PROLONGATION BY BILE SALTS OF THE DURATION OF ACTION OF A STEROIDAL NEUROMUSCULAR BLOCKING AGENT

R. J. Vonk, P. Westra, M. C. Houwertjes and S. Agoston

SUMMARY
The influence of bile salts on the duration of action of the steroidal non-depolarizing neuromuscular blocking agent ORG 6368 was investigated in cats. The intravenous administration of ORG 6368 (100 μg/kg body wt) caused a maximum neuromuscular blockade of 71 ± 6% with a duration of action of 3.4 ± 0.1 min. However, intraportal administration of the same dose caused no significant neuromuscular blockade. Following an infusion of dehydrocholate 320 μmol, lasting for 8 min, the magnitude and the duration of action of the neuromuscular blockade produced by ORG 6368 were increased markedly. This effect of the bile salt is possibly a result of inhibition of the hepatic uptake of ORG 6368, thereby retarding its disappearance from the plasma and consequently prolonging the neuromuscular blockade. The neuromuscular blocking effect of intraportally administered gallamine (1 mg/kg body wt) was not influenced significantly by the infusion of dehydrocholate.

The durations of action of neuromuscular blocking agents are determined by a number of factors including drug–receptor interactions, possible cholinesterase inhibition by the relaxant drug (Foldes, 1971, 1975—personal communications; Schuh, 1977) and their pharmacokinetic behaviour. In particular, renal and hepatic clearance (Marsh, 1952; Kalow, 1953; Cohen, Corbascio and Fleischli, 1965; Agoston, Kersten and Meijer, 1973; Agoston et al., 1973) and binding to “acceptor tissue depots” (Chagas, 1962; Cohen, Hood and Golling, 1968; Asghar and Roth, 1971; Shindo et al., 1974) are important pharmacokinetic factors in determining the magnitude and duration of the neuromuscular blockade. Pharmacokinetic studies of various non-depolarizing muscle relaxants indicated that gallamine (Mushin et al., 1949; Agoston et al., 1978) and alcuronium (Raaflaub and Frey, 1972) are excreted mainly by the kidneys. The liver appears to play a role in the disappearance, from the plasma, of tubocurarine (Cohen, Brewer and Smith, 1967; Meijer and Scaf, 1968), pancuronium (Agoston et al., 1973; Buzello, 1975) and hexafluorocurarine (Meijer and Kwant, 1971). In the cat, hepatic uptake is an important factor in the plasma disappearance of a number of steroidal neuromuscular blocking agents, such as pancuronium, dacturonium and ORG 6368 (2β,16β-dipiperidino-5α-androstan-3α-ol acetate dimethobromide) (Agoston, Kersten and Meijer, 1973; Agoston et al., 1977). Therefore, alterations in hepatic function may influence the pharmacokinetics, and consequently the duration of action, of these drugs. It was reported that, in patients with cholestasis, the duration of action of pancuronium was prolonged (Somogyi, Shanks and Triggs, 1977). Recently, Vonk and others (1978a, b) observed that bile salts inhibited the hepatocellular transport of the organic cations tubocurarine and acetylprocainamide ethobromide in intact rats, isolated perfused rat livers and isolated hepatocytes.

The aim of this study was to investigate the possible role of bile salts in the hepatic transport of neuromuscular blocking agents, which could lead to an increase in the duration of action of these compounds. The experiments were performed with ORG 6368 and gallamine triethiodide. ORG 6368 is a short-acting analogue of pancuronium bromide (Sugrue and Duff, 1973). It has been shown (Agoston et al., 1977) that its duration of action is determined mainly by hepatic clearance. In contrast, the plasma disappearance of gallamine triethiodide, which is determined by renal function, theoretically should not be changed by alterations in the hepatic transport systems of drugs.

