THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 186, No. 3

Printed in U.S.A.

THE BRONCHODILATOR AND CARDIAC STIMULANT EFFECTS OF Th1165a, SALBUTAMOL AND ISOPROTERENOL

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Accepted for publication May 3, 1973

ABSTRACT

GILES, RALPH E., JOSEPH C. WILLIAMS AND MARTIN P. FINKEL: The bronchodilator and cardiac stimulant effects of Th1165a, salbutamol and isoproterenol. J. Pharmacol. Exp. Ther. **186:** 472–481, 1973.

There were differences among the *beta* adrenoceptor stimulants Th1165a (3,5-dihydroxy-α-{[(*p*-hydroxy-α-methylphenethyl)amino] methyl}-benzyl alcohol hydrobromide; hydroxyphenylorciprenaline), isoproterenol and salbutamol in bronchodilator and cardiac stimulant potency. *In vitro,* the agonists were similar in potency in relaxing guinea-pig trachea but the order of potency for the chronotropic response of guinea-pig atria was isproterenol > Th1165a > salbutamol. *In vivo,* drugs were evaluated in both guinea pig and dog. Each of the agonists administered i.p., p.o. or by aerosol protected against histamine-induced collapse in guinea pigs; isoproterenol produced a greater tachycardia than either Th1165a or salbutamol. By mouth, salbutamol was more potent than Th1165a or isoproterenol in preventing histamine-induced collapse. In the anesthetized dog, Th1165a or salbutamol, i.p., protected against histamine-induced bronchospasm at doses which caused minimal cardiac stimulation, but isoproterenol caused a pronounced tachycardia, even at doses affording weak protection. The bronchoconstrictor effects of pilocarpine in the dog were significantly reduced by i.v. administration of each of the agonists; duration of the effect of Th1165a or salbutamol was longer than that of isoproterenol. Th1165a and salbutamol produced long-lasting bronchodilatation with less cardiac stimulation than did the shorter acting agonist isoproterenol.

Beta adrenoceptors have been divided into subclasses based on the relative activities of various sympathomimetic amines on different target tissues (Lands and Brown, 1964; Lands *et al.,* 1967a,b). *Beta*-1 receptors are found in the heart and *beta*-2 receptors are found in bronchiolar and other smooth muscle. The potent *beta* adrenoceptor stimulant isoproterenol, a common agent for treatment of asthma, is nonselective and causes undesirable cardiac stimulation. Drugs are now available which are said to stimulate the *beta*-2 receptor subclass selectively, thus reducing undesirable cardiac side effects. Salbutamol (Cullum *et al.,* 1969) and MJ 1992 (Dungan *et al.,* 1968), both structural analogs of isoproterenol and trimetoquinol (Iwasawa and Kiyomoto, 1967), a tetrahydroisoquinoline derivative, are reported to be selective *beta*-2 adrenoceptor

Received for publication January 8, 1973.

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agonists. O'Donnell (1970), using isolated guinea-pig atria and trachea to evaluate relative *beta* agonist activities, reported that Th1165a, an analog of orciprenaline, was a selective *beta*-2 stimulant. However, assessment of *beta* agonist selectivity *in vitro* in one species cannot be considered conclusive. For example, trimetoquinol, which had less than $\frac{1}{10,000}$ the activity of isoproterenol on guinea-pig atria (Brittain, 1971), was one-fifth as potent in producing tachycardia in the anesthetized dog (Sato *et al.*, 1967) and caused an immediate and prolonged tachycardia in the anesthetized cat (Fogelman and Grundy, 1970). Further *in vivo* comparison of Th1165a with isoproterenol and salbutamol is required to define the degree of selectivity for *beta*-1 and *beta*-2 responses. In the present study, pulmonary resistance and dynamic compliance measurements in dogs and prevention of histamine-induced collapse in guinea pigs were used to evaluate *beta*-2 bronchodilator responses while *beta*-1 effects were based upon heart rate responses. Studies were also done *in vitro*, using guinea-pig trachea and atria. Structures of the *beta* agonists evaluated in the present study are shown in figure 1.

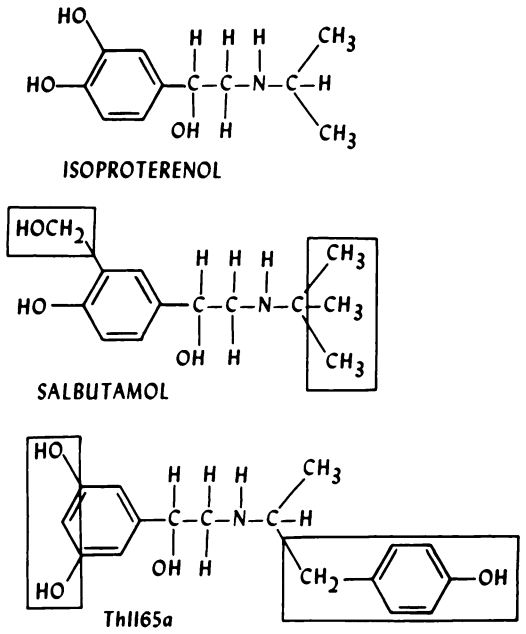

FIG. 1. Structures of *beta* adrenoceptor agonists selected for comparison.

