

Formoterol fumarate, a new β_2 -adrenoceptor agonist

Acute studies of selectivity and duration of effect after inhaled and oral administration

C.-G. LÖFDAHL & N. SVEDMYR

Departments of Clinical Pharmacology and Pulmonary Medicine, University of Göteborg, Sweden

Four double-blind, double-dummy, randomized, crossover studies were performed in nine asthmatic patients to evaluate β_2 -adrenoceptor selectivity and the duration of effect of formoterol. One study with cumulatively increasing doses of formoterol and salbutamol showed that with the inhaled route, formoterol was 5-15 times more potent than salbutamol with respect to bronchodilation. In a second study, 6 μ g formoterol and 0.1 mg salbutamol were given for comparison of effect duration. Five hours after salbutamol inhalation the FEV₁ values were back to basal. Eight hours after formoterol inhalation about 75% of the maximum bronchodilation remained and the FEV₁ was significantly higher than after salbutamol inhalation. By oral route, a study with cumulatively increasing doses showed that as a bronchodilator formoterol was about 50 times more potent than salbutamol. The same potency difference was seen for increase of heart rate and decrease of diastolic blood pressure, indicating that the clinical selectivity for β_2 -adrenoceptors is equal for the two drugs. In a fourth study comparable doses by oral route did not show any difference in the duration of bronchodilation. We conclude that inhaled formoterol is 5-15 times more potent than salbutamol. Inhaled formoterol produces longer duration of bronchodilation, with clinically relevant bronchodilation for at least 8 h. The prolonged duration of formoterol was not seen with oral treatment.

Key words: formoterol; salbutamol; bronchodilation; cardiovascular effect; skeletal muscle tremor.

Accepted for publication 4 December 1988

β_2 -adrenoceptor agonists are considered a cornerstone of current asthma therapy (7, 20, 21). The main route of administration is via inhalation, with several advantages over systemic treatment (14, 25). One of the disadvantages of the currently available β_2 -adrenoceptor agonists is their relatively short duration of effect, about 4 h after inhalation (4, 6, 8, 15, 23). This has made it impossible to retain a continuous effect during the night without repeated inhalations.

Formoterol fumarate is a new catecholamine analogue possessing a potent β -adrenoceptor agonist effect. Selectivity studies in isolated tissue samples have shown that formoterol fumarate is at least as β_2 -adrenoceptor selective as salbutamol and terbutaline (5, 11). It has been shown in *in vivo* animal experiments to

have a high potency and long duration of effect; longer than salbutamol. The potency was about 50 times higher than that of salbutamol (10).

Japanese studies in asthmatics have indicated that oral treatment with this drug produces longer effect duration than salbutamol (13, 18). To our knowledge no studies have been performed to evaluate the effect duration of inhaled treatment with this drug.

A study with the following objectives was therefore planned:

1. To establish equipotent bronchodilating doses for formoterol and salbutamol when given by inhaled and oral route, and evaluate the β_2 -adrenoceptor selectivity by comparing the effects on heart rate and skeletal muscle tremor.

2. To study the duration of bronchodilation for formoterol, compared with salbutamol given by inhaled and oral route.

METHODS

Experimental design

Four studies were performed as double-blind, double-dummy, randomized, crossover comparisons between formoterol and salbutamol in patients with stable endogenous asthma. In two studies cumulatively increasing doses of the drugs were given to establish the dose-response curves for bronchodilation, circulatory effect and skeletal muscle tremor with formoterol and salbutamol by oral or inhaled route. In two other studies one dose of each drug, shown in the dose-response experiments to be equipotent doses, were given and the ventilatory capacity was studied during a period of 8 h for both inhaled and oral treatment.

Patients

Nine patients with endogenous asthma in a stable phase and reproducible obstruction of the airways when off treatment were studied (Table 1). In the two oral studies the same eight asthmatics took part. The patients showed a reversibility of FEV₁ of 20–63% during the salbutamol dose-response experiment. They were

included in the study after giving informed consent to participation in the study, which had previously been approved by the Ethics Committee at the University of Gothenburg.

