Bronchodilatory and Cardiovascular Effects of Metaproterenol

By L. DIAMOND*

Until recently, the methods which were available for evaluating bronchodilator drugs in the intact animal did not yield quantitative data which were primarily related to the smooth muscle tone of the airways. In the present study a technique was employed which yielded direct measurements of the total lung resistance to airflow. Since pulmonary resistance values depend directly on the dimensions of the airways, their magnitude is very sensitive to changes in the smooth muscle tone of the tracheobronchial tree. Metaproterenol [1-(3,5-dihydroxyphenyl)-2-isopropyl-aminoethanol sulfate], a new beta-adrenergic stimulant, inhibited the increase in total lung resistance evoked by histamine aerosol. The drug was effective in anesthetized, spontaneously breathing dogs when administered as a 2 percent aerosolized solution. A series of experiments was designed to compare the cardiovascular effects which followed inhalation of a therapeutic dose of metaproterenol with those which occurred following a similar dose of isoproterenol in anesthetized dogs. The positive chronotropic effect and the increase in pulse pressure were more pronounced after metaproterenol inhalation than after isoproterenol inhalation. However, the hypotensive effect of isoproterenol was somewhat greater than that of metaproterenol.

DRUGS CAPABLE of dilating the smooth musculature of the tracheobronchial tree have been employed extensively in the symptomatic treatment of obstructive diseases of the lungs. In recent years, oral inhalation has become a preferred means of administering bronchodilator drugs. Bronchospasmolytic agents administered *via* this route can provide rapid relief from acute episodes of bronchospasm.

The sympathomimetic amine, isoproterenol, is probably the most popular drug in use today for the management of asthma, emphysema, and related conditions. However, whether inhaled or given by some other route, isoproterenol has a limited duration of action and with repeated administration various undesirable systemic effects may develop, such as palpitations, tachycardia, hypotension, and central nervous system stimulation.

A new compound, purported to be as potent a bronchodilator as isoproterenol, but possessing a longer duration of action and producing fewer and milder side effects, has recently been made available. The new compound, metaproterenol,[1] differs chemically from isoproterenol by having the hydroxyl groups on the phenyl nucleus in the *meta* position. The general pharmacodynamic properties of metaproterenol were first studied by Engelhardt *et al.* (1) in 1961.

The method used by the latter workers, as well as many other investigators to assess the bronchodilatory activity of a compound in the intact animal, measures changes in the capacity of the respiratory tract as expressed by an increase or decrease in the volume of air taken up by the lungs under constant pressure inflation (2). This technique provides only a qualitative indication of the bronchiolar resistance. Moreover, it has been shown that at the usual frequencies of respiration this method is sensitive to changes in lung compliance but relatively less sensitive to changes in lung resistance (3).

The purpose of the present study was to obtain quantitative data relating to the bronchodilating efficacy of metaproterenol by utilizing direct determinations of the total lung resistance to airflow. A method similar to the one employed in this study has been previously used for the pharmacological evaluation of bronchodilator drugs by Familiar *et al.* (4). Since pulmonary resistance values depend directly on the caliber of the airways, their magnitude is very sensitive to changes in the smooth muscle tone of the tracheobronchial tree. The cardiovascular side effects which followed inhalation of a therapeutic dose of metaproterenol were also studied and compared with those which occurred after inhalation of a similar dose of isoproterenol.

EXPERIMENTAL

Ten mongrel dogs of either sex were anesthetized with an intraperitoneal injection of a solution containing 100 mg. of allobarbital, 400 mg. of urethan, and 400 mg. of monoethylurea in each ml. (0.6 ml./Kg.). The dogs were suitably restrained upon a surgical table and allowed to breathe spontaneously. A Murphy endotracheal tube was inserted into the trachea and secured there by inflation of its cuff. A

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[1] Alupent, 1-(3,5-dihydroxyphenyl)-2-isopropyl-aminoethanol sulfate, brand of metaproterenol sulfate, Geigy Pharmaceuticals, Ardsley, N. Y.

