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ROLE OF METHAMPHETAMINE METABOLISM IN THE DEVELOPMENT OF CNS TOLERANCE TO THE DRUG

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Key words: *Methamphetamine (METH); Amphetamine (AMP); Tolerance; Metabolism.*

ABSTRACT

We have previously observed that pretreatment with increasing doses of methamphetamine (METH) attenuates the effects of METH on the dopaminergic and serotonergic systems as compared with the ones observed in nonpretreated controls. In order to understand the mechanism of this tolerance, untreated rats (naive) and rats pretreated with METH (daily doses of 2.5, 5.0, 7.5, and 10 mg/kg, s.c., at 6h intervals with a 24h drug-free period between each dose) were challenged with the administration of 5 doses of METH (15 mg/kg, s.c., at 6h intervals). The metabolism of METH in brain, liver, and blood was studied by measuring the concentration of METH and its metabolites 2, 4, 6, 8 and 10h after the last dose by gas chromatography/mass spectrometry techniques.

The forebrain concentrations of METH in the pretreated animals were significantly lower than those observed in the forebrain of naive animals. Liver concentrations of METH in the pretreated animals were not significantly modified as compared to the ones of naive animals, but in the liver amphetamine and the p-hydroxylated metabolites, p-hydroxyamphetamine (p-OH-AMP) and p-hydroxymethamphetamine (p-OH-METH), were significantly greater than those observed in the naive group. Blood levels of METH, AMP and their p-hydroxylated metabolites were also greater in the pretreated animals. The involvement of an altered distribution of methamphetamine in the CNS and the development of tolerance is discussed.

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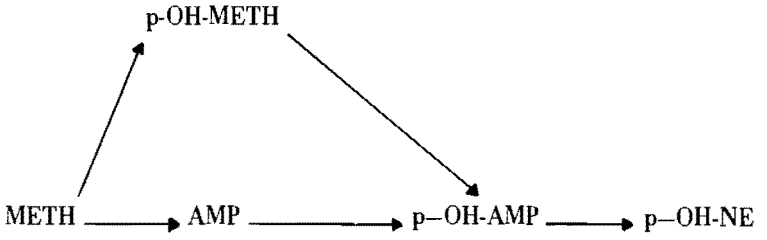
INTRODUCTION

Methamphetamine undergoes N-demethylation and aromatic hydroxylation metabolic reactions. This metabolic pathway of METH in the rat is shown below (3).



Some authors (2, 21) have shown that many of the effects of AMP disappear following chronic administration. These include the anorectic, hyperthermic, and hypertensive effects. It is possible that the effects of AMP which disappear with chronic administration are due to the metabolite of AMP, p-hydroxynorephedrine (p-OH-NE), which acts as an antagonist to the actions of the drug by functioning as a pseudotransmitter. In this way the metabolite seems to contribute to the development of tolerance to amphetamine-induced hyperthermia and increases in free fatty acids (1). However, there has been much controversy as to the mechanisms of tolerance to the CNS actions of AMP.

It has been demonstrated that administration of high doses of METH to rats depresses tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activities in the neostriatum and reduces the concentration of dopamine (DA) and 5-hydroxytryptamine, 5-HT, (11, 12). However, Schmidt et al., (23) found that when rats were pretreated with increasing doses of METH, METH-induced changes in the monoaminergic system were attenuated.

The purpose of this study was to determine the profiles of METH metabolites in forebrain, liver, and blood in order to determine if METH metabolism plays an important role in the development of tolerance to METH effects in the body.

MATERIALS AND METHODS

Drug schedule

The drug administration schedule used was that of Schmidt et al., (23) with one

minor modification, a dose of 10 mg/kg of METH, was added to the schedule.

Male Sprague-Dawley rats (200-300 g) were initially divided into saline and METH-pretreated groups. On day 1, the pretreament group was given 2.5 mg/kg METH every 6h (5 doses). The animals were then allowed a 24h drug-free period. On days 3,5 and 7, doses were increased to 5.0 mg/kg, 7,5 mg/kg, and 10 mg/kg respectively, under the same schedule. On day 9, the saline group was subdivided into a saline control and a naive METH group. All animals were reweighed at this time. Naive and METH-pretreated animals were then challenged with 15 mg/kg METH every 6h for one day (5 doses). Animals were sacrificed 2, 4, 6, 8 and 10h after the last dose If METH. Bood was collected by exsanguination into a beaker. Liver and forebrain were quickly removed and frozen at -70° C until analysis.

