Br. J. Pharmac. (1984), **83**, 607–614

Modification by betamethasone of the effects of bronchodilator drugs on cholinergic bronchoconstriction in rats

M.J. Mattila & R.O. Salonen

Department of Pharmacology, University of Helsinki, Siltavuorenpenger 10, SF-00170 Helsinki 17, Finland

1 Effects of betamethasone (BM), a long-acting glucocorticoid, given alone and in combination with bronchodilator drugs, terbutaline (Ter), theophylline (Theo), or ipratropium bromide (Ipra), were studied on dose-related methacholine (MeCh 2, 3, and 4.5 μg i.v.)-induced bronchoconstriction in anaesthetized rats. BM (0.4 or 2 mg kg^{-1}) was given intraperitoneally 24 h before the experiment followed by a similar dose intravenously, 12 min before MeCh challenge. The bronchodilator drugs were given i.v. as acute single doses.

2 BM 0.4 mg kg^{-1} counteracted significantly MeCh-induced bronchoconstriction without modifying MeCh-induced transient bradycardia and hypotension. BM 2 mg kg^{-1} failed to improve that response. A time interval of 24 h after pretreatment proved mandatory to produce these effects.

3 Combined treatment with BM 0.4 mg kg^{-1} + Ter 20 μg kg^{-1} antagonized the MeCh-induced bronchoconstriction in an additive manner at 2 and 3 μg of MeCh, but a synergistic interaction was found at the largest MeCh dose. The effects of the other combinations (BM 0.4 mg kg^{-1} + Theo 20 mg kg^{-1} and BM 0.4 mg kg^{-1} + Ipra 0.5 μg kg^{-1}) on airways failed to exceed the expected sum of the individual drugs.

4 The combination of BM + Ter was selective to the airways only, whereas BM + Theo also counteracted MeCh-induced bradycardia and BM + Ipra counteracted both hypotension and bradycardia.

5 It is concluded that combined treatment with glucocorticoid and β_2-adrenoceptor agonist may result in a synergistic interaction on severe airway obstruction without significant influence on cardiovascular system.

Introduction

Glucocorticoids are important drugs in the treatment of bronchial asthma, being life-saving in severe acute attacks. The anti-inflammatory action of glucocorticoids is probably their major therapeutic mechanism in asthma (Melby, 1977; Stevenson, 1977), but they also have a permissive effect on β-adrenoceptor stimulation in the airways (Ellul-Micallef & Fenech, 1975; Middleton, 1975). Corticosteroids are known to potentiate the relaxant action of catecholamines on animal and human isolated airway smooth muscles (Lefcoe *et al.*, 1975; Rinard, 1978). A synergistic effect between corticosteroids and isoprenaline has also been seen in asthmatic patients (Franklin *et al.*, 1958; Hume & Jones, 1960; Shenfield *et al.*, 1975). Holgate *et al.* (1977) showed that intravenous hydrocortisone could restore the bronchial responsiveness to salbutamol, which had been lost after regular high-dose inhalations in healthy volunteers.

The present study was conducted in order to investigate the actions of a glucocorticoid in combination with bronchodilator drugs with different mechanisms of action. The bronchodilators used were a β_2-selective agonist terbutaline, theophylline, and a quaternary antimuscarinic agent, ipratropium bromide (Bauer *et al.*, 1976). Betamethasone, a long-acting glucocorticoid without significant mineralocorticoid activity (Melby, 1977), was chosen to represent modern glucocorticoids. Single and combined bronchodilator actions of these drugs were studied on dose-related bronchoconstriction with methacholine (MeCh) in anaesthetized rats as previously reported (Salonen *et al.*, 1982). The use of MeCh seems appropriate since cholinergic mechanisms play an important role in the regulation of bronchomotor tone in normal and asthmatic states (Reed, 1974; Widdicombe, 1979; Boushey *et al.*, 1980).

Methods

Anaesthetized rat preparation

Female Sprague-Dawley rats weighing 200–250 g (Tuohilampi farm, Finland) were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.) and relaxed with pancuronium bromide (2 mg kg^{-1} i.p.) to prevent spontaneous respiration. During the experiment no extra doses of anaesthetic or relaxant were given. The animals, which occasionally failed to show full anaesthesia with complete muscle relaxation after this medication, were discarded.

Polyethylene catheters were inserted in the right jugular vein and the left carotid artery, and a metal cannula through the tracheostomy. The animals were artificially ventilated with a Palmer constant volume respiration pump and the intratracheal pressure was measured as previously described (McCulloch *et al.*, 1967; Salonen & Mattila, 1981). The outputs of a tracheal pressure transducer (Statham P 23 BC) and a blood pressure transducer (Statham P 23 Db) were fed to preamplifiers of a Grass Model 7 D Polygraph. The animals were ventilated (stroke volume 1.4–2.2 ml; 72 strokes min^{-1}) with a maximal intratracheal pressure of 5 mmHg at rest. The heart rate was recorded with a Nihon Kohden one-channel electrocardiograph using a limb connection.

