

ORIGINAL ARTICLE

2Salbutamol-Induced Desensitization and Attempts to Resensitize In Vitro

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8 ABSTRACT

9 The study was carried out to desensitize spontaneously active isolated chick rectum with salbutamol in 10 log doses starting from 100 nanogram and resensitize with various drugs as a result to revive the 11 desensitized tissue and respond to Salbutamol. The tissue response after desensitization to alpha, beta 12 adrenergic and muscarinic acetylcholine receptor antagonists was isotonically recorded for 10 minutes 13 using thermostatically-controlled organ bath with aeration. The results with prazosin showed that the 14 tissue recovered from desensitization and exhibited spontaneous motility and responded to salbutamol 15 faster.

16 Keywords: Salbutamol, Chick rectum, Desensitization, Prazosin, Resensitization

18 common chronic inflammatory disease of the airways 43 tolerance [1-4], an increase in airway hyper-19 characterized by variable and recurring symptoms, 44 responsiveness to allergen [5], poor asthma control [6] 20 airflow obstruction and bronchial spasm. Symptoms 45 and even increased mortality [7]; effects which may be 21 include wheezing, coughing, chest tightness and 46 secondary to beta2-AR desensitization. SABAs are the 22 shortness of breath. Medications used to treat asthma 47 mainstay for the acute symptomatic treatment of asthma 23 are divided into 2 general classes:

25 symptoms including Short acting beta-2 agonists 50 medicines too frequently, the efficiency may decline, 26 (SABA) such salbutamol as 27 levosalbutamol, terbutaline and bitolterol.

29 further exacerbations including inhaled corticosteroids 54 have much longer side chains resulting in a 12-hour 30 such as hydrocortisone and beclomethasone; inhaled 55 effect. While patients report improved symptom control, 3 long acting beta-2 agonists (LABA) such as salmeterol 56 these drugs do not replace the need for routine rescuers and formeterol; inhaled anti-cholinergics such as 57 and their slow onset means the short acting dilators are 33 ipratropium and tiotropium; leukotriene modifiers such 58 still be required. However for the past 4 decades, there 34 as montelukast and zafirlukast; mast cell stabilizers such 59 has been a continuing debate concerning whether s as sodium cromoglicate and nedocromil sodium; methyl 60 regular chronic treatment with these drugs may be doing 36 xanthenes such as theophylline and imunomodulators 61 more harm than good [8]. In 2005, the USFDA released 37 such as omalizumab.

39 most-commonly used bronchodilators in both the acute 64 symptoms and in some cases death. In 2008, members 40 rescue and maintenance therapy of asthma. However, 65 of USFDA recommended withdrawing approval for 41 chronic mono-therapy with long-acting and/or short- 66 these medications in children. In 2010, USFDA gave

Asthma, from the Greek (asthma) meaning gasp, is a 42 acting beta₂-AR agonists have been associated with 48 and provide effective bronchial protection to a wide 1. Quick-relief medications used to treat acute 49 range of bronchial constrictor agents. By using these (Albuterol), 51 producing desensitization resulting in an exacerbation 52 of symptoms which may lead to refractory asthma and 2. Long-term control medications used to prevent 53 death. LABAs are similar in structure to SABAs but 62 a health advisory alerting the public to findings that Beta₂-adrenoceptor (beta₂-AR) agonists are the 63 show the use of LABA could lead to worsening of

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Fig 1. Effect of various concentrations of salbutamol (SAL) on isolated chick rectum in vitro



Fig 2. Effect of salbutamol (SAL) on isolated chick rectum and influence of prazocin (PRA) in vitro

67 new safety requirements for LABA that is, use of 106 Drug Solutions 68 LABAs are contraindicated without the use of an 69 asthma controller medication such as an inhaled 70 corticosteroid. Single-ingredient LABAs should only be 71 used in combination with an asthma controller 72 medication; they should not be used alone. The role of 73 beta-2 adrenoceptor in both the pathogenesis and 74 treatment of asthma has become a subject of intense 75 speculation and investigation for the last 25 years. This 76 study was carried out to resensitize the salbutamol-77 induced desensitization in spontaneously active isolated 78 chick rectum.

MATERIALS AND METHOD

80 Animals

Freshly-removed intestine of chick slaughtered at a 82 local chicken shop was immediately put into cold 500 83 ml Krebs solution, transferred to laboratory and aeration 84 provided immediately.

85 Methods

87 tract, was identified; 2-3 cm portion was cut and 127 relaxation. Salbutamol (300 µg) produced slight 88 transferred into Petri dish containing Krebs solution, 128 relaxation. Salbutamol (1 mg) did not produce any 89 trimmed off from the mesentery and other tissues. Krebs129 response showing desensitization (Fig 1). 90 solution was slowly passed through the lumen to flush130 91 out any contents. The rectum was mounted in a131 brief contraction followed by relaxation; with washings 92 thermostatically controlled organ bath and aerated. The132 the tone of the tissue went up to half the original 93 tissue response was isotonically recorded (tension 133 baseline. salbutamol (30 µg) produced relaxation, with 94 weight 1 gm, magnification 10 times) in non-cumulative 134 washings the tone did not regain its baseline. 95 and cumulative manner that this tissue invariably had 135 Salbutamol (100 µg) did not produce any response 96 spontaneous motility. The rectum was exposed to136 showing desensitization. Prazocin (10 µg) produced 97 salbutamol in log doses starting from 100 nanogram for137 tone and motility, then 10 µg salbutamol produced some 981 min each to record the tissue responses, until tissue138 relaxation, with washings the tone regained its baseline 99 stopped responding which is said to be desensitized.139 and 10 µg salbutamol produced prominent relaxation. 100 Continuing further, tissue responses with prazosin140 With washing, the tone did not rise and finally 101 (PRA) in different microgram concentrations were 141 salbutamol 10 µg produced slight relaxation. Second 102 observed for 5-10 minutes. Finally, once the tissue142 dose of prazocin (10 µg) reproduced tone and motility, 103 regained the original baseline and motility which can be143 and subsequent doses of 10 µg, 30 µg and 100 µg 104 resensitized, salbutamol in microgram concentrations144 salbutamol produced relaxations. Prazocin (30 µg) did 105 produced responses.

