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# SHORT COMMUNICATIONS

## Effect of carbohydrate intake on phenobarbital-, polychlorinated biphenyl- and 3-methylcholanthrene-induced enhancement of drug oxidation in rat liver

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We have recently reported that when ethanol is administered to rats in combination with a low-carbohydrate (low-CHO) diet, the latter highly augments the ethanol-induced enhancement of hepatic monooxygenase activities for variety of volatile hydrocarbons [1]. Phenobarbital (PB), polychlorinated biphenyl (PCB) and 3-methylcholanthrene (MC) are well known inducers of these enzymes, each stimulating *de novo* synthesis of different forms of cytochrome P-450 [2–4]. It is therefore an interesting question, by analogy with the case of ethanol, whether lowered CHO intake also affects the enzyme induction due to these enzyme inducers. In attempting to answer the question, rats maintained on various diets each containing different amounts of carbohydrate were treated with PB, PCB or MC, and the activity of hepatic monooxygenase for eight volatile hydrocarbons was assessed.

### *Materials and methods*

*Animals and diet.* Male Wistar rats, purchased from Shizuoka Laboratory Animal Center, Japan, were used throughout. All animals were fed pellet food (Nippon Clea CE-2) and water *ad libitum* until they reached twelve weeks of age. The rats were then switched to a nutritionally adequate liquid diet (basal diet), the composition of which was reported elsewhere [5]. Rats were fed the basal diet (80 ml, 1 kcal/ml) daily at 4 p.m. After 2 weeks on the diet, they were allotted to four test diets, which differed from each other only in the amount of CHO independently of the others (1.74, 3.50, 9.72 and 13.90 g sucrose in 80 ml of the liquid diet, respectively), from the day before PB-, PCB- or MC-treatment to the day prior to sacrifice. The total caloric intake a day hence varied according to the CHO content (Table 1). Rats were administered PB (80 mg/kg per day) for 3 days, p.o., MC (20 mg/kg per day) for 3 days, p.o., or PCB (a single dose, 500 mg/kg, of tetrachlorinated biphenyl, purchased from Wako Chemical Industrials, Osaka, Japan), i.p. Every treatment was performed at 10 a.m.

*Monooxygenase activity.* Rats were killed by decapitation at 10 a.m. on the fifth day after starting of PB, PCB or MC treatment. A 10% (w/v) crude homogenate of the liver was prepared with a 1.15% KCl–0.01 M phosphate buffer, pH 7.4, in a glass homogenizer. It was then homogenized at 10,000 *g* for 10 min at 0°. One fifth (liver of PB-treated rats for styrene), one-half (liver of PB- and PCB-treated rats for toluene and styrene) or 1 ml (the others) of the supernatant fraction was used as the enzyme source. The enzyme activity was assessed by measuring the disappearance rate of substrate acccording to the method of Sato and Nakajima [6].

Table 1. Microsomal protein and cytochrome P-450 contents in liver after PB, PCB and MC treatments

