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Formate Metabolism in Young Swine

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Formate Metabolism in Young Swine. MAKAR, A. B., TEPHLY, T. R., SAHIN, G., AND OSWEILER, G. (1990). *Toxicol. Appl. Pharmacol.* **105**, 315–320. Formate generated from methanol metabolism *in vivo* is the chemical entity responsible for the development of the methanol toxicity syndrome in the monkey. Compared to rats, monkeys are in a state of folate deficiency. This leads to a decreased ability to dispose of formate generated leading to its accumulation and the subsequent development of the classic symptoms of methanol toxicity. Rats possess a more efficient folate system; therefore, they metabolize formate very readily and do not exhibit methanol toxicity symptoms. In this report, the hepatic folate content and the ability to handle a formate "load" were evaluated in another animal species, the pig. The results obtained indicate that the pig, compared to all other species studied, has extremely low levels of folates and very low levels of a key enzyme in the folate pathway, namely 10-formyl H_4folate dehydrogenase. Also the pig's ability to dispose of formate was extremely limited and slower than that observed in rats or monkeys. These results suggest that the pig may be a suitable animal model for studying formate metabolism and possibly methanol toxicity. © 1990 Academic Press, Inc.

Formic acid plays a major role in the toxicity of methanol. It accounts largely for the metabolic acidosis (Tephly *et al.*, 1979) and the ocular pathological changes (Martin-Amat *et al.*, 1978) associated with methanol poisoning in monkeys. The species differences in susceptibility to methanol poisoning are well known (Gilger and Potts, 1955) and can be explained from an understanding of a species' capacity to metabolize formate to carbon dioxide. Monkeys metabolize formate at low rates and demonstrate the classic signs of metabolic acidosis and blindness seen in humans (Tephly *et al.*, 1979), whereas rats oxidize formate readily and are insensitive to methanol.

The rate of formate oxidation is dependent on adequate levels of hepatic folic acid, particularly hepatic tetrahydrofolate (H_4folate) (Tephly and McMartin, 1974; Johlin *et al.*, 1987). Formate is converted to 10-formyltetrahydrofolate (10-HCO H_4folate) via 10-HCO H_4folate synthetase. 10-HCO H_4folate is then oxidized to carbon dioxide through the mediation of 10-HCO H_4folate dehydrogenase (Johlin *et al.*, 1989). Species demonstrating sensitivity to methanol poisoning and formate accumulation (e.g., monkeys) possess low hepatic H_4folate although total hepatic folate does not differ from hepatic folate levels seen in rats (Johlin *et al.*, 1987; Tephly and McMartin, 1974). Thus, a species ability to metabolize formate correlates well with hepatic H_4folate, the important folate derivative involved in formate oxidation.

Methanol use will probably increase in the future because it is seen as an important energy alternative especially as an automobile fuel source. However, experimental animal models suitable for use in understanding

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dose–response relationships and mechanisms of toxicity are relatively limited. At this time, only monkeys appear to be appropriate. In the current study we report findings on formate metabolism and hepatic folate levels in swine. The potential for studying this species is implicit since this species appears to demonstrate a severe deficit in formate metabolizing capacity and in hepatic folates.

MATERIALS AND METHODS

Animals. Six crossed (Yorkshire × Duroc × Hampshire) pigs ranging in weight from 9.5 to 14 kg were used. They were obtained from a closed herd maintained by the Department of Veterinary Clinical Sciences, Iowa State University. They were housed individually in stainless steel cages. They were fed *ad lib.* a balanced diet meeting National Research Council nutritional specifications for swine.

Male Sprague–Dawley rats (200–250 g) were used in this study. They were obtained from Sasco/King Animal Laboratories (Omaha, NE) and were housed in wire-bottomed cages. Purina Laboratory Chow and water were provided *ad lib.*

Chemicals. The cation-exchange resin Cellex P was purchased from Bio-Rad Laboratories (Richmond, CA), [^{14}C]sodium formate (57 mCi/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Preblend 3a70B scintillation cocktail was purchased from Research Products International Corp. (Elk Grove, IL). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were of the highest available purity.

Metabolic experiments. Animals were injected intraperitoneally with [^{14}C]sodium formate (500 mg/kg; 12,000 dpm/mg). At various time intervals thereafter, blood samples were collected into vacutainers by the ocular sinus technique described previously (Huhn *et al.*, 1969). A protein free supernatant was prepared from these samples by the use of zinc sulfate and sodium hydroxide described previously (Makar *et al.*, 1975).

Blood formate assay. In samples obtained from the first two pigs, formate was determined by two methods: the enzymatic method of Makar and Tephly (1982), and radioactivity measurements. Both methods gave identical values. Therefore, in the samples obtained from the subsequent four pigs, formate was determined using only the radioactive assay. The radioactive assay was performed as follows: An aliquot of the protein free supernatant (0.6 ml) was mixed with 10 ml of Preblend 3a70B in a scintillation vial and counted in a Packard counter (Model 3310). Formate concentrations were then calculated from sample counts as well as from the counts of suitable standards and controls.