METHODS

Animal experiments

All experiments were carried out on adult cats (2–4 kg body wt) of either sex under pentobarbitone anaesthesia. After orotracheal intubation with a
cuffed tube, mechanical ventilation with air was maintained at a rate of 30 b.p.m. with a tidal volume of 34 ml. In order to check the general condition of the cat, e.c.g. and mean arterial pressure were monitored. Mean arterial pressure was not less than 100 mg Hg in all experiments. Body temperature was kept constant at 37-38 °C by heating the operating table. To replace fluid loss, glucose 2.5% and saline 0.45% were infused through a polythene cannula via an external jugular vein. Neuromuscular studies (isometric twitch tension) were performed on the tibialis anterior muscle which was stimulated through its motor nerve with supramaximal square wave pulses of 0.2 ms duration at a frequency of 0.1 Hz. Following the administration of heparin 2500 i.u., a silicon catheter was placed in the hepatic portal vein to permit the administration of drugs into the portal system. After the administration of the neuromuscular blocking drugs, arterial blood samples were taken at the time when twitch tension approached 50% of control. Bolus injections of the neuromuscular blocking agents were made within periods of 5 s. The bile salts, dehydrocholate (Fluka A. G.) and chenodeoxycholate (Falk GmbH & Co), were administered by continuous infusions through the catheter placed in the portal vein. In order to avoid accumulation, ORG 6368 (Organon Ltd) was administered at intervals of at least 30 min. Gallamine triethiodide (Flaxedil, Specia) was injected at intervals of 60 min.

Chemical analysis
ORG 6368 in plasma was estimated according to the procedures described before (Kersten, Meijer and Agoston, 1973; Agoston et al., 1977).

Statistical analysis
Statistical comparisons were made using Student's t test with a 95% significance level. The values given are mean values ± SEM.

RESULTS
The i.v. administration of ORG 6368 100 μg/kg body wt produced a maximal depression of the control twitch height to 71 ± 6% (n = 6) (fig. 1A; table I). The time to 90% recovery of control twitch tension was 3.4 ± 0.1 min. When the same dose of ORG 6368 was administered through the catheter in the portal vein, a neuromuscular blockade of only 2 ± 2% was

<table>
<thead>
<tr>
<th>Administration</th>
<th>Maximal neuromuscular blockade (%)</th>
<th>Duration of action (min)</th>
<th>ORG 6368 concn at 50% recovery (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.v.</td>
<td>71 ± 6</td>
<td>3.4 ± 0.1</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Intraportal</td>
<td>2 ± 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intraportal (+ dehydrocholate)</td>
<td>70 ± 12</td>
<td>5.5 ± 0.6</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>Intraportal</td>
<td>7 ± 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I.v.</td>
<td>71 ± 6</td>
<td>4.0 ± 0.1</td>
<td>—</td>
</tr>
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Fig. 1. Influence of dehydrocholate on the pattern of action of ORG 6368, a steroidal non-depolarizing neuromuscular blocking agent. A: i.v. injection of ORG 6368; B: intraportal injection of ORG 6368; C: intraportal injection of ORG 6368 immediately after intraportal infusion of dehydrocholate 320 μmol in 8 min; note the lack of any change in the twitch height during the bile salt infusion; D: intraportal injection of ORG 6368 about 2 h after dehydrocholate infusion; E: i.v. injection of ORG 6368.
produced (fig. 1b). The influence of bile salts was subsequently investigated. Dehydrocholate was infused at a rate of 40 μmol min⁻¹ over 8 min. The intraportal injection of ORG 6368 100 μg/kg body wt immediately after the infusion of the bile salt resulted in 70 ± 12% depression of twitch height and a duration to 90% recovery of 5.6 ± 0.6 min (fig. 1c). This potentiating effect of dehydrocholate was reversible and lasted from 30 min to approximately 180 min.

