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ORIGINAL ARTICLE

Salbutamol-Induced Desensitization and Attempts to Resensitize In Vitro

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ABSTRACT

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

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

Asthma, from the Greek (asthma) meaning gasp, is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, airflow obstruction and bronchial spasm. Symptoms include wheezing, coughing, chest tightness and shortness of breath. Medications used to treat asthma are divided into 2 general classes:

1. Quick-relief medications used to treat acute symptoms including Short acting beta-2 agonists (SABA) such as salbutamol (Albuterol), levosalbutamol, terbutaline and bitolterol.

2. Long-term control medications used to prevent further exacerbations including inhaled corticosteroids such as hydrocortisone and beclomethasone; inhaled long acting beta-2 agonists (LABA) such as salmeterol and formeterol; inhaled anti-cholinergics such as ipratropium and tiotropium; leukotriene modifiers such as montelukast and zafirlukast; mast cell stabilizers such as sodium cromoglicate and nedocromil sodium; methyl xanthenes such as theophylline and imunomodulators such as omalizumab.

Beta$_2$-adrenoceptor (beta$_2$-AR) agonists are the most-commonly used bronchodilators in both the acute rescue and maintenance therapy of asthma. However, chronic mono-therapy with long-acting and/or short-acting beta$_2$-AR agonists have been associated with tolerance [1-4], an increase in airway hyper-responsiveness to allergen [5], poor asthma control [6] and even increased mortality [7]; effects which may be secondary to beta2-AR desensitization. SABAs are the mainstay for the acute symptomatic treatment of asthma and provide effective bronchial protection to a wide range of bronchial constrictor agents. By using these medicines too frequently, the efficiency may decline, producing desensitization resulting in an exacerbation of symptoms which may lead to refractory asthma and death. LABAs are similar in structure to SABAs but have much longer side chains resulting in a 12-hour effect. While patients report improved symptom control, these drugs do not replace the need for routine rescuers and their slow onset means the short acting dilators are still be required. However for the past 4 decades, there has been a continuing debate concerning whether regular chronic treatment with these drugs may be doing more harm than good [8]. In 2005, the USFDA released a health advisory alerting the public to findings that show the use of LABA could lead to worsening of symptoms and in some cases death. In 2008, members of USFDA recommended withdrawing approval for these medications in children. In 2010, USFDA gave

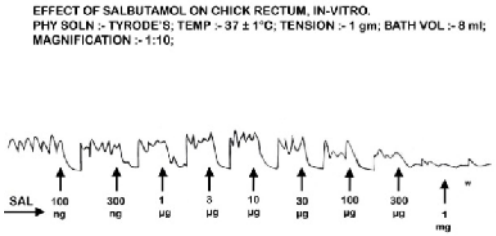

Fig 1. Effect of various concentrations of salbutamol (SAL) on isolated chick rectum in vitro

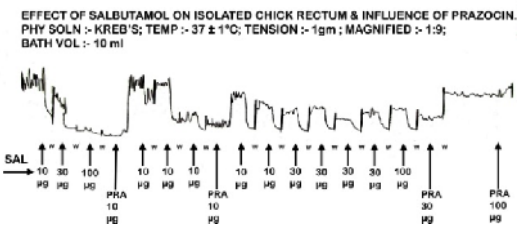

Fig 2. Effect of salbutamol (SAL) on isolated chick rectum and influence of prazocin (PRA) in vitro

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

MATERIALS AND METHOD

Animals

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

Methods

The rectum, the end part of the gastro-intestinal tract, was identified; 2-3 cm portion was cut and transferred into Petri dish containing Krebs solution, trimmed off from the mesentery and other tissues. Krebs solution was slowly passed through the lumen to flush out any contents. The rectum was mounted in a thermostatically controlled organ bath and aerated. The tissue response was isotonically recorded (tension weight 1 gm, magnification 10 times) in non-cumulative and cumulative manner that this tissue invariably had spontaneous motility. The rectum was exposed to salbutamol in log doses starting from 100 nanogram for 1 min each to record the tissue responses, until tissue stopped responding which is said to be desensitized. Continuing further, tissue responses with prazosin (PRA) in different microgram concentrations were observed for 5-10 minutes. Finally, once the tissue regained the original baseline and motility which can be resensitized, salbutamol in microgram concentrations produced responses.

Drug Solutions

Tyrodes solution (composition: sodium chloride 8.0 gm, potassium chloride 0.2 gm, magnesium chloride 0.1 gm, calcium chloride 0.2 gm, sodium bicarbonate 1.0 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 0.29 gm, potassium/sodium di-hydrogen phosphate 0.15 gm, dextrose 2.0 gm, distilled water 1 litre).

Salbutamol obtained as Asthalin respiratory solution purchased from drug store and prepared dilutions of 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 water, filtered and prepared different concentrations in micrograms.