Inhaled β_2 -adrenoceptor agonist therapy and inhaled anticholinergics were withdrawn at least 12 h before the start of an experiment. Oral β_2 -adrenoceptor agonist therapy given in slow-release preparations was withdrawn 24 h and theophylline derivatives 36 h before each experiment. Corticosteroids, inhaled or oral, were unchanged during the study period.

If the difference in each study between mean basal values for one individual was greater than 15%, that day's experiment was repeated.

Experimental details

1. *Inhaled cumulative dose-response study.* The patients arrived at the laboratory at 7.30 a.m. after a light breakfast without coffee or tea at home 1 h earlier. After 50 and 70 min rest in a semi-recumbent position, basal measurements of the following variables were performed:
- 1) Heart rate from continuous ECG recordings.
 - 2) Blood pressure by the cuff method.
 - 3) Skeletal muscle tremor from a single plane accelerometer according to a previously described method (24).
 - 4) Forced expiratory volume in 1 s (FEV₁) measured with a Collin's survey spirometer. At least two recordings were made and the highest value was used for calculation.

Table 1
Patient characteristics

Patient	Age	Sex	Height (cm)	Basal FEV ₁ (l)	Reversibility (%)	Treatment
1	56	M	176	1.66	41	IB, IC
2	57	F	168	1.36	63	IB, OB, IC, OC, T
3	54	M	185	2.34	30	IB, IC, OC, T
4	62	M	170	1.98	46	IB, OB, IC, OC, T
5	64	M	167	1.88	29	IB, OB, IC, T
6	66	M	184	2.54	32	IB, OB, IC
7	67	M	186	1.97	49	IB, OB, IC, OC
8	60	M	167	2.13	42	IB, OB, IC, T
9	71	M	176	2.72	20	IB, OB

Patient No. 3 took part only in the inhalation studies, patient No. 9 only in the oral studies. Abbreviations: I = inhaled, O = oral, B = β -adrenoceptor agonist, C = corticosteroid, T = oral theophylline.

After basal measurements, four increasing doses of each drug were given at 70-min intervals, as a pilot study had shown that an almost maximum bronchodilating effect was seen within 1 h for both drugs. Fifteen min after each inhalation, all measurements except blood pressure were performed, and 30 min after inhalation a new spirometry was performed and 60 min after inhalation all measurements were repeated. The patients did not eat during the time of the experiment.

The doses chosen for formoterol were 3 µg in one inhalation, 9 µg given as 3 inhalations, 27 µg given as 2 inhalations of 12 µg and one of 3 µg, and 84 µg given as 7 inhalations of 12 µg each. For salbutamol the doses were 100 µg in one inhalation, 300 µg in 3 inhalations, 900 µg as 9 inhalations and 2,800 µg as 7 inhalations from powder aerosol capsules (400 µg Ventoline Rotahaler®). Matching placebo canisters/capsules without active drug were given together with the active drug to ensure blindness. Doses with several inhalations were given as alternating inhalations between placebo and active drug. The same spacer device, Volumatic®, was used for inhalations of both drugs to decrease inhalation errors and to improve bronchial deposition. A special adapter for the formoterol dose inhalers was built in the laboratory. The inhalations were supervised by a trained technician and the patients rinsed their mouths with water after each inhalation.

2. *Inhaled duration study.* The patients arrived at the laboratory as in the dose-response study and basal measurements were performed after 50 and 70 min. The following measurements were made:

- 1) Heart rate from continuous ECG recordings.
- 2) FEV₁ measured with a Collin's survey spirometer. At least two recordings were made and the highest value was used for calculation.

After basal measurements, one dose of active formoterol or salbutamol with matching placebo was given. The dose of formoterol was 6 µg and of salbutamol 100 µg. New measurements were

performed 15, 30 and 60 min after inhalation and then exactly once every hour up to 8 h after inhalation. After the 8-h measurement the patient inhaled 1.25 mg terbutaline (5 inhalations). The inhalations of formoterol and salbutamol were performed as in the dose-response experiment and administered via a Volumatic® spacer device.

