Alterations of Plasma Neurotransmitters in Heroin and Amphetamine-addicted Subjects

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ABSTRACT

Thailand and many countries are facing a very serious health problem of heroin and amphetamine abuse. Both of drugs affect directly to neurotransmitters in our body. The alterations of plasma neurotransmitters in abusers are fewly reported in Thailand. This study was designed to investigate plasma neurotransmitters in heroin (H), heroin-combined amphetamine (HA), amphetamine-addicted (A) and amphetamine withdrawal (AW) subjects by using HPLC and ECD. The results showed significant decrease ($p<0.05$) of plasma DA in HA and A groups, no changes in H and AW subjects. Plasma 5-HT was significant increased in H ($p<0.05$) and HA ($p<0.01$), no changes in A and AW subjects were observed. These results suggest that heroin altered both dopaminergic and serotonergic systems while amphetamine affected only on the dopaminergic system.

บทคัดย่อ

การติดสารเสพติดชนิดแอมเฟตามีนและเฮโรอีนเป็นปัญหาต่อสุขภาพที่สำคัญมากปัญหาหนึ่งของประเทศไทยและหลายๆ ประเทศ โดยสารเสพติดทั้งสองชนิดนี้มีผลต่อการเปลี่ยนแปลงของระดับสารสื่อประสาท การศึกษาดังกล่าวในมนุษย์ยังมีผู้ทำการวิจัยน้อย จึงทำการวัดสารสื่อประสาทในพลาสมาของผู้เสพสารเสพติดชนิดเฮโรอีน (H), สารผสมเฮโรอีนและแอมเฟตามีน (HA), สารแอมเฟตามีน(A) รวมทั้งกลุ่มของแอมเฟตามีนที่อยู่ในระยะถอนยา (AW) โดยใช้ High Performance Liquid Chromatography (HPLC) ร่วมกับ electrochemical Detector (ECD) ผลการศึกษาพบว่ามีการลดลงของ dopamine (DA) ($p<0.05$) ในกลุ่ม A และ HA แต่ไม่พบการเปลี่ยนแปลงในกลุ่ม H และ AW มีการเพิ่มขึ้นสูงของระดับ 5-hydroxytrytamine (5-HT) ในกลุ่ม HA ($p<0.01$) และ H ($p<0.05$) แต่ไม่พบการเปลี่ยนแปลงในกลุ่ม A และ AW การศึกษานี้แสดงให้เห็นว่า ผู้ที่เสพติดสารเฮโรอีนมีการเปลี่ยนแปลงทั้งระบบ dopamine และ serotonin ในขณะที่พบการเปลี่ยนแปลงเฉพาะระบบ dopamine ในผู้เสพสารเสพติดแอมเฟตามีน

Key words: Heroin, Amphetamine, Neurotransmitter, Abuse
คำสำคัญ: เฮโรอีน แอมเฟตามีน สารสื่อประสาท เสพติด

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Introduction

The problem of drug addicts has increased in Thailand. The total numbers of patients at the Thanyarak Hospital were increased from 7,595 cases in 1989 to 10,661 cases in 1995. Most of patients were labourers or unemployed. Nowadays student addicts were increased from 1.3% in 1995 to 4.0, 8.0 and 17.0% in 1996-1998 respectively. Heroin addiction was markedly decreased from 80.6-92.4% to 38% in 1998. Methamphetamine addiction was markedly increased from 0.4%to 51.5% (Verachai et al., 2001). Drug abuse directly affects to patients and then induces to family and social problems subsequently. Heroin is the most important drug that is abused and more potent than opioid 50-100 folds (Unakelarb, 1998). Opioid drugs are used primarily for the treatment of severe pain, later it acts as CNS suppressants resulting in analgesia and positive change in mood (euphoria). Amphetamine is one of the most potent sympathomimetic amines in stimulating CNS. It stimulates the medullary respiratory center and produces the signs of stimulation of the CNS. These effects are thought to be due to cortical stimulation of the reticular activating system (RAS) which results in increasing of motor activity and mental alertness, decreasing of sense of fatique and mild euphoria. Both of drugs produce alterations of brain function that result in positive changes in mood (euphoria) and others. These changes are affected by interactions with neurochemical processes that usually increases the action of endogenous neurotransmitters. They increase extracellular fluid dopamine level in nucleus accumbens (Nac) (Herz, 1998). However the alteration of plasma neurotransmitter in abuser with mixed drugs between heroin and amphetamine is fewly reported in Thailand. Time course of withdrawal heroin and amphetamine addicted subjects is important in index craving states. It is very interesting to study the alteration of plasma neurotransmitters in various withdrawal periods of those abusers, index the brain function and may be contribute to the investigation of mechanisms of craving and drug addiction.