Determination of methamphetamine and its metabolites in forebrain, liver and blood.

Concentrations of METH, AMP, p-OH-AMP and p-OH-METH were determined by gas chromatography-mass spectrometry (Alburges et al., in preparation) using a Hewlett-Packard 5970A gas chromatograph-mass selective detector. Forebrain (1 g) and liver (5 g) were homogenized in 4 and 10 ml of 0.1 M perchloric acid, respectively. To 1 ml of blood, 3 ml of 0.1 M perchloric acid was added. After centrifugation, an aliquot of the supernatant was taken for the assay of METH and AMP, and an aliquot for the assay of p-hydroxy-metabolites as follows: brain, 0.5 and 2 ml; liver, 2 and 4 ml; and blood, 1 and 2 ml respectively. Solid sodium carbonate: sodium chloride (4: 1) was added (0.25 g/ml of supernatant) and extracted with 5 ml of n-butyl chloride. Pseudoephedrine (0. 3 ug) and phentermine (2 ug) were used as internal standard in the METH, AMP assay and in the p-hydroxy-metabolites assay, respectively. After centrifugation, the organic layer was removed and the volume was adjusted to 2 ml and trifluoroacetylated with 0.2 ml of trifluoroacetic anhydride (TFA), at 70 °C for 15 min. After cooling, the organic layer was evaporated to dryness at room temperature. The residues obtained from each fraction were dissolved in 50 ul of ethyl acetate. One ul was injected separately onto a gas chromatograph/mass spectrometer equipped with a 12.5-meter cross-linked dimethylsilicone (0.2 mm) capillary column. Different ion current detectors were used in the electron multiplier, 2200 V with the detector on at 7.5 min, and 1600 V with the detector on at 5 min for METH, AMP assay and for p-hydroxy-metabolites assay, respectively. The ions monitored for TFA derivatives were as follows: AMP 140.0 m/z; METH 154.0 m/z; pseudoephedrine 154.0 m/z; p-OH-AMP 140.0 m/z; p-OH-METH 154.0 m/z; and phentermine 154.0 m/z. The sensitivity of the assay for each compound was approximately 0.125 ng/mg or 0.125 ng/ml.

All results were analyzed by means of Student's t-test with the level of significance at $p < 0.05$.

RESULTS

Figure 1, shows the time-course of methamphetamine and its metabolites concentrations in forebrain or naive and pretreated rats. During the first 4h period the mean brain concentrations of METH in the naive animals were significantly higher ($p < 0.001$) than in the pretreated animals. The p-hydroxylated metabolites, p-OH-METH and p-OH-AMP were present in brain samples in trace concentrations.

The liver concentrations of methamphetamine and of each metabolite, are shown in figure 2. The major metabolite present was p-hydroxymethamphetamine. METH concentrations in the pretreated animals were not significantly different from the ones of naive animals, but amphetamine, p-hydroxyamphetamine and p-hydroxymethamphetamine concentrations, were significantly greater than those observed in the untreated animals at times 2, 4 and 6h after administration of the last challenging dose of methamphetamine. Methamphetamine, amphetamine and their p-hydroxylated metabolites in the naive animals show a slight increase at 6h.

Blood levels of METH, AMP and their p-hydroxy-metabolites were significantly higher at 2h after administration of the last dose of METH in the pretreated group compared to the naive animals, as shown in figure 3. As in the liver, the small 6h increase in the concentrations of METH, AMP, and their p-hydroxylated metabolites was present.

Figure 4 shows concentrations of METH, AMP, p-OH-METH and p-OH-AMP in forebrain, liver and blood of naive and pretreated rats 2h after the last injection of the high challenging dose of METH. Concentrations of METH and its metabolites in blood were higher in the pretreated than in the naive group. However, concentrations of METH and AMP in forebrain were significantly lower in pretreated than in the naive rats. Even though the concentrations of METH metabolites were significantly higher in the liver of the pretreated animals as compared to the ones of controls, the concentrations of METH itself did not show any significant difference between groups.