cannula, consisting of an 18-gauge (3.8 cm., 1.5 in.) hypodermic needle attached to a length of polyethylene tubing, was introduced through one of the intercostal spaces into the intrapleural space. The intrapleural cannula was connected to one port of a Sanborn differential pressure transducer. A polyethylene tube leading from the endotracheal tube was connected to the other port of the transducer. This procedure permitted the pressure at the mouth to be subtracted from the intrapleural pressure to provide values for the transpulmonary pressure. A Sanborn pneumotachograph and differential strain gauge were used to measure the flow of air into and out of the lungs. A volume signal was obtained by electrical integration of the flow signal. The three parameters of pressure, flow, and volume were simultaneously recorded on a Sanborn direct-writing oscillograph.

Pulmonary Mechanics—The total lung resistance to airflow was calculated by selecting points along the ascending and descending limbs of the tidal volume record which represented points of equal inflation and deflation. Vertical lines were then drawn from these points through the pressure and flow curves. The difference in pressure and flow between the two points provided the data for the equation: pulmonary resistance = pressure difference in cm. H_2O/flow difference in L./sec. This method provides values which represent the average inspiratory and expiratory resistance near peak inspiratory and expiratory flows. Although the values thus obtained do not express the change of resistance with flow rate, they do closely approximate the average resistance during the respiratory cycle (5).

The compliance of the lungs was calculated from the following formula: pulmonary compliance = volume change in ml./pressure change in cm. H_2O. The necessary data were obtained by measuring volume and pressure changes at corresponding points of no airflow (beginning and end of inspiration) as seen from the flow-rate curve. The details of these methods have been described in an earlier report from this laboratory (6).

Evaluation of Bronchodilating Activity of Metaproterenol—Approximately 1 ml. of a 2% solution of histamine base was placed in a DeVilbiss No. 42 nebulizer which was modified with connectors and placed between the endotracheal tube and the pneumotachograph screen (Fig. 1). Control conditions were recorded and then histamine was administered by squeezing the hand bulb of the nebulizer at the outset of five consecutive inspirations. Immediately thereafter data for pulmonary mechanics calculations were recorded at 30-sec. to 2-min. intervals for a period of 5 min.

After allowing adequate time for the animals to recover from the bronchoconstricting effects of histamine, 10 inhalations of a 2% metaproterenol sulfate solution were administered. Two minutes were allowed for the drug to exert its activity after which time the respiratory patterns of the animals were again recorded. Histamine inhalation was then repeated, and the protection afforded by metaproterenol was evaluated by again measuring changes in pulmonary mechanical values over a 5-min. period.

Comparison of Cardiovascular Side Effects of Isoproterenol and Metaproterenol—A test group of 10 dogs was used to study and compare the cardiovascular side effects produced by inhalation of either isoproterenol or metaproterenol. Systemic blood