Methods

Isolated trachea. Male albino guinea pigs (250–300 g) were sacrificed by a blow on the head and the trachea was removed. Spiral strips of the tracheas, cleaned of connective tissue by careful dissection, were suspended in Krebs-Henseleit solution (37°C; 95% O_2–5% CO_2). The strip was attached to a Statham G10B transducer and contractile activity was recorded on a Grass polygraph. The strips were placed under 1.5 g of tension and allowed to equilibrate for two hours before drugs were used. One agonist was evaluated on each strip. Cumulative concentration effect curves were obtained by consecutive additions of a drug without change of the bath medium. Drugs were allowed to reach equilibrium at each concentration and responses were expressed as percentage of maximum relaxation of the tissue.

Guinea-pig atria. Hearts of male albino guinea pigs (400–500 g) were removed. The atria were suspended in Krebs-Henseleit solution (37°C; 95% O_2–5% CO_2). Spontaneous contractions were recorded with a Statham G10B transducer on a Beckman Dynograph. Atria were placed under a tension of 500 mg and allowed to equilibrate for one hour before drugs were added. Cumulative concentration effect curves were determined. Each dose was allowed to produce its equilibrium effect, and responses were expressed as percentage of maximum tachycardia.

Treatment of *in vitro* data. Regression analysis of percentage of maximum response *vs.* the log of drug concentration provided a slope and EC50 value (the molar concentration producing 50% of the maximum relaxation of trachea or rate increase of atria) for each tracheal strip and atrial preparation. The maximum efficacy was assessed by comparing the maximum relaxation of trachea (in milligrams of tension) and the maximum tachycardia of atria (in beats per minute) after administration of each of the *beta* agonists. Mean values for isoproterenol were arbitrarily assigned the value of 1; all individual values were then equated to this arbitrary value.

Conscious guinea pigs. Male albino guinea pigs (250–300 g) were continuously exposed to a histamine aerosol (0.1%) for 10 minutes; delivery was by means of two De Vilbiss no. 40 nebulizers positioned at the back of a closed, six-unit Plexiglas chamber (19 × 12½ × 9 inches). The time from onset of aerosol to collapse of each animal was recorded. Guinea pigs that did not collapse during the 10-minute period were removed from the chamber and a maximum score of 10 was recorded. Preliminary experiments, in which guinea pigs were pretreated with aerosolized saline or isoproterenol (1 mg/ml) and treated with histamine aerosol 15 minutes later, indicated that time to

collapse did not differ among the six compartments (10 guinea pigs per treatment were tested in each compartment). The compartments used for guinea pigs on a specific dosage of a drug were assigned in a consecutive numerical sequence to ensure that each position was employed with similar frequency; *i.e.*, no compartment was used $1 + x$ times until all compartments were used x times. In any single histamine treatment, two or more drugs or saline were evaluated simultaneously. Heart rates were determined from electrocardiograph tracings, obtained from two pin electrocardiograph leads affixed to the sides of the guinea pigs; printout was on a physiograph recorder. The average of two heart rate measurements obtained 5 and 10 minutes before administration of the *beta* agonists served as control. *Beta* agonists were administered i.p., p.o. or by aerosol and heart rates were monitored at 5-minute intervals until the time of histamine treatment. Various time intervals were used between administration of the *beta* agonist and histamine. Aerosol administration of the *beta* agonists was by means of two DeVilbiss no. 40 nebulizers driven by an air pressure of 10 pound/in^2; guinea pigs were exposed to the aerosolized *beta* stimulant for one minute. In certain experiments the *beta* adrenoceptor antagonist bunolol, 2 mg/kg i.p., was administered 15 minutes before the *beta* agonists.

Anesthetized Dogs

Anesthesia. Mongrel dogs of either sex (9–13 kg) were given morphine sulfate, 2 mg/kg s.c., and were anesthetized 30 minutes later with pentobarbital sodium, 30 mg/kg i.v. They were allowed to breathe spontaneously.

Physiologic considerations. Pulmonary resistance is due primarily to frictional resistance caused by the flow of gas molecules through the airways and also to frictional resistance in the pulmonary and thoracic tissues. It is measured under dynamic conditions (airflow). Compliance represents the distensibility of the lungs and thorax and is ideally measured under static conditions (no airflow) (Comroe *et al.*, 1962). However, since static measurements are not possible in a spontaneously breathing animal, lung distensibility must be measured during the respiratory cycle at specific zero flow points. This measurement, dynamic compliance, reflects both static recoil tendency and resistive properties of small airways and may be lower than static compliance. In the present study, resistance and dynamic compliance were evaluated by relating tidal volume, flow rate and transpulmonary pressure at specified points in the respiratory cycle. During a respiratory cycle, transpulmonary pressure overcomes both frictional resistance and elastic recoil tendency. These elastic and flow-resistive components can be separately evaluated by proper sampling procedures. Specifically, compliance is measured by relating the volume change (tidal volume) to the transpulmonary pressure change at zero flow points (beginning and end of inspiration). At these zero points, the flow-resistive component of pressure may be neglected leaving only the elastic component. At isovolumetric points on the inspiratory and expiratory portion of a tidal volume curve, elastic forces should be equal and changes in pressure should reflect the flow-resistive component. Resistance is thus measured by comparing the pressure changes to the flow change at isovolumetric points of the tidal volume curve (Amdur and Mead, 1958).

