DUAL EFFECTS OF TETRACHLORVINPHOS ON PROCAINE TOXICITY AND PROCAINESTERASE ACTIVITY IN RATS

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Abstract—The effect of tetrachlorvinphos (TCVP) on liver procainesterase (PROCase) and procaine toxicity was studied in rats. TCVP is an organophosphate with an inducible effect on drug metabolizing enzymes. A single oral dose of 500 mg/kg of TCVP caused a remarkable decrease in PROCase (40% of control) 24 hr later and increased the mortality after injection of procaine (250 mg/kg, i.p.) from 54% to 87%. Conversely, it was observed that PROCase elevated to 140% of the control and mortality decreased from 54% to 25% on day 3. With repeated administration of TCVP (500 mg/kg/day) for 5 days, the PROCase activity that was inhibited on day 1 was gradually restored to normal levels by 5 days and the mortality altered to 25%. The inducible effect on PROCase was examined using desmethyl-TCVP, a metabolite of TCVP without inhibitory effect on the enzyme; PROCase activity was enhanced to 1.6-fold of the control and procaine concentration in the brain was reduced to 30% of the control, accompanied with no death of rats after procaine injection. Electrophoresis of the solubilized liver microsomal fraction confirmed the inducible effect of TCVP on PROCase; microsomal protein from the TCVP-treated rat was more deeply stained than that from the control, and the PROCase activity of two anodic bands increased in the TCVP-treated microsomes. These results indicate that TCVP has a dual action on PROCase, inducible and inhibitory, and that the direct inhibitory effect of TCVP might mask the increased amount of the enzyme induced by repeated administration of TCVP. The dual effect of TCVP on PROCase would cause the change in procaine toxicity.

Organophosphates are known to inhibit not only acetylcholinesterase, but also other hydrolases including carboxylesterases. Some studies have been done on the toxicologic interaction between those organophosphates and ester or amide drugs which are metabolized by carboxylesterases (1–3). In previous papers, we have demonstrated that liver procainesterase (PROCase), which is responsible for hydrolysis of procaine, was one of the liver microsomal carboxylesterases and that it was strongly inhibited by organophosphates and induced by phenobarbital treatment (4, 5). In addition, these treatment caused a marked change in procaine toxicity; that is, pretreatment with organophosphates potentiated procaine toxicity; but in contrast, phenobarbital treatment of rats reduced the toxicity (6).

Tetrachlorvinphos (TCVP) is one of vinylphosphate insecticides, and the repeated administration of TCVP to rats resulted in the
dose-dependent induction of the liver microsomal drug metabolizing enzymes (7). This suggests the possibility that TCVP might have both inhibitory and inducible effects on PROCase activity as well as procaine toxicity.

The present study was undertaken to determine whether or not TCVP has both effects on PROCase, and in addition, the relation between PROCase activity and procaine toxicity after a single and repeated administration of TCVP in rats.

MATERIALS AND METHODS

Materials: TCVP (98% pure) was kindly supplied by Shell Kagaku K.K., Tokyo, Japan. Procaine hydrochloride was obtained from Iwaki Seiyaku Co. Ltd., Tokyo. Glucose 6-phosphate, NADP, dl-ethionine, acetylthiocholine iodide, 5,5-dithiobis (2-nitrobenzoic acid), α-naphthylacetate, N-1-naphthylethenediamine dihydrochloride, Fast Blue RR, and Coomassie blue G250 were purchased from the Nakarai Chemical Co. Ltd., Kyoto. Desmethyl TCVP (2-chloro-1-(2,4,5-trichlorophenyl)vinyl methylhydrogen phosphate) was synthesized in our laboratory by the hydrolysis of TCVP with H2SO4 as described by Akintonwa and Hutson (8). All the chemicals were of reagent grade and used without further purification.

