CHAPTER IV

RESULTS AND DISCUSSION

1. Antidepressant activity in animal models

CU 763-14-07 and CU 763-14-10 are the chemically synthesized agents that were used in the primary screening for antidepressant properties. Behavioral despair (forced swimming) test, locomotor activity and rotarod test are the animal models that were chosen in this study.

Forced swimming test is one of the most widely used animal models of depression (Geyer and Markou, 1995). It is a behavioral model developed to predict the efficacy of antidepressant drugs. Few studies have been aimed at evaluating the mechanism of action of antidepressants by this model (Redrobe, MacSweeney, and Bourin, 1996).

Locomotor activity test and rotarod test were used to assess whether changes in immobility time (in forced swimming model) were associated with changes in motor behavior, motor coordination or fatigue resistance.

Test substances were administered to all experimental animals by intraperitoneal injection.

1.1 Behavioral despair (forced swimming) test

The purpose of this test was to determine anti-immobility effect of substances. The mean of immobility time was used as an indicator in this model. The posture of mice which judged to be immobile was whenever it remained floating passively in the water in a slightly hunched but upright position, its head just above the surface (Persolt *et al*,1979), as shown in Figure 10.

All groups of mice were forced to swim for 15 min in a restricted space from which they cannot escape. Twenty-four hours later, they were measured the immobility again. Mice were pretreated with agents/or vehicle at 30 min before the duration of immobility was recorded during the last 5 min of the 6-min testing period.

Experimental results obtained with amitriptyline, pargyline, CU 763-14-07 and CU 763-14-10, are shown in Table 5, Figure 13, and Figure 14. The result reveals that amitriptyline (30 mg/kg i.p.) and pargyline (150 mg/kg i.p.) greatly reduced the immobility time. A decrease in immobility was also observed with the two synthesized agents, CU 763-14-07 and CU 763-14-10. For both of them, a statistically significant effect was observed at 10 mg/kg and/or 20 mg/kg.

Pretreatment with amitriptyline (30 mg/kg i.p.) produced a decrease in the immobility time comparing with the saline-treated controls. This result confirmed the observation that amitriptyline possessed an antidepressant property in mice (Persolt, Bertin, Deniel, and Jalfre, 1977). Amitriptyline is the tricyclic antidepressant and it has been known to be a mixed amine-reuptake inhibitor. It inhibited both NE and 5-HT reuptake into the nerve terminal, therefore, blockage of the NE and 5-HT uptake pump may relate with the anti-immobility effect of this drug.

Pretreatment with pargyline (150 mg/kg i.p.) caused changes in the forced swimming test. It appeared to decrease immobility time. Pargyline has been known to be a nonselective monoamine oxidase inhibitor. MAO enzymes are responsible for oxidative deamination of endogenous monoamines such as noradrenaline, dopamine, and serotonin. Thus, the effect of pargyline in decreasing immobility may relate with the inhibition of MAO enzymes and potentiation of monoamines in the brain.

The result from experiment of antidepressants (Amitriptyline and Pargyline) revealed that they have positive effect to this behavioural despair test so the immobility time of unknowns can be assessed the antidepressant activity by this test.

CU 763-14-07 produced significant effects at doses of 10 and 20 mg/kg (Figure 13 and Table 5) while CU 763-14-10 decreased the duration of immobility only at a dose of 20 mg/kg. These results suggested that CU 763-14-07 and CU 763-14-10 might have an antidepressant property as determined by a forced swimming test. In addition, CU 763-14-07 had anti-immobility effect in dose that lower than CU 763-14-10 so CU 763-14-07 may be better index of antidepressant activity than CU 763-14-10.

In the former studies (Ratanachol, 1997; Wongsomnuk, 1998), CU 763-14-07 and CU 763-14-10 were shown to inhibit MAO activity of both MAO-A and MAO-B *in vitro*. It is conceivable that inhibition of MAO enzymes in the brain would lead to increased brain monoamine levels and thus decreased immobility time. Therefore, it is possible that antidepressant-like effects of CU 763-14-07 and CU 763-14-10 may be due to inhibition of monoamine oxidase enzymes.

Table 5. Effects of CU 763-14-07, CU 763-14-10 and some antidepressant drugs on the total duration of immobility time. Drugs were given in i.p. injections thirty minutes before the test. Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Treatment and Dose	No. of mice Per Dose	Immobility time (sec)	
		mean ± S.E.M.	% Control
CU 763-14-07			
- Control	8	267.75 ± 4.66	100.00 ± 1.74
- 10 mg/kg i.p	11	219.36 ± 14.01**	81.93 ± 5.23
- 20 mg/kg i.p	11	246.54 ± 6.17*	92.08 ± 2.31
- 40 mg/kg i.p	9	256.44 ± 5.70	95.78 ± 2.13
CU 763-14-10			
- Control	9	267.00 ± 3.77	100.00 ± 1.42
- 10 mg/kg i.p	12	254.50 ± 4.38	95.32 ± 1.64
- 20 mg/kg i.p	10	231.70 ± 13.88*	86.78 ± 5.20
- 40 mg/kg i.p	11	268.63 ± 4.09	100.61 ± 1.53
Amitriptyline			
- Control	8	267.75 ± 4.66	100.00 ± 1.74
- 30 mg/kg i.p	11	138.45 ± 11.89***	51.71 ± 4.44
Pargyline			
- Control	8	267.75 ± 4.66	100.00 ± 1.74
- 150 mg/kg i.p	10	214.00 ± 15.83**	79.93 ± 5.91

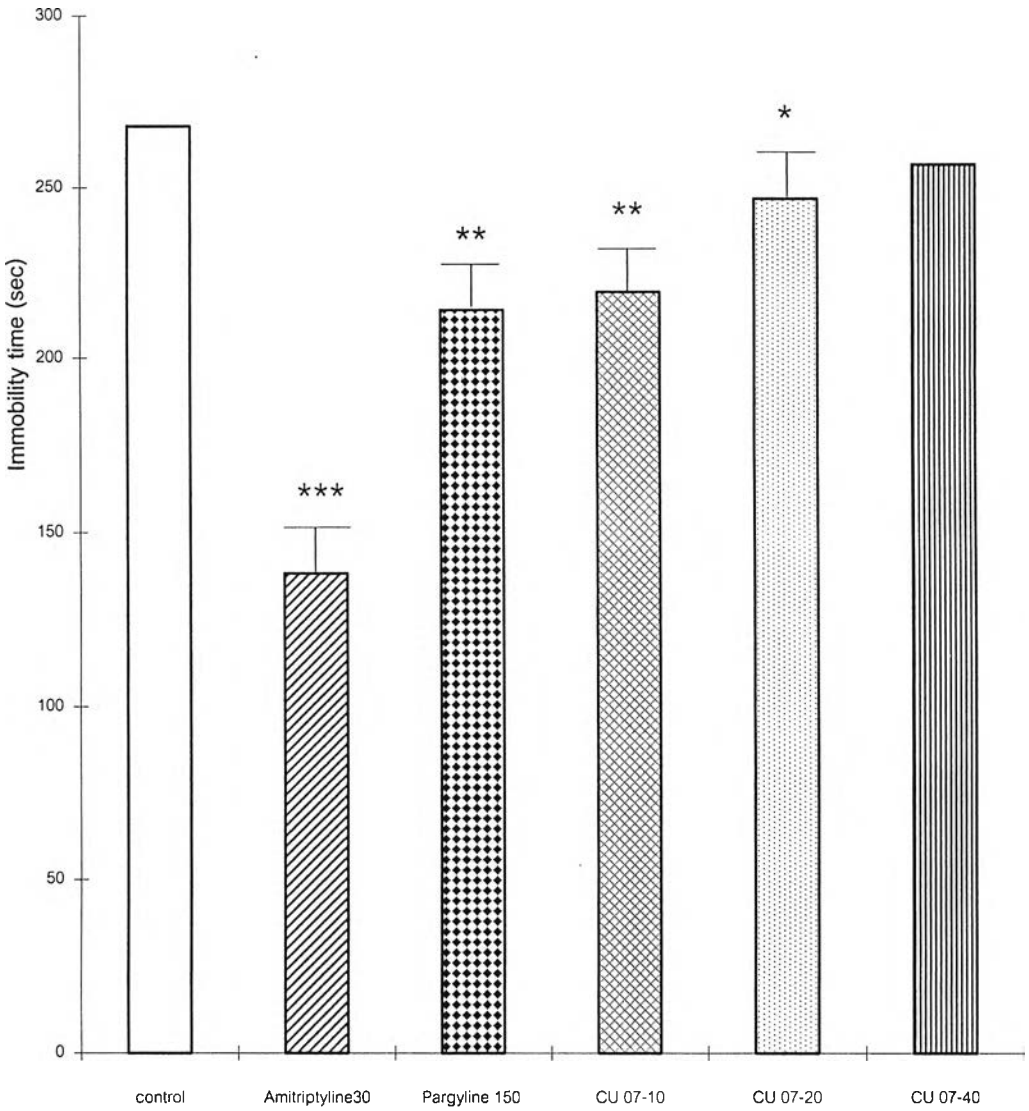

Figure 13. Effects of CU 763-14-07 (10,20 and 40 mg/kg i.p), Amitriptyline (30 mg/kg i.p.) and pargyline (150 mg/kg i.p.) on immobility time in the forced swimming test in mice. Control mice were given vehicle instead of drugs. Data represent mean ± S.E.M., n = 8-12. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

