Reduced Sensitivity to some Drugs 48 h after Chlorpromazine Treatment

Previous experiments demonstrated that, in rats pretreated with electroshock, the sleeping-time induced by barbiturates (48–72 h later) is much longer than in normal animals[1].

It was supposed that this effect was not specific for electroshock treatment, but that also a pretreatment with certain substances could modify the effect of other drugs administered some time later.

Using chlorpromazine, the opposite effect to that shown after electroshock was observed. A single dose of chlorpromazine was able to reduce the sleeping-time when the barbiturates are given 48 h later.

This unexpected result was studied under different conditions. The following points are particularly stressed in the present research: (1) time between chlorpromazine administration and the onset of shortened barbiturate sleeping-time; (2) quantitative relation between the doses of chlorpromazine and the effects obtained; (3) possible different effects between single and repeated doses; (4) if this effect was specific for barbiturates or reproducible using other hypnotic agents; (5) possible hormonal interference; (6) a possible relation between the shortening of the sleeping-time and a faster breakdown of the hypnotic drugs.

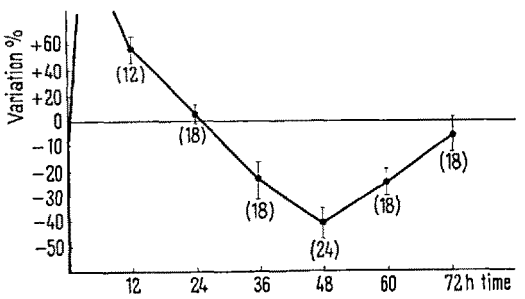
Sprague-Dawley female rats, weighing 200 g were used. Chlorpromazine was administered intraperitoneally at the dose of 15 mg/kg. Room temperature was 18–22° C.

The Figure shows the duration of sleep when pentobarbital was given at different intervals after chlorpromazine administration.

The synergistic effect of chlopromazine, when administered with barbiturates, is well known. This synergism is still noticeable after 12 h, but after 24 h has completely disappeared, until 72 h after the chlorpromazine administration, a markedly decreased sleeping-time is observed which reached a peak after 48 h. All further experiments were carried out using this time.

Our results on the relation between the dose of chlorpromazine used and the sleeping-time reduction are reported in Table I. In some experiments, chlorpromazine was administered for 5 days, before an interruption of 48 h.

The sleeping-time decreases with increasing doses of chlorpromazine. When chlorpromazine is given for 5 successive days and the treatment is then discontinued, as usual, for 48 h; the shortening of the sleeping-time is even more remarkable.



Variation of pentobarbital induced sleeping-time after a single injection of chlorpromazine
The groups after 36 and 60 h are significantly different (versus controls) at the level of $p < 0.01$

In Table II results are collected concerning the animals pretreated with chlorpromazine under the usual experimental conditions and injected after a 48 h interval, with 'Doriden', 'Viadril', ethyl alcohol, and hexobarbital.

The effect observed with pentobarbital is also evident in the case of the hexobarbital. Neither with 'Doriden' nor 'Viadril' and ethyl alcohol there was any noticeable variation of the sleeping-time observed. It may be deduced that the chlorpromazine pretreatment does not induce an aspecific resistance against narcotic drugs. There is a specific effect against pentobarbital and hexobarbital, consistent with the hypothesis that the chlorpromazine pretreatment is able to modify the metabolic pathways of barbiturates.

Tab. I. Effect of different doses of chlorpromazine pretreatment on the sleeping time of pentobarbital.

Dose (i. p.)	No. of animals	Sleeping-time (min) ± S.E.	% variation	p
I Controls	18	84 ± 3·9	—	—
2·5 mg/kg 1 day	8	78 ± 4·4	− 7	n. s.
5 mg/kg 1 day	18	64 ± 2·4	− 24	$p < 0.001$
10 mg/kg 1 day	18	53 ± 3·8	− 37	$p < 0.001$
15 mg/kg 1 day	18	49 ± 4·5	− 42	$p < 0.001$
II Controls	14	74 ± 5·7		$0.02 >$
2·5 mg/kg 5 days	14	57 ± 3·4	− 23	$p > 0.01$
5 mg/kg 5 days	13	46 ± 4·0	− 37	$p < 0.001$
10 mg/kg 5 days	14	39 ± 4·4	− 46	$p < 0.001$

25 mg/kg pentobarbital were injected i. p. 48 h after the last injection of chlorpromazine.