3. *Oral cumulative dose-response study.* After basal measurements performed as in the inhaled cumulative study, three increasing doses of each drug were given at 130-min intervals, as a pilot study showed that the maximum effect was achieved within 2 h after both drugs given by the oral route. 30, 60, 90 and 120 min after each dose all measurements were repeated. 130 min after the third dose inhalations of terbutaline were given (1.25 mg), and FEV₁ was measured 15 min after inhalation. The patients were given a light lunch 70 min after the second oral dose and soup 70 min after the third dose.

The doses for formoterol were 40 µg, 120 µg and 360 µg, and for salbutamol 2 mg, 6 mg, and 18 mg. Matching placebo tablets were given together with the active drug.

4. *Oral duration study.* The same measurements were performed as in the inhaled cumulative dose-response study. After placebo measurements one dose of formoterol 80 µg or salbutamol 4 mg with matching placebo was given in randomized order. Measurements were performed after 30 and 60 min and then every hour up to 8 h after oral intake. Terbutaline was then inhaled (1.25 mg), and FEV₁ was performed 15 min after inhalation.

Statistics

Values are presented as means and standard errors of the mean (SE). Bronchodilatory responses are presented as per cent of bronchodilating capacity (BDC). Per cent of BDC = [(Actual value - Basal value achieved on experiment day)/(Maximum value during study - Basal value achieved on experiment day)] * 100. Changes from basal value were

