Missbildungen bei Rattenfeten nach gleichzeitiger Verabreichung von Äthylharnstoff (ÄH) und Nitrit an tragende Muttertiere

Gruppe	Substanzbeimengungen	Zahl der graviden Ratten	Zahl der implant. Embryonen	Zahl der abgestorb. Embryonen	Lebende Feten Total	Lebende Feten Mit Missbildungen	Art der Missbildungen (zum Teil kombiniert)
I	1% Nitrit im Futter 0,5% ÄH im Wasser 200 mg/kg ÄH durch Magensonde	3	33	20 (60,6%)	13	10 (76,9%)	Hydrozephalus 6 Exenzephalie 3 Anophthalmie 3
II	0,3% Nitrit im Futter 0,5% ÄH im Wasser 100 mg/kg ÄH durch Magensonde	9	82	13 (15,8%)	69	21 (30,4%)	Hydrozephalus 21 Anophthalmie 9
III	0,3% Nitrit im Futter 0,5% ÄH im Wasser	8	78	6 (7,7%)	72	3 (4,2%)	Hydrozephalus 2 Anenzephalie 1 Anophthalmie 1 Eventeration der Bauchorgane 1
IV	Kontrolle 0,5% ÄH im Wasser 200 mg/kg ÄH durch Magensonde	9	76	8 (10,5%)	68	0	
V	Kontrolle 0,5% ÄH im Wasser 100 mg/kg ÄH durch Magensonde	7	82	6 (7,3%)	76	0	
VI	Kontrolle 0,5% ÄH im Wasser	8	76	3 (3,9%)	73	0	
VII	Kontrolle 1% Nitrit im Futter	4	30	0	30	0 (6 weitere Muttertiere 1 Tag nach Nitritfütterung verstorben)	
VIII	Kontrolle 0,3% Nitrit im Futter	10	92	5 (5,4%)	87	0	
IX	Kontrolle unbehandelt	15	118	8 (6,8%)	110	0	

Zusatz von 1% Natrium-nitrit zum Futter allein (Gruppe VII) verursacht eine Intoxikation der Muttertiere, die bei 6 von 10 tragenden Ratten tödlich verlief, aber keinen embryotoxischen oder teratogenen Effekt an der Nachkommenschaft der überlebenden Muttertiere erkennen liess. Mit abnehmender Konzentration von Äthylharnstoff und Nitrit im Futter bzw. Trinkwasser verschwindet die embryotoxische Wirkung, denn die Zahl der abgestorbenen Früchte unterscheidet sich nicht signifikant von den Werten der Kontrollgruppen. Die teratogene Aktivität bleibt dagegen noch erkennbar (Gruppen II und III).

Die Resultate zeigen, dass die Menge des endogen entstehenden ÄNH für eine teratogene Wirkung ausreicht, während die Vorstufen allein keine Missbildungen hervorrufen. Es ist möglich, dass auch im menschlichen Organismus eine endogene Synthese derartiger Stoffe aus Nahrungsbestandteilen stattfindet. Damit ergeben sich Ansatzpunkte für eine Verminderung der teratogenen Gefährdung durch Eliminierung von Vorstufen teratogener Substanzen aus der Nahrung von Schwangeren.

Summary. Simultaneous peroral application of ethylurea and sodium nitrite to pregnant Sprague-Dawley rats, on the 9th and 10th days of gestation, causes embryotoxic and/or teratogenic effects. Each substance alone does not afflict the fetuses, whereas the combined addition to the food permits the formation of ethyl nitroso-urea in the digestive tract. The latter readily passes the placenta and develops the teratogenic effect. It is to be considered that teratogenic substances can occur from food constituents in the human organism also.