At the end of each experiment the lungs were dissected free, and major bronchi and blood vessels were cut off. The lungs were weighed (wet weight), dried for 5 s under firm finger pressure with filter paper, and reweighed (dry weight), in order to estimate leakage of fluid from blood vessels and possible changes in the pulmonary vascular bed.

Measurement of bronchoconstriction

About 10 min after the administration of pancuronium, the test animals usually showed no spontaneous breathing. Bronchoconstriction was induced with three consecutive doses (2, 3, and 4.5 μg) of methacholine (MeCh) as hydrochloride injected at 2 min intervals into the right jugular vein. The ECG was recorded at the time of peak intratracheal pressure response (PIPR) and the heart rate was calculated.

In order to quantitate bronchoconstriction in response to MeCh, the difference in the penwriter amplitude between the peak response and the basal resting values was measured in mm and determined as PIPR for every MeCh dose. An increase in the response area (RA) was also measured for the 2 min period after each MeCh dose, using transparent millimeter paper and expressing the values in mm^2 (Salonen & Mattila, 1981).

Bronchodilator treatment

Two groups of six rats each received 0.4 or 2 mg kg^{-1} of betamethasone (BM) infused i.v. 12 min before the MeCh challenge. Twelve rats out of 60 were injected with 0.2 ml of saline i.v. and served as controls. The other groups each consisting of six rats received terbutaline (Ter) 20 μg kg^{-1}, theophylline (Theo) 20 mg kg^{-1}, or ipratropium bromide (Ipra) 0.5 μg kg^{-1} i.v. 5 min before the MeCh challenge in combination with either saline or 0.4 mg kg^{-1} of BM given in similar manner as above.

Glucocorticoid pretreatment

In addition to BM (0.4 or 2 mg kg^{-1}) given i.v. during the experiment, the rats were also pretreated by injecting a similar dose of BM i.p. 24 h before the experiment. Other rats not receiving BM were given 0.5 ml of saline i.p., respectively. The order of rats allocated to different treatment groups was balanced.

Drugs

The following drugs were used: betamethasone disodiumphosphate (Betnesol, Glaxo, United Kingdom), ipratropium bromide (Boehringer Sohn, Germany), methacholine hydrochloride (Sigma, U.S.A.), pancuronium bromide (Pavulon, Organon, Holland), pentobarbitone sodium (Apodan, Denmark), terbutaline sulphate (Draco, Sweden), theophylline ethylenediamine (Theophyllaminum, Medica, Finland).

Statistics

The results of pulmonary and cardiovascular measurements were tested with one-way analysis of variance (ANOVA) at every MeCh dose. The final comparisons between treatment groups were done with Duncan's new multiple-range test (Duncan, 1955).

Results

Effects of betamethasone

Infusion of BM 0.4 or 2 mg kg^{-1}, did not have any immediate effects on pulmonary or cardiovascular parameters. BM 2 mg kg^{-1} failed to counteract MeCh-induced bronchoconstriction, bradycardia, or hypotension, when given as an acute single dose or after a 12 h pretreatment with a similar dose. When BM was administered 24 h before the experiment and a similar acute dose was given 12 min prior to the MeCh challenge, both 0.4 and 2 mg kg^{-1} of BM

counteracted significantly (Duncan's test; $P<0.05$) MeCh-induced bronchoconstriction at the two lowest MeCh doses (Figure 1). BM 0.4 mg kg^{-1} seemed to give a maximal response, because no further bronchodilatation was seen after BM 2 mg kg^{-1}. The PIPR values at 2, 3, and 4.5 μg of MeCh were 1.3, 3.6, and 7.8 mmHg for BM 0.4 mg kg^{-1} as well as 1.7, 3.7, and 8.8 mmHg for BM 2 mg kg^{-1}, respectively. Neither dose of BM counteracted significantly MeCh-induced transient bradycardia or hypotension.

Additional experiments ($n=3$ in each group) were performed in order to check the significance of the acute administration to the effects of BM. The antagonism of the MeCh-induced bronchoconstriction by BM 2 mg kg^{-1} was similar, irrespective of whether it was given only once 24 h before the experiment or in combination with a similar acute dose 12 min prior to the MeCh challenge. The PIPR values at 2, 3, and 4.5 μg of MeCh were 1.1, 2.4, and 5.6 mmHg for subacute BM treatment, and 1.0, 2.3, and 5.1 mmHg for subacute + acute BM treatment. The PIPR values of the respective saline controls were 1.8, 4.2, and 8.3 mmHg.

