Effect of Diazinon PLUS on rapidly adapting receptors in the rabbit

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Campbell, Hillary, Krishnan Ravi, Emigdio Bravo, and C. Tissa Kappagoda. Effect of Diazinon PLUS on rapidly adapting receptors in the rabbit. J. Appl. Physiol. 81(6): 2604-2610, 1996.—The effects of Diazinon PLUS aerosol on the activities of rapidly adapting receptors (RARs) and slowly adapting receptors (SAR) of the airways were investigated in anesthetized rabbits. The effects on both the baseline activity and the responses to stimulation by increasing mean left atrial pressure were examined. Action potentials were recorded from the left cervical vagus nerve. Aerosols (particle size 3 µm) were generated by a Mini-HEART nebulizer. We observed that an aerosol of Diazinon PLUS (1:10 vol/vol dilution in normal saline) decreased the baseline RAR activity (n = 10) significantly (P < 0.05) from 209 ± 77 to 120 ± 40 impulses/min. In the post-Diazinon PLUS control period, the RAR activity recovered partially to 185 ± 75 impulses/min and decreased significantly to 131 ± 52 impulses/min (P < 0.05) after a second exposure of Diazinon PLUS (undiluted) aerosol. Aerosols of normal saline in the control state did not produce a significant change in the RAR activity. A group of SAR (n = 8) were examined under similar conditions, and it was found that only the exposure to Diazinon PLUS (undiluted) aerosol decreased the activity significantly (P < 0.05) from 1,536 ± 206 to 1,367 ± 182 impulses/min. The effect of Diazinon PLUS on the response to increasing mean left atrial pressure was examined in seven RARs. In the control state, RAR activity increased significantly (P < 0.05) during elevation of mean left atrial pressure. This response was abolished after exposure to Diazinon PLUS. These findings suggest that Diazinon may interfere with airway defense mechanisms by reducing the activity of RARs.

Methods

General Methods

Experiments were performed on New Zealand White rabbits (weight 3.1–3.8 kg). Anesthesia was induced with ketamine hydrochloride (50 mg/kg im; Ketaset, Fort Dodge Laboratories, Fort Dodge) and xylazine (5 mg/kg im; AnaSed, Lloyd Laboratories). Anesthesia was maintained with pentobarbital sodium (0.5 mg/kg; Fisher Scientific) given as an intravenous bolus every 30 min. The trachea was cannulated, and the animals were artificially ventilated with a tidal volume of 7–9 ml/kg and respiratory rate of 20 breaths/min. The inspired air was supplemented with 100% oxygen delivered at a rate of 1 l/min. The tracheal pressure was recorded by using a catheter introduced into the endotracheal tube and advanced into the trachea. The respiratory muscles were paralyzed with injections of gallamine triethiodide (1 mg/kg; Sigma) given every hour or as required. The adequacy of anesthesia was assessed by testing for the absence of a withdrawal reflex or a spontaneous increase in systemic arterial blood pressure before gallamine was administered.

The chest was opened in the midline in all animals, and the end of the expiratory line from the ventilator was placed under 1–2 cm H2O to prevent collapse of the lung. A cannula (1.67 mm ID; Intramedic polyethylene tubing) was introduced into the left atrium through the auricular appendage and used for measuring left atrial pressure. In some animals, a cannula equipped with a balloon at its tip was also introduced into the left atrium. Stepwise inflation of this balloon obstructed the mitral valve and caused a graded increase in left atrial pressure.

The arterial Pco2 and pH were maintained within the physiological range by adjusting the ventilator and/or by

ORGANOPHOSPHATE INSECTICIDES are commonly employed as a means of controlling pests in both domestic and agricultural settings. Widespread usage of this class of insecticides has resulted in a significant increase in the number of reports of poisoning. For instance, in 1974 the World Health Organization compiled data from 19 countries and estimated that ~500,000 instances of poisoning occurred annually, resulting in 9,000 deaths. By 1983, these figures had risen to 2 million and 40,000, respectively (8). The majority of these deaths occur in the Third World where, even today, organophosphate is one of the agents most commonly used for committing suicide. As a result, much of the scientific and clinical interest has been directed at defining the signs, symptoms, and treatment of acute organophosphate poisoning (11, 21).

In the United States, the majority of instances of pesticide poisoning are accidental and often occur away from the workplace. In view of the easy availability of these compounds (900 chemicals with 25,000 brand names registered as pesticides), increasing attention is being paid to the health effects of mild exposure to organophosphates (11, 14). Many of the later studies have focused on increasing the sensitivity of diagnostic tests and investigating the toxicology of organophosphates. Relatively little attention has been paid to the potential effects of sublethal doses of these compounds on physiological function, particularly in the respiratory tract. Such a study is of particular interest in the case of organophosphate insecticides for two reasons. First, acute poisoning with these compounds is associated with a variety of respiratory symptoms, such as increased bronchial secretion, bronchospasm, cough, and pulmonary edema, and, second, they are used in the form of aerosols (17).