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Species Differences in the Influence of Chlorothiazide on Curarization

Potentiation by chlorothiazide and other sulphonamides of D-tubocurarine induced (-) neuromuscular blockade in the rabbit has been reported; displacement of D-tubocurarine from nonspecific binding sites by the sulphonamides was proposed as the mechanism underlying the observed potentiation[1,2]. However, it was found[3] that chlorothiazide antagonized the curarization produced by D-tubocurarine in the dog and also in the isolated phrenic-diaphragm preparation of the rabbit. These conflicting results prompted the present study of the influence of chlorothiazide on the curarization induced by D-tubocurarine and other paquicurares in different mammalian species. Experiments were also performed to investigate whether interaction of D-tubocurarine

with plasma proteins could influence its neuromuscular blocking effects in the rabbit. For this purpose phenylbutazone was employed since it is highly bound to plasma proteins and has a high displacement potential[4-6].

Methods. Experiments were performed using the sciatic nerve-tibialis anterior muscle preparation of dogs anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.), of cats and rabbits anaesthetized with a mixture of chloralose (80 mg/kg, i.p.) and sodium pentobarbitone (15 mg/kg, i.p.), and of guinea-pigs and rats under urethane anaesthesia (150 mg/100 g, i.p.). In all animals a tracheal cannula was inserted to allow prompt artificial respiration when necessary.

Maximal twitches of the tibialis anterior muscle were elicited once every 10 sec by rectangular pulses (0.1 to 0.2 msec duration) applied to the sciatic nerve. The strength of the pulses was twice that necessary to evoke

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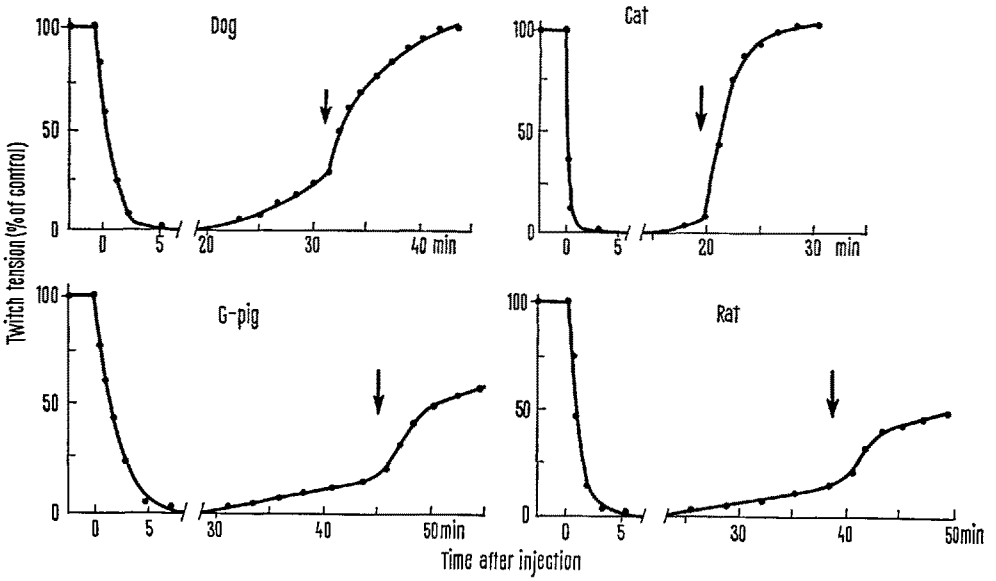

Fig. 1. Effect of chlorothiazide on D-tubocurarine-induced curarization of the tibialis anterior muscle in different animal species. In this and in the following figures, the abscissae indicate the time after injection of the curarizing agent and the ordinates represent twitch tension expressed as percent of the initial value. In the experiments plotted in this figure, D-tubocurarine was injected at zero time in doses of either 100 (rat) or 300 μg/kg (dog, cat and guinea-pig). The arrows indicate administration of chlorothiazide (50 mg/kg). Notice that in all instances chlorothiazide enhanced twitch tension; reversal of the block was observed in the dog and the cat.