DISCUSSION

Other studies have demonstrated that d-amphetamine is hydroxylated in rat liver to form p-hydroxyamphetamine. It is metabolized by the enzyme dopamine-B-hydroxylase (DBH) into p-hydroxynorephedrine within the noradrenergic neurons (5, 10). Accumulation of p-hydroxynorephedrine as a false transmitter has been suggested as a mechanism for the development of tolerance to some effects of amphetamine (2, 5, 6 14). Since DBH is not present in dopaminergic neurons, this false transmitter could not be formed in these neurons. This and the poor permeability of the blood brain barrier for phenolic amines (16) could be responsible for the trace concentrations of the

p-hydroxylated metabolites present in forebrain samples.

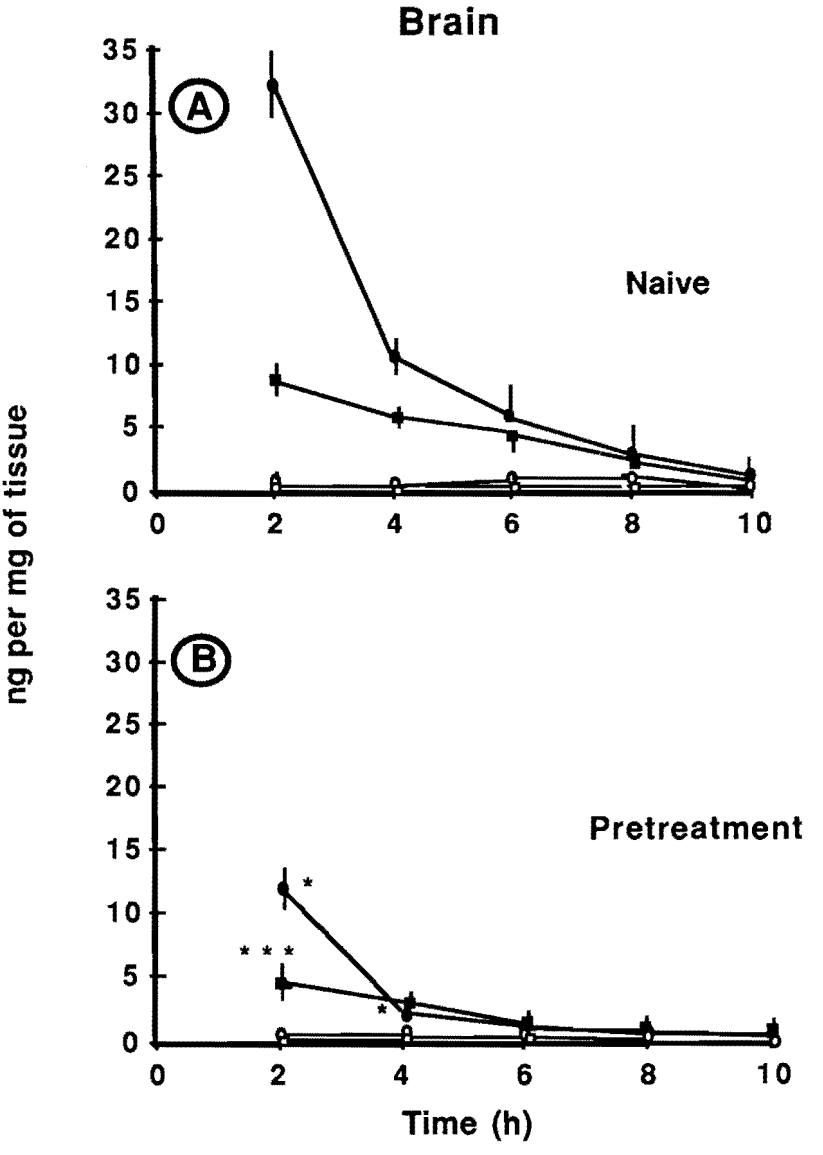


Fig. 1. Forebrain concentrations of METH (*), AMP (■), p-OH-METH (0) and p-OH-AMP (□) in naive (A) and METH-pretreated rats (B) 2, 4, 6, 8, and 10h after the last injection of the high challenging dose of METH. Each point is the average of 3 rats with ± SEM. (total number of animals, 15 naive and 15 pretreated) *P< 0.01 compared to the corresponding interval in the naive group.