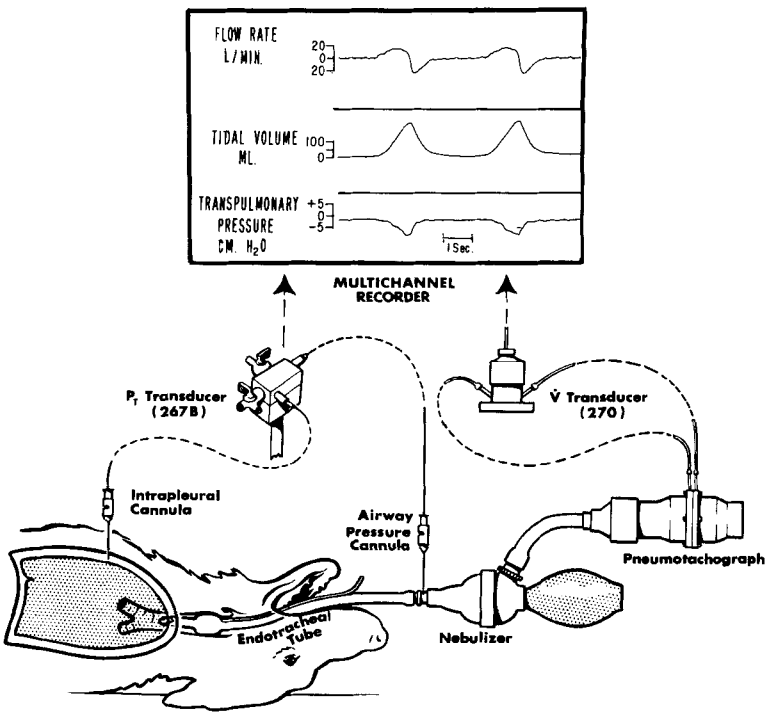

Fig. 1—Apparatus for administration of drug solutions and measurement of pulmonary mechanics.

pressure was measured from the abdominal aorta *via* the left femoral artery using a polyethylene catheter attached to a Sanborn pressure transducer. Pulse pressure was determined by subtracting the diastolic from the systolic pressure. Mean pressure was obtained by switching the pressure preamplifier to a filter circuit having a 1-sec. time constant. This procedure allowed the average value of the load on the transducer to be displayed on the oscillograph. A Sanborn high-gain preamplifier was used to record lead I of the electrocardiogram. Since the chart speed of the recorder was known, the heart rate was easily calculated.

Sufficient time was allowed for the animals' blood pressures to stabilize before control conditions were recorded. Each dog then received 10 inhalations of a 0.5% aqueous solution of isoproterenol hydrochloride. Drug-induced changes in the cardiovascular status of the animals were recorded at 1–3 min. intervals for a period of 15 min. After the isoproterenol effects had subsided, 10 inhalations of a 2% aqueous solution of metaproterenol sulfate were administered and the same data recording procedure repeated.

RESULTS AND DISCUSSION

The results of the first series of experiments, designed to evaluate the bronchodilator potential of metaproterenol, are presented in Tables I and II. The control value of the mean pulmonary resistance was 8.0 ± 0.4 (Table I). Following inhalation of histamine the mean pulmonary resistance increased sharply. The peak of the reaction usually occurred within 1 min. after administration of the spasmogen. Bronchoconstriction subsided in all of the test subjects within 20 min. at which time metaproterenol was administered. Two minutes thereafter the pulmonary resistance was again measured and the mean value found to be 9.4 ± 0.7. The latter value did not differ significantly from the initial control value ($p > 0.2$).

In each of the 10 experimental animals, metaproterenol afforded various degrees of protection

TABLE I—EFFECT OF HISTAMINE INHALATIONS ON PULMONARY RESISTANCE OF ANESTHETIZED, SPONTANEOUSLY BREATHING DOGS BEFORE AND AFTER TREATMENT WITH METAPROTERENOL