RESULTS

Salbutamol (SAL) in log dose range of 100 ng to 30 µg produced dose dependent relaxations; 100 µg of Salbutamol produced initial contraction followed by relaxation. Salbutamol (300 µg) produced slight relaxation. Salbutamol (1 mg) did not produce any response showing desensitization (Fig 1).

As shown in Fig 2, salbutamol (10 µg) produced a brief contraction followed by relaxation; with washings the tone of the tissue went up to half the original baseline. salbutamol (30 µg) produced relaxation, with washings the tone did not regain its baseline. Salbutamol (100 µg) did not produce any response showing desensitization. Prazocin (10 µg) produced tone and motility, then 10 µg salbutamol produced some relaxation, with washings the tone regained its baseline and 10 µg salbutamol produced prominent relaxation. With washing, the tone did not rise and finally salbutamol 10 µg produced slight relaxation. Second dose of prazocin (10 µg) reproduced tone and motility, and subsequent doses of 10 µg, 30 µg and 100 µg salbutamol produced relaxations. Prazocin (30 µg) did not produce any response, with wash, the tone went up

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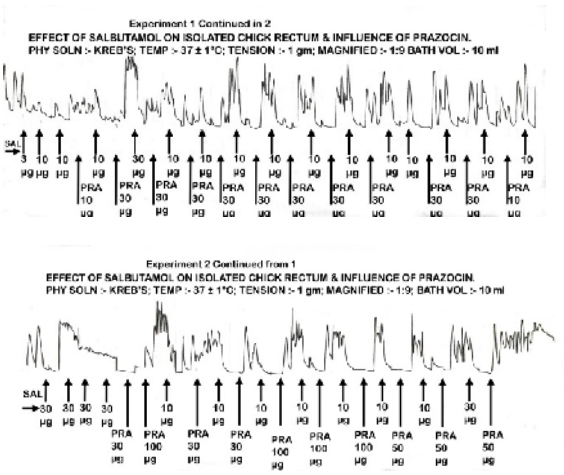

Fig 3. Effect of salbutamol (SAL) on isolated chick rectum and influence of various concentration of prazocin (PRA) in vitro

and finally prazocin 100 µg did not produce any response.

As shown in Fig 3, salbutamol (3 µg) produced relaxation, with washings the tone did not regain to baseline. Subsequent addition of two doses of salbutamol (10 µg) produced slight relaxations. Prazocin (10 µg) produced tone and motility and in the presence of prazocin, SAL (10 µg) produced relaxation. Similarly prazocin in several fixed doses of 30 µg and a single dose of 10 µg produced tone and motility followed by prominent relaxation with salbutamol (10 µg). Continuing in second tracing in the Fig 3, four doses of salbutamol 30 µg were added with intermittent washings, the first dose did not produce any response, the second dose produced some relaxation, third and fourth doses did not produce any response. Prazocin (30 µg) did not produce any response, a second higher dose of prazocin (100 µg) produced tone and motility and in the presence of 10 µg salbutamol-produced relaxation. Similarly prazocin in different doses was added and produced tone and motility and in its presence salbutamol-produced relaxations.

As shown in Fig 4, first dose of 10 µg salbutamol produced relaxation; second dose of salbutamol (10 µg) produced slight relaxation. Subsequent three cumulative doses of 10 µg salbutamol did not produce any response could be due to desensitization. Prazocin (100 µg) did not produce any response, second dose of 30 µg prazocin produced contraction. Salbutamol (10 µg) produced relaxation. Subsequent addition of three cumulative fixed doses of 10 µg salbutamol did not produce any response but the tone fell down. Two sets of prazocin and cumulative doses of salbutamol produced contractions followed by relaxations respectively.

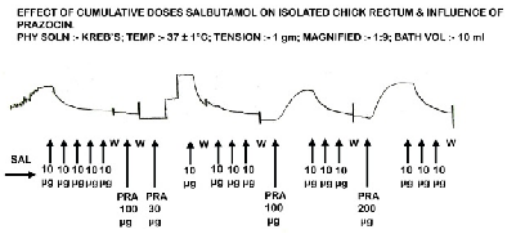

Fig 4. Effect of cumulative doses of salbutamol (SAL) on isolated chick rectum and influence of various concentration of prazocin (PRA) in vitro

DISCUSSIONS

Salbutamol produced desensitization at beta-2 receptor in Fig 1. Many of our experiments showed that salbutamol is not specific beta-2 adrenergic receptor agonist, it acts on both alpha and beta receptors i.e., producing immediate contraction followed by a slower relaxation and this could be the component which is responsible for sudden deaths in asthma patients [9-14]. Salbutamol produced response by acting on alpha-1 and beta-2 receptors till receptor saturation, Prazosin per se 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 in tissue resensitization is subject of further research.

It is concluded that to certain extent we succeeded in achieving our goal of finding out the possible combination of prazocin with salbutamol which can help the asthma patient in getting relief without any danger or emergencies.

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