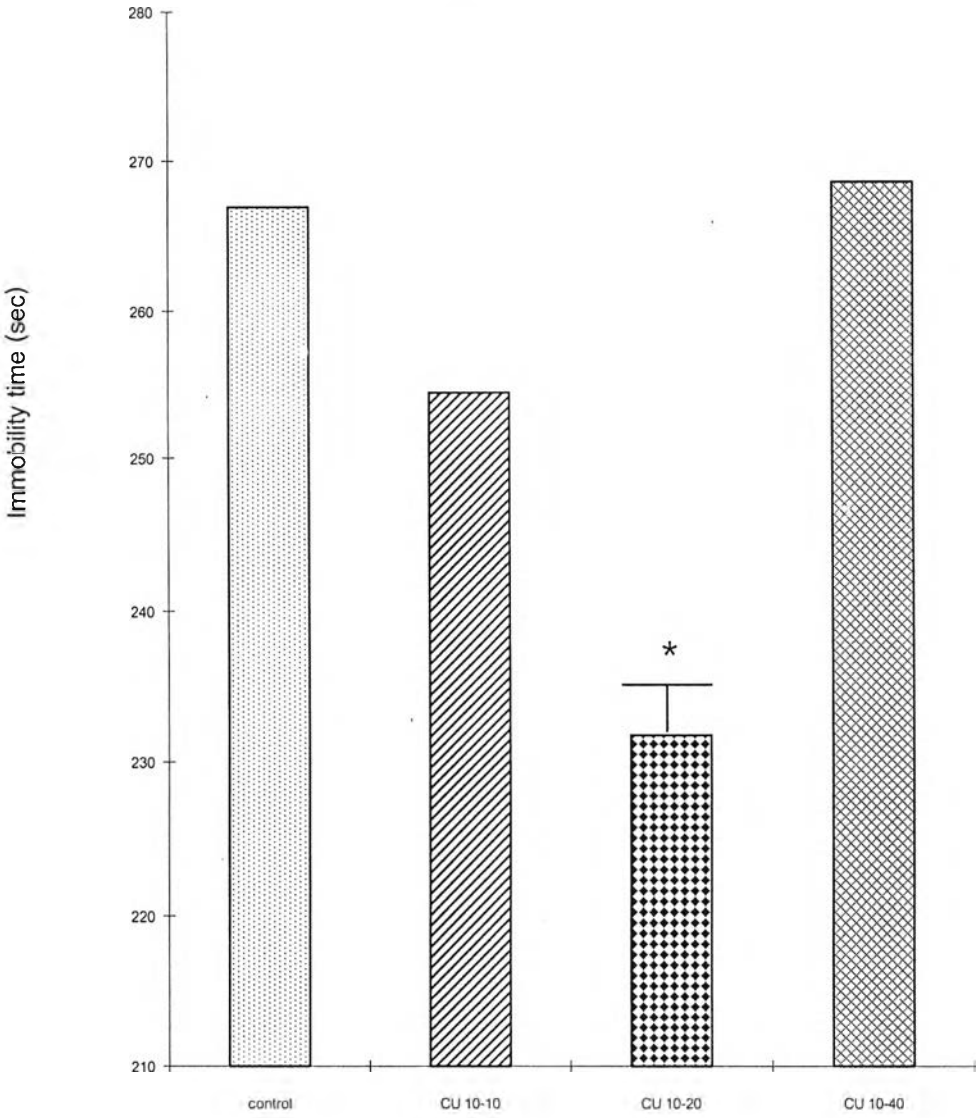

Figure 14. Effects of CU 763-14-10 on immobility time in the forced swimming test in mice. Control mice were given vehicle instead of drugs. Data represent mean ± S.E.M., n = 8-12. * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$. Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

1.2 Locomotor activity test

After preconditioning (the animal was placed in the activity cage for 45 min), the test substance was administered and the animal was placed immediately in the cage again and the sum of activity count was recorded in 1 h.

Effects of treatments with CU 763-14-07 and CU 763-14-10 on locomotor activity in activity cage were shown in Table 6, Figure 15, and Figure 16.

CU 763-14-07 appeared to induce hypolocomotor activity by decreasing total number of count but the effect was significant only at a dose of 40 mg/kg. However at doses of 10 and 20 mg/kg, which apparently decreased the immobility time in behavioral despair model, it did not affect motor activity. This result indicated that antidepressant effect of this substance as determined by the improved performance of animal is not due to increased motor activity. Apparently, CU 763-14-07 at a dose of 40 mg/kg, which did not decrease immobility time in forced swimming test, decrease the motor activity in activity test. Therefore, reduced performance at high dose of this substance may be due to the decrease in motor activity, not the absence of antidepressant activity.

CU 763-14-10 at lower doses (10 and 20 mg/kg i.p.) had no significant effects on locomotor activity. Therefore, antidepressant activity of CU 763-14-10 at a dose of 20 mg/kg as determined by decreased immobility time in forced swimming test is not due to increased motor activity. In similar to CU 763-14-07, CU 763-14-10 at a high dose (40 mg/kg i.p.) produced hypolocomotor activity that was significantly different from the control. Thus, an increase in immobility time at 40 mg/kg may relate to decreased motor activity, not the lack of antidepressant activity.

It is possible that CU 763-14-07 and CU 763-14-10 have antidepressant activity both low dose and high doses of testing but in higher dose, the activity may be hindered by their depressive effect on locomotor activity.

Table 6. Effects of CU 763-14-07 and CU 763-14-10 on locomotor activity in mice. The mice were placed singly in activity cages and locomotor activity was measured before and after drug administration over a period of 45 and 60 min, respectively. Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

Treatment and Dose	No. of mice per dose	Activity counts (60 min)	
		mean ± S.E.M.	% control
CU 763-14-07			
Control	8	802.62 ± 154.88	100.00 ± 19.30
10 mg/kg i.p	8	792.62 ± 103.09	98.75 ± 12.85
20 mg/kg i.p	8	760.50 ± 172.56	94.75 ± 21.50
40 mg/kg i.p	7	412.14 ± 53.06*	51.35 ± 6.61
CU 763-14-10			
Control	8	683.37 ± 131.84	100.00 ± 19.29
10 mg/kg i.p	7	768.42 ± 211.19	112.45 ± 30.90
20 mg/kg i.p	9	658.88 ± 121.97	96.42 ± 17.85
40 mg/kg i.p	9	237.66 ± 31.67*	34.78 ± 4.64

