Yonsei Medical Journal
Vol. 34, No. 3, 1993

Regional Differences in the Levels of Biogenic Amines and Their Metabolites in Rat Brain after Tricyclic Antidepressant Treatments

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Changes in the levels of biogenic amines in different brain regions and the cerebrospinal fluid in rats were measured after acute or chronic treatment with tricyclic antidepressants. After single or 3 weeks' treatment with imipramine or desipramine, blocks of tissues were obtained from seven regions of the brain (frontal cortex, corpus striatum, hippocampus, thalamus, hypothalamus, substantia nigra and cerebellum) immediately after collection of the cerebrospinal fluid (CSF) from the cisterna magna. The concentrations of biogenic amines and their metabolites (norepinephrine, epinephrine, dopamine, 5-hydroxytryptamine (5-HT), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA)) in brain tissues and the CSF were measured using the high performance liquid chromatography-electrochemical detection system (HPLC-ECD). Treatment with desipramine or imipramine caused major alterations in the concentrations of central norepinephrine or 5-HT and its metabolite, respectively. Brain regional responses were variable according to the kind of tricyclic antidepressants and the duration of treatment. It is noteworthy that chronic treatment with both desipramine and imipramine caused altered hippocampal concentrations of norepinephrine and/or 5-HT and its metabolites. Striatal DOPAC concentrations were also changed after acute or chronic treatment with both drugs. These results suggest that tricyclic antidepressants altered neurotransmission according to the brain region, and the hippocampal norepinephrine and 5-HT and/or the striatal dopamine may have a significant role for the expression of antidepressant action of tricyclic antidepressants.

Key Words: Depression, biogenic amines, tricyclic antidepressants, HPLC-ECD

Treatment with reserpine has been used as an experimental model of depression (Costa *et al.* 1960), since it was observed that reserpine depleted monoamines in the brain (Brodie *et al.* 1956) which had relations with the depressive behavioral alterations (Brodie and Shore, 1957). It has also been observed that drugs which increased the availability of catecholamines at receptor sites improved depressive symptoms, and that a lack of the monoamine neuronal function caused sedation and a decreased motor activity (Carlsson *et al.* 1959; Quitkin *et al.* 1979). These findings established the monoamine hypothesis that affective disorders result from either a decrease in the availability of catecholamine at the receptor site or a change of physiologic equilibrium between adrenergic and serotonergic neuronal functions (Bunny and Davis, 1965; Schildkraut, 1965; Schildkraut, 1978; Van Praag, 1978; Kostowski, 1981). Imipramine and similar tricyclic antidepressants were believed to exert their antidepressant

Received June 10, 1993
Accepted September 1, 1993
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actions through the blockade of reuptake of norepinephrine and 5-HT at nerve terminals, which results in an increase of monoamine receptor activity at synapses (Dengler *et al.* 1961; Carlsson *et al.* 1968; Ross and Renyi, 1975). However, the blockade of monoamine reuptake appeared immediately after the treatment with tricyclic antidepressants although their antidepressant effects appeared at least two weeks after the initiation of treatment (Baldessarini, 1991). The monoamine hypothesis as the mechanism of action of tricyclic antidepressants have also been challenged by other observations such as cocaine or amphetamine showing only a mild antidepressant effect although they have the ability to block monoamine reuptake, and atypical antidepressants, such as mianserin, not having a strong ability to block norepinephrine reuptake (Stahl and Palazidou, 1986). These evidences raises doubts about the monoamine hypothesis.

Although imipramine and similar tricyclic antidepressants inhibit reuptake of 5-HT and norepinephrine, the concentration of norepinephrine in the brain was not changed by acute treatment (Sedlock and Edwards, 1985). However 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), a major metabolite of norepinephrine, was decreased (Schildkraut *et al.* 1976; Tang *et al.* 1978; Nielsen and Braestrup, 1977a), which implied a decreased turnover rate of norepinephrine after acute treatment of tricyclic antidepressants (Rosloff and Davis, 1978; Sedlock and Edward, 1985). On the other hand, the concentrations of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were not changed after acute treatment of tricyclic antidepressants. However, it is reported that the rate of disappearance of 5-HT at the presynaptic neuron was delayed (Segawa and Mizuta, 1980).