The aim of the present investigation was to determine whether administration of a commonly used pesticide in the form of an aerosol influences the activities of vagal sensory endings in airways that are most likely to be involved in the symptomatology of pesticide poisoning. The studies were undertaken in New Zealand White rabbits, and the pesticide used was Diazinon PLUS, which is available commercially. It is a solution of the organophosphate diazinon in petrolatum-based solvents (25:75 wt/wt).

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infusing sodium bicarbonate (7.5% wt/vol iv). Both femoral arteries were cannulated, one for recording arterial blood pressure and the other for obtaining samples for blood-gas analysis. Both femoral veins were cannulated also. One of these cannulas was advanced as far as the right atrium and used for recording the right atrial pressure. The other venous cannula was used for infusions of drugs. The temperature of the animals was maintained at 37°C by using heating pads.

The cannulas used for measuring pressures were connected to strain-gauge manometers (model P23 DB, Statham Instruments, Hato Rey, PR), and the outputs were then amplified and recorded on light-sensitive paper (model TA11, Gould Instrument Systems, Valley View, OH).

Recording of action potentials. The cervical vagus on the left side was exposed and dissected away from the carotid sheath. A pool was made around the vagus from the surrounding skin. This pool was then filled with warm mineral oil. The nerve was placed on a plastic platform, and thin fibers were dissected away from the nerve until a single unit was found. These methods have been described in detail previously (2).

Action potentials originating from rapidly adapting receptors (RARs) and slowly adapting receptors (SARs) were recorded. RARs were identified by their rapid adaptation to maintained lung inflations and by their conduction velocities. SARs were identified by their slow rates of adaptation to maintained lung inflations (2, 5). The conduction velocities were measured by electrical stimulation (2–6 V, 1.5-ms duration) of the vagus 2–3 cm caudal to the recording site. Receptors were grossly localized at the end of each experiment by carefully probing the external surface of the airways by using a blunt glass rod that was 3 mm in diameter.

Introduction of aerosol. Aerosols of the pesticides were introduced into the inspiratory line of the ventilator by using a medication nebulizer (Mini-HEART, Votran Medical Technology, Sacramento, CA). The nebulizer was filled with 5 ml of the pesticide solution, and oxygen was bubbled through the solution at a flow rate of 2 l/min. Under these conditions, particles of ~3 µm in diameter were produced. The expiratory line from the tracheal cannula was then clamped, and the nebulized airflow was directed into the lungs until the tracheal pressure rooftop 10–15 mmHg. The lungs were kept inflated in this way for 10 s ± 3°C. Subsequently, the clamp on the expiratory line was released, and the animal was reconnected to the ventilator for three breaths. After this period, the maneuver was repeated twice. Three solutions were introduced into the airways: 1) normal saline, 2) 1:10 dilution vol/vol of Diazinon PLUS in normal saline (Diazinon PLUS Insect Spray, Ortho Chemical, San Ramon, CA), and 3) undiluted Diazinon PLUS.

Estimation of distribution of aerosol. To estimate the distribution of the aerosol particles in the airways, 5 ml of carbon black dispersion (Saber-Castell, Newark, NJ) were placed in the Mini-HEART nebulizer and the aerosol particles were introduced into the airways in the same manner as was the Diazinon PLUS. The animals were killed, and the trachea and the airways were dissected and the epithelial surface was examined under a dissecting microscope to determine the extent of the distribution of the carbon particles.

Estimation of potential absorption of organophosphates. Samples of blood were collected before and 5 min after introduction of the aerosolized Diazinon PLUS into the airways, and samples of blood were collected for estimation of cholinesterase activity in whole blood. Fresh whole blood was kept in Vacutainer tubes at 4°C. Ten microliters of well-mixed whole blood were diluted to 10 µl with phosphate buffer (pH 8.00, 25°C). The sample was mixed well, and the cholinesterase activity was determined by using a modification of the Ellman method (24).

Experimental Protocols

Protocol 1: effect of Diazinon PLUS on baseline activity of RARs and SARs. Action potentials and pressures were recorded continuously. When a receptor was identified as an RAR or SAR, action potentials were recorded during the following sequence of events: 1) initial control, 2) saline aerosol exposure, 3) postsaline control, 4) 1:10 vol/vol dilution of Diazinon PLUS aerosol exposure, 5) post-Diazinon PLUS control, 6) undiluted Diazinon PLUS exposure, and 7) final control. Action potentials were recorded for 5 min during each control period and after each aerosol exposure.

