

Effects of amobarbital on pterins and dinucleotides contents in rat brain¹

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ABSTRACT The effects of amobarbital (50 mg · kg⁻¹ ip) on the brain concentrations of biopterin, pterin, reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD) were studied by a reverse phase high performance liquid chromatography with fluorescent detector. Amobarbital increased FAD concentrations in 5 brain areas and pterin concentration in the cortex, but decreased biopterin concentrations in the cortex and hippocampus.

KEY WORDS amobarbital; high pressure liquid chromatography; pterins; NAD; FAD; biogenic amines; brain; dinucleoside phosphates

Barbiturates produce several effects in brain, in particular a general slowing of oxidative metabolism⁽¹⁾. Tryptamine excites spinal neurons in unanesthetized spinal cats, but it had no effect when barbiturate anesthesia is employed⁽²⁾. Barbiturate anesthesia reduces the turnover rate of brain catecholamines⁽³⁾. It would be interesting to observe the effect of barbiturates on monoamine biosynthesis and energy metabolism. The mechanism of barbital action was studied by determining pterin cofactor as an index of monoamine biosynthesis and dinucleotide coenzymes (NADH, FAD) as indices of energy metabolism.

Although various methods for analyzing pterins⁽⁴⁾ and pyridine dinucleotides⁽⁵⁾ were known, it would be useful to develop a new method to detect the brain coenzymes

simultaneously. The purpose of this study was to inquire into the relationship between the dinucleotide coenzymes and pterins.

MATERIALS AND METHODS

Shimadzu LC-3A high performance liquid chromatograph, sample injector SIL-IA, Shimadzu fluorescent HPLC monitor model RF-530, and XWT-164 recorder (made by Shanghai Second Factory for Automatic Instruments).

Reduced nicotinamide adenine dinucleotide (NADH, Boehringer), Flavin adenine dinucleotide (FAD, Serva), pterin (Z-4-amino-4-hydroxy-pteridine, Sigma), biopterin (Fluka AR).

Chromatographic conditions YWG-CH₁₈, 10 μm (Tianjin Chemical Reagent Factory) column 25 × 0.5 cm ID, mobile phase methanol: water 15:85, flow rate 1 ml · min⁻¹, fluorescent monitor worked at sensitivity 10 mV, excited wavelength 340 nm, emission wavelength 460 nm. The recorder worked at 1 mV output range with paper speed at 4 mm · min⁻¹.

Standard solutions Distilled water was used to dissolve NADH and FAD (2 nmol · L⁻¹), as well as biopterin and pterin (2 mg · L⁻¹). The standard solutions were stored at 4°C, and were changed once a month. NADH 2 μl, FAD 6 μl, biopterin 2 μl, and pterin 1 μl were diluted respectively in phosphate buffer 0.5 ml as an external standard or in whole brain homogenate 0.5 ml as an internal standard. The samples of discrete brain areas were treated the same.

Brain samples Twelve rats weighing 200 ± SD 25 g were randomly divided into 2 groups of 6 each and saline (1 ml) or

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amobarbital ($12.5 \text{ mg} \cdot \text{ml}^{-1}$, 1 ml) were injected to the rats in each group. The rats were decapitated and brains were dissected quickly 15 min after the medication. Frontal cortex, hippocampus, caudate nucleus, diencephalon, cerebellum and brain stem were separated according to known methods^(6,7). The brain homogenates were made by phosphate buffer in the ratio of 1:10 (wt:vol). The test tubes containing 0.5 ml of brain homogenate and the internal standard sample were heated at 100°C for 20 min.

The cooled homogenate was added trichloroacetic acid $6 \mu\text{l}$ and centrifuged at $2800 \times g$ for 20 min. The homogenate supernatant was added I_2/KI solution $30 \mu\text{l}$. The mixture was left at room temperature ($15\text{--}20^\circ\text{C}$) for 1 h under reduced light. Then, the oxidative sample was centrifuged again at $2800 \times g$ for 20 min. The supernatant was applied directly to the HPLC system.