Materials and Methods

Human studies and subject selection

Human subjects consisted of parenteral heroin abusers and inhalation amphetamine and amphetamine withdrawal abusers from the Thanyarak Hospital. All subjects were males, aged between 16-45 years, and none had recent infections, active inflammatory disease, a positive HIV or HbsAg. All subjects were divided into 5 groups; the normal control group (C), the heroin-addicted group (H), the heroin-combined amphetamine-addicted group (HA), the amphetamine-addicted group (A) and the 1 day amphetamine withdrawal group (AW1), the 2-4 days amphetamine withdrawal group (AW2) and the 5-20 days amphetamine withdrawal group (AW3). Studies have been reviewed and approved by the ethic committee of the Khon Kaen University, based on the Declaration of Helsinki and written informed consent was obtained from all subjects.

Plasma neurotransmitters study

The levels of plasma neurotransmitters of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) were measured by using high performance liquid chromatography with electrochemical detector (HPLC & ECD). All subjects were collected 5 ml of blood samples. Whole blood was centrifuged at 1500 rpm. Took 1 ml of plasma for protein precipitation with 50 μl of 18.1 M perchloric acid, 50 ml of 5 μM $Na_2O_5S_2$ and 50 μl of 500 ng/ml of internal satadard (ISOPR), vortex and let it precipitated for at least 2 hours, centrifuged at 15000 rpm. The clear supernatant was then passed through filter before injected 50 μl in HPLC system. Mobile phase was composed of 100 vols of 0.1 M monobasic sodium phosphate containing 1 mM disodium EDTA and 1 mM sodium octanesulfonate mixed with 4 vols of acetonitrile and 4 vols of methyl alcohol (adjusted pH 4 with saturate citric acid). All separations were performed isocratically at flow rate 1.2 ml/min. The potential od ECD was maintained at 0.8 V&Ag/AgCl reference electrode.

Statistical analysis. Result of mean were expressed as mean values ± standard error (SEM). Data analysis was carried out with one-way ANOVA with appropriate post hoc tests (Duncan's Multi-Range). A p-values of less than 0.05 and 0.01 were considered significant and highly significant, respectively. The recovery of all neurotransmitters from plasma approximately was 70%.

Results

The baseline characteristics of all subjects

The baseline characteristics of àll subjects in the study were shown in Table 1. The average ages of the all subjects ranged from 21 to 28 years. The dosage range of heroin that used by heroin and heroin-combined amphetamine addicts was 150-600 mg/day. The dosage of amphetamine that used by amphetamine addicts, amphetamine withdrawal subjects and heroin combined amphetamine subjects was 90-180 mg/day. The duration of heroin abuse ranged from 5 to 6 years. The duration of amphetamine abuse ranged from 3 to 6 years. Urine morphine concentration more than 5.5 μg/ml was detected in the heroin and heroin-combined amphetamine-addicted groups. All subjects were negative test of HIV and HBsAg.

Chromatograms of standard samples and plasma neurotransmitters in all subjects

Chromatograms of 250 ng NE, DOPAC, DA, 5-HIAA, HVA and 5-HT samples were shown the peaks at various retention times. Isoproterenol (ISOPR) was acted as internal standard. Figure 1 showed the chromatogram of 250 ng of all standard samples.

Chromatograms representation of 250 ng internal standard (ISOPR) in control (C) plasma, heroin-addicted (H) and amphetamine-addicted (A) plasma were shown in the same manifestation peak of standard samples. The chromatograms representation of C, H and A plasma were shown in Figure 2, 3 and 4, respectively.

Alterations of plasma neurotransmitters

The plasma levels of DA, DOPAC and HVA in control group, heroin-addicted (H), heroin-combined amphetamine-addicted (HA), amphetamine-addicted, amphetamine withdrawal subjects are shown in Figure 5, 6 and 7, respectively.

There was no different of plasma DA levels in heroin-addicted group when compared to normal subjects. However there was significant decrease ($p<0.05$) in DA level of heroin-combined amphetamine-addicted subjects when compared with control. The DA level of heroin-combined amphetamine addicts was 21.68 ± 6.9 ng/ml while it was 56.29 ± 9.42 ng/ml in control subjects. Plasma DA level in amphetamine-addicted (A) subjects was significant decrease ($p<0.05$) when compared to control (C) group. While there was no different among amphetamine withdrawal (AW) groups when compared to control group.

