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# Influence of Anticholinesterase on Distribution of Ventilation and Gas Exchange

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MODELL, H. I. *Influence of anticholinesterase on distribution of ventilation and gas exchange.* PHARMACOL BIOCHEM BEHAV **40**(1) 17–20, 1991.—This project was designed to titrate the influence of pyridostigmine injected intraarterially on pulmonary resistance and gas exchange in pigs and dogs. Pyridostigmine at 1 mg/kg reduced red blood cell cholinesterase activity 28–35% that was not significantly reduced further with doses up to 9 mg/kg. Plasma cholinesterase was reduced by 80% in the dog and 40% in the pig with 1 mg/kg of pyridostigmine and with 3 mg/kg it was reduced to 40% in the pig and 10% in the dog. Higher doses had no further significant effect. Breathing resistance (cm $H_2O$/l/s) in the pig was doubled as a linear function with 9 mg/kg pyridostigmine. In the dog, breathing resistance went to a maximum of 8 cm $H_2O$/l/s from a control value of 1 cm $H_2O$/l/s with 3 mg/kg of pyridostigmine but did not go higher with doses up to 9 mg/kg. $PaO_2$ was reduced by approximately 20% in the pig and 15% in the dog with pyridostigmine doses of 6–9 mg/kg. These experiments indicate that significant alterations in pulmonary function do not occur until acute dosages in the range of 3–6 mg/kg are reached. Furthermore, acute administration of large doses of pyridostigmine results in salivation and gastrointestinal stimulation well in advance of any impairment of respiratory function.

Anticholinesterase Pulmonary resistance Pig Pyridostigmine Gas exchange Dog

THE threat of enemy employment of chemical warfare agents is a priority area of concern for the U.S. Air Force (USAF). Prophylactic use of anticholinesterase compounds is one strategy being considered for environments where chemical warfare nerve agents are a potential threat. These compounds are used clinically in the treatment of myasthenia gravis (1, 4, 11, 13) and in surgical settings for reversal of muscle relaxants used in conjunction with anesthesia (5, 6, 9, 14). Reported adverse reactions for these compounds include bronchial constriction and increased bronchial secretions (2, 3, 15). Although these reactions are generally assumed to be associated with overdosage, these anticholinesterases are contraindicated in patients with bronchial asthma (2). There is evidence of pulmonary edema formation with clinical doses of neostigmine (12). Hence, there is a potential risk of pulmonary complications and impaired gas exchange when anticholinesterases are used therapeutically or as a prophylactic measure to combat chemical warfare nerve agents (7,10).

Little data are available in the literature relating dosage of pyridostigmine to the onset of pulmonary complications. Furthermore, it is not clear at what point the degree of bronchial constriction is sufficient to cause gas exchange impairment. If compounds such as pyridostigmine are to be used as a prophylactic chemical defense agent, two questions must be answered: 1) At what dosage are aircrew members at risk for increased bronchial constriction and/or bronchial secretions? and 2) Is there a "safety zone" where bronchial constriction may occur, but gas exchange remains unaffected? This study was designed to provide information that will help answer these questions.

## METHOD

The pig was chosen as the primary experimental model for this study because the pig model is commonly used in studies involving cardiopulmonary responses to acceleration. To obtain an estimate of species variation in the response of the respiratory system to pyridostigmine, experiments conducted in pigs were repeated in 4 dogs.

Eleven Yorkshire barrows weighing $23.2 \pm 4.96$ kg were anesthetized with 18 mg/kg ketamine and 2 mg/kg xylazine administered intramuscularly. Pentobarbital sodium was administered intravenously as supplemental anesthesia when required. In each animal, a tracheostomy was performed, a carotid artery was cannulated, and a catheter was passed through the right internal jugular vein to the level of the pulmonary artery. Catheter placement in the pulmonary artery was determined from the observed pressure profile measured at the catheter tip.

