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Short communication

Salbutamol-induced airway hyperreactivity in guinea pigs is not due to a loss of its bronchodilator effect

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Abstract

Guinea pigs were treated for 10 days with (±)-salbutamol (0.2 mg/kg/day, delivered from subcutaneously implanted osmotic minipumps). Airway reactivity to intravenously administered histamine, methacholine and bombesin was substantially increased in salbutamol-treated guinea pigs relative to controls. In the same animals, the potency of intravenously administered salbutamol to reverse bombesin-induced bronchoconstriction remained unchanged thus exactly reflecting effects in man. In conclusion, subchronic administration of salbutamol at low doses to guinea pigs increases airway reactivity. Since the bronchorelaxant effect of salbutamol remained unchanged, desensitisation of β-adrenoceptors on airway smooth muscle is unlikely to account for this effect.

Keywords: Airway hyperreactivity; β-Adrenoceptor agonist; Salbutamol; β-Adrenoceptor; Receptor desensitisation; (Guinea pig)

1. Introduction

Recent evidence suggests that frequent use of inhaled β-adrenoceptor agonists can increase airway responsiveness, decrease stability of asthma, and increase morbidity and mortality by adversely affecting the severity of the disease (for review see Taylor and Sears, 1994). Moreover, studies in man show that β-adrenoceptor agonists, even after short or medium term administration of therapeutic doses, lose their protective effect against bronchoconstrictor stimuli but not their ability to induce bronchodilatation (O'Connor et al., 1992; Cheung et al., 1992).

Increased airway reactivity has also been observed in guinea pigs following acute, parenteral administration of isoprenaline or salbutamol (Sanjar et al., 1990; Galland and Blackman, 1993) or subchronic inhalation of fenoterol (Wang et al., 1994). However, whether these results fully resemble those in man is not clear since it was not established whether increased airway reactivity was associated with desensitisation of β-adrenoceptors and whether the bronchodilator effects of the β-adrenoceptor agonists were maintained in these animals.

We have approached this question by treating guinea pigs subchronically with low doses of the β-adrenoceptor agonist, salbutamol, and by subsequent measurement of airway reactivity to various spasmogens. In the same animals, the ability of salbutamol to reverse bronchoconstriction was investigated as an index of desensitisation of the β-adrenoceptors.

2. Materials and methods

2.1. Pretreatment

Male guinea pigs (390–510 g, BRL, Füllinsdorf, Switzerland) were equipped with osmotic minipumps (Alzet model 2002; volume 218 μl, Alza Corp., Palo Alto, USA) which released either (±)-salbutamol (0.2 mg/kg/day) or its vehicle (1% tartaric acid in 0.9% saline). The minipumps were implanted subcutaneously in the neck of the guinea pigs under light general anaesthesia with isoflurane. After 10 days, the minipumps were removed using the same anaesthetic protocol and the animals were allowed to recover for 1

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day. The minipumps were checked for proper release of their contents by measurement of the volume of the fluid remaining.

2.2. Measurement of lung function

Guinea pigs were anaesthetized with phenobarbital (100 mg/kg i.p.) and pentobarbital (30 mg/kg i.p.), then paralyzed with gallamine (10 mg/kg i.m.). Animals were ventilated via a tracheal cannula (8 ml/kg, 1 Hz) with a mixture of air and oxygen (45:55 v/v). Ventilation was monitored by a flow transducer (Fleisch type 0000, Zabona, Switzerland) in line with the respiratory pump (KTR5, Alfos Instruments, Biel-Benken, Switzerland). Pressure changes within the thorax were monitored directly via an intrathoracic cannula so that the pressure difference between the trachea and the thorax could be measured and displayed. From these measurements of flow and differential pressure, both resistance ($R_{L)}$ and compliance (C_{dyn}) were calculated with a digital electronic respiratory analyser (PMS 300, Mumed, London, UK) for each respiratory cycle.