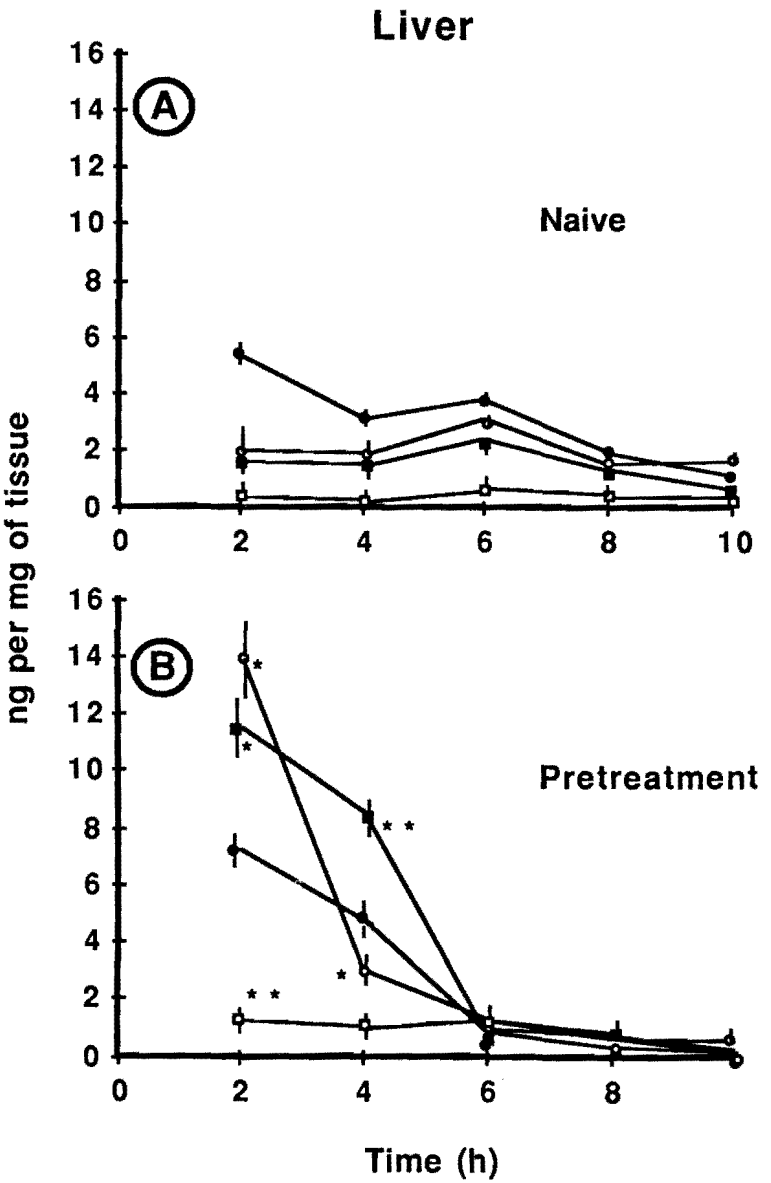


Fig. 2. Liver concentrations of METH (*), AMP (■), p-OH-METH (o) and p-OH-AMP (□) in the naive (A) and METH-pretreated rats (B). See legend of figure 1 for details.

