Rats were exposed to trichloroethylene (TriCE) and tetrachloroethylene (TetraCE) at concentrations of 200, 400, and 800 ppm for one month. The effects of inhalation exposure on neurotransmitter candidates of the rat brain were studied. Changes in the acetylcholine (ACh) content of the striatum were investigated by pyrolysis gas chromatography. The dopamine (DA) in the striatum, norepinephrine (NE) in the hypothalamus, NE and serotonin (5TH) in brain regions containing cortex and hippocampus were analyzed by high-performance liquid chromatography. (1) The DA in the striatum was slightly increased by TriCE and decreased by TetraCE, but the change were not statistically significant. (2) The ACh in the striatum was markedly reduced dose-dependently by these two organic solvents. The decreases in ACh at 800 ppm of TriCE and TetraCE were significant (p<0.05). (3) The NE was slightly increased by these solvents, except that the NE in the cortex and hippocampus was reduced by TriCE. (4) The 5HT in the cortex and hippocampus was increased by TriCE and TetraCE, but the increase was not statistically significant. It is suggested that long-term exposure to organic solvents may cause some disturbance of the cholinergic neurons of the central nervous system.
activity of dopaminergic neurons\textsuperscript{15,16}. DA is also involved in the development of emotional behavior\textsuperscript{17}. Norepinephrine (NE) and serotonin (5HT) containing neurons are distributed throughout the brain\textsuperscript{18}. Sleep and emotional behavior are regulated by these neurons\textsuperscript{19,20}. NE containing neurons play an important role in the regulation of the autonomic nervous system\textsuperscript{21}.

The present report considers the influence of inhalation exposure to TriCE and TetraCE on the monoamine contents of the rat brain, as well as the changes in the activity of monoaminergic neurons in different brain areas.

\textbf{Materials and Methods}

(1) Animals

Male albino rats (SD strain), weighing 250–300 g (8 weeks old) at the beginning of exposure, were used. Five to six rats were grouped in an inhalation chamber and allowed free access to food and water throughout the experiment.

(2) Exposure procedure

Inhalation exposure was performed in a room with a barriar system at $23 \pm 1^\circ C$ and $55 \pm 5\%$ humidity with lights on from 8 a.m. to 8 p.m. TriCE and TetraCE were changed to vapour by saturation method and introduced into inhalation chambers. The Inhalation exposure was continued for one month at 0, 200, 400, and 800 ppm.

(3) Extraction and assay of monoamines

On termination of the exposure, post mortem changes in the substances in the brain were prevented by rapidly raising the brain temperature using a microwave applicator (Muromachi Kikai). The brain was dissected into 7 areas (right and left striatum, hypothalamus, midbrain, cerebellum, pons-medulla and the residual brain containing the hippocampus and cortex) as described by Glowinski and Iversen\textsuperscript{22}, and stored at $-80^\circ C$. For analysis of the DA, NE, and 5HT, the tissues were homogenized in 1.5–4.0 ml of 0.1 N HClO$_4$ solution containing 5 mM EDTA using a polytron homogenizer. The homogenate was centrifuged, and 20 $\mu l$ of the supernatant was applied to a Zipax-SCX column (2.1 $\times$ 1000 mm, Du Pont) in a high-performance liquid chromatograph (Shimazu-Du Pont) by an automatic sampling system (Kyowa Seimitsu). The column temperature was 40$^\circ C$ and the flow rate was 0.6 ml/min. To estimate the DA content in the striatum, 0.075 M NH$_4$H$_2$PO$_4$ solution was employed as the mobile phase, and the DA was converted to fluorophore by reaction with ethylenediamine. The NE in the hypothalamus and residual brain was analyzed using 0.025 M NaH$_2$PO$_4$ solution as the mobile phase. NE was reacted with ferricyanide to form fluorophore. The 5HT in the residual brain was separated using 0.125 M NH$_4$H$_2$PO$_4$ as the mobile phase and converted to fluorophore by reaction with o-phthalaldehyde. The intensity of fluorescence of the DA, NE, and 5HT derivatives was measured with an autoanalyzer. The ACh in the striatum was assayed by
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pyrolysis-gas chromatography. The tissue was homogenized in 1 ml of 1 N formic acid-acetone (15:85) in a polytron homogenizer with 100 µl of 0.1 mM butyrylcholine iodide added as an internal standard. The homogenate was centrifuged and the supernatant was transferred to a test tube. Ether (3 ml) was then pipetted into the tube, which was immediately closed, shaken for 10 min on a shaker, and briefly centrifuged to separate the phases. The upper phase (ether) was aspirated. This shaking procedure was repeated twice. The aqueous phase was then transferred to a small plastic tube and 20 µl of KI-I₂ solution was pipetted into the tube. After centrifugation, the supernatant was discarded. The precipitate was dissolved in 50 µl of methanol. To remove excess iodine, approximately 5 mg of macroporous AG1-X8-C1⁻ was added to the tube. Choline esters were pyrolyzed on the filament of a pyrolysis apparatus (Kotaki), and separated with a gas chromatograph (YHP) equipped with a glass column, packed with 5% OV-101, 5% Jenden phase, on 100/120 mesh Gas Chrom Q, and detected with a flame ionization detector.