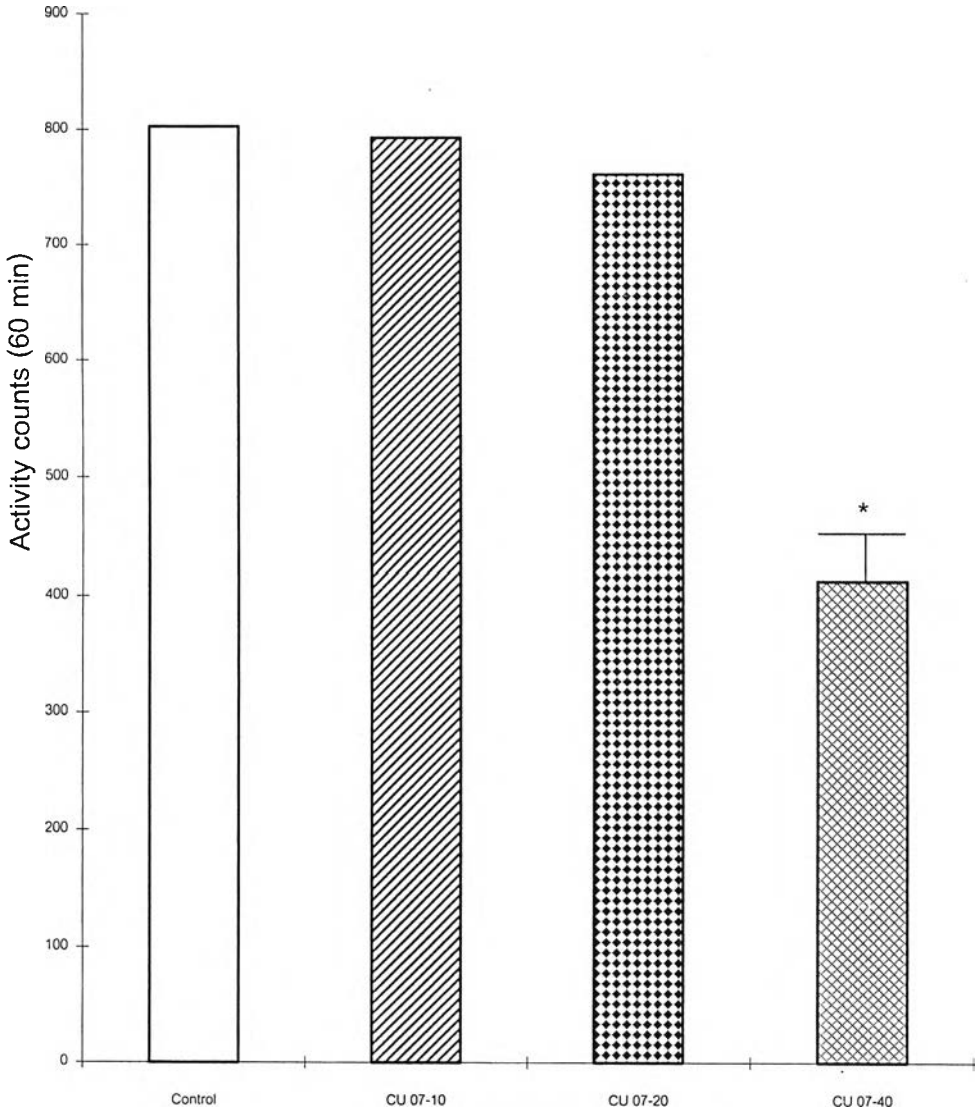

Figure 15. Effects of CU 763-14-07 (10, 20, 40 mg/kg i.p.) on locomotor activity of mice. Values represent mean ± S.E.M. of 8-11 mice * $p < 0.05$. Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

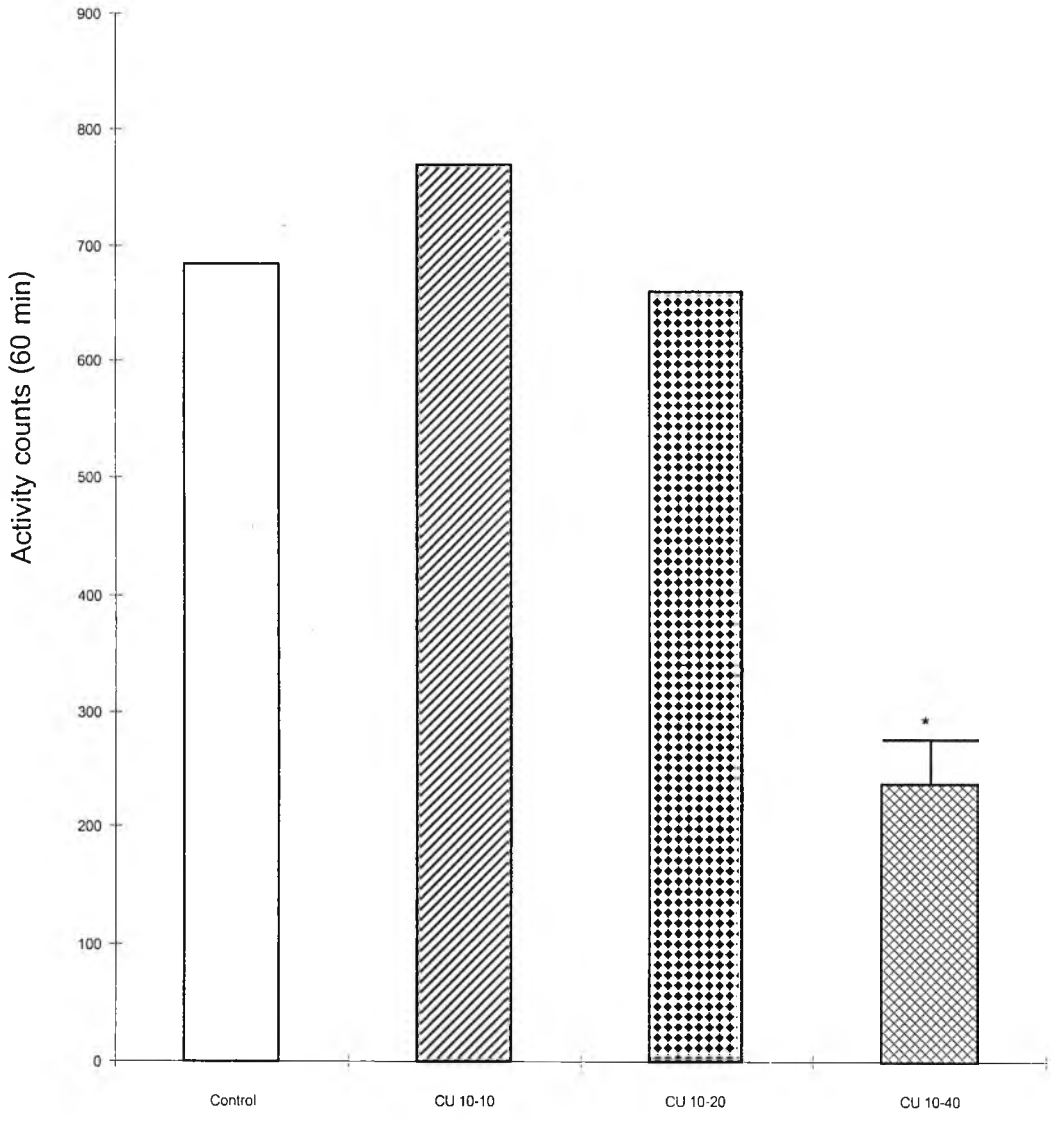

Figure 16. Effects of CU 763-14-10 (10, 20, 40 mg/kg i.p.) on locomotor activity of mice. Values represent mean ± S.E.M. of 8-11 mice * $p < 0.05$ Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

1.3 Rotarod test

Motor incoordination in mice was indicated by an inability of them to maintain their equilibrium on the apparatus. Number of falling mice would be counted in comparison with controls that received vehicle only.

Table 7 demonstrated the effect of CU 763-14-07 and CU 763-14-10, at the same dose range used in the forced swimming test and locomotor activity (10, 20, and 40 mg/kg i.p.), on rotarod test. Mice were tested at 30 min after intraperitoneal injection of test agents.

In this test, control mice receiving NSS or 2% tween 80 in NSS were able to maintain their equilibrium for at least 1 min on the rotarod apparatus in three successive trials.

None of test substances (CU 763-14-07 and CU 763-14-10) was found to affect motor coordination or motor deliberation in mice (Table 7, Figure 17)

The data of this study confirm that the anti-immobility of the substances not related to neurological deficit especially the motor coordination or muscle relaxation. The longer in immobility time may cause by depress in locomotor activity or no activity of antidepressant. However, both compounds may depress locomotor activity in higher dose but it is not severe to neurotoxicity and lead to destroy in motor system.

