TOXICOLOGY AND APPLIED PHARMACOLOGY 78, 139–146 (1985)

Pulmonary Responsiveness to Methacholine and Disodium Hexachloroplatinate (Na_2PtCl_6) Aerosols in Cynomolgus Monkeys (*Macaca fascicularis*)[1]

RAYMOND E. BIAGINI,*,[2] WILLIAM J. MOORMAN,* TRENT R. LEWIS,* AND I. LEONARD BERNSTEIN†

*Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, 4676 Columbia Parkway, Cincinnati, Ohio 45226 and †The University of Cincinnati Medical Center, Department of Internal Medicine, Division of Immunology, 231 Bethesda Avenue, Cincinnati, Ohio 45267

Received July 6, 1984; accepted November 13, 1984

Pulmonary Responsiveness to Methacholine and Disodium Hexachloroplatinate (Na_2PtCl_6) Aerosols in Cynomolgus Monkeys (*Macaca fascicularis*). BIAGINI, R. E., MOORMAN, W. J., LEWIS, T. R., AND BERNSTEIN, I. L. (1985). *Toxicol. Appl. Pharmacol.* **78**, 139–146. Hyperreactivity of the airways is a common finding in human asthma, and responsiveness to inhaled methacholine aerosols is routinely used for assessing airway irritability. Workers in precious metal refineries demonstrate pulmonary signs suggestive of asthma, presumably related to exposure to soluble platinum salts. In these workers, evidence of physiologic dysfunction precedes immunologic evidence (skin test) of disease, suggesting an initial pharmacologic mechanism. With a primate animal model for the screening of occupational asthmogens, 24 Cynomolgus monkeys were evaluated for their comparative pulmonary responsiveness to inhaled aerosols of methacholine and sodium hexachloroplatinate (Na_2PtCl_6). Average pulmonary flow resistance (R_L), dynamic compliance ($C_{L\ dyn}$), maximum expiratory flow volume (MEFV), and respiratory frequency changes were evaluated after bronchoprovocation challenge. Both agents produced dose-dependent increases in R_L, dose-dependent decreases in $C_{L\ dyn}$ and MEFV, and no effect on respiratory rates. Analyses of the correlation between concentration effects of the two agents showed no association between cholinergic airway irritability status and Na_2PtCl_6-induced bronchoconstriction. Na_2PtCl_6 bronchoprovocation produced significantly greater flow impairment at lower lung volumes when compared to methacholine concentrations with equipotent effects on R_L and $C_{L\ dyn}$. These compounds have differential effects on peripheral airway function. The lack of respiratory rate change seen on bronchoprovocation with these compounds, in comparison to the rapid shallow breathing in anesthetized monkeys following irritant or histamine challenge, indicates that neither aerosol stimulated pulmonary irritant receptors. © 1985 Academic Press, Inc.

Platinum metal refining is done by sequential solubilization and precipitation of platinum-rich ores or metal concentrates. This process yields purified platinum metal salts, which can be further processed to the pure elemental metal. Through these processes, workers employed in platinum refineries have potential exposure to platinum salt and metal aerosols.

There have been numerous reports in the literature concerning the incidence of pulmonary and dermal hypersensitivity reactions in workers exposed to the soluble salts of

[1] Parts of this work were presented before the American Physiological Society, San Diego, Calif., November 1982. This research was conducted in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

[2] To whom requests for reprints should be addressed.

0041-008X/85 $3.00
Copyright © 1985 by Academic Press, Inc.
All rights of reproduction in any form reserved.

platinum (Karasek and Karasek, 1911; Roberts, 1951; Pepys *et al.*, 1972; Dalley *et al.*, 1980; Biagini *et al.*, 1981). Hypersensitivity reactions to soluble platinum salts appear to be mediated by platinum-specific reaginic (IgE) antibodies (Cromwell *et al.*, 1979; Biagini *et al.*, 1984). Workers in precious metal refineries demonstrate pulmonary symptoms suggestive of asthma prior to any evidence of an immune mechanism (Dalley *et al.*, 1980). This reaction suggests that the initial pulmonary responses may be a hyperreactive pharmacologic effect rather than an immune effect. Upon continued exposure, large numbers of exposed workers will develop positive platinum salt skin tests, indicating peripheral immune involvement.