RESULTS AND DISCUSSION

The validity and reliability of the method The retention time of the external standard sample was very stable in different conditions including both heating and oxidative procedures. As shown in Fig 1, the retention time was as following: NADH 3.5 min, FAD 4 min 45 s, biopterin 6.5 min, pterin 7.5 min. NADH, FAD, biopterin and pterin were thermostable, the amplitude of chromatogram was not changed in different heating times (5, 10, 20, 30, 40 min) at 100°C . The results were consistent with reference⁽⁸⁾ for pterins and reference⁽⁹⁾ for dinucleotides. The detection limits for NADH, FAD, biopterin and pterin were 3.5 ng, 13 pg, 2.3 pg and 1 pg, respectively.

Studying the method to assay pterins and dinucleotides in brain samples simultaneously, we found that the peaks of dinucleotides in chromatogram increased from time to time, because 350 kinds of enzymes are served to dinucleotide coenzymes in tissues⁽⁹⁾. In order

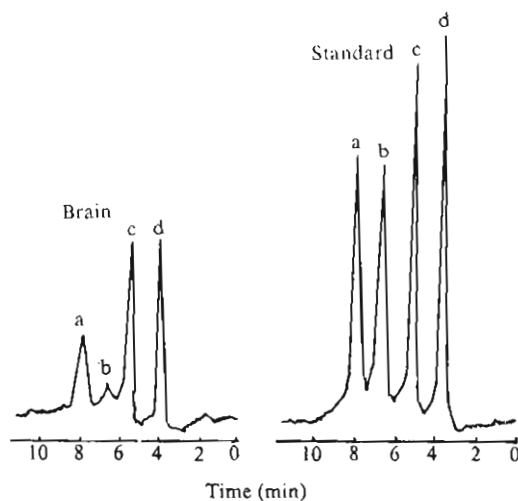


Fig 1. Chromatogram of brain sample and internal standard solution. Samples injected: $10 \mu\text{l}$. a) Pterin; b) Biopterin; c) Flavin adenine dinucleotide (FAD); d) Nicotinamide adenine dinucleotide (NADH).

to destroy the oxidoreductases, we heated the brain homogenate to 100°C for 20 min. The peaks of NADH, FAD in chromatogram had good reproducibility in 24 h, but the peak of pterin increased as the temperature was raised due to the release of conjugated pterins⁽⁸⁾, and the peak of biopterin was hardly visible. The brain homogenate was oxidized by I_2/KI solution⁽⁴⁾, and the peak of biopterin became visual. Although Dowex 1 [OH^-] column and Dowex 50 [H^+] column could concentrate biopterin in the sample, the peaks of dinucleotides disappeared from chromatogram. We deleted resin column treatment from the method⁽⁴⁾, but retained the I_2/KI oxidative procedure.

The recovery rates were 86.2% for NADH, 77.1% for FAD, 98.4% for biopterin and 90.3% for pterin. There were linear relationships between concentrations and peak heights of the chromatograms for pterins and dinucleotides (Fig 2). The correlation coefficients (r) were 0.765–0.853.

It should be mentioned that the

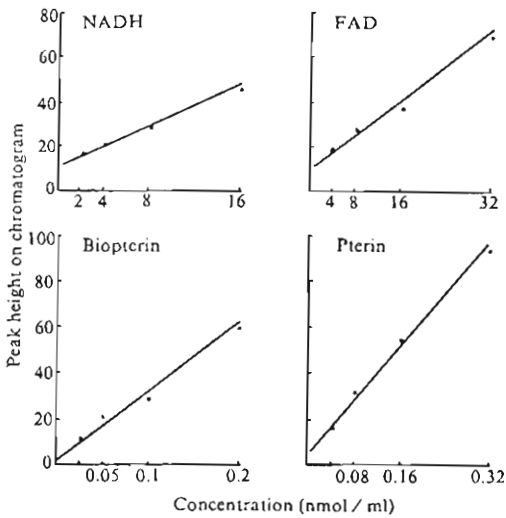


Fig 2. Concentrations and peak heights in chromatogram. The mixed standards are dissolved into a series of 0.5 ml supernatants of the whole brain homogenate.

concentrations of pterins and dinucleotides determined by different methods were in comparable. For example, NADH and biopterin concentrations in rat brain determined by enzyme assay^(7,10) may be as high as 100 times of those by HPLC^(4,11). The concentrations of pterins and dinucleotides in control brain samples assayed by our method were consistent with HPLC methods of other authors.