Protocol 2: effect of Diazinon PLUS on the response of RARs to pulmonary venous congestion. When an RAR was identified, the following protocol was performed on some of the units used in protocol 1: 1) control period, 2) balloon inflation to increase the mean left atrial pressure by 3 mmHg, 3) balloon inflation to increase the mean left atrial pressure by 6 mmHg, and 4) balloon deflation. Action potentials were recorded for 3 min during the control period and during each balloon inflation or deflation. This part of the protocol was carried out before administration of Diazinon PLUS. This sequence was repeated after the administration of the Diazinon PLUS aerosol.

Statistical Methods

Action potentials were counted in 3-s bins, and group data were averaged on a minute-by-minute basis. Responses of the receptors to increases in left atrial pressure, saline aerosol, or Diazinon PLUS aerosol were compared with the respective control periods. Tracheal pressure, heart rate, mean arterial pressure, and right atrial pressure were recorded continuously throughout the experiment. However, for the present purpose, the data are presented as follows: 1) tracheal pressure analyzed every minute and averaged for each phase of the protocol and 2) heart rate, mean arterial pressure, and right atrial pressure analyzed at minutes 1 and 5 during the control periods and aerosol exposure (protocol 1) at minute 2 during balloon inflation and deflation (protocol 2).

Group data were expressed as means ± SE. Comparisons among means were made by using an analysis of variance for repeated measurements followed by Scheffé’s F-test where appropriate. A P value of <0.05 was taken as significant.

RESULTS

The experiments were performed on 14 open-chest rabbits. At the commencement of the recordings, the pH, PCO₂, and PO₂ were 7.4 ± 0.01, 30.4 ± 1.0 Torr, and 393.6 ± 26.8 Torr, respectively. The tracheal pressure, heart rate, mean arterial pressure, and right atrial pressure were 5.2 ± 0.64 mmHg, 211 ± 8 beats/min, 82.5 ± 3.1 mmHg, and 4.1 ± 1.02 mmHg, respectively.

Protocol 1: Effect of Diazinon PLUS on Baseline Activity of RARs and SARs

RARs. Ten RARs were examined in nine rabbits. Their average conduction velocity was 19 ± 2.0 m/s. All of the RARs were located in the bronchus within 1 cm of the hilum. Three were located in the left upper lobe, and seven were located at the lower lobe. In one of the animals, two RARs were examined. The second unit, which was identified ~2 h after the first, was
tested to determine whether the effect of Diazinon PLUS was sustained. Because the second unit behaved in the same manner as the first, it was included in the final analysis. Exposure to Diazinon PLUS resulted in a reduction in baseline activity of RARs. An example is shown in Fig. 1.

Group data plotted minute by minute are shown in Fig. 2. The average receptor activity was 180 ± 45 impulses/min during the control period, 163 ± 50 impulses/min after saline exposure, and 209 ± 77 impulses/min during the postsaline control period (P > 0.05). After 1:10 (vol/vol) Diazinon PLUS aerosol exposure, the receptor activity fell significantly from the control value of 209 ± 77 to 120 ± 40 impulses/min (P < 0.05). During the post-Diazinon PLUS control period, the activity recovered partially to 185 ± 75 impulses/min. After undiluted Diazinon PLUS exposure, the activity fell significantly from 185 ± 75 to 131 ± 52 impulses/min (P < 0.05). During the final control period, the activity recovered partially to 150 ± 60 impulses/min. The response times to both undiluted and diluted Diazinon PLUS were within 10 s of introduction of the aerosol.

The changes in heart rate, mean arterial blood pressure, and tracheal pressure are summarized in Table 1. There were no significant changes in heart rate and mean arterial blood pressure throughout the experimental run. (P > 0.05 for both). Tracheal pressure fell from 5.2 ± 0.6 mmHg during the control period to 4.4 ± 0.4 mmHg after the 1:10 (vol/vol) Diazinon PLUS aerosol exposure, and it fell from 5.1 ± 0.6 to 4.3 ± 0.4 mmHg after the undiluted Diazinon PLUS exposure. These changes were significant (P < 0.05).

The spontaneous variation in the activity of RARs with time was investigated in a previous investigation in the rabbit (3). In that study, the activity of seven RARs was recorded for a period of 60 min without any interventions. There was no significant change in RAR activity with time (P > 0.05). In the present study, it took ~35 min for the completion of the protocols. When the results were replotted (Fig. 3), it was observed that the spontaneous activity of RARs did not change significantly over a period of 35 min (P > 0.05).