Soluble platinum salts increase bronchomotility and cause release of histamine or other mediators *in vitro* and *in vivo* in animals (Parrot *et al.*, 1969; Saindelle and Ruff, 1969). We have recently shown (Biagini *et al.*, 1983) that monkeys exposed by nose-only inhalation of sodium hexachloroplatinate (Na_2PtCl_6) aerosols show significant pulmonary hyperreactivity on subsequent bronchoprovocation challenge.

Normal bronchomotor tone is established primarily from the combined effects of the antagonistic actions of parasympathetic, sympathetic, and nonadrenergic inhibitory neurons (Hogg, 1982). Parasympathetic stimulation, inhibition of sympathetic or antagonism of nonadrenergic systems, and various other stimuli can lead to bronchoconstriction. Although the role of pulmonary mast cells and their released mediators in the control of bronchomotor tone is not clear at this time, they are believed to be important in allergic bronchoconstriction.

A characteristic feature of human reactive airway disease is augmentation of bronchial responsiveness (bronchial hyperreactivity) from a variety of nonspecific stimuli (Simonsson *et al.*, 1967), histamine (Curry, 1946), or parasympathomimetics, such as methacholine (Fish *et al.*, 1976). Affected individuals may have pulmonary signs of bronchoconstriction at concentrations of these agents which do not affect normal individuals (Gandevia, 1970).

The purpose of the present report was to investigate the association between airway cholinergic status and Na_2PtCl_6 pulmonary reactivity in naive monkeys. These data could potentially be used to evaluate the predictive value of methacholine challenge for the screening of new workers who may become exposed to platinum salts.

METHODS

Animals. Twenty-four adult male Cynomolgus monkeys (*Macaca fascicularis,* Charles River Primate Research, Inc., Port Washington, N.Y.)[3] were randomly selected from the NIOSH animal colony (weight, 4.5 $\pm$ 0.5 kg). The animals were in excellent health, and routine screening tests for tuberculosis and fecal parasites were negative. The monkeys were fed standard chow daily (Monkey Chow Jumbo, Ralston Purina Co., St. Louis, Mo.), and once weekly fresh fruit (oranges, apples, and bananas). Water was provided *ad libitum.* The monkeys were maintained on a 12-hr photoperiod (lights on 7 AM to 7 PM).

Anesthesia. To perform pulmonary function testing and bronchoprovocation challenges, the monkeys were anesthetized (im) with a mixture (Banknieder *et al.*, 1978) of 70 mg/ml ketamine hydrochloride and 6 mg/ml xylazine (Ketaset, Bristol Labs, Syracuse, N.Y., and Rompun, Bayvet Division of Cutter Labs, Shawnee, Kan.) at 0.15 ml/kg body wt.

Bronchoprovocation challenges and pulmonary function evaluations. Na_2PtCl_6 (99.99%, Pfaltz and Bauer, Inc., Division of Aceto Chemical Co., Stamford, Conn.) and acetyl-β-methacholine chloride (methacholine chloride, Sigma Chemical Co., St. Louis, Mo.) were used in these studies. Na_2PtCl_6 aerosols were generated for bronchoprovocation challenges (Patterson and Talbot, 1969) using a micronebulizer (output 0.065 ml/min) and a positive pressure ventilator respirator (Bird Mark 7, Bird Inc., Palm Springs, Calif.). The diameters of wet test droplet aerosols used for bronchoprovocation in this study were determined using a Model 3302 diluter (TSI, Inc., St. Paul, Minn.) and particle size analyzer (APS 33 aerodynamic particle sizer, TSI, Inc.). All Na_2PtCl_6 and methacholine aerosols had mass median aerodynamic diameters (MMADs) of 1.0 to 1.5 μm with standard

[3] Mention of a product or company name does not constitute endorsement by NIOSH.

geometric deviations (SDGs) of 1.7 to 2.0. Freshly prepared solutions of Na_2PtCl_6 in 0.9% NaCl (saline) and methacholine in 0.02 M phosphate-buffered saline (PBS), pH 7.4, were aerosolized by this system. Individual animals were randomly selected and bronchoprovocation challenged with serially increasing concentrations of methacholine. Two to three weeks later the same animals were challenged in a similar manner with Na_2PtCl_6. Bronchoprovocation challenges were performed for 1 min (15 breaths) in the following sequence: PBS, 0.1, 0.5, 1.05, and 6.25 mg/ml solutions of methacholine and saline, 0.5, 2.5, 25, and 50 mg/ml solutions of Na_2PtCl_6. Increasing bronchoprovocation challenge concentrations with each agent were administered at 10-min intervals. Preliminary studies demonstrated that equipotent effects on pulmonary mechanics would be observed using these challenge concentrations.

