

The debate on S-enantiomers of β -agonists: Tempest in a teapot or gathering storm?

A certain drama is unfolding in the nearly 30-year attempt to clarify any serious adverse effects of β -agonists. Could the d-(S-) enantiomer in the racemic d/l (rac-, S/R) mixtures of these synthesized drugs be the culprit behind the purported increased morbidity and mortality envisioned by some?¹ Nearly inactive as β -agonists, the S-enantiomers have their own unique actions in complex systems and their own individual pharmacokinetic properties. Similar concerns are being voiced with other drug families possessing stereoisomers, including the β -antagonists.² Some of them are detailed in an editorial similar to this one.³

These concerns are not frivolous. The study presented in this issue (Nelson et al. *J Allergy Clin Immunol* 1998;102:943-52) is the first large-scale effort in asthmatic subjects to evaluate the long-term effects of these compounds by comparing thrice daily nebulized doses of the d/l-racemate of albuterol to the pure R (levo)-enantiomer administered over 4 weeks. At the very least, and in contrast to a single-dose study by another group,⁴ the study demonstrates greater potency of R-albuterol given alone than an equivalent amount given in the racemate, implying some interfering effect of the S-albuterol. If this were all, we could compensate with a larger dose of the racemate, but there are more insidious questions in the study. Over 4 weeks, the baseline FEV₁ rose slightly but insignificantly (approximately 0.1 L) in the placebo group and the 2 levalbuterol groups but not in the 2 racemate groups. These differences were greater and significant if confined to patients not receiving inhaled corticosteroids. Are these differences spurious or meaningful, arising perhaps from a deleterious effect of the S-albuterol? When referred to the placebo response over 8 hours, all drug groups also show a trend of declining area under the curve at the 4-week interval. Is this tachyphylaxis or rather a consequence of the rising placebo baseline curve? We are not given the absolute FEV₁ values to assist in making this judgement.

Abbreviation used

MDI: Metered-dose inhaler

Studies in the guinea pig have given us leads and helped define the issues. Like human airways, the smooth muscle of guinea pig airways contains β_2 -adrenoreceptors, and prejunctional autoinhibitory M₂-muscarinic receptors exist at cholinergic terminals. Dysfunction of these M₂-receptors can be produced by viral infection⁵ and allergic reactions⁶ with resulting bronchospasm. M₂-receptors are also believed to be dysfunctional in asthma.^{7,8} However, there are differences. The guinea pig has a prominent sympathetic innervation to its airway smooth muscle, whereas in humans such innervation is scarce or absent. As assessed by functional means, β_2 -adrenoreceptors have been presumed to be inhibitory to acetylcholine output in airway smooth muscle of several species, including humans.^{9,10} Yet when acetylcholine production is measured directly, β -agonists paradoxically are stimulatory in the guinea pig airway but have no discernible effect in the human airway.¹¹

By using data from studies of the guinea pig, one can model and develop ideas but can only speculate on their human application until many facets of human work converge on a consensus. We are not at that point with the S-enantiomers of β -agonists. A case in point is tachyphylaxis. In 1971, Conolly et al¹² proposed that the preceding epidemic of asthma deaths was due to airway receptor tachyphylaxis produced by the very large doses of isoproterenol then marketed in the metered-dose inhaler (MDI). This conclusion was based on findings that the protective action of isoproterenol against histamine in guinea pigs was lost after 48 hours of its intraperitoneal injections. Tachyphylaxis has been subsequently produced and studied in intact animals, airway smooth muscle, and smooth muscle cultures, as well as at the molecular level. Yet it has been difficult to reach agreement until recently concerning its existence in the stable asthmatic subject receiving treatment, and virtually no data exist in the acutely ill patient.

Among the most important findings emerging are that (1) the airway β_2 -adrenoreceptors of asthmatic subjects in a fatal attack are distinctly impaired compared with those from nonasthmatic subjects, although with a greater response to contractile agents¹³; (2) full agonists (isoproterenol, fenoterol, formoterol) produce tachyphylaxis

Received for publication Aug 13, 1998; revised Aug 25, 1998; accepted for publication Aug 27, 1998.

Reprint requests: John W. Jenne, MD, 54 PAA-KO Dr, Sandia Park, NM 87047.

J Allergy Clin Immunol 1998;102:893-5.

Copyright © 1998 by Mosby, Inc.

