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Diabetes alters drug metabolism – in vivo studies in a streptozotozin-diabetic rat model

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Summary. The influence of experimental streptozotozin-induced diabetes on hepatic drug metabolism in vivo has been studied in rats, using $^{14}CO_2$-exhalation after ^{14}C-aminopyrine injection. Male diabetic rats showed a decreased (−18%), females an increased (+19%) $^{14}CO_2$-exhalation compared to controls, indicating altered hepatic drug metabolism due to diabetes.
Key words. Aminopyrine breath test; diabetes; in vivo; NADPH; phenobarbital induction; streptozotozin.

Several publications have demonstrated altered oxidative drug metabolism in vitro in experimentally induced diabetes. These studies involved microsomes[1–6], isolated hepatocytes[7,8] and extrahepatic tissue[9–11]. The extent of the diabetic alteration in drug metabolism was dependent on sex and substrate[1,3,7,12]. The relevance of these in vitro studies for the in vivo situation, however, is ill-defined and controversial. Some workers have suggested a good correlation between in vitro and in vivo studies[13], whereas others found discrepancies between findings in microsomes and isolated hepatocytes[7]. In order to evaluate the influence of diabetes on drug metabolism in the intact animal, the oxidative demethylation of aminopyrine, labeled with ^{14}C at its two methyl groups, was measured. In this model, the cleaved methyl groups are metabolized to $^{14}CO_2$ and eventually exhaled in the breath. After trapping the CO_2 in ethanolamine, the radioactivity is counted[14]. This 'breath test' has been shown to be a good indicator of altered liver function in experimental animals and in man[14–16]. Studies were performed, therefore, in male and female control and diabetic rats. In order to get some insight into possible mechanisms of the effect of diabetes on hepatic aminopyrine demethylation, similar studies were also performed in rats whose drug metabolizing enzymes, the cytochrome P-450, had been induced by phenobarbital administration. The results showed alterations in aminopyrine demethylation in vivo qualitatively similar to the ones previously obtained in vitro.

Material and methods. Male and female Sprague-Dawley rats (150–210 g b.wt) were used. Diabetes was induced by i.v.-injection of streptozotozin (Sigma, St. Louis, USA), 75 mg/kg freshly dissolved in 0.3 ml of citric buffer pH 4.5[1]. Control animals received buffer alone. Only diabetic animals exhibiting urine glucose concentrations > 100 mmol/l and blood glucose levels > 30 mmol/l at the time of the experiments were used. Ketonuria could not be detected (Keto-Diastix, Ames, GB). All diabetic rats exhibited polydipsia, growth retardation, and increased liver to body weight ratio. These parameters indicating severity of diabetes were not different in males and females. A group of rats received phenobarbital-Na 80 mg/kg i.p. on days 7, 8 and 9 after streptozotozin. Experiments were performed on day 10 after induction of diabetes.

Aminopyrine breath test: (Dimethylamine-^{14}C)-aminopyrine (Amersham, Buckinghamshire, GB), specific activity 120 mCi/mmol, was used. The dose was 1 µCi/kg i.v., dissolved in 3 ml NaCl. To prevent adsorption to the tubing, 0.2 mg/ml of unlabeled aminopyrine was added. The breath test was performed as described earlier[14] by quantitatively collecting exhaled CO_2 in 10-min.-portions, in the unanesthetized animal.

Calculations. Since it has been demonstrated that peak exhalation rate as well as the $^{14}CO_2$ elimination rate constant in breath correlate well with in vitro aminopyrine demethylase activity[15], these two parameters were chosen to characterize the amino-

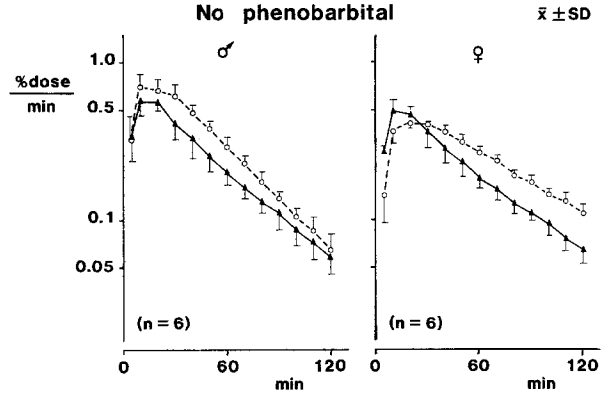

Figure 1. $^{14}CO_2$ exhalation after i.v. injection of a tracer dose of ^{14}C-aminopyrine (1 µCi/kg b.wt) in control and streptozotozin-diabetic rats not receiving phenobarbital. Diabetes decreased peak exhalation rate in male rats by 18%, whereas in females, there was an increase by 19%.
○---○ Controls; ▲———▲ Diabetics.