For pulmonary function testing, each anesthetized monkey was transorally intubated with a cuffed endotracheal tube (Rusch, Inc., New York, N.Y.) of maximum diameter (20 to 22 Fr). An esophageal balloon was placed into the lower third of the esophagus and adjusted to demonstrate the most negative end tidal transpulmonary pressures. The esophageal and mouth pressures were sensed by a PM 131TC differential pressure transducer (Statham Labs, Inc., Hato Rey, Puerto Rico). Flow at the mouth was measured by observing differential pressures generated across a pneumotachograph (Fleisch No. 0, Dynasciences Medical Products, Blue Bell, Pa.) and were electrically transduced (PM-5 transducer, Statham Labs Inc.). Volume was obtained by electrical integration of airflow with a variable time constant. Measures were made with the monkeys in a supine position inside a variable-pressure plethymograph–respirator previously described (Moorman *et al.*, 1975). Forced breathing maneuvers were produced by rapid (0.168-sec) external pressure changes producing adequate (>35 cm H_2O intrapleural) driving pressures required for maximum flow and volume maneuvers.

Pulmonary mechanics (Frank *et al.*, 1957) were obtained from simultaneous volume, flow, and transpulmonary pressure tracings displayed on a 12-channel photographic recorder (Model DR-12, Electronics for Medicine, White Plains, N.Y.). Forced maneuvers were also followed with this recording system. The pulmonary function parameters studied were average pulmonary flow resistance (R_L), dynamic compliance ($C_{L\ dyn}$), peak expiratory flow rate (PEFR), forced vital capacity (FVC), forced expiratory volume in 0.5-sec/FVC ($FEV_{0.5}$/FVC), and forced expiratory flows at 50 and 25% of vital capacity normalized for forced vital capacity (FEF50/FVC and FEF25/FVC). Respiratory rates after bronchoprovocation challenges were measured by timed direct visualization. All data sampling, storage, and calculations were performed by computer (Healthgarde CPT-5, Healthgarde Inc., Salt Lake City, Utah). A minimum 18 breaths were analyzed for the evaluation of R_L and $C_{L\ dyn}$. Flow–volume parameters were determined from a forced vital capacity maneuver conducted after each serial bronchoprovocation challenge.

Data reporting. Reactivity to bronchoprovocation challenges with increasing concentrations of each agent was calculated by dividing pulmonary function performance after agonist challenge by postvehicle (PBS or saline) pulmonary function results. This value was multiplied by 100, yielding the percentage of postvehicle challenge for each pulmonary function parameter tested. Preliminary studies in two or three monkeys had shown that five sequential saline or PBS bronchoprovocation challenges at 10-min intervals had no effect on baseline pulmonary function.

Statistical analyses. All hypothesis tests were performed by distribution-free (nonparametric) methods (Hollander and Wolfe, 1973), as no assumptions of the normality of the data were made. Each variable was analyzed individually. Saline and PBS (postvehicle) homogeneity was investigated by performing two-tailed sign tests for each pulmonary function variable. This test was also used to investigate the homogeneity of pulmonary function changes from corresponding bronchoprovocation challenge concentrations of Na_2PtCl_6 and methacholine. For visual simplification, the abscissae of the figures displaying these comparisons have been adjusted (Figs. 1, 3, and 4; i.e., 0.5 mg/ml Na_2PtCl_6 challenge results were compared with 0.1 mg/ml methacholine challenge results; 2.5 mg/ml Na_2PtCl_6 challenge results were compared with 0.5 mg/ml methacholine challenge results, etc.). Challenge responses were compared to corresponding post-vehicle values by one-tailed sign tests. Spearman rank correlation coefficients (r_S) were calculated to determine if correlations existed between Na_2PtCl_6 and methacholine challenges. Results were considered significant at a Type 1 error level of $p \leq 0.05$.