Animal experiments: Male Wistar rats weighing 180–230 g were used in all experiments. They were obtained from the Nippon Rat Co. Ltd., Urawa, and they were kept in an air conditioned room and given standard chow and water ad libitum. Freshly prepared TCVP or desmethyl TCVP suspended in corn oil was given to the animals and an equivalent volume of corn oil to the control rats. Procaine and ethionine were dissolved in 0.9% NaCl solution. At definite time intervals after TCVP treatment (500 mg/kg, p.o.), animals were sacrificed for measurement of enzyme activity or were subjected to procaine toxicity experiments. Paralysis, convulsion, and death were used as indices of procaine toxicity. Onset of paralysis was defined only when rats began to show loss of the righting reflex. After the animals were sacrificed by decapitation, livers and brains were quickly removed and homogenized in ice-cold 1.15% KCl solution to obtain 20% liver or 10% brain homogenate. The liver homogenate were centrifuged at 10,000 g for 20 min at 2°C, and the resulting supernatant was used as the enzyme source for PROCase and drug metabolizing enzyme assays. The supernatant was further centrifuged at 100,000 g for 60 min to separate the microsomal fraction. For determination of tissue procaine and p-aminobenzoic acid (PABA) content, brain and liver were homogenized in 10% trichloroacetic acid (TCA) solution as soon as possible after sacrifice. Serum was separated by centrifugation of blood at 2,000 rpm for 20 min.

Enzyme assay: All enzyme activities were spectrophotometrically measured in a Hitachi Perkin-Elmer 139 spectrophotometer. PROCase activity was assayed by measuring the amount of PABA formed from procaine during incubation (1). The incubation mixture consisted of 0.5 ml of buffered substrate (2×10^-3 M in Tris-HCl buffer, pH 8.0), 0.2 ml of the 10,000 g supernatant, and water to make a total volume of 1.0 ml. It was incubated for 30 min at 37°C, and the enzyme activity was stopped by addition of 1.0 ml of 10% TCA solution. Procaine and PABA were determined by the method of Ting et al. (9). Cholinesterase (ChE) activities of serum or brain were determined by the method of Voss and Sachsse (10) with the minor modification described by Moroijn et al. (7). Serum and brain homogenates were diluted 10-fold with 1.15% KCl before and incubated for 15 min at 37°C with
acetylthiocholine as the substrate. Drug metabolizing enzyme activity (aminopyrine demethylase activity) was assayed by measuring formaldehyde formation from aminopyrine used as the substrate (7, 11). Enzyme activity was expressed as a percentage of the control or μ moles of products formed/g tissue weight or ml/min. Statistical significance was determined by the Student's t-test (two-tailed).

Polyacrylamide gel electrophoresis: Disc gel electrophoresis was carried out according to the method of Davis (12) using 7.5% gels. Rat liver microsomes were solubilized by adding Triton X-100 (final concentration, 0.2%) and the centrifuged at 100,000 g for 70 min. The supernatants were applied to polyacrylamide gels and electrophorised for 2 hr at 2 mA/gel. After electrophoresis, the gels were stained with Coomassie blue for protein or with 2 ml of 0.1 M phosphate buffer containing Fast blue RR (4 mg) and α-naphthylacetate (10⁻³ M) dissolved in acetone. The gels were densitometrically scanned at 600 nm (protein) or 480 nm (α-naphthylacetate esterase activity) in a Shimazu dual wavelength Chromatoscanner (Model CS-910).

To determine PROCase activity, gels were cut by a gel-slicer (Hotta Rika K.K., Tokyo) into 1.0 mm thin slices and two pieces per tube were incubated with procaine as substrate for 3 hr, following the course of incubation to estimate the PABA formation.

RESULTS

Effect of a single and repeated administration of tetrachlorvinphos on liver procainesterase in rats: Figure 1 shows the liver microsomal PROCase activity of rats treated with TCVP at a dose of 500 mg/kg p.o. singly (—●—) or for 5 successive days (—Ο—). Arrows show the treatment with TCVP, and the enzyme activity was determined on days 1, 3 and 5 after treatment. Each point represents the mean percentage of the control ±S.E. from 5 or more rats. The control value of procainesterase is 0.073 ± 0.006 μmoles p-aminobenzoic acid/g liver/min. *P<0.05 as compared with the control.

![Fig. 1. Liver procainesterase activity after treatment with TCVP in a dose of 500 mg/kg p.o. singly (—●—) or for 5 successive days (—Ο—). Arrows show the treatment with TCVP, and the enzyme activity was determined on days 1, 3 and 5 after treatment. Each point represents the mean percentage of the control ±S.E. from 5 or more rats. The control value of procainesterase is 0.073 ± 0.006 μmoles p-aminobenzoic acid/g liver/min. *P<0.05 as compared with the control.](image-url)
dual effects on PROCase activity: one is a direct inhibitory effect and the another is probably an inducible one. In fact, PROCase was inhibited to 20% of the control by $10^{-5} \text{ M}$ TCVP in vitro. Furthermore the phenomenon observed in the repeated administration of TCVP suggests that the enzyme activity induced by repeated treatment might be masked by the inhibitory effect of TCVP on PROCase.