Measurement of pulmonary resistance and dynamic compliance. Pulmonary resistance and compliance were estimated by a modification of the method of Amdur and Mead (1958). Values of transpulmonary pressure, flow and tidal volume were necessary for the calculation. Transpulmonary pressure was determined by monitoring the difference between pressure in the external end of a tracheal cannula and pressure in the pleural cavity by means of a Statham differential transducer (PM 5TC ± 0.3–350). Intrapleural pressure measurements were performed with a small spear-like cannula (containing two large holes) introduced through the 7th intercostal space into the pleural cavity and held by a tight suture. A Fleisch pneumotachograph (7319 No. 1) was used to monitor respiratory flow rate. Tidal volumes were obtained by electrical integration of the flow signal.

Flow, pressure and volume signals were fed into an on-line analog computer (Giles *et al.*, 1971) which performed the necessary calculations after each breath. The computer output of resistance and compliance values (as well as flow, volume and pressure) was recorded on a Beckman Dynograph. Previous manual calculations had provided a calibration table so that output signals on the Beckman Dynograph could be converted to numerical resistance and compliance values.

Blood pressure was routinely monitored from

the femoral artery, using a Statham pressure transducer (P23Db).

Experimental protocol. Bronchoconstriction was induced by histamine or pilocarpine. The *beta* agonists were administered i.p. in experiments using histamine and were administered i.v. in experiments using pilocarpine.

Histamine was delivered to coincide with inspiration by means of a metered nebulizer which fitted into the tracheal cannula and delivered 150 μg of histamine base per actuation; eight actuations were used to produce bronchoconstriction 30 minutes before and 15 and 60 minutes after the i.p. administration of the *beta* agonists. Protection *vs.* histamine-induced bronchoconstriction was calculated by the formula $[(a-b)/a] \times 100$, where a equals percent change in resistance or compliance due to histamine before administration of the *beta* agonists and b equals percent change in resistance or compliance due to histamine after administration of the *beta* agonists. Heart rates were monitored by a cardiotrachometer triggered by the R wave of the lead II electrocardiogram and displayed on the recorder. The maximum change in heart rate that occurred between the administration of the *beta* agonist and the administration of histamine (15-minute period) was noted.

Beta receptor agonists were injected into the femoral vein 15 minutes after the administration of pilocarpine (0.3 mg/kg i.p.) and percent changes in resistance and compliance were measured. Maximum change in heart rate was recorded.

Drugs. Doses and concentrations of all drugs used represent the amount as base. Drugs were *dl*-isoproterenol hydrochloride (Winthrop Laboratories, Inc., New York, N.Y.), *dl*-Th1165a hydrobromide (supplied by Ciba-Geigy Corporation, Summit, N.J.), *dl*-salbutamol (Schering Corporation, Bloomfield, N.J.), histamine diphosphate (Nutritional Biochemicals Corporation, Cleveland, Ohio), *dl*-propranolol (Ayerst Laboratories, New York, N.Y.), *dl*-bunolol (Warner-Lambert Products Division, Morris Plains, N.J.) and pilocarpine nitrate (Nutritional Biochemicals). Drugs were dissolved in saline immediately prior to the experiment. Volumes for injection were kept constant at 1 ml for dogs and ½ ml for guinea pigs. Volumes added to tissue bath fluid never exceeded 3% of total volume.

The molecular weights (base) of the *beta* agonists used in this study are: isoproterenol, 211.2; salbutamol, 239.3; Th1165a, 303.4.

Statistics. Mean values were compared using Student's t test. Potency ratios were determined only when the dose-response lines did not differ from parallelism. The lines were adjusted to a common slope and the ratio of activity, based on an arbitrary response, was calculated; confidence limits were determined as described by Finney (1952).

Results

Guinea-pig trachea. The effects of the *beta* agonists on tracheal spirals are shown in table 1. Each agonist produced a dose-related relaxation of spontaneous tone; mean values for maximal relaxation, EC50 concentrations and slope of the log concentration *vs.* percentage of maximum response were similar for each drug. Time to reach maximum response usually took 1 to 1½ minutes for isoproterenol, 5 to 6 minutes for salbutamol and 3 to 4 minutes for Th1165a.

