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A NEW INSIGHT ON CARBON TETRACHLORIDE EFFECT ON TRIGLYCERIDE TRANSPORT

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Abstract—The administration of CCl_4 is known to induce a rapid increase of triglycerides (TGL) in the liver and a decrease of plasma TGL concentrations, but the mechanism of action of this agent on TGL transport has been not completely clarified. Evidence is now presented that CCl_4 has a direct effect on the liver systems responsible for TGL release into the plasma and that its action is still present without adrenocortical hormones and catecholamines, provided that body temperature is kept at normal levels. The implications of these findings are discussed.

THE administration of CCl_4 in mammals rapidly induces fatty infiltration of the liver, combined with a sharp decrease of plasma lipids and particularly of the triglyceride fraction.[1, 2]

This effect on lipid transport is quite precocious and it may be detected before the biochemical and morphological lesions of liver mitochondria,[3–5] indicating an early and specific impairment of the mechanisms involved in secretion of liver triglycerides into plasma, which include a blockade of liver biosynthesis and/or release of triglyceride-rich lipoproteins.[6, 7]

The reduced liver triglyceride release into plasma, the accumulation of triglycerides in the liver and the blocked synthesis of liver lipoproteins are well demonstrated, but considerable disagreement still exists on CCl_4 mechanism in inducing the impairment of lipid transport across the liver.

According to Brody,[8, 9] the effect of CCl_4 is primarily an indirect one: this agent induces a massive discharge of peripheral catecholamines, liver anoxia and fatty infiltration.[10] The administration of anti-adrenergic drugs[8] or transection of the spinal cord at a level high enough to discontinue liver sympathetic innervation,[11] markedly reduce CCl_4-induced fatty liver. Other investigators, however, disagree with these hypotheses: Heimberg, working with isolated perfused rat liver, has shown a pronounced effect of CCl_4 on liver triglyceride transport, when liver is taken from CCl_4-treated rats, or after injection of minute amounts of this agent in the blood perfusing the isolated liver.[12, 13]

In addition to that, experiments carried out in this laboratory demonstrated that CCl_4 normally inhibits triglyceride release from the liver in rats when the peripheral sympathetic function is blocked,[14] after surgical removal of the adrenal medulla and administration of bretylium-like drugs, in order to induce a 'chemical sympathectomy'.[15] In adrenalectomized rats, however, CCl_4 does not induce the early fatty infiltration of the liver and the morphological lesions of the endoplasmic reticulum are also absent.[5] These findings may be interpreted as an absolute requirement for the

adrenal hormones for triglyceride accumulation in the liver induced by CCl_4 or that, in adrenalectomized animals, CCl_4 cannot exert its toxic activity, for modified environmental conditions.

In favor of this second hypothesis, there is a recent observation by Larson and Plaa[16] confirming that the section of the spinal cord protects rat liver against the lesions induced by administration of CCl_4. However, if the cordotomized animals are kept in a thermostatic room, the effect of CCl_4 is similar to that occurring in normal animals. These morphological data suggest that the protection observed in cordotomized CCl_4-treated animals is related to a fall of body temperature and not necessarily to a blockade of the sympathetic function.

A similar possibility had to be tested in adrenalectomized animals and for this reason experiments with CCl_4 have been carried out, both at room temperature and in a thermostatic chamber. The possible effect of CCl_4 on triglyceride transport in normal and adrenalectomized animals has been investigated using, as an experimental model, rats treated with a surface active agent, Triton W 1339, able to interfere with the uptake of plasma lipoproteins by peripheral tissues.[17, 18]

Triglycerides secreted from the liver to the plasma, as lipoproteins, are therefore retained in plasma compartment and their measurement represents a direct index of the rate of triglyceride secretion by the liver and it gives an easy way to evaluate the effect of CCl_4 on this mechanism.[19]

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 300 $\pm$ 20 g maintained on a standard diet and fasted for 16 hr before the experiment, were used. In some groups of animals, bilateral adrenalectomy was performed 72 hr before the experiment and animals kept on 1% saline.

Corticosterone (Mann Research Laboratories, New York, U.S.A.) suspended in mineral oil, was administered s.c. at the dosage of 5 mg/kg in two subsequent daily doses, and at 10 mg/kg in a final dose at the same time as CCl_4.

Carbon tetrachloride (Merck Company, Germany) was dissolved in mineral oil (1 : 1, v/v) and administered in a single dose, 5 ml/kg, by gastric intubation. Control animals received 2·5 ml/kg of mineral oil.

Triton WR 1339 (a *p*-iso-octyl polyoxyethylene phenol polymer, supplied by Winthrop Labs., New York, U.S.A.) was dissolved in saline and administered i.v. at the dose of 200 mg/kg. Controls received the same amount of saline.