Tab. III. Influence of pretreatment with chlorpromazine on 'Doriden', 'Viadril', ethylalcohol, hexobarbital narcosis.

Pretreatment	Narcosis	No. of animals	Sleeping-time (min) ± S.E.	p
	Doriden	7	80 ± 8·7	n. s.
Chlorpromazine	Doriden	6	76 ± 6·5	
	Viadril	8	49 ± 9·6	n. s.
Chlorpromazine	Viadril	8	66 ± 9·6	
	Alcohol	7	31 ± 4·8	n. s.
Chlorpromazine	Alcohol	7	46 ± 5·9	
	Hexobarbital	15	69 ± 3·2	$0.01 >$
Chlorpromazine	Hexobarbital	16	53 ± 4·5	$p > 0.001$

'Doriden' (85 mg/kg), 'Viadril' (70 mg/kg), 'ethyl alcohol' (40%, 10 cm³/kg), and 'hexobarbital' (95 mg/kg) were injected intraperitoneally.

[1] S. Garattini, R. Kato, and L. Valzelli, Symposium Bad Nauheim (1959), in press.
[2] H. Remmer, Arch. exp. Path. Pharmak. *233*, 184 (1958).
[3] B. B. Brodie, J. Pharm. Pharmacol. *8*, 1 (1956).
[4] R. Kato, Atti Soc. Lomb. Sci. Med. Biol. *14*, 771 (1959).
[5] H. Remmer, Arch. exp. Path. Pharmak. *237*, 296 (1959).
[6] R. Kato, to be published.
[7] Research fellow from Keio University, Tokyo (Japan).

Tab. III. Influence of pretreatment with chlorpromazine on pentobarbital narcose in adrenalectomized[a] or immature[b] rats.

Treatment	No. of animals	Sleeping-time (min) ± S.E.	% variation	p
I Controls	16	18 ± 3·9	—	—
Adrenalectomized rats	14	35 ± 5·0	—	—
Adrenalectomized rats + Chlorpromazine	12	11 ± 3·2	−68	$0{\cdot}01 > p > 0{\cdot}001$
II Immature rats	14	41 ± 2·9	—	—
Immature rats + Chlorpromazine	14	26 ± 2.9	−37	$p < 0.001$

[a] Female rats weighing 180 g, adrenalectomized 7 days before narcose and treated daily with 1 mg/kg cortisone acetate (s. c.). All rats were kept at 25°C room temperature and received 1% NaCl solution, as drinking water. Chlorpromazine was injected at the dose of 15 mg/kg and pentobarbital at the dose of 20 mg/kg i. p.

[b] Female immature rats (30 days old, average body weight 100 g) are used. The treatment dose of chlorpromazine was 10 mg/kg (i. p.) pentobarbital (22 mg/kg) was injected i. p.

It is well known that the barbiturate breakdown is modified by the cortical[2] and sex[3] hormones. For this reason we studied the late effect of chlorpromazine in adrenalectomized and immature rats. Our results are reported in Table III.

From our data it is possible to conclude that chlorpromazine pretreatment is able to reduce pentobarbital sleeping-time after 48 h also in the adrenalectomized and immature rats.

A possible decrease of barbiturate levels in brain, when the sleeping-time was reduced, was also investigated. In experiments injecting 15 mg/kg of chlorpromazine, and 48 h later, 25 mg/kg of pentobarbital, it was observed that the barbiturate concentration in brain of animals killed 1 h after the administration, was only 60% in comparison with that of the controls[4].

Our results can be explained with a decreased penetration of barbiturates into the brain or with an enhanced breakdown. The second effect is more probable. Recently, REMMER[5] observed an increase of hexobarbital oxidation by the liver microsome enzymes in rats pretreated with barbiturates. On the other hand, in very recent experiments, we were able to observe that chlorpromazine pretreatment can decrease the toxicity of strychnine and picrotoxin and the pharmacological effects of meprobamate and myanesin[6].

