the prolongation of action of Org 6368 and Org NC 45 during extrahepatic cholestasis in vivo and during bile salt infusion in vivo. Inhibition of the (primary) hepatic uptake of the muscle relaxants pancuronium bromide, Org 6368, Org NC 45 and hexafluorenium in cats by infused bile salts, resulted in a prolongation of action of these drugs (Vonk, Westra et al., 1979a; Westra, Houwertjes et al., 1981). Animal studies showed that inhibition of hepatocellular uptake of pancuronium bromide and Org 6368 in extrahepatic cholestasis might largely explain the varying pharmacokinetic behaviour of these drugs (Westra et al., 1980). We have shown in man a decreased biliary excretion of pancuronium bromide during extrahepatic cholestasis (Westra, Vermeer et al., 1981). Vonk, Scholtens and others (1978) and Vonk, Jekel and others (1978) observed that increased bile salt concentrations inhibited hepatic uptake of the organic cations tubocurarine and acetyl-procainamide ethobromide in intact rats, isolated rat livers and isolated hepatocytes. Hitherto the reversibility of this inhibition of hepatic uptake of drugs by bile salts was not reported. The various bile salts used showed a different potency in inhibition of the hepatic uptake of the muscle relaxants; the conjugated bile salts were more potent than the unconjugated ones. Considering the data of Greim and others (1972) on chemical structure–detergent activity relationships, no correlation with the detergent action of the various bile salts could be concluded for this phenomenon. This indicates that this bile salt effect, which has also been observed for organic anions (Vonk, Danhof et al., 1979), is not a result of general damaging effects. The present observation of complete reversibility of this effect supports this contention. The cholestatic rat livers exhibited a clearance which was still 50% of the normal value during perfusion with Krebs–bicarbonate medium without external addition of bile salts. During high concentrations of bile salt in the medium, however, clearance was only 15% of the normal value. These findings strongly suggest that bile salts are, at least partially, responsible for the strong inhibition of the hepatic uptake of the drugs in intact animals. The microscopic examination of the cholestatic rat livers showed only occasional slight degeneration of the hepatocytes. The presence of mitotic nuclei might indicate the occurrence of a higher degree of degeneration in the earlier phase of cholestasis. This regeneration might be sustained by the higher 5’-nucleotidase activity of the plasma membrane of the hepatocytes. The systematic presence of large, less intensively coloured areas in the centrolobular zone of the perfused livers of rats with cholestasis may indicate a decreased functional capacity in these parts of the liver. This damage may be a result of the increased perfusion pressure of the cholestatic livers in an attempt to ensure a standard flow of 36 ml min⁻¹.

**Alteration of tissue binding or neuromuscular blocking activity of the muscle relaxants**

Waser (1973) reported a significant tissue binding of pancuronium bromide by connective tissues. A plausible explanation for the potentiation of the neuromuscular blockade of pancuronium bromide, Org 6368 and gallamine by a high concentration of taurocholate in the hemidiaphragm preparation in the present study could be such an impairment of tissue binding by bile salts for these drugs, although a direct potentiating activity cannot be excluded. However, this effect of the bile salts on the muscle contractions in vitro was not seen with Org NC 45, while the effect of this compound was clearly increased by cholestasis and bile salts in vivo. We conclude, therefore, that it is unlikely that the prolongation of action of the muscle relaxants in cholestasis and
during bile salt infusion is largely determined by interference with tissue binding or drug effect.

The present study indicates that the inhibition of hepatic uptake and consequently the prolonged duration of action of the curare-like agents Org 6368 and Org NC 45 in rats with extrahepatic cholestasis, a pathological condition involving increased plasma concentrations of bile salts, is mainly a result of an interaction of these naturally occurring bile components with the curare-like agents during hepatic uptake. In addition, a general decrease in liver transport function as a result of cholestasis may play a role.

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REFERENCES


CHOLESTASIS AND MUSCLE RELAXANTS


MECANISMES SOUS-JACENTS DE LA DUREE D'ACTION PROLONGEE DES RELAXANTS MUSCULAIRES CAUSES PAR UNE CHOLESTASE EXTRAHEPATIQUE

RESUME
On a fait des études sur les effets des blocages neuromusculaires causés par Org 6368 et Org NC 45, à l'aide de rats soumis à une cholestase expérimentale et de témoins. Les effets d'Org NC 45 ont été surveillés pendant le perfusion de taurocholat. Sur les rats soumis à une cholestase, on a observé une augmentation équivalente à trois fois la durée d'action d'Org 6368 et d'Org NC 45. On a fait la même observation en ce qui concerne Org NC 45 et le taurocholat. Dans une préparation phrénique de nerf d'hémidiaphragme de rat, le taurocholat a renforcé le blocage neuromusculaire provoqué par Org 6368, le pancuronium et la gallamine, mais pas celui causé par Org NC 45. Les concentraciones de sel dans la bile qui avaient augmenté, ont entraîné une forte inhibition de la fixation hépatique de même que l'excrétion biliaire d'Org 6368 et de tubocurarine sur des foies isolés et soumis à une perfusion. Le taurocholat et le glycocholat ont été plus puissants que le cholat et le chénodéoxycholat. Les foies soumis à la cholestase ont fait ressortir un coefficient d'épuration d'Org 6368 qui a été de 50% des valeurs témoins. Nous en concluons que la prolongation de la durée d'action de certains relaxants musculaires, du fait de la cholestase, proviennent à la fois de l'inhibition de la fixation hépatique par les sels de la bile qui se sont accumulés et d'une détérioration de la fonction "transport" du foie.

MECHANISMEN, DIE DER AUSGEDEHNTEN WIRKUNGSDAUER VON MUSKELENTSPANNUNGSMITTELN INFOLGE VON EXTRAHEPATISCHER CHOLESTASE ZUGRUNDE LIEGEN

ZUSAMMENFASSUNG

MECANISMOS QUE SOPORTAN LA PROLONGADA DURACION DE LA ACTIVIDAD DE LOS RELAJANTES MUSCULARES CAUSADA POR COLESTASIS EXTRAHEPATICA

SUMARIO
Se estudiaron los efectos de bloqueo neuromuscular del Org 6368 y del Org NC 45 en ratas con colestasis experimental y en otras de control. Se investigó el efecto del Org NC 45 durante la infusión de taurocolata. En las ratas colestásicas se observó un triple incremento de la duración de la actividad del Org 6368 y del Org NC 45. Lo mismo se observó en los tocantes al Org NC 45 con taurocolata. En la preparación del hemidiaphragma del nervio frénico, la taurocolata potenció el bloqueo neuromuscular del Org 6368, del pancuronio y de la gallamina, pero no del Org NC 45. Las concentraciones salines con incremento de bilis ocasionaron una fuerte inhibición de la admisión hepática y de la excreción biliar del Org 6368 y de la tubocurarina en hígados inundados aislados. La taurocolata y la glicocolata fueron más potentes que la colata y la quenodeoxicolata. Los hígados colestásicos presentaron una eliminación de Org 6368 que fue el 50% de la del control. Nuestra conclusión es que la prolongada duración de la actividad de ciertos relajantes musculares debida a colestasis es el resultado tanto de la inhibición de la admisión hepática como de la acumulación de sales biliares y de una deterioración general de la función de transporte del hígado.