| Carbohydrate intake (g/day) | Caloric intake (kcal/day) | | Liver wt (g) | Liver/body (%) | Microsomal protein (mg/g liver) | Cytochrome P-450 (nmole/mg protein) |
|---|---|---|---|---|---|---|
| A 1.74 | 47.3 | Control | 8.6 ± 0.3 | 2.95 ± 0.09 | 23.2 ± 1.8 | 0.85 ± 0.06 |
| | | PB | 11.1 ± 0.6† | 3.88 ± 0.27† | 30.6 ± 2.2† | 2.10 ± 0.16† |
| | | PCB | 15.2 ± 0.6† | 4.88 ± 0.09† | 31.2 ± 1.0† | 2.45 ± 0.39† |
| | | MC | 9.5 ± 0.7 | 3.29 ± 0.18† | 23.0 ± 1.4 | 1.21 ± 0.08† |
| B 3.50 | 54.5 | Control | 9.6 ± 0.3 | 3.14 ± 0.04 | 23.2 ± 1.2 | 0.82 ± 0.12 |
| | | PB | 11.2 ± 0.5† | 3.93 ± 0.13† | 30.5 ± 1.2† | 1.77 ± 0.20† |
| | | PCB | 14.9 ± 1.1† | 4.87 ± 0.38† | 32.0 ± 2.8† | 2.40 ± 0.12† |
| | | MC | 10.4 ± 0.7 | 3.49 ± 0.08† | 23.5 ± 0.9 | 1.14 ± 0.03† |
| C* 9.72 | 80.0 | Control | 11.7 ± 0.8 | 3.62 ± 0.19 | 22.3 ± 1.8 | 0.81 ± 0.04 |
| | | PB | 13.5 ± 0.3† | 4.37 ± 0.16† | 27.9 ± 2.2† | 1.50 ± 0.13† |
| | | PCB | 16.8 ± 1.0† | 5.17 ± 0.17† | 27.5 ± 0.7† | 1.73 ± 0.15† |
| | | MC | 12.2 ± 0.2 | 4.03 ± 0.05† | 22.7 ± 2.5 | 0.99 ± 0.04† |
| D 13.90 | 97.1 | Control | 13.0 ± 0.7 | 3.95 ± 0.08 | 20.2 ± 2.7 | 0.79 ± 0.09 |
| | | PB | 14.1 ± 2.8 | 4.75 ± 0.25† | 24.4 ± 0.1† | 1.37 ± 0.14† |
| | | PCB | 16.1 ± 0.6† | 4.93 ± 0.19† | 25.5 ± 1.7† | 1.57 ± 0.41† |
| | | MC | 12.2 ± 0.6 | 3.99 ± 0.09 | 21.2 ± 1.3 | 0.98 ± 0.07† |

Values represent the means ± S.D. for five rats.
* This diet is the basal diet.
† Significantly different ($P < 0.05$) from the paired controls.

Table 2. Effects of carbohydrate intake on the metabolism of hydrocarbons after treatment with PB, PCB and MC

| Diet | | Metabolic rates, nmole/g/min* | | | |
|---|---|---|---|---|---|
| | | Toluene | Styrene | Chloroform | Trichloroethylene |
| A | Control | 17.6 ± 2.7 | 24.8 ± 3.5 | 18.8 ± 2.2 | 18.0 ± 3.0 |
| | PB | 129.1 ± 13.0† | 154.6 ± 10.1† | 23.2 ± 1.1† | 39.4 ± 4.1† |
| | PCB | 54.1 ± 9.5† | 104.3 ± 13.8† | 13.9 ± 1.7† | 31.9 ± 1.0† |
| | MC | 23.7 ± 2.2† | 42.4 ± 4.0† | 19.0 ± 1.7 | 25.2 ± 2.7† |
| B | Control | 14.8 ± 2.6 | 23.7 ± 2.3 | 13.2 ± 3.6 | 14.4 ± 1.2 |
| | PC | 94.2 ± 9.2† | 133.6 ± 10.1† | 21.7 ± 2.3† | 35.6 ± 1.5† |
| | PCB | 55.6 ± 3.7† | 108.7 ± 16.6† | 11.6 ± 3.0 | 29.7 ± 1.0† |
| | MC | 15.8 ± 2.1 | 36.5 ± 5.8† | 13.9 ± 3.8 | 15.9 ± 1.4 |
| C | Control | 10.5 ± 1.0 | 20.0 ± 3.9 | 7.8 ± 1.1 | 9.4 ± 0.3 |
| | PB | 78.9 ± 3.2† | 111.9 ± 10.9† | 16.0 ± 1.3† | 28.4 ± 1.1† |
| | PCB | 33.8 ± 7.8† | 68.4 ± 6.8† | 8.3 ± 1.6 | 22.1 ± 3.4† |
| | MC | 11.6 ± 3.4 | 23.0 ± 2.7† | 7.7 ± 2.7 | 10.1 ± 1.9 |
| D | Control | 8.4 ± 2.9 | 18.1 ± 2.4 | 7.2 ± 1.3 | 9.0 ± 2.0 |
| | PB | 60.1 ± 5.0† | 66.3 ± 11.5† | 15.4 ± 3.0† | 24.8 ± 2.1† |
| | PCB | 25.4 ± 0.1† | 45.1 ± 4.8† | 7.9 ± 0.2 | 18.9 ± 1.2† |
| | MC | 10.3 ± 1.2 | 20.4 ± 2.8 | 5.2 ± 3.9 | 8.7 ± 2.2 |

* The metabolic rate was determined from the disappearance rate of substrate. Values represent the mean ± S.D. for five rats. See legend to Table 1 for details.