To further investigation of plasma DOPAC levels in all groups, there was no different among heroin-addicted group, heroin-combined amphetamine-addicted group when compared to control groups. However, ratio of plasma DOPAC /DA was shown significant decrease ($p<0.05$) in heroin-addicted group and heroin-combined amphetamine-addicted group when compared to control group. In amphetamine-addicted (A), 1-day (AW1), 2-4 days (AW2) and 5-20 days (AW3) amphetamine withdrawal subjects were shown significant decrease ($p<0.05$) in DOPAC and DOPAC/DA ratio when compared to control (C) group. Plasma DA level in A group was shown 34.11 ± 7.39 ng/ml ($p<0.05$) when compared to control group, 56.29 ± 9.42 ng/ml. The mean values ± SEM of plasma DOPAC in C, A, AW1, AW2 and AW3 groups were 40.64 ± 3.55, 30.23 ± 1.80, 32.31 ± 3.76, 24.71 ± 2.49 and 24.59 ± 2.88 ng/ml respectively. The ratios of DOPAC/DA in C, A, AW1, AW2 and AW3 groups were calculated at 0.34 ± 0.07, 0.12 ± 0.01, 0.01 ± 0.01, 0.01 ± 0.10 and 0.13 ± 0.03, respectively.

Homovanillic acid (HVA), one of the metabolic products of DA was investigated. There was significant increase ($p<0.05$) of plasma HVA in heroin and heroin-combined amphetamine groups when compared to control group. The level of plasma HVA in heroin and heroin-combined amphetamine group were

54.14 ± 9.9 and 42.62 ± 18.55 ng/ml while the control group was 29.13 ± 3.61 ng/ml. The ratio of plasma HVA/DA was shown significant decrease ($p<0.05$) only in heroin-combined amphetamine-addicted subjects when compared to control group. The mean values ± SEM of the HVA/DA ratio in control (C), heroin (H) and heroin-combined amphetamine (HA) groups were 1.21 ± 0.21, 1.33 ± 0.29 and 0.31 ± 0.12, respectively. There was no different of HVA/DA ratio in the heroin group when compared to control group. Plasma HVA levels in A, AW1, AW2 and AW3 groups were 73.3 ± 9.24, 70.93 ± 9.78, 71.31 ± 11.17 and 97.59 ± 12.18 ng/ml, respectively. Those values were significantly increase ($p<0.05$) when compared to the value of control subjects, 29.13 ± 3.61 ng/ml. The ratios of HVA/DA in A, AW1, AW2 and AW3 groups were calculated at 2.31 ± 0.38, 1.64 ± 0.13, 1.70 ± 0.38 and 2.21 ± 0.54, respectively. Those ratios were significant increase ($p<0.05$) when compared to control group at the ratio of 0.78 ± 0.12.

Plasma serotonin (5-HT) and its metabolite, 5-HIAA was furthering investigated in this study (Figure 8 and 9). There was shown significant increase ($p<0.05$) of plasma 5-HT level in heroin group and highly significant increase ($p<0.01$) of plasma 5-HT level in heroin-combined amphetamine group when compared to control group. The mean values ± SEM of plasma 5-HT levels in heroin and heroin-combined amphetamine were 134.56 ± 27.44 and 289.47 ± 57.47 ng/ml. respectively, when compared to control group, 81.51 ± 8.77 ng/ml. The ratio of plasma 5-HIAA/5-HT was shown significant decrease ($p<0.05$) only in heroin-combined amphetamine-addicted subjects when compared to control group. The mean values ± SEM of the 5-HIAA/5-HT ratio in control (C), heroin (H) and heroin-combined amphetamine (HA) groups were 0.09 ± 0.02, 0.05 ± 0.02 and 0.02 ± 0.005, respectively. There was no different of 5-HIAA/5-HT ratio in the heroin group when compared to control group.