Effects of terbutaline, theophylline and ipratropium bromide

Infusion of Ter 20 μg kg^{-1} within 2 min decreased blood pressure and increased heart rate transiently. It also counteracted significantly ($P<0.05$ to 0.01) MeCh-induced bronchoconstriction assessed in terms of PIPR and RA (Figure 1). Infusion of Theo 20 mg kg^{-1} caused a transient decrease in blood pressure but a sustained increase in heart rate. Theo counteracted significantly ($P<0.05$ to 0.01) MeCh-induced changes in PIPR and RA values (Figure 2).

Ipra 0.5 μg kg^{-1} infused in a similar manner to Ter and Theo had no effect on blood pressure or heart rate. It antagonized significantly ($P<0.05$ to 0.01) the bronchoconstrictor effects of MeCh at every MeCh dose (Figure 3). Infusion of Ter, Theo, or Ipra did not change basal bronchial tone. None of these drugs counteracted significantly MeCh-induced dose-dependent hypotension or bradycardia (Table 1). Nor did they modify the lung weight or leakage of fluid from blood vessels.

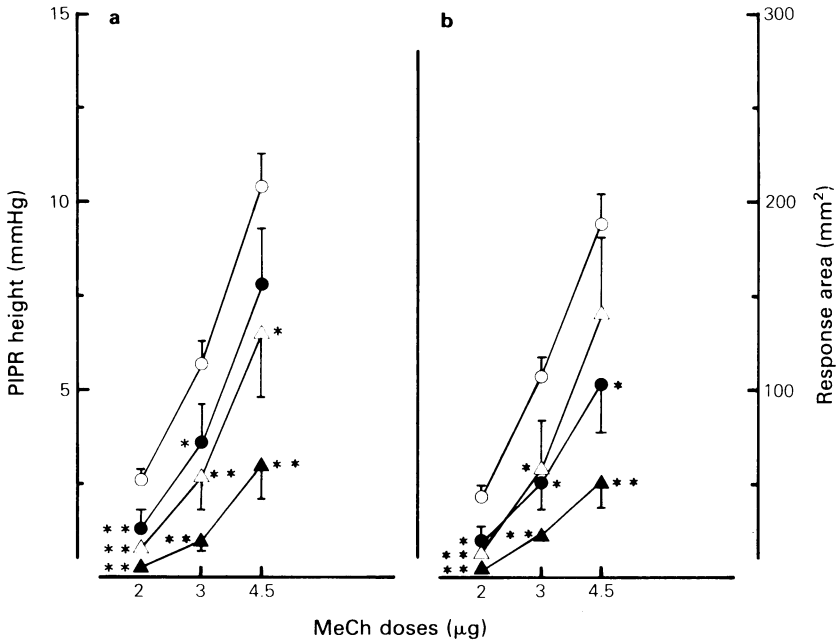

Figure 1 Effects of betamethasone (BM) and terbutaline (Ter), alone and in combination, on methacholine (MeCh)-induced bronchoconstriction in anaesthetized and relaxed rats assessed in terms of peak intratracheal pressure responses (PIPR) and response areas (RA) for 2, 3, and 4.5 μg of MeCh given i.v. All animals receiving BM i.v. were also pretreated with a similar dose of BM i.p. 24 h before the experiment. Other rats were each given 0.5 ml saline, i.p. Symbols: (○) controls given 0.2 ml of saline i.v. ($n=12$); (●) BM 0.4 mg kg^{-1} i.v. ($n=6$); (△) Ter 20 μg kg^{-1} i.v. ($n=6$); (▲) BM 0.4 mg kg^{-1} + Ter 20 μg kg^{-1} i.v. ($n=6$). Mean values are presented for each group; vertical lines show s.e.mean. Statistical significance (Duncan's test; $*P<0.05$; $**P<0.01$) refers to differences from the control group.