In the case of the chronic treatment with tricyclic antidepressants, which has more than two weeks, changes of the turnover rate of norepinephrine were inconsistently reported (Sedlock and Edwards, 1985; Schildkraut *et al.* 1970, 1971; Rosloff and Davis, 1978; Nielsen and Braestrup, 1977b). However, the turnover rate of 5-HT was invariably decreased (Meek and Werdinius, 1970; Sugrue *et al.* 1976; Hyttel, 1977).

The influence of tricyclic antidepressants on the dopaminergic system was minimal. It has been known that there was no significant changes in the binding capacity of the dopamine receptors (Rehavi *et al.* 1980; Spyraki and Fibiger, 1981). However, chronic treatment caused decreased sensitivity of the autoreceptor (Serra *et al.* 1979, 1980; Chiodo and Antelman, 1980; Holcomb *et al.* 1982).

According to these previous findings, it may be certain that tricyclic antidepressants affect the activity of central biogenic amines in a complicated manner. However, the brain function may not be investigated systematically since most studies dealt with the whole brain or only a couple of structures of the brain with specific amines. Therefore, the present study aimed to investigate different effects of the duration of treatment (single or chronic) and to investigate specific responses of the different brain regions after treatment with tricyclic antidepressants on the concentrations of biogenic amines (norepinephrine, 5-HT, dopamine) and their metabolites in discrete brain regions and the CSF. Two tricyclic antidepressants were used for these purposes; imipramine which has a strong 5-HT reuptake-blocking ability (Carlsson *et al.* 1968; Langer *et al.* 1980; Langer, 1987), and desipramine which has a highly selective norepinephrine reuptake-blocking ability (Ross and Renyi, 1975).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats which had been adjusted to the experimental environments for more than one week were used. Their weights were about 200 g each at the beginning of the experiment.

Imipramine and desipramine (10 mg/kg, Sigma Chemical Co., USA; respectively) were dissolved in physiological saline and injected intraperitoneally at 9:00 a.m. of the experimental day for the single treatment group, and at 9:00 a.m. and 5:00 p.m. for 3 weeks for the chronic treatment group. The CSF and blocks of brain tissues were obtained 5 hours after the last injection of imipramine or desipramine.

Measurement of biogenic amines and their metabolites

Rats were anesthetized with secobarbital (30 mg/kg, ip) and placed in a stereotaxic apparatus. The skin of the posterior neck was incised and the neck muscles (M. auricularis longus, M. platysma, M. trapezius, M. biventa, M. rectus capitis dorsalis major, M. rectus capitis dorsalis minor) were dissected in order, then the atlantooccipital membrane was exposed. By puncturing the atlantooccipital membrane with a 25G needle, cerebrospinal fluid was obtained from the cisterna magna without blood contamination at a velocity of 5-10 μl/sec. Immediately after obtaining the CSF, the rats were killed by decapitation and the brains were collected. The brains were dissected into 7 brain regions (frontal cortex, corpus striatum, hippocampus, thalamus, hypothalamus, substantia nigra, and cerebellum) on the ice using the modified method of Glowinski and Iversen's (1966).

The modified method of Wagner's (1982) was used for the measurement of the concentrations of biogenic amines and their metabolites in the CSF and blocks of brain tissues. Briefly, the same amount of 0.1 M perchloric acid (containing 0.25% disodium EDTA) as the volume of CSF was added to the CSF, and the mixture was centrifuged at 12,500 g for 15 minutes, then the supernatant was obtained. For the processing of the brain tissues, more than 20 ml of 0.1 M perchloric