Following catheter placement, mechanical ventilation with a tidal volume of 15 ml/kg was instituted using a ventilator that required the animal to generate $-5$ cm $H_2O$ airway pressure (assisted ventilation). During the course of the experiment, the animal was "sighed" periodically with a large tidal volume to minimize development of atelectasis. After a stabilization period, arterial and mixed venous blood were sampled for blood gas analysis; $P_{CO_2}$ in mixed expired gas was determined; and arterial blood was drawn into a vacutainer tube containing EDTA for determination of cholinesterase activity in whole blood, plasma and red blood cells. A period of hyperventilation was then imposed using the controlled ventilation mode of the venti-

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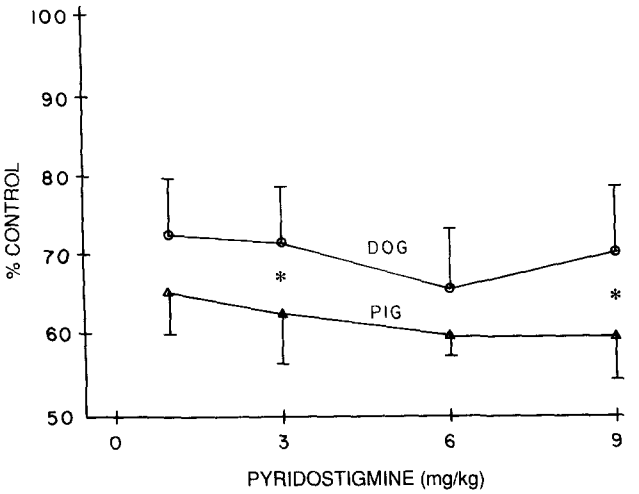


FIG. 1. Normalized RBC cholinesterase activity as a function of cumulative pyridostigmine dose in 4 pigs and 4 dogs. Differences between species response ($p<0.05$, Student's $t$-test) are noted by *. Standard error of the mean is indicated.

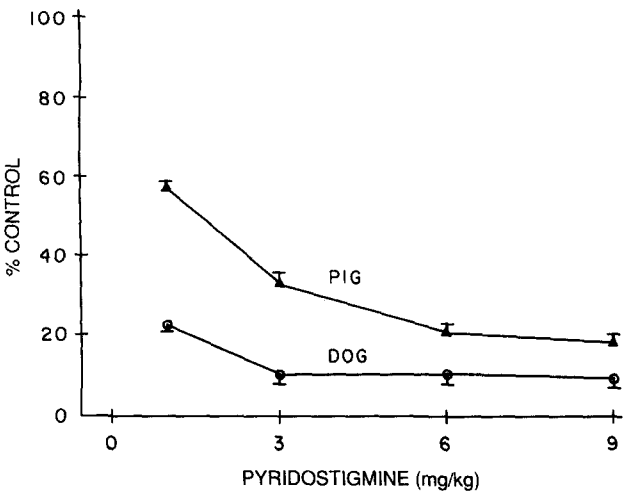


FIG. 2. Normalized plasma cholinesterase activity as a function of cumulative pyridostigmine dose in 4 pigs and 4 dogs. Differences between species response ($p<0.05$, Student's $t$-test) existed at all pyridostigmine levels. Standard error of the mean is indicated.

lator. Total pulmonary resistance during a period of apnea at functional residual capacity was determined using the forced oscillation method (6) after which assisted ventilation was reinstated. A test dose of pyridostigmine bromide (Mestinon or Regonol) was administered intra-arterially over a period of 1–2 min, and, after a stabilization period of 15 min, the determinations were repeated.

The protocol was modified slightly in the dog study. In the experiments in which 4 mongrel dogs (weight = $21.9 \pm 3.18$ kg) were used, anesthesia was induced with pentobarbital sodium (30 mg/kg), and tracheal access was provided by an endotracheal tube rather than by tracheostomy.

In the initial experimental design, the end-point for the titration was to be the point at which the animal could no longer generate the $-5$ cm $H_2O$ airway pressure necessary to trigger the ventilator. After several pilot experiments, however, in which massive doses of pyridostigmine were given without achieving the desired end-point, the maximum cumulative dose used was 9–12 mg/kg administered over a maximum time period of 3 hours.

## RESULTS

The influence of pyridostigmine on red blood cell (RBC) and plasma cholinesterase activities in both pig and dog are shown in Figs. 1 and 2. Mean RBC cholinesterase activity, normalized to control value, in 4 pigs and 4 dogs is shown in Fig. 1 as a function of pyridostigmine dose. Mean cholinesterase activity in RBC before administration of pyridostigmine was found to be $5220 \pm 367$ (S.D.) mU/ml at 25°C in the pigs and $2858 \pm 276$ mU/ml in the dogs. Significant differences in species response to pyridostigmine, as determined by Student's $t$-test, were evident only at the 3 and 9 mg/kg levels ($p<0.05$).