Effect of a single administration of TCVP on procaine toxicity and procaine concentration in the brain: In order to determine the effect of TCVP on procaine toxicity, rats were treated with TCVP singly in a dose of 500 mg/kg, and on days 1 (24 hr), 3 (72 hr) and 5 (120 hr) after treatment, they were injected with procaine (250 mg/kg, i.p.). The selection of this procaine dose was based on preliminary studies which demonstrated that it caused about 50% mortality in control rats. Procaine toxicity (paralysis, convolution and death) was observed over 1 hr after procaine treatment. In control rats, loss of the righting reflex occurred at 4.0 min after procaine injection and lasted 20 min as indicated in Table 1. Death was observed in the range from 8 min to 16 min after procaine injection. Table 1 shows that an administration of TCVP tended to increase procaine toxicity on day 1; only one rat survived after long paralysis. The rest of the group died soon after convulsions in 8–10 min after procaine injection. Conversely, there was a tendency for procaine toxicity to decrease on day 3 after treatment with TCVP: the time from procaine injection to loss of righting reflex lengthened, duration of paralysis shortened, and mortality decreased. On day 5, pretreatment with TCVP did not affect the toxicity. This alteration of the toxicity after procaine injection in TCVP-pretreated rats seemed to be closely related to the change in liver PROCase activity after TCVP treatment as shown in Fig. 1. Therefore, liver PROCase, brain procaine concentration, and serum ChE activities, as indicators of the anticholinesterase action of TCVP, were determined at the moment of death (Died) or at 1 hr after procaine injection (Survived) in the rats which recovered from the toxicological signs. The results are shown in Table 2. Both in the control and the treated rats, the rats that died showed high brain procaine concentrations, but there were no differences among these groups. PROCase activity was 40% lower in dead rats as compared to rats which survived, and significant differences in serum ChE activity was not observed between the live and dead animals. TCVP treatment caused a decrease in PROCase and serum ChE activities in the dead rats on day 1, but not on days 3 and 5.

Table 1. Effect of a single administration of tetrachlorvinphos on procaine toxicity in rats

| Treatment | Time (day) | Paralysis | Convulsion (%) | Mortality (%) |
|-----------|------------|-----------|----------------|--------------|
| None      | –          | 4.0±0.2 (13/13) | 20.2±2.8 (6/13) | 77.0 (10/13) | 54.0 (7/13) |
| TCVP      | 1          | 3.9±0.4 (8/8)  | 37.0 (1/8)      | 100.0 (8/8)  | 87.0 (7/8)  |
| TCVP      | 3          | 6.0±0.9 (7/8)  | 12.9±2.4 (5/8)  | 63.0 (5/8)   | 25.0 (2/8)  |
| TCVP      | 5          | 4.4±0.8 (8/9)  | 19.0±5.2 (3/9)  | 83.0 (8/9)   | 55.0 (5/9)  |

Procaine (250 mg/kg, i.p.) was administered 1, 3 and 5 days after TCVP-treatment (500 mg/kg, p.o.). Onset is equal to the time from injection to loss of the righting reflex and duration is equal to the time between the loss and the regaining of the righting reflex. Values in table represent the mean±S.E. Numbers in parentheses indicate the number of rats showing toxicity/number of rats used.
Table 2. Effect of a single administration of tetrachlorvinphos on brain procaine concentration, liver carboxylesterase and serum cholinesterase activities in rats

| Treatment | Time (day) | Brain procaine conc. | Liver procainesterase | Serum cholinesterase |
|-----------|------------|----------------------|-----------------------|----------------------|
|           |            | Survived             | Died                  | Survived             | Died                  |
| None      | –          | 0.070±0.010(6)       | 0.345±0.029(7)        | 0.068±0.004(6)       | 0.044±0.005(7)        | 2.554±0.195(6)        | 2.415±0.143(7)        |
| TCVP      | 1          | 0.20 (1)             | 0.410±0.010(7)        | 0.052 (1)            | 0.010±0.004(7)*       | 0.61                  | 0.660±0.043(7)*       |
| TCVP      | 3          | 0.070±0.004(6)       | 0.350±0.008(2)        | 0.074±0.002(6)       | 0.044±0.008(2)        | 2.090±0.089(5)        | 1.984±0.197(2)        |
| TCVP      | 5          | 0.076±0.012(4)       | 0.369±0.022(5)        | 0.055±0.005(4)       | 0.035±0.004(5)        | 2.405±0.253(4)        | 2.707±0.103(5)        |