When an intraportal injection of ORG 6368 100 μg/kg body wt was given, a neuromuscular blockade of 7 ± 3% (fig. 1f) was subsequently produced, a pattern which was comparable to that before the administration of the bile salt. The i.v. administration of ORG 6368 caused approximately the same neuromuscular blockade as before the dehydrocholate administration (table I: 71 ± 6%, lasting 4.0 ± 1 min).

In contrast to the observations obtained with ORG 6368, the neuromuscular blocking effect of intraportally administered gallamine 1 mg/kg body wt was not influenced significantly by dehydrocholate infusion. Gallamine administered in this dose caused a 71 ± 8% depression of twitch height with a duration to 90% recovery of 8.4 ± 1.0 min; after dehydrocholate infusion these values were 74 ± 5% and 9.5 ± 1.3 min respectively (n = 4).

The influence of dehydrocholate on the relationship between plasma concentration of ORG 6368 and the neuromuscular blocking effect is shown in table I. At 50% recovery of the twitch tension, the concentration of ORG 6368 was significantly higher in the presence of dehydrocholate.

**Discussion**

The marked decrease in the effectiveness of ORG 6368 when injected into the portal vein, rather than into the general circulation, is explained by the high hepatic clearance of this drug (Agoston et al., 1977). Differences in the patterns of efficacy of neuromuscular blocking agents injected by different routes have also been described by Hughes (1972). In contrast to that of ORG 6368, gallamine’s efficacy was not reduced when it was injected intraportally—an observation compatible with the absence of any important role of the liver in the plasma clearance of gallamine (Feldman, Cohen and Galling, 1969; Agoston et al., 1978). During the infusion in the present study, and in separate in vitro experiments (unpublished data) in the rat phrenic nerve–diaphragm preparation, dehydrocholate (concentration up to 2.0 mmol) and chenodeoxycholate (concentration up to 0.2 mmol) did not influence the indirectly evoked twitch contractions.

The effects of the semi-synthetic bile salt dehydrocholate were studied in these experiments because this substance has previously been found to modify the hepatic transport of drugs (Vonk et al., 1978a, b) and, in the doses used, it is free from complicating cardiovascular actions. Its effects on the actions of neuromuscular blocking drugs were compared with, and found to be qualitatively similar to, those of the naturally occurring bile salt chenodeoxycholate.

Either bile salt, when infused into the portal vein, potentiated the neuromuscular blocking action of ORG 6368 injected by the same route. That the potentiating action was not the result of a peripheral sensitizing action of the bile salts at the neuromuscular junction was shown by the observation that gallamine was not similarly potentiated. Furthermore, the increased efficacy of intraportally injected ORG 6368 in the presence of bile salts was associated with an increased plasma concentration of the drug. Thus, it seems clear that the potentiating action of bile salts on ORG 6368 takes place in the liver, and occurs because the bile salts impair the hepatic uptake of the neuromuscular blocking drug. The mechanism underlying this effect is not fully established. Inhibition of biliary excretion is unlikely, because biliary excretion of ORG 6368 in the cat is a relatively unimportant route of elimination (12% of the dose in 8 h; Agoston et al., 1977).

The bile salts do not appear to displace ORG 6368 from the liver since, in unpublished experiments, we have shown that after previous loading of the liver with ORG 6368, infusions of bile salts do not produce neuromuscular block. Therefore, we conclude that the bile salts act by inhibiting the primary hepatic uptake process of ORG 6368, a conclusion which is in agreement with earlier studies in which the interactions of bile salts with tubocurarine or acetylprocainamide ethobromide were studied (Vonk et al., 1978a, b). The results suggest that in patients with an increased plasma concentration of bile salts, neuromuscular blocking agents that are cleared from the plasma by the liver may have an unusually prolonged duration of action.