Effects of amobarbital on dinucleotides

The differences in brain concentrations of dinucleotide coenzymes (NADH, FAD) between barbital-treated rats and control rats ($n=6$) were shown in Fig 3A. Although NADH concentrations in 6 brain areas of barbital-treated rats were higher than those of control rats, the differences did not reach statistically significant level ($P > 0.05$). FAD concentrations in all of the studied brain structures of barbital-treated rats were higher than those of control rats ($P < 0.05$) except in diencephalon.

While amobarbital did not change NADH concentrations in 6 brain areas

significantly, it increased FAD concentrations in the brain structures except diencephalon. The results suggest that barbital anesthesia blocks citric acid cycle of energy metabolism at the point of succinate dehydrogenation, except diencephalon which plays a very important role in keeping life. Amobarbital anesthesia appears to be a specific blockader to succinate dehydrogenase which catalyzes the reaction of succinate oxidation, and reduces FAD to FADH₂. FAD concentrations were increased because succinate dehydrogenase was depressed by amobarbital.

Effects of amobarbital on pterins

The differences in pterins (biopterin, pterin) concentrations in brains between amobarbital-treated rats and control rats ($n=6$) were shown in Fig 3B. The changes of biopterin concentrations in 6 brain areas of barbital-treated rats were different. The concentrations were decreased in the cortex, hippocampus, diencephalon and brain stem. increased in caudate nucleus and cerebellum, but the differences were significant only in the cortex and hippocampus ($P < 0.01$). Amobarbital increased pterin concentrations in all of the studied structures, but this effect was significant in the cortex ($P < 0.05$) only.

While amobarbital blocked citric acid cycle, it decreased biopterin concentrations in the cortex and hippocampus, and increased pterin concentration in cortex. Since biopterin is in oxidative form of tetrahydrobiopterin, which is an important cofactor for monoamine biosynthesis⁽¹²⁾, the results suggest that amobarbital depresses monoamine biosynthesis in the cortex and hippocampus, while energy metabolism decreases in the brain structures except diencephalon. The folate cofactors were called "conjugated" pteridins referring to the linkage of *p*-amino-benzoylglutamates to the pterin⁽¹²⁾. Pterin was increased by heat-related release from the folate cofactors⁽⁸⁾ which was a unique carrier of one-carbon units, and it is known to have a