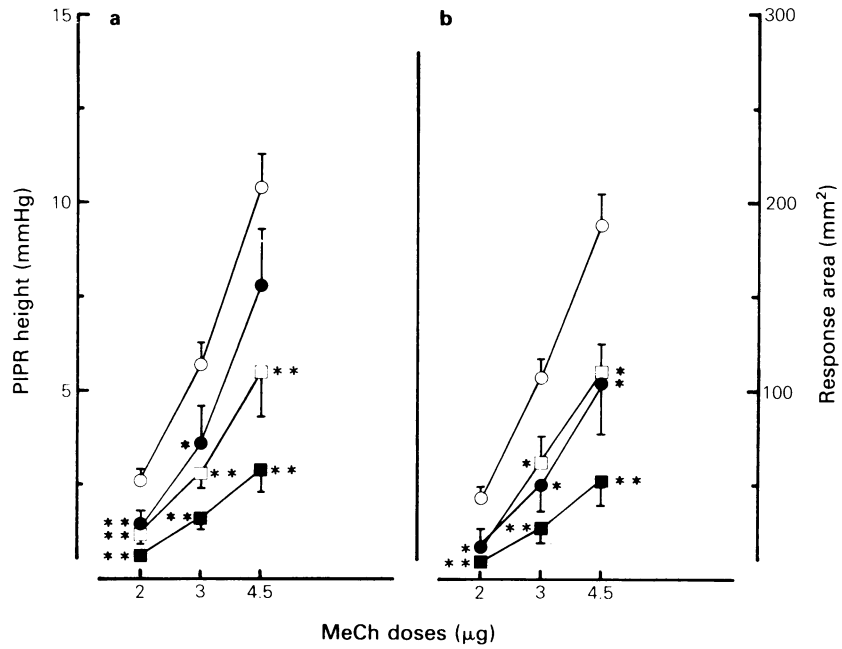

Figure 2 Effects of betamethasone (BM) and theophylline (Theo), alone and in combination, on methacholine (MeCh)-induced bronchoconstriction. Symbols: (○) controls given 0.2 ml of saline i.v. ($n = 12$); (●) BM 0.4 mg kg^{-1} i.v. ($n = 6$); (□) Theo 20 mg kg^{-1} i.v. ($n = 6$); (■) BM 0.4 mg kg^{-1} + Theo 20 mg kg^{-1} i.v. ($n = 6$). Mean values are presented for each group; vertical lines show s.e.mean. Statistical significance (Duncan's test; $*P < 0.05$; $**P < 0.01$) refers to differences from the control group. For further details see Figure 1 and Methods.

Table 1 Cardiovascular effects of consecutive doses of methacholine (MeCh) in anaesthetized rats, as modified by saline (controls), betamethasone (BM), terbutaline (Ter), theophylline (Theo), and ipratropium bromide (Ipra) given i.v. alone and in combinations

Treatment	*Baseline*	*MeCh doses* (μg)		
		2	3	4.5
Controls	BP: 173 ± 5	65 ± 5	45 ± 7	34 ± 4
	HR: 470 ± 13	412 ± 14	377 ± 16	347 ± 15
BM 0.4 mg kg^{-1}	BP: 174 ± 7	77 ± 7	65 ± 8	45 ± 7
	HR: 480 ± 15	470 ± 14	427 ± 21	387 ± 17
BM 2 mg kg^{-1}	BP: 169 ± 5	65 ± 4	54 ± 7	35 ± 8
	HR: 473 ± 25	453 ± 25	420 ± 27	347 ± 17
Ter 20 μg kg^{-1}	BP: 163 ± 9	69 ± 11	57 ± 9	44 ± 8
	HR: 457 ± 16	430 ± 24	403 ± 26	350 ± 28
BM 0.4 + Ter 20	BP: 185 ± 5	73 ± 7	58 ± 9	43 ± 8
	HR: 473 ± 7	450 ± 12	400 ± 27	377 ± 19
Theo 20 mg kg^{-1}	BP: 186 ± 4	64 ± 11	38 ± 6	33 ± 4
	HR: 477 ± 6	450 ± 48	417 ± 40	367 ± 40
BM 0.4 + Theo 20	BP: 175 ± 4	78 ± 6	64 ± 7	55 ± 6
	HR: 477 ± 6	507 ± 4*	480 ± 14*	470 ± 15**
Ipra 0.5 μg kg^{-1}	BP: 178 ± 3	68 ± 12	58 ± 11	44 ± 6
	HR: 493 ± 13	427 ± 30	400 ± 37	403 ± 8
BM 0.4 + Ipra 0.5	BP: 182 ± 5	98 ± 8*	88 ± 10**	80 ± 9**
	HR: 500 ± 12	473 ± 17	460 ± 17	453 ± 16**

Mean ± s.e.mean values of blood pressure (BP, mmHg) and heart rate (HR, beats min^{-1}) are presented for each group. The number of experiments was twelve in the control group and six in the other groups. Statistical significance (Duncan's test; $*P < 0.05$; $**P < 0.01$) refers to differences from the controls.