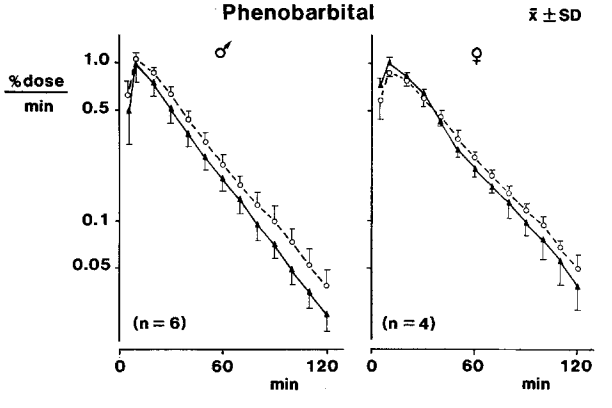

Figure 2. $^{14}CO_2$ exhalation after i.v. injection of a tracer dose of ^{14}C-aminopyrine (1 µCi/kg b.wt) in phenobarbital treated rats. Diabetes altered peak exhalation rate in a similar direction as in non-induced animals.
○---○ Controls; ▲———▲ Diabetics.

Peak exhalation rate and $^{14}CO_2$-elimination rate constant of not phenobarbital-treated and phenobarbital-treated rats

	No phenobarbital				Phenobarbital			
	Male		Female		Male		Female	
	Controls (n = 6)	Diabetics (n = 6)	Controls (n = 6)	Diabetics (n = 6)	Controls (n = 6)	Diabetics (n = 6)	Controls (n = 4)	Diabetics (n = 4)
Peak exhalation rate (% dose·min^{-1})	0.74 ± 0.11	0.61 ± 0.06	0.42 ± 0.02	0.50 ± 0.08	1.04 ± 0.10	0.98 ± 0.21	0.85 ± 0.03	1.03 ± 0.11
p (compared to controls)	< 0.05		< 0.05		n.s.		< 0.05	
p (sex difference)			< 0.001	< 0.025			< 0.01	n.s.
$^{14}CO_2$ elimination rate constant (h^{-1})	1.5 ± 0.2	1.3 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.1	1.6 ± 0.1	1.9 ± 0.3
p (compared to controls)	n.s.		n.s.		n.s.		n.s.	
p (sex difference)			< 0.001	n.s.			< 0.025	n.s.

pyrine breath test. Peak exhalation rate was defined as the fraction with the highest output of $^{14}CO_2$, expressed as a percentage of the dose exhaled as $^{14}CO_2$ per minute (% dose/min). The $^{14}CO_2$ elimination rate constant was calculated by log-linear regression analysis. Differences were calculated by Student's t-test, $p < 0.05$ being considered as statistically significant.

Results. In control rats not receiving phenobarbital, the peak exhalation rate as well as the $^{14}CO_2$ elimination rate constant showed a strong sex dependency, male rats exhibiting about 70% higher values than females (fig. 1). A statistically significant reduction of peak exhalation rate was observed in diabetic males, whereas in female rats, peak exhalation rate was significantly increased (fig. 1). The $^{14}CO_2$ elimination rate constant showed a tendency to decrease in male and to increase in female diabetic animals without the changes reaching statistical significance in either sex. Phenobarbital induction caused the expected increase in peak exhalation rate and $^{14}CO_2$ elimination rate constant, but it decreased the sex difference since the females showed a much greater inducibility than the males (fig. 2). Phenobarbital increased peak exhalation rate to a similar degree percentagewise in both control and diabetic animals (fig. 2). A summary of the data is provided in the table.

Discussion. This study shows for the first time to our knowledge an influence of diabetes on the aminopyrine breath test in vivo. The observed changes in diabetic animals, as well as the sex differences, are qualitatively similar to the alterations reported in vitro[1,3]. Diabetes increased in vivo demethylation rate in females (represented by an increased peak exhalation rate), whereas in males, the opposite effect was observed. A similar sex difference has been reported for aminopyrine and hexobarbital metabolism in vitro. It seems to be unique for the rat and can be eliminated by castration of male rats[12].

The observed influence of diabetes on the aminopyrine breath test was relatively small, compared to published in vitro results. This may be related to the experimental conditions; in the present study, a tracer dose of aminopyrine was used, whereas in vitro, aminopyrine demethylase capacity is usually determined under saturating conditions. It may also be influenced by the fact that the in vitro demethylase assay measures only formation of the initial demethylase product, formaldehyde, whereas in vivo, formaldehyde undergoes two further oxidative steps to formic and carbonic acid, before being exhaled as CO_2. It has been shown that factors like physical exercise may profoundly affect the kinetics of the C-1 fragments[17]. Although data are not available for rats, in man only a relatively small portion (15–55%) of the demethylated C-1-label appears eventually in the breath as $^{14}CO_2$[18,19]. It is conceivable, therefore, that a multifactorial metabolic disorder like diabetes may alter the kinetics of the C-1-fragments, thus masking any possible changes in the initial demethylation reaction.