Table 7. Effects of CU 763-14-07 and CU 763-14-10 on Rota-Rod test in mice. The mice were tested 30 min,after intraperitoneal injection. Differences from control were assessed statistically using Pearson Chi-Square test

compounds	An ability of mice that maintain on the rotarod apparatus		
	Yes	No	Total
CU 763-14-07			
Control	8	2	10
10 mg/kg i.p	9	1	10
20 mg/kg i.p	8	2	10
40 mg/kg i.p	8	2	10
CU 763-14-10			
Control	7	3	10
10 mg/kg i.p	7	3	10
20 mg/kg i.p	7	3	10
40 mg/kg i.p	6	4	10

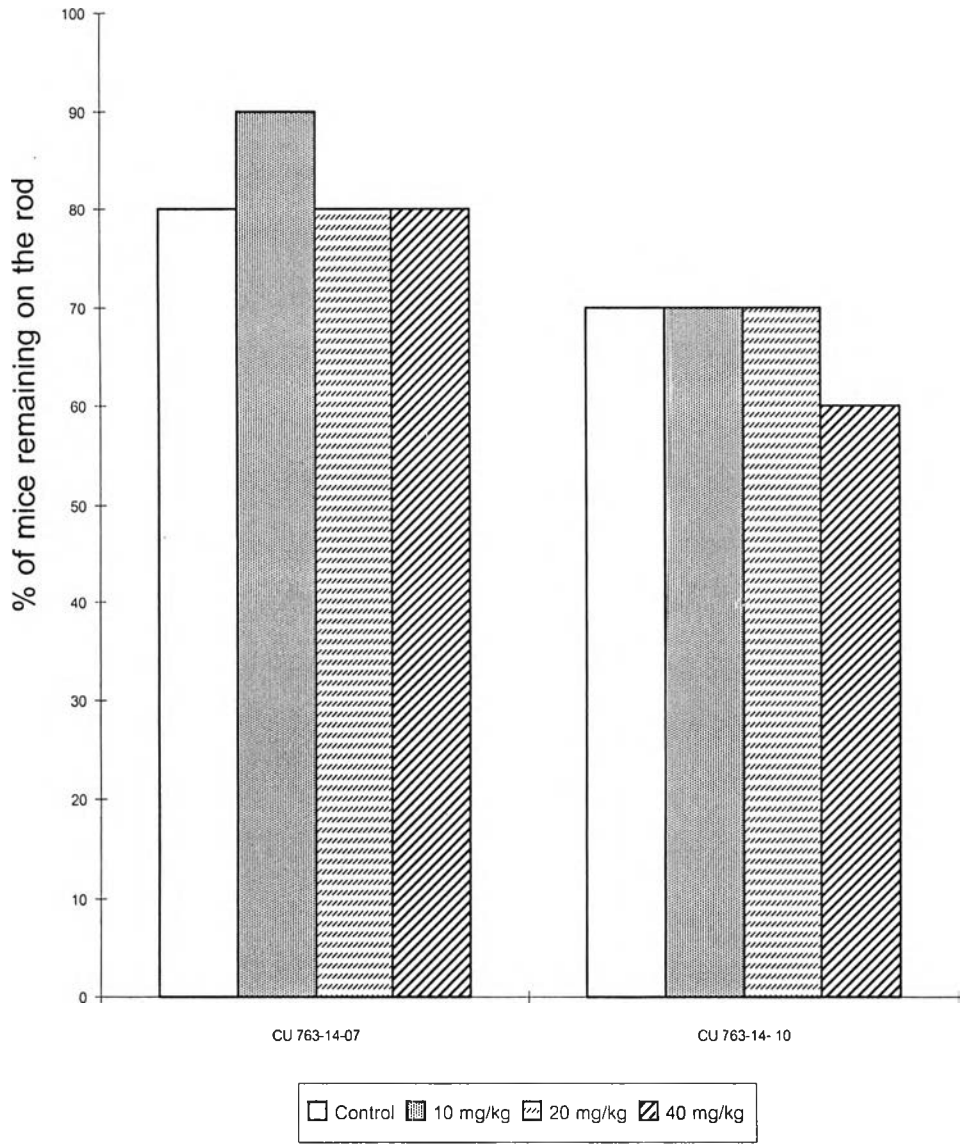

Figure 17. Percent of mice that received CU 763-14-07 (10,20 and 40 mg/kg i.p) and CU 763-14-10 (10,20 and 40 mg/kg i.p) and could be maintaining equilibrium on rotating rod

2. Effects on monoamine neurotransmitter levels in the whole rat brain

Effects of intraperitoneal injections of CU 763-14-07 and CU 763-14-10 at once-daily doses of 20 and 40 mg/kg for 7 days, on brain levels of noradrenaline, dopamine, and 5-hydroxytryptamine (serotonin) were determined by measuring the amount of those amines in the whole rat brain. This study used amitriptyline as positive control. Quantitative analysis of the neurotransmitter was accomplished by HPLC

Amitriptyline administration could be significantly increasing monoamine levels especially noradrenaline and serotonin. In addition, CU 763-14-07 and CU 763-14-10 induced significant increase in the brain levels of these amines (Table 8, Table 9). After CU 763-14-07 administration, only noradrenaline levels significantly increased. No change was seen in dopamine and 5-hydroxytryptamine levels following CU763-14-07 treatment (Figure 21).

NE, DA, and 5-HT levels in the whole rat brain were increased by CU 763-14-10 administration (Figure 22). These experimental results demonstrated that CU 763-14-07 and CU 763-14-10 induced an increase in monoamine levels in the whole rat brain. It is possible that increased levels of DA, 5-HT, and NA may be due to inhibition of brain MAO activity.

The inhibition of enzyme monoamine oxidase which deaminates biogenic amine such as noradrenaline, dopamine and serotonin, can increase the amount of these amines. In rat brain, type A MAO preferentially deaminates 5-hydroxytryptamine (5-HT) and noradrenaline, and is selectively inhibited by clorgyline and harmaline, whereas type B MAO prefers β-phenethylamine (PEA) as substrate and is highly sensitive to the specific inhibitor L-deprenyl. In addition, dopamine seems to be deaminated by both forms of MAO (Kan, Mouget-Goniot, Worms, and Biziere, 1985).

Drug-induced increase in brain monoamine levels may be one of the possible mechanism to explain antidepressant activity of these two compounds. It is notable that CU 763-14-07 showed a profile of effect in accordance to an MAO-A inhibitor while CU 763-14-10 displayed a profile of effect similar to non-specific MAO inhibitors.

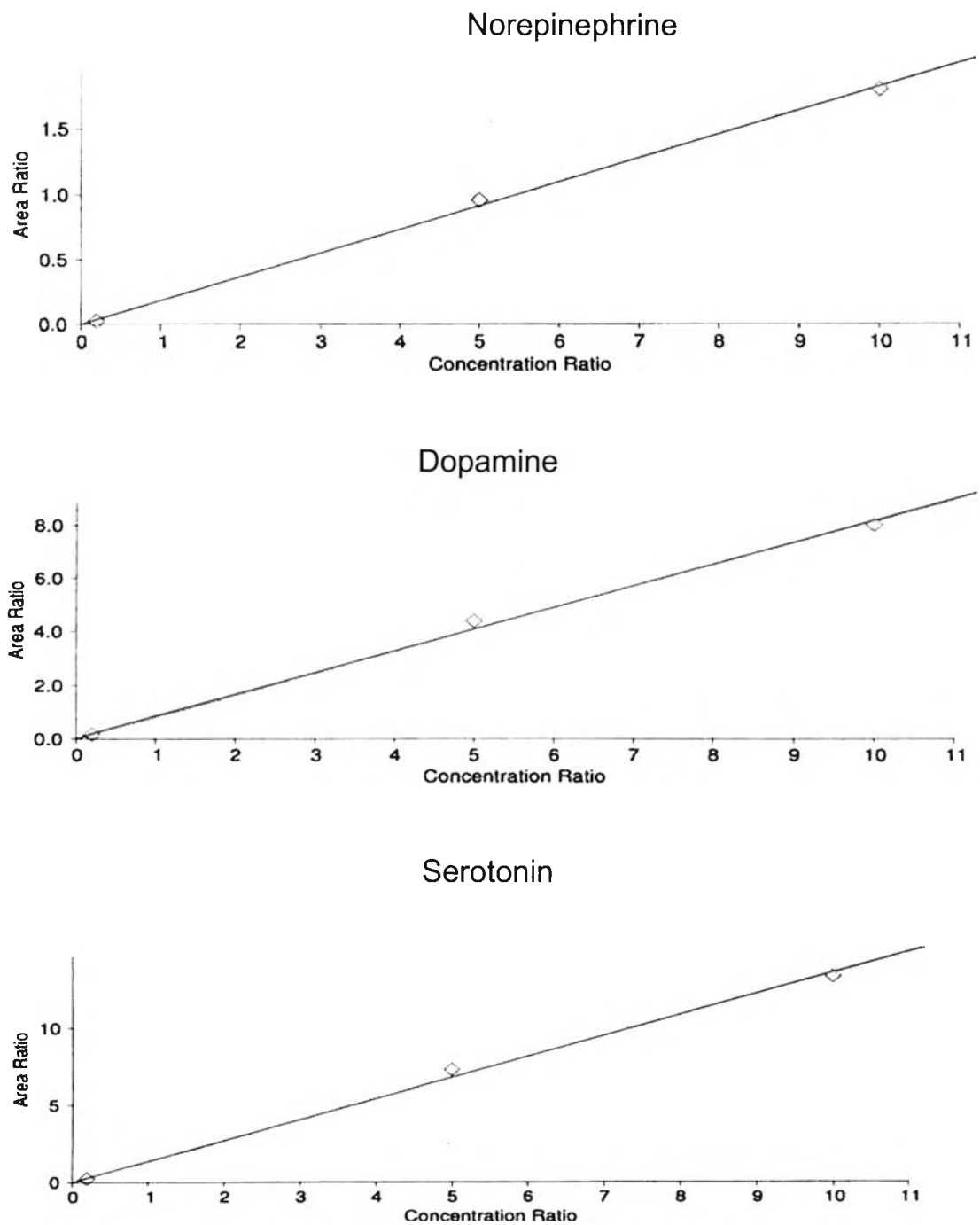

Figure 18. Standard calibration curve for norepinephrine (top panel), Reliability=98.705%; dopamine (middle panel), Reliability=96.363%; serotonin (5-HT) (bottom panel), Reliability=96.802%.