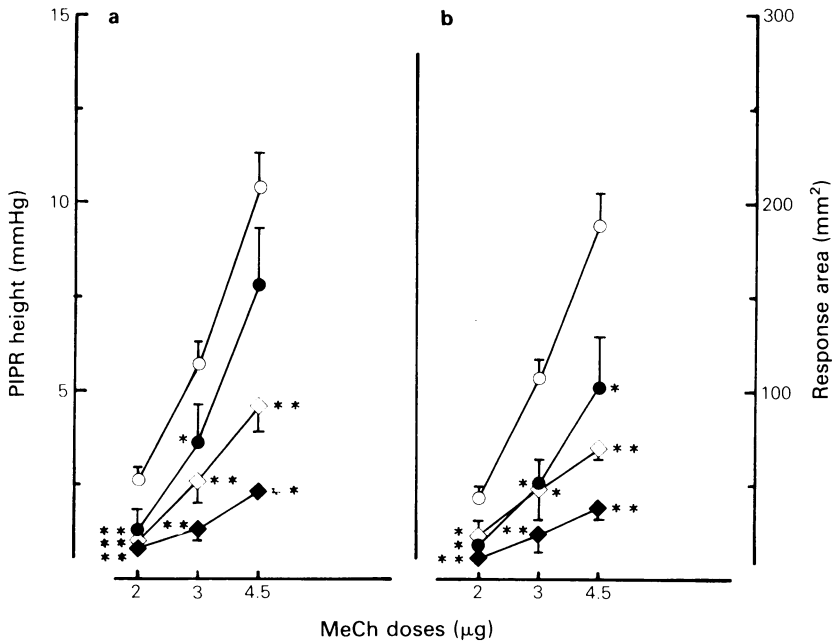

Figure 3 Effects of betamethasone (BM) and ipratropium bromide (Ipra), alone and in combination, on methacholine (MeCh)-induced bronchoconstriction. Symbols: (○) controls given 0.2 ml of saline i.v. ($n = 12$); (●) BM 0.4 mg kg^{-1} i.v. ($n = 6$); (◇) Ipra 0.5 μg kg^{-1} i.v. ($n = 6$); (◆) BM 0.4 mg kg^{-1} + Ipra 0.5 μg kg^{-1} i.v. ($n = 6$). Mean values are presented for each group; vertical lines show s.e.mean. Statistical significance (Duncan's test; $*P < 0.05$; $**P < 0.01$) refers to differences from the control group. For further details see Figure 1 and Methods.

Terbutaline, theophylline and ipratropium in betamethasone pretreated rats

The immediate cardiovascular effects after infusion of Ter, Theo, and Ipra in the BM (0.4 mg kg^{-1})-treated animals were similar to those recorded in animals receiving Ter, Theo, or Ipra alone. The combined actions of BM 0.4 mg kg^{-1} + Ter 20 μg kg^{-1} on PIPR depended on the severity of airway obstruction, being additive at 2 and 3 μg of MeCh and more than the expected sum of the individual drugs at MeCh 4.5 μg (Figure 1). The PIPR differences between BM and BM + Ter were statistically significant ($P < 0.05$ to 0.01) at all MeCh doses, while the differences between Ter and the combination were significant ($P < 0.05$) only at the largest dose of MeCh. No more than additive effects were seen on RA at any MeCh dose (Figure 1), and the only significant ($P < 0.05$) difference between drug treatments was between Ter and BM + Ter at MeCh 4.5 μg. The combination did not modify MeCh-induced reversible hypotension or bradycardia (Table 1).

BM 0.4 mg kg^{-1} + Theo 20 mg kg^{-1} counteracted MeCh bronchoconstriction more than did the respective doses of BM and Theo alone (Figure 2), but the combined actions on PIPR and RA were only additive at all MeCh doses (Figure 2). The PIPR values between BM and the combination differed significantly ($P < 0.05$ to 0.01) from each other at 3 and 4.5 μg of MeCh, while the differences between Theo and BM + Theo were not significant. As to the RA values there were no significant differences between the combination and the single drugs. BM + Theo counteracted significantly MeCh-induced bradycardia but not hypotension at every MeCh dose when compared to the controls (Table 1). The heart rate values after the combination also differed significantly ($P < 0.05$ to 0.01) from those after treatment with BM or Theo alone at the largest MeCh dose.

Combined treatment with BM 0.4 mg kg^{-1} + Ipra 0.5 μg kg^{-1} antagonized MeCh-induced bronchoconstriction better than did the single drugs, but the effects on PIPR and RA did not exceed the expected sum of the individual drug treatments at any MeCh dose (Figure 3). The PIPR but not the RA values after BM + Ipra differed significantly ($P < 0.05$ to 0.01) from those after BM alone at 3 and 4.5 μg of MeCh, while the differences between Ipra and BM + Ipra were not significant. BM + Ipra antagon-

ized significantly MeCh-induced hypotension at every MeCh dose as well as bradycardia at the largest MeCh dose (Table 1). The blood pressure values after the combination also differed significantly ($P<0.05$ to 0.01) from those measured after Ipra alone at every MeCh dose, and from those measured after BM alone at 4.5 μg of MeCh. The heart rate values after BM + Ipra were significantly ($P<0.05$) different from those recorded after BM at MeCh 4.5 μg but not from those after Ipra.