RESULTS

Effects on Pulmonary Mechanics

Postvehicle R_L or $C_{L\ dyn}$ were not significantly changed over the 2- to 3-week interval between methacholine and Na_2PtCl_6 challenges. With both Na_2PtCl_6 and methacholine serial bronchoprovocation challenges, R_L was increased and $C_{L\ dyn}$ decreased in concentration-dependent fashions (Fig. 1). When R_L and $C_{L\ dyn}$ challenge responses to increasing methacholine or Na_2PtCl_6 concentrations were evaluated, no statistically significant differences were observed. The respective concentrations (noncumulative) necessary for a

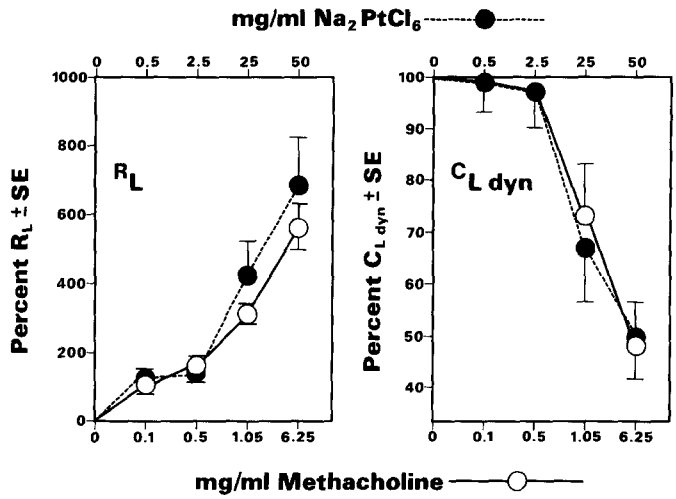

FIG. 1. Effect of acute serial bronchoprovocation challenge with Na_2PtCl_6 (●) and methacholine (○) on average pulmonary flow resistance (R_L) and dynamic compliance ($C_{L\,dyn}$). Data points are $\bar{x}$ ± SE for 24 individual monkey bronchoprovocation challenges at each concentration for each agent. The abscissae have been adjusted for visual simplification.

400% increase in postvehicle R_L (effective concentration, EC400 R_L) for the two compounds were Na_2PtCl_6, 23 mg/ml, and methacholine 2 mg/ml. The correlation between an individual animal's response to methacholine and a subsequent response to Na_2PtCl_6 was quite variable and not statistically significant when responses were compared at the 1.05 vs 25 mg/ml (r_S = 0.31) and 6.25 vs 50 mg/ml (r_S = 0.22) challenge concentrations (methacholine vs Na_2PtCl_6). In addition, challenge with both agents yielded variable individual R_L responses. For example, percentages of postvehicle R_L values following the 50 mg/ml Na_2PtCl_6 challenge concentration ranged from 188 to 2906%, while values after the 6.25 mg/ml methacholine challenge concentration ranged from 144 to 1419% (see Fig. 2).

Effects on Maximal Expiratory Flow Volume (MEFV) Performance

Postvehicle maximal effort flow-volume (MEFV) performance parameters (PEFR, $FEV_{0.5}$/FVC, FEF50/FVC, and FEF25/FVC) were not significantly changed over the 2- to 3-week period between methacholine and Na_2PtCl_6 challenges. When serial bronchoprovocation challenges with the two agonists were investigated for their effects on MEFV performance, regional pulmonary effects (central vs peripheral airways) were noted. When comparisons of agonist challenge effects on these variables were made at Na_2PtCl_6 and methacholine concentrations that had equipotent effects on R_L and $C_{L\,dyn}$, significant differences were observed (Figs. 3 and 4). Statistically significant reductions ($p < 0.05$) in $FEV_{0.5}$/FVC were seen at Na_2PtCl_6 concentrations greater than 0.5 mg/ml when these results were compared to methacholine challenge effects on this variable. Marked reductions were also observed in PEFR performance after challenge with the two agonists, with a statistically significant difference ($p < 0.05$) between the agents at the 50 mg/ml Na_2PtCl_6 vs 6.25 mg/ml methacholine challenge concentrations (Fig. 3). Na_2PtCl_6 challenge yielded significantly greater reductions ($p < 0.05$) in flows (FEF50/FVC, and FEF25/FVC) at concentrations greater than 2.5 mg/ml Na_2PtCl_6 when compared to methacholine challenge effects for these variables (Fig. 4).