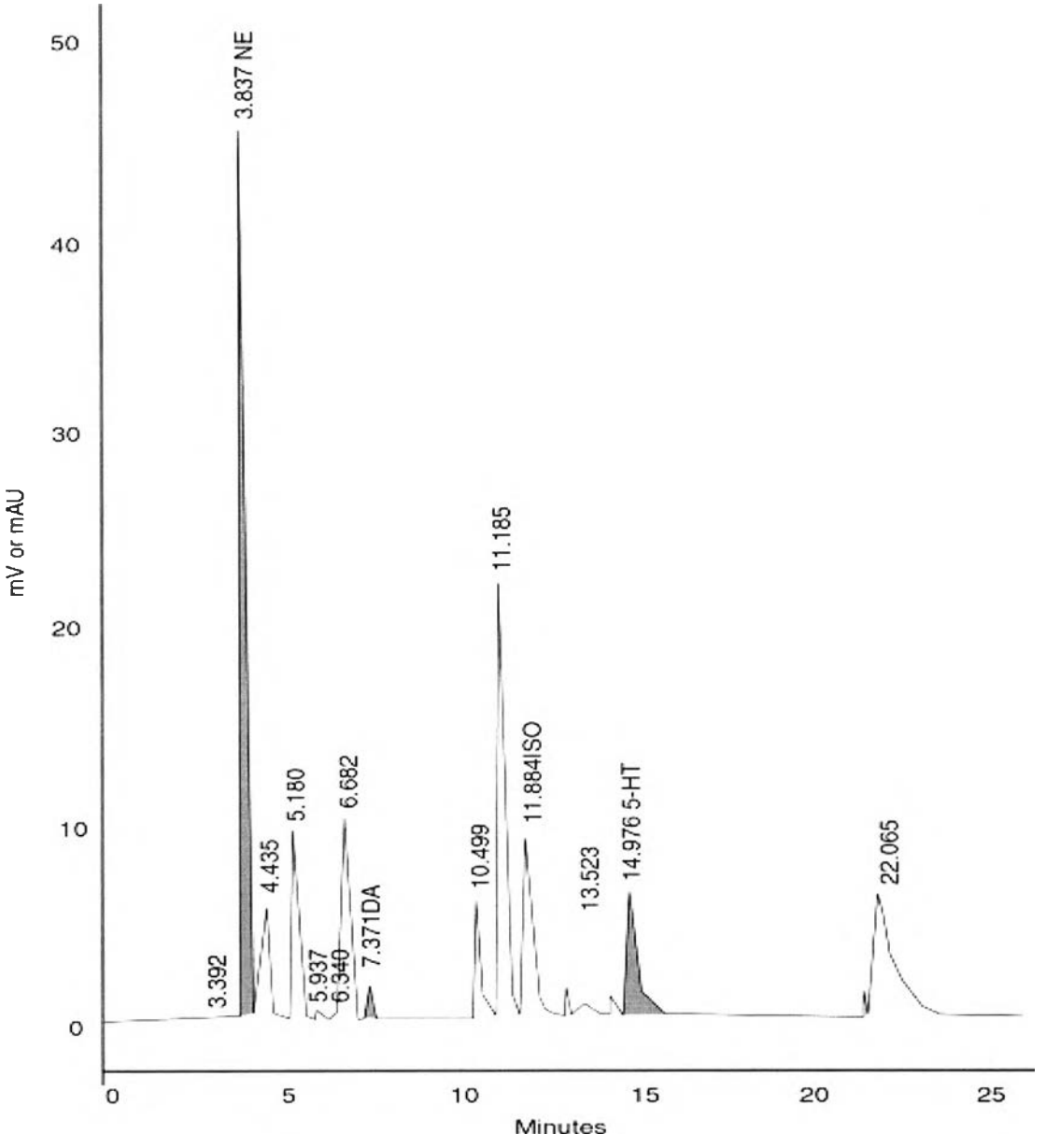

Figure 19. HPLC chromatograms of monoamines in the whole rat brain, using isoproterenol as an internal standard. 2%Tween80 in NSS was used as a control. Rats were treated daily for 7 day (subchronic)

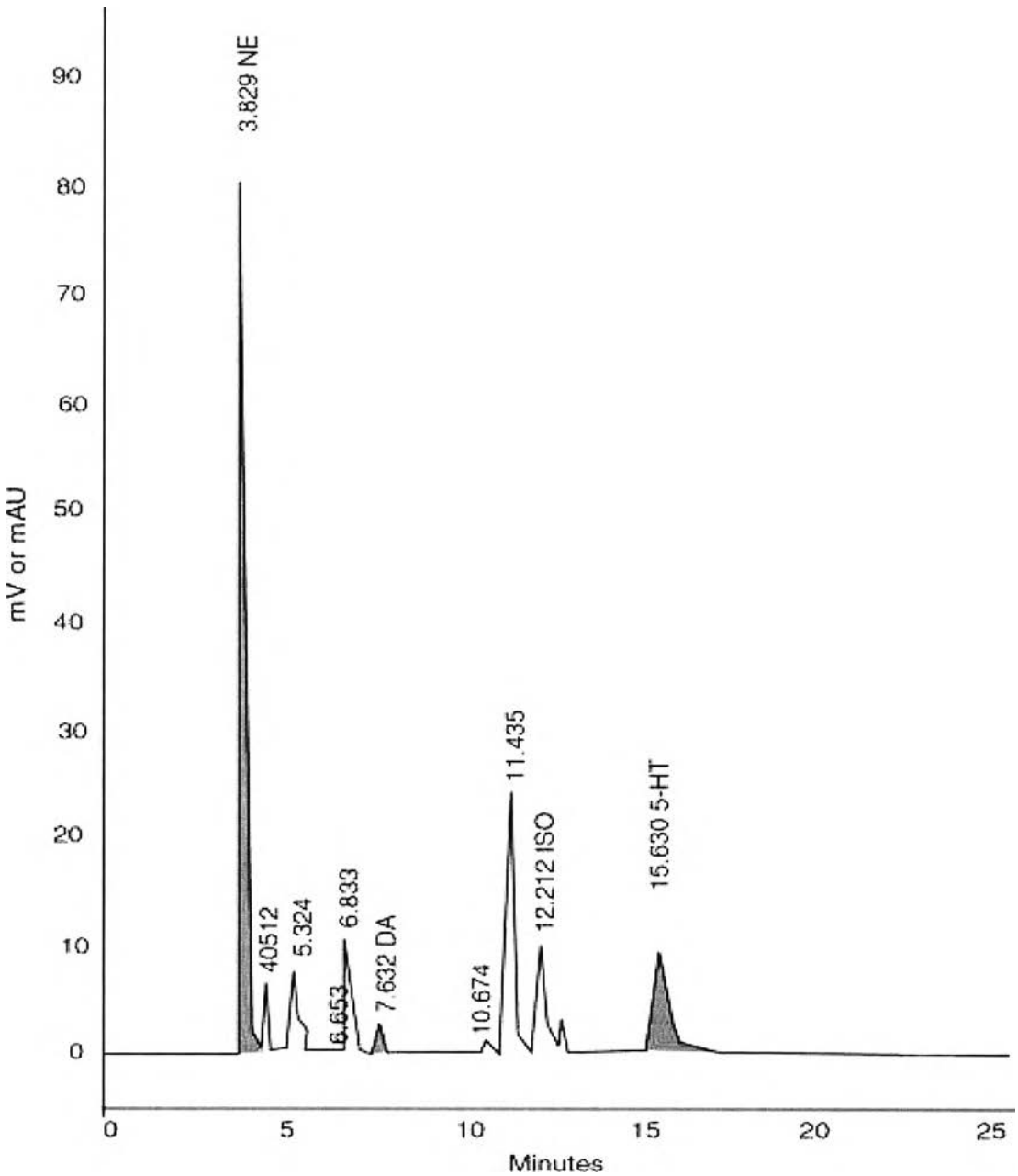

Figure 20. HPLC chromatograms of monoamines (NE, DA, and 5-HT) in the whole rat brain, using isoproterenol as an internal standard. Rats were treated daily with CU 763-14-07 for 7 day (subchronic).