There were no different in the plasma 5 HT and 5 HIAA levels and the ratio of 5 HIAA/5 HT of amphetamine-addicted and all periods of amphetamine withdrawal subjects when compared to control subject. The plasma 5-HT levels of A, AW1, AW2 and AW3 groups were 115.52 ± 18.09, 104.94 ± 17.11, 107.09 ± 17.62 and 103.42 ± 16.06 ng/ml, respectively. The plasma 5-HIAA levels were 6.41 ±1.05, 4.26 ± 0.67, 4.59 ± 0.53, 5.02 ± 0.73 and 4.07 ± 0.80 ng/ml in C, in A, AW1, AW2 and AW3 groups, respectively. The ratio of 5-HIAA/5-HT was 0.09 ± 0.02 in control subject, while they were ranged from 0.04 ± 0.1 to 0.06 ± 0.02 in amphetamine-addicted and all periods of amphetamine withdrawal subjects.

Summary and Discussion

The dopamine system is implicated in the control of locomotion and cognition. The relative constitution of the various dopamine-related components is not well established mainly because drugs that target the dopaminergic system often lack selectivity. (Jaber M et al., 1997) The present study showed reduce in plasma DA levels but elevate in plasma HVA levels while no changes in plasma DOPAC levels in heroin-addicted and heroin-combined amphetamine-addicted subjects. The fall in extracellular DA concentration and marked reduction of DOPAC level were shown in amphetamine addicts. We has also studied subjects during withdrawal from chronic amphetamine used, and found a significant decrease in plasma DOPAC levels in 1 day, 2-4 days and 5-20 days of withdrawal periods but significant increase in HVA in all groups. These results are consistent with the study of Kooncumchoo (1997) that found lower plasma DA levels in heroin addicts. Moreover these study were consistent with the study of Rossetti et al. (1992) who reported significant decrease in extracellular DA in the ventral striatum for 5 days following this continuation of chronic amphetamine treatment in rat and the studies of Segal and Kuzenski (1997) found that rats, exposed to chronic doses of amphetamine, showed a significant decrease in extracellular DA levels in the caudate-putamen within three days to three weeks of drug withdrawal. Brain DA level in chronic amphetamine animals is decreased in the caudate nucleus in the study of Swerdlov NR et al (1991).

In the present study we found that plasma 5-HT levels in heroin-addicted and heroin-combined amphetamine subjects were elevated during the addiction period. The levels of 5-HT metabolite, 5-HIAA, were reduced during the addiction period. Serotonin turnover in heroin addicts and heroin-combined amphetamine addicts were reduced during the addiction period. These results was different from a microdialysis study in morphine-dependent rats, which reported that there was no changes in basal 5-HT released during chronic morphine administration (Silverstone et al., 1993). We also found no difference in plasma 5-HT and its metabolite, 5-HIAA and its turnover in amphetamine-addicted subjects and all periods of withdrawal. This result was inconsistent with the study of Kooncumchoo (1997) that found increase in plasma 5-HT levels in amphetamine withdrawal period and increase in plasma 5-HIAA levels during the addiction period and the study of Piazza (1991) found lower levels of 5-HT and 5-HIAA levels in chronic amphetamine administered rats.

In conclusion, the heroin addicts were shown the alterations of both dopaminergic and serotonergic systems while the amphetamine addicts seems to affect clearly in dopaminergic system.

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Table 1 Baseline characteristics (mean ± SEM) of control, heroin-addicts, heroin plus amphetamine addicts, amphetamine addicts and amphetamine withdrawal subjects.

Subject groups / Characteristics	Control (C)	Heroin addicts (H)	Heroin plus amphetamine addicts (HA)	Amphetamine addicts (A)	Amphetamine withdrawal subjects		
					(AW1)	(AW2)	(AW3)
Number of subjects	26	18	17	19	21	18	13
Sex	Male	Male	Male	Male	Male	Male	Male
Age (years)	28.96 ± 1.58	28.48 ± 1.31	27.07 ± 1.37	22.61 ± 1.03	23.0 ± 1.06	21.9 ± 0.9	21.8 ± 1.32
Urine morphine (μg/ml)	Negative	>5.50	>5.50	Negative	Negative	Negative	Negative
Urine amphetamine	Negative	Negative	Positive	Positive	Positive	Positive	Negative
Dosage of heroin abuse (mg/day)	-	150-600	150-600	-	-	-	-
Dosage of amphetamine (mg/day)	-	-	90-180	90-180	90-180	90-180	90-180
Duration of heroin abuse (years)	-	5.6 ± 0.73	5.53 ± 1.18	-	-	-	-
Duration of amphetamine abuse (years)	-	-	5.53 ± 1.18	3.17 ± 0.34	3.67 ± 0.71	3.3 ± 0.44	3.36 ± 0.64
HIV test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
HBsAg test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Withdrawal periods	-	-	-	-	1 day	2-4 days	5-20 days

