Korean J. Zool. 35:23-28, 1992

# Effects of an Antimetabolite 6-aminonicotinamide on Carbohydrate, Nucleotide and Catecholamine Metabolism in Mouse Brain

**Heon Keun Jung* and In Kook Park**

**Department of Clinical Pathology, Taejon Junior College, Taejon 302-210, Korea; Department of Agrobiology Dongguk University, seoul 100-715, Korea*

**The effects of an antimetabolite, 6-aminonicotinamide (6-AN) on the levels of glucose, glycogen, catechoamines and mucleotides in mice brain were investigated. The level of glucose in the blood starts increasing from 3 h after administration of 6-AN while those in the brain tissue start increasing from 9 h after administration of 6-AN. The concentration of brain glycogen remained unchanged at all time points except 11h. The level of epinephrine in the brain was found to reach maximum value at initial 3 h following 6-AN administration, after which it started decreasing significantly. The level of brain norepinephrine remained virtually unchanged before 24 h time point at which it starts decreasing significantly. ATP, CTP, UMP and UTP levels were significantly reduced but AMP and CMP levels were not affected.**

**KEW WORDS: 6-aminonicotinamide, Carbohydrate, Nucleotide, Epinephrine, Norepinephrine**

Certain structural analogs of nicotinic acid or nicotinamide have been known to interfere with the synthesis or action of nicotinamide coenzymes (Balaban, 1985). As an analog of nicotinamide, 6-aminonicotinamide (6-AN) is substantially incorporated into Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP) by glycohydrolase to form 6-amino-NAD and 6-amino-NADP derivatives which are incapable of transferring hydrogen in oxido-reduction (Herken and Neuhoff, 1964).

In addition, this drug was demonstrated to lower NAD and ATP (Park and Shin, 1991), purine and pyrimidine nucleotide, and poly (ADP-ribose) synthesis (Hunting *et al.*, 1985). Furthermore, 6-AN gave rise to a marked hyperglycemia effect and a significant reduction of alkaline phosphatase (Park *et al.*, 1990) and glyceraldehyde-3-phosphate dehydrogenase and malic enzyme activities metabolically associated with energy generation (Park and Shin, 1991). Since little information is available regarding effects of 6-AN on overall nucleotide and catecholamine metabolism, the current investigation was carried out to determine effects of 6-AN on levels of glycogen, nucleotides and catecholamines in the brain of mouse.

## Materials and Methods

### Materials

All chemicals used were purchased from Sigma Chemical Co. st. Louis, Mo. and were of the highest purity grade available.

### Treatment of animals

Male mice (ICR strain) weighing 20-25 g were randomly divided into two groups, 30 control and 30 test. Mice were bred under controlled conditions (defined light-dark rhythm, 10 h light, 14 h dark, 26°C, 60% air humidity). All animals were allowed free access to food and water. Initially

mice in the test group received intraperitoneally the injection of 1 ml 6-AN (15 mg/kg of body weight), whereas those in the control group received 1 ml saline solution (0.9% NaCl).

At designated time intervals immediately following the administration of 6-AN or saline solution the animals were sacrificed by decapitation and the blood was collected and the brain was quickly chiselled out of the skull. The serum was obtained by centrifuging the blood at 1,000 × g for 10 min. The serum and brain were stored at −70°C until further analysis.

**Determination of Glucose and Glycogen**

The determination of glucose was essentially based on the oxidation of glucose by glucose oxidase method (Falis, 1963). glycogen was measured by the method of Hutchins and Rogers (1970).

**Determination of Nucleotides**

The analysis of nucleotides was performed using reverse phase HPLC with UV-absobance detector (Brown *et al.*, 1982). After appropriate protion of brain tissue was homogenized in ice cold 5% $HClO_4$, the homogenate was centrifuged for 10 min at 10,000 rpm at 4°C. The supernatant was neutralized to pH 6.8-7.0 with 2 M $KHCO_3$ and precipitated $KClO_4$ was removed by centrifugation at 3,000 rpm at 4°C. Then the clear supernatant was stored at −70°C until analyzed.

In order to fractionate nucleotides from base and nucleoside contaminants, silica chromatography using Sepak silica cartridge was utilized. After the Sepak eluate was filtered through a 0.2 $\mu$m column guard HV filter (Milipore, USA), the resultant free nucleotides were then chromatographed on $\mu$ Novapak $C_{18}$ column.