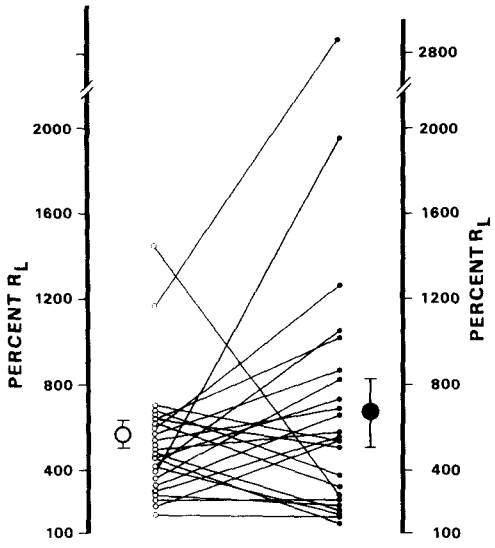

FIG. 2. Acute pulmonary responsiveness (methacholine vs Na_2PtCl_6). Data are reported as individual (N = 24 monkeys) percentages of postvehicle average pulmonary flow resistance (R_L) at the 6.25 mg/ml methacholine (○) and 50 mg/ml Na_2PtCl_6 (●) challenge concentrations. The individual animal responses to each agent are connected (Spearman correlation, r_S = 0.22, NS). Group mean results (±SE) for each challenge agent are displayed at the sides of the figure.

Effects on Respiratory Rates after Challenge

There were no statistically significant changes in respiratory rates observed with increasing bronchoprovocation challenge concentrations of either agent when these results were compared to postvehicle challenge respiratory rates.

DISCUSSION

The bronchoconstrictive effects of inhaled methacholine aerosols on pulmonary mechanics at tidal breathing in anesthetized monkeys (Michoud *et al.*, 1978) and of Na_2PtCl_6 on pulmonary mechanics and maximal expiratory flow–volume (Biagini *et al.*, 1983) have been previously reported. The present study is one of the first reports of the effects of methacholine challenge on monkey MEFV. From reports in the literature and our data, both agents produce peripheral and central airway bronchoconstriction. The wide variability in individual monkey responses to both methacholine and Na_2PtCl_6 bronchoprovocation challenge found here is consistent with other reports showing widely variable individual bronchoprovocation responses to aerosol challenge with histamine or methacholine in dogs (Snapper *et al.*, 1978), monkeys (Michoud *et al.*, 1978), or guinea pigs (Douglas *et al.*, 1973). The individual animal variabilities in the present experiment then are probably not due to random error from the cumulative bronchoprovocation regimens or other sources. Pulmonary function parameters which assess airway mechanical status at tidal breathing (R_L and $C_{L\,dyn}$; Fig. 1) are insensitive to the differential effects of the two agents that become apparent during MEFV maneuvers (Figs. 3 and 4). When the effects of the two agents were compared using pulmonary function parameters which assess maximal effort volume or flow performance in both central and peripheral airways ($FEV_{0.5}$/FVC and PEFR) or flows at low lung volumes (FEF50/FVC and FEF25/FVC), significantly greater reductions in these parameters were observed following Na_2PtCl_6 challenge compared to methacholine challenge.

The lack of correlation between observed effects, i.e., pulmonary mechanics at tidal breathing vs forced expiratory maneuvers (MEFV performance), is probably not due to differing regional pulmonary deposition patterns (large central vs small peripheral airways), since the particle sizes of the two aerosols were essentially equal. The observed regional bronchoconstriction differences probably lie in different mechanisms of pharmacologic action for the two compounds. Methacholine is thought to act directly at the smooth muscle cholinergic receptor (Scanlon. 1984), while Na_2PtCl_6 is thought to act on mast cells (causing histamine or other mediator release) or directly on smooth muscle tissue (Saindelle and Ruff, 1969). Histamine is thought to act both on smooth muscle directly and on afferent nerve endings (irritant