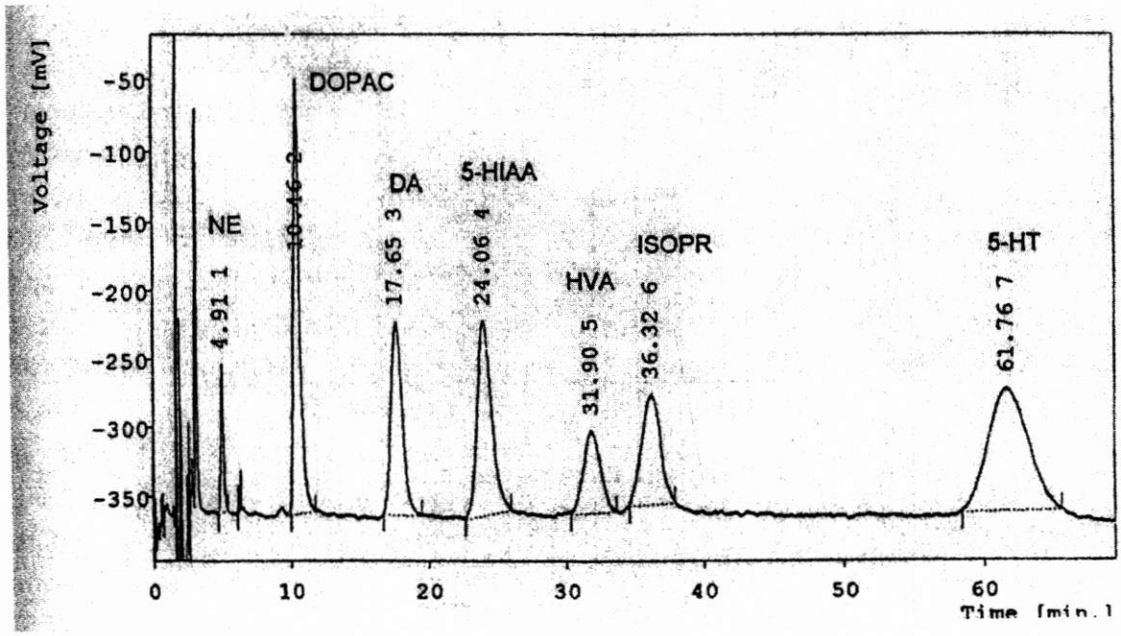

Figure 1 Chromatogram of 250 ng standard samples, the peaks of NE (norepinephrine), DOPAC (3, 4-dihydroxyphenylacetic acid), DA (dopamine), 5-HIAA (5-hydroxyindole-3-acetic acid), HVA (homovanillic acid), ISOPR (isoproterenol) and 5-HT (serotonin) were shown in various retention times.