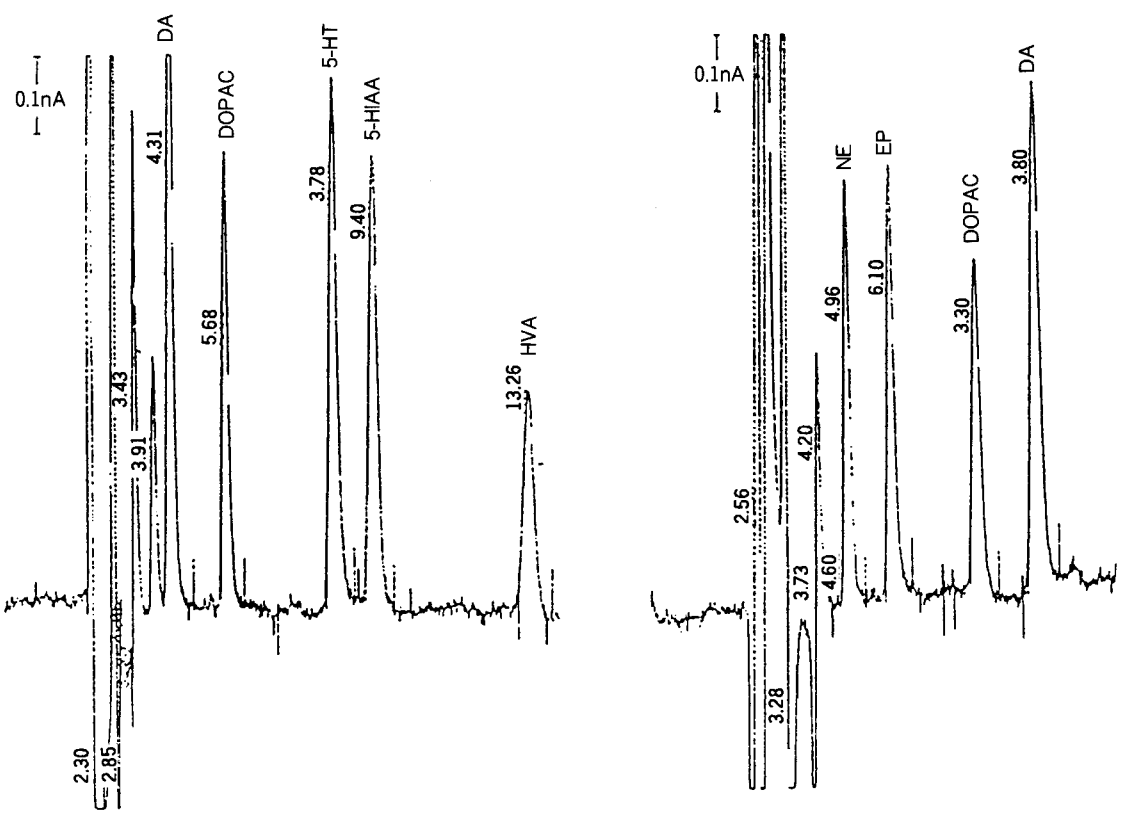

Fig. 1. *Standard chromatogram of biogenic amines and their metabolites.*
NE, norepinephrine; DA, dopamine; 5-HT, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid

acid/mg tissue (containing 0.25% disodium EDTA) was added to the tissues and the mixture was homogenized, centrifuged at 12, 500 g for 15 minutes and the supernatant was obtained. These supernatants were filtered with a nitrocellulose membrane filter (pore size: 0.22 mm, Bioanalytical System Inc., USA), and 10 ml of filtered fluid was injected into the high performance liquid chromatograph (HPLC) system and the amounts of 5-HT, 5-HIAA, and HVA were measured. Meanwhile, 500 ml of 3 M Tris EDTA buffer (pH 8.6) and 12 mg of alumina were added to 200 ml of filtrate and shaken for absorbing catecholamines into the alumina. The alumina was washed with distilled water and dried, then catecholamines were extracted by adding 0.1 N HCl 60 ml and 10 ml of extraction solution was injected into the HPLC system to measure the amount of norepinephrine, epinephrine, DOPAC and dopamine. Substances separated by HPLC were analyzed by an electrochemical detector and the area of the corresponding peak of each substance were measured (Fig. 1).

