Changes in the Amounts of Neurotransmitters Released from the Striatum and Spontaneous Motor Activity in Rats Exposed to High Doses of Toluene

Hiroyuki AIKAWA, Takahiko YOSHIDA and Sadayoshi SHIGETA

Department of Environmental Health, Tokai University School of Medicine, Isehara

Abstract

We studied the neurotoxicological effects of high-dose toluene exposure by measuring neurotransmitter release from the striatum and spontaneous motor activity in free-moving Tokai High Avoider (THA) male rats. The rats were exposed to 1,000, 2,000, and 4,000ppm toluene for 4 hours. During the 4-hour exposure period and each one hour pre- and post-exposure periods, acetylcholine (ACh), 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the microdialysate from the striatum of each rat were measured continuously using microdialysis. Simultaneously, spontaneous motor activity of each rat was monitored using an Aimex device. The amounts of neurotransmitters released and spontaneous motor activity showed similar changes for every concentration of toluene exposure during those periods. Although minimal changes in neurotransmitters and motor activity were observed in the 1,000 ppm group, increases in neurotransmitter release and motor activity were observed in the 2,000 ppm group during the entire exposure period and also in the 4,000 ppm group during the early period of exposure. On the other hand, decreases in neurotransmitter release and motor activity were observed in the 4,000 ppm group during the late period of exposure. These findings indicate that the striatum influences motor activity via changes in the amounts of neurotransmitters released during the period of high-dose toluene exposure.

Key words: Toluene, Neurotransmitter, Rat, Striatum, Spontaneous motor activity

Introduction

We have been studying the effects of chemicals such as toluene on the central nervous system by examining learning activity in animals¹⁻⁴. Neurotransmitters are closely involved in learning activity, and there are an increasing number of studies on the effects of chemicals on neurotransmitters. However, many of the studies are based on fragmentary observations after exposure of animals to the chemicals.

Recently, continuous measurement of neurotransmitters in the brains of experimental animals has become possible under unrestricted conditions due to the development of the microdialysis method⁵.

It is well-known that high doses of toluene cause changes in activity such as excitement and anesthesia by suppressing the activities of the central nervous system. The amounts of neurotransmitters released from the striatum are correlated with behavior in rats exposed to high doses of toluene^{6,7}. However, there are no studies observing continuous changes in the neurotransmitter release from the striatum and spontaneous motor activity during exposure to toluene simultaneously. In this study, serial changes in the amounts of neurotransmitters released in the brain and spontaneous motor activity were examined in rats exposed to toluene at 1,000, 2,000 and 4,000 ppm, concentrations known to cause obvious changes in behavior^{8,9}. In addition, the relationship between the changes of two parameters, the amounts of acetylcholine and monoamine metabolites released from the striatum and spontaneous motor activity, was investigated.

Materials and Methods

1. Experimental animals

We used male rats (mean age: 8-9 weeks, body weight: 275-290g)

Reprint requests to:

Hiroyuki Aikawa,
Department of Environmental Health, Tokai University School of Medicine,
Bohseidai, Isehara 259-11, Japan
Telephone number; 0463-93-1121, FAX number; 0463-93-8796

of the 42nd generation of Tokai High Avoider (THA) rats¹⁰⁾, an inbred line derived from the JCL-Wistar rat (Japan Clea, Tokyo), showing hereditary high ability in the Sidman electric shock avoidance learning test. Seven rats were used for the 4,000 ppm toluene-exposure group and 5 rats each for the 2,000 and 1,000 ppm toluene-exposure groups and control group. Animals in which measurement could not be performed because of obstruction of the dialysis probe or the connection tube were excluded.

Animals (4 animals per cage) were kept at the temperature of $23\pm 2^\circ\text{C}$ and the humidity of $50\pm 10\%$ in a Clean Rack (CL-5412, Japan Clea, Tokyo) in a rearing facility. The lighting conditions were lights on from 8 a.m. to 8 p.m. and off from 8 p.m. to 8 a.m. Animals were fed with solid chow (CE-2, Japan Clea, Tokyo) and given tap water ad libitum.