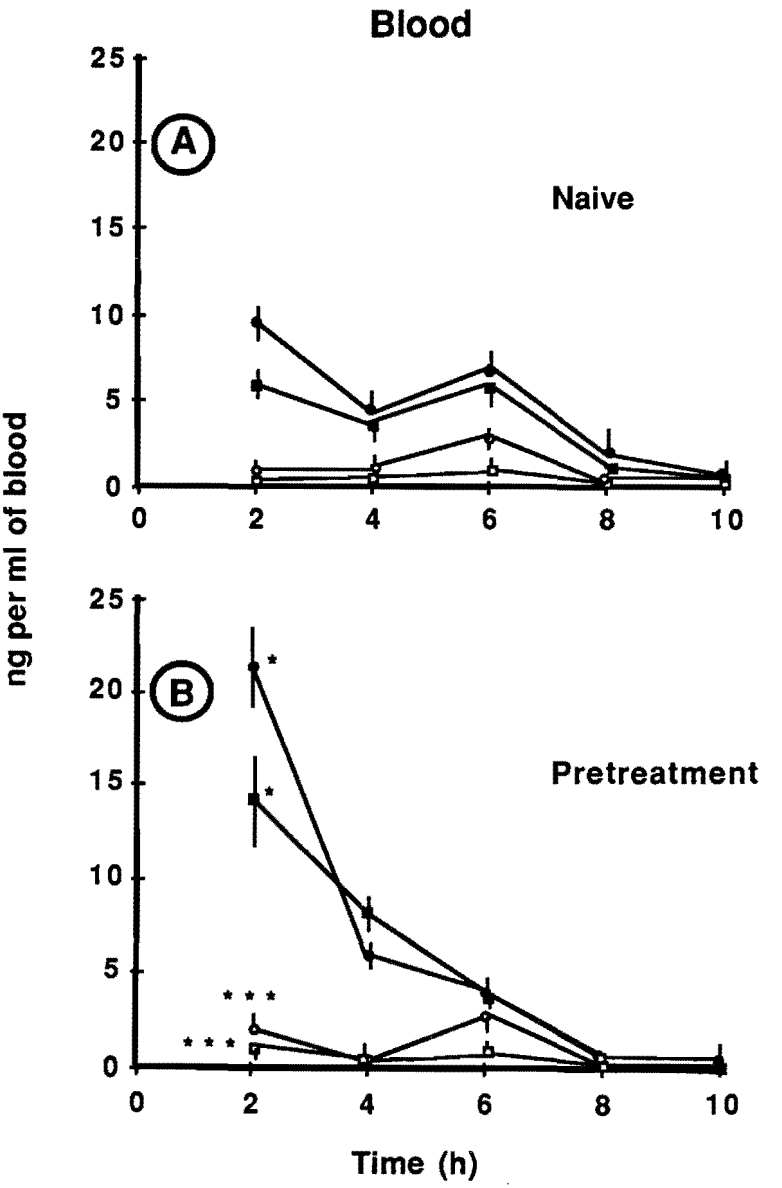


Fig. 3. Blood concentrations of METH (*), AMP (■), p-OH-METH (o) and p-OH-AMP (□) in the naive (A) and METH-pretreated rats (B). See legand of figure 1 for details.

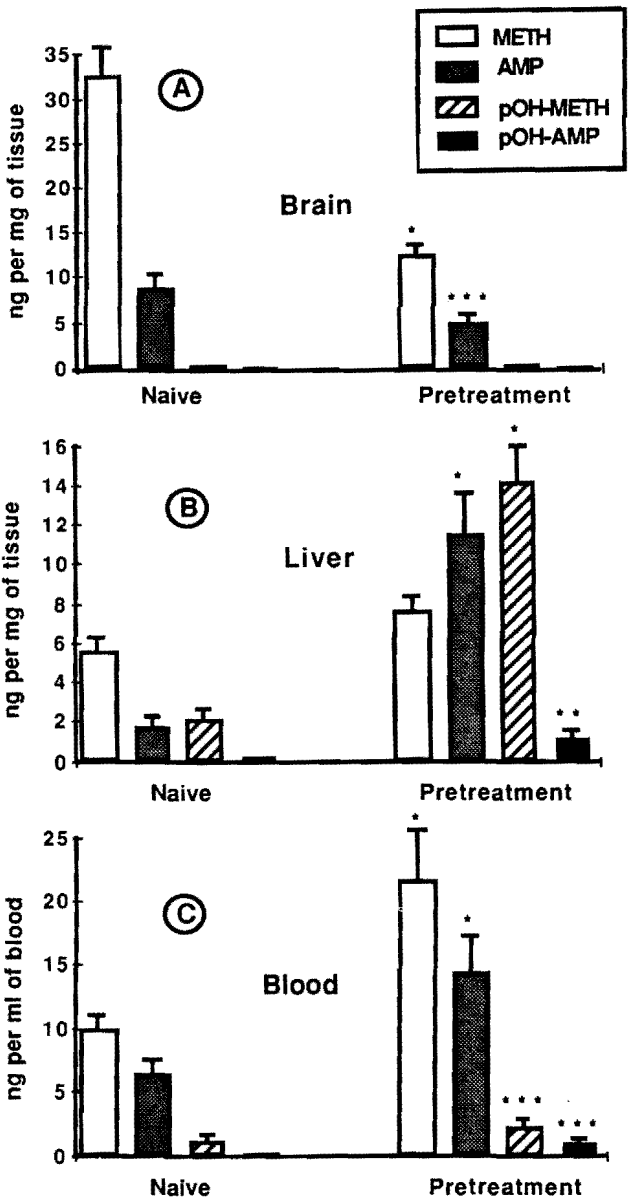


Fig. 4. Forebrain (A), liver (B) and blood (C) concentrations of METH, AMP, p-OH-METH and p-OH-AMP in naive and METH-pretreated rats 2h after the last injection of high dose challenge with METH. Each value is the average of 3 rats with ± SEM *P < 0.001, **P < 0.01, ***P < 0.05 compared to the corresponding time in the naive group.