The HPLC system and conditions used were as follows:

HPLC: High Performance Liquid Chromatography (Waters Associates, Model 441, USA)

Column: Biophase ODS 5 μm (250×5 mm) (Bioanalytical Systems Inc., USA)

Mobile Phase: Measurement of 5-HT, 5-HIAA, and HVA ; 6.5% acetonitrile and 93.5% 0.15 M monochloroacetic acid buffer (containing 0.17 mM sodium octyl sulfonate and 2 mM disodium EDTA) was adjusted to pH 2.5 and the flow rate of this mobile phase was 1.4 ml /min. Measurement of norepinephrine, epinephrine, dopamine and DOPAC; 5% acetonitrile and 95% 0.15 M monochloroacetic acid buffer (containing 1.38 mM sodium octyl sulfonate and 2 mM disodium EDTA) was adjusted to pH 2.8 and the flow rate of this mobile phase was 1.2 ml/min. Reagents used for the mobile phase were purchased from Sigma Chemical Co., USA.

Detector: LC 4B/17 Electrochemical Detector with TL-5 glassy carbon working electrode (Bioanalytical System Inc., USA).

Applied Potential: +800 mV vs. Ag/AgCl

Controller Sensitivity: 1 nA/V

Statistical analysis

The effects of tricyclic antidepressants were analyzed by one way ANOVA for each brain region, and preplanned comparisons of treated groups with the control group were made using Dunnett's t statistic (Winer, 1971).

RESULTS

The concentrations of catecholamines, 5-HT and their metabolites in the normal rat brain

The concentration of norepinephrine was the highest in the hypothalamus (2,599.9±230.0 ng/g), and norepinephrine was detectable in the thalamus, substantia nigra, hippocampus, frontal cortex, cerebellum, and corpus striatum in descending order. Epinephrine was not detectable in any part of the brain tissues. The concentration of dopamine was the highest in the corpus striatum (10,408.5±486.6 ng), and dopamine was detectable in the substantia nigra, hypothalamus, thalamus, hippocampus, and frontal cortex in descending order. Dopamine was not detectable in the cerebellum. Large amount of dopamine metabolites were also detectable in the corpus striatum; the concentration of DOPAC was 7, 770.1±491.1 ng/g, and that of HVA was 1,411.1 59.0 ng/g. Dopamine metabolites was not detectable in the other brain regions except the hypothalamus in which the concentration of DOPAC was 245.5±18.8 ng/g (Table 1).

The concentration of 5-HT was the highest in the substantia nigra (1,597±99.5 ng/g), and 5-HT was detectable in the hypothalamus, frontal cortex, thalamus, corpus striatum, hippocampus, and cerebellum in descending order. The concentration of 5-HT metabolite, 5-HIAA, was the highest in the substantia nigra (2,347.7±126.7 ng/g) and 5-HIAA was detectable in the hypothalamus, thalamus, substantia nigra, hippocampus, and frontal cortex in descending order. 5-HIAA was not detectable in the cerebellum (Table 1).

Table 1. Effects of single or chronic treatment with imipramine on the levels of biogenic amines and their metabolites in rat brain