(4) Statistics

Statistical treatment was performed by two-tailed t-test. When the variance of the

![Graph](173)
exposure group was significantly different from that of the control group, Aspin-Welch method was used.

**Results**

Fig. 1 illustrates the effects of exposure to TriCE and TetraCE on the DA content of rat striatum. The DA was slightly increased by the exposure to TriCE, whereas a dose-dependent reduction of DA was induced by TetraCE. However, these changes were not statistically significant. The ACh in the striatum was reduced dose-dependently by both TriCE and TetraCE, as shown in Fig. 2. The reduction in ACh at 800 ppm of these two solvents was statistically significant \((p<0.05)\). TriCE and TetraCE produced a slight change in the NE content of the hypothalamus (Fig. 3) and residual brain (Fig. 4). The NE content of these brain regions was slightly increased, except for an NE decrease in the residual brain induced by TriCE, but the differences between the control and experimental groups were not statistically significant. The 5HT in the residual brain was increased by TetraCE as shown in Fig. 5. The increase in 5HT in the rat group exposed to 200 ppm of TetraCE was about 30\%, but was not significant compared to

![Graph showing effects of inhalation exposure of rats to TriCE and TetraCE on the ACh content of the striatum.](image-url)
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Fig. 3. Effects of TriCE and TetraCE on the NE content of the hypothalamus.

Fig. 4. Effects of TriCE and TetraCE on the NE content of the residual brain.
The mean values of monoamine contents (nmole/wet weight tissue) in the control group were as follows: DA in the striatum 67.3, ACh in the striatum 60.4, NE in the hypothalamus 10.3, NE in the residual brain 2.68, and 5HT in the residual brain 2.43.

**DISCUSSION**

The ACh in the striatum was significantly reduced by exposure to TriCE and TetraCE. Such findings have not previously been reported in the literature insofar as we are aware. The level of endogenous ACh in the rat brain was reduced by excitation and convulsion, but increased by sleep and anesthesia. It would be expected that ACh is reduced by inhibition of ACh synthesis but increased by inhibition of choline esterase. The effects of TriCE and TetraCE on ACh synthesis or choline esterase are not known. TriCE and TetraCE exhibit anesthetic activity. It may be considered therefore that these organic solvents bear some similarity to anesthetic drugs, such as barbiturates, as regards their effects on the CNS. It has been reported by Nordberg et al. that oral administration of pentobarbital sodium for 33-44 weeks significantly decreased the ACh content of the striatum. They found that the rate of ACh synthesis, however, showed no change in the striatum, while single injection of pentobarbital led
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to an increase in the ACh content, although the rate of ACh synthesis was markedly reduced\(^{27,28}\). Based on these results, the decrease in ACh content induced by the continuous exposure to TriCE and TetraCE for one month might not be explained by lowered activity of ACh synthesis. The activities of cholinergic neurons have relevance to many functions of the brain, such as the regulation of sleep, memory, excitability and motor function\(^{8-13}\). Alteration of the activity of cholinergic neurons would produce some dysfunction of the CNS, although the mechanism of action of TriCE and TetraCE has remained obscure.

Further studies on the metabolism of ACh in the rat brain after exposure to various organic solvents, are now in progress.

The changes in catecholamine contents were not remarkable, although a DA decrease in the striatum induced by TetraCE and an NE decrease in the residual brain induced by TriCE were observed. TriCE and TetraCE may cause only little change in the activity of catecholaminergic neurons in the CNS. The possibility remains, however, that these organic solvents alter the metabolism of catecholamines without any change in the levels of endogenous DA and NE.

TriCE and TetraCE produced a slight increase in the 5HT content of the brain regions containing the hippocampus and cortex. The most marked increase was observed at 200 ppm of TetraCE. 5HT is thought to be involved in the regulation of sleep\(^{29}\). The disturbance of sleep in human beings induced by organic solvents might have some relation with changes in serotonergic neurons.

Both TriCE and TetraCE lowered the level of ACh in the striatum remarkably. In our previous experiments\(^{30}\), which were carried out using the same kinds of brain samples as those employed in the preset study, we observed that both of these organic solvents significantly increased the endogenous glutamine in the midbrain. Such phenomena as a decrease in ACh and an increase in glutamine may be common to many organic solvents including TriCE and TetraCE, even if there are differences in relative potency; for example, TriCE was more effective in reducing the ACh content than TetraCE but the former was weaker in increasing the glutamine content.

More detailed examinations of the effects of organic solvents on neurotransmitter substances in the CNS are now in progress.

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References

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