Altered composition and content of cytochrome P-450[3,20–22], as well as decreased hepatic levels of NADPH[7,23,24] have been proposed as a cause of metabolic alterations in the intact diabetic animal. In vitro, NADPH is supplied from exogenous sources at saturating concentrations. If a deficiency of NADPH in this diabetic model is of significance, it should be more accentuated by increasing the demand for NADPH. This may be achieved by inducing the mono-oxygenase system by phenobarbital[25]. Any limitation in NADPH supply should result in a lower inducibility of the aminopyrine breath test in diabetic animals, compared to non-diabetics. Our results, however, do not support this hypothesis, since diabetic rats showed the same inducibility by phenobarbital as non-diabetic animals: In both groups, phenobarbital increased the peak exhalation rate and $^{14}CO_2$-elimination rate to a similar degree. It appears, therefore, that at least in the rat, differences in cytochrome P-450 content and composition are more important for demethylase activity than a limitation in NADPH supply.

In conclusion, our study using ^{14}C-aminopyrine as model compound has shown that experimental diabetes in the rat alters oxidative drug metabolism in vivo. Further studies in diabetic patients are needed to evaluate the relevance of our results for man.

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Serotonin in the human infant carotid body

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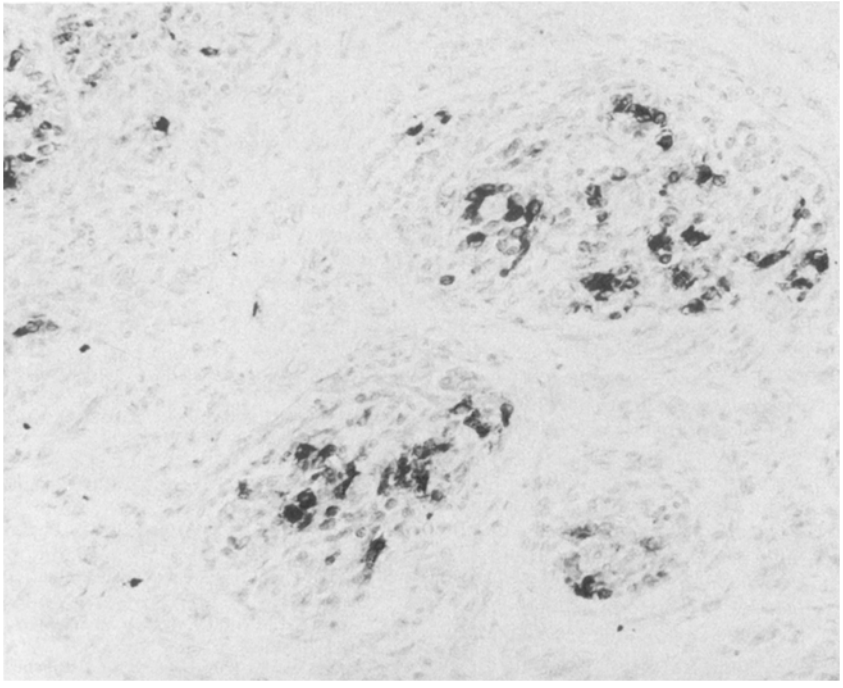
Summary. By immunocytochemistry serotonin was localized in the chief cells of the carotid body in human infants. Radioenzymatic measurement of the serotonin concentration revealed that it represents a significant proportion of the total amine content of the carotid body.
Key words. Carotid body; serotonin; human infant.

The carotid bodies (CB) are principal peripheral chemoreceptors involved in respiratory responses to hypoxia, hypercapnea and acidosis[1]. The morphology and physiology of CB has been reviewed previously[1-7]. The principal cells of CB are glomus or chief cells surrounded by sustentacular cells. The chief cells, considered to be the chemosensory element of CB, contain biogenic amines and peptides stored within the cytoplasmic dense core vesicles[8-16]. Dopamine and norepinephrine are thought to be the predominant amines in the CB of most animal species[8-14]. Although serotonin has been detected in CB of some species, including human, its concentration has been reported to be low compared to the other amines[15,16]. The content and cellular localization of serotonin in the human CB has received only limited study[16]. To our knowledge serotonin levels in the infant CB have not been reported. In this study, we report on the localization and measurement of serotonin in CB of normal infants using immunocytochemistry and a highly specific and sensitive radioenzymatic assay for serotonin.

Materials and methods. The CB were obtained from eight infants at autopsy 11–48 h after death (21.5 ± 4.1 h; mean ± standard error of mean). The infants died as a result of accidents or due to acute illnesses, and either were dead on arrival (DOA) at hospital or were treated for a short time prior to death. The age of infants ranged from 1–11 months (4.9 ± 1.3 months) and included five females and three males. In three patients the cause of death was acute meningitis (one of them DOA), two died from gastroenteritis (one of them DOA), and one each died from cold exposure, cardiac tamponade and a brain tumor.



Immunoperoxidase staining for serotonin in the carotid body of a 3-month-old infant who died from cold exposure. Note that both single and clusters of chief cells within the glomic area stain positively. The surrounding stroma is negative. × 75.