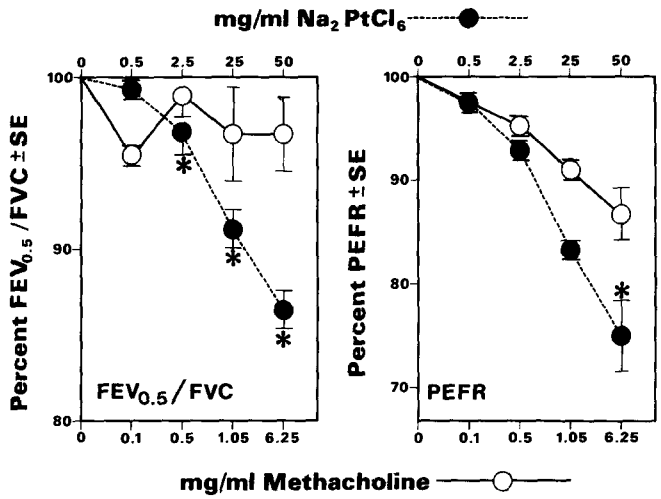

FIG. 3. Effect of acute serial bronchoprovocation challenge with Na_2PtCl_6 (●) and methacholine (○) on forced expiratory volume in 0.5-sec/forced vital capacity ($FEV_{0.5}/FVC$) and peak expiratory flow rate (PEFR). Data points are $\bar{x} \pm SE$ for 24 individual monkey bronchoprovocation challenges at each concentration for each agent. The abscissae have been adjusted for visual simplification. *Statistically significant difference ($p < 0.05$) between Na_2PtCl_6 and methacholine effects at the concentrations indicated.

receptors), causing reflex bronchoconstriction (Casterline and Evans, 1977).

The lack of respiratory rate changes upon methacholine challenge in anesthetized monkeys has been reported (Michoud *et al.*, 1978). However, these same investigators reported increased respiratory rates in anesthetized monkeys challenged with antigen or

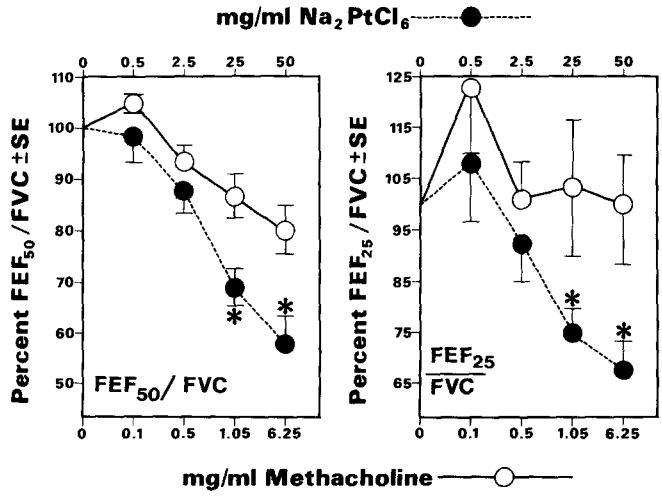

FIG. 4. Effect of acute serial bronchoprovocation challenge with Na_2PtCl_6 (●) and methacholine (○) on forced expiratory flows at 50 and 25% of forced vital capacity/FVC (FEF50/FVC and FEF25/FVC). Data points are $\bar{x} \pm SE$ for 24 individual monkey bronchoprovocation challenges at each concentration for each agent. The abscissae have been adjusted for visual simplification. *Statistically significant difference ($p < 0.05$) between Na_2PtCl_6 and methacholine effects at the concentrations indicated.

histamine aerosols. These respiratory rate changes were hypothesized to be due to irritant receptor stimulation. The pulmonary functional deficits of inhaled methacholine and histamine aerosols have recently been studied in normal human volunteers (Savoy *et al.*, 1984). These investigators found that challenge with both agents produced similar levels of bronchospasm without significant changes in respiratory rates. The lack of respiratory rate changes on challenge with Na_2PtCl_6 aerosols observed in monkeys in our work suggests that this compound does not stimulate irritant receptors (afferent nerve endings) directly at these concentrations. The observed bronchoconstriction is due either to a direct action on smooth muscle or to a release of mediators which induces bronchoconstriction without stimulating irritant receptors. Alternatively, maximal inspirations used for forced vital capacity maneuvers may have limited the cumulative effects of repeated serial bronchoprovocations or distributed the agents more uniformly throughout the lungs. This change possibly could have led to lowered local concentrations of the challenge agents. The "therapeutic" effects of deep inspirations have been reported in humans challenged with bronchoconstrictive agents (Nadel and Tierney, 1961).