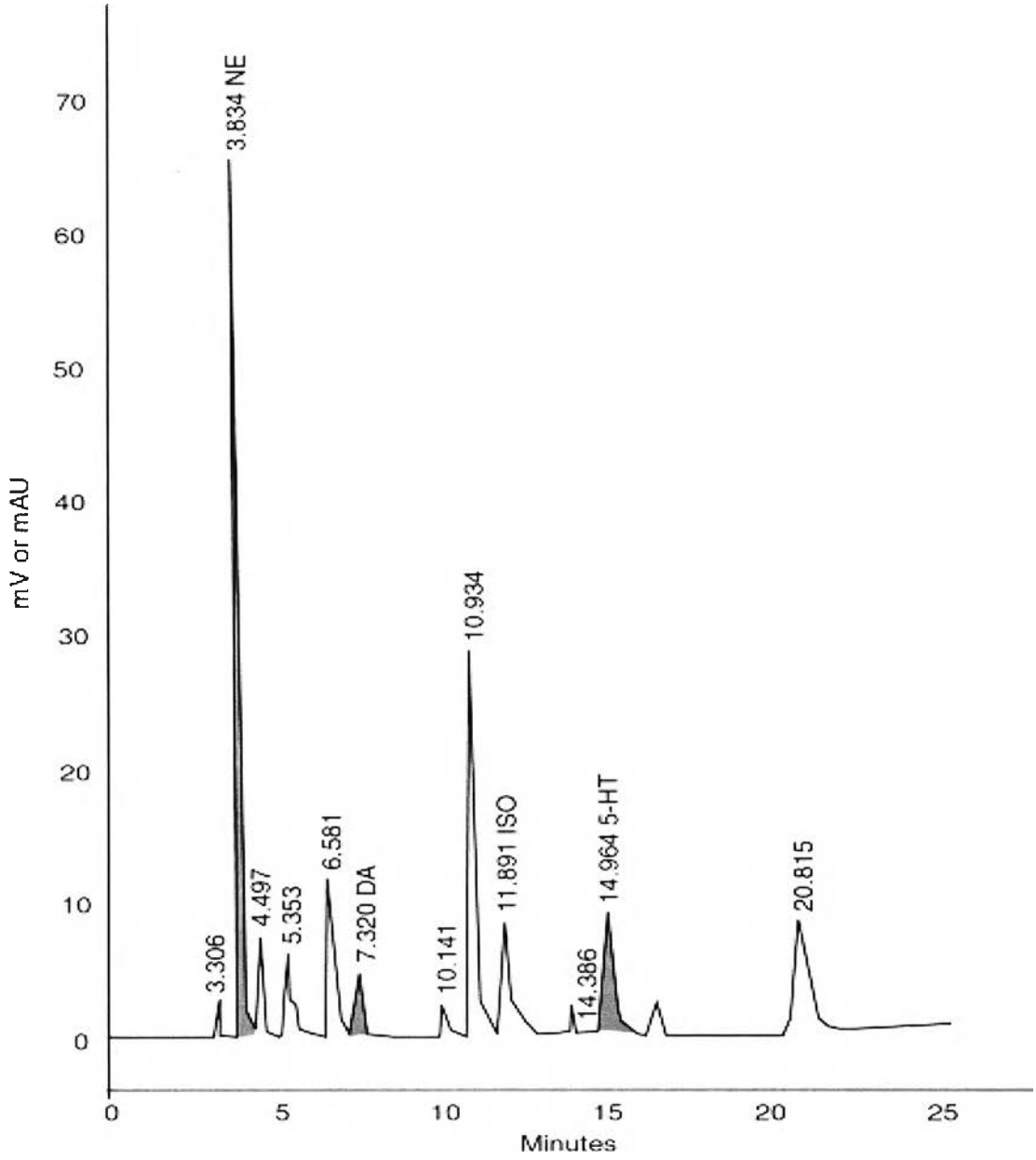

Figure 21. HPLC chromatograms of monoamines (NE,DA, and 5-HT) in the whole rat brain, using isoproterenol as an internal standard. Rats were treated daily with CU 763-14-10 for 7 day (subchronic).

Table 8. Concentrations of norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT) in the whole rat brain treated with various agents. Data represent percent of control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

Agent	Dose (mg/kg)	Number of rat	Monoamine concentration (μg/g tissue)		
			NE	DA	5-HT
NSS	-	8	64.64 ± 1.30	2.23 ± 0.03	72.51 ± 0.85
CU 76314-07	20	8	78.91 ± 3.69 *	2.15 ± 0.09	72.00 ± 9.80
CU 76314-07	40	8	106.36 ± 9.63 **	2.43 ± 0.09	73.10 ± 1.80
Amitryptyline	15	10	127.70 ± 6.57 ***	2.14 ± 0.08	175.70 ± 12.36 ***
2% Tween 80 in NSS	-	8	81.20 ± 0.40	1.52 ± 0.04	78.62 ± 4.13
CU 76314-10	20	8	115.82 ± 0.88 ***	1.86 ± 0.04 **	117.80 ± 3.44 ***
CU 76314-10	40	10	90.89 ± 2.88 *	1.47 ± 0.03	93.32 ± 4.02 *

Table 9. Percent of control of norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT) levels in the whole rat brain treated with various agents. Values represent percent of control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ Differences from control were assessed statistically using one way ANOVA and Fisher's PLSD test.

Agent	Dose (mg/kg)	Number of rat	Monoamine levels (percent of control)		
			NE	DA	5-HT
NSS	-	8	100.00 ± 2.01	100.00 ± 1.43	100.00 ± 1.17
CU 76314-07	20	8	122.07 ± 5.70*	96.08 ± 3.93	99.30 ± 13.50
CU 76314-07	40	8	164.53 ± 14.89**	108.85 ± 3.91	100.82 ± 2.48
Amitryptylline	15	10	197.55 ± 10.16***	95.86 ± 3.51	242.31 ± 17.04***
2 % Tween 80 in NSS	-	8	100.00 ± 0.50	100.00 ± 2.86	100.00 ± 5.26
CU 76314-10	20	8	142.64 ± 1.08***	122.37 ± 2.36**	149.83 ± 4.37***
CU 76314-10	40	10	111.93 ± 3.55*	96.45 ± 2.25	118.69 ± 5.11*