2.3. Evaluation of airway reactivity and bronchorelaxation

Transient bronchoconstriction (measured as increases in R_L in cm $H_2O/(l/s)$) was induced by i.v. injection of 3 doses of methacholine (3.2, 4.2, 5.6 μg/kg) at 10 min intervals. This was followed by 3 i.v. injections of histamine (1.0, 1.8, 2.4 μg/kg). Thirty minutes later, sustained bronchoconstriction was induced by continuous i.v. infusion of bombesin (75–180 ng/kg/min). The dose of bombesin was adjusted to obtain increases in R_L between 450–550 cm $H_2O/(l/s)$. After submaximal, stable bronchoconstriction had been obtained (30–45 min after the start of the bombesin infusion), (±)-salbutamol was given cumulatively (1 ng/kg–10 μg/kg i.v.). The dose was increased when the effect of the previous dose had reached a plateau.

The bronchorelaxant effect of (±)-salbutamol was determined as reversal of bombesin-induced bronchoconstriction. The potency to reverse bronchoconstriction was expressed as ED_{50}, the dose which caused a 50% reduction of bronchoconstriction. The ED_{50} was determined using a non-linear curve fitting programme of the ORIGIN software package (MicroCal Software, Northampton, MA, USA).

2.4. Statistics

All data are expressed as means ± S.E.M. ($n = 5–6$). Comparison of means was performed using a two-tailed t-test for unpaired observations of the EXCEL software package (Microsoft, USA). Statistical significance was assumed at the 95% level of confidence ($P < 0.05$).

2.5. Drugs

(±)-Salbutamol base was dissolved in 0.9% saline containing 1% tartaric acid. Histamine dihydrochloride and methacholine chloride were dissolved in 0.9% saline; bombesin was dissolved in ethanol and diluted with phosphate buffer pH 7.4. All compounds were purchased from Sigma (Munich, Germany).

3. Results

In control animals (pretreated for 10 days with the vehicle of salbutamol, delivered from subcutaneously implanted minipumps), the basal value for airway resistance (R_L) was 128 ± 5 cm $H_2O/(l/s)$. Both histamine (1.0–2.5 μg/kg i.v.) and methacholine (3.2–5.6 μg/kg i.v.) increased R_L in those animals. The dose-response curves were flat and the increases obtained at the highest doses were 117.5 ± 44 cm $H_2O/(l/s)$ and 82.4 ± 13 cm $H_2O/(l/s)$ for histamine and methacholine, respectively (Fig. 1).

In animals pretreated for 10 days with salbutamol (0.2 mg/kg/day, delivered continuously from minipumps), the basal value for R_L was 115 ± 7 cm $H_2O/(l/s)$ and not significantly different from basal R_L in vehicle-treated animals. In salbutamol-treated animals, histamine and methacholine caused markedly greater increases in R_L than in control animals; at each dose of each spasmogen, the response was signifi-

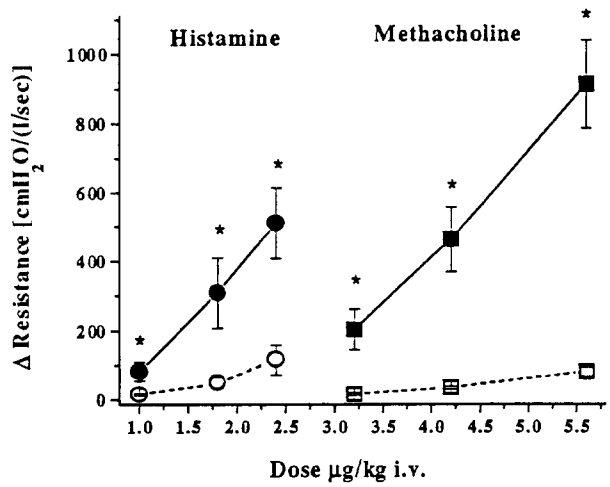

Fig. 1. Bronchoconstriction, measured as increase in airway resistance (R_L) caused by intravenous injection of histamine (circles) and methacholine (squares) in anaesthetized guinea pigs which were pretreated for 10 days with (±)-salbutamol (0.2 mg/kg/day, filled symbols) or its vehicle (open symbols), delivered by osmotic minipumps. Shown are means ± S.E.M. ($n = 5–6$); * Significant differences from control ($P < 0.05$).