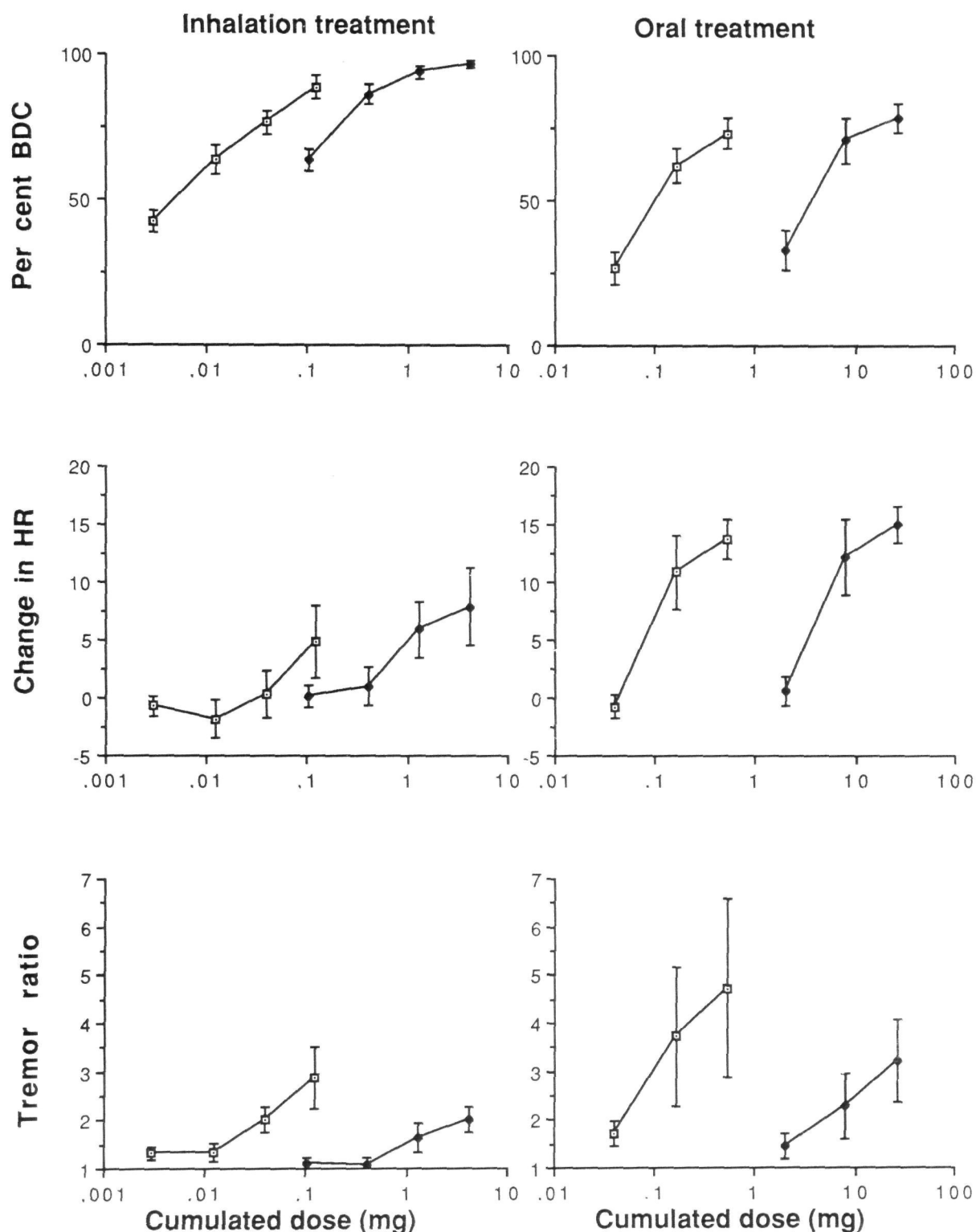


Fig. 1. Cumulative dose-response curves for formoterol (□) and salbutamol (●) given by inhaled and oral routes.

used to evaluate heart rate and diastolic blood pressure. Skeletal muscle tremor was expressed as the tremor ratio, which is the relationship between the observed accelerometer value and the basal value on the actual experiment day (24). In the dose response study the maximum value after each dose was

used for calculation. Statistical testing of significance was performed with Student's *t*-test for paired comparisons. As differences were tested at four time points, 5, 6, 7 and 8 h after inhalation, the Bonferroni method of adjustment of the critical values was used (26).

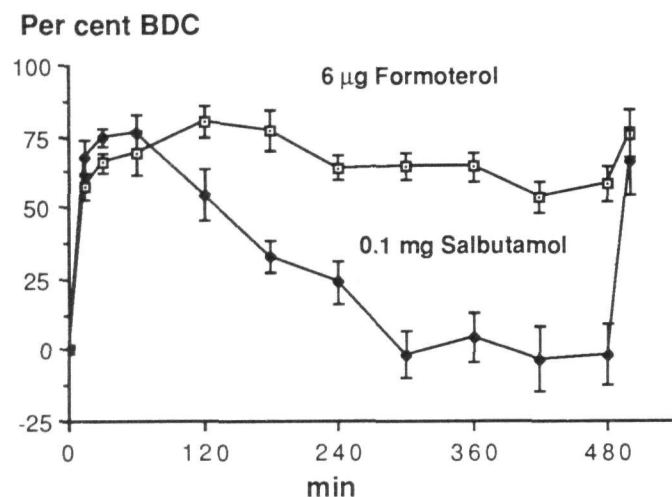


Fig. 2. Duration study of inhaled formoterol, 6 µg (□) and salbutamol 100 µg (●) during a period of 8 h. After 8 h 1.25 mg terbutaline was inhaled from a metered dose inhaler.

RESULTS

Inhaled cumulative dose-response study

Respiratory studies (Fig. 1). Mean basal FEV_1 values before the treatments did not differ significantly. Before formoterol it was 2.02 ± 0.15 l and before salbutamol it was 2.04 ± 0.15 l.

The mean dose-response curves showed that with 3 µg formoterol, the FEV_1 increased with $42 \pm 4\%$ of BDC, whereas with 100 µg salbutamol the increase was $64 \pm 4\%$ of BDC. The mean dose-response curves showed the same effect when 5–15 times higher doses of salbutamol than formoterol were given.

Circulatory studies (Fig. 1). Only minor changes in heart rate were observed during the study. After the highest dose of formoterol the heart rate increase was 4.8 ± 3.1 bpm and after salbutamol 7.9 ± 3.1 bpm. The difference was not significant. Diastolic blood pressure showed no statistically significant changes.