2. Toluene exposure

Medical compressed air was allowed to pass through a bubbler containing toluene to generate toluene gas at a high concentration, which was then diluted with medical compressed air to yield concentrations of 1,000, 2,000, and 4,000 ppm, which were monitored by gas chromatography (Type 163, Hitachi, Tokyo). The control group was exposed to only medical compressed air. Exposure was performed by ventilating a free-moving box (30 cm $\times$ 30 cm $\times$ 30 cm) with toluene gas about 10 times per hour for 4 hours between 11 a.m. and 3 p.m. for all groups.

3. Measurement of neurotransmitters in the brain

For simultaneous measurement of neurotransmitters requiring different measurement conditions at the same site of the brain without sacrificing sensitivity, a microdialysis method⁵⁾ using a W-probe was adopted. Twenty-four hours before the start of exposure, the 3-mm tip housing the dialysis membrane of the W-probe was inserted into the striatum (0 mm anterior from the pregyra, 2.7 mm right external from the median line, and 6.5 mm vertical from the dura to the base of brain) of the right hemisphere of each animal under anesthesia with 2.5% halothane, and fixed with dental resin. As the dialysis fluid, physiological saline supplemented with 10^{-5}M eserine for acetylcholine (ACh) and physiological saline without addition for monoamine metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were separately perfused at 2 $\mu\text{l}/\text{min}$. Perfused solution was pooled, collected every 20 min, and quantified by high-performance liquid chro-

matography with an electrochemical detector (HPLC-ECD, KK Eicom, Kyoto). The measurement conditions for HPLC-ECD are shown in Table 1.

4. Measurement of spontaneous motor activity in rats

We used an Animex device (MK-Animex SE-type, Muromachi Science KK, Tokyo) for measurement of spontaneous motor activity because it can monitor vertical motion as well as horizontal motion of animals without affecting circadian rhythm and spontaneous motor activity over a long period of observation while performing measurements of neurotransmitters in the brain by the microdialysis method. Spontaneous motor activity was measured for 6 hours from 1 hour before the initiation of exposure to 1 hour after the end of exposure, and recorded every 20 min in accordance with the interval of the neurotransmitter measurement.

5. Statistical analysis

Differences in the amount of neurotransmitters released and spontaneous motor activity between exposure concentrations were examined by two-way analysis of variance (ANOVA) and comparisons between exposure concentrations in each exposure time and the entire exposure period were performed by Bonferroni's multiple comparative method. The "Stat View" statistical program was used.

Results

1. Amounts of neurotransmitters released

1-1. ACh

The amounts of ACh released before exposure were 2.52 ± 0.35 ng/40 $\mu\text{l}/20$ min in the control group, and 2.90 ± 0.76 , 2.82 ± 0.52 and 2.60 ± 0.54 ng/40 $\mu\text{l}/20$ min in the 1,000, 2,000 and 4,000 ppm groups, respectively. There were no significant differences among the groups. In the control group exposed to air, the mean amount of ACh released ($2.49\text{--}2.69$ ng/40 $\mu\text{l}/20$ min) showed small fluctuations throughout the examination period.

Changes in the amount of ACh released in each toluene-exposure group relative to the control group are shown in Fig. 1. In the 1,000 ppm group, the amount of ACh released showed only small fluctuations throughout the examination period, similar to the control group. In the 2,000 ppm group, the amount of