*Liver microsomal protein and cytochrome P-450.* The microsomal protein content was measured according to the method of Lowry *et al.* [7] as modified by Miller [8]. The amount of cytochrome P-450 was assayed according to the spectrophotometric method of Omura and Sato [9].

*Results and discussion*

PCB-treatment of rats caused a significant increase in liver weight at every level of CHO intake, and PB increased the weight at CHO intake of 9.72 g/day or less, whereas MC did not affect the weight at any level of the intake. The increase of liver weight in PB- and PCB-treated rats was increasingly distinct with decreasing CHO intake. In addition, the lower the intake of CHO, the more remarkably PB and PCB increased microsomal protein and cytochrome P-450 contents. MC caused no significant effect on the protein content, but it tended to increase the cytochrome content with decreasing intake of CHO (Table 1).

In agreement with the previous report [5], a low-CHO diet enhanced the metabolism of all the hydrocarbons studied (the results with toluene, styrene, chloroform and trichloroethylene are shown in Table 2 as the representatives). However, although causing a remarkable increase in microsomal protein and cytochrome P-450 contents, PB and PCB induced only a restricted range of enzyme activity: PB increased the metabolism of toluene, styrene, trichloroethylene and chloroform, and PCB those of toluene, styrene and trichloroethylene. The enhancement of toluene and styrene metabolism was particularly remarkable. Both PB- and PCB-treatment, when combined with a lower-CHO diet (A or B), caused much more remarkable increase in the metabolism of toluene and styrene than when combined with a high-CHO diet (C or D). The combined effect of PB or PCB with a low-CHO diet cannot be explained as mere additive effects since the value for combination was much greater than the sum of the effects produced by either treatment alone. MC-treatment also accelerated the metabolism of toluene, styrene and trichloroethylene when MC was administered to rats in combination with a low-CHO diet, although the extent was not comparable to that in the case of PB or PCB. These results indicate that a low-CHO diet potentiates the enzyme-inducing abilities of PB, PCB and MC.

Recently, Tu and Yang [10] have reported that fasting of rats induces a low-$K_m$ form of cytochrome P-450 isozyme which has a high affinity for dimethylnitrosamine (apparent $K_m$, 0.07 mM). We have also shown that 1-day food deprivation accelerates the metabolism of a variety of volatile hydrocarbons [11] and that the lack of CHO is primarily responsible for the acceleration. The $K_m$ values for volatile hydrocarbons such as toluene and trichloroethylene are much lower than that for dimethylnitrosamine (2.8 and 2.4 $\mu$M, respectively). Therefore caloric restriction (lowered CHO intake) may possibly induce certain forms of enzymes with low $K_m$ which are highly active for such volatile hydrocarbons as used in the present study.

PB has been shown to induce several cytochrome P-450-linked enzymes more effectively in fasting rats than in normally fed rats [12–14]. Assuming that caloric restriction mimics the effect produced by food deprivation, this observation is consistent with our finding that lowered CHO intake enhances the inducibility of certain forms of cytochrome P-450. The underlying mechanism of how dietary CHO affects the enzyme induction remains unknown.

In summary, dietary CHO intake affects the enzyme-inducing effect of PB, PCB or MC in a dose related manner: a low-CHO diet augmented both the increase in microsomal cytochrome P-450 content and the enhancement of toluene and styrene metabolism caused by these enzyme inducers, whereas a high-CHO diet suppressed the effects.

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# Succinylcholine—tissue distribution and elimination from plasma in the dog

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According to Taylor [1] the brief duration of action of SCh** is due to a rapid enzymatic hydrolysis by pseudocholinesterase. Its presence in blood is indeed very short-lived as recently proven by chemical determinations [2]. Other means of disposition seem to have been largely neglected in the discussion of its evanescent action. The experiments reported here were undertaken to throw further light on the role of enzymatic hydrolysis as compared to redistribution in tissues and excretion.