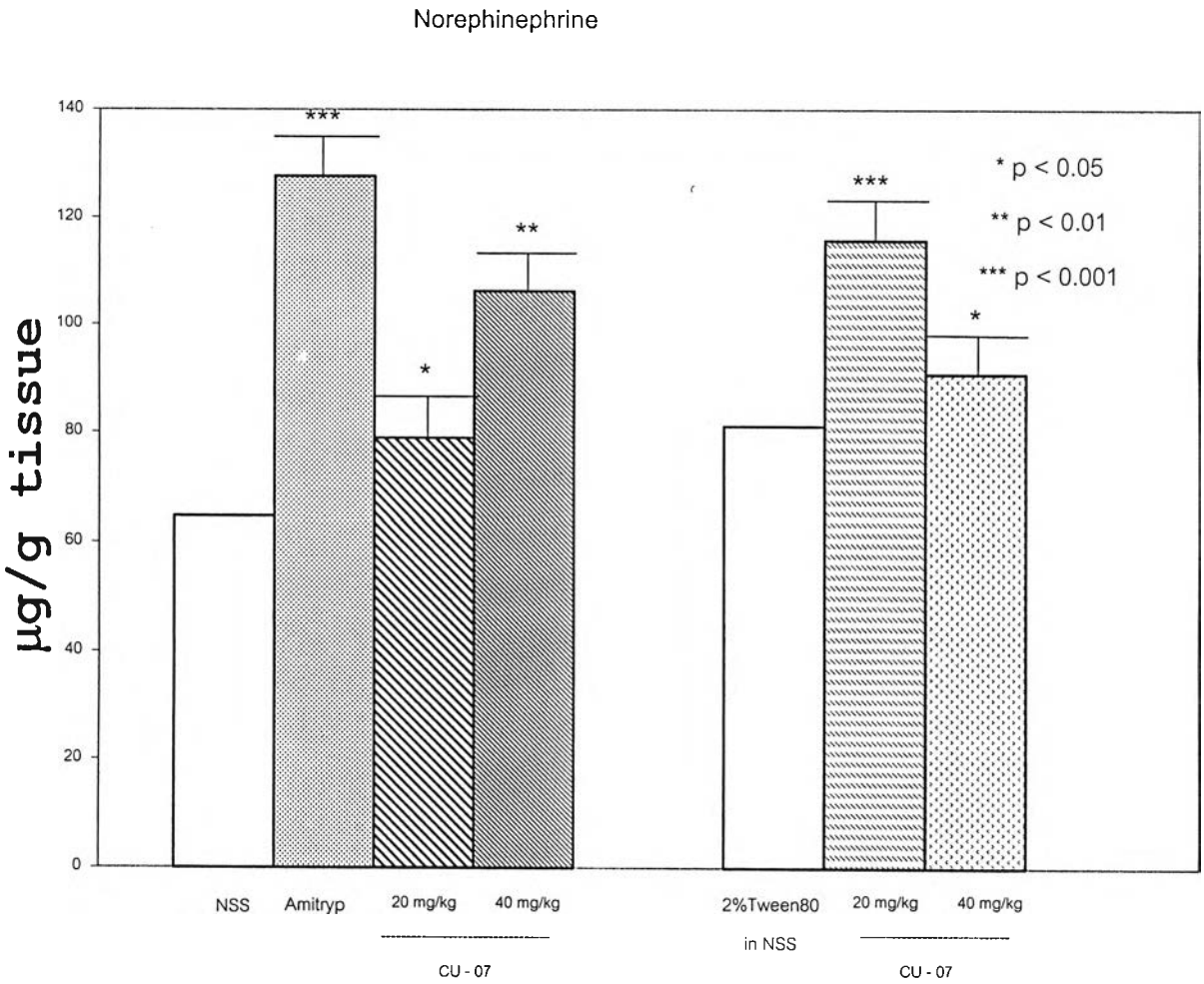

Figure 22. Effects of intraperitoneal administration of various agents on norepinephrine levels in the whole rat brain. Data are express as mean ± S.E.M. Numbers of rats used were the same as table 8.

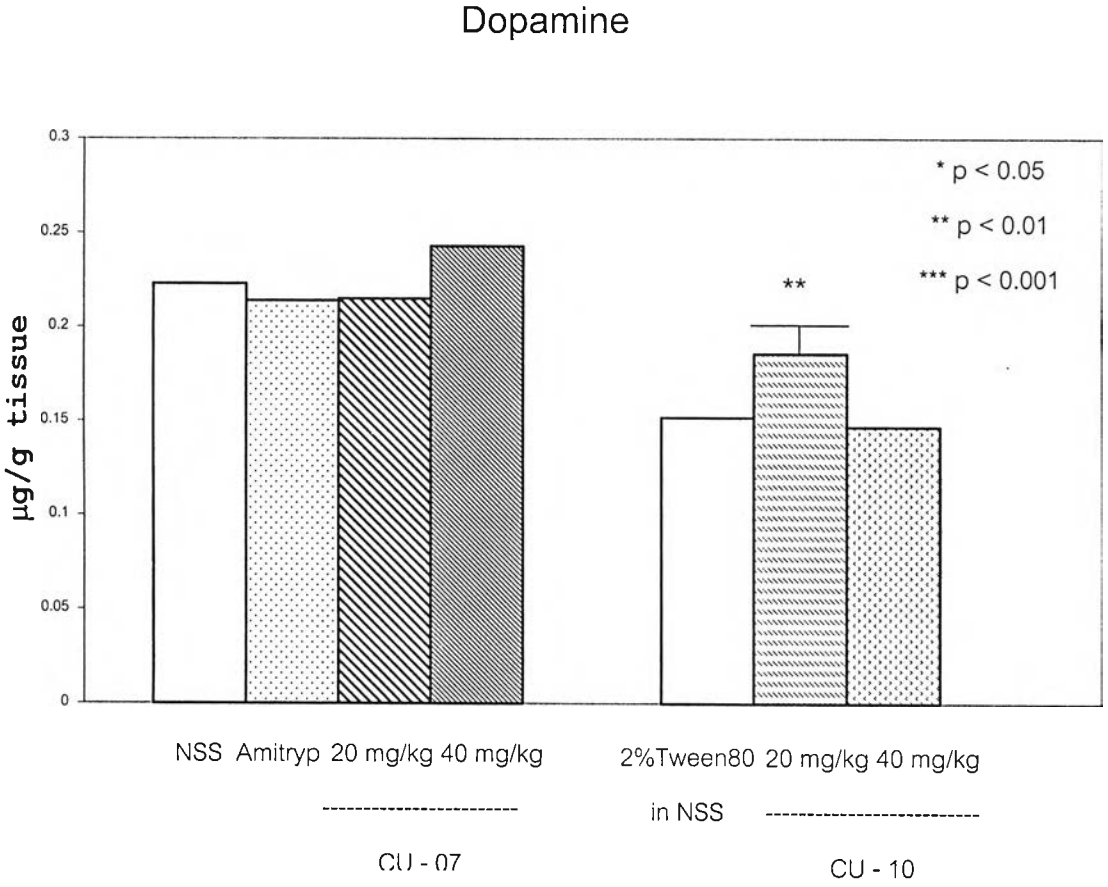

Figure 23. Effects of intraperitoneal administration of various agents on dopamine levels in the whole rat brain. Data are express as mean ± S.E.M. Numbers of rats used were the same as table 8.

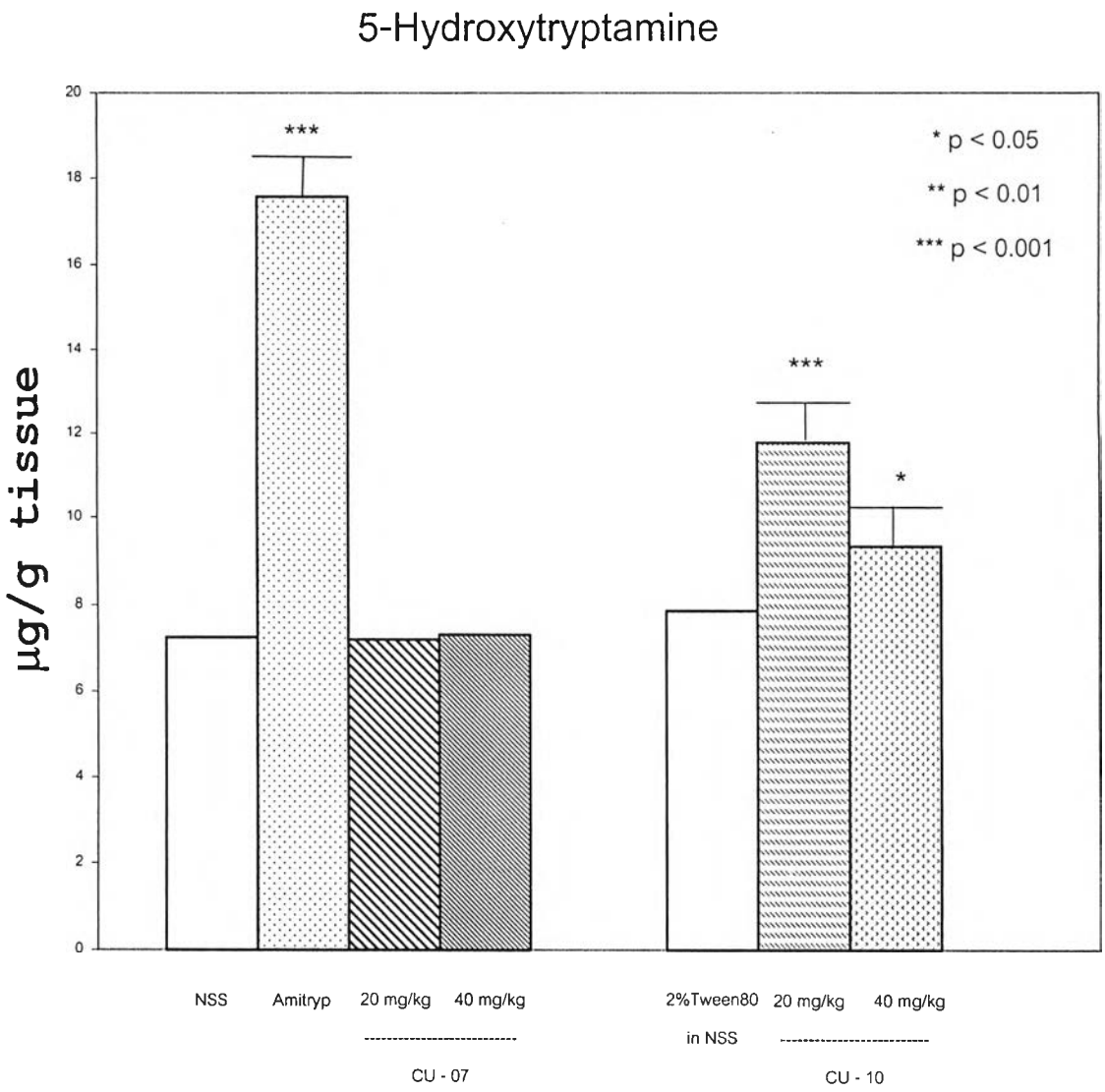

Figure 24. Effects of intraperitoneal administration of various agents on serotonin levels in the whole rat brain. Data are express as mean ± S.E.M. Numbers of rats used were the same as table 8.