SARs. Eight SARs were examined in six rabbits. Their conduction velocities were 25 ± 4 m/s. One receptor was located in the carina, and the remaining were located in a lobar bronchus within 1 cm of the hilum. One receptor was located in the left upper lobe, and six were located in the left lower lobe. Diazinon PLUS at a dilution of 1:10 vol/vol did not significantly alter the receptor activity (Fig. 4). The average activity was 1,623 ± 236 impulses/min during the control period, and it was 1,657 ± 281 impulses/min during the saline aerosol exposure. During the postsaline control period, the activity was 1,545 ± 264 impulses/min, and it 1,525 ± 232 impulses/min after 1:10 vol/vol Diazinon PLUS exposure (P > 0.05). When the receptor was exposed to undiluted Diazinon PLUS, the activity changed from 1,536 ± 206 impulses/min in the control period to 1,367 ± 182 impulses/min. In the final control period, the activity was 1,389 ± 221 impulses/min.

Tracheal pressure, mean arterial pressure, right atrial pressure, and heart rate did not change significantly during the experimental runs (Table 1).

Protocol 2: Effect of Diazinon PLUS on the Responses of RARs to Pulmonary Venous Congestion

Seven RARs were examined in this protocol. The mean left atrial pressure in the control period was
4.3 ± 0.9 mmHg. An example of a response is shown in Fig. 5. The activity of the RARs increased when the left atrial pressure was raised by 3 and 6 mmHg, respectively. This response was not evident after administration of Diazinon PLUS aerosol. In the seven receptors, the average activity in the control period before administration of Diazinon PLUS was 179 ± 64 impulses/min. The activity increased to 288 ± 96 and 395 ± 136 impulses/min when the left atrial pressure was raised by 3 (left atrial pressure 6.8 ± 1.0 mmHg) and 6 mmHg (left atrial pressure 10.9 ± 1.2 mmHg), respectively (P < 0.05). When the left atrial pressure was lowered to control values (4.1 ± 0.7 mmHg), the activity returned to 179 ± 59 impulses/min. After exposure to undiluted Diazinon PLUS, elevation of left atrial pressure did not stimulate the RARs. The RAR activity during the post-Diazinon PLUS control period was 149 ± 60 impulses/min, and the left atrial pressure was 2.7 ± 1.0 mmHg. When the left atrial pressure was raised by 3 mmHg (left atrial pressure 6.5 ± 1.0 mmHg) and 6 mmHg (left atrial pressure 10.2 ± 1.2 mmHg), the activities were 165 ± 85 and 145 ± 71 impulses/min, respectively. After the left atrial pressure was restored to control values (5.4 ± 1.5 mmHg), the activity was 103 ± 54 impulses/min. These values were not statistically significant (P > 0.05). These changes are shown in Fig. 6.

The values of mean arterial blood pressure, heart rate, and tracheal pressure are summarized in Table 2. The changes in heart rate and mean arterial blood pressure were not significant (P > 0.05). The tracheal pressure increased during graded pulmonary venous congestion in the control state (Table 2).

### Distribution of the Aerosol

In these experiments (n = 3), it was possible to discern the presence of carbon particles in the trachea, the left and right main bronchi, and the proximal 1 cm of the intrapulmonary airways. These are regions...
where the receptors studied in the present investigation were located.

**Cholinesterase Measurements in Blood**

Cholinesterase in blood was measured in three animals before and after inhalation of undiluted Diazinon PLUS. The average value before inhalation was 0.95 (range 0.8–1.21) µmol·ml⁻¹·min⁻¹ and that after was 0.96 (range 0.78–1.18) µmol·ml⁻¹·min⁻¹.

**DISCUSSION**

The findings reported in this paper demonstrated that exposure to an aerosol of Diazinon PLUS, a pesticide that is available commercially, attenuated the activity of pulmonary receptors connected to myelinated vagal afferents in the rabbit. In RARs, the aerosol attenuated both the baseline activity and the activity when the receptors were stimulated by pulmonary venous congestion (Figs. 1 and 4). In the case of SARs, undiluted Diazinon PLUS alone caused a small transient attenuation in baseline activity. In contrast, the RARs were affected by both the undiluted form and by the 1:10 vol/vol dilution in saline. Also, there were no associated changes in mean arterial blood pressure and heart rate during exposure to the aerosol.