Tremor studies (Fig. 1). Skeletal muscle tremor showed only minor changes with the two lowest doses of both drugs. However, with the two highest doses there was a similar increase in tremor for both drugs. After formoterol there was a tendency towards more pro-

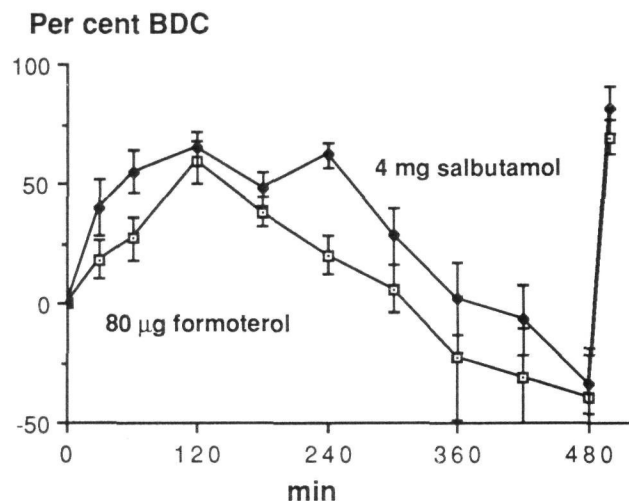


Fig. 3. Duration study of oral formoterol, 80 µg (□) and salbutamol, 4 mg (●) during a period of 8 h. After 8 h 1.25 mg terbutaline was inhaled from a metered dose inhaler.

nounced tremor, although the difference was not significant.

Inhaled duration study (Fig. 2)

The basal mean value of FEV_1 before formoterol was 1.90 ± 0.16 l and before salbutamol 1.86 ± 0.16 l. There was a rapid increase after 100 µg salbutamol, and maximum bronchodilation, ($76 \pm 5\%$ BDC) was attained after 1 h. For 6 µg formoterol the maximum increase, $80 \pm 5\%$ BDC, was attained after 2 h. There was a rapid decline after salbutamol treatment and after 5 h the FEV_1 measurements were back to basal level. With formoterol there was a very small decrease during 8 h of follow-up. The difference between the two treatments 5 h after inhalation was $67 \pm 10\%$ BDC ($P < 0.01$); after 6 h $60 \pm 11\%$ BDC ($P < 0.01$), after 7 h $57 \pm 11\%$ BDC ($P < 0.01$), and after 8 h $60 \pm 13\%$ BDC ($P < 0.01$). Eight hours after formoterol inhalation about 75% of the maximum effect remained.

There were no statistically significant changes in heart rate during this study.

The area under the % BDC curve (AUC) for the two drugs during the first 2 h were equal in size. The AUC during 8 h for formoterol was more than twice that of sal-

butamol and the difference was highly significant ($P < 0.001$).

Oral cumulative dose-response study

Respiratory studies (Fig. 1). Basal FEV₁ values before the two treatments did not differ significantly. Before formoterol it was 1.89 ± 0.19 l and before salbutamol 2.00 ± 0.19 l. The individual maximum value after each dose was used to establish the dose-response curves for the two drugs. The dose response curves run parallel to one another. It was found that formoterol was about 50 times more potent than salbutamol so that 80 μ g of formoterol was equipotent to 4 mg of salbutamol.

Circulatory studies. The basal mean heart rate value before formoterol treatment was 62.5 ± 1.7 bpm and before salbutamol 62.1 ± 1.5 . The individual maximum value after each dose was then used to establish the dose-response curves (Fig. 1). The dose-response curves run in parallel and also with respect to heart rate, the difference in potency is similar to that found in FEV₁ measurements, i.e. formoterol is about 50 times more potent than salbutamol.

Diastolic blood pressure showed a similar decrease with the high doses of both drugs, with a tendency towards a more pronounced decrease after formoterol treatment.