The chromatography was performed at a flow rate of 0.6 ml/min under 1,200 p.s.i. and monitored at 254 nm with recorder sensitivity of 100 mV. The mobile phase consists of two buffer solutions: buffer solution I, 2.5% $CH_3CN$/65 mM $KH_2PO_4$ containing 1 mM tetrabutyl ammonium phosphate, and buffer II, 5% $CH_3CN$/65 mM $KH_2PO_4$ contaning 1 mM tetrabutyl ammonium phosphate.

**Determination of Epinephrine and Norepinephrine**

HPLC with amperometric detection was employed to measure epinephrine and norepinephrine following purification on alumina (Kawasaki *et al.*, 1989). The brain tissue was homogenized in cold 5% $HClO_4$ for 2 min and the homogenate was centrifuged at 15,000 × g for 20 min. The clear supernatant was absorbed on acid-washed alumina and norepinephrine was eluted with 0.05 M $H_3PO_4$ and 0.1 mM sodium metabisulfite. The chromatography consisted of Model 45 delivery system (Waters Assoc., Milford, Mass., U.S.A.), a manual injector and $\mu$ Bondapak $C_{18}$ column. The mobile phase was composed of 8 parts of methanol and 92 parts of 0.1 M $NaH_2PO_4$. 0.1 mM EDTA and 1 mM sodium octylsulfate, pH 5.5. Norepinephrine was detected by means of carbon paste electrode at a sensitivity of 5 $nAV^{-1}$ and at +7.0 V against an Ag/AgCl reference electrode.

**Statistical Analysis**

The student t-test was employed for the determination of statistical significance (Smith, 1962). Differences between means which give probability value(p) smaller than 0.05 are considered to be significant.

## Results and Discussion

Male ICR mice, which are administered the antimetabolite 6-aminonicotinamide start showing a slight reduction in body weight after 24 h experiment whereas that of the control group remained virtually unchanged (Fig. 1). Likewise, the blood glucose level in the control group remained constant throughout the experiment. However, the blood glucose level in 6-An administrered mice starts increasing at 5 h, eventually reaches the maximum value at 13 h and decreases until 24 h of experiment.

This type of hyperglycemia was clearly demonstrated in other species such as rabbit (Park *et al.*, 1990) and quail (Park and shin, 1991) when animals were administered the same level of 6-AN (15 mg/kg body weight) on the basis of body weight

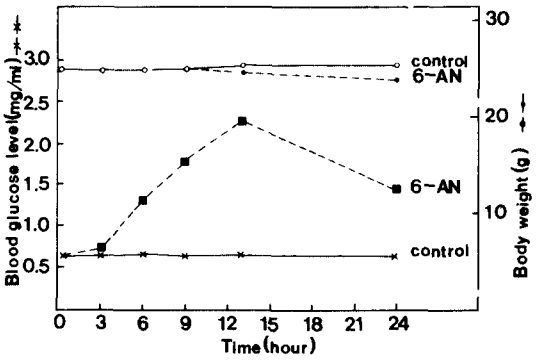


**Fig. 1.** Effect of 6-aminonicotinamide (6-AN) on body weight change and blood glucose level in mouse. Saline or 6-AN solution was administrated intraperitoneally as described in 3Meterials and Methods". Body weight; control (○ -- ○), 6-AN (● -- ●). Blood glucose; control. Valuse are given as means ± S.D. of 7 mouse.

(Park *et al.*, 1991; Park and Shin, 1991). 6-AN was strongly believed to trigger either the decrease of insulin (Tanese *et al.*, 1983) or the stimulation of epinephrine release from adrenal medulla which may be responsible for the enhancement of blood glucose concentration. Similarly, the level of glucose in the brain increased starting from 9 h after 6-AN administration.

Changes in the levels of glycogen in the brain at different time after 6-AN admimistration were presented in Table 1. 6-AN appeared to decrease glycogen concentration in the brain after 3 h and this lowering effect continued to stay on up to 24 h. In particular, glycogen content is significantly lower at 11 h ($p < 0.01$). The rapid breakdown of brain glycogen caused by 6-AN could also make an additional contribution to the enhancement of brain glucose level (Baba *et al.*, 1978). Thus it can be postulated that 6-AN stimulates glycogenolysis and blocks glycolysis through the inhibition of glyceraldehyde-3-phosphate dehydrogenase in Embden-Mayerhoff pathway and pentose phosphate pathway in the brain (Kolbe *et al*, 1977).