Table 1 HPLC-ECD conditions

	Monoamine metabolites	Acetylcholine
Injection volume	40 μl	40 μl
Mobile phase	0.1M-Phosphate buffer (pH3.5) +Methyl alcohol (20%) +160 mg/L, Sodium 1-octansulfonic acid +1 μM , EDTA-2Na	0.1M-Phosphate buffer (pH8.0) +300 mg/L, Sodium 1-decansulfonic acid +65mg/L, tetramethyl ammonium chloride
Column (precolumn) (analytical) (enzyme reactor)	Eicom PC-05 (4.0 ϕ $\times$ 5mm) Eicompak MA-50DS (4.6 ϕ $\times$ 150mm)	Eicom PC-05 (4.0 ϕ $\times$ 5mm) Eicompak AC-Ge (6.0 ϕ $\times$ 150mm) Eicom AC-Enzympak (4.0 ϕ $\times$ 5mm)
Column temperature	25 $^\circ\text{C}$	33 $^\circ\text{C}$
Flow rate	1.0ml/min	1.0ml/min
Detector	Eicom ECD-100	Eicom ECD-100
Working electrode	Graphite, WE-3G	Platinum, WE-PT
Applied potential	750mV	450mV
Reference electrode	Ag/AgCl	Ag/AgCl

Fig. 1 Changes in ACh release in the microdialysate output from the striatum during and after 4hr toluene exposure against control (n=4).

○: 1000ppm (n=4), △: 2000ppm (n=5), □: 4000ppm (n=7). Values are shown as mean $\pm$ SD. The statistical significance of differences between groups was calculated by two-way ANOVA followed by Bonferroni's multiple comparison procedure.

*: Significant difference between control and 2000ppm ($p < 0.05$).

†: Significant difference between 1000ppm and 2000ppm ($p < 0.05$).

‡: Significant difference between 2000ppm and 4000ppm ($p < 0.05$).

ACh released started to increase 40 min after the initiation of exposure. In the 4,000 ppm group, ACh release increased immediately after the initiation of exposure and started to decrease 80 min thereafter, becoming lower than the control group level at 180 min after the initiation of exposure, and slightly decreased further. Sixty minutes after the end of exposure, the ACh release returned to the level before exposure.

There were significant differences in ACh release among the toluene exposure groups in the examination by the binary configuration method [$F_{3, 195} = 28.655$ ($p < 0.0001$)]. Comparisons of the results throughout the experimental period by the multiple comparative method showed significant differences between the control group and the 2,000 ppm group, between the 1,000 and 2,000 ppm groups, between the 1,000 and 4,000 ppm groups, and between the 2,000 and 4,000 ppm groups ($p < 0.05$). For each exposure period, there were no significant differences between the 1,000 ppm group and the control group. There were significant differences between the 2,000 and 4,000 ppm groups from 180 min after the initiation of exposure to 60 min after the end of exposure ($p < 0.05$). At 200 min after the initiation of exposure, there were significant differences between the control group and the 1,000 ppm group ($p < 0.05$). The 4,000 ppm group showed significant differences from the 2,000 ppm group in some exposure periods as described above, but not from the control group and the 1,000 ppm group.

1-2. DOPAC

The amounts of DOPAC released before exposure were 45 ± 14 ng/40 μ l/20 min in the control group, and 48 ± 17 , 45 ± 12 and 45 ± 11 ng/40 μ l/20 min in the 1,000, 2,000 and 4,000 ppm groups, respectively, showing no significant differences among the groups. In the control group, the mean amount of DOPAC released (38 – 45 ng/40 μ l/20 min) slightly decreased with time.

Changes in DOPAC release in each toluene exposure group relative to the control group are shown in Fig. 2. In the 1,000 ppm group, the DOPAC release slightly decreased up to 80 min after the initiation of exposure, and slightly increased with time.

Fig. 2 Changes in DOPAC release in the microdialysate output from the striatum during and after 4hr toluene exposure against control (n=4).

○: 1000ppm (n=4), △: 2000ppm (n=5), □: 4000ppm (n=7). Values are shown as mean $\pm$ SD. The statistical significance of differences between groups was calculated by two-way ANOVA followed by Bonferroni's multiple comparison procedure.

*: Significant difference between 1000ppm and 4000ppm ($p < 0.05$).

In the 2,000 ppm group, it increased between 100 min and 200 min after the initiation of exposure, and then slightly decreased until 60 min after the end of exposure. In the 4,000 ppm group, DOPAC release slightly increased up to 160 min after the initiation of exposure, and then decreased.