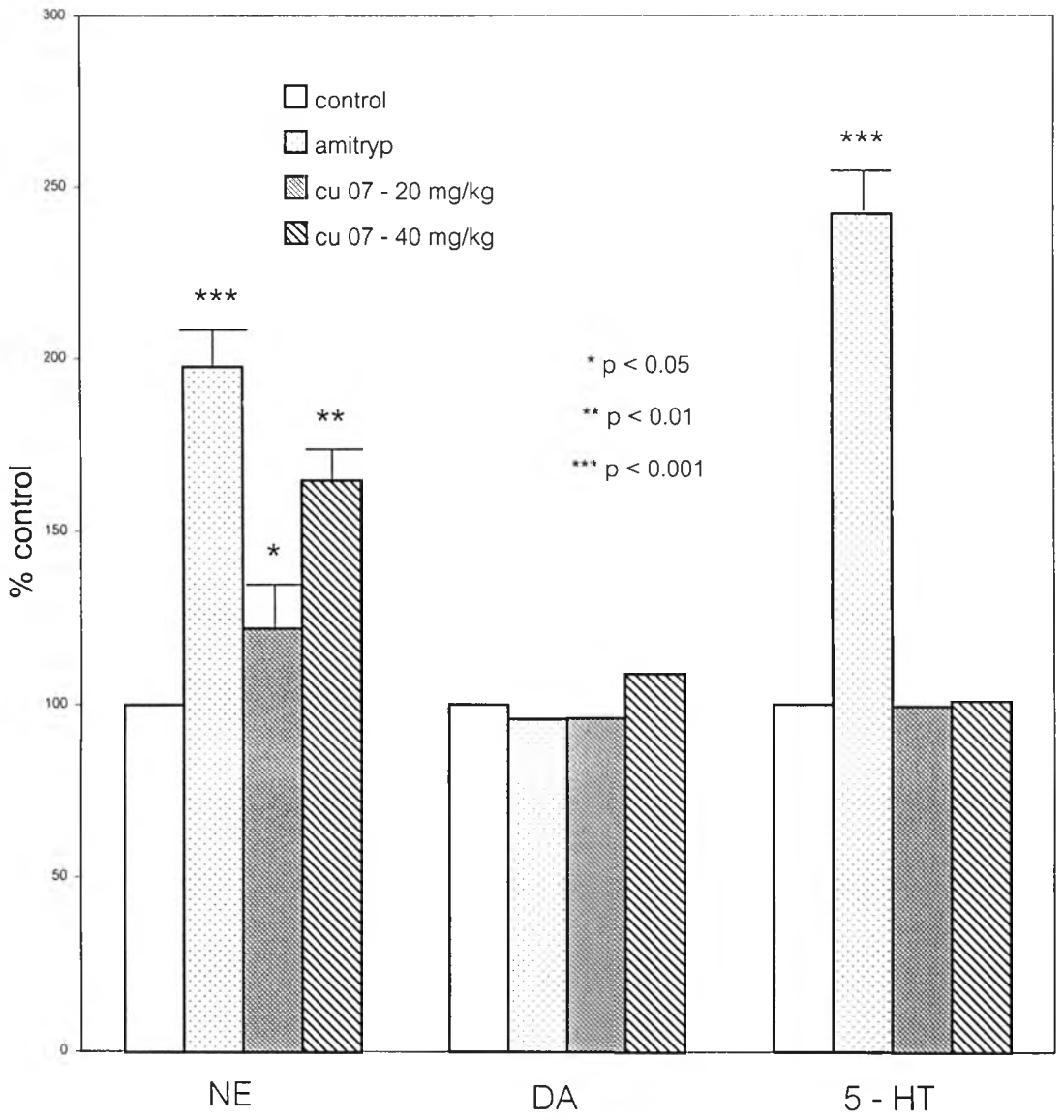

Figure 25. Effects of CU 763-14-07 and Amitriptyline on some neurotransmitter levels in whole rat brain. Data are express as percent of control. Numbers of rats used were the same as table 9.

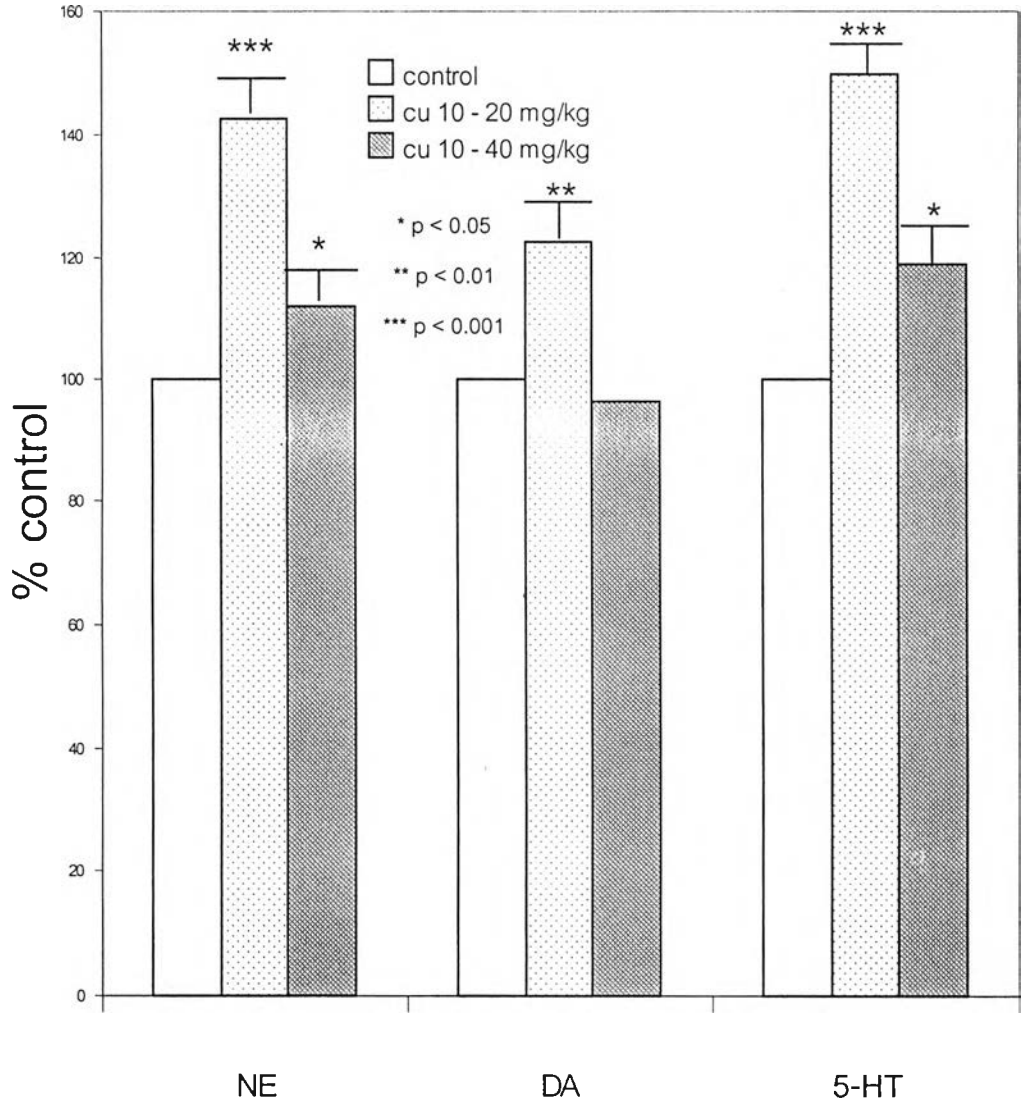

Figure 26. Effects of CU 763-14-10 on some neurotransmitter levels in whole rat brain. Data are express as percent of control. Numbers of rats used were the same as table 9.