Non-Cholinergic Mechanisms Underlying the Acute Lethal Effects of P=S Type Organophosphorus Insecticides in Rats

Takeshi KOJIMA*, Shuji TSUDA, and Yasuhiko SHIRASU

Mitsukaido Laboratories, Institute of Environmental Toxicology, 4321 Uchimoriya, Mitsukaido-shi, Ibaraki 303, Japan

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ABSTRACT. Intravenous administration of the lethal dose of diazinon or fenthion, P=S type organophosphates, to urethan anesthetized rats induced bradycardia and transient apnea followed by a decline of blood pressure, and death. We investigated the mechanisms of the lethal action of these organophosphates in rats through measurements of blood pressure, heart rate, and respiratory pattern. We compared their cardiorespiratory effects in the five different conditions under anesthesia; 1) normal (without treatment), 2) artificially ventilated, 3) vagotomized, 4) atropinized, 5) pithed, vagotomized and atropinized. It was found that the administration of 200 mg/kg of fenthion and 100 mg/kg of diazinon, caused sudden bradycardia, transient apnea and gradual decline of blood pressure in the anesthetized normal rat, and the rat died. The rats in other conditions also died except the artificially ventilated rats, in which 400 mg/kg of fenthion was administered to cause hypotension and subsequent death. Hypotension was observed consistently even after the cardiac effect such as bradycardia was eliminated by atropine treatment. In the pithed rats which were further vagotomized and atropinized, these organophosphates also caused hypotension. These results may indicate that hypotension is the main cause of death which resulted from intravenous administration of the P=S types. Hypotension may be caused by peripheral cardiovascular effect of the P=S types, which is unrelated to cholinergic mechanisms.—KEY WORDS: cardiovascular action, diazinon, fenthion, hypotension, organophosphate.

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Organophosphorus insecticides are categorized into two main types of P=O type and P=S type by their chemical structures. The former type has direct anti-cholinesterase actions, while the latter needs to be activated in the animal body, mainly in the liver, to acquire anti-cholinesterase function [4]. Toxic actions of P=O and P=S type insecticides have been attributed to anti-cholinesterase actions [6], and the direct cause of death by the cholinesterase inhibitors was attributed to a respiratory failure induced by central nervous system depression [2] or by an impairment of neuromuscular transmission [8]. In addition to an interference of respiratory movement due to convulsion by anti-cholinesterase action, a combination of respiratory obstruction due to bronchoconstriction by anti-cholinesterase action and cardiac failure unrelated to cholinesterase inhibition was also suggested to be the cause of death [7]. Wolthuis and Meeter [11] also reported that rats given diisopropyl fluorophosphate (DFP) died of cardiac failure through its direct inhibition, unrelated to cholinesterase inhibition, on the heart muscle.

We have reported [10] the effects of chlorfenvinphos (CVP) and dichlorvos (DDVP) as the representatives of the P=O type organophosphorus insecticides, and diazinon and fenthion as those of the P=S types, on cholinesterase activity and cardiorespiratory system as follows. Oral or intravenous administration of lethal doses of CVP or DDVP in rats caused anti-cholinesterase symptoms and death. Oral administration of diazinon or fenthion also exhibited the same lethal effects. In these cases cholinesterase was markedly inhibited. In contrast, intravenous administration of the P=S types caused death in a short progress with a slight anti-cholinesterase symptoms. In this case cholinesterase activity was scarcely inhibited and cardiorespiratory effects induced were not antagonized by atropine treatment.

In the acute lethal action of the P=S types administered intravenously, mechanisms other than cholinesterase inhibition may be involved because the cardiorespiratory responses were not antagonized by atropine. The purpose of this experiment was to examine the role of non-cholinergic actions as underlying mechanisms for the lethal effects of the P=S types.

MATERIALS AND METHODS

Animals: Thirty-four male specific-pathogen free

* CORRESPONDENCE TO: KOJIMA, T., 1–25–9 Hakusan, Bunkyo-ku, Tokyo 113, Japan.

rats of Sprague-Dawley strain in 8–9 weeks of age, weighing 270–340 g, were employed in this study. Though only two to four rats were examined in each experiment, the consistent results were obtained and were considered to reflect the mode of action of the drugs examined.