There were significant differences in amounts of DOPAC released among the toluene-exposure groups as determined by the binary configuration method [$F_{3, 195} = 5.091$ ($p < 0.005$)]. Analysis of the results throughout the experimental period by the multiple comparative method showed a significant difference only between the control group and the 2,000 ppm group ($p < 0.05$). For each exposure period, the 4,000 ppm group showed a significantly larger amount of DOPAC 40 min after the initiation of exposure than the 1,000 ppm group ($p < 0.05$), but there were no significant differences among the groups in any other exposure period.

1-3. HVA

The amounts of HVA released before exposure were 44 ± 28 ng/40 μ l/20 min in the control group, and 36 ± 15 , 49 ± 21 and 42 ± 5 ng/40 μ l/20 min in the 1,000, 2,000 and 4,000 ppm groups, respectively, showing no significant differences among the groups. In the control group, the mean amount of HVA released was 43 – 45 ng/40 μ l/20 min, showing small fluctuations.

Changes in HVA release in each toluene-exposure group relative to the control group are shown in Fig. 3. At 240 min after the initiation of exposure, the HVA release in the 1,000 ppm group was 10% lower than in the control group, while at the other times, it was constantly lower by about 5%. In the 2,000 ppm group, HVA release started to increase at 120 min after the initiation of exposure, and remained at a high level after 180 min. The amount of HVA released in the 4,000 ppm group was 6–11% lower than that in the control group from 220 min after the initiation of exposure to 60 min after the end of exposure.

The binary configuration method [$F_{3, 195} = 32.797$ ($p < 0.0001$)] revealed significant differences in HVA release among the toluene-exposure groups. Analysis of the results throughout the experimental period by the multiple comparative

Fig. 3 Changes in HVA release in the microdialysate output from the striatum during and after 4hr toluene exposure against control (n=4).

○: 1000ppm (n=4), △: 2000ppm (n=5), □: 4000ppm (n=7). Values are shown as mean $\pm$ SD. The statistical significance of differences between groups was calculated by two-way ANOVA followed by Bonferroni's multiple comparison procedure.

*: Significant difference between control and 2000ppm ($p < 0.05$).

†: Significant difference between 1000ppm and 2000ppm ($p < 0.05$).

‡: Significant difference between 2000ppm and 4000ppm ($p < 0.05$).

method showed significant differences between the 2,000 ppm group, the control group and the 1,000 and 4,000 ppm groups ($p < 0.05$). There were no significant differences in the amount of HVA released among the control group and the 1,000 and 4,000 ppm groups in any exposure period. However, the 2,000 ppm group showed significant differences from the control group from 180 to 200 min after the initiation of exposure, from the 1,000 ppm group from 180 min after the initiation of exposure to the end of exposure, and from the 4,000 ppm group from 180 min after the initiation of exposure to 20 min after the end of exposure ($p < 0.05$).

1-4. 5-HIAA

The amounts of 5-HIAA released before exposure were 48 ± 12 ng/40 μ l/20 min in the control group, and 51 ± 32 , 48 ± 12 and 34 ± 10 ng/40 μ l/20 min in the 1,000, 2,000 and 4,000 ppm groups, respectively, showing no significant differences among the groups. In the control group, the mean amount of 5-HIAA released (48 – 64 ng/40 μ l/20 min) slightly increased with time.

Changes in amount of 5-HIAA released in each toluene exposure group relative to the control group are shown in Fig. 4. The amount of 5-HIAA released relative to the control group continuously increased up to 200 min after the initiation of exposure in the 1,000 ppm group, up to 180 min in the 2,000 ppm group and up to 160 min in the 4,000 ppm group, and then gradually decreased in all three toluene-exposure groups.

Significant differences in the amounts of 5-HIAA released among the toluene-exposure groups were revealed with in statistical analysis by the binary configuration method [$F_{3, 195} = 5.091$ ($p < 0.005$)]. Analysis of the results throughout the experimental period by the multiple comparative method showed a significant difference only between the control group and the 2,000 ppm group ($p < 0.05$). There were no significant differences among any groups for any individual exposure periods.