The effect of 6-AN on the levels of epinephrine and norepinephrine in the brain was also examined (Table 2). 6-AN was found to increase the concentration of epinephrine in the brain at 3 h and 9 h, but it was found to reduce epinephrine concentration at 13 h and 24 h. whereas the levels of norepinephrine was not significantly

**Table 1.** Effect of 6-aminonicotinamide (6-AN) on levels of glucose and glycogen in mous brain.

| Treatment | Glucose (mmol/g brain) | Glycogen (mg/g brain) |
|---|---|---|
| Control | 0.60 ± 0.08 | 0.51 ± 0.03 |
| 6-AN treatment | | |
| 3 h | 0.62 ± 0.07 | 0.45 ± 0.03 |
| 5 h | — | 0.46 ± 0.04 |
| 9 h | 1.83 ± 0.1*** | 0.43 ± 0.03 |
| 11 h | 3.10 ± 0.2*** | 0.37 ± 0.04** |
| 24 h | — | 0.44 ± 0.33 |

Values are given as means ± S.D. of 7 mice.
**$p < 0.01$, when compared with control.
***$p < 0.001$, when compared with control.

**Table 2.** Effect of 6-aminonicotinamide (6-AN) on levels of epinephrine and norepinephrine in mous brain.

| Treatment | Catecolamine (n mol/g brain) | |
|---|---|---|
| | Epinephrene | Norepinephrine |
| Control | 0.07 ± 0.01 | 1.79 ± 0.16 |
| 6-AN treatment | | |
| 3 h | 0.42 ± 0.05** | 1.52 ± 0.25 |
| 9 h | 0.14 ± 0.02** | 1.86 ± 0.18 |
| 13 h | 0.04 ± 0.01** | 1.61 ± 0.24 |
| 24 h | 0.02 ± 0.01** | 0.73 ± 0.15** |

Valuese are given as means ± S.D. of 5 mice.
**$p < 0.01$, when compared with control.

changed at all time intervals examined except 24 h. The increase of epinephrine in the initial time is due to release from the adrenal medulla (Kawasaki *et al.*, 1989).

Our current results may indicate that the hyperglycemic action of 6-AN in the brain and blood is triggered by releasing of epinephrine from the adrenal medulla. 6-AN may interfere with the biosynthesis of catecholamines, which blocks the pentose phosphate pathway by decreasing the supply of reducing equivalents in the form of NADPH which are necessary for the synthesis of tetrahydropteridine cofactors of tyrosine hydroxylase (Jansson *et al.*, 1977). A change in the metabolic activity of actecholamine -containing neurons in the brain can also influence cerebral glycogen levels (Hutchins and Rogers, 1973). This effect was mediated by an increase of

epinephrine release from the adrenal medulla and not by direct sympathetic innervation of the $\beta$ cell (Frohman *et al.*, 1973). Further studies will be needed to shed light on an overall glycogen metabolism in relation to epinephrine secretion under metabolic stresses.

A typical chromatogram of a solution containing standard amounts of authentic nucleotides is presented in Fig. 1. The nucleotides are eluted in the order of decreasing polarity, the times being calculated as 2.5 min for CMP, 3.4 min for CDP, 4.1 min for AMP, 5.0 min for UMP, 5.7 min for GMP, 9.2 min for CTP, 9.6 min for ATP, 13.8 min for GDP and 14.7 min for UTP. The complete elution of all nucleotides was accomplished within 15 minutes of injection. Almost identical patterns of separation were observed with brain nucleotide extract.

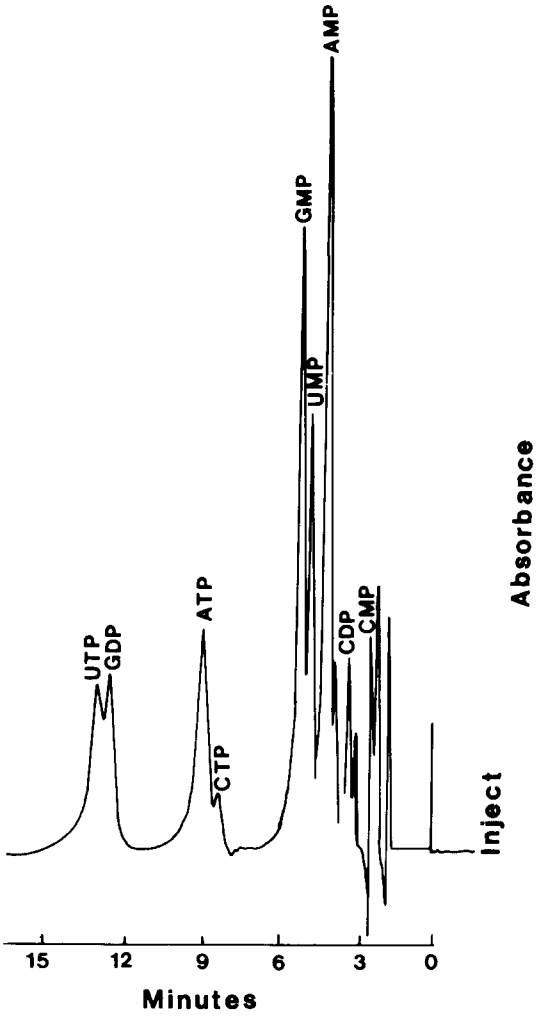


**Fig. 2.** A typical chromatogram of authentic standard nucleotides. Ten $\mu$l containing 100 n moles each of nucleotides was injected. Experimental details were described in methods.