Some groups of normal and adrenalectomized rats were kept in a thermostatic room and maintained at constant physiological body temperature for the duration of the experiment. Body temperature (rectal) was measured using a thermocouple with a probe for small animals.

All the animals were killed by decapitation, blood collected in heparinized tubes and centrifuged and livers rapidly excised and weighed. Plasma and liver lipids were extracted and triglycerides separated on Florisil columns, as described by Blankenhorn[20] and measured according to Van Handel and Zilversmit.[21]

RESULTS AND DISCUSSION

The effect of carbon tetrachloride in Triton-treated normal rats is quite evident. As shown by Recknagel *et al.*[19] the increased level of plasma triglycerides induced by

Triton is completely blocked by administration of carbon tetrachloride. A completely different picture, however, is evident when adrenalectomized rats are treated with Triton and CCl_4. In these conditions the effect of Triton on plasma triglycerides is not affected at all by CCl_4 and at the same time there is no CCl_4-induced fatty liver (Table 1), which is in agreement with our previous findings.[5, 14] These results

TABLE 1. EFFECT OF CCl_4 IN NORMAL AND ADRENALECTOMIZED TRITON-TREATED RATS

Group	Treatment	No. of animals	Liver triglycerides (mg/g ± S.E.)	Plasma triglycerides (mg/100 ml ± S.E.)
I	None	10	3·83 ± 0·27	31·60 ± 2·1
II	CCl_4	10	11·45 ± 0·84	18·30 ± 0·7
III	Triton	10	4·07 ± 0·24	259·00 ± 11·0
IV	Triton + CCl_4	10	13·48 ± 0·65	52·80 ± 4·3
V	Adrenalectomy	14	3·53 ± 0·30	30·80 ± 2·4
VI	Adrenalectomy + CCl_4	14	3·64 ± 0·30	30·50 ± 0·6
VII	Adrenalectomy + Triton	14	3·87 ± 0·20	92·30 ± 6·8
VIII	Adrenalectomy + Triton + CCl_4	14	4·14 ± 0·18	86·70 ± 6·8
Significance levels:		I–II	$p < 0·01$	$p < 0·01$
		III–IV	$p < 0·01$	$p < 0·001$
		V–VI	p n.s.	p n.s.
		VII–VIII	p n.s.	p n.s.

CCl_4 (2·5 ml/kg) given by gastric intubation 4 hr and Triton WR 1339 (200 mg/kg i.v.) 2 hr before the death of the animal.

may indicate that CCl_4 is completely inactive in triglyceride transport in absence of the adrenal hormones. It is therefore interesting to investigate if the administration of corticosterone may antagonize the protection of the adrenalectomy against the CCl_4 effects on triglyceride transport. This is the case, as shown in Table 2. In corticosterone-

TABLE 2. EFFECT of CCl_4 IN ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS

Group	Treatment	No. of animals	Liver triglycerides (mg/g ± S.E.)	Plasma triglycerides (mg/100 ml ± S.E.)
I	Corticosterone	10	3·83 ± 0·38	33·00 ± 2·6
II	Corticosterone + CCl_4	10	11·34 ± 0·94	22·00 ± 2·0
III	Corticosterone + Triton	10	3·71 ± 0·09	84·6 ± 5·1
IV	Corticosterone + Triton + CCl_4	10	12·07 ± 0·79	38·5 ± 6·4
Significance levels:		I–II	$p < 0·01$	$0·02 > p > 0·01$
		III–IV	$p < 0·01$	$p < 0·01$

CCl_4 (2·5 ml/kg) by gastric intubation 4 hr and Triton WR 1339 (200 mg/kg i.v.) 2 hr before the death of the animals.
Corticosterone in mineral oil, 5 mg/kg s.c. for 2 days; 10 mg/kg s.c. at the same time as CCl_4.

treated adrenalectomized rats, the treatment with Triton induces an important increase of plasma triglycerides, which is completely blocked by administration of CCl_4 The data reported in Tables 1 and 2 suggest an important role for the adrenocortical hormones in the mechanism of action of CCl_4, but it was decided, at this point, to investigate if in adrenalectomized animals treated with CCl_4 the general metabolic patterns were impaired and if other changes had some significance with the lack of

effect of CCl_4. For this reason it was decided to measure body temperature in normal and in adrenalectomized rats treated with CCl_4, corticosterone or both agents (Table 3). Our results clearly show that the administration of a large single dose of CCl_4 by gastric intubation has a significant effect in decreasing body temperature in normal animals and one much more pronounced in adrenalectomized rats, where a decrease of about 6° takes place in the 4 hr following CCl_4 administration. When the adrenalectomized animals are pretreated with corticosterone, there is no significant decrease of body temperature, after CCl_4 administration.