Fig. 4 Changes in 5-HIAA release in the microdialysate output from the striatum during and after 4hr toluene exposure against control (n=4).

○: 1000ppm (n=4), △: 2000ppm (n=5), □: 4000ppm (n=7). Values are shown as mean $\pm$ SD. The statistical significance of differences between groups was calculated by two-way ANOVA followed by Bonferroni's multiple comparison procedure.

2. Spontaneous motor activity

Spontaneous motor activities before exposure were 278 ± 178 counts/20 min in the control group, and 590 ± 334 , 489 ± 213 and 381 ± 173 counts/20 min in the 1,000, 2,000 and 4,000 ppm groups, respectively, showing no significant differences among the groups. In the control group, the mean spontaneous motor activity showed small fluctuations within the range of 60–342 counts/20 min over the experimental period.

Changes in spontaneous motor activity in each toluene-exposure group relative to the control group are shown in Fig. 5. Spontaneous motor activity in the 1,000 ppm group, as well as in the control group, was low with small fluctuations throughout the experiment period. Spontaneous motor activity in the 2,000 ppm group started to increase at 60 min after the initiation of exposure and remained at a high level after the exposure period. In the 4,000 ppm group, spontaneous motor activity started to increase at 20 min after the initiation of exposure, then began to decrease at 140 min and reached the minimum level just before the end of exposure, following which it slightly increased.

There were significant differences in spontaneous motor activity among the toluene-exposure groups demonstrated by statistical evaluation using the binary configuration method [$F_{3, 252} = 27.034$ ($p < 0.001$)]. Analysis of the results for the entire experimental period by the multiple comparative method showed significant differences between the control group and the 2,000 ppm group, between the control group and the 4,000 ppm group, between the 1,000 and 2,000 ppm groups, and between the 1,000 and 4,000 ppm groups ($p < 0.05$). There were no significant differences in spontaneous motor activity between the control group and the 1,000 ppm or 2,000 ppm group in any single exposure period. However, there were significant differences at 100 and 120 min after the initiation of exposure between the 4,000 ppm group and the control and 1,000 ppm groups ($p < 0.05$). There were also significant differences at 200 and 220 min after the initiation of exposure between the 4,000 ppm group and the control group, and at 220 and 240 min between the 4,000 and 1,000 ppm groups ($p < 0.05$).

Fig. 5 Changes of spontaneous motor activity before, during and after of 4hr toluene exposure against control (n=4).

○: 1000ppm (n=4), △: 2000ppm (n=5), □: 4000ppm (n=7). Values are shown as mean $\pm$ SD. The statistical significance of differences between groups was calculated by two-way ANOVA followed by Bonferroni's multiple comparison procedure.

*: Significant difference between control and 2000ppm ($p < 0.05$).

†: Significant difference between control and 4000ppm ($p < 0.05$).

‡: Significant difference between 1000ppm and 2000ppm ($p < 0.05$).

§: Significant difference between 1000ppm and 4000ppm ($p < 0.05$).

¶: Significant difference between 2000ppm and 4000ppm ($p < 0.05$).

Discussion

In this study, We investigated the effects of high-dose toluene exposure on the central nervous system based on movement of neurotransmitters in the brain by using the microdialysis method with a W-probe and by examining changes in spontaneous motor activity using an Animex device at the same time in THA rats exposed to toluene at high concentrations under unrestricted conditions.

Since toluene exposure in this study was performed in the daytime, spontaneous motor activities of every exposure group before exposure and of the control group during exposure to air were at a low level, reflecting the characteristics of nocturnal animals in which spontaneous motor activity, including feeding and drinking, is high during the dark period and low during the light period^{11, 12}. The small fluctuations of spontaneous motor activity in the control group throughout the experimental period indicated that exposure to air did not affect the spontaneous motor activity of rats.