Pulmonary cholinergic receptors are preferentially located in the central airways (Barnes *et al.*, 1983) and bronchoconstriction after cholinergic agonist challenge occurs preferentially in central airways (Colebatch *et al.*, 1966). The number and distribution of mast cells in monkey lungs have been studied (Guerzon *et al.*, 1979), and, by far, the majority are located in the peripheral airways (generations 5 through 15). Therefore, the peripheral airway effects of Na_2PtCl_6 bronchoprovocation challenge observed in the present experiments may be due to effects on mast cells.

In conclusion, methacholine pulmonary reactivity was not predictive of Na_2PtCl_6 reactivity in naive monkeys, and both agents yielded quite variable individual pulmonary responses in a relatively large group of monkeys. MEFV performance parameters were more sensitive to the differential airway effects of the two agonists than the analysis of pulmonary mechanical parameters (R_L and $C_{L\ dyn}$). These data indicate that there are differential mechanisms of pharmacologic action for the bronchoconstrictive effects of these two compounds in monkeys.

The initial incidence of asthma-like pulmonary signs reported for platinum refinery workers without evidence of immune-mediated disease (Dalley *et al.*, 1980) may be due to pharmacologic airway hyperreactivity to the soluble platinum salts, presumably from a mast cell-mediated release mechanism. Variability in the intrinsic susceptibility of individuals to histamine or other mediators (Habib *et al.*, 1979) may explain why some individuals are affected while others are not. Prior airway cholinergic irritability status does not seem predictive of subsequent airway reactivity in naive monkeys challenged with soluble platinum salts.

ACKNOWLEDGMENTS

The authors acknowledge the expert assistance of John C. Clark for the conduct of monkey pulmonary function evaluations, Randall Smith for statistical analyses, and Dennis Lynch for editing the final manuscript. In addition, the authors express their appreciation to Dr. Paul Barron of the Division of Physical Science and Engineering of NIOSH for aerosol sizing measurements.

REFERENCES

BANKNIEDER, A. R., PHILLIPS, J. M., JACKSON, K. T., AND VINAL, S. I. (1978). Comparison of ketamine with the combination of ketamine and xylazine for effective anesthesia in the rhesus monkey (*Macaca mulatta*). *Lab. Animal Sci.* **28**, 742–745.

BARNES, P. J., BASBAUM, C. B., AND NADEL, J. A. (1983). Autoradiographic localization of autonomic receptors in airway smooth muscle. *Amer. Rev. Respir. Dis.* **127**, 758–762.

BIAGINI, R. E., CLARK, J. C., GALLAGHER, J. S., BERNSTEIN, I. L., AND MOORMAN, W. J. (1981). Passive transfer in the monkey of human immediate hypersensitivity to complex salts of platinum and palladium. *Fed. Proc.* **41**, 827.

BIAGINI, R. E., GALLAGHER, J. S., BERNSTEIN, I. L., AND MOORMAN, W. J. (1984). The use of a platinum (Pt) inhibitable enzyme as a protein conjugate for a platinum RAST system. *Fed. Proc.* **43,** 1936.

BIAGINI, R. E., MOORMAN, W. J., SMITH, R. J., LEWIS, T. R., AND BERNSTEIN, I. L. (1983). Pulmonary hyperreactivity in cynomolgus monkeys (*Macaca fascicularis*) from nose-only inhalation exposure to disodium hexachloroplatinate, Na_2PtCl_6. *Toxicol. Appl. Pharmacol.* **69,** 377–384.

CASTERLINE, C. L., AND EVANS, R. (1977). Further studies on the mechanism of human histamine induced asthma. *J. Allergy Clin. Immunol.* **59,** 420–424.

COLEBATCH, H. J. H., OLSEN, C. R., AND NADEL, J. A. (1966). Effect of histamine, serotonin and acetylcholine on peripheral airways. *J. Appl. Physiol.* **21,** 217.

CROMWELL, O., PEPYS, J., PARISH, W. E., AND HUGHES, E. G. (1979). Specific IgE antibodies to platinum salts in sensitized workers. *Clin. Allergy* **9,** 109–117.

CURRY, J. J. (1946). The action of histamine on the respiratory tract of normals and asthmatic subjects. *J. Clin. Invest.* **25,** 785–791.

DALLEY, M. B., HUNTER, J. V., HUGHES, E. G., STEWART, M., AND NEWMAN-TAYLOR, A. J. (1980). Hypersensitivity to platinum salts: A population study. *Amer. Rev. Respir. Dis.* **4**(Suppl), 230.