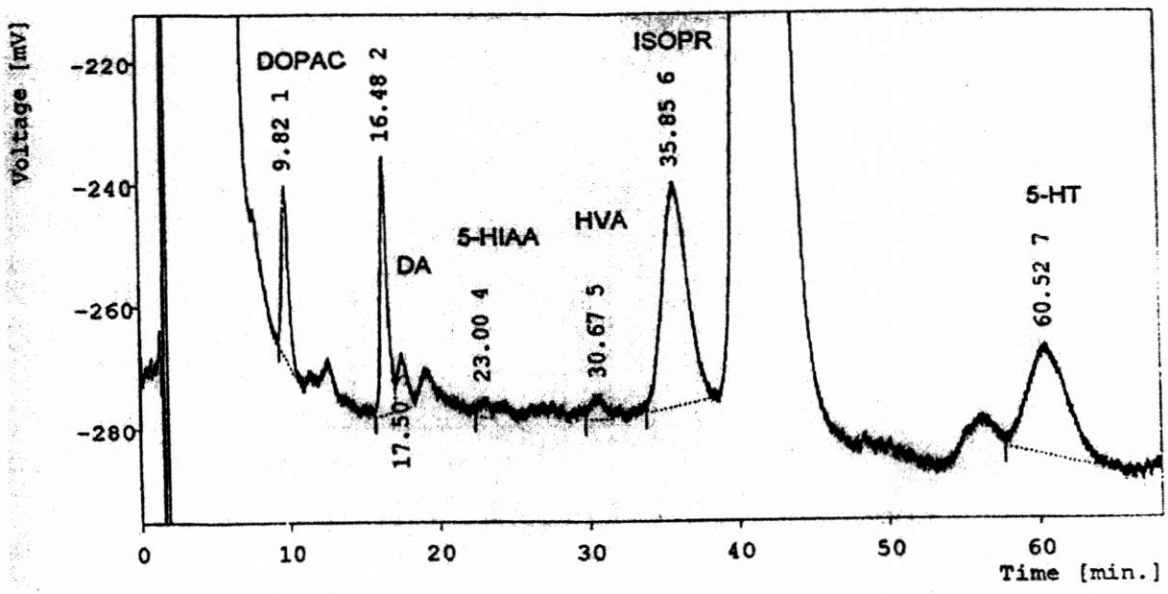

Figure 2 Chromatogram representation of control plasma added internal standard, the peaks of NE (norepinephrine), DOPAC (3,4-dihydroxyphenylacetic acid), DA (dopamine), 5-HIAA (5-hydroxyindole-3-acetic acid), HVA (homovanillic acid), ISOPR (isoproterenol) and 5-HT (serotonin) were shown in various retention times.

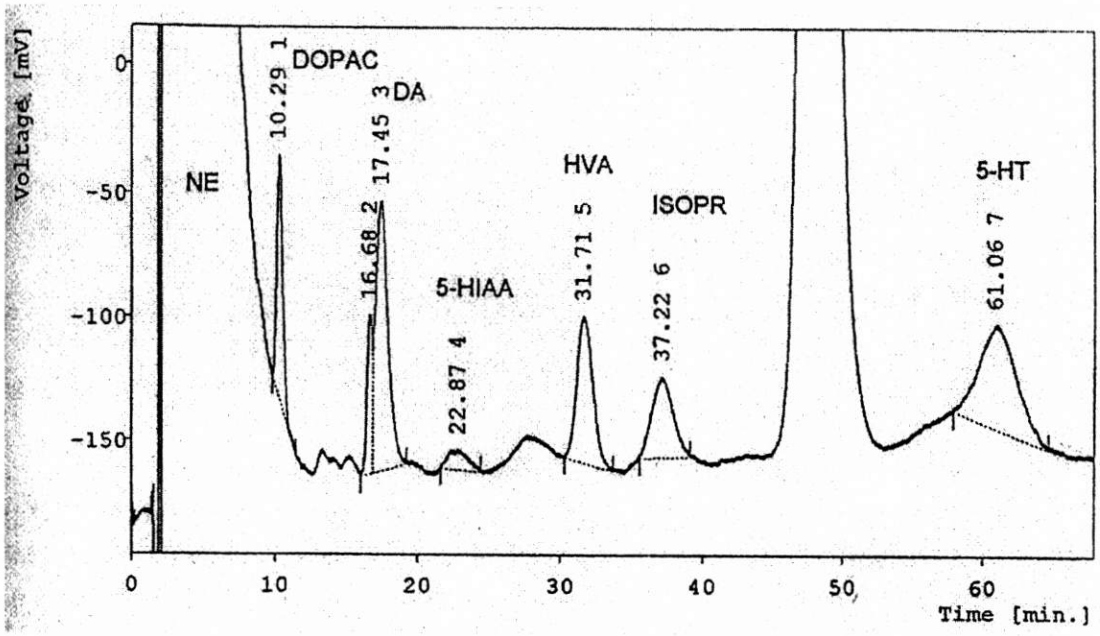

Figure 3 Chromatogram representation of heroin addicts plasma added internal standard, the peaks of NE (norepinephrine), DOPAC (3,4-dihydroxyphenylacetic acid), DA (dopamine), 5-HIAA (5-hydroxyindole-3-acetic acid), HVA (homovanillic acid), ISOPR (isoproterenol) and 5-HT (serotonin) were shown in various retention times.