	NE			DA			5-HT			DOPAC			HVA			5-HIAA		
	C(13)	S(5)	Ch(6)	C(13)	S(4)	Ch(6)	C(13)	S(5)	Ch(6)	C(13)	S(4)	Ch(5)	C(14)	S(5)	Ch(5)	C(14)	S(5)	Ch(6)
Fcx	329.5 ±27.2	340.7 ±26.6	322.8 ±39.8	69.0 ±6.4	77.8 ±5.9	82.9 ±12.6	670.9 ±25.6	686.0 ±92.3	718.9 ±40.3	ND	ND	ND	ND	ND	ND	560.8 ±23.1	464.4* ±17.6	487.0 ±24.1
Str	103.9 ±16.9	119.9 ±20.7	135.5 ±26.1	10408.5 ±486.6	11837.6 ±591.1	11763.0 ±768.2	557.0 ±17.1	517.1 ±36.5	536.1 ±46.7	7770.1 ±49.1	5135.4* ±979.1	5532.5* ±871.0	1411.1 ±59.0	1384.7 ±93.5	1078.4** ±95.4	825.9 ±24.3	683.6* ±29.7	717.7 ±53.8
Hip	496.9 ±26.8	527.9 ±46.8	372.5 ±76.4	74.7 ±10.3	75.4 ±4.5	68.0 ±3.5	415.7 ±17.3	508.2* ±18.6	376.4 ±16.2	ND	ND	ND	ND	ND	ND	659.1 ±20.3	656.7 ±62.8	569.8* ±34.7
Tha	609.5 ±34.3	526.5 ±46.5	462.8 ±72.9	87.6 ±9.0	75.1 ±14.2	58.8 ±8.9	574.2 ±41.3	670.9 ±106.3	664.3 ±23.3	ND	ND	ND	ND	ND	ND	1002.9 ±44.3	895.1 ±8.8	965.5 ±73.6
Hyp	2599.9 ±230.0	2466.7 ±265.1	2094.3 ±230.2	425.7 ±20.4	426.0 ±31.9	437.6 ±41.4	891.0 ±60.4	962.3 ±150.9	913.8 ±69.3	245.5 ±18.8	290.7 ±77.5	295.5 ±45.3	ND	ND	ND	1013.7 ±46.9	810.5* ±82.5	994.6 ±57.6
SN	504.6 ±60.6	393.1 ±96.6	409.6 ±64.8	958.8 ±89.9	884.8 ±106.7	812.8 ±179.6	1597.5 ±99.5	1731.9 ±130.1	1691.8 ±195.3	ND	ND	ND	ND	ND	ND	2347.7 ±126.7	2023.7 ±142.7	2541.7 ±224.7
Cbl	281.9 ±15.6	239.0 ±23.1	252.8 ±37.6	ND	ND	ND	84.7 ±12.2	90.2 ±14.3	75.1 ±8.3	ND	ND	ND	ND	ND	ND	ND	ND	ND

Values are means±S.E. expressed as ng/g tissue. Numbers in parentheses denote the number of animals.

ND, not detectable; C, control; S, single treatment; Ch, chronic treatment; NE, norepinephrine; DA, dopamine; 5-HT, 5-hydroxytryptamine; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; Fcx, frontal cortex; Str, corpus striatum; Hip, hippocampus; Tha, thalamus; Hyp, hypothalamus; SN, substantia nigra; Cbl, cerebellum.

*$p<0.05$, **$<p<0.01$ (Dunnett's test)

Table 2. Effects of single or chronic treatment with desipramine on the levels of biogenic amines and their metabolites in rat brain