## *Materials and methods*

*Chemicals.* All chemicals were of analytical grade. The reagents were prepared as described by Nordgren *et al.* [2].

*Animal experiments.* Mongrel dogs were injected i.v. or i.m. with doses of SCh ranging between 2 and 106 mg/kg body weight. The dogs were pretreated with pentobarbital. Blood was collected from an indwelling catheter in heparinized tubes containing eserine (final concentration $10^{-4}$ M) at intervals ranging between 0.5 and 36 min after drug administration. Tissues were collected 1–45 min after administration of SCh. In some cases the tissues were embalmed by injection and soaking in FAX (Champion Chemical Co., Ontario, Canada), a commercially available glutaraldehyde embalming fluid diluted 1:8 with water. Two of the dogs received artificial ventilation from a Harvard Model 613 Dual Phase Respirator throughout the experiments. The others were left to die from the dose of SCh (no artificial respiration).

*Determination of SCh.* The analytical method used has been described by Forney *et al.* [3] and Nordgren *et al.* [2]. SCh is extracted from plasma or tissue homogenate (in 0.4 N perchloric acid) into dichloromethane as an ion pair with hexanitrodiphenylamine. To enable gas chromatography SCh is demethylated with sodium benzenethiolate to form the corresponding tertiary amine which is quantitated by gas chromatography–mass spectrometry using deuterium labeled SCh as internal standard.

## *Results and discussion*

To throw further light on the importance of enzymatic hydrolysis of SCh as compared to tissue distribution and excretion, the elimination of SCh from dog plasma was studied in animals kept alive by artificial respiration and in animals that were left to die from the SCh dose. Dog plasma is known to have ChE activity [4]. There is no reason to believe that the plasma ChE activity stops when the animals die, since it is well known that the esterase activity can be kept under test tube conditions for a considerable time.

The results are demonstrated in Fig. 1. Panel A and B show the elimination from plasma during artificial respiration. A dog was injected with SCh 2 mg/kg i.v. (panel A). A second dose of SCh (106 mg/kg) was administered i.v. 1.5 hr after the first dose (panel B). Artificial ventilation was maintained throughout the experiment. Even after the higher dose the SCh is rapidly eliminated. This was expected and is in accordance with studies in humans [1, 2].

Panel C and D show the plasma curves from two dogs receiving no artificial ventilation. The dose administered was 10 mg/kg i.v. (panel C) and 67 mg/kg i.v. (panel D), respectively. In both dogs the respirations ceased after approximately 30 sec, followed by a decrease in blood pressure and cessation of regular heart beats after about 4 min. In these dogs, the decrease in plasma SCh stopped upon circulatory failure, which indicates that the rapid disappearance of SCh from plasma is not only due to enzymatic hydrolysis. Tissue distribution and excretion also play an important role.

The initial concentration of SCh (30 sec after injection) is very high, but in all cases except one in accordance with a rapid and even distribution to the whole plasma volume. In the dog given the high dose and no artificial ventilation, the initial concentration is higher than expected, probably due to the rapid circulatory failure.

The concentration of SCh in tissues, under the above mentioned conditions, was also studied in samples collected at different times after administration (from one up to 45 min). Because of the analysis of forensic cases performed simultaneously some of the tissues were embalmed. The results are summarized in Table 1. In all cases, without comparison, the kidney has the highest levels, and the elimination seems to be rather slow (No. 1). The relative distribution between the organs studied show roughly the same relationship in all dogs, with or without artificial respiration and irrespective of the dose administered. However, the tissue concentrations seem to be lower for animals kept under artificial respiration (No. 1 and 5), even though a lower dose of SCh naturally results in lower tissue levels. I.m. administration results in considerably lower tissue levels compared to a similar i.v. dose (No. 3 and 4). After injection of 10 mg/kg i.m. cessation of respiration occurred after about one min.

The present study shows that the rapid disappearance of SCh from plasma is not only due to hydrolysis by ChE,

** Abbreviations: SCh, succinylcholine; ChE, cholinesterase.