Procaine concentration and enzyme activity were determined at the moment of death (Died) or 1 hr after procaine injection (Survived). Brain procaine concentration, procainesterase, and serum cholinesterase activities were expressed as μmoles procaine/g brain, μmoles p-aminobenzoic acid/g liver/min, and μmoles thiocholine/ml serum/min, respectively. Values in the table represent the mean±S.E. Numbers in parentheses indicate the number of rats used. *P<0.01, significantly different from the control.

Table 4. Effect of repeated administration of tetrachlorvinphos and desmethyl tetrachlorvinphos on brain procaine concentration, liver procainesterase and serum cholinesterase activities in rats

| Treatment | Time (day) | Brain procaine Conc. | Liver procainesterase | Serum cholinesterase |
|-----------|------------|----------------------|-----------------------|----------------------|
|           |            | Survived             | Died                  | Survived             | Died                  |
| None      | –          | 0.070±0.010(7)       | 0.354±0.019(5)        | 0.076±0.005(7)       | 0.041±0.007(5)        | 2.468±0.092(7)        | 2.21±0.074(5)         |
| TCVP      | 5          | 0.079±0.014(6)       | 0.315±0.056(2)        | 0.096±0.007(6)*      | 0.036±0.004(2)        | 1.970±0.144(6)*       | 1.935±0.085(2)        |
| Desmethyl-TCPV | 5  | 0.023±0.004(8)**   | –                     | 0.126±0.006(8)**     | –                     | 3.084±0.108(8)**      | –                     |

Procaine concentration and enzyme activity were determined at the moment of death (Died) or 1 hr after procaine injection (Survived). Brain procaine concentration, procainesterase, and serum cholinesterase activities were expressed as μmoles procaine/g brain, μmoles p-aminobenzoic acid/g liver/min, and μmoles thiocholine/ml serum/min, respectively. Values in table represent the mean±S.E. Numbers in parentheses indicate the number of rats showing toxicity/number of rats used. * and **: significantly different from the control at P<0.05 and P<0.01, respectively.
Effect of repeated administration of TCVP on procaine toxicity and brain procaine concentration: Table 3 shows the effect of repeated administration of TCVP on procaine toxicity when procaine was injected 24 hr after the last treatment with TCVP. There were no differences in time of onset and duration between control and TCVP-treated rats. A change in mortality was observed from 58% in the control to 25% in the TCVP-treated rats. As indicated in Table 4, procaine content 1 hr after procaine injection and at the moment of death were respectively the same value both in the control and the TCVP-treated rats. PROCase activity of rats that survived in the treated group increased to 120% of the control, suggesting that this is a possible reason for change in mortality after treatment with TCVP.

Effect of repeated administration of desmethyl-TCVP on procaine toxicity, liver procainesterase and brain procaine concentration: In order to reveal the inducible effect of TCVP by repeated administration and the relation between induction of PROCase and change in procaine toxicity, rats were pretreated with desmethyl-TCVP (480 mg/kg/day) in an equal molar dose as TCVP for 5 days. Desmethyl-TCVP is a monomethyl analogue of TCVP which does not inhibit liver PROCase and ChE activities. As shown in Table 3, desmethyl-TCVP treatment tended to decrease procaine toxicity; no death was observed and duration of paralysis was shortened from 22 min to 16 min. Concurrently, it reduced brain procaine content to 30% of the procaine-injected control and enhanced PROCase and serum ChE activities to 160% and 125% of the control, respectively (Table 4). Increases in drug metabolizing enzyme activity and in liver weight were also observed. This inducible effect of desmethyl-TCVP strongly supported that TCVP had an inducible effect on PROCase and that the enzyme activity induced by repeated administration of TCVP might be inhibited directly by the anti-cholinesterase action of TCVP.