Guinea-pig atria. Table 1 shows that isoproterenol was more potent than Th1165a ($P < .05$) which in turn was more potent than salbutamol, ($P < .05$). The maximum tachycardia produced by salbutamol was also considerably less than that of either isoproterenol or Th1165a. The slope of the log concentration *vs.* percentage of maximum response curve for isoproterenol was much steeper than those of either of the other two agents.

i.p. administration to conscious guinea pigs. The effects of the *beta* agonists on heart rate and histamine-induced bronchospasm are shown in figure 2. Fifteen minutes after administration both salbutamol and Th1165a protected against histamine. The time to collapse was increased about 2½-fold at doses which did not increase heart rate. However, doses of salbutamol or Th1165a required to prolong collapse time further caused a tachycardia. Isoproterenol also protected against histamine, but produced a significant tachycardia at all such doses. Maximum heart rate response usually occurred 10 to 15 minutes after drug administration. Both actions of the *beta* agonists were blocked by prior treatment with the *beta* adrenoceptor antagonist, bunolol (2 mg/kg i.p.). Slopes of the log dose-effect lines (for both heart rate or antagonism of histamine-induced collapse) for the three *beta* agonists were not significantly different and

TABLE 1

Mean EC50 and relative maximum efficacy for isoproterenol, salbutamol or Th1165a on guinea-pig atria and trachea

Drug	Mean EC50 ± S.E.[a]		Relative Maximum Efficacy[b]	
	Trachea	Atria	Trachea	Atria
Isoproterenol	$6.14 \pm 2.11 \times 10^{-9}$[c]	$9.10 \pm 0.74 \times 10^{-9}$	1.00 ± 0.15	1.00 ± 0.06
Salbutamol	$8.70 \pm 1.92 \times 10^{-9}$	$1.35 \pm 0.22 \times 10^{-6}$[d]	0.85 ± 0.11	0.48 ± 0.04[d]
Th1165a	$4.53 \pm 1.42 \times 10^{-9}$	$1.41 \pm 0.31 \times 10^{-7}$[d,e]	1.07 ± 0.19	0.89 ± 0.05[e]

[a] Molar concentration producing 50% of the maximum response.

[b] Mean maximum relaxation of trachea and mean maximum tachycardia of atria were evaluated for each of the *beta* agonists; isoproterenol was arbitrarily assigned the value of 1. Standard errors are indicated.

[c] Values represent 7 to 10 experiments.

[d] Significantly different from isoproterenol, $P < .05$.

[e] Significantly different from salbutamol, $P < .05$.

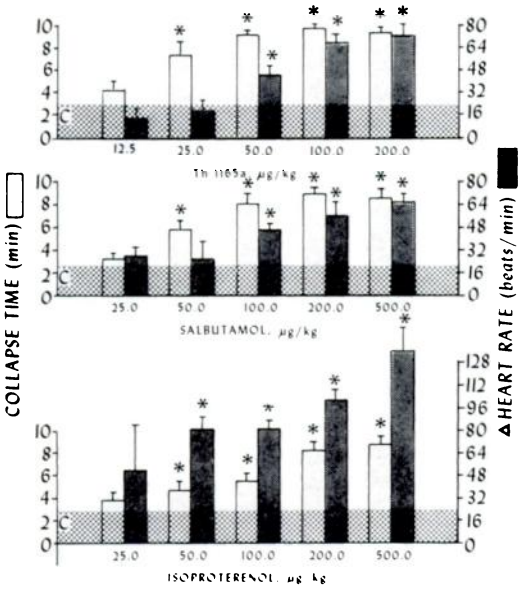

FIG. 2. Comparison of the effects of the *beta* adrenoceptor agonists and saline administered i.p. on histamine-induced collapse and on heart rate in guinea pigs. *Beta* adrenoceptor agonists were administered 15 minutes before histamine. Each set of bars represents the mean value for 9 to 15 animals. The top of the hatched area represents the mean values for both collapse time and heart rate for saline-treated animals. *Significantly different from saline, $P < .05$.

in vivo potency ratios (table 2) indicated both salbutamol and Th1165a to be relatively more selective for bronchiolar (*beta*-2) receptors than for cardiac (*beta*-1) receptors.

Two hours after administration of 200 μg/kg of each of the *beta* agonists, times (minutes, mean ± S.E., $N = 6$) from onset of histamine aerosol to collapse for the various treatment groups were: Th1165a, 6.3 ± 1.0; salbutamol, 3.2 ± 0.6; isoproterenol, 3.1 ± 0.7; and saline, 2.2 ± 0.6.

Aerosol administration to conscious guinea pigs. The effects of the *beta* agonists on heart rate and histamine-induced collapse are shown in figure 3. Each *beta* agonist, at the lower aerosol concentrations, delayed collapse time significantly but produced only minor changes in heart rate. At higher concentrations, isoproterenol produced a greater ($P < .05$) tachycardia than either of the other two agonists. The solution containing 6 mg/ml of salbutamol formed a white precipitate in the nebulizer during use so that the amount delivered may have been less than represented.