The normalized response of dog and pig plasma cholinesterase activity to pyridostigmine administration is shown in Fig. 2. Mean cholinesterase activity at 25°C prior to pyridostigmine was $516 \pm 3.8$ mU/ml in the pigs and $1427 \pm 377$ mU/ml in the dogs. At each pyridostigmine level, the relative plasma cholinesterase inhibition was greater in the dogs than the pigs ($p<0.001$, Student's $t$-test).

Observed changes in pulmonary resistance are shown as a function of pyridostigmine dose for the 4 pigs in Fig. 3 and for the 4 dogs in Fig. 4. An analysis of variance (ANOVA) performed on the pig data shown in Fig. 3 indicates that the general trend of increased pulmonary resistance is statistically significant ($p<0.005$). However, comparison of the resistance values following pyridostigmine to control values by paired $t$-test indicates that a significant increase in resistance was not evident until at least 3 mg/kg was administered.

A similar analysis of the corresponding dog data did not yield statistically significant differences, probably because of the small number of animals and large scatter in the data. Nevertheless, a trend toward pyridostigmine-induced increased pulmonary resistance in the dog is also apparent.

The gas exchange data show a similar pattern. Arterial $Po_2$

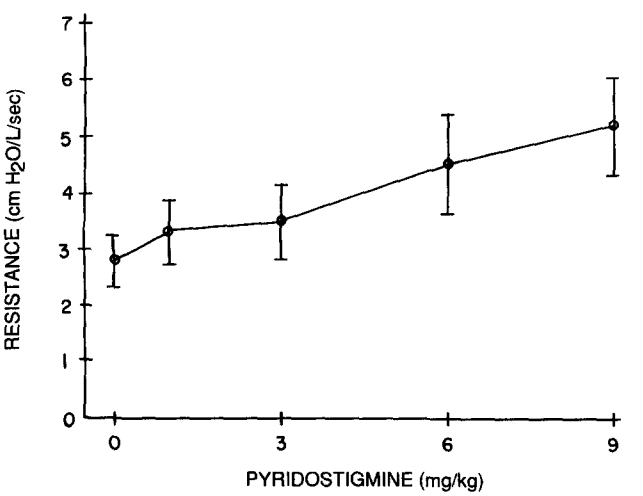


FIG. 3. Observed changes in pulmonary resistance as a function of cumulative pyridostigmine dose in 4 pigs. Standard error of the mean is indicated.

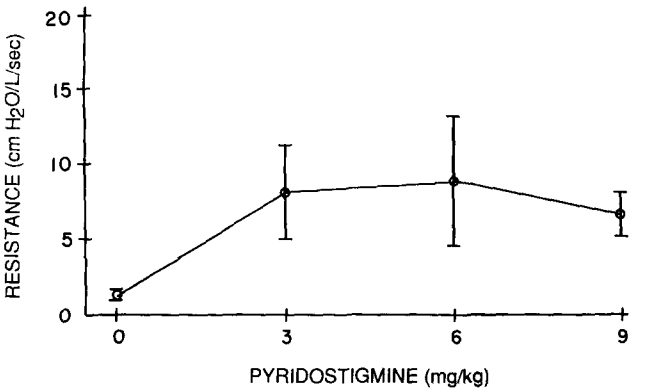


FIG. 4. Observed changes in pulmonary resistance as a function of cumulative pyridostigmine dose in 4 dogs. Standard error of the mean is indicated.

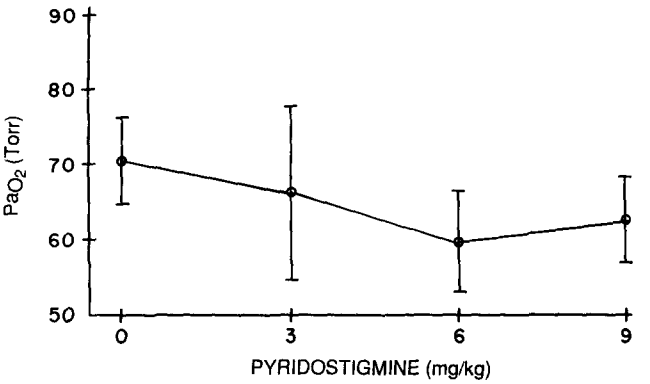


FIG. 6. Arterial $P_{O_2}$ as a function of cumulative pyridostigmine dose in 4 dogs. Standard deviations are indicated (see text).

as a function of pyridostigmine administered to 4 pigs is shown in Fig. 5. An ANOVA performed on these data indicates that a significant decrease in arterial $P_{O_2}$ occurs with increasing doses of pyridostigmine ($p<0.005$). However, a paired $t$-test comparison of $P_{O_2}$ control data with values at each dosage level indicates that a significant impairment of gas exchange did not occur until the pyridostigmine dose reached 6 mg/kg.