Change of brain procaine concentration in rats treated with tetrachlorvinphos: As indicated in Tables 2 and 4, there was no difference in the brain procaine concentration 1 hr after treatment (in survived rats) of the control and the TCVP-treated rats, even on days 3 or 5 when change in mortality was observed. In order to clarify the relationship between change in procaine toxicity and brain procaine concentration or liver PROCase activity, the tissue concentrations of procaine and its metabolite, p-aminobenzoic acid (PABA), was determined at selected time

| Table 3. Effect of repeated administration of tetrachlorvinphos and desmethyl-tetrachlorvinphos on procaine toxicity in rats |
|---------------------------------------------------------------|
| **Treatment** | **Time (day)** | **Paralysis** | **Convulsion** | **Mortality** |
|               |                | Onset (min) | Duration (min) | %       | %       |
| None          | 1              | 4.45±0.6   | 22.2±2.8      | 75.0    | 58.0    |
| TCVP          | 5              | 5.1±0.4    | 20.8±1.8      | 75.0    | 25.0    |
| Desmethyl-TCVP| 5              | 5.3±0.3    | 16.2±1.3      | 75.0    | 0       |

Procaine (250 mg/kg, i.p.) was administered 24 hr after TCVP (500 mg/kg, p.o.) or desmethyl-TCVP (480 mg/kg, p.o.) treatment for 5 days. Onset is equal to the time from injection to loss of the righting reflex and duration is equal to the time between the loss and the regaining of the righting reflex. Values in the table represent the mean±S.E. Numbers in parentheses indicate the number of rats showing toxicity/number of rats used.
intervals after procaine injection. Procaine was given at a dose of 200 mg/kg to prevent animals from death produced by procaine. As shown in Fig. 2, procaine content in control rats reached maximum levels within 10 min after treatment, and then rapidly decreased. A single treatment with TCVP caused accumulation of brain procaine content at all times tested on day 1, and, conversely, low concentrations of brain procaine was observed over 30 min in rats treated with TCVP for 5 days. Most animals showed highest procaine concentration around 10 min after procaine injection which reflected the finding that severe toxicity (convulsion and death) appeared at this period. This also indicated that procaine toxicity resulted from an accumulation of brain procaine.

Figure 3 demonstrated the alteration of liver procaine and PABA concentration after procaine injection. Liver procaine (A) concentration decreased rapidly to minimum levels 1 hr after injection both in the control and in the TCVP-treated rats for 5 days. On the other hand, high procaine content was observed in rats treated with TCVP on day 1. The change in PABA content (B) was
opposite to that of procaine content; the liver from rats treated with TCVP for 5 days showed a high content of PABA which suggested rapid hydrolysis of procaine to PABA. The low concentration of PABA in the liver observed for the single administration of TCVP was closely related to the inhibition of PROCase activity on day 1. These results confirmed our presumption that modification of liver PROCase activity by TCVP caused a change of tissue procaine concentration, which resulted in altered procaine toxicity.

Fig. 4. Densiometric scans of polyacrylamide gels after electrophoresis of rat solubilized microsomes. (A-C) Protein bands stained with Coomassie blue and scanned at 600 nm. (D-F) Enzyme activity bands stained with α-naphthylacetate and scanned at 480 nm. The bands of activity were numbered from the cathodic end of the gels. Procainesterase activity (—▲—) was determined by incubating the sliced gels with procaine for 3 hr. * shows the bands induced.
In addition, the result of Fig. 3 (B) showing the increase in PABA content in rats treated with TCVP for 5 days demonstrated the enhancement of liver PROCase activity after repeated administration of TCVP, although the apparent enzyme activity was not activated on day 5 in TCVP-treated rats as shown in Fig. 1.