Anesthetized normal rats: Under anesthesia with urethan (1.2 mg/kg, i.p.) the femoral artery was cannulated with a polyethylene tubing of PE-50 standards for measurement of blood pressure. This tubing filled with saline containing 10 U/m*l* of heparine sodium was connected to a pressure transducer (TP-300T, Nihon Kohden, Tokyo) through a microinfusion/flush device (Gould, California). The saline was continuously infused through this device by a syringe pump at a constant slow rate of 0.28 m*l*/hr. A grass cannula was inserted into a cut opening of the trachea and a thermistor sensor was attached to the opening of the cannula for recording of respiratory patterns. Body temperature was maintained by a homeothermic blanket (KN-474, Natsume, Tokyo). Blood pressure (B.P.), mean blood pressure (M.B.P.), heart rate (H.R.), respiratory pattern (RESP.) and respiratory rate (R.R.) were recorded on a polygraph (RM-6000, Nihon Kohden, Tokyo). H.R. and R.R. is calculated from the pulse interval just before. If the pulse interval becomes longer than 5 seconds (12 c/min), a rate counter indicates 0 level.

All the rats of the following groups were treated as mentioned above.

Artificially ventilated rats: An artificial ventilation apparatus for rats (Muromachi, Tokyo) was connected to the cannula inserted into the trachea instead of the thermistor sensor. Ventilation volume was 10 m*l*/kg rat body weight and the cycle was 1/sec. Artificial ventilation was started immediately after the preparation was finished.

Vagotomized rats: The vagus nerve was cut bilaterally in the neck just before the tracheal cannula was inserted.

Atropinized rats: Rats were given 1 mg/kg of atropine intravenously 10 min before organophosphates administration.

Pithed rats: Rats were pithed according to the method of Gillespie [5]. After a guide needle (10 cm long, 2 mm in diameter) pierced from an orbit to the atlas, a metal rod was inserted through the guide needle into the spinal canal. The animal was artificially ventilated immediately after pithing. In this preparation also the vagus nerve was cut and atropine was administered.

Chemicals: Diazinon and fenthion (Wako Pure Chemical Industries, Osaka) were homogeneously emulsified in a saline solution containing 1% Tween 80 by a polytron homogenizer. Atropine sulfate (Wako Pure Chemical Industries) was dissolved in a physiological saline. The drugs were administered through a polyethylene cannula inserted into the jugular vein. Administration volume was 1 m*l*/kg for all the drugs.

RESULTS

Anesthetized normal rats: Administration of fenthion with a dose of 100 mg/kg (n=2) or diazinon with a dose of 50 mg/kg (n=2) did not cause death, though transient bradycardia and a transient decline of blood pressure were observed.

Administration of 200 mg/kg of fenthion (n=3) or 100 mg/kg of diazinon (n=2) induced bradycardia 5–6 sec after the beginning of administration, but