0092-6749/98 \$5.00 + 0 1/1/94136

more readily than partial agonists (albuterol terbutaline, salmeterol) both *in vitro*¹⁴ and *in vivo*¹⁵; (3) systemic corticosteroids rapidly reverse acutely produced tachyphylaxis,¹⁵⁻¹⁷ but inhaled corticosteroids in large doses do not prevent it¹⁸; (4) provocation challenge techniques with methacholine or histamine can quantitate tachyphylaxis from as little as 200 μ g albuterol per day,¹⁹ or salmeterol²⁰ (even from a single dose²¹) and can detect a 2-fold difference in delivered drug²²; and (5) protection against complex airway challenges (eg, exercise,²³ allergen,²⁴ and adenosine monophosphate²⁵) may even more readily show tachyphylaxis, perhaps because additional, more susceptible β_2 -adrenoceptors are involved.²⁵ Although the practical importance of such tachyphylaxis may be negligible when recommended treatment guidelines are followed, scenarios can certainly be visualized in which it could make a difference.

The peak bronchodilator response is poorly suited to detect tachyphylaxis because, as stressed by Ahrens et al^{26,27} and the Food and Drug Administration Proceedings,²⁸ even a single puff from the MDI in the subjects with mild-to-moderate asthma studied quickly increases airway caliber to the insensitive plateau range of its dose-response curve. Differences may then only appear in the area under the curve and duration of response. It is yet to be shown, however, that protection from challenge and bronchodilation are mechanistically any different as far as smooth muscle relaxation is concerned.

Guinea pig studies now lead to the cholinergic system, and sensitization to ovalbumin is usually required. Functional antagonism to isoproterenol or prostaglandin E₂ by carbacholine is enhanced 10-fold by ovalbumin sensitization, although not by histamine.²⁹ Fenoterol instilled intratracheally for 6 weeks, repeated ovalbumin injection, or both enhance responses to acetylcholine both *in vitro* and *in vivo*, whereas the response to fenoterol is unaffected as tested 72 hours later.³⁰ After rechallenge with ovalbumin, the sensitized guinea pig shows an increased cholinergic contractile response despite a normal response to β -agonists, probably because of events distal to the M₃-receptor.³¹ Such challenge releases mediators, which evidently act synergistically with the cholinergic response.³²

For more than a decade, Morley and coworkers^{33,34} have pursued the role of β -agonists in producing airway hyperresponsiveness in the guinea pig, and they are now focusing on S-enantiomers. In ovalbumin-sensitized animals, infusion of rac-albuterol for less than an hour totally suppresses bronchial obstruction produced by inhaled antigen, but when continued for 48 hours, the animals succumb to challenge.³⁵ These researchers discount tachyphylaxis of β_2 -adrenoceptors as the explanation, preferring to incriminate S-albuterol because inhaled isoproterenol or rac-albuterol is still protective, although tachyphylaxis is clearly only relative to the dose used and could have been overridden by larger effective doses. Infusion of S-albuterol over 1 hour enhances responsiveness to inhaled histamine, as does tracheal deposition of particulates containing S-albuterol, S-terbutaline, or S-isoproterenol. In all cases hyperresponsiveness requires an intact vagus nerve.

Tissue studies also point to the cholinergic system, but now direct measurement of acetylcholine after electric field stimulation avoids the uncertainties involved in previous functional studies (eg, from interfering NaNc neuropeptide release). In the purely β_2 action of cAMP production, the receptor affinity of the S-enantiomer of albuterol is several orders of magnitude less than that of the R-enantiomer,³⁶ and stimulation of acetylcholine release in equine respiratory smooth muscle is correspondingly much weaker.³⁷ However, when used in roughly equal concentration (as in the racemate), S-albuterol paradoxically seems to counteract the calcium-lowering effect of R-albuterol in uncontracted bovine trachea, actually increasing measured Ca⁺⁺ in smooth muscle cells and by itself causing cell shortening.³⁸ Earlier workers³⁹ also found a paradoxical rise in Ca⁺⁺ with addition of either isoproterenol or forskolin to uncontracted bovine trachea cells. But when contracted by methacholine or histamine, bovine tracheal smooth muscle cells are relaxed by isoproterenol, forskolin, and 8-Br-cAMP, and this correlates with calcium lowering.^{39,40} S-albuterol is found to have the characteristics of a contractile agonist operating through the phospholipase C pathways, reacting or cross-reacting at the M₃-muscarinic receptor.³⁸ If S-albuterol levels sufficiently exceed R-albuterol on cumulative dosing,^{34,41} it is conceivable that relaxation would be seriously impaired.

Returning to the article by Nelson et al in this issue, it is difficult to make any sense of the baseline variations at 4 and 5 weeks. The authors quite reasonably suggest that the relative failure of the 2 rac-albuterol baselines to rise with the others was due to the presence of S-albuterol. But should we uncritically accept the rising baselines in the placebo and levalbuterol groups? By history, all groups used their MDI rac-albuterol to a modest and similar degree (about 4 puffs/day), including the preliminary wash-out week. Were all patients previously on a larger intake, with the S-albuterol now subsiding to a lower level in the non-rac subjects? Or were rising baselines due to some extraneous factor, such as drop outs, seasonal effects, training, or holdover of drug from the last nebulized dose 8 or more hours previously? Examination of the 8-hour bronchodilation responses, particularly to 1.25 mg levalbuterol, raises this possibility. And why should inhaled corticosteroids mitigate these changes to the point of insignificance ($P = .41$)?