Electrophoresis of microsomal procainesterase: In order to reveal induction of PROCase by repeated treatment with TCVP, the following experiment was carried out using the electrophoretic method. After the rat microsomes were solubilized by addition of 10% Triton X-100 to a final concentration of 0.2%, the solutions were centrifuged at 100,000 g for 70 min to obtain the solubilized supernatant. The concentration of Triton X-100 was selected to solubilize whole PROCase activity but not drug metabolizing enzyme activity. The resulting extracts containing an equivalent amount of 10 mg of liver by weight were subjected to disc polyacrylamide gels and electrophoresed for 2 hr. Figure 4 shows the distribution of protein bands stained with Coomassie blue (A–C) and bands showing a-naphthylacetate (a-NA) esterase activity (D–F) after densitometry. Figure 4 (D) demonstrated that microsomes had at least five esterase activities toward a-NA. To investigate the relation between the PROCase and the a-NA esterases, the gels were cut into 1.0 mm slices and two slices per tube were incubated for 3 hr with procaine. The finding that the PROCase activity migrated to the same distance as the bands of a-NA esterase activity as shown in Fig. 4(D) indicated that a-NA esterases were responsible for hydrolysis of procaine. It was observed as shown in Fig. 4 (B, C) that microsomal protein from rats pretreated with TCVP were more deeply stained with Coomassie blue, suggesting the increase in microsomal protein. In fact, the microsomal protein from TCVP treated rats increased to 134% on day 3 (single treatment) and to 165% of the control levels on day 5 (repeated treatment). In addition, in the microsomes treated with TCVP, three anodic bands (* in Fig. 4), two of them having esterase activity, appeared with high protein content; and the anodic esterase activities markedly increased in rats treated with TCVP, particularly with the single treatment (F). The microsomes treated with TCVP for 5 days (B, E) showed high protein content at anodic bands, but the esterase activities of them were lower than those of single treated microsomes, indicating the inhibition of increased enzyme protein by TCVP. At 24 hr, after a single treatment with TCVP, the protein pattern of the electrophoretogram was not affected. But the esterase activities which migrated toward the anodic side were almost completely inhibited (Fig. 4 B, E).

Effect of ethionine on procainesterase induced by tetrachlorvinphos treatment: As the induction of PROCase activity by TCVP was considered to accelerate protein synthesis of the enzyme, ethionine, an inhibitor of protein synthesis, was given i.p. to rats simultaneously with a single administration of TCVP in a dose of 200 mg/kg twice a day for 3 days. Seventeen hours after the last injection of ethionine, the enzyme assay was carried out. As shown in Table 5, ethionine treatment completely suppressed the increase in PROCase activity by TCVP. Drug metabolizing enzyme activity also decreased from 230% to 150% of the control levels, and the ratio of liver weight per body weight returned to the control levels. These results indicated that the induction of PROCase by TCVP resulted from acceleration of enzyme protein synthesis by TCVP. The same finding was observed in the electrophoretic pattern: the increase in microsomal protein content and in esterase activities by TCVP treatment was suppressed by an
Table 5. Effect of ethionine and tetrachlorvinphos on procainesterase and aminopyrine demethylase activities and liver weight/body weight ratio

| Treatment | Procainesterase  | Aminopyrine demethylase | liver wt./body wt. ×100 |
|-----------|------------------|-------------------------|-------------------------|
| None      | 100.0± 6.88 (6)  | 100.0± 4.71 (7)         | 4.80±0.08 (7)           |
| TCVP      | 145.4± 5.90 (4)*** | 223.9±19.59 (4)***     | 5.21±0.14 (4)*          |
| Ethionine | 66.8±13.12 (7)   | 137.7±7.63 (7)**        | 4.82±0.09 (7)           |
| Ethionine | 71.8±12.17 (5)   | 50.8±10.98 (5)**        | 4.65±0.17 (5)           |

Ethionine was injected into rats i.p. at a dose of 200 mg/kg twice a day for 3 days. TCVP (500 mg/kg) was given to rats po simultaneously with the first injection of ethionine. Enzyme assay was carried out 17 hr after the last injection of ethionine. Enzyme activities were expressed as percentage of control values: mean±S.E. Numbers in parentheses indicate the numbers of rats used. Procainesterase and aminopyrine demethylase activities in the control rats were 0.073±0.010 and 0.029±0.002 tmoles products/g liver wt./min, respectively. * and **: significantly different from the control at P < 0.05 and P < 0.01, respectively.

application of ethionine (Fig. 4C, F).

