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Influence of Sex Hormones upon Disturbances Caused by Ethanol on Mitochondrial Oxidations in Rat

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Previously we observed that prolonged alcohol treatment of rats strongly lowered the ability of the liver mitochondria from males, but not from females, to oxidize succinate. The present work deals with attempts to abolish the inhibition in the male rats and to develop it in the females by changing the levels of sex hormones. Thus male rats were castrated and female rats were either impregnated or injected with progesterone.

In male rats castration nearly completely abolished the ethanol inactivation of the succinate oxidation.

In female rats neither the pregnant state nor the injected progesterone contributed towards the development of an ethanol impairment of the succinate oxidation analogous to that in ethanol treated male rats.

Rats given ethanol instead of water for a long time produce liver mitochondria with certain abnormal qualities as to their function[1,2] and appearance.[3] Among others their capacity to oxidize pyruvate and glutamate is reduced in both sexes,[1,2] whereas the oxidation rate of succinate is strongly decreased in male, but hardly at all in female rats.[2]

As to the succinate oxidation this may indicate a participation of sex hormones in the development of the abnormality in the male rat.

This paper contains results from experiments with ethanol on castrated male rats and on female rats during pregnancy or with a frequent high level of progesterone caused by injections.

EXPERIMENTAL

At the age of 6 months 24 male rats were castrated. When recovered from the operation, they were divided into two groups, one getting exclusively a 15 % (v/v) ethanol solution to drink, and the other water. Twelve rats forming a third group also got the same ethanol solution, but were not castrated. The litters used were equally distributed

into the three experimental groups. In addition to water or ethanol for drinking they had free access to adequate solid food.

After another 5—7 months the animals were sacrificed, mitochondria prepared from liver and brain, and the pyruvate and succinate oxidation determined in the same way as previously described.[2]

In the other types of experiments 75 days old female rats were used. Irrespective of whether they belonged to the pregnancy or the progesteron injection experiments, they were distributed into three groups according to the same principle regarding litter and alcohol treatment as for the males. In the first type of experiment the rats belonging to the alcohol groups were given ethanol for 40 days after which the animals of one alcohol drinking group and of the water drinking one were impregnated whereas those belonging to the second alcohol-drinking group were kept nonimpregnated. After another 18 days the mitochondrial activities were studied in the same way as described for male rats. In the progesterone injection experiments the females of two groups, *viz.* the one given water and one of the alcohol drinking groups, were, instead of being impregnated, injected with 7—8 mg progesterone, while the second alcohol group was injected with the solvent not containing progesterone. This was repeated three times a week for four to five weeks, after which the animals were treated in the same way as in the other experiments.

The progesterone was a commercial preparation from Pharmacia, Uppsala Sweden (Progestin 25 mg/ml solution for intramuscular injection. The solvent consisted of peanut oil).

RESULTS

Does castration prevent a decrease in succinate oxidation caused by ethanol? The answer is found in Table 1. In the liver mitochondria from the non-castrated alcohol drinking animals the oxidation of succinate is obviously low compared with the water drinking castrated rats. In the castrated animals, however, this decrease after alcohol consumption does not occur.

Although the succinate oxidation in the castrated rats used in these experiments (mean value 37.6 μat. O_2/60 min/10 mg protein) is slower than in non-

Table 1. Oxidation of succinate in liver mitochondria from male rats pretreated by castration and with ethanol. The figures are mean values of ten experiments. The oxygen consumptions have been compared by means of t-test. p-Values are given in the lower part of the table, the types of animals compared being given within brackets. In the head of the table μat. O_2 indicates mitochondrial oxygen consumption expressed as μat. O_2 consumed per 60 min per 10 mg mitochondrial protein, respiration control (resp. contr.) means the ratio between oxygen consumption in the presence and the absence of hexokinase and glucose and P/O oxidative phosphorylation expressed as the ratio of number of micromoles of esterified phosphate to the number of microatoms of oxygen consumed.

Pretreatment of the rats	μat.O_2	Resp. contr.	P/O
Castrated	37.6 ± 1.9	4.6	1.8
Alcohol	25.9 ± 1.1	4.4	1.7
Castrated + alcohol	35.0 ± 1.8	5.2	1.6

p (castrated/castrated + alcohol) > 0.3
p (castrated/alcohol) < 0.001
p (castrated + alcohol/alcohol) = 0.001

castrated, previously studied rats (mean value about 50 μat. O_2), the results in Table 1 show that castration almost completely eliminates the ethanol decreasing effect.

The effect of combined ethanol and progesterone treatment of female rats on their mitochondrial respiration. Experience from experiments with rats consuming ethanol instead of water has shown that three months is the minimum time to bring about measurable effects of the ethanol on liver mitochondrial oxidation of pyruvate and glutamate in males and females, and of succinate in males.

The sex difference with regard to the sensitivity of the succinate oxidation system towards prolonged alcohol treatment of the rats [2] and the abolishment of this effect by castration (Table 1) opened the question, whether or not mitochondrial functions in female rats could be influenced more rapidly by ethanol during periods with high, but still physiological doses of progesterone.

In order to elucidate this question female rats were treated as described in "Experimental", *i.e.* the alcohol drinking groups were kept on ethanol two weeks before treatment with progesterone during which time their daily alcohol consumption reached a steady value.

After four to five weeks on progesterone parallel with the previously started ethanol treatment the rats were sacrificed, and the muscle, liver and brain mitochondrial oxidations of pyruvate and succinate studied (Table 2).

