Salbutamol-Induced Desensitization and Attempts to Resensitize In Vitro

MOHAMMED SULTAN MOHIDDIN SAJID

For author affiliations, see end of text.
Received February 9, 2012; Revised June 29, 2012; Accepted August 12, 2012

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
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 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) in 1 gm, magnification 10 times) in non-cumulative and cumulative manner that this tissue invariably had a brief contraction followed by relaxation. Salbutamol (300 μg) produced slight relaxation. Salbutamol (1 mg) did not produce any relaxation. Salbutamol produced initial contraction followed by relaxation. Salbutamol (10 μg) produced a 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 regained its baseline. As shown in Fig 2, salbutamol (10 μg) produced a second relaxation, with washings the tone did not regain its baseline. Salbutamol (100 μg) did not produce any response. Salbutamol in log doses starting from 100 nanogram for 1 min each to record the tissue responses, until tissue regained its baseline and cumulative manner that this tissue invariably had a brief contraction followed by relaxation. Salbutamol (300 μg) produced slight relaxation. Salbutamol (1 mg) did not produce any relaxation.

**Drug Solutions**

Krebs 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).

Tyrodes solution (composition: sodium chloride 8.0 gm, potassium chloride 0.2 gm, magnesium chloride 0.1 gm, calcium chloride 0.2 gm, potassium dihydrogen phosphate 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 relaxation showing desensitization (Fig 1).

As shown in Fig 2, salbutamol (10 μg) produced a second relaxation, with washings the tone did not regain its baseline. Salbutamol (100 μg) did not produce any response showing desensitization. Prazosin (10 μg) produced salbutamol produced slight relaxation; 100 μg of salbutamol produced prominent relaxation. Second dose of prazosin (10 μg) reproduced tone and motility, regained the original baseline and motility which can be observed for 5-10 minutes. Finally, once the tissue regained its baseline and subsequent doses of 10 μg, 30 μg and 100 μg produced relaxations. Prazosin (30 μg) did not produce any response, with wash, the tone went up.

**Discussion**

One possible mechanism for the salbutamol-induced desensitization is that repeated exposure to the agonist stimulates the receptor stimulating the G protein, which then binds to and activates a downstream effector cascade. This cascade can result in decreased receptor expression or increased receptor turnover, leading to a decrease in receptor density and a decrease in response to continued stimulation. It is possible that the repeated exposure to salbutamol in this study caused a similar mechanism to occur, leading to decreased receptor expression and a decrease in response to continued stimulation. This could explain why the tissue did not respond to subsequent doses of salbutamol.
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 relaxations. 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.

### DISCUSSIONS

Salbutamol produced desensitization at beta-2 receptor in Fig 1. Many of our experiments showed that salbutamol is not specific beta-2 adrenergic 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 a 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.

### REFERENCES


CURRENT AUTHOR ADDRESSES

Mohammed Sultan Mohiddin Sajid, E-mail: sultanmsajidm@gmail.com