The study will undoubtedly be repeated in some form, perhaps with fewer dose groups, particularly to restudy the baseline changes and the effects of corticosteroids, which were both key to the study. Comparison of the 2 preparations on inherent responsiveness to methacholine challenge and other constrictants is crucial to the hyperresponsiveness issue. Examination of other β_2 -agonists such as terbutaline, the S-enantiomer of which does not accumulate and which also produces some tachyphylaxis,⁴² will be necessary. Certainly the idea of an adversely acting S-enantiomer, with all its implications for treatment, particularly of the acute attack, is novel and powerful, but it deserves thorough study before passing

judgement on the racemic preparations we now use so widely and satisfactorily.

John W. Jenne, MD
54 PAA-KO Dr
Sandia Park, NM 87047

REFERENCES

1. Sears MR, Taylor DR, Print CG, et al. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet* 1990;336:1391-6.
2. Stoschitzky K, Klein W, Lindner W. Time to reassess chiral aspects of β -adrenoceptor antagonists. *Trends Pharmacol Sci* 1997;18:306-7.
3. Ind PW. Salbutamol enantiomers. Early clinical evidence in humans. *Thorax* 1997;52:839-40.
4. Cockcroft DW, Swystun VA. Effect of single doses of S-salbutamol, R-salbutamol, racemic salbutamol and placebo on the airway response to methacholine. *Thorax* 1997;52:845-8.
5. Fryer AD, Jacoby DB. Parainfluenza virus infection damages inhibitory M_2 -muscarinic receptors on pulmonary parasympathetic nerves in the guinea pig. *Br J Pharmacol* 1991;102:267-71.
6. Fryer AD, Wills-Karp M. Dysfunction of M_2 -muscarinic receptors in pulmonary parasympathetic nerves after allergen challenge. *J Appl Physiol* 1991;71:2255-61.
7. Ayala LE, Ahmed T. Is there loss of a protective mechanism in asthma. *Chest* 1989;96:1285-91.
8. Minette PAH, Lammers J-WJ, Dixon CMS, McKusker MT, Barnes PJ. A muscarinic agent inhibits reflex bronchoconstriction in normal but not in asthmatic subjects. *J Appl Physiol* 1989;67:2461-5.
9. Minette PA, Barnes PJ. Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways. *J Appl Physiol* 1988;64:2532-7.
10. Rhoden KJ, Meldrum LA, Barnes PJ. Inhibition of cholinergic neurotransmission in human airways by β_2 -adrenoceptors. *J Appl Physiol* 1988;65:700-5.
11. Belvissi MG, Patel HJ, Takahashi T, Barnes PJ, Gienbycz MA. Paradoxical facilitation of acetylcholine release from parasympathetic nerves innervating guinea-pig trachea by isoprenaline. *Br J Pharmacol* 1996;117:1413-20.
12. Conolly ME, Davies DS, Dollery CT, George CF. Resistance to β_2 -adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). *Br J Pharmacol* 1971;43:389-402.
13. Bai TR, Prasad FW. Abnormalities in airway smooth muscle in fatal asthma. *Am Rev Respir Dis* 1990;141:552-7.
14. January B, Seibold A, Whaley B, et al. β_2 -Adrenergic receptor desensitization, internalization, and phosphorylation in response to full and partial agonists. *J Biol Chem* 1997;272:23871-9.
15. Ellul-Micallef R, Fenech FF. Effects of intravenous prednisolone in asthmatics with diminished adrenergic responsiveness. *Lancet* 1975;2:1269-71.
16. Stephan WC, Chick TW, Avner BP, Jenne JW. Tachyphylaxis to inhaled isoproterenol and the effect of methylprednisolone in dogs. *J Allergy Clin Immunol* 1980;65:105-9.
17. Tan KS, Grove A, McLean E, Gnosspeilus Y, Hall IP, Lipworth BJ. Systemic corticosteroid rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. *Am J Respir Crit Care Med* 1997;156:28-35.
18. Yates DH, Kharitonov SA, Barnes PJ. An inhaled glucocorticoid does not prevent tolerance to the bronchoeffective effect of a long-acting inhaled β_2 -agonist. *Am Rev Respir Crit Care Med* 1996;154:1603-7.
19. Bhagat R, Swystun VA, Cockcroft DW. Salbutamol-induced increased airway responsiveness to allergen and reduced protection versus methacholine: dose response. *J Allergy Clin Immunol* 1996;97:47-52.