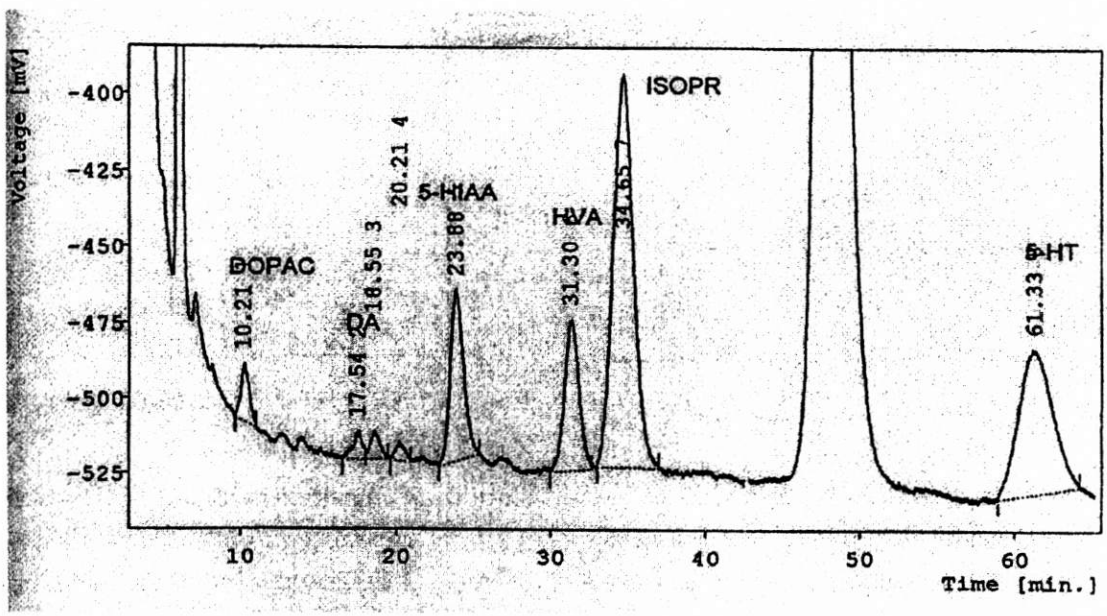

Figure 4 Chromatogram of representation amphetamine addicts plasma standard, the peaks of NE (norepinephrine), DOPAC (3,4-dihydroxyphenylacetic acid), DA (dopamine), 5-HIAA (5-hydroxyindole-3-acetic acid), HVA (homovanillic acid), ISOPR (isoproterenol) and 5-HT (serotonin) were shown in various retention times.

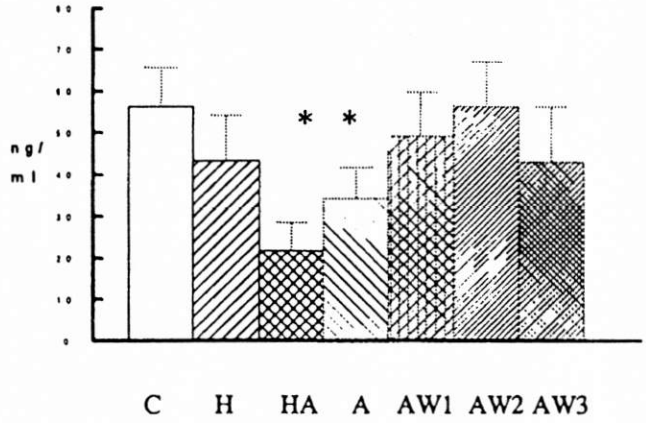

Figure 5 Comparison of plasma dopamine levels among control (C; n=26), heroin addicts (H; n= 18), heroin-combined amphetamine (HA; n=17), amphetamine addicts (A; n=19) 1 day amphetamine withdrawal (AW1; n= 21), 2-4 days amphetamine withdrawal (AW2; n=18) and 5-21 days amphetamine withdrawal (AW3; n= 13). * Significant difference from control group ($p<0.05$)

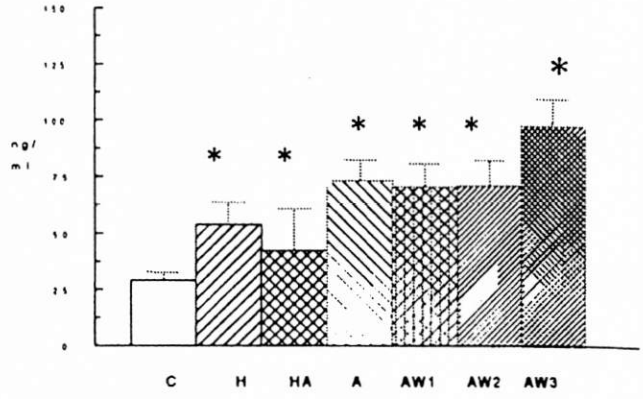

Figure 7 Comparison of plasma HVA levels among control (C; n=26), heroin addicts (H; n= 18), heroin-combined amphetamine (HA; n= 17), amphetamine addicts (A; n=19) 1 day amphetamine withdrawal (AW1; n= 21), 2-4 days amphetamine withdrawal (AW2; n=18) and 5-21 days amphetamine withdrawal (AW3; n= 13). * Significant difference from control group ($p<0.05$)