The duration of bronchodilator and cardiac stimulant effects was compared with aerosols of

TABLE 2

Relative activities of the beta agonists administered i.p. on heart rate and histamine-induced collapse in guinea pigs

Drug	Antagonism of Histamine Collapse (Potency)	Heart Rate Increase (Potency)	Selectivity[a] Ratio
Isoproterenol	1.00	1.00	1.00
Salbutamol	2.06[b] (1.11–4.47)[c]	0.10[b] (0.04–0.18)	20.6
Th1165a	7.29[b] (4.20–16.89)	0.25[b] (0.12–0.44)	29.2

[a] Ratio of potency in antagonizing histamine/potency in increasing heart rate. Isoproterenol arbitrarily assigned value of 1.00.

[b] $P < .05$ *vs.* corresponding potency of isoproterenol.

[c] 95% CL.

solutions each containing 3 mg/ml of one of the compounds. Duration of protective activity is shown in figure 4, and the tachycardia in figure 5. Both Th1165a and salbutamol produced a longer bronchodilator response and a shorter, less severe tachycardia than isoproterenol.

p.o. administration to conscious guinea pigs. The effects of the *beta* agonists on histamine-induced collapse are shown in table 3. Salbutamol was more potent than the other agonists. The tachycardia produced by Th1165a or salbutamol was of short duration, while that produced by isoproterenol persisted over the 90-minute period (not shown).

Antagonism of histamine bronchospasm in the anesthetized dog. Resting control resistance values did not vary significantly among the various groups. Control resistance and compliance values for all dogs fell within the range of 0.72 to 2.28 cm H_2O/liters per second and 34 to 79 ml/cm H_2O, respectively. The mean increase in resistance and mean decrease in compliance due to histamine for all control responses were 158.6 ± 8.7% and 38.2 ± 2.0%, respectively, (mean ± S.E., $N = 55$). No tolerance to histamine was observed in this experiment (histamine was given 30 minutes before and 15 and 60 minutes after the *beta* agonists). The response to histamine did not appear to be affected by the weight of the animal. The results in table 4 show that the *beta* agonists exerted protective effects against changes in both pulmonary resistance and dynamic compliance produced by histamine aerosol, but isoproterenol was less potent than either of the other two agonists. As to duration of the protection, Th1165a exerted a similar protective effect when histamine was administered 15 or 60 minutes after the *beta* agonists; in contrast, the bronchodilator activity of isoproterenol or salbutamol was reduced at the final administration of histamine (*i.e.*, 60 minutes after administration of the *beta* agonist). Protection against increases in pulmonary resistance was greater than protection against decreases in dynamic compliance.

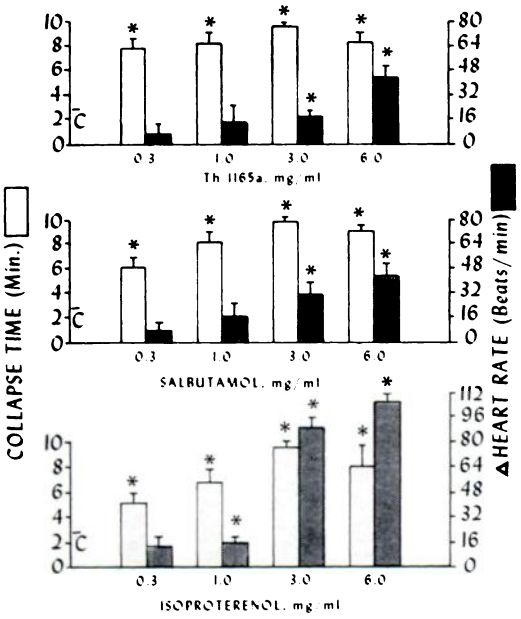

FIG. 3. A comparison of the effects of the *beta* adrenoceptor agonists and saline administered by aerosol on histamine-induced collapse and on heart rate in guinea pigs. *Beta* adrenoceptor agonists were administered 15 minutes before histamine. Each set of bars represents the mean value for 9 to 15 animals with standard errors. Animals treated with aerosolized saline had a heart rate change of −5 ± 5.5 (mean ± S.E. for 15 animals). Collapse time of animals that received saline is indicated by C. *Significantly different from saline, $P < .05$.

Each of the *beta* agonists increased heart rate and decreased mean and diastolic blood pressure. Maximum changes usually occurred 10 to 15 minutes after drug administration. Heart rate changes for isoproterenol were not dose related and a pronounced tachycardia was seen at dose levels which produced only a weak protection against histamine bronchospasm.

Antagonism of pilocarpine-induced bronchoconstriction in the anesthetized dog. Pilocarpine elevated resistance 188.7 ± 16.2% and decreased compliance 32.8 ± 2.7% (mean ± S.E., $N = 16$ dogs). The peak effect occurred 15 to 25 minutes after i.p. administration. The effects of each of the *beta* agonists, (1.56 μg/kg i.v.) are shown in figures 6 and 7. All produced an initial decrease in pulmonary resistance and an increase in dynamic compliance. Isoproterenol-induced bronchodilatation was shorter than that of either of the other two agonists. The effects of the *beta* agonists again appeared to be greater on resistance than on compliance. Each *beta* agonist also reduced resistance and increased compliance in the absence of bronchoconstrictor drugs, but this effect was less pronounced than that in the bronchoconstricted state.