DOUGLAS, J. S., DENNIS, M. W., RIDGWAY, R., AND BOUHUYS, A. (1973). Airway constriction in guinea pigs: Interaction of histamine and autonomic drugs. *J. Pharmacol. Exp. Ther.* **184,** 169–179.

FISH, J. E., ROSENTHAL, R. R., BATRA, G., MENKES, H., SUMMER, W., PERMUTT, S., AND NORMAN, P. (1976). Airway responses to methacholine in allergic and nonallergic subjects. *Amer. Rev. Respir. Dis.* **113,** 579–586.

FRANK, N. R., MEAD, J., AND FERRIS, B. G. (1957). The mechanical behavior of the lungs in healthy elderly persons. *J. Clin. Invest.* **36,** 1680–1687.

GANDEVIA, B. (1970). Occupational asthma. Part 1. *Med. J. Austr.* **2,** 332–335.

GUERZON, G. M., PARE, P. D., MICHOUD, M. C., AND HOGG, J. C. (1979). The number and distribution of mast cells in monkey lungs. *Amer. Rev. Respir. Dis.* **119,** 59–66.

HABIB, M. P., PARE, P. D., AND ENGEL, L. A. (1979). Variability of airway responses to inhaled histamine in normal subjects. *J. Appl. Physiol.* **47,** 51–58.

HOGG, J. C. (1982). The pathophysiology of asthma. *Chest* **82,** 8S–12S.

HOLLANDER, H., AND WOLFE, D. A. (1973). *Nonparametric Statistical Methods.* Wiley, New York.

KARASEK, S. R., AND KARASEK, M. (1911). *Report of Illinois State Commission of Occupational Disease, Springfield,* p. 97.

MICHOUD, M. C., PARE, P. D., BOUCHER, R., AND HOGG, J. C. (1978). Airway responses to histamine and methacholine in *Ascaris suum* allergic rhesus monkeys. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **45,** 846–851.

MOORMAN, W. J., LEWIS, T. R., AND WAGNER, W. D. (1975). Maximum expiratory flow-volume studies on monkeys exposed to bituminous coal dust. *J. Appl. Physiol.* **39,** 444–448.

NADEL, J. A., AND TIERNEY, D. F. (1961). Effects of a previous deep inspiration on airway resistance in man. *J. Appl. Physiol.* **16,** 717–719.

PARROT, J-L., HEBERT, R., SAINDELLE, A., AND RUFF, F. (1969). Platinum and platinosis: Allergy and histamine release due to some platinum salts. *Arch. Environ. Health* **19,** 685–691.

PATTERSON, R., AND TALBOT, C. H. (1969). Respiratory responses in subhuman primates with immediate type hypersensitivity. *J. Lab. Clin. Med.* **73,** 924–933.

PEPYS, J., PICKERING, C. A. C., AND HUGHES, E. G. (1972). Asthma due to chemical agents—Complex salts of platinum. *Clin. Allergy* **2,** 391–396.

ROBERTS, F. (1951). Platinosis; A five year study of the effects of soluble platinum salts on employees in a platinum laboratory and refinery. *Arch. Ind. Hyg.* **4,** 549–559.

SAINDELLE, A., AND RUFF, F. (1969). Histamine release by sodium chloroplatinate. *Brit. J. Pharmacol.* **35,** 313–321.

SAVOY, J., ALLGOWER, E., COURTEHEUSE, C., AND JUNOD, A. F. (1984). Ventilatory response to bronchospasm induced by methylcholine and histamine in man. *Respir. Physiol.* **56,** 195–203.

SCANLON, R. T. (1984). Asthma: A panoramic view and hypothesis. *Ann. Allergy* **53,** 203–212.

SIMONSSON, B. G., JACOBS, F. M., AND NADEL, J. A. (1967). Role of the autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with constrictive airway disease. *J. Clin. Invest.* **46,** 1812–1818.

SNAPPER, J. R., DRAZEN, J. M., LORING, S. H., SCHNEIDER, W., AND INGRAM, R. H. (1978). Distribution of pulmonary responsiveness to aerosol histamine in dogs. *J. Appl. Physiol. Respir. Environ. Exercise Physiol.* **44,** 738–742.