The amounts of the neurotransmitters ACh, HVA, DOPAC and 5-HIAA released in the 1,000 ppm group showed minimal differences, by less than 10%, from those in the control group, suggesting that such a low concentration of toluene had little effect on the release of neurotransmitters in the rat brain. Changes in the spontaneous motor activity were also small in the control group and the 1,000 ppm group where the rats were almost somnolent during the experiment period in a curled-up position.

In the 2,000 ppm group, the amounts of ACh and the three monoamine metabolites released increased with exposure time, and in the second half of the exposure period, the amounts of ACh and HVA released were significantly larger than those of other groups. Although the differences were not significant, the amount of DOPAC released increased by 15% at maximum and that of 5-HIAA increased by 20% at maximum, showing the

greatest increases among all toluene-exposure groups. On the other hand, the spontaneous motor activity in the 2,000 ppm group started to increase at 60 min after the initiation of exposure and in the second half of the exposure period. It was significantly higher than in the control group and the 1,000 ppm group, which showed no changes during the experiment, and than in the 4,000 ppm group, in which the spontaneous motor activity increased between the first and second halves of the exposure period but then decreased. Thus, both the release of neurotransmitters and spontaneous motor activity were increased at the same time by exposure to 2,000 ppm toluene.

In the 4,000 ppm group, the amounts of ACh and DOPAC released slightly increased in the first half of the exposure period, and those of DOPAC and 5-HIAA slightly increased in the middle of the exposure period. In the second half of the exposure period or before the end of exposure, the amounts of each of the four neurotransmitters released were the smallest in the experimental groups. On the other hand, the spontaneous motor activity in the 4,000 ppm group increased in the first half of the exposure period and in the middle of the exposure period, while in the second half of the exposure period, between 220 and 240 min after the start of exposure, it decreased to reach the level of the control group, rats of which were somnolent. Thus, neurotransmitter release and spontane motor activity changed with similar time courses.

As shown above, the changes in the release of neurotransmitters paralleled, though they did not exactly coincide with, the change in the spontaneous motor activity in each exposure group. There was little change in the 1,000 ppm group, a slight increase in the 2,000 ppm group, and dual-phase changes in the 4,000 ppm group, which showed a constant increase during the exposure period followed by a decrease after the end of exposure.

We found that the spontaneous motor activity increased in the 2,000 ppm group, whereas it decreased to a low level in the second half of the exposure period in the 4,000 ppm group while the rats curled up and slept. These observations agreed with the report by Miyagawa et al.¹³ that the rate of the lever-pressing response in a learning test for obtaining food increased after exposure to toluene at 1,700 ppm for 4 hours, and decreased after exposure to toluene at 3,400 and 5,100 ppm for 4 hours. Such changes in spontaneous motor activity may be caused by the suppressive action of toluene on the central nervous system¹⁴⁻¹⁶. In the early stage of exposure in the 2,000 and 4,000 ppm groups and after exposure in the 4,000 ppm group, the toluene level in the animals might have been such that it caused excitation due to its weak suppressive action on the central nervous system, and as a result increases of the spontaneous motor activity occurred. In the second half of the exposure to 4,000 ppm toluene, the increased level of toluene in the animals exerted an anesthetic effect due to its strong suppressive action on the central nervous system, resulting in decreased spontaneous motor activity. The changes in spontaneous motor activity observed in this study were thus due to the excitation and anesthetic effects induced by suppressive actions of toluene on the central nervous system.

The increase in spontaneous motor activity paralleled the increase in the release of DOPAC, a metabolite of dopamine, in the first half of the exposure period in the 4,000 ppm group and throughout the exposure period in the 2,000 ppm group, and the increase in the release of HVA, a metabolite of DOPAC, in the second half of the exposure period in the 2,000 ppm group.

