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ACUTE EFFECT OF METHAMPHETAMINE ON HEME OXYGENASE ACTIVITY AND ON CYTOCHROME P-450 CONTENT IN THE LIVER OF FEMALE RATS

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After a single treatment with *d*-methamphetamine (philopon) at a dose of 8 mg/kg in 10 weeks old female Wistar rats, heme oxygenase activity was enhanced at 3 hr and reached its peak level at 12 hr, whereas cytochrome P-450 content showed the lowest level at 12 hr. However, activity of δ -aminolevulinic acid synthetase was not affected. Twenty-four hr later, all measured parameters returned to nearly initial levels at zero time. The pretreatment with cycloheximide blocked the increase of heme oxygenase activity in philopon-treated animals.

Keywords— *d*-methamphetamine; philopon; heme oxygenase; δ -aminolevulinic acid synthetase; cytochrome P-450; cycloheximide; aminopyrine demethylase; aniline hydroxylase; rat liver

Increased intoxication in nonmedical abuse of stimulants arise recently again as a social problem. Central stimulant effects of amphetamine or methamphetamine[1-3] and the pharmacokinetics[4-6] have been widely studied in experimental animals and humans. Although many chemicals induce a mixed function oxidase which locates in the microsomes of the liver, lung, kidney and so on, several drugs including amphetamine were reported to have no significant effects on such inductive action.[7] However, we found that *d*-methamphetamine enhanced the activity of heme oxygenase, the rate-limiting enzyme in heme degradation, whereas decreased the cytochrome P-450 content in liver microsomes when measured after acute treatment. Therefore, we have further investigated the relationship between the enzyme activities in heme metabolic pathway and cytochrome P-450 content by the treatment with *d*-methamphetamine.

Ten weeks old female Wistar rats were used in all experiments and fed on a commercial diet and tap water *ad libitum*. *d*-Methamphetamine hydrochloride (philopon) was intraperitoneally injected at a single dose of 8 mg/kg in 0.9% saline solution at 2 ml per kg. Control animals were treated with equivalent volumes of 0.9% saline solution. Methods of determination were as follows —heme oxygenase activity: Maines and Kappas[8]; cytochrome P-450 content: Omura and Sato[9]; δ -aminolevulinic acid (δ -ALA) synthetase activity: Marver *et al.*[10]; drug metabolizing enzyme activity: Ariyoshi *et al.*[11]

The typical results in the time course experiments are summarized in Table I. Heme oxygenase activity reached peak level at 12 hr, whreas cytochrome P-450 content showed the lowest level, although the δ -ALA synthetase activity was not affected. In addition, the activities of aminopyrine demethylase and aniline hydroxylase at 12 hr showed 63% and 68% to level of the zero time, respectively (data not shown). An approximate parallelism was observed between decreases of drug metabolizing

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enzyme activities and cytochrome P-450 content, and these measured activities and content at 24 hr returned again to nearly levels at the zero time. These findings suggest that the stimulation of hepatic heme oxygenase is linked to the degradation of microsomal cytochrome P-450 heme. However, there is a question why philopon did not affect the δ -ALA synthetase activity in this study. In general, chemicals which promote a degradation of liver heme may stimulate more effectively δ -ALA synthetase, because heme is known to exercise a negative feedback control on the activity of porphyrin biosynthetic pathway at the level of δ -ALA synthetase.

TABLE I. *Time-Course Effects of d-Methamphetamine on the Activities of δ -ALA Synthetase and Heme Oxygenase and on the Content of Cytochrome P-450*

Time after treatment (hr)	Rat No.	Enzyme activity		Content of cytochrome P-450[c]
		δ -ALA synthetase[a]	Heme oxygenase[b]	
0	6	41.6±2.3	1.21±0.16	0.78±0.04
3	4	36.3±4.8	1.72±0.24[d]	0.57±0.02[e]
6	4	39.7±3.8	2.16±0.21[d]	0.55±0.06[d]
12	6	38.5±4.0	2.42±0.27[d]	0.53±0.02[e]
24	4	35.2±4.0	1.34±0.13	0.82±0.08

Animals were injected intraperitoneally with d-methamphetamine at a single dose of 8 mg/kg, and were killed at the indicated times after the administration.
Each value represents the mean ± S.E. of 4 to 6 rats.
a) nmol δ -ALA/g liver/hr.
b) nmol bilirubin/mg protein/hr.
c) nmol/mg protein.
d) Significantly different from corresponding mean of the zero time, $p < 0.05$.
e) Significantly different from corresponding mean of the zero time, $p < 0.01$.

TABLE II. *Effects of Pretreatment with Cycloheximide on the Heme Oxygenase Activity and on the Cytochrome P-450 Content in d-Methamphetamine-Treated Rats*

Treatment	Heme oxygenase activity (nmol bilirubin/mg protein/hr)	Cytochrome P-450 content (nmol/mg protein)
Control	1.52±0.14	0.64±0.02
Cycloheximide	1.21±0.13	0.51±0.03[a]
d-Methamphetamine	2.18±0.22[a]	0.48±0.02[b]
Cycloheximide + *d*-methamphetamine	1.32±0.18	0.40±0.02[b]

Animals were pretreated with cycloheximide (0.5 mg/kg, i.p.) 2 hr prior to the administration of d-methamphetamine at a single dose of 8 mg/kg, and were killed 12 hr after the injection of d-methamphetamine.
Each value represents the mean ± S.E. of 6 rats.
a) Significantly different from corresponding mean of control, $p < 0.05$.
b) Significantly different from corresponding mean of control, $p < 0.01$.

A single pretreatment with cycloheximide (0.5 mg/kg, *i.p.*) 2 hr prior to a single dose of philopon blocked almost the elevation of heme oxygenase activity, as shown in Table II. This finding suggests that the enhancement of heme oxygenase by philopon may be due to induction of protein moiety. In this experiments, however, cycloheximide treatment alone showed the tendency to decrease the heme oxygenase activity and indicated significant decrease of the cytochrome P-450 content. This is presumably a result of inhibition of protein moiety in heme oxygenase and cytochrome P-450. Therefore, a combined injection of cycloheximide and philopon seemed to indicate additive effects on heme oxygenase and cytochrome P-450 content. However, it is unknown as to whether philopon induces at first heme oxygenase and secondarily degradates cytochrome P-450 heme or philopon initiates destruction of cytochrome P-450 heme and thereby inducing heme oxygenase in order to utilize the released heme.

Further studies will be needed to elucidate this relationship by a direct measurement of cytochrome P-450 heme, and to investigate the increased formation of bilirubin which leads to a stimulation of heme oxygenase activity in the liver.

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