The figures in the Table show that progesterone administered to the rats during simultaneous alcohol treatment did not impair the mitochondrial respiration rate in any of the three types of tissue in comparison with those from animals treated with progesterone or ethanol alone. On the contrary there was a 40 % stimulation of the pyruvate oxidation in muscle mitochondria ($p < 0.1$).

Table 2. Oxidation of pyruvate and succinate by mitochondria from female rats pretreated with ethanol and progesterone. Muscle, liver, and brain mitochondria have been isolated according to Chappell and Perry,[4] Schneider and Hogeboom,[5] and Brody and Bain,[6] respectively. The figures are mean values of eight experiments and standard errors. For definition of the headings see Table 1.

Tissue and substrate	Treatment of the rats					
	Progesterone		Alcohol		Progesterone + alcohol	
	μat. O_2	resp. contr.	μat. O_2	resp. contr.	μat. O_2	resp. contr.
Muscle:						
puruvate	26.5 ± 2.0	2.8	26.9 ± 2.5	2.6	36.6 ± 3.8	2.6
Liver:						
pyruvate	27.7 ± 1.8	5.8	26.7 ± 2.0	4.7	26.2 ± 2.0	5.6
succinate	39.8 ± 1.3	4.1	36.3 ± 2.7	3.6	38.6 ± 2.3	4.2
Brain:						
pyruvate	30.6 ± 2.6	3.5	29.7 ± 1.7	3.3	34.0 ± 2.6	3.7
succinate	24.1 ± 1.9	2.3	25.0 ± 1.3	2.1	24.0 ± 1.5	2.1

Table 3. Oxidation of pyruvate and succinate in liver mitochondria from pregnant rats drinking alcohol. The figures are mean values of eight experiments and standard errors. For statistical treatment see Table 1. *Alcohol* means that the rats had been given ethanol (15 % v/v) to drink instead of water, but were not being impregnated. *Pregnant* that they have had water for drinking during pregnancy, and were killed 18—20 days after impregnation, and *alcohol + pregnant* that they were pregnant and treated like the last mentioned group, but had ethanol to drink instead of water.

Substrate	Treatment of the rats					
	Alcohol		Pregnant		Alcohol + pregnant	
	μat. O_2	resp. contr.	μat. O_2	resp. contr.	μat. O_2	resp. contr.
Pyruvate	23.9 ± 1.9	6.7	19.4 ± 1.1	5.1	20.7 ± 1.1	5.0
	p(pregnant/alcohol + pregnant) > 0.4					
Succinate	37.5 ± 3.0	3.6	42.3 ± 3.9	4.0	38.2 ± 2.7	3.9
	p(pregnant/alcohol + pregnant) > 0.4					

The effect of ethanol treatment on mitochondrial respiration in pregnant rats. The intention with these experiments was to find out, whether or not a period with increased production of female sex hormones should more rapidly evoke the ethanol effect on the reduced mitochondrial oxidation capacity otherwise typical for a long period of alcohol treatment. As was the case in experiments with progesterone also these animals were given ethanol for such a short time (40 days before impregnation and 18 to 20 days after) that no visible effect on mitochondrial respiration, resembling those after 6—12 months on alcohol [1,2] should have appeared.

Results, given in Table 3 do, however, not suggest that ethanol should produce these effects more rapidly during pregnancy than normally.

DISCUSSION

In the present investigation attempts are made to find out, whether or not alterations in the content of sex hormones in rats influence the time necessary for the evocation of metabolic disturbances by ethanol. Behind these attempts lies the previous observation [2] that ethanol, given to rats during a long time gives rise to a decrease in the oxidation rate of succinate in liver mitochondria from males, but not from females.

Thus a decrease in the level of sex hormones in male rats was brought about by castration. In females, not showing any remarkable change in the succinate oxidation during alcohol treatment,[2] a change in sex hormones had to be produced. In the present investigation we chose to increase the concentration of progesterone by allowing the rats to become pregnant or by administrating progesterone.

The results in Table 1 strongly support the conclusion that removal of testes, and thereby testosterone, at the same time removes or delays the inhibiting effect of ethanol on the succinate oxidation system. Increase of sex hormones in the females, however, does not, or at a rate below possibility of detection in the present type of experiments, accelerate the effect of ethanol on the mitochondrial respiration capacity (Tables 2 and 3).

An attempt to explain the effect of ethanol on the mitochondrial respiration by the sex difference is of course very hazardous. The mechanism by which steroid hormones exert their influence on particular enzymes or enzyme systems is still very little known.

A report by Maxwell and Topper [7] may, however, have some bearing on our observations. They found that a partially purified diphosphopyridine nucleotide-linked aldehyde dehydrogenase from rabbit liver was obviously inhibited by 10^{-5} M testosterone and progesterone. How this inhibition may retroact on the succinate oxidazing system is only a matter for speculation. The previous observation, *viz.* that acetaldehyde is an inhibitor of the pyruvate oxidation [8] might be of importance. According to Haslam and Krebs [9] between 15 and 40 % of the pyruvate in liver is transformed into dicarboxylic acids, among others oxaloacetic acid. Oxaloacetic acid is a strong inhibitor of the succinic dehydrogenase.

Thus a continuous production of the actual sex hormone may exert an inhibition of the aldehyde dehydrogenase which, normally, is of less importance, but with a steady formation of acetaldehyde from ethanol may give rise to an increased aldehyde level. As a consequence more pyruvate will be transformed to oxaloacetic acid, and less will be left for oxidative decarboxylation and further succinate formation. One or both of these factors may ultimately lead to an enzyme adaptation.

This is pure speculation, but may serve as a working hypothesis. No explanation is, however, available for the lacking effect, when attempts were made to increase the level of progesterone in the female body unless that the time of treatment might have been too short.

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