Yamawaki et al. investigated the relationship between neurotransmitters and spontaneous motor activity and found a slight increase in dopamine in the brain, except for the cerebellum, and a significant increase in spontaneous motor activity after exposure to 7,000 ppm toluene for 15 min¹⁷⁾. There are several reports demonstrating a close relationship between the activity of dopaminergic neurons in the striatum and spontaneous motor activity¹⁸⁻²¹⁾. Therefore, the changes in the spontaneous motor activity of the animals exposed to toluene at high concentrations observed in this study were likely affected by the increase of dopamine metabolites released from the striatum during exposure to toluene.

Exposure to toluene also induced an increase in the amount of ACh released from the striatum. This observation suggested that exposure to toluene affected not only dopaminergic neurons but also cholinergic neurons, or that there was a relationship between both types of neurons. Therefore it is possible that the changes in ACh release induced by exposure to toluene also affected the observed changes in the spontaneous motor activity. The similarity in the patterns of changes of dopamine-metabolite release and ACh noted in this study is consistent with the reported observations that the spontaneous motor activity is increased by administration of scopolamine, which inhibits cholinergic neurons²²⁾ and that cholinergic neurons are controlled by dopaminergic neurons in the rat striatum²³⁻²⁵⁾.

The present study suggested that dopaminergic neurons in the striatum were closely related to changes in spontaneous motor activity induced by exposure to toluene, and that cholinergic neurons were also affected by exposure to toluene and involved in changes in spontaneous motor activity.

The experimental system used in this study allowed simultaneous measurement of neurotransmitters and spontaneous motor activity during exposure to toluene, and the relationship between

the effects of toluene on neurotransmitters and the spontaneous motor activity could be studied.

In the future, we will further study the involvement of the two types of neurons in the changes in spontaneous motor activity induced by exposure to toluene using specific inhibitors of each neuron.

Conclusions

Neurotransmitter release and spontaneous motor activity were simultaneously measured in THA rats exposed to toluene at 1,000, 2,000 and 4,000 ppm for 4 hours.

1. The amounts of neurotransmitters released from the striatum and the spontaneous motor activity exhibited similar changes in each group, those in the 1,000 ppm group being at the same level as in the control group, those in the 2,000 ppm group increasing with time, and those in the 4,000 ppm group showing a decrease in the second half of the exposure period following an increase in the first half.
2. Exposure to toluene affected the release of ACh as well as dopamine.
3. Different levels of suppression of the central nervous system were observed, as reflected by neurotransmitter release and spontaneous motor activity, as a result of exposure to various concentrations of toluene.

Acknowledgments

We would like to thank Mr. Tomoyasu Hagino in the Department of Environmental Health for the care of animals.

This study was supported in part by a grant (No. 05670367) from the Ministry of Education, Science and Culture, Japan.