Tremor studies (Fig. 1). There was a dose-dependent increase in skeletal muscle tremor with both drugs. There was a tendency toward higher values after formoterol than after salbutamol.

Oral duration study

The basal mean value of FEV₁ before 80 μ g formoterol was 2.01 ± 0.13 l and before 4 mg salbutamol 2.03 ± 0.12 l. Fig. 3 shows the mean values of changes in FEV₁ as per cent of BDC at different times after tablet intake. The figure shows that the curves of 80 μ g formoterol and 4 mg salbutamol run in parallel and the maximum effects were achieved 2 h after intake. For 80 μ g formoterol the increase after 2 h was $59 \pm 9\%$ BDC, and after 4 mg salbutamol

$65 \pm 7\%$ BDC. The decline was similar for the two drugs with a small deviation after 4 h, after lunch, when the salbutamol curve showed a small increase again. The values were back to baseline 5–6 h after intake. As shown in the graph there was good bronchodilatory potential after 8 h as there was a substantial increase with inhaled terbutaline.

Heart rate, diastolic blood pressure and skeletal muscle tremor showed no significant effects after the treatments with 80 μ g formoterol or 4 mg salbutamol.

DISCUSSION

These studies have evaluated the β_2 -adrenoceptor selectivity and duration of bronchodilation of formoterol, compared with salbutamol. The most important finding was sustained bronchodilation up to 8 h after inhalation of formoterol, which is considerably longer than for salbutamol.

To study the selectivity of the drug we compared the dose-response curves for effects on both β_1 and β_2 adrenoceptors (16). This was done as cumulative dose-response experiments (3,16). A problem with cumulative studies of this kind could be that the time to reach the maximum effect may be different for the two drugs. This problem did not occur with oral treatment as the maximum effect was achieved at the same time with both drugs. For inhalation the maximum bronchodilation with salbutamol occurred after 60 min, whereas the maximum effect with formoterol was seen after 120 min. However, the bronchodilation of formoterol after 60 min was 86 % of the maximum effect seen after 2 h. We therefore believe that the difference in the time course for the bronchodilation has not had a major influence on the potency evaluation in this study. For the other parameters there were only small effects with inhaled treatment, and they had the same time-effect relationship as bronchodilation. The cumulative dose-response curves for bronchodilation, changes in heart rate and skeletal muscle tremor showed a similar potency difference between salbutamol and formoterol.

This is true both for oral and inhaled treatment. This shows that the β_2 -adrenoceptor selectivity of formoterol is the same as that of salbutamol.

To make it possible to evaluate the duration of bronchodilation, it is important to use equipotent bronchodilating doses (16, 22). There was a good possibility of arriving at equipotent doses in this study as the duration study was performed in the same patients as in the cumulative dose-response study, from which the doses were chosen. In order not to overestimate the duration of the new drug in the inhaled duration study, we chose a dose of formoterol which tended to be lower than the salbutamol dose.

However, with inhalation there was marked prolongation of the bronchodilating effect. After 8 h, about 75% of the maximum bronchodilating effect still remained. This study did not evaluate how long the effect lasted after 8 h. Some other studies indicate that the effect lasts up to 12 h (1, 19).

The mechanism behind this prolonged effect is not fully understood. Either it can be explained by local pharmacokinetic properties in the airways or by the general pharmacokinetic properties of the drugs. A surprising finding in this study with regard to the duration of the effect of formoterol, was the discrepancy between the oral and inhaled route, although a study of this question had not been intended. This discrepancy could have several explanations, but could also be due to local pharmacokinetic properties. With oral treatment, the drug reaches the smooth muscle directly from the serosal side, whereas with inhalation the drug has to pass the epithelium. It has been shown that there is a difference between the potency of a bronchodilating drug when administered on the serosal side, compared with intraluminal administration (28). Most drugs show a considerably higher potency when given on the serosal side (12, 27). However, recent experiments show that this discrepancy is less pronounced with some of the new β_2 -adrenoceptor agonists, such as formoterol (12). Thus, it seems likely that there is a difference in the local pharmacokinetics of these drugs in the airway which merits further investigation.