	NE			DA			5-HT			DOPAC			HVA			5-HIAA		
	C(13)	S(5)	Ch(6)	C(13)	S(4)	Ch(6)	C(13)	S(5)	Ch(6)	C(13)	S(4)	Ch(5)	C(14)	S(5)	Ch(5)	C(14)	S(5)	Ch(6)
Fcx	329.5 ±27.2	321.2 ±14.4	257.4 ±15.5	69.0 ±6.4	72.8 ±10.7	84.4 ±9.0	670.9 ±25.6	665.3 ±50.6	656.1 ±38.3	ND	ND	ND	ND	ND	ND	560.8 ±23.1	469.7* ±19.5	471.5 ±32.4
Str	103.9 ±16.9	118.6 ±15.8	194.7* ±25.8	10408.5 ±486.6	9804.2 ±532.7	10841.5 ±636.6	557.0 ±17.1	523.4 ±59.1	475.2* ±29.9	7770.1 ±491.1	3943.2** ±764.5	5560.0* ±246.1	1411.1 ±59.0	1320.7 ±53.1	1422.9 ±75.3	825.9 ±24.3	751.4 ±24.2	694.3* ±49.8
Hip	496.9 ±26.8	431.2 ±31.2	367.9* ±46.0	74.7 ±10.3	77.2 ±36.4	77.1 ±0.7	415.7 ±17.3	416.6 ±23.4	521.9* ±67.9	ND	ND	ND	ND	ND	ND	659.1 ±20.3	581.3 ±42.6	669.5 ±25.6
Tha	609.5 ±34.3	643.2 ±114.1	379.0** ±23.7	87.6 ±9.0	97.4 ±27.6	98.4 ±19.4	574.2 ±41.3	581.5 ±23.7	582.2 ±19.3	ND	ND	ND	ND	ND	ND	1002.9 ±44.3	950.4 ±34.8	872.2 ±33.3
Hyp	2599.9 ±230.0	2171.8 ±164.8	2254.0 ±154.1	425.7 ±20.4	350.5 ±40.7	460.5 ±43.2	891.0 ±60.4	922.7 ±63.6	880.6 ±91.6	245.5 ±18.8	204.8 ±17.8	245.4 ±22.0	ND	ND	ND	1013.7 ±46.9	907.5 ±51.6	789.0* ±42.7
SN	504.6 ±60.6	389.1 ±63.8	389.7 ±63.1	958.8 ±89.9	865.0 ±84.5	933.3 ±126.9	1597.5 ±99.5	1729.8 ±200.1	1794.7 ±140.6	ND	ND	ND	ND	ND	ND	2347.7 ±126.5	2423.8 ±251.5	2197.9 ±179.5
Cbl	281.9 ±15.6	264.0 ±23.1	238.4 ±18.1	ND	ND	ND	84.7 ±12.2	69.4 ±7.2	77.4 ±3.3	ND	ND	ND	ND	ND	ND	ND	ND	ND

Legends are the same as Table 1.

Effect of single treatment with imipramine on the concentrations of catecholamines, 5-HT and their metabolites in brain tissues (Table 1, 2)

No significant alterations in the concentration of norepinephrine were found in 7 brain regions studied in this experiment. Changes of the concentration of 5-HT was found only in the hippocampus where the concentration of 5-HT increased by 22.3% after a single injection. On the other hand, the reduced concentration of 5-HIAA, a major metabolite of 5-HT, was found in 3 brain regions, such as the hypothalamus, corpus striatum and frontal cortex. The ratio of 5-HIAA/5-HT was decreased in all structures but the cerebellum, which implies a generalized decrease of the 5-HT turnover rate in the cerebral hemisphere.

No significant changes of the concentration of dopamine were found in the brain regions after the single treatment with imipramine. The concentration of dopamine metabolite, DOPAC, was decreased in the corpus striatum. However, the concentration of HVA, another metabolite of dopamine, was not changed in the corpus striatum.

Effects of chronic treatment with imipramine on the concentrations of catecholamines, 5-HT and their metabolites in brain tissues (Table 1, 2)

There were no significant alterations in the concentration of norepinephrine after three weeks' treatment with imipramine in the brain regions. Similarly, no changes in the concentrations of 5-HT and 5-HIAA were found in the brain regions. This finding suggested that decreased concentrations of 5-HIAA after the single treatment recovered after repeated treatment with imipramine.

There were no changes in the concentration of dopamine after the chronic treatment of imipramine. However, the concentrations of both dopamine metabolites, DOPAC and HVA, were significantly decreased in the corpus striatum.

Effects of the single treatment with desipramine on the concentrations of catecholamines, 5-HT and their metabolites in brain tissues (Table 1, 2)

There were no significant changes in the concentrations of norepinephrine, 5-HT and 5-HIAA in the brain regions except in the frontal cortex in which the concentration of 5-HIAA was decreased. The concentrations of dopamine and HVA were not changed in the brain regions. However, the concentration of DOPAC was significantly reduced in the corpus striatum.

Effects of the chronic treatment with desipramine on the concentrations of catecholamines, 5-HT, and their metabolites in brain tissue (Table 1, 2)

Unlike the case of the imipramine treatment, significant alterations in the concentration of norepinephrine were found in three brain areas after chronic treatment with desipramine. The concentration of norepinephrine was decreased in the hippocampus and thalamus, and increased in the corpus striatum. The alterations of the concentrations of 5-HT and 5-HIAA depended on the brain regions. The concentration of 5-HT was reduced in the corpus striatum and increased in the hippocampus, whereas 5-HIAA was reduced in the corpus striatum and in the hypothalamus.