References

- 1) Aikawa H, Suzuki K, Misawa T, Momotani H, Shigeta S. Effects of peri- and postnatal lead administration on learning of high avoider rats. *Jpn J Ind Health* 1986; **28**: 428-37. (in Japanese)
- 2) Shigeta S, Misawa T, Aikawa H, Momotani H, Suzuki K, Yoshida T. Effects of low level toluene exposure during the developing stage of the brain on learning in high avoider rats. *Jpn J Ind Health* 1986; **28**: 445-54. (in Japanese)
- 3) Miyake K, Misawa T, Aikawa H, Yoshida T, Shigeta S. The effects of prenatal trimethyltin exposure on development and learning in high avoider rats. *Jpn J Ind Health* 1989; **31**: 363-71. (in Japanese)
- 4) Aikawa H, Miyake K, Momotani H, Shigeta S. Sex difference in operant behavior following single exposure to carbon monoxide in adult rats. *Jpn J Ind Health* 1990; **32**: 26-32. (in Japanese)
- 5) Aikawa H, Shigeta S. W-probe for microdialysis method in freely moving rat brain. *Jpn J Psychopharmacol* 1992; **12**: 215-21. (in Japanese)
- 6) Sugita R, Sawa U, Nakazawa T, Yamauchi T. Changes of monoamine metabolites in the nucleus accumbens and locomotor activity by reserpine. — An in vivo microdialysis study —. *Jpn J Psychopharmacol* 1988; **453-62**. (in Japanese)
- 7) Salamone JD, Keller RW, Zigmond MJ, Stricker EM. Behavioral activation in rats increases striatal dopamine metabolism measured by dialysis perfusion. *Brain Res* 1989; **487**: 215-24.
- 8) Shigeta S, Aikawa H, Misawa T, Kondo A. Effect of single exposure to toluene on Sidman avoidance response in rats. *J Toxicol Sci* 1978; **3**: 305-12.
- 9) Shigeta S, Misawa T, Aikawa H. Effects of concentration and duration of toluene exposure on Sidman avoidance in rats. *Neurobehav Toxicol* 1980; **2**: 85-8.
- 10) Shigeta S, Miyake K, Misawa T, Aikawa H, Katoh H. A new inbred rat strain "THA". *Rat News Letter* 1990; **23**: 9-11.
- 11) Kuribara H, Tadokoro S, Miura T. Changes in circadian rhythm of drinking in hypophysectomized rats. *Clin Endocrinol* 1979; **27**: 259-64. (in Japanese)
- 12) Zucker I. Light-dark rhythms in rat eating and drinking behavior. *Physiol Behav* 1971; **6**: 115-26.
- 13) Miyagawa M, Honma T, Sato M, Hasegawa H. Behavioral change after single exposure to toluene, and brain toluene levels in rats. *Ind Health* 1986; **24**: 157-61.
- 14) World Health Organisation. Toluene, WHO (Environmental Health Criteria 52): Geneva, 1985.
- 15) Patty's Industrial Hygiene and Toxicology, Third revised edition. John Wiley and Sons Inc. USA, 1981.
- 16) Tadokoro S. Abuse of thinner and other volatile organic solvents: Focusing on thinner sniffing. *Jpn J Clin Pharmacol* 1977; **8**: 347-54. (in Japanese)
- 17) Yamawaki S, Sarai K. Effects of toluene inhalation on locomotor activity and brain catecholamine levels in rats. *Jap Psychopharmacol* 1982; **2**: 57-9.
- 18) Cools AR, Honig WMM, Pijnenburg AJJ, Van Rossum JM. The nucleus accumbens of rats and dopaminergic mechanisms regulating locomotor behaviour. *Neuroscience Letters* 1976; **3**: 335-9.
- 19) Cools AR. Mesolimbic dopamine and its control of locomotor activity in rats: Differences in pharmacology and light/dark periodicity between the olfactory tubercle and the nucleus accumbens. *Psychopharmacology* 1986; **88**: 451-9.
- 20) Svensson L, Ahlenius S. Suppression of exploratory locomotor activity in the rat by the local application of 3-ppp enantiomers into the nucleus accumbens. *Europ J Pharmacol* 1983; **88**: 393-7.

- 21) Wachtel H, Ahlenius S, Anden NE. Effects of locally applied dopamine to the nucleus accumbens on the motor activity of normal rats and following α -methyltyrosine or reserpine. *Psychopharmacology* 1979; **63**: 203-6.
- 22) Toide K. Effects of scopolamine on extracellular acetylcholine and choline levels and spontaneous motor activity in freely moving rats measured by brain dialysis. *Pharmacol Biochem Behav* 1989; **33**: 109-13.
- 23) Arnfred T, Randrup A. Cholinergic amphetamine and induced stereotyped behaviour. *Acta Pharmacol Toxicol* 1968; **26**: 384-94.
- 24) Bryan KS, Ellison G. Cholinergic modulation of an opposed effect of d-amphetamine and methylphenidate on the rearing response. *Psychopharmacologia* 1975; **43**: 169-73.
- 25) Joseph MH, Frith CD, Waddington JL. Dopaminergic mechanisms and cognitive deficit in schizophrenia, A neurobiological model. *Psychopharmacology* 1979; **63**: 273-80.

(Received Aug. 18, 1995/Accepted Oct. 14, 1996)