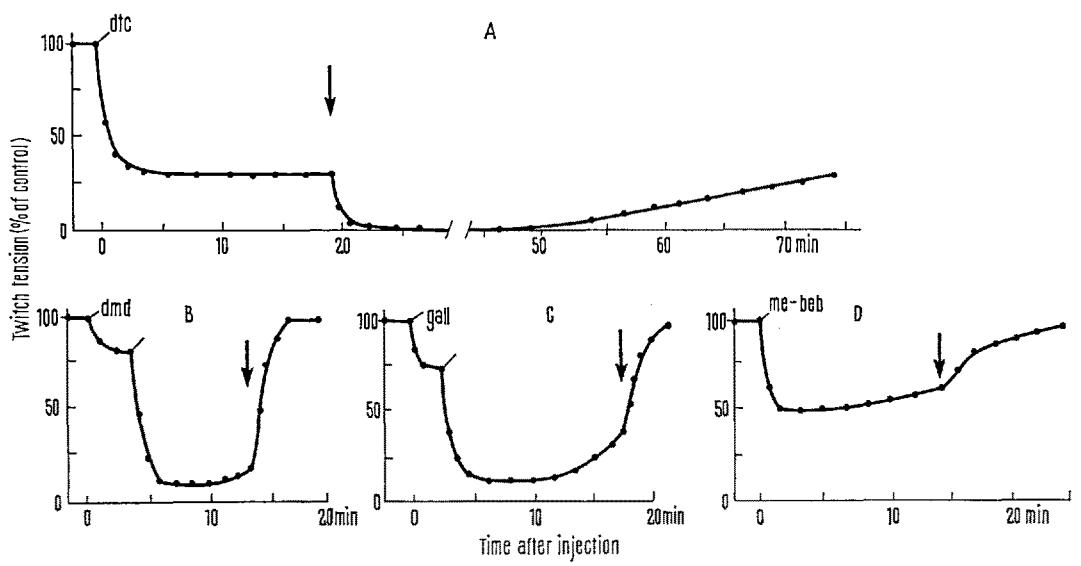

Fig. 2. Effect of chlorothiazide on the neuromuscular blockade induced by different paquicurares on the tibialis anterior muscle of the rabbit. A) D-tubocurarine, 300 μg/kg. B) Dimethyl-D-tubocurarine injected twice in doses of 10 μg/kg. C) Triethiodide of gallamine, injected twice in doses of 500 μg/kg. D) Dimethylether of methyl-beberine, 100 μg/kg. The arrows indicate administration of chlorothiazide (50 mg/kg, i.v.). Notice that only in the case of D-tubocurarine did chlorothiazide potentiate the block. In all other instances the neuromuscular blockade was reversed by chlorothiazide.

a maximal twitch. The muscle twitches were recorded isometrically on a smoked drum.

The drugs used were chlorothiazide (Specia), D-tubocurarine chloride (Lilly), dimethyl-D-tubocurarine chloride (Lilly), dimethylether of methylbeberine (Kondrocurare, Instituto Vital Brasil), gallamine triethiodide (Flaxedil, Rhodia), and phenylbutazone (Butazona, De Angeli). The doses of the curarizing drugs are expressed in terms of their salts.

Results. Preliminary experiments have shown that in the dose range used (10–50 mg/kg, i.v.), chlorothiazide per se does not affect the amplitude of twitch of the tibialis anterior muscle in the species studied. However, chlorothiazide modified the twitch tension of the tibialis anterior muscle partially curarized by D-tubocurarine (100–300 µg/kg, i.v.). This effect varied with the animal species: In the dog, cat, rat and guinea-dig, administration of chlorothiazide (25–50 mg/kg, i.v.) during recovery from the block produced an increase in twitch tension and in most instances completely reversed the block. Results of typical experiments are plotted in Figure 1. In contrast, chlorothiazide (10–50 mg/kg, i.v.) administered to the rabbit during partial curarization by D-tubocurarine (300 µg/kg, i.v.) induced a complete neuromuscular block (Figure 2). This effect was reversible. Recovery of neuromuscular transmission began after 18 to 40 min (6 rabbits).

To determine whether potentiation of neuromuscular blockade in the rabbit was specific to D-tubocurarine, we studied the influence of chlorothiazide on the curarization induced by other paquicurares in this species. In Figure 2 it can be seen that i.v. injection of chlorothiazide (25 mg/kg) reversed the block caused by gallamine, dimethyl-D-tubocurarine and dimethylether of methylbeberine. Failure of chlorothiazide to potentiate the neuromuscular blockade produced by depolarizing agents in the rabbit has also been reported[2].