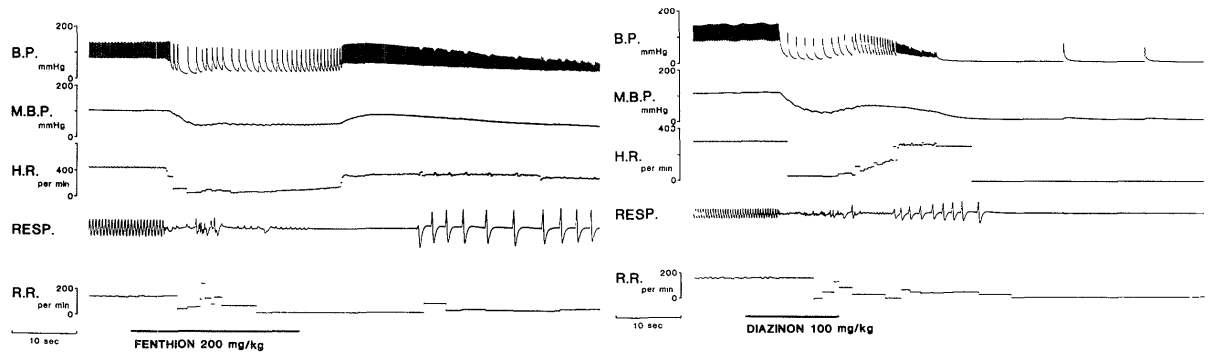

Fig. 1. Effects of 200 mg/kg of fenthion (left) and 100 mg/kg of diazinon (right) on blood pressure (B.P.), mean blood pressure (M.B.P.), heart rate (H.R.), respiratory pattern (RESP.) and respiratory rate (R.R.) of the urethan anesthetized normal rats. The upward movement indicates inspiration in RESP. The administration period of organophosphates was indicated in a boldline at the bottom of the figure.

pulse rate recovered 6–7 sec after the end of administration. Transient apnea was observed but respiration recovered within 10–40 sec (Fig. 1). In the case of fenthion, transient bradycardia and a decline of blood pressure occurred coincidently and recovered after the end of administration. But blood pressure declined again gradually in the following phase (Fig. 1). The rat died 1–1.5 min after the administration. Respiration ceased after heart beat disappeared. As for diazinon, transient bradycardia was followed by a decline of blood pressure, and heart beat stopped after blood pressure became lower than 30–40 mmHg. Breathing disappeared after cessation of heart beat. These observations were consistent with the results reported previously [10].

Artificially ventilated rats: Artificial ventilation was adopted in order to eliminate the lethal influence of respiratory failure which might result from anti-cholinesterase poisoning. Administration of 200 mg/kg of fenthion (n=2) caused remarkable hypotension but the rat survived under artificial ventilation. Administration of 400 mg/kg of fenthion to other two rats caused transient bradycardia and a gradual decrease in blood pressure, resulting in final death (Fig. 2). The pattern until death was similar to that of the anesthetized normal rats described above. Diazinon with a dose of 100 mg/kg (n=2) also caused bradycardia and hypotension and death (Fig. 2), as was observed in the anesthetized normal

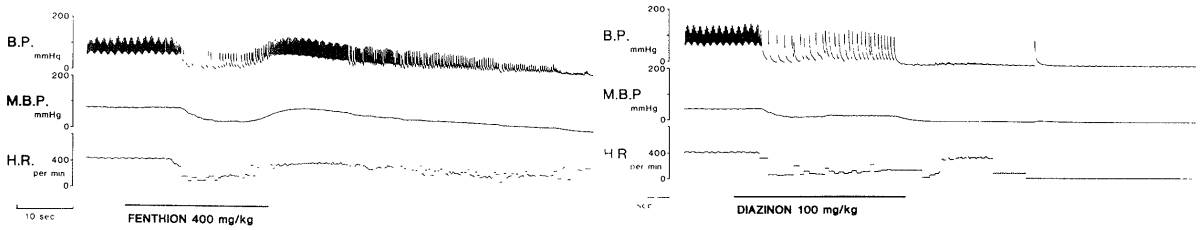

Fig. 2. Effects of 400 mg/kg of fenthion (left) and 100 mg/kg of diazinon (right) in the artificially ventilated rat under urethan anesthesia.