There are several possible explanations for these findings. The tracheal pressure declined slightly after exposure to Diazinon PLUS aerosol in some experiments and merits further comment. When the effects of the aerosols on baseline activity of RARs were examined, there was a small concomitant decrease in peak tracheal pressure (see Table 1), leading to the possibility that the effects on baseline activity were secondary to this change. However, the results obtained during pulmonary venous congestion did not support such a contention. Before administration of aerosol, elevation of left atrial pressure by 3 mmHg increased RAR activity despite a small drop in tracheal pressure (Table 2). Thus changes in tracheal pressure were unlikely to have been a major determinant of the findings of the present study. Additionally, previous observations in the dog (6) and rabbit (2) have demonstrated that the stimulus-response curves relating left atrial pressure and RAR activity did not change significantly after an interval of 20–45 min. Thus the attenuation of the responses of RARs to increments in left atrial pressure could not have been due to a natural variation in the stimulus-response relationship with time.

Another possibility is that diazinon, being an anticholinesterase, caused accumulation of acetylcholine locally in the vicinity of the receptors, which in turn inhibited the receptors. Indeed there is evidence to show that acetylcholine injections into the carotid sinus region (20–200 µg) inhibited the discharge from carotid sinus baroreceptor (10). The present investigation was not designed to address these issues.

Finally, it could be argued that the aerosols attenuated the activity of RARs through a direct effect on the receptors. Being highly lipid soluble (23), diazinon may gain direct access to RARs, which are located in the superficial layers of the airways (7). The small but significant attenuation in the baseline activity of SARs, which are located in the deeper layers of the airways, was observed only when undiluted Diazinon PLUS was used. This quantitative difference in the responses of RARs and SARs may be due to the greater sensitivity of the former to airway irritants (26) and to a diminished accessibility of the latter.
Although a solvent effect cannot be excluded, it seems unlikely. For instance, diazinon is known to be miscible in alcohol, ether, petroleum ether, cyclohexane, benzene, and related hydrocarbons (12). RARs have been shown to be activated by both ether and alcohol (1). Also, alcohol has been shown to increase the amplitude of the action potentials (15). In the present study, Diazinon PLUS reduced the frequency and amplitude of the action potentials. Thus it is suggested that the solvents would have been more likely to blunt any inhibitory effect of diazinon on RARs. Taken collectively, these considerations indicate that the inhibitory effect on the receptors is probably attributable to diazinon.

Diazinon PLUS is a commercially available product containing the organophosphate diazinon. The latter is an anticholinesterase and is present as a 25% wt/wt solution in petroleum-based solvents. Unfortunately, the identity of the solvent is not in the public domain. Although a solvent effect cannot be excluded, it seems unlikely. For instance, diazinon is known to be miscible in alcohol, ether, petroleum ether, cyclohexane, benzene, and related hydrocarbons (12). RARs have been shown to be activated by both ether and alcohol (1). Also, alcohol has been shown to increase the amplitude of the action potentials (15). In the present study, Diazinon PLUS reduced the frequency and amplitude of the action potentials. Thus it is suggested that the solvents would have been more likely to blunt any inhibitory effect of diazinon on RARs. Taken collectively, these considerations indicate that the inhibitory effect on the receptors is probably attributable to diazinon.

Physiological Implications of Attenuation of RAR Activity

The toxic effects of acute exposure to organophosphates include nasal hyperemia and watery discharge, cough, chest discomfort, dyspnea, and wheezing due to increased bronchial secretions and bronchoconstriction (17). Stimulation of RARs causes cough (9), chest discomfort (26), dyspnea (26), tachypnea (6), increased bronchial secretions (27), bronchoconstriction (9), and an increase in tracheal tone (4). Thus the respiratory symptoms observed after the exposure to toxic levels of organophosphates may be due, in part, to the stimulation of RARs.

However, in the present study, exposure to sublethal doses of the organophosphate Diazinon PLUS reduced the activity of RARs. A reduction in the activity of RARs is likely to impair the airway defense reflexes mentioned above. One consequence of this effect is that subjects could inhale additional quantities of aerosol. The effects of chronic inhalations of such low levels of organophosphates on lung receptors have not been examined in a systematic manner.

With respect to massive acute exposure to organophosphates used in agriculture, the symptoms (cough, wheezing, increased bronchial secretion, and bronchoconstric-
tion) suggest a strong stimulation of RARs. The frequent occurrence of blood-stained frothy sputum associated with pulmonary edema (22) in this situation indicates damage to capillaries in the respiratory tract. The latter findings resemble the effects of chemicals used to generate experimental pulmonary edema, such as alloxan (16). Previous studies have shown that in such experimental models there is a strong activation of both RARs and pulmonary C-fiber receptors (19). It is suggested that the respiratory syndromes resulting from chronic low-level exposure to organophosphates differ from those that occur after massive exposure.

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