Discussion

The present results demonstrate that BM, a potent glucocorticoid, counteracted MeCh-induced bronchoconstriction in rats. The combined actions of BM and Ter on peak intratracheal pressure response (PIPR) seemed to depend on the severity of airway obstruction, being additive on mild or moderate states and synergistic on the most severe bronchoconstriction (Figure 1). A similar response pattern has previously been observed by us using combinations of Theo with Ter or Ipra (Salonen *et al.*, 1982). In the present study, only an additive effect was seen with BM + Ter on response area (RA), i.e. an integral of the bronchoconstrictor response. The effects of the other drug combinations, BM + Theo (Figure 2) and BM + Ipra (Figure 3), on PIPR or RA failed to exceed the expected sum of the individual drug treatments at any MeCh dose. Furthermore, combined actions of BM + Ter were selective to the airways only, whereas the other combinations had synergistic interactions on the cardiovascular system especially at the largest MeCh dose (Table 1). BM was not effective after an acute single dose, but a time interval of 24 h was necessary to provide these effects. After this pretreatment acute administration did not seem to make any significant contribution to the effects of BM. The time lag of 24 h in response suggests an action at the level of protein synthesis.

Our findings tally well with some previous results. Aviado & Carrillo (1969) showed that high acute doses of dexamethasone failed to counteract bradykinin- or 5-hydroxytryptamine-induced bronchoconstriction in rats, but after a pretreatment of seven days it reduced the bronchoconstrictor response to inhalation of cigarette smoke. Church (1975) demonstrated that dexamethasone, 5 mg kg^{-1}, given 24 h before the experiment counteracted bradykinin- or acetylcholine-induced bronchoconstriction in pithed rats. A time interval of 8–24 h after the corticosteroid treatment was needed to counteract effectively anaphylactic bronchoconstriction in rats (Church *et al.*, 1972) and to reduce airway sensitivity to inhaled histamine in guinea-pigs (Brink *et al.*, 1977). There seems to be species differences in response to corticosteroids, because Carrillo & Aviado (1968) have reported a fall in pulmonary resistance after an acute i.v. injection of dexamethasone to nonsensitized rabbits.

The mechanism of the airway actions of BM, given alone or in combination with bronchodilator drugs, has remained obscure. Hydrocortisone reduces α-adrenoceptor-mediated contraction and enhances β-adrenoceptor-induced relaxation in isolated airway smooth muscle (Townley *et al.*, 1970; 1972). In vascular tissues adrenal cortical steroids increase the contractile responses to α-adrenoceptor agonists (Fritz & Levine, 1951; Zweifach *et al.*, 1953); Besse & Bass, 1966; Kalsner, 1969). Corticosteroids could enhance the effects of endogenous or exogenous adrenoceptor agonists by reducing their elimination when blocking the extraneuronal uptake (Iversen & Salt, 1970; Salt, 1972; Pun *et al.*, 1973) and/or catechol-*O*-methyltransferase (COMT) (Kalsner, 1969). Other possible mechanisms include an accumulation of cyclic AMP due to stimulation of adenylate cyclase (Logsdon *et al.*, 1972; Parker *et al.*, 1973), inhibition of cyclic AMP phosphodiesterase (Senft *et al.*, 1968), or increase in the number of pulmonary β-adrenoceptors (Mano *et al.*, 1979).

In the present study, inhibition of extraneuronal uptake and/or COMT by BM seems unlikely. Corticosteroids inhibit extraneuronal uptake within a few min after an acute treatment (Salt, 1972; Pun *et al.*, 1973; Geddes *et al.*, 1974), which suggests that this action is not mediated by the synthesis of new proteins. Extraneuronal uptake and metabolism by COMT is specific to catecholamines only (Kalsner, 1969; Geddes *et al.*, 1974), which means that blocking these events does not explain why the bronchodilator action of Ter was enhanced. Furthermore, it seems that selective glucocorticoids like BM and dexamethasone do not inhibit extraneuronal uptake (Salt, 1972; Geddes *et al.*, 1974). Iversen & Salt (1972) and Salt (1973) have shown that the endogenous corticosteroid corticosterone, but not hydrocortisone, blocks the extraneuronal uptake of catecholamines in the rat heart.

The influence of BM on the airways could be directly associated with β-adrenoceptors. Andersson & Kövesi (1974) and Brink *et al.* (1977) have reported that the bronchodilator action of hydrocortisone can be abolished by β-receptor antagonists. Hydrocortisone increases cyclic AMP concentration by stimulating adenylate cyclase in human lymphocytes (Logsdon *et al.*, 1972; Parker *et al.*, 1973), but according to Andersson & Kövesi (1974) the increase of cyclic AMP in human tracheal smooth muscle does not result from the stimulation of adenylate cyclase or inhibition of cyclic AMP phosphodiesterase. A direct elevation of intracellular cyclic AMP in the airways does not seem to be the probable

mechanism by which BM influenced the MeCh-induced bronchoconstriction in the present study, because that effect is seen within 1 to 5 min after the corticosteroid treatment both in human lymphocytes and tracheal tissue. Another possibility might be an increased number of functional β-adrenoceptors in the lung after subacute glucocorticoid treatment. Mano *et al.* (1979) have shown that hydrocortisone (50 mg kg^{-1} given i.p. for nine days) increases the total number of β-adrenoceptor binding sites by 70% in rat lung tissue.