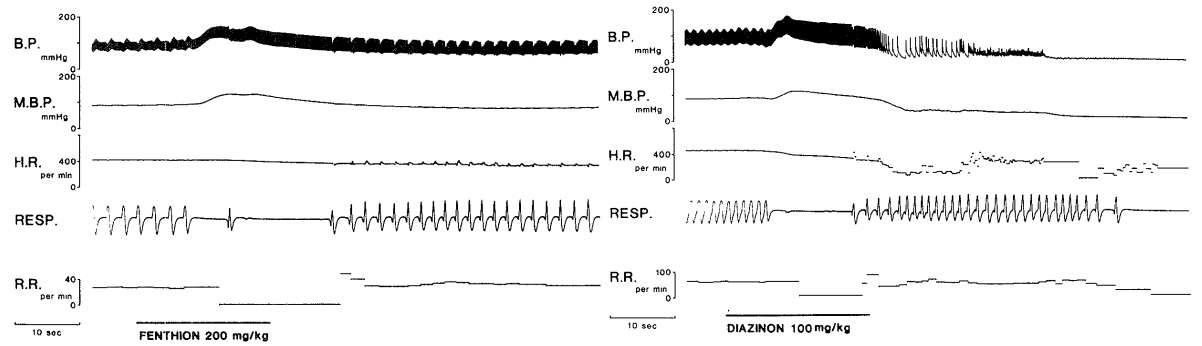

Fig. 3. Effects of 200 mg/kg of fenthion (left) and 100 mg/kg of diazinon (right) in the bilaterally vagotomized rat under urethan anesthesia.

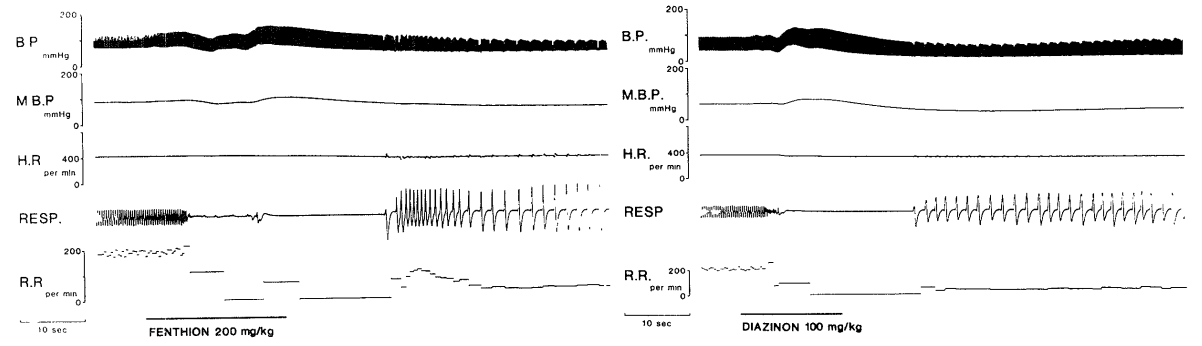

Fig. 4. Effects of 200 mg/kg of fenthion (left) and 100 mg/kg of diazinon (right) in the atropinized rat under urethan anesthesia. Atropine was treated 10 min before the administration of the organophosphate.

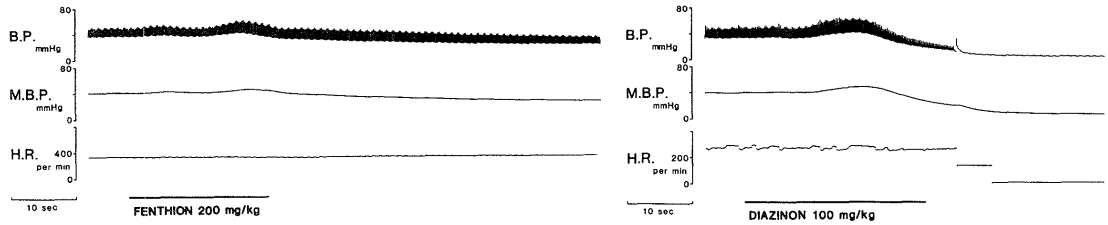

Fig. 5. Effects of 200 mg/kg of fenthion (left) and 100 mg/kg of diazinon (right) in the pithed rat under urethan anesthesia. This preparation was artificially ventilated after the rat was pithed, vagotomized and pretreated with atropine.

rats.

Vagotomized rats: Since the vagus nerve plays a dominant role in cardiac response such as bradycardia, the vagus nerve was cut bilaterally to eliminate vagal influence. Bradycardia which was observed soon after the initiation of administration of fenthion or diazinon in the anesthetized normal or artificially ventilated rats was not generated in the vagotomized rats (Fig. 3) at the same dosage (fenthion, 200 mg/kg, n=2; diazinon, 100 mg/kg, n=2). This suggests that bradycardia was of vagus origin. A transient increase in blood pressure was observed from the midst of the administration coincidently with apnea. In the case of fenthion, blood pressure declined gradually, and the rat died 4 min after administration. In the case of diazinon, a certain fraction of bradycardia appeared in the recovery phase of elevated blood pressure, followed by a decline of blood pressure. Respiration became deep and slow after transient apnea as observed in the anesthetized normal rats.