Each of the *beta* agonists (1.56 μg/kg i.v.) increased heart rate. Mean changes in beats/min were 39.5 ± 10.4 for isoproterenol, 16.4 ± 5.6 for

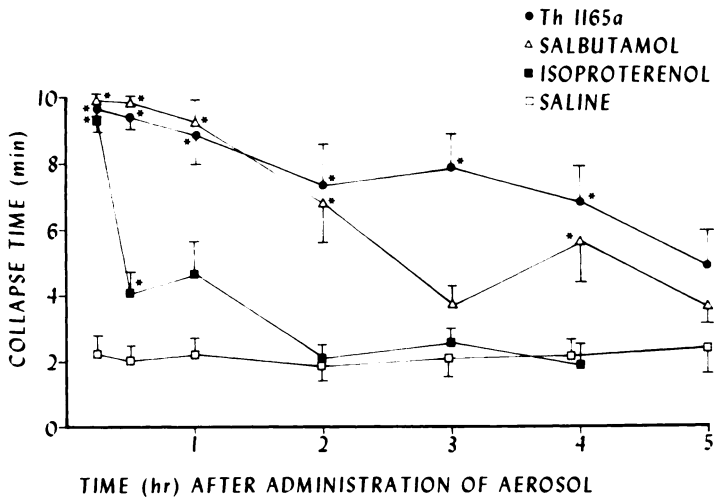

FIG. 4. A comparison of the duration of effects of the *beta* agonists and saline administered by aerosol, on collapse time due to histamine. Each *beta* agonist was used at a concentration of 3 mg/ml. Each point is the mean value for six guinea pigs. * Significantly different from saline, $P < .05$.

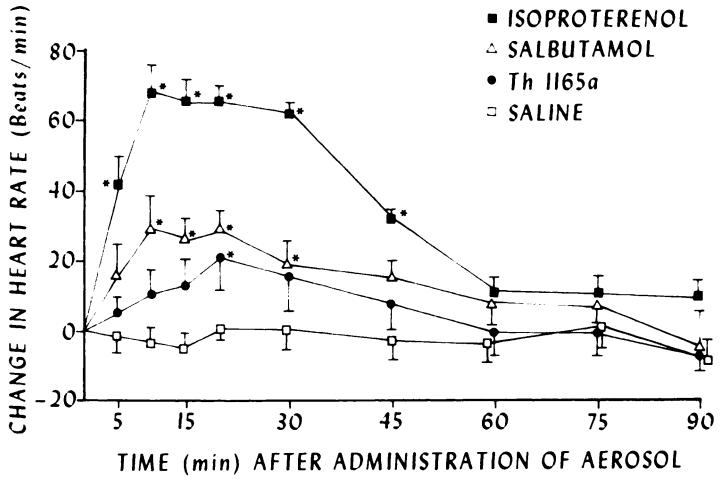

FIG. 5. A comparison of the duration of the effects of the *beta* agonists and saline administered by aerosol on heart rate. Each *beta* adrenoceptor agonist was used at a concentration of 3 mg/ml. Each point is the mean value for six guinea pigs. * Significantly different from saline, $P < .05$.

Th1165a and 9.0 ± 4.58 for salbutamol ($N = 4$). All caused decreases in diastolic blood pressure, but in all dogs receiving isoproterenol and in one dog each receiving Th1165a or salbutamol, increases in systolic pressure were sufficient to cause an increase in mean blood pressure.

Discussion

The *beta* adrenoceptor agonists compared in this study protected guinea pigs from histamine-induced collapse and prevented histamine-or pilocarpine-induced changes in resistance and dynamic compliance in the anesthetized dog. The greater selectivity of Th1165a or salbutamol for the bronchioles as compared to the heart was clearly demonstrated. Although the bronchodilator response to isoproterenol was equal to or less than that of Th1165a or salbutamol, the cardiac response was more pronounced in the guinea pig and dog. Quantitative selectivity ratios for bronchodilatation *vs.* tachycardia were usually difficult to determine because of differences in maximum response, duration of action and slope of dose-effect curves. Nonetheless, the

bronchoselectivity ratios were calculated from i.p. data in guinea pigs (table 2) and indicated that both Th1165a and salbutamol exerted relatively greater effects than isoproterenol on bronchial (*beta*-2) receptors as compared to cardiac (*beta*-1) receptors.

There were differences among the *beta* agonists in bronchodilator potency depending on the route of administration. Salbutamol, given p.o. to guinea pigs, was a more potent bronchodilator

TABLE 3

Duration of the effect of beta agonists administered p.o. on histamine-induced collapse

Treatment	Dose	Collapse Time		
		30[a]	60[a]	90[a]
	mg/kg p.o.	*min*		
Saline		2.3 ± 0.5[b]	2.2 ± 0.6	2.4 ± 0.4
Th1165a	10	10+[c]	10+	7.3 ± 0.9
	5	6.8 ± 1.2	9.5 ± 0.5	3.3 ± 0.4
Salbutamol	5	10+	10+	8.7 ± 0.9
	2.5	9.2 ± 0.8	7.7 ± 1.65	8.5 ± 1.4
Isoproterenol	10	10+	10+	5.7 ± 0.8
	5	5.7 ± 0.6	7.1 ± 1.6	5.5 ± 0.9

[a] Time (minutes) between administration of *beta* agonists or saline and histamine challenge.