It is concluded that combined treatment with glucocorticoid and the β_2-adrenoceptor agonist, terbutaline, may result in a synergistic interaction on severe airway obstruction without any significant effect on the cardiovascular system. The combinations of glucocorticoid with theophylline or ipratropium were additive only, and they also influenced circulation. A time interval of 24 h after betamethasone pretreatment was required to produce these effects suggesting the necessity of new protein synthesis.

This study was supported by the Astra Research Foundation (Södertälje, Sweden), the Finnish Antituberculosis Association, and Väinö and Laina Kivi's Foundation.

References

ANDERSSON, R.G.G. & KÖVESI, G. (1974). The effect of hydrocortisone on tension and cyclic AMP metabolism in tracheal smooth muscle. *Experientia*, **30,** 784–786.

AVIADO, D.M. & CARRILLO, L.R. (1969). Hormones and pulmonary effects of tobacco. I. Corticosteroids and their antiasthmatic action. *Arch. Environ. Health*, **18,** 925–933.

BAUER, R., KUHN, F.-J., STOCKHAUS, K. & WICK, H. (1976). Allgemeine pharmakologie und sekretionshemmende wirkung von (8r) - 3α - hydroxy - 8 - isopropyl - 1αH, 5αH - tropaniumbromid - (±) - tropat (ipratropiumbromid). *Arzneimittelforsch.*, **26,** 974–980.

BESSE, J.C. & BASS, A.D. (1966). Potentiation by hydrocortisone of responses to catecholamines in vascular smooth muscle. *J. Pharmac. exp. Ther.*, **154,** 224–238.

BOUSHEY, H.A., HOLTZMAN, M.J., SHELLER, J.R. & NADEL, J.A. (1980). Bronchial hyperreactivity. *Am. Rev. Respir. Dis.*, **121,** 389–413.

BRINK, C., RIDGWAY, P. & DOUGLAS, J.S. (1977). Modification of airway smooth muscle responses in the guinea pig by hydrocortisone, in vitro and in vivo. *J. Pharmac. exp. Ther.*, **203,** 1–11.

CARRILLO, L.R. & AVIADO, D.M. (1968). Mechanisms for the bronchodilator effects of corticosteroids in the sensitized rabbit. *J. Pharmac. exp. Ther.*, **164,** 302–311.

CHURCH, M.K. (1975). Response of rat lung to humoral mediators of anaphylaxis and its modification by drugs and sensitization. *Br. J. Pharmac.*, **55,** 423–430.

CHURCH, M.K., COLLIER, H.O.J. & JAMES, G.W.L. (1972). The inhibition by dexamethasone and disodium cromoglycate of anaphylactic bronchoconstriction in the rat. *Br. J. Pharmac.*, **46,** 56–65.

DUNCAN, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, **11,** 1–42.

ELLUL-MICALLEF, R. & FENECH, F.F. (1975). Effect of intravenous prednisolone in asthmatics with diminished adrenergic responsiveness. *Lancet*, **ii,** 1269–1270.

FRANKLIN, W., MICHELSON, A.L., LOWELL, F.C. & SCHILLER, I.W. (1958). Bronchodilators and corticosteroids in the treatment of obstructive pulmonary emphysema. *N. Engl. J. Med.*, **258,** 774–778.

FRITZ, I. & LEVINE, R. (1951). Action of adrenal cortical steroids and norepinephrine on vascular responses of stress in adrenalectomized rats. *Am. J. Physiol.*, **165,** 456–465.

GEDDES, B.A., JONES, T.R., DVORSKY, R.J. & LEFCOE, N.M. (1974). Interaction of glucocorticoids and bronchodilators on isolated guinea pig tracheal and human bronchial smooth muscle. *Am. Rev. Respir. Dis.*, **110,** 420–427.

HOLGATE, S.T., BALDWIN, C.J. & TATTERSFIELD, A.E. (1977). β-adrenergic agonist resistance in normal human airways. *Lancet*, **ii,** 375–377.

HUME, K.M. & RHYS JONES, E. (1960). Bronchodilators and corticosteroids in asthma. *Lancet*, **ii,** 1319–1322.