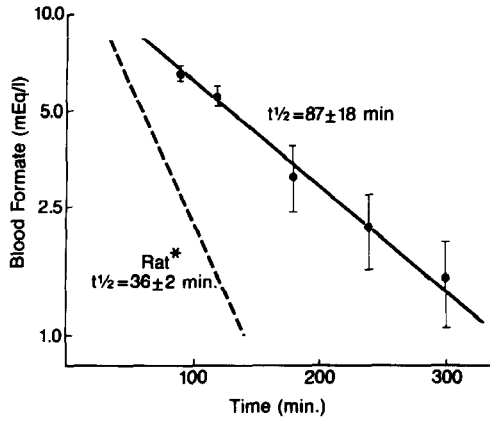

FIG. 1. Formate elimination from the blood of pigs. Sodium formate (500 mg/kg) was administered ip. Each point represents the mean ± SEM for six animals. The half-life ($t\frac{1}{2}$) represents the mean value ± SEM for the value obtained from regression analysis of the disappearance curve for each animal. *Data from Eells *et al.* (1981).

Hepatic S-adenosylmethionine (AdoMet) assay. Livers were obtained from pigs euthanized by using T-61 euthanasia solution (obtained from Hoechst-Roussel, Sommerville, NJ). Thin slices of liver were obtained and immediately immersed in liquid nitrogen. They were stored at −80°C until used. Rat livers were obtained as described by Makar and Tephly (1983). AdoMet was determined using the method of Eloranta *et al.* (1976) as modified by Makar and Tephly (1983).

Folate determination. Hepatic folates were determined in pig livers obtained as described under Hepatic AdoMet assay. Folate analysis was performed using the method described by McMartin *et al.* (1981). Folates were assayed in their monoglutamate form after hydrolysis with hog kidney polyglutamate hydrolase. HPLC separation of various forms was carried out and fractions were collected directly into folate deficient *Lactobacillus casei* growth media. Then they were inoculated and incubated as previously described (McMartin *et al.*, 1981).

Hepatic 10-formyl H_4folate dehydrogenase activity. Liver samples from pigs and rats were obtained as described under Hepatic AdoMet assay. The 10-formyl H_4folate dehydrogenase activity was measured in hepatic cytosol as described by Kutzbach and Stokstad (1968). Hepatic cytosol was prepared as described by Johlin *et al.* (1989) and reactions were carried out at room temperature and at protein concentrations that led to linear rates of product formation.

Statistical evaluation. Statistical evaluations were made using the Student's *t* test for unpaired data. Linear

TABLE 1

LEVELS OF FOLATE INTERMEDIATES IN THE LIVER

Folate derivative	Species				
	Pig (8)[b]	Mouse[a] (4)	Rat[a] (6)	Monkey[a] (7)	Human[a] (5)
H_4folate	3.3 ± 1.1	42.9 ± 1.2	11.4 ± 0.8	7.4 ± 0.8	6.5 ± 0.3
5-CH_3-H_4folate	1.0 ± 0.2	11.6 ± 0.4	9.3 ± 0.6	7.6 ± 0.6	6.0 ± 0.7
HCO-H_4folate[c]	0.7 ± 0.1	6.4 ± 0.6	4.6 ± 1.3	10.5 ± 0.8	3.3 ± 0.5
Total folate	5.1 ± 1.2	60.9 ± 2.1	25.3 ± 0.9	25.5 ± 1.2	15.8 ± 0.8

Note. Values are expressed as nmol of folate/g of liver. Values represent the means ± SEM.

[a] Data from Johlin *et al.* (1987).

[b] Number of animals used.

[c] Values represent the sum of 10-HCO-H_4folate and 5-HCO-H_4folate.

regression analysis was performed by using the least-squares fit method. A p value of <0.05 was considered statistically significant.

RESULTS

Rate of formate disappearance from the blood of pigs. Following the ip injection of 500 mg/kg sodium formate, blood formate concentrations were followed over a period of 5 hr. The results are presented in Fig. 1. Note that the disappearance of formate from the blood of pigs takes place at a much slower rate than that observed in rats.

Hepatic folate concentrations in pigs. A very strong correlation has been shown between the rate of formate oxidation and hepatic tetrahydrofolate content (Eells *et al.*, 1982; Johlin *et al.*, 1987). Since the rate of formate disappearance is very slow in the pig, it was expected that hepatic folate concentrations might also be low in this species. Table 1 shows that H_4folate concentrations were extremely low compared to those in other species.

Hepatic 10-formyl H_4folate dehydrogenase activity in rats and pigs. 10-Formyl H_4folate dehydrogenase is the enzyme that is ultimately responsible for the conversion of formate to carbon dioxide (Johlin *et al.*, 1987). The activity of this enzyme was determined in liver samples obtained from rats and pigs. Table 2 summarizes the results obtained. The activity in pig liver is less than half that found in rat liver.