The nucleotides present in the brain tissues were identified by comparison with retention times of each peak of standard solution.

Effects of 6-AN on level of AMP, CMP, CTP, UMP and UTP in mice brain were presented in Table 3. AMP and CMP levels were not affected by 6-AN forms an abnormal NAD-analogue which consequently inhibits with the production of ATP (Sheffield and Seegmiller, 1980). In addition, our previous results strongly suggest that a reduction of NAD concentration along with a low activity of glyceraldehyde-3-phosphate dehydrogenase in the pectoral muscle of quail could be a major contributing factor for the subsequent reduction of ATP (Park and Shin, 1991).

In de novo synthesis of UMP, ATP is utilized as one of substrates in the initial reaction (Voet and Voet, 1990). Thus the low level of UMP may arise from the lower supply of ATP available in the brain. In the same vein, the low UMP concentration would presumably account for the low UTP concentration. As demonstrated in Table 1, it can be speculated that this low level of UTP could be responsible for the decrease of glycogen contents in mice brain since the first step in glycogen synthesis requires UTP as one of major substrates (Voet and Voet, 1990). Also the decreased level of UTP

**Table 3.** Effect of 6-aminonicotinamide (6-AN) on levels of nucleotides in mouse brain.

| | Nucleotide ($\mu$mol/g brain) | | |
|---|---|---|---|
| | Control | 6-An 7 h | 6-AN 13 h |
| AMP | 3.23 ± 0.1 | 3.10 ± 0.3 | 2.92 ± 0.2 |
| ATP | 3.29 ± 0.14 | 2.69 ± 0.22* | 1.70 ± 0.2** |
| CMP | 3.68 ± 0.17 | 3.60 ± 0.1 | 3.14 ± 0.29 |
| CTP | 0.43 ± 0.05 | 0.35 ± 0.07 | 0.31 ± 0.04* |
| UMP | 1.99 ± 0.07 | 2.05 ± 0.07 | 1.31 ± 0.06* |
| UTP | 2.05 ± 0.07 | 1.84 ± 0.04 | 1.32 ± 0.16* |

Values are given as means ± S.D. of 7 mice.
*$p < 0.05$, when compared with control.
**$p < 0.01$, when compared with control.

would lead to the decrease of CTP level since CTP is formed from UTP in the biosynthetic pathway of pyrimidine (Voet and Voet, 1990). Furthermore, the reduced concentration of ATP, CTP and UTP caused by 6-AN could eventually lead to the inhibition of RNA synthesis (Ritter *et al.*, 1975; Knoll-Kohler *et al.*, 1980).

In conclusion, 6-AN appears to exert an inhibitory action on energy metabolism as well as nucleotide metabolism.

## References


Baba, A., T. Baba, T. Matsuda, and H. Iwata, 1978. Centrally mediated hyperglycemia by 6-aminonicotinamide. *J. Nutr. Sci. Vitaminol.* **24:** 429-435.

Balaban, C. D., 1985. A critical review of central 3-acetylpyridine neurotoxicity. *Brain Res. Reviews* **9:** 21-42.

Brown, E. G., R. P. Newton, and N. M. Shaw, 1982. Analysis of the free neucleotide pools of mammalian tissues of high pressure liquid chromatography. *Anal. Biochem.* **123:** 378-388.

Falis, F. W., 1963. Standard Methods of clinical Chemistry (Seligson, D. ed.). vol. 4, *Academic Press,* New York, pp. 401.

Frohman, L. A., E. E. Muller, and D. Cocchi, 1973. Central nervous system mediated inhibition of insulin due to 2-deoxyglucose. *Horm. Metab. Res.* **5:** 21-26.

Herken, H. and V. Neuhoff, 1964. Spektrofluorometrische bestimmung des einbaus von 6-aminonicotisauremid in die oxydiertex pyridinnuclestide der niere. *Naunyn Schmidebergs Arch. Exp. Path. Phamark.* **247:** 187-191.