In the rabbit, phenylbutazone (20–100 mg/kg, i.v.) has no effect per se on the twitch tension of the tibialis anterior muscle indirectly stimulated. However, administration of phenylbutazone (50 mg/kg) to rabbits partially curarized by D-tubocurarine resulted in complete neuromuscular block (Figure 3). This effect was reversible. Recovery of neuromuscular transmission began after 25–42 min (4 animals). Injection of chlorothiazide (50 mg/kg) during the phase of partial recovery resulted in immediate increase of twitch tension but did not completely reverse the block.

Discussion. The results show that chlorothiazide can potentiate or antagonize neuromuscular blockade. Potentiation was specific to D-tubocurarine in the rabbit. This effect has been attributed to displacement of D-tubocurarine from non-specific binding sites on plasma proteins[1]. Affinity of D-tubocurarine for binding to serum albumin has been demonstrated, extensive binding resulting in lack of curarizing activity[7]. Although we do not have direct evidence for displacement of D-tubocurarine from plasma proteins by chlorothiazide, our findings with phenylbutazone give indirect support for such a mechanism underlying the potentiation of D-tubocurarine. Phenylbutazone which competes with sulphonamides for binding to serum albumin[4,8], potentiated D-tubocurarine-induced curarization in the rabbit and reversed the effect of chlorothiazide.

Chlorothiazide and phenylbutazone are anionic drugs; thus, it is unlikely that they interact competitively with D-tubocurarine for binding to plasma proteins. It has been proposed[9] that conformational changes in plasma proteins induced by chlorothiazide underlie its influence on the binding of pempidine to serum albumin. A similar mechanism might be involved in the potentiation of D-tubocurarine by chlorothiazide in the rabbit.

The specificity of potentiation of D-tubocurarine by chlorothiazide may indicate that, among the species studied, only in the rabbit is the association constant of the D-tubocurarine-plasma protein complex high enough to have a significant influence on the drug distribution[10]. Variation in drug binding to plasma proteins occurs among species and the 'superior binding properties of the rabbit serum towards several drugs has been almost universally noted'[11]. Accordingly, failure of chlorothiazide to potentiate other paquicurares in the rabbit could be attributed to low values of the association constants for the binding of these drugs to plasma proteins.

Chlorothiazide has been shown to have anti-cholinesterase-activity[3]. Thus, its antagonism of curarization may be due to inhibition of endplate acetylcholinesterase[12,13].

Résumé. Chez le Chien, le Chat, le Cobaye et le Rat, le chlorothiazide est antagoniste de la D-tubocurarine. Chez le Lapin, le chlorothiazide potentialise la paralysie D-tubocurarinique, mais exerce une action antagoniste vis-à-vis d'autres curarimimétiques. L'administration préalable de phénylbutazone transforme en antagonisme la synergie entre le chlorothiazide et la D-tubocurarine.

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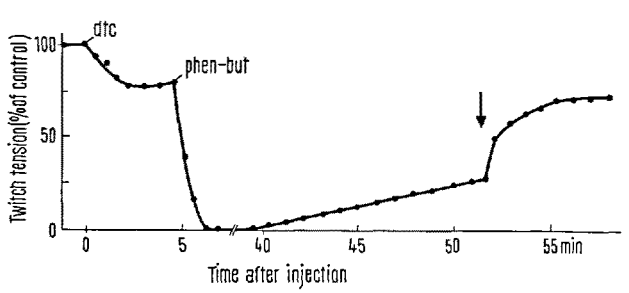

Fig. 3. Effect of phenylbutazone on D-tubocurarine-induced curarization of the tibialis anterior muscle of the rabbit. dtc, D-tubocurarine, 300 µg/kg; phen-but, phenylbutazone, 50 mg/kg. The arrow indicates the administration of 50 mg/kg of chlorothiazide. Notice that the treatment with phenylbutazone reversed the effect of chlorothiazide on the curarization induced by D-tubocurarine in the rabbit.

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