One speculation about the sustained effect of another new inhaled β_2 -agonist, salmeterol, has been the possible presence of an exoreceptor for these drugs in the airway smooth muscle (2). Another factor which is correlated to the duration could be the lipophilicity, as shown recently (12). Formoterol is more lipophilic than salbutamol, which could increase the unspecific binding of the drug to the cell membrane, thereby establishing a depot of the drug in the vicinity of the receptor.

Systemic pharmacokinetic properties of the drugs do not seem to influence the prolonged duration of bronchodilating effect seen with inhaled formoterol, as the serum half-life of formoterol and salbutamol is about the same (17). Other β_2 -adrenoceptor agonists, e.g. clenbuterol, have a very long plasma half-life without any substantial prolongation of the duration of bronchodilation with inhaled route.

To sum up, this study shows that formoterol is a potent bronchodilator; by inhalation 5–15 times more potent than inhaled salbutamol, and that inhaled formoterol produces a considerably longer lasting bronchodilatory effect. This last effect is of great clinical interest as these results indicate that the drug may give good bronchodilatory effect during the night, and that the number of hours between doses can be increased, thus perhaps resulting in better compliance.

REFERENCES

1. v Berg, A., Berdel, D. & Buhr, W.: Ein inhalatives β_2 -Adrenergikum mit Langzeitwirkung in der Akut- und Dauertherapie beim Asthma bronchiale im Kindesalter. *Atemw.- Lungenanerkh.* 14 Suppl. 2, S128–S132, 1988.
2. Bradshaw, J., Brittain, R. T., Coleman, R. A., et al.: The design of salmeterol, a long-acting selective β_2 -adrenoceptor agonist. *Br. J. Pharmacol.* Suppl. 91, 590P, 1987.
3. Britton, J. & Tattersfield, A. E.: Comparison of cumulative and non-cumulative techniques to measure dose-response curves for β -agonists in patients with asthma. *Thorax* 39, 597–599, 1984.
4. Choo-Kang, Y. F. J., MacDonald, H. L. & Horne, N. W.: A comparison of salbutamol and terbutaline aerosols in bronchial asthma. *Practitioner* 211, 801–804, 1973.
5. Decker, N., Quennedey, M. C., Rouot, B., Schwartz, J.