20. Cheung D, Timmers MC, Zwinderman AH, Bel EH, Dijkman JH, Sterk PJ. Long-term effects of a long-acting β_2 -adrenoreceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma. *N Engl J Med* 1992;327:1198-20.
21. Verbeke AAPH, Hop WCJ, Creyghton FBM, et al. Airway hyperresponsiveness after a single dose of salmeterol and during four months of treatment in children with asthma. *J Allergy Clin Immunol* 1996;97:938-46.
22. Simons RER, Soni NR, Watson WTA, Bechner AB. Bronchodilator and bronchoprotective effects of salmeterol in young patients with asthma. *J Allergy Clin Immunol* 1992;90:840-6.
23. Ramage L, Lipworth BJ, Ingram CG, Cree JA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. *Respir Med* 1994;88:363-8.
24. Cockcroft DW, Swystun VA, Bhagat R. Interaction of inhaled β_2 -agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. *Am J Respir Crit Care Med* 1995;152:1485-9.
25. O'Connor BJ, Aikman SL, Barnes PJ. Tolerance to the non-bronchodilator effects of inhaled β_2 -agonists in asthma. *N Engl J Med* 1992;327:1204-8.
26. Ahrens RC. On comparing inhaled beta adrenergic agonists. *Ann Allergy* 1991;67:296-8.
27. Ahrens RC, Bonham AC, Annis L, Ries R. Use of bronchial provocation with histamine to compare the pharmacodynamics of inhaled albuterol and metaproterenol in asthmatic patients. *J Allergy Clin Immunol* 1987;79:876-82.
28. Proceedings of the FDA Joint Advisory Committee on Pharmacokinetics/Pharmacodynamics of Beta Agonists. Gaithersburg (MD): Food and Drug Administration; Aug 16, 1996.
29. Wills-Karp M, Gilmour MI. Increased cholinergic antagonism underlies impaired β_2 -adrenergic response in ovalbumin-sensitized guinea pigs. *J Appl Physiol* 1993;74:2729-35.
30. Wang Z, Bramby AM, McNamera A. Chronic fenoterol exposure increases in vivo and in vitro airway responses in guinea pigs. *Am J Respir Crit Care* 1994;149:960-5.
31. Whicker SD, Compton MR, Seale JP, Black JL. The effect of sensitization and aerosol antigen challenge in guinea pigs—studies of airway receptor function and characteristics. *Pulm Pharmacol* 1990;3:129-36.
32. Udem BJ, Adams GK III. An analysis of the functional interactions of selected contractile agonists in the guinea pig isolated trachea. *J Pharmacol Exp Ther* 1988;246:47-53.
33. Morley J. Anomalous effects of albuterol and other sympathomimetics in the guinea pig. *Clin Rev Allergy Immunol* 1996;14:65-89.
34. Handley DA, McCullough JR, Crowther SD, Morley J. Sympathomimetic enantiomers and asthma. *Chirality* 1998;10:262-72.
35. Mazzone L, Naef R, Chapman ID, Morley J. Hyperresponsiveness of airways following exposure of guinea pigs to racemic mixtures and diastomers of β_2 -selective sympathomimetics. *Pulm Pharmacol* 1994;7:367-76.
36. Penn RB, Frielle T, McCullough JR, Aberg G, Benovic JL. Comparisons of R-, S-, and RS-albuterol interaction with human β_2 - and β_2 -adrenergic receptors. *Clin Rev Allergy Immunol* 1996;14:37-45.
37. Zhang X-Y, Zhu F-X, Olszewski MA, Robinson NE. Effects of enantiomers of β_2 -agonists on ACh release and smooth muscle contraction in the trachea. *Am J Physiol* 1998;274:L32-8.
38. Mitra S, Ugur M, Ugur O, Goodman HM, McCullough JR, Yamaguchi H. (S)-Albuterol increases intracellular free calcium by muscarinic receptor activation and a phospholipase C-dependent mechanism in airway smooth muscle. *Mol Pharmacol* 1998;53:347-54.
39. Takuwa Y, Takuwa N, Rasmussen H. The effect of isoproterenol on intracellular calcium concentration. *J Biol Chem* 1998;263:762-8.
40. Hoiting BH, Meurs H, Schuiling M, Kuipers R, Elzinga CRS, Zaagsma J. Modulation of agonist-induced phosphoinositide metabolism, Ca^{++} signalling and contraction of airway smooth muscle by cyclic AMP-dependent mechanisms. *Br J Pharmacol* 1996;117:419-26.
41. Eaton EA, Walle UK, Wilson HM, Aberg G, Walle T. Stereoselective sulfate conjugation of salbutamol by human lung and bronchial epithelial cells. *Br J Clin Pharmacol* 1996;41:201-6.
42. Weber RW, Smith JA, Nelson HS. Aerosolized terbutaline in asthmatics: development of subsensitivity with long-term administration. *J Allergy Clin Immunol* 1982;70:417-22.