Atropinized rats: Rats were treated intravenously with 1 mg/kg of atropine 10 min before organophosphates administration, to investigate the involvement of muscarinic receptor in the action of fenthion and diazinon. Bradycardia was not observed in the atropinized rats by fenthion administration (n=3) (Fig. 4) as in the vagotomized rats. A gradual decline of blood pressure was observed and the rats died 2–13 min after the administration. In the case of diazinon (n=2), delayed bradycardia, remained in the vagotomized rats, was not observed in the atropinized rats (Fig. 4). Blood pressure declined gradually and the rats died 6–8 min after the administration.

Pithed rats: The influence of sympathetic discharge via the spinal cord was eliminated in the pithed rats. Administration of 100 mg/kg of fenthion (n=2) or 50 mg/kg of diazinon (n=2) did not cause death.

Administration of 200 mg/kg of fenthion (n=4) or 100 mg/kg of diazinon (n=2) caused no bradycardia, but induced hypotension and led to death in spite of the artificial ventilation (Fig. 5). Diazinon administration caused hypotension in a rapid progress and led to death (Fig. 5). As for fenthion, the rats died 2–13 min after the administration.

DISCUSSION

Administration of the P=O type organophosphates in urethan anesthetized rats generally caused a transient increase in blood pressure [3], and the respiration was inhibited severely and ceased at lethal doses [6]. A transient increase in blood pressure was considered to be of central origin [1, 9]. Since respiratory failure preceded hypotension and bradycardia, it was considered to be the primary cause of death [2].

On the other hand, the P=S type organophosphates induced bradycardia and transient apnea followed by a gradual decline of blood pressure. In contrast with the P=O types, no transient increase in blood pressure was observed in the anesthetized normal rats.

The rats under artificial ventilation survived after administration of 200 mg/kg of fenthion which was a lethal dose in the rat without artificial ventilation. Respiratory inhibition may therefore be partly involved in the cause of death. The rats, however, died after hypotension even under artificial ventilation. The cardiovascular action of fenthion seems to contribute in greater portion to the lethal cause. Artificial ventilation did not influence the lethality in the case of diazinon.

Bradycardia soon after the initiation of diazinon administration disappeared by vagotomy but a certain fraction of bradycardia remained for a shorter period after the end of administration. This fraction disappeared after atropine treatment. Since the appearance of this delayed bradycardia was too early to be explained by the conversion of diazinon

in the liver to acquire the anti-cholinesterase activity, it is suggested that diazinon exerts a direct cardiac effect via muscarinic receptor.

When bradycardia was eliminated with vagotomy and atropine treatment, development of hypotensive effect, after the end of organophosphorus administration, became somewhat milder. Since bradycardia may have enhanced the hypotensive effect, it might be partly involved in the cause of death induced by the P=S types. But the rat died of hypotension even after bradycardia was eliminated. This might suggest the involvement of vascular effect in the hypotension.

After pithing the spinal cord, blood pressure was kept depressed because of the lack of sympathetic participation to vascular tone. Administration of the P=S types made blood pressure still lower and led the rat to death. This result suggests a direct action of the drug on the peripheral organs, although this cannot exclude the possible involvement of the central hypotensive effect. This action comes other than via a muscarinic receptor because the pithed rats were treated with enough dose of atropine to prevent hypotension by exogenous acetylcholine in this experiment.

In conclusion the P=S type organophosphorus insecticides, different from the P=O types, have a hypotensive action which contributes mainly to the lethal cause. This action was found to remain even after the cardiac effect such as bradycardia was eliminated by vagotomy or atropine treatment. Even if a sympathetic regulation of the blood vessels was eliminated, the rat died of hypotension. Thus, the hypotensive effect was suggested to be elicited through the non-cholinergic action on the peripheral organ. We are to make further research on isolated organs to identify whether this peripheral effect is on heart or on blood vessels.

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