- & Velly, J.: Effects of N-aralkyl substitution of β -agonists on alpha and β -adrenoceptor subtypes: pharmacological studies and binding assays. *J. Pharm. Pharmacol.* **34**, 107-112, 1982.
6. Gray, B. J., Frame, M. H. & Costello, J. F.: A comparative double-blind study of the bronchodilator effects and side effects of inhaled fenoterol and terbutaline administered in equipotent doses. *Br. J. Dis. Chest* **76**, 341-350, 1982.
7. Hardy, C. & Holgate, S. T.: Bronchodilator management of the adult in the United Kingdom. In Jenne, J. W. & Murphy, S. (eds.): *Drug therapy for asthma, research and clinical practice*, pp. 779-836. Marcel Dekker, New York, Basel, 1987.
8. Hartnett, B. J. S. & Marlin, G. E.: Comparison of terbutaline and salbutamol aerosols. *Aust. N. Z. J. Med.* **7**, 13-15, 1977.
9. Hume, K. M. & Gandieva, B.: Forced expiratory volume before and after isoprenaline. *Thorax* **12**, 276-278, 1957.
10. Ida, H.: Cardiorespiratory activities of 3-formylamino-4-hydroxy-alpha-(N-1-methyl-2p-methoxyphenethoxyamine-omethyl)-benzylalcohol-hemifumarate (BD40 A) and some other β -adrenoceptor stimulants in conscious guinea pigs. *Arzneim. Forsch. (Drug Res.)* **26**, 1337-1340, 1976.
11. Ida, H.: Comparison of the action of BD 40 A and some other β -adrenoceptor stimulants on the isolated trachea and atria of the guinea pig. *Arzneim.-Forsch. (Drug Res.)* **26**, 839-842, 1976.
12. Jeppsen, A. B., Löfdahl, C. G., Waldeck, B. & Widmark, W.: Predictive value of in vitro experiments evaluating the effect duration of bronchodilator drugs for local administration (submitted).
13. Kono, O. & Morita, M.: Evaluation of duration of action and clinical effect of formoterol (BD 40) in single doses for bronchial asthma. *Jpn. Pharmacol. Therapeut.* **11**, 4405-4416, 1983.
14. Larsson, S. & Svedmyr, N.: Bronchodilating effect and side effects of β_2 -adrenoceptor stimulants by different modes of administration (tablets, metered aerosol, and combinations thereof). A study with salbutamol in asthmatics. *Am. Rev. Respir. Dis.* **116**, 861-869, 1977.
15. Lawford, P., Dowd, D. E. & Palmer, K. N. V.: A comparison of the duration of action of fenoterol and salbutamol in asthma. *Curr. Med. Res. Opin.* **7**, 349-351, 1981.
16. Löfdahl, C. G.: Selectivity of β -adrenoceptor agonists and antagonists in asthmatics. *Eur. J. Respir. Dis.* **63**, Suppl. 120, 1-53, 1982.
17. Löfdahl, C. G. & Svedmyr, N.: Bronchodilators. In Wilson, J. W., (ed.): *Respiratory pharmacology*, pp. 60-77. Adis Press, Sydney, 1987.
18. Nakajima, S.: Dose-response evaluation of bronchodilator effect of formoterol (BD 40) in single doses in bronchial asthma. *J. Med. Pharmacol. Sci. (Japan)* **10**, 571-582, 1983.
19. Nolte, D. & Rolke, M.: Comparison of the acute bronchospasmolytic effect of formoterol versus fenoterol over 12-hours. In Sybrecht, G. (ed.): *Formoterol - a new long-acting bronchodilator*, pp. 24-31. H. Huber Publishers, Toronto, 1988.
20. Reed, C. E.: Bronchodilator management of the adult in the United States pp. 761-778. Marcel Dekker, New York, Basel, 1987.
21. Svedmyr, N. & Löfdahl, C. G.: Physiology and pharmacodynamics of beta-adrenergic agonists, pp. 177-211. In Jenne, J. W. & Murphy, S. (eds.): *Drug therapy for asthma*, Marcel Dekker, New York, Basel, 1987.
22. Tashkin, D. P.: Measurement and significance of bronchodilator response. In Jenne, J. W. & Murphy, S. (eds.): *Drug therapy for asthma*. pp. 535-613. Marcel Dekker New York-Basel, 1987.
23. Tattersfield, A. E. & McNicol, M. W.: Salbutamol and isoproterenol. A double-blind trial to compare bronchodilator and cardiovascular activity. *N. Engl. J. Med.* **281**, 1323-1326, 1969.
24. Thiringer, G. & Svedmyr, N.: Evaluation of skeletal muscle tremor due to bronchodilator agents. *Scand. J. Respir. Dis.* **56**, 93-102, 1975.
25. Thiringer, G. & Svedmyr, N.: Comparison of infused inhaled terbutaline in patients with asthma. *Scand. J. Respir. Dis.* **57**, 17-24, 1976.
26. Wallenstein, S., Zucker, C. L. & Fleiss, J. L.: Some statistical methods useful in circulation research. *Circ. Res.* **47**, 1-9, 1980.
27. Widmark, E. & Waldeck, B.: An experimental model for the study of transport of bronchodilating drugs through the tracheobronchial epithelium. *Acta Pharmacol Toxicol.* **59**, Suppl. V, 335, 1986.
28. Widmark, E. & Waldeck, B.: Physiological and pharmacological characterization of an in vitro vagus nerve-trachea preparation from guinea-pig. *J. Auton. Pharmacol.* **6**, 187-194, 1986.

Address:

Claes-Göran Löfdahl, M.D.

Dept. of Clinical Pharmacology

Sahlgren Hospital

Göteborg,

Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.