Hepatic S-adenosylmethionine concentrations. The flux of one-carbon units through the folate biochemical pathway, in part, leads to *S*-adenosylmethionine (Johlin *et al.*, 1987). Thus one might expect that if folate intermediates are low, a relatively low hepatic AdoMet level might be observed. Indeed, Eells *et al.* 1982) have demonstrated an excellent correlation between hepatic H_4folate and AdoMet concentrations. Since pig liver contains low H_4folate concentrations, it was of interest to determine whether AdoMet concentrations were also low. Table 3 shows the

TABLE 2

LEVELS OF HEPATIC 10-HCO-H_4FOLATE DEHYDROGENASE ENZYME ACTIVITY IN THE PIG AND THE RAT

Rat	Pig
12.0 ± 0.4	5.8 ± 0.2[a]

Note. Values are expressed as nmol product formed/min/mg protein. Values are means ± SEM of five livers.

[a] Value is significantly different from that of rat liver ($p < 0.001$).

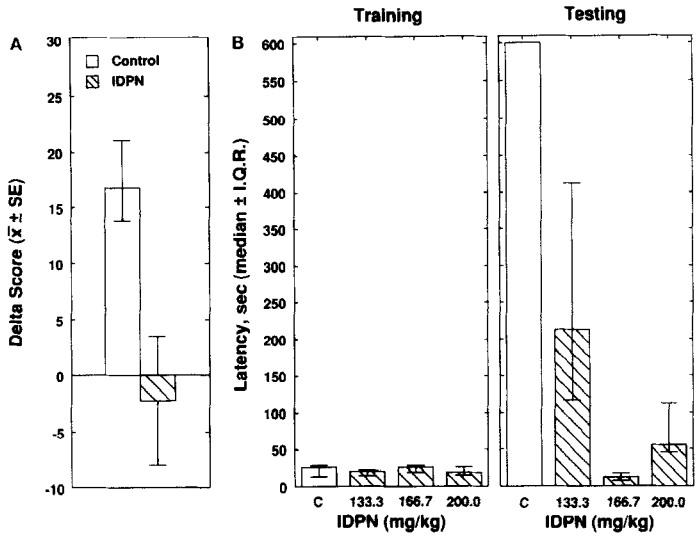

FIG. 2. Effects of IDPN (200 mg/kg/day) on olfactory discrimination learning (A) and PA conditioning (B). The data presented from the olfactory discrimination study are mean ± SE (n = 22/group) delta (δ) scores, derived from the difference in odor preferences prior to and following discrimination training (pretraining preference for CS^+ − post-training preference for CS^+). Positive scores represent an acquired avoidance response to the odor paired with shock. Dosage–effect determinations of IDPN on performance of a PA conditioning task are shown in B. Each bar represents the median (± interquartile range) step-through latencies for rats (n = 12/group) receiving the saline vehicle (C) or 133.3, 166.7, or 200 mg/kg/day. Bars on the left and right panels of B represent median latencies during training and testing, respectively. An upper limit of 600 sec was imposed on step-through latencies.

charin intake)/(saccharin intake + water intake), constituted the data of interest. A two-way analysis of variance (ANOVA) was used to statistically evaluate treatment effects (delay and IDPN) on saccharin preference. When appropriate, post hoc comparisons were made using Tukey's studentized range statistic. For both tests, the α level was set at 0.05.

RESULTS

General Observations

The effects of IDPN (200 mg/kg/day) on body weight are shown in Fig. 1. During the 7 days following the first injection, rats treated with IDPN showed a 9.6% decrease in body weight while rats receiving saline showed a 10.8% increase. During the following 3 weeks, both groups showed nearly equal growth curves. Four weeks after the final injection, the mean body weight for IDPN-treated rats was 9.7% less than that of their saline-treated counterparts. The reduction in body weight at 200 mg/kg/day was not seen in the lower dosages (data not shown). Rats in the high-dosage group also exhibited several behavioral symptoms including head bobbing, occasional retropulsion, and slight hyperactivity particularly after being removed from their home cages. These effects were largely absent in rats receiving lower dosages.

Screening Tests

Olfactory discrimination. The effect of pairing odor stimuli with footshock on subsequent odor preferences in control and treated rats is shown in Fig. 2A. During the pretest phase, treated and control rats spent 53.7 and 56.5% of their time, respectively, over the odor that eventually served as CS^+. Following training, controls reduced the time spent over the CS^+ by a mean of 17.5% (δ score). Rats receiving IDPN showed a 2.5% increase in

finding was not unexpected in the liver of species where hepatic H_4folate concentrations are low (monkeys, human) and 10-HCO H_4folate dehydrogenase activity is also low (Johlin *et al.*, 1987). Thus the observation in pig liver is consistant with data obtained from other species. This enzyme has recently been purified to homogeneity from rat and human liver (Johlin *et al.*, 1989) and its NH_2-terminal amino acid sequence has been determined and found to be similar through the first 15 amino acids. Also the molecular mass on SDS–PAGE for each protein was similar (96,000 Da). The only difference observed between the rat and human protein was a decrease in the enzyme protein present as determined by immunoblotting experiments. The purified pig liver enzyme was also observed to react immunochemically on SDS–PAGE with a molecular weight of 96,000 Da (data not shown). Therefore, the pig liver enzyme appears to be similar in nature to the rat and human enzyme.

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