IVERSEN, L.L. & SALT, P.J. (1970). Inhibition of catecholamine uptake by steroids in the isolated rat heart. *Br. J. Pharmac.*, **40,** 528–530.

KALSNER, S. (1969). Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circulation Res.*, **24,** 383–395.

LEFCOE, N.M., TOOGOOD, J.H. & JONES, T.R. (1975). In vitro pharmacologic studies of bronchodilator compounds; interactions and mechanisms. *J. Allergy Clin. Immunol.*, **55,** 94.

LOGSDON, P.J., MIDDLETON E. Jr., & COFFEY, R.G. (1972). Stimulation of leukocyte adenyl cyclase by hydrocortisone and isoproterenol in asthmatic and nonasthmatic subjects. *J. Allergy Clin. Immunol.*, **50,** 45–56.

MANO, K., AKBARZADEH, A. & TOWNLEY, R.G. (1979). Effect of hydrocortisone on beta-adrenergic receptors in lung membranes. *Life Sci.*, **25,** 1925–1930.

McCULLOCH, M.W., PROCTOR, C. & RAND, M.J. (1967). Evidence for an adrenergic homeostatic bronchodilator reflex mechanism. *Eur. J. Pharmac.*, **2,** 214–223.

MELBY, J. (1977). Clinical pharmacology of systemic corticosteroids. *A. Rev. Pharmac. Tox.*, **17,** 511–527.

MIDDLETON E. Jr. (1975). Mechanism of action of corticosteroids. In *New Directions in Asthma.* ed. Stein, M. pp. 433–446. Park Ridge, Illinois, U.S.A.: American College of Chest Physicians.

PARKER, C.W., HUBER, M.G. & BAUMANN, M.L. (1973). Alterations in cyclic AMP metabolism in human bronchial asthma. III. Leukocyte and lymphocyte responses to steroids. *J. clin. Invest.*, **52,** 1342–1348.

PUN, L.Q., McCULLOCH, M.W. & RAND, M.J. (1973). The effect of hydrocortisone on the bronchodilator activity of sympathomimetic amines and on the uptake of isoprenaline in the isolated guinea-pig trachea. *Eur. J. Pharmac.*, **22,** 162–168.

REED, C.E. (1974). Abnormal autonomic mechanisms in asthma. *J. Allergy Clin. Immunol.*, **53,** 34–41.

RINARD, G.A. (1978). Relaxation of airway smooth muscle in response to catecholamines is enhanced by glucocorticosteroids. *Am. Rev. Respir. Dis.*, **117,** 75.

SALONEN, R.O. & MATTILA, M.J. (1981). Dose-related bronchoconstriction with methacholine in rats, as modified by terbutaline alone and in combination with theophylline. *Acta Pharmac. Tox.*, **48,** 193–198.

SALONEN, R.O., MATTILA, M.J. & EDHOLM, L.-E. (1982). Actions of theophylline, terbutaline, and ipratropium bromide alone and in combinations on the methacholine induced bronchoconstriction in rats. *Acta Pharmac. Tox.*, **51,** 309–320.

SALT, P.J. (1972). Inhibition of noradrenaline uptake in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. *Eur. J. Pharmac.*, **20,** 329–340.

SENFT, G., SCHULTZ, G., MUNSKE, K. & HOFFMANN, M. (1968). Effects of glucocorticoids and insulin on 3′,5′-AMP phosphodiesterase activity in adrenalectomized rats. *Diabetologia*, **4,** 330–335.

SHENFIELD, G.M., HODSON, M.E., CLARKE, S.W. & PATERSON, J.W. (1975). Interaction of corticosteroids and catecholamines in the treatment of asthma. *Thorax*, **30,** 430–435.

STEVENSON, R.D. (1977). Mechanism of anti-inflammatory action of glucocorticosteroids. *Lancet*, **I,** 225–226.

TOWNLEY, R.G., HONRATH, T. & GUIRGIS, H.M. (1972). The inhibitory effect of hydrocortisone on the alpha responses of human and guinea pig isolated respiratory smooth muscle. *J. Allergy Clin. Immunol.*, **49,** 88.

TOWNLEY, R.G., REEB, R., FITZGIBBONS, T. & ADOLPHSON, R.L. (1970). The effect of corticosteroids on the beta-adrenergic receptors in bronchial smooth muscle. *J. Allergy Clin. Immunol.*, **45,** 118.

WIDDICOMBE, J.G. (1979). The parasympathetic nervous system in airways disease. *Scand. J. Respir. Dis.* (Suppl.), **103,** 38–43.

ZWEIFACH, B.W., SHORR, E. & BLACK, M.M. (1953). The influence of the adrenal cortex on behaviour of terminal vascular bed. *A. N.Y. Acad. Sci.*, **56,** 626–633.

(Received December 30, 1983.
Revised May 24, 1984.)