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REFERENCES


PROLON©ATION DE LA DUREE DE L'ACTION D'UN AGENT DE BLOCAGE NEUROMUSCULAIRE STEROIDE PAR LES SELS BILIAIRES

RESUME

On a fait des recherches sur des chats pour étudier l'influence des sels biliaires sur la durée de l'action de l'agent de blocage neuromusculaire stéroïde non dépolarisant ORG 6368. L'administration intraveineuse d'ORG 6368 (à raison de 100 µg/kg de poids du corps) a provoqué un blocage neuromusculaire maximum de 71 ± 6% avec une durée d'action de 3,4 ± 0,1 mn. Cependant, l'administration intraportale de la même dose n'a provoqué aucun blocage neuromusculaire significatif. Après l'infusion de 320 µmol de déhydrocholate, d'une durée de 8 mn, l'importance et la durée d'action du blocage musculaire produit par l'ORG 6368 ont été distinctement et fortement accrues. Ces effets des sels biliaires sont probablement le résultat de l'inhibition de la fixation hépatique de l'ORG 6368, qui en a retardé la disparition du plasma et a par conséquent prolongé le blocage neuromusculaire. L'effet de blocage neuromusculaire de la gallamine administrée par voie intraportale (à raison de 1 mg/kg de poids du corps) n'a pas été influencé d'une manière significative par l'infusion de déhydrocholate.

VERLÜNGERUNG DER WIRKUNGSDAUER EINES AUF STEROIDEN AUFGEBAUTEN NEUROMUSKULÄREN BLOCKIERUNGSMITTELS DURCH GALLENSALZ

ZUSAMMENFASSUNG

Es wurde der Einfluss von Gallensalz auf die Wirkungsdauer des auf Steroiden aufgebauten, nicht depolarisierenden neuromuskulären Blockierungsmittels ORG 6368 bei Katzen untersucht. Die intravenöse Verabreichung von ORG 6368 (100 µg/kg Körpergewicht) bewirkte eine maximale neuromuskuläre Blockierung von 71 ± 6% mit
einer Wirkungsdauer von 3,4 ± 0,1 Min. Eine Eingabe derselben Dosis in die Pfortader verursachte jedoch keine bedeutsame neuromuskuläre Blockierung. Nach einer 8 Min dauernden Infusion von 320 μmol Dehydrocholat war die Stärke und Wirkungsdauer der durch ORG 6368 hervorgerufenen neuromuskulären Blockierung bedeutend erhöht. Diese Wirkung des Gallensalzes ergibt sich möglicherweise aus einer Hemmung der hepatischen Aufnahme von ORG 6368, wodurch sein Verschwinden aus dem Blutplasma verzögert und die neuromuskuläre Blockierung dementsprechend verlängert wird. Die neuromuskuläre Blockierungswirkung von in die Pfortader eingegebenem Gallamin (1 mg/kg Körpergewicht) wurde durch die Infusion von Dehydrocholat nicht wesentlich beeinflusst.

PROLONGACION DE LA DURACION DE ACCION DE UN AGENTE DE BLOQUEO NEUROMUSCULAR ESTEROIDAL POR SALES BILIARES

SUMARIO
Se investigó en gatos la influencia de la sal biliar sobre las duraciones de acción del agente bloqueador neuromuscular no-depolarizante esteroidal ORG 6368. La administración intravenosa de ORG 6368 (100 μg/kg de peso corporal) causó un bloqueo neuromuscular máximo de 71 ± 6%, con una duración de acción de 3,4 ± 0,1 min. Sin embargo, la administración intraportal de la misma dosis no causó bloqueo neuromuscular significativo. Después de una infusión de 320 μmol de dehidrocolato, de 8 min de duración, aumentaron notable y significativamente la magnitud y la duración de acción del bloqueo neuromuscular producido por el ORG 6368. Posiblemente, este efecto de la sal biliar resulte de la inhibición de la captación hepática de ORG 6368, demorando por causa de ello su disparición de la plasma y prolongando por ende el bloqueo neuromuscular. La infusión de dehidrocolato no influenció de manera significativa el efecto bloqueador neuromuscular de la galamina administrada por vía intraportal (1 mg/kg de peso corporal).