[b] Each value is the mean (±S.E. where appropriate) of six guinea pigs.

[c] All guinea pigs in group remained in chamber for full 10-minute period.

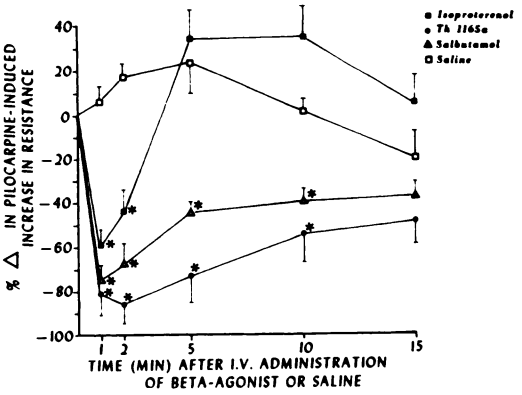

FIG. 6. A comparison of the effects of the *beta* adrenoceptor agonists (1.56 μg/kg i.v.) and saline on pulmonary resistance when administered 15 minutes after pilocarpine, 300 μg/kg i.p. Each *beta* adrenoceptor agonist was administered to four dogs. Each point represents the mean percentage of change (S.E. indicated) in resistance. *Significantly different from saline, $P < .05$.

than the other two agonists. This may be due to differences in biotransformation or absorption. Isoproterenol is inactivated by both sulfate conjugation in the gut (Davies *et al.*, 1969) and by catechol-O-methyltransferase (COMT) in the liver and other organs (Giles and Miller, 1967; Conway *et al.*, 1968), but salbutamol is not a substrate for either enzyme (Martin *et al.*, 1971). The lesser potency of Th1165a may be due

TABLE 4

Effect of beta agonists on histamine-induced changes in resistance and dynamic compliance and on heart rate and mean blood pressure in the anesthetized dog

Treatment	Dose	No. of Dogs	% Protection				Heart Rate[b]	Mean Blood Pressure[b]
			Resistance		Compliance			
			15[a]	60[a]	15[a]	60[a]		
	μg/kg i.p.						*Δbeats/min*	*Δ mm Hg*
Saline		4	8.7 ± 6 2	9.2 ± 7.0	4.3 ± 5.0	−5.0 ± 6.2	−0.2 ± 1.1	0.25 ± 0.8
Th1165a	3.12	5	85.9 ± 4.1[c,d]	83.7 ± 6.6[d]	75.3 ± 16.0[d]	65.5 ± 11.2[d]	45.6 ± 14.2[d]	+1.0 ± 3.9
	1.56	6	76.5 ± 4.9[d]	79.8 ± 5.7[d]	57.0 ± 4.8[d]	60.3 ± 3.2[d]	36.0 ± 14.5[d]	−7.6 ± 2.4[d]
	0.78	7	67.6 ± 10.4[d]	63.9 ± 10.4[d]	47.9 ± 6.6[d]	28.6 ± 9.1[d]	11.5 ± 6.0	−10.0 ± 3.0[d]
	0.39	3	70.2 ± 7.2[d]	53.0 ± 15.2[d]	48.3 ± 18.1[d]	22.0 ± 11.5	7.0 ± 7.0	−1.3 ± 1.1
Salbutamol	6.25	7	82.3 ± 2.8[d]	64.1 ± 6.9[d]	65.2 ± 12.7[d]	50.0 ± 12.9[d]	28.0 ± 6.9[d]	−3.4 ± 2.7
	3.12	6	85.0 ± 3.4[d]	48.1 ± 10.0[d]	60.6 ± 8.6[d]	28.5 ± 8.8[d]	17.4 ± 4.6[d]	−8.0 ± 3.4
	1.56	3	74.0 ± 3.3[d]	32.0 ± 1.1[d]	41.5 ± 11.9[d]	14.5 ± 5.1	6.0 ± 6.0	−3.5 ± 3.7
	0.39	3	33.0 ± 16.6	7.7 ± 7.7	26.0 ± 7.0[d]	9.0 ± 4.1	1.0 ± 1.0	−1.7 ± 1.3
Isoproterenol	12.5	4	82.0 ± 6.9[d]	29.5 ± 5.5	54.2 ± 15.2[d]	18.3 ± 9.0	43.1 ± 11.1[d]	−6.2 ± 2.8[d]
	6.25	4	71.8 ± 3.2[d]	41.0 ± 15.0	49.0 ± 3.0[d]	16.7 ± 6.9	46.5 ± 7.3[d]	−8.8 ± 2.8[d]
	3.12	3	38.3 ± 7.6[d]	7.7 ± 7.7	20.6 ± 10.0	−3 ± 4.5	41.4 ± 11.3[d]	−9.2 ± 2.1[d]

[a] Time (minutes) between administration of *beta* agonist and histamine.