Tyrodes solution (composition: sodium chloride 8.0 08 gm, potassium chloride 0.2 gm, magnesium chloride 0.1 09 gm, calcium chloride 0.2 gm, sodium bicarbonate 1.0 10 gm, dextrose 1.0 gm, distilled water 1 litre).

Krebs solution (composition: sodium chloride 6.9 gm, potassium chloride 0.35 gm, calcium chloride 0.28 gm, sodium bicarbonate 2.1 gm, magnesium sulphate 40.29 gm, potassium/sodium di-hydrogen phosphate 0.15 15 gm, dextrose 2.0 gm, distilled water 1 litre).

Salbutamol obtained as Asthalin respiratory solution 117 purchased from drug store and prepared dilutions of 118 100ng, 300ng, 1µg, 3 µg, 10 µg, 30 µg, 100 µg, 300 µg and 1mg using distilled water. Prazosin tablets purchased from local drug store, dissolved in distilled 121 water, filtered and prepared different concentrations in 122 micrograms.

RESULTS

Salbutamol (SAL) in log dose range of 100 ng to 30 125 µg produced dose dependent relaxations; 100 µg of The rectum, the end part of the gastro-intestinal126 Salbutamol produced initial contraction followed by

> As shown in Fig 2, salbutamol (10 µg) produced a 145 not produce any response, with wash, the tone went up

Resensitizing Salbutamol-Induced Desensitization



ence of various concentration of prazocin (PRA) in vitro

147 response.

As shown in Fig 3, salbutamol (3 µg) produced 149 relaxation, with washings the tone did not regain to 150 baseline. Subsequent addition of two doses of 151 salbutamol (10 μ g) produced slight relaxations. 152 Prazocin (10 µg) produced tone and motility and in the 153 presence of prazocin, SAL (10 µg) produced relaxation. 154 Similarly prazocin in several fixed doses of 30 µg and a 155 single dose of 10 µg produced tone and motility 156 followed by prominent relaxation with salbutamol (10^{-1}) 157 μg). Continuing in second tracing in the Fig 3, four₂₀₁ achieving our goal of finding out the possible 158 doses of salbutamol 30 µg were added with intermittent202 combination of prazocin with salbutamol which can 159 washings, the first dose did not produce any response,203 help the asthma patient in getting relief without any 160 the second dose produced some relaxation, third and 204 danger or emergencies. 161 fourth doses did not produce any response. Prazocin (30

- 162 µg) did not produce any response, a second higher dose
- 163 of prazocin (100 µg) produced tone and motility and in205 REFERENCES
- 164 the presence of 10 μg salbutamol-produced relaxation.2061. 165 Similarly prazocin in different doses was added and²⁰⁷ 166 produced tone and motility and in its presence 2092
- 167 salbutamol-produced relaxations. As shown in Fig 4, first dose of 10 µg salbutamol 113. 169 produced relaxation; second dose of salbutamol $(10 \ \mu g)_{212}$ 170 produced slight relaxation. Subsequent three cumulative²¹³ 171 doses of 10 µg salbutamol did not produce any response 154 172 could be due to desensitization. Prazocin (100 µg) did 173 not produce any response, second dose of 30 μg_{217} 174 prazocin produced contraction. Salbutamol (10 µg)2185. 175 produced relaxation. Subsequent addition of three219 176 cumulative fixed doses of 10 μ g salbutamol did not²²⁰ 177 produce any response but the tone fell down. Two sets²²¹⁶ 178 of prazocin and cumulative doses of salbutamol 179 produced contractions followed by relaxations224

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EFFECT OF CUMULATIVE DOSES SALBUTAMOL ON ISOLATED CHICK RECTUM & INFLUENCE OF PHY SOLN -- KREB'S; TEMP -- 37 ± 1°C; TENSION -- 1 gm; MAGNIFIED -- 1:9; BATH VOL -- 10 m



Fig 4. Effect of cumulative doses of salbutamol (SAL) on isolated chick rectum and influence of various concentration of prazocin (PRA) in vitro

DISCUSSIONS

Salbutamol produced desensitization at beta-2 183 receptor in Fig 1. Many of our experiments showed that Fig 3. Effect of salbutamol (SAL) on isolated chick rectum and influE84 salbutamol is not specific beta-2 adrenergic receptor 185 agonist, it acts on both alpha and beta receptors i.e., 186 producing immediate contraction followed by a slower 187 relaxation and this could be the component which is 188 responsible for sudden deaths in asthma patients [9-14]. 146 and finally prazocin 100 µg did not produce any¹⁸⁹ Salbutamol produced response by acting on alpha-1 and 190 beta-2 receptors till receptor saturation, Prazosin per se 91 produced tone and motility, and it seems to facilitate relaxation. Combination of salbutamol-prazosin by alternate administration showed beneficial effects. This is fairly satisfactory combination which might help in preventing the desensitization. The numerous experiments are quite supportive that salbutamol and prazosin combination could be a suitable combination in the therapy of asthma. The actual mechanism involved 9 in tissue resensitization is subject of further research.

It is concluded that to certain extent we succeeded in

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