The results of the present study indicate that although the initial accumulation of the methamphetamine metabolites (AMP and p-OH-METH) in liver tissue of pretreated animals were present in the first 4h, considerable differences with further accumulation between naive and pretreated animals do not exist. Methamphetamine concentration in liver at different times was not significantly different between the groups. It suggests that chronic methamphetamine administration does not result in an increase in the rate of methamphetamine metabolism, as was reported in early studies (8, 13, 15).

The significant increase in blood methamphetamine and amphetamine in the pretreated group during the first 2h might have resulted from a decrease in the rate of excretion of these compounds. Any condition which impairs the flow of blood to the kidneys could decrease their excretion, and it is well known that the abuse of several drugs, including amphetamine, produces acute renal failure (4, 9, 20).

Methamphetamine, amphetamine and their p-hydroxylated metabolites in liver and blood samples from naive animals show a 6h peak. This could be due to an enterohepatic circulation of the parent drug and its metabolites, as was reported in earlier studies (18, 19).

Pardridge et al., 1973 (17) heve found that amphetamine gains access to the brain by a combination of two different mechanisms: free diffusion and a carrier-mediated process. The latter is saturable and pH-dependent. In addition, amphetamine decreases the permeability of the blood-brain barrier at high concentrations (8). In our study, forebrain concentrations of methamphetamine and amphetamine were lower in the pretreated group than in the naive group. These results were consistent with previous studies in our laboratory (22) and probably are the basis for the attenuation of the neurochemical effects of methamphetamine in the neostriatal monoaminergic systems of pretreated animals. In addition, we found no differences in the forebrain concentration of the p-hydroxy-metabolites between naive and pretreated groups, which follows the same line of reasoning.

In the periphery (i.e., blood and hepatic tissue), concentrations of methamphetamine, amphetamine and their p-hydroxylated metabolites were actually higher in the pretreated group than in the naive group. These results suggest that differences in hepatic metabolism of methamphetamine are not responsible for the development of tolerance to methamphetamine by CNS systems. A possible explanation for our finding is that a CNS transport mechanism for methamphetamine and amphetamine is altered in the pretreated animals leading to a reduction in the passage of the agents into the brain and a diminished drug effect.

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RESUMEN

Rol del metabolismo de la Metanfetamina en el desarrollo de tolerancia del sistema nervioso central a los efectos de la Droga. *Alburges, M.E. (Cátedra de Toxicología, Escuela de Bioanálisis, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela), Hanson, G. R. and Gibb, J. W.* Invest. Clin. *31(4): 165-176, 1990.*– Previamente hemos observado que el pretratamiento con dosis progresivamente crecientes de metanfetamina (METH) atenúa los efectos de dosis ulteriores de METH sobre los sistemas dopaminérgicos y serotonérgicos, en comparación con los efectos observados en controles no-pretratados. Para entender el mecanismo de esta tolerancia, animales no-pretratados y pretratados con METH (dosis diarias de 2.5; 5.0; 7.5 y 10 mg/kg,-s.c., con 6h de intervalo por 24h, con un período de 24h sin administración de droga entre cada incremento de dosis) fueron injectados con 5 dosis de METH (15 mg/kg,-s.c., con 6h de intervalo). El metabolismo de la METH en cerebro, hígado y sangre fue estudiado midiendo concentraciones de METH y sus metabolitos 2, 4, 6, 8 y 10 después de la última dosis de la droga, utilizando cromatografía de gases/espectrometría de masa.

Las concentraciones de METH en prosencéfalo de los animales pretratados fueron significativamente menores respecto de aquellas observadas en animales no-pretratados. Las concentraciones de METH en el hígado de animales pretratados no fueron significativamente diferentes de las concentraciones de animales no-pretratados; pero las concentraciones de anfetamina (ANF) y los metabolitos p-hidroxilados, p-hidroxianfetamina y p-hidroximetanfetamina, fueron significativamente mayores que aquellas en el grupo no-pretratado. Los niveles sanguíneos de METH, ANF y de sus metabolitos p-hidroxilados también fueron significativamente incrementados en animales pretratados. Un acceso alterado de la metanfetamina al SNC puede estar involucrado en la tolerancia a la droga.

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