Dog	Sex	Wt., Kg.	Pulmonary Resistance (cm. H_2O/L./sec.)[a]						
			Drug	Control	Drug	30 sec.	1 min.	2 min.	3 min.	5 min.
1	F	12.2		8.4	Hist. (1)[b]	10.9	12.8	11.3	10.5	8.2
			Meta.	7.1	Hist. (2)[c]	8.4	9.7	9.3	9.4	7.4
2	M	6.7		8.3	Hist. (1)	16.7	21.8	19.6	18.2	14.4
			Meta.	10.6	Hist. (2)	11.1	12.1	11.6	10.8	11.3
3	F	6.0		8.7	Hist. (1)	31.2	31.1	28.4	23.6	19.5
			Meta.	12.9	Hist. (2)	13.4	15.8	16.1	16.4	16.4
4	M	10.0		6.3	Hist. (1)	13.9	14.1	12.8	11.6	9.9
			Meta.	5.3	Hist. (2)	6.5	6.0	7.1	7.9	7.7
5	F	12.2		8.2	Hist. (1)	ap[d]	23.1	19.3	15.6	13.6
			Meta.	9.8	Hist. (2)	11.3	11.1	11.1	10.6	10.4
6	F	11.4		7.6	Hist. (1)	9.4	11.2	12.8	12.7	11.3
			Meta.	8.6	Hist. (2)	10.4	11.5	11.4	11.0	9.6
7	F	11.4		9.6	Hist. (1)	ap[d]	ap[d]	ap[d]	62.2	31.2
			Meta.	13.0	Hist. (2)	16.4	16.9	15.9	15.6	12.8
8	F	9.0		5.8	Hist. (1)	ap[d]	60.0	57.1	48.0	38.3
			Meta.	9.1	Hist. (2)	9.8	10.8	11.0	9.4	9.8
9	F	10.0		9.8	Hist. (1)	ap[d]	51.7	28.0	33.1	41.6
			Meta.	9.9	Hist. (2)	8.4	11.9	10.3	9.3	10.0
10	M	13.6		7.7	Hist. (1)	13.1	15.8	15.9	14.4	11.9
			Meta.	7.9	Hist. (2)	8.3	9.2	9.1	8.8	8.6
Mean ± S.E.				8.0 ± 0.4	Hist. (1)	15.9 ± 2.9	26.8 ± 5.0	22.8 ± 4.5	25.0 ± 5.3	20.0 ± 3.1
Mean ± S.E.			Meta.	9.4 ± 0.7	Hist. (2)	10.4 ± 0.9	11.5 ± 0.9	11.3 ± 0.9	10.8 ± 0.9	10.4 ± 0.8

[a] Each value represents the average of 3 consecutive measurements. [b] Hist. (1) refers to the initial histamine administration. [c] Hist. (2) refers to the second administration of histamine to the same animal following treatment with metaproterenol. [d] ap denotes apnea.

TABLE II—EFFECT OF HISTAMINE INHALATIONS ON TIDAL VOLUME, RESPIRATORY RATE, MINUTE VOLUME, AND PULMONARY COMPLIANCE OF ANESTHETIZED, SPONTANEOUSLY BREATHING DOGS BEFORE AND AFTER TREATMENT WITH METAPROTERENOL

	Vol. (ml.)		Rate ($min.^{-1}$)		Min. Vol. (ml./min.)		Compliance (ml./cm. H_2O)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Control	157	75–265	25	7–60	3393	1210–7800	38	23–52
Histamine administered								
30 sec.	69	32–110	75	15–108	4692	1650–3400	11	2–27
1 min.	72	30–143	78	18–125	4747	1920–10010	9	3–20
2 min.	89	37–164	72	18–120	5963	2448–13302	11	6–21
3 min.	98	39–190	59	18–95	6043	2574–12240	12	4–25
5 min.	100	40–156	48	9–80	4354	1848–12000	18	7–31
Meta.[a]	142	83–228	33	10–75	3898	2508–8025	35	15–78
Histamine administered								
30 sec.	125	56–256	53	18–100	5653	2800–10400	27	9–67
1 min.	126	63–240	57	18–103	6150	3950–10800	25	8–51
2 min.	137	75–260	55	20–100	6523	3720–11284	26	11–51
3 min.	144	74–260	53	18–90	6648	4512–10992	27	11–59
5 min.	130	64–261	51	15–84	5582	3000–8568	27	12–41

[a] The tabulated values were obtained 2 min. after metaproterenol had been administered.

against subsequent challenging doses of histamine. The percent protection at each of the measurement intervals was computed utilizing the following formula: $P = (I_c/R_c - I_t/R_t)/I_c/R_c \times 100$ where P is the percent protection, I_c is the increase in mean pulmonary resistance after histamine administration, R_c is the control mean pulmonary resistance before histamine had been given, I_t is the increase in mean pulmonary resistance after histamine administration to the metaproterenol treated dogs, and R_t is the mean pulmonary resistance 2 min. after the administration of metaproterenol. The percent protection values 30 sec., 1, 2, 3, and 5 min. after the second series of histamine inhalations were 89.2, 90.6, 89.2, 92.7, and 93.3%, respectively.