[b] Maximum change recorded within 15 minutes after administration of *beta* agonists or saline.

[c] Mean ± S.E.

[d] Significantly different from saline treated group, $P < .05$.

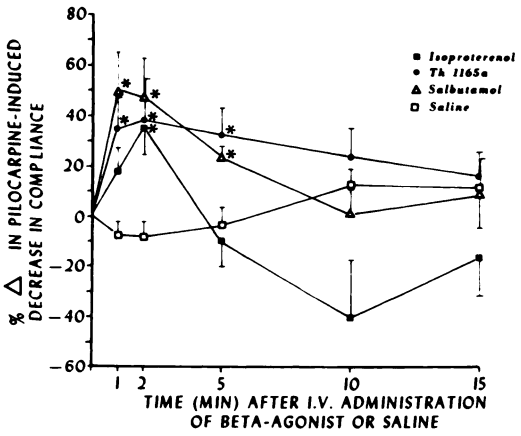

FIG. 7. A comparison of the effects of the *beta* adrenoceptor agonists (1.56 μg/kg i.v.) and saline on dynamic compliance when administered 15 minutes after pilocarpine, 300 μg/kg i.p. Each *beta* adrenoceptor agonist was administered to four dogs. Each point represents the mean percentage of change (S.E. indicated) in compliance. *Significantly different from saline, $P < .05$.

to differences in absorption or metabolism in the gut, since metabolism by COMT or by MAO is unlikely. COMT methylates compounds with hydroxyl groups on the 3,4 position of benzene ring (Axelrod and Tomchick, 1958; Axelrod, 1966) but terbutaline, an adrenergic agonist having hydroxyl groups on the 3,5 positions of the benzene ring, as does Th1165a, was reported not to be metabolized by COMT (Persson and Olsson, 1970). Metabolism by MAO is not likely to occur to any degree since a large substituent on the nitrogen makes the molecule resistant to MAO (Blaschko, 1952). Also, Th1165a was more potent than salbutamol after i.p. administration.

Aerosol administration of each of the *beta* agonists to guinea pigs protected against histamine-induced bronchospasm at doses which produced little cardiac stimulation. Localization of the drug in the lungs probably minimizes the cardiac response. The ability of aerosolized isoproterenol to produce a bronchodilatation without cardiac stimulation has been observed in humans (Minette, 1970). The longer duration of the bronchodilator response to aerosol administration of Th1165a or salbutamol, as compared with that to isoproterenol, could again be explained by the resistance of the former drugs to COMT. Such differences, however, could also be attributed to a slow absorption of either Th1165a or salbutamol from lung sites. Salbutamol has been reported to be slowly absorbed from the lungs of both humans and dogs (Martin *et al.*, 1971).

The bronchoconstrictor properties of histamine have been well documented (Dale and Laidlaw, 1910; Sollman and von Oettinger, 1929; Yonkman *et al.*, 1947; Douglas *et al.*, 1972). We have interpreted the protection against collapse produced by the *beta* adrenoceptor stimulants as a physiological antagonism (bronchodilatation) mediated through *beta* adrenoceptors. Drugs having an antihistaminic action could protect against histamine-induced bronchospasm (Yonkman *et al.*, 1947). However, since the specific *beta* adrenoceptor antagonist, bunolol (Robson and Kaplan, 1970) blocked the protective actions of these three bronchodilator agents, the response is most likely mediated through *beta* adrenoceptors.

Each of the *beta* agonists prevented or reduced histamine- or pilocarpine-induced increases in pulmonary resistance or decreases in dynamic compliance in the anesthetized dog. The greater effect on resistance, as compared to dynamic compliance, suggests that the major site of muscle relaxation was the relatively larger airways, since it has been reported that the larger airways are mainly responsible for airway resistance (Nadel *et al.*, 1964; Colebatch *et al.*, 1966; Macklem and Mead, 1967; Hogg *et al.*, 1968). Such a finding is in agreement with the work of Douglas *et al.* (1972) who suggested that the major bronchodilator effect of isoproterenol was on the relatively larger airways in guinea pigs. The greater effect of the *beta* agonists on resistance observed in the present study could also be due to a preponderant action of the bronchoconstricting drugs on peripheral airways, since the latter primarily affects compliance. However, since a more pronounced effect of the bronchoconstrictors on compliance was not observed in the absence of the *beta* agonists, this latter explanation seems unlikely.

While a reflex component due to a fall in blood pressure could be present in the tachycardia response to the *beta* agonists, there was no evidence to suggest that differences in blood pressure response could account for the difference in heart rate response. For example, isoproterenol increased mean blood pressure in pilocarpine-treated dogs and still produced a significantly

greater increase in heart rate than did Th1165a or salbutamol.

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