Table 3. Effects of single or chronic treatment with imipramine or desipramine on the levels of HVA and 5-HIAA in rat CSF

Treatment	HVA	5-HIAA
Control	24.1±4.2(5)	158.8± 9.2(14)
Imipramine		
Single	24.4±3.2(4)	168.5±33.6(4)
Chronic	20.8±9.2(6)	162.1±23.6(8)
Desipramine		
Single	18.7±2.3(5)	137.2±19.6(5)
Chronic	21.1±3.9(4)	141.6±17.7(4)

Values are means±S.E. expressed as ng/ml CSF. Numbers in parentheses denote the number of animals. HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid.

The concentrations of dopamine and HVA were not changed in the brain regions. However, the concentration of DOPAC was significantly reduced in the corpus striatum.

Effects of treatment with imipramine or desipramine on the concentrations of the metabolites of catecholamines and 5-HT in the CSF (Table 3)

Among several compounds measured in the present study, only metabolites of biogenic amines, HVA and 5-HIAA, were detectable in the CSF. No alterations in the concentrations of 5-HIAA and HVA were found after single or chronic treatment with imipramine or desipramine.

DISCUSSION

The results of the present study clearly showed uneven alterations of the concentrations of monoamine neurotransmitters and their metabolites among different brain regions after single or chronic treatment with imipramine or desipramine. In the present study, we used a highly sensitive method for the measurement of biogenic amines and their metabolites, HPLC-ECD, which enabled us to measure these compounds simultaneously and regionally. These advantages of HPLC-ECD strengthened the validity of the data.

The concentration of norepinephrine was not changed after the single treatment with imipramine or desipramine. This finding was consistent with other reports (Schildkraut *et al.* 1970, 1971; Sedlock and Edwards, 1985) which observed alterations in the concentration of brain norepinephrine only after 2 weeks' treatment. After the chronic treatment with desipramine, the concentration of norepinephrine was decreased in the hippocampus and thalamus, and increased in the corpus striatum. In the case of the chronic imipramine treatment, no significant alterations in the concentrations of norepinephrine were found in the various brain regions. This result suggested that the area of the brain which was affected by desipramine was broader than by imipramine. Although the meaning of the direction of the alteration can not be speculated, an altered concentration of the hippocampal neurotransmitter may be of importance because the hippocampus is involved in important brain functions, such as emotion, motivation, learning and memory which may be related to the expression of depression (Kostowski *et al.* 1986). It is reported that the hippocampus had high density binding sites for ^{3}H-imipramine and ^{3}H-desipramine (Sherman and Allers, 1980), and that the concentration of norepinephrine metabolite, MHPG, was increased (Miyauchi *et al.* 1982) and the basal firing rate of hippocampal neurons was accelerated (Huang, 1979) after the chronic treatment of desipramine. Moreover, the density of hippocampal β-adrenergic receptor was found to be decreased in the animal model of depression developed by forced-running stress (Nakamura *et al.* 1992). Our results and these observations of others' are highly supportive to the notion that the hippocampus is the major site of action of tricyclic antidepressants.

Chronic treatment of tricyclic antidepressants elicit adaptive responses of the central adrenergic receptors. Down-regulation of β-receptors (Sulser *et al.* 1978), upregulation of α_1-receptors, down-regulation of α_2-receptors (Cambell and Mckerman, 1982; Vetulani, 1984), increased sensitivity of α_1-receptors and decreased sensitivity of α_2-receptors (Hong *et al.* 1986) were reported. It is possible that the decreased concentration of hippocampal norepinephrine observed in the present study resulted from the enhanced metabolism and/or from the augmented depletion of the stored norepinephrine by the decreased density and sensitivity of the presynaptic α_2 receptors which inhibit norepinephrine release upon stimulation of norepinephrine.