The effects of histamine inhalations on tidal volume, respiratory rate, minute volume, and pulmonary compliance, before and after treatment with metaproterenol, are summarized in Table II. The untreated animals experienced a decrease in tidal volume and pulmonary compliance and an increase in respiratory rate and minute volume following histamine. When the histamine inhalations were repeated in the metaproterenol-treated animals, the parameters again changed in the same direction but the magnitude of the changes differed. For instance, the maximum decrease in mean tidal volume of the unprotected animals was 88 ml., whereas the treated animals showed a maximum decrease of 17 ml. The mean respiratory rate of the unprotected animals increased from 25/min. to a maximum of 78/min., while the animals which had received metaproterenol experienced an increase of from 33/min. to a maximum of 57/min. The increase in minute volume of the untreated animals was only slightly less than the increase observed in the treated animals. The mean pulmonary compliance of the unprotected animals fell from the control value of 38 ml./cm. H_2O to a low of 9 ml./cm. H_2O while that of the treated animals fell from the control value of 35 ml./cm. H_2O to a low of 25 ml./cm. H_2O.

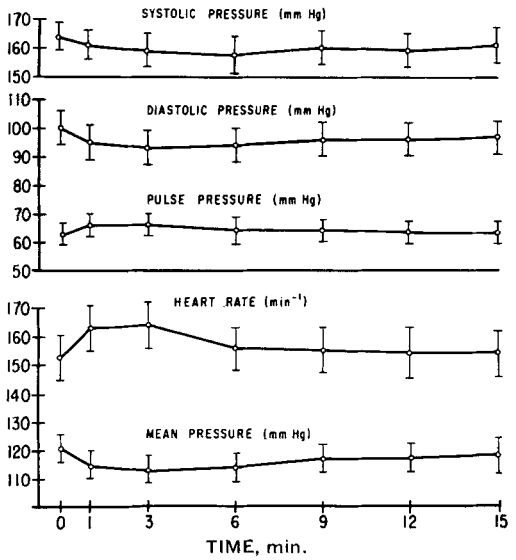

Fig. 2—Cardiovascular effects following 10 inhalations of isoproterenol hydrochloride (0.5%). The standard error is represented by vertical lines.

There are several possible explanations for the increase in respiratory frequency which was observed in both the unprotected and protected animals. Inhalation of histamine has been shown to reduce the systemic blood pressure of anesthetized dogs (7). The latter observation was confirmed by the present investigation. It is possible then that the respiratory stimulation was compensatory in nature and mediated by a reflex response evoked from baroreceptors in the carotid sinus and/or the aortic arch. In this connection it should be noted that in order for an inhaled drug to produce systemic effects it must first undergo transpulmonary absorption. Lovejoy and his co-workers (8) presented evidence to show that systemic effects frequently result after administering drugs from standard commercial nebulizers (such as the one used in this study) owing to their inherent property of delivering particles which are not exclusively submicronic. Robillard *et al.* (9) reported observing tachypnea and hypotension in human patients receiving carbachol from a commercial nebulizer. An increase in the respiratory rate of rabbits following carbachol inhalation was also observed by Karczewski and Dautrebande (10). These authors attributed the increase to the inhibiting effect of increased activity of pulmonary stretch receptors on the inspiratory center.

It is doubtful that the decrease in pulmonary compliance which followed histamine inhalation was caused by an actual lessening of the elastic qualities of the lungs. The changes in lung compliance followed the same time course and were proportional to the changes in total lung resistance. It is likely that histamine caused a marked constriction of several pulmonary units and as a consequence some alveoli collapsed due to surface forces. If such did occur, the decrease in compliance could be accounted for by atelectasis secondary to airway constriction.