Figure 6 shows similar data obtained from the 4 dogs. Although the trend is again evident, the data failed to exhibit statistical significance.

Arterial $P_{CO_2}$ and physiological dead space, calculated from arterial and mixed expired $P_{CO_2}$ data, did not show physiologically significant alterations as a function of pyridostigmine dose.

## DISCUSSION

The data from these experiments indicate not only that during acute exposure to pyridostigmine significant increases in pulmonary resistance can be detected at dosage levels in the 3 mg/kg range, but also that significant gas exchange impairment does not occur at levels below 6 mg/kg. Since these levels are 10 to 60 times the recommended clinical intravenous dose, it is doubtful that gas exchange abnormalities would result from the small prophylactic oral doses being considered for pilots.

The data also suggest some interesting species variation with respect to cholinesterase distribution and responses to anticholinesterase administration. In pigs, there was a 10:1 ratio of red blood cell cholinesterase activity to plasma activity; however, in the dogs, the ratio was 2:1.

The degree of RBC cholinesterase inhibition shown in Fig. 1 suggests that the pyridostigmine is not well distributed among blood components. To confirm that circulating plasma levels of pyridostigmine continued to increase during continued administration of pyridostigmine, we sent plasma samples from 2 pigs to the USAF School of Aerospace Medicine (USAFSAM) where Dr. Faust Parker (Rothe Development, Inc.) analyzed the samples for pyridostigmine concentration (8). The resulting data, shown in Table 1, demonstrates that the pyridostigmine was not sequestered and that the circulating levels did, indeed, increase as was the intent in the experimental design.

Figure 1 suggests that, with the increasing plasma pyridostigmine levels, the inhibition of cholinesterase in RBCs was limited as it was in the plasma above 3 mg/kg pyridostigmine in the dog and 6 mg/kg in the pig (Fig. 2). However, the overall systemic response was not the same. The primary systemic response observed in the pigs was increased salivation. In the dogs, there was also increased salivation along with more severe muscarinic effects including increased peristaltic activity, vomit-

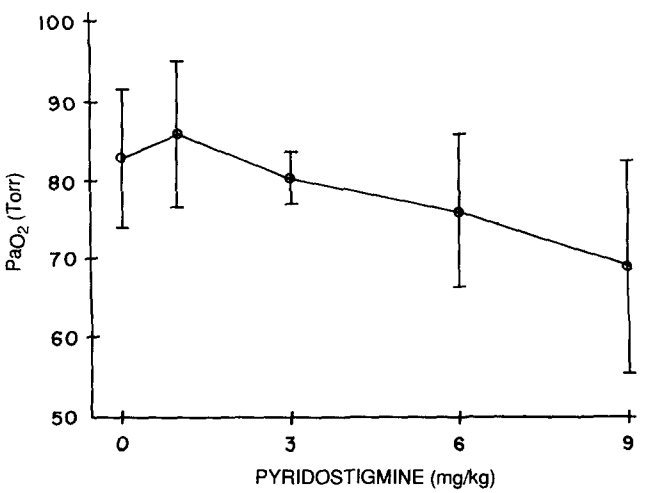


FIG. 5. Arterial $P_{O_2}$ as a function of cumulative pyridostigmine dose in 4 pigs. Standard deviations are indicated (see text).

TABLE 1

PLASMA PYRIDOSTIGMINE LEVELS IN PIGS

| Pyridostigmine Dose (mg/kg) | Pig 1 Plasma Concentration ng/ml | Pig 2 Plasma Concentration ng/ml |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 711 | 614 |
| 3 | 1202 | 1217 |
| 6 | 2011 | 2064 |
| 9 | 2932 | 2817 |

ing, and diarrhea. These systemic responses suggest that the dogs were more sensitive to the actions of the drugs, or that a sudden drop in the circulating plasma level of cholinesterase rather than total blood cholinesterase is responsible for these effects.

In conclusion, this study indicates that acute administration of large doses of pyridostigmine bromide results in salivation and gastrointestinal stimulation well in advance of detrimental effects involving the respiratory system and the muscles of respiration.

ACKNOWLEDGEMENT

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