TABLE 3. EFFECT OF CCl_4 ON BODY TEMPERATURE IN NORMAL AND ADRENALECTOMIZED ANIMALS

Group	Pretreatment	No. of animals	Treatment	Body temperature (°C ± S.E.)	
				Initial	After 4 hr
I	None	10	None	36·8 ± 0·28	37·0 ± 0·21
II	None	10	CCl_4	37·0 ± 0·19	35·6 ± 0·43
III	Adrenalectomy	14	None	36·1 ± 0·58	35·5 ± 0·25
IV	Adrenalectomy	14	CCl_4	35·8 ± 0·58	29·9 ± 1·46
V	Adrenalectomy	14	Corticosterone	37·8 ± 0·15	36·8 ± 0·40
VI	Adrenalectomy	14	Corticosterone + CCl_4	36·8 ± 0·21	34·7 ± 0·29
Final temperature, significance levels:				I–II	$p < 0·01$
				III–IV	$p < 0·01$
				V–VI	$p < 0·01$

Corticosterone in mineral oil 5 mg/kg s.c. for 2 days and 10 mg/kg s.c. at the same time as CCl_4. CCl_4 (2·5 ml/kg) by gastric intubation 4 hr before the death of the animals.

These results strongly suggest that CCl_4 is inactive on triglycerides transport in adrenalectomized animals, because of lowered body temperature in agreement with the observation in cordotomized rats reported by Larson and Plaa.[16] In order to test this possibility a series of experiments was designed using adrenalectomized animals kept at constant body temperature for the duration of the experiment. In these animals the effect of CCl_4 on hypertriglyceridemia induced by Triton has been measured in conditions identical to those of the experiment reported in Table 2. The results are quite univocal. When body temperature is normal, CCl_4 is normally active also in adrenalectomized animals and Triton hyperlipemia is blocked almost completely (Table 4).

Our data give a direct demonstration that CCl_4 is active directly on the liver mechanisms responsible for the release of liver triglycerides into the plasma. The presence of adrenal hormones is only important because it abolishes the fall in body temperature induced by CCl_4 administration in adrenalectomized animals and it is non-essential when animal body temperature is kept normal with physical means, for instance in a thermostatic room.

These findings are in agreement with the recent observations of Heimberg[13] that CCl_4 is active on lipid transport in the isolated perfused liver kept at 37°.

New suggestions for the possible prevention of the toxic effects of CCl_4 arise from these studies. The observation that a complete protection against CCl_4 effects on liver and plasma lipids and on liver morphological structure may be obtained by reducing

TABLE 4. EFFECT OF CCl_4 IN TRITON-TREATED RATS KEPT AT CONTROLLED BODY TEMPERATURE

Group	Treatment	No. of animals	Body temperature (°C ± S.E.)		Liver triglycerides (mg/g ± S.E.)	Plasma triglycerides (mg/100 ml ± S.E.)
			Initial	4 hr after CCl_4		
I	Triton	6	36·3 ± 0·1	36·8 ± 0·3	5·77 ± 0·38	176·0 ± 12·1
II	Triton + CCl_4	6	36·4 ± 0·1	37·0 ± 0·4	12·13 ± 0·78	52·8 ± 4·3
III	Adrenalectomy	6	36·1 ± 0·1	36·4 ± 0·3	3·07 ± 0·30	31·2 ± 2·8
IV	Adrenalectomy + CCl_4	6	36·1 ± 0·1	36·6 ± 0·4	5·07 ± 0·64	23·3 ± 1·2
V	Adrenalectomy + Triton	9	35·8 ± 0·05	37·1 ± 0·2	2·76 ± 0·27	115·7 ± 6·4
VI	Adrenalectomy + Triton + CCl_4	9	36·1 ± 0·10	36·2 ± 0·3	6·44 ± 0·57	50·1 ± 7·1
Significance levels:				I–II	$p < 0·01$	$p < 0·001$
				III–IV	$0·05 > p > 0·02$	$0·02 > p > 0·01$
				V–VI	$p < 0·001$	$p < 0·001$

Rats kept from 2 hr before CCl_4 administration at controlled body temperature.
CCl_4 (2·5 ml/kg) by gastric intubation 4 hr and Triton WR 1339 (200 mg/kg i.v.) 2 hr before the death of the animals.

body temperature seems particularly promising and further investigations along this line may show if this is a practical way of treatment for experimental animals and patients, intoxicated with carbon tetrachloride.

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