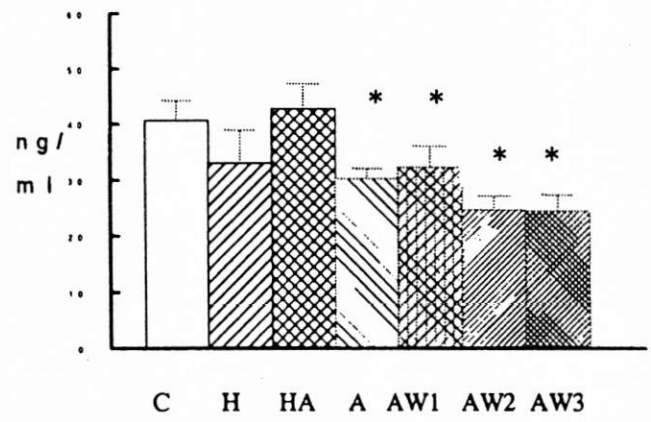

Figure 6 Comparison of plasma DOPAC levels among control (C; n=26), heroin addicts (H; n= 18), heroin-combined amphetamine (HA; n= 17), amphetamine addicts (A; n=19) 1 day amphetamine withdrawal (AW1; n= 21), 2-4 days amphetamine withdrawal (AW2; n=18) and 5-21 days amphetamine withdrawal (AW3; n= 13). * Significant difference from control group ($p<0.05$)

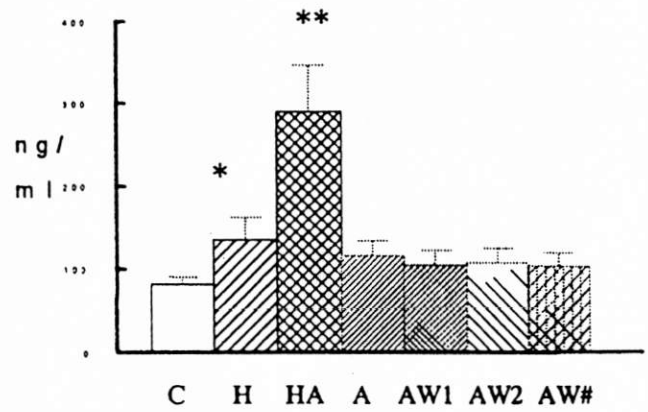

Figure 8 Comparison of plasma 5-HT levels among control (C; n=26), heroin addicts (H; n= 18), heroin-combined amphetamine (HA; n=17), amphetamine addicts (A; n=19) 1 day amphetamine withdrawal (AW1; n= 21), 2-4 days amphetamine withdrawal (AW2; n=18) and 5-21 days amphetamine withdrawal (AW3; n= 13). * Significant difference from control group ($p<0.05$) ** Significant difference from control group ($p<0.01$)

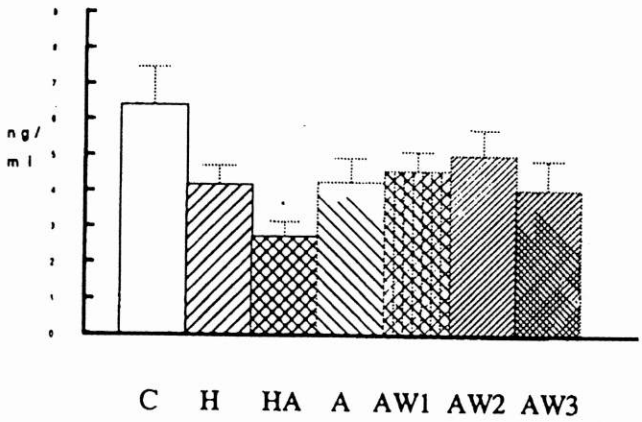

Figure 9 Comparison of plasma 5-HIAA levels among control (C; n=26), heroin addicts (H; n= 18), heroin-combined amphetamine (HA; n= 17), amphetamine addicts (A; n=19) 1 day amphetamine withdrawal (AW1; n= 21), 2-4 days amphetamine withdrawal (AW2; n=18) and 5-21 days amphetamine withdrawal (AW3; n= 13). * Significant difference from control group ($p<0.05$)