Hunting, D., B. Gowans, and J. F. Henderson, 1985. Effects of 6-aminonicotinamide on cell growth, poly (ADP-ribose) synthesis and nucleotide metabolism. *Biochem. Pharmacol.* **34:** 3999-4003.

Hutchins, D. A. and K. J. Rogers, 1970. Effect of depletion of cerebral catecholamines on the concentration of glycogen and on amphetamine-induced glycogenolysis in the brain. *Brit. J. Pharmacol.* **48:** 19-29.

Jansson, S. E., J. Gripenberg, and M. Harkoen, 1977. The effect of 6-aminonicotinamide blockade of the pentose phosphate pathway on catecholamine in the adrenal medulla, superior cervical ganglion, hypothalamus and synaptosomal fractions. Acta Physiol. Scand. **99:** 467-475.

Kawasaki, T., T. Higuchi, K. Imai, and O. S. Wong, 1989. Determination of dopamine, norepinephrine, and related trace amines. *Anal. Biochem.* **180:** 279-285.

Knoll-kohler, E., F. Wonjnorowicz, and H. J. Sarkander, 1980. Correlated changes in neural cerebral rat brain RNA systhesis by 6-aminonicotinamide. *Exp. Brain Res.* **38:** 173-179.

Kolbe, H., K. Keller, K. Lange, and H. Herken, 1977. Glucose metabolism in C-1300 neuroblastoma cells after inhibition of hexose monophosphate pathway. *Naunyn-Schmiedeber's Arch. Pharmacol.* **296:** 123-130.

Park, I. K., C. S. Lee, S. H. Lee, Y. K. Song, and S. Shin, 1990. Effect of 6-aminonicotinamide on the levels of some metabolites and related enzymes in rabbit serum, *Korean J. Zool.* **33:** 493-498.

Park, I. K., Y. D. Kwon, J. H. Lee, and S. Shin, 1991. Effect of 6-aminonicotinamide on levels of enzymes and metabolites in mice. (in press) *korean J. Gerontol.*

Park, I. K. and S. Shin, 1991. Effect of an antimetabolite 6-aminonicotinamide on levels of coenzymes and enzymes in various tissues of quail. (in press) *Korean Bicohem. J.*

Ritter, E. J., W. J. Scott, and J. G. Wilson, 1975. Inhibition of ATP synthesis associated with 6-aminonicotinamide. *Teratology* **12:** 233-236.

Sheffield, V. C. and R. E. Seegmiller, 1980. Impaired energy metabolism as an initial step in mechanism for 6-aminonicotinamide-induced limb malformation. *J. Embryol. Exp. Morph.* **59:** 217-222.

Smith, G. M., 1962. A Simplified Guide to Statistics. Holt, Reinfart, and Winston, *New York,* pp. 35-50.

Tanese, T., M. Nariyama, H. Yamada, I. Matsuda, T. Sasaki, A. Tsuruoka, K. Ishi, K. Ustunomoya, Y. Ikeda, and M. Abe, 1983. Hyperglycemia and inhibition of glucose-induced insulin release in 6-aminonicotinamide-treated rats. *Folia Endocrinol.* **59:** 1752-1758.

Voet, D. and J. G. Voet, 1990. *Biochemistry, John Wiley and Sons, New York,* pp. 740-768.


(Accepted November 30, 1991)

## 항 대사물질 6-aminonicotinamide(6-AN)가 생쥐 뇌의 탄수화물, 뉴클레오티드 및 카테콜라민 대사에 미치는 영향

정현근* · 박인국(*대전전문대학 임상병리학과, 동국대학교 농업생물학과)

항 대사물질 6-aminonicotinamide(6-AN)가 생쥐뇌의 포도당, 글리코겐, 뉴클레오티드 및 카테콜라민 대사에 미치는 영향에 관하여 조사하였다. 6-AN투여 13시간 후 혈당량은 증가하기 시작하였고 뇌의 포도당은 7시간 후 증가하였다. 글리코겐 농도는 투여 후 11시간 후를 제외한 모든 시간에서 변화하지 않았다. 에피테프린은 투여 후 처음 3시간에 최대로 증가한 후 시간 경과와 함께 감소하여 24시간에는 유의하게 감소하였다. 반면에 노르에피네프린은 투여 후 24시간만 유의한 감소를 보일뿐 13시간까지는 유의한 변화를 보이지 않았다. ATP, CTP, UMP 및 UTP 함량은 현저히 감소하였으나 AMP와 CMP 함량은 변화하지 않았다.