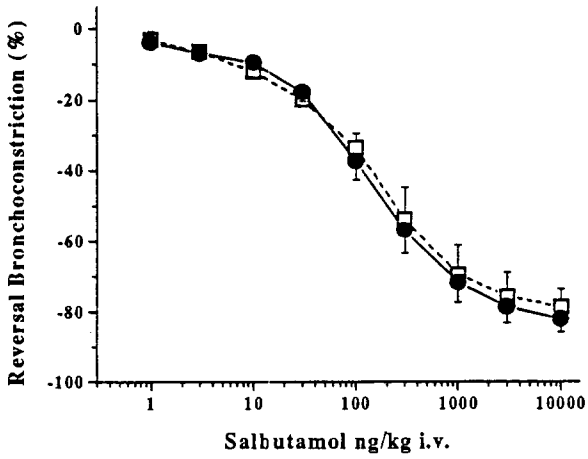

Fig. 2. Reversal of bombesin-induced bronchoconstriction by cumulative intravenous doses of (±)-salbutamol in anaesthetized guinea pigs pretreated for 10 days with either (±)-salbutamol (0.2 mg/kg/day, filled circles) or its vehicle (open squares), delivered by osmotic minipumps. Shown are means ± S.E.M. ($n = 5$–6).

cantly greater in salbutamol- than in vehicle-treated animals ($P < 0.05$). The increases at the highest doses used were 512.7 ± 103 cm H_2O/(l/s) and 911.8 ± 126 cm H_2O/(l/s) for histamine and methacholine, respectively (Fig. 1). In addition, the slope of the dose-response curves for histamine and methacholine increased markedly (Fig. 1).

In vehicle-treated animals, bombesin had to be infused at a dose of 142 ± 13 ng/kg/min to obtain an increase in R_L of 430–490 cm H_2O/(l/s). After salbutamol pretreatment, the dose required for a similar increase in R_L was 77 ± 15 ng/kg/min, which was significantly smaller than the dose used in control animals ($P < 0.05$).

(±)-Salbutamol administered i.v., reversed bombesin-induced bronchoconstriction dose-dependently (Fig. 2). There were no differences in the potency and efficacy between the control- and the salbutamol-pretreated groups (ED_{50}, E_{max} values were 385 ng/kg, 77.8% reversal and 363 ng/kg, 82% reversal for vehicle and (±)-salbutamol-pretreated animals, respectively). Small increases in heart rate (maximal increase at 10 μg/kg; 14.3 ± 1.7%) and mean arterial blood pressure (maximal increase at 10 μg/kg; 12.0 ± 2.1%) were recorded after i.v. administration of (±)-salbutamol in vehicle-treated animals. Identical effects were obtained in (±)-salbutamol-pretreated animals (maximal increases for heart rate and mean arterial blood pressure at 10 μg/kg were 12.6 ± 2.8% and 10.5 ± 2.9%, respectively.

4. Discussion

The results demonstrate that guinea pigs treated subchronically with low doses of (±)-salbutamol, continuously released from subcutaneously implanted minipumps, develop increased reactivity to histamine, methacholine and bombesin in vivo. Significantly, in the same animals, (±)-salbutamol reversed bombesin-induced bronchoconstriction and the dose-response curve for this effect was indistinguishable from that in vehicle-treated animals. This demonstrates that desensitisation of β-adrenoceptors on airway smooth muscle cannot be the cause of the increased airway reactivity induced by subchronic administration of (±)-salbutamol.

Increased airway reactivity has been described previously after *acute* administration of β-adrenoceptor agonists. In those experiments much higher doses of the β-adrenoceptor agonists were needed for an increase in airway reactivity. Thus, Sanjar et al. (1990) infused (±)-isoprenaline at a rate of 100 μg/kg for 1 h. Galland and Blackman (1993) used (±)-isoprenaline and (±)-salbutamol at a rate of 0.4 μmol/kg/h (equivalent to approximately 100 μg/kg/h for each compound) for 30 min. Our dose of 0.2 mg/kg/day (8 μg/kg/h) increased airway reactivity to a similar extent, thus demonstrating that much lower doses of salbutamol are needed to increase airway reactivity when administered over several days rather than acutely.