Alterations of the concentrations of 5-HT and/or 5-HIAA were found in the frontal cortex, corpus striatum, hippocampus and hypothalamus after single treatment with imipramine; however, only the frontal cortex showed altered concentrations of 5-HT and/or 5-HIAA after single treatment with desipramine. This result suggested that more diffuse regions of the brain were affected by the acute treatment with imipramine than with desipramine. The fact that no significant alterations, except in the hippocampus, in the concentrations of 5-HT and 5-HIAA

after the chronic treatment with imipramine suggested an existence of a certain neuronal adaptation mechanism. Our result was consistent with the reports which observed a decrease in the 5-HT turnover rate after acute treatment with Lu-10-170, a selective 5-HT uptake inhibitor (Hyttel, 1977), and which observed no significant changes in the concentrations of 5-HT (Hall *et al.* 1985) and 5-HIAA (Segawa and Mizuta, 1980) after chronic treatment with imipramine. After chronic treatment with imipramine, only the hippocampus showed a significantly altered concentration of 5-HIAA. This observation with the altered hippocampal concentration of norepinephrine after chronic treatment with desipramine strengthened the importance of the hippocampus as a common site of action of tricyclic antidepressants. Abnormality of the hippocampus has also been reported in a human magnetic resonance imaging study in which shortened T1 spin-lattice relaxation times were observed in patients of major depression (Krishnan *et al.* 1991). A decrease in the ratio of 5-HIAA/5-HT, which implies turnover rate of the 5-HT, after single treatment with imipramine disappeared after chronic treatment. This finding may result from adaptive responses of the 5-HT receptors after treatment with tricyclic antidepressants. It is reported that treatment with tricyclic antidepressants caused a decrease in the density of 5-HT receptors (Kendall *et al.* 1981; Peroutka and Snyder, 1980) and/or a decrease in the sensitivity of the presynaptic 5-HT receptors (Svensson, 1978).

Although a recent observation showed a reduced volume of the caudate nucleus, where dopamine is the major neurotransmitter, in patients of major depression (Krishnan *et al.* 1992), dopaminergic neurotransmission has been neglected as a mechanism of action of tricyclic antidepressants since high concentrations of tricyclic antidepressants were needed to block dopamine reuptake. However, Serra *et al.* (1979, 1980) proposed the involvement of dopaminergic presynaptic receptors to the antidepressant action. They observed that chronic treatment with tricyclic antidepressants reduced the effect of a small dose of apomorphine which decreased the concentration of DOPAC by stimulating dopaminergic presynaptic receptors. Moreover, it has been reported that amineptine, which blocks dopamine reuptake, had the ability of antidepressive action (Simoni *et al.* 1986) and that tricyclic antidepressants itself could decrease the concentration of DOPAC (Holcomb *et al.* 1982; Diggory and Buckett, 1984). In the present study, a decreased concentration of striatal DOPAC was observed invariably after single or chronic treatment with imipramine or desipramine. A blockade of dopamine reuptake may have a role for this reduced concentration of DOPAC after tricyclic antidepressants since there was no alterations in the concentration of HVA, an extracellular metabolite, in the corpus striatum.

In the present study, HVA and 5-HIAA were detectable in the CSF. There were no significant alterations in these detectable metabolites after treatment with imipramine or desipramine. No alterations in the concentrations of HVA and 5-HIAA were also observed in human CSF (Koslow, 1986) after tricyclic antidepressants. However, it is premature to draw conclusions because neurochemical changes of the collected CSF reflects only those of the adjacent brain tissue (Wood, 1980).

In conclusion, acute or chronic treatment with imipramine or desipramine caused alterations in the concentrations of monoamine neurotransmitters and their metabolites according to the brain regions. Hippocampal adrenergic and serotonergic neurotransmission may be deeply involved in the expression of the antidepressant effects of tricyclic antidepressants, and dopaminergic neurotransmission in the corpus striatum may be also important. The relationship between the activity of the biogenic amines and the activity of histamine (Kanof and Greengard, 1978) and/or muscarinic receptors (Rehavi *et al.* 1980), which is affected by tricyclic antidepressants, remains a matter for future experiments.

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