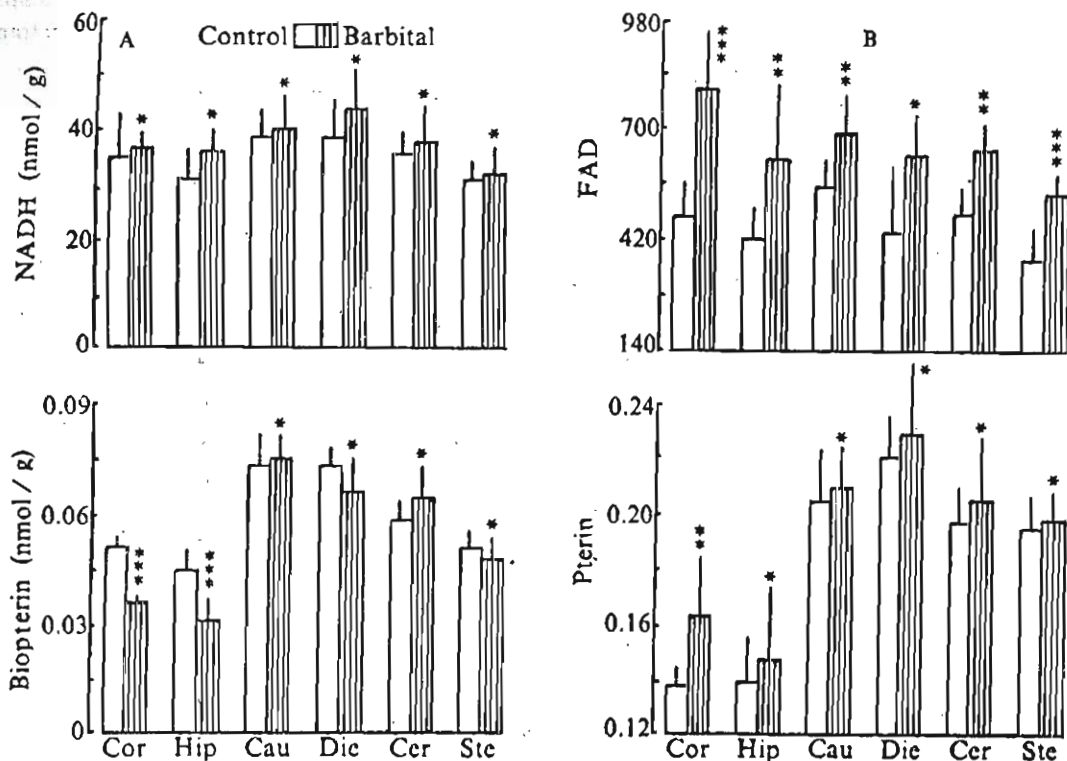


Fig 3. Concentrations of dinucleotides and pterins after ip amobarbital (50 mg · kg⁻¹), Cor=cortex; Hip=hippocampus; Cau=caudate nucleus; Die=diencephalon; Cer=cerebellum; Ste=brain stem. n=6, $\bar{x} \pm SD$, *P>0.05, **P<0.05, ***P<0.01.

very important role in the conversion of serine to glycine⁽¹³⁾. Amobarbital increased pterin concentration in cortex, facilitated biosynthesis of glycine, which is an inhibitory neurotransmitter. The results were consistent with a report⁽¹⁴⁾, which reported that amytal anesthesia increases ATP/ADP ratio as well as GABA concentration in isolated rat brain synatosomes.

The results indicate that amobarbital anesthesia decreases the brain energy metabolism by means of blocking succinate dehydrogenase, decrease biosynthesis of monoamines in the cortex and hippocampus, and facilitates glycine generation in the cortex.

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异戊巴比妥对大鼠脑内蝶呤类和二核苷酸类含量的影响

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提要 利用 HPLC 反相色谱法及荧光检测技术, 研究了异戊巴比妥($50 \text{ mg} \cdot \text{kg}^{-1}$, 腹腔下注射)在 12 只大鼠脑的 6 个区内对生物蝶呤、蝶呤、还原型烟酰胺二核苷酸(NADH)和黄素腺苷二核苷酸(FAD)含量的影响。结果表明, 异戊巴比妥对 6 个脑区内 NADH 的含量不发生显著性影响; 除间脑以外, 在其他 5 个脑区内 FAD 的含量却显著增高。对蝶呤类而言, 异戊巴比妥引起大脑皮层和海马内生物蝶呤含量的降低, 却增加了大脑皮层内蝶呤的含量。这一结果, 说明异戊巴比妥降低了间脑以外其它脑区内的能量代谢过程, 并在皮层和海马中抑制了单胺类物质的生物合成。与此同时, 异戊巴比妥却易化了大脑皮层内甘氨酸这种抑制性神经递质的生物合成过程。

关键词 异戊巴比妥; 高压液相色谱法; 蝶呤类; NAD; FAD; 生物胺类; 脑; 磷酸二核苷

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普罗托品松弛平滑肌的作用

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Relaxant effects of protopine on smooth muscles

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ABSTRACT The relaxant effects of protopine (Pro) on smooth muscles were studied by recording isotonic contraction and radioimmunoassay. Pro relaxed the contraction of rabbit thoracic aorta, mesenteric artery, portal vein and guinea pig ileum and taenia colon induced by high K^+ ($70 \text{ mmol} \cdot \text{L}^{-1}$). Pro also in-

hibited the contraction of rabbit thoracic aorta, mesenteric artery, portal vein induced by NE ($0.3 \mu\text{mol} \cdot \text{L}^{-1}$) and guinea pig taenia colon induced by BaCl_2 ($1 \text{ mmol} \cdot \text{L}^{-1}$). Pro inhibited the intracellular Ca^{2+} release, but did not inhibit Ca^{2+} influx induced by NE. These results suggested that the smooth muscle relaxant mechanism of action of Pro may be the inhibition of intracellular Ca^{2+} release.

KEY WORDS protopine; verapamil; nor-epinephrine; calcium; potassium; barium; ileum; radioimmunoassay; vascular smooth muscle

提要 应用平滑肌等张收缩和放射免疫方法, 研究了普罗托品(protopine, Pro)对平滑肌的作用。结果, Pro