A comparison of our data with those from human studies (O'Connor et al., 1992; Cheung et al., 1992) is difficult since in clinical studies long-acting β-adrenoceptor agonists were administered by inhalation. Nevertheless, our results – at least qualitatively – fully resemble the situation in man where, after prolonged use of β-adrenoceptor agonists, a loss of the protective effect (O'Connor et al., 1992; Cheung et al., 1992) or even an increase in airway reactivity (Van Schayck et al., 1990) was observed which was not associated with receptor desensitisation since the bronchorelaxant effect of the β-adrenoceptor agonists was preserved. Based on these similarities, the present model could prove useful in the evaluation of the propensity of bronchodilators to induce airway reactivity upon long-term use.

The present results provide no explanation for the mechanism underlying the salbutamol-induced increase in airway reactivity. In man, long-term use of β-adrenoceptor agonists was suggested to enhance reactivity by increasing the exposure to inhaled particles such as allergen (Taylor et al., 1993). This cannot explain our results since non-allergic guinea pigs were employed. A potential explanation might be that subchronic or chronic use of β-adrenoceptor agonists causes a reduction of the concentration of circulating catecholamines thus leading to increased sensitivity to spasmogens. This and other hypotheses, however, remain to be evaluated.

In conclusion, our results indicate that subchronic

administration of low doses of a β-adrenoceptor agonist increases airway reactivity to spasmogens in guinea pigs. The effect is not associated with desensitisation of those β-adrenoceptors which reverse bronchoconstriction, as indicated by normal bronchodilator sensitivity, but otherwise awaits further elucidation. Our model could serve to estimate the propensity of routinely used bronchodilators to induce airway hyperreactivity as a serious adverse effect.

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References

Cheung, D., M.C. Timmers, A.H. Zwinderman, E.H. Bel, J.H. Dijkman and P.J. Sterk, 1992, Long-term effects of a long-acting β_2-adrenoceptor agonist, salmeterol, on airway hyperresponsiveness in patients with mild asthma, New Engl. J. Med. 327, 1198.

Galland, B.C. and J.G. Blackman, 1993, Enhancement of airway reactivity to histamine by isoprenaline and related β-adrenoceptor agonists in the guinea-pig, Br. J. Pharmacol. 108, 1016.

O'Connor, B.J., S.L. Aikman and P.J. Barnes, 1992, Tolerance of the nonbronchodilator effects of inhaled β_2-agonists in asthma, New Engl. J. Med. 327, 1204.

Sanjar, S., A. Kristersson, L. Mazzoni, J. Morley and E. Schaeublin, 1990, Increased airway reactivity in the guinea-pig follows exposure to intravenous isoprenaline, J. Physiol. 425, 43.

Taylor, D.R. and M.R. Sears, 1994, Regular beta-adrenergic agonists. Evidence, not reassurance, is what is needed, Chest 106, 552.

Taylor, D.R., M.R. Sears, G.P. Herbison, E.M. Flannery, C.G. Print, D.C. Lake, D.M. Yates, M.K. Lucas and Q. Li, 1993, Regular inhaled β agonist in asthma: effects on exacerbations and lung function, Thorax 48, 134.

Van Schayck, C.P., S.J. Graafsma, M.B. Visch, E. Dompeling, C. Van Weel and C.L.A. Van Herwaarden, 1990, Increased bronchial hyperresponsiveness after inhaling salbutamol during 1 year is not caused by subsensitization to salbutamol. J. Allergy Clin. Immunol. 86, 793.

Wang, Z.-L., A.M. Bramley, A. McNamara, P.D. Paré and T.R. Bai, 1994, Chronic fenoterol exposure increases in vivo and in vitro airway responses in guinea pigs, Am. J. Respir. Crit. Care Med. 149, 960.