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Riassunto

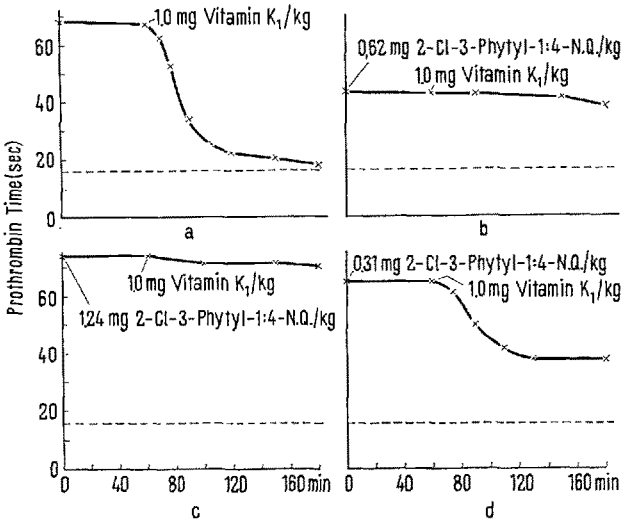
L'autore osserva che un trattamento fatto 48 h prima con cloropromazina induce nei ratti una diminuzione nel tempo di sonno da pentobarbital e da esobarbital, che si accompagna a una diminuzione della concentrazione encefalica dei barbiturici. Il fenomeno non si avvera per il sonno da «Doriden», nè da «Viadril», nè da alcool.

The Inhibition of the Antidotal Activity of Vitamin K_1 against Coumarin Anticoagulant Drugs by its Chloro Analogue[1]

The replacement of a methyl group by a chlorine atom in a biologically active compound may result in an analogue with inhibitory activity. The application of this principle has led to the synthesis of chlorine analogues of riboflavine which are competitive inhibitors[2,3]. Since vitamin K_1, 2-methyl-3-phytyl-1:3-naphthoquinone, contains a methyl group, its chloro analogue, 2-chloro-3-phytyl-1:4-naphthoquinone, has been synthesized and tested for its ability to inhibit the antidotal activity of vitamin K_1 against coumarin anticoagulant drugs.

Rabbits weighing from 2·5 to 3·5 kg were fed Warfarin [3-(α-phenyl-β-acetylethyl)-4-hydroxycoumarin] using a dose of 15 mg/kg of body weight/day for two days. This increased the prothrombin time to approximately 70 sec compared to a value of 15 to 17 sec before treatment. In such animals, the intravenous administration of 1 mg/kg of body weight of vitamin K_1 resulted in a significant reduction of the prothrombin time within 40 to 60 min (Fig. a). When the chloro analogue was administered intravenously 60 min before, 1·24 mg and 0·62 mg/kg completely blocked (Fig. b and c), whereas 0·31 mg/kg significantly reduced the antidotal effect of vitamin K_1 (Fig. d).

The precise site or mode of action of vitamin K is not known. QUICK[4] and ALMQUIST[5] have suggested that certain plasma clotting factors (prothrombin, factors VII, IX, X) are synthesized by an enzyme system of which the vitamin is a coenzyme. Because all compounds with Vitamin K activity are para-quinones, they may function as components of an electron transport system. Coumarin anticoagulaut drugs must interfere with this system by depleting it of the coenzyme, as their effect is reversed readily



a) Vitamin K_1 alone, b) c) d) 1·24 mg, 0·62, 0·31 mg respectively o 2-Chloro-3-Phythl-1:4-Naphthoquinone 60 min before the administration of Vitamin K_1.

[1] This work was supported by a grant from the National Research Council of Canada.

[2] D. W. WOOLLEY, Proc. Soc. exp. Biol. Med., N. Y. *75*, 745 (1950).

[3] R. KUHN, F. WEYGAND, and E. F MOLLER, Ber. dtsch. chem. Ges. *76*, 1044 (1943).

[4] A. J. QUICK and G. E. COLLENTINE, Amer. J. Physiol. *164*, 716 (1951).

[5] H. J. ALMQUIST, Arch. Biochem. Biophys. *35*, 464 (1952).

[6] J. LOWENTHAL, to be published.