**DISCUSSION**

The present research provided a systematic study of the toxicologic interaction between TCVP and procaine. TCVP, an organophosphate showed dual effects on liver microsomal PROCase, an inhibitory one and an inducible one, which altered metabolism of procaine and resulted in the change in the change in acute toxicity of procaine in rats treated with TCVP singly or repeatedly. First, TCVP strongly inhibited PROCase as well as ChE activity 24 hr after treatment with TCVP, and the resultant accumulation of brain procaine content and tendency for procaine toxicity to increase were observed in the TCVP-pretreated rats (Fig. 2 and Table 1). This result is consistent with the report by Cohen and Orzech (3) that pretreatment with triorthotolyl phosphate (TOTP), which significantly inhibited liver carboxylesterase with less inhibition of serum ChE activity, enhanced the acute toxicity of procaine in mice and that there was a good correlation between esterase inhibition by TOTP and increased procaine toxicity. Subsequently, a single administration of TCVP was observed to elevate PROCase activity on day 3 and procaine toxicity tended to decrease: the onset of the righting reflex was a little delayed, duration of paralysis was shortened, and mortality was changed from 54% to 25%. The repeated administration of TCVP for 5 days neither caused accumulative inhibition of PROCase nor potentiated procaine toxicity, and the rapid recovery of the enzyme activity and tendency for toxicity to decrease were observed compared to the procaine-injected control. (Fig. 1, Table 3), suggesting that the PROCase activity was probably induced by repeated administration of TCVP, but the increased enzyme activity may be overcome by a direct inhibitory effect of TCVP on this enzyme. In fact, high concentration of PABA in the rat with administration of TCVP for 5 days (Fig. 3 (B)) demonstrated that procaine was rapidly hydrolyzed to PABA in the liver which was the major responsible for procaine hydrolysis and it showed enlargement due to TCVP pretreatment. Consequently, procaine was quickly eliminated from the liver and the brain (Figs 2 and 3).

The inducible activity was more significant in rats treated with desmethyl-TCVP, which induced PROCase as well as liver microsomal drug metabolizing enzymes without inhibition.
of the esterases. No death produced by procaine injection was observed in the rat pretreated with desmethyl-TCVP. The result indicated that the two effects of TCVP, one its inhibitory action as an organophosphate such as EPN, and another its inducible one like phenobarbital, behaved independently of each other. The methyl group seems to be essential to its anticholinesterase action, and it is likely that the leaving group of TCVP, containing 2, 4, 5-trichlorophenacyl chloride which has been speculated to be a metabolite of TCVP by Akintonwa and Hutson (8), may be involved in the enzyme induction. Furthermore, as indicated in Table 5, the inducible effect of TCVP on PROCase was completely suppressed by simultaneous treatment with ethionine, so TCVP may accelerate protein synthesis of this enzyme.

The results of electrophoreses confirmed the enzyme induction by TCVP: in the microsomes from TCVP treated rats on day 3 (singly) and 5 (repeatedly), high protein containing bands appeared in anodic positions and the esterase activity of two anodic bands increased markedly in rats treated with TCVP. The similarity of the electrophoretogram of microsomes pretreated with ethionine and TCVP to that of the control microsomes demonstrated that ethionine suppressed the increase in enzyme protein by TCVP treatment.

Kaneko et al. (13) have reported similar results concerning the induction of liver microsomal butyrylcholinesterase after phenobarbital treatment using the cellulose acetate membrane electrophoresis technique. The most anodic anodiconomerase activity, which has low activity in normal adult liver and was located in smooth microsomal fractions, increased markedly in rats after phenobarbital treatment. It is very interesting that they have observed an increase in the most anodic anodiconomerase activity in infant rat liver 3-4-week after birth, in precancerous liver, in differentiated type of hepatoma, and in generating liver than in adult rat liver.

This paper has demonstrated the modification of the PROCase by the organophosphate TCVP and the relationship between a change in procaine toxicity and liver PROCase activity after TCVP treatment. These findings confirm results shown in a previous paper which demonstrated that occurrence of paralysis, convulsion, and death induced by procaine was dose-dependent on brain procaine concentration, and these were modified by liver PROCase activity (3). It is possible that enzymes or tissues other than liver PROCase are also involved in procaine toxicity. Thus, the induction of drug metabolizing enzyme activity by repeated administration of TCVP has been found to accelerate metabolism of TCVP and to decrease its toxicity (7). A decrease in the amount of TCVP which should inhibit PROCase activity may, therefore, contribute in part to procaine toxicity. In addition, the facts that the accumulation of kidney procaine and the elevation of blood urea nitrogen content to 120% of the control levels were observed in rats treated with TCVP suggest a role of kidney damage by TCVP administration in a consequent change in procaine toxicity. In fact, there are a number of reports concerning kidney damage by halogenated hydrocarbons (14-16).

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