The results of the experiments designed to compare the cardiovascular effects produced by inhalation of therapeutic doses of isoproterenol or metaproterenol are presented graphically in Figs. 2 and 3. Statistical analysis of the data was performed by using paired samples and the results were subjected to Student's analysis. A measured difference between control and postdrug values was considered to be significant and used to form conclusions only if $p < 0.05$.

Isoproterenol produced a significant drop in systolic blood pressure and a significant elevation of the heart rate at the 3-min. and 6-min. observation points. The diastolic and mean pressures were significantly depressed 1–6 min. after the drug had been administered. Isoproterenol had no significant effect upon pulse pressure. None of the cardiovascular responses to isoproterenol inhalation persisted for more than 6–9 min.

Metaproterenol produced a slight, insignificant increase in systolic blood pressure. The diastolic and mean pressures fell somewhat, but the decreases were significant only at the 9-min. postdrug administration time. The slight rise in systolic pressure coupled with the small decrease in diastolic pressure was sufficient to produce a significant increase in pulse pressure at each of the measurement intervals. The most pronounced cardiovascular effect of meta-

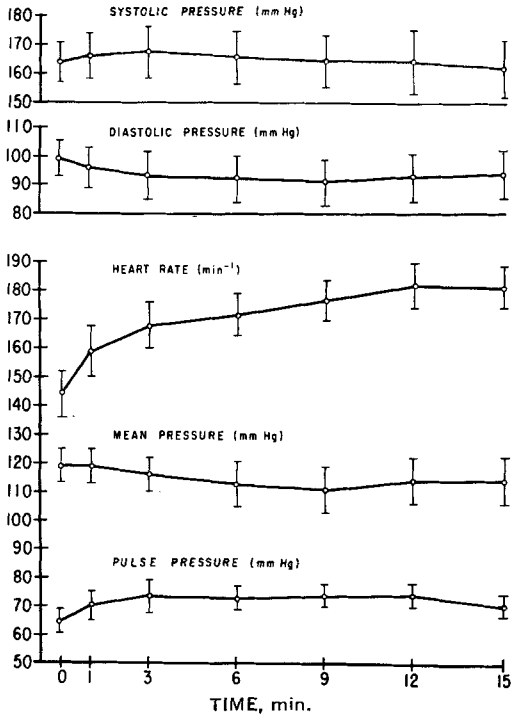

Fig. 3—Cardiovascular effects following 10 inhalations of metaproterenol sulfate (2%). The standard error is represented by vertical lines.

proterenol was observed on the heart rate which showed significant elevation at every observation time. Whereas the maximum increase in heart rate after isoproterenol inhalation was 7% and occurred 1 min. after the drug had been administered, the maximum increase following metaproterenol inhalation was 26% and occurred 12 min. after the drug had been administered.

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Keyphrases

Metaproterenol—β-adrenergic stimulant
Bronchodilatory effects—metaproterenol
Pulmonary resistance, complicance—determined
Cardiovascular effects—metaproterenol
Histamine antagonism—metaproterenol

Effect of Deuterium Oxide on the Culturing of *Penicillium janczewskii* II

Isolation of Fully Deuterated Griseofulvin

By D. A. NONA, M. I. BLAKE, H. L. CRESPI, and J. J. KATZ

Fully deuterated griseofulvin was isolated in pure form from *P. janczewskii* grown in a completely deuterated medium. Direct fermentation and a replacement culture technique were used. The isolated antibiotic was purified by thin-layer chromatography and characterized by ultraviolet, infrared, and nuclear magnetic resonance spectra. The extent of incorporation of deuterium during biosynthesis at the various proton sites in the molecule was determined.

In a previous paper (1) the effect of heavy water both on the growth of the mold *Penicillium janczewskii* and on its production of antibiotic was reported. Nutritional requirements for optimal growth in D_2O and the general culture techniques were described in detail. Griseofulvin production was severely impaired when the organism was grown in pure D_2O media by the usual surface culture method, but the griseofulvin titer was improved when the D_2O culture medium was supplemented with vitamin B complex. In the present study a replacement culture method was employed to produce deuterated griseoful-

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