

1 ORIGINAL ARTICLE

2 **Salbutamol-Induced Desensitization and Attempts to**
3 **Resensitize In Vitro**

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7 This paper is available online at <http://ijpt.iums.ac.ir>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 isotonicly 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*

17 Asthma, from the Greek (asthma) meaning gasp, is a 42 acting beta₂-AR agonists have been associated with
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: 48 and provide effective bronchial protection to a wide

24 1. Quick-relief medications used to treat acute 49 range of bronchial constrictor agents. By using these
25 symptoms including Short acting beta-2 agonists 50 medicines too frequently, the efficiency may decline,
26 (SABA) such as salbutamol (Albuterol), 51 producing desensitization resulting in an exacerbation
27 levosalbutamol, terbutaline and bitolterol. 52 of symptoms which may lead to refractory asthma and

28 2. Long-term control medications used to prevent 53 death. LABAs are similar in structure to SABAs but
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,
31 long acting beta-2 agonists (LABA) such as salmeterol 56 these drugs do not replace the need for routine rescuers
32 and formoterol; 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
35 as sodium cromoglicate and nedocromil sodium; methyl 60 regular chronic treatment with these drugs may be doing
36 xanthenes such as theophylline and immunomodulators 61 more harm than good [8]. In 2005, the USFDA released
37 such as omalizumab. 62 a health advisory alerting the public to findings that

38 Beta₂-adrenoceptor (beta₂-AR) agonists are the 63 show the use of LABA could lead to worsening of
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

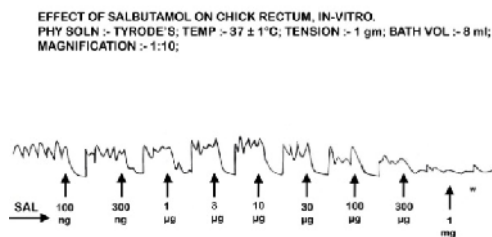


Fig 1. Effect of various concentrations of salbutamol (SAL) on isolated chick rectum in vitro

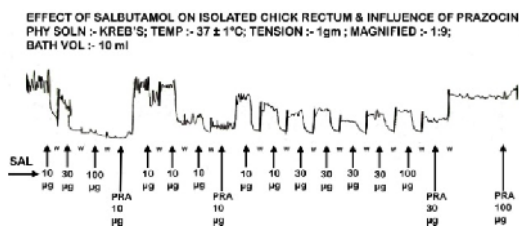


Fig 2. Effect of salbutamol (SAL) on isolated chick rectum and influence of prazosin (PRA) in vitro

67 new safety requirements for LABA that is, use of
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

Animals

81 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.

Methods

86 The rectum, the end part of the gastro-intestinal
87 tract, was identified; 2-3 cm portion was cut and
88 transferred into Petri dish containing Krebs solution,
89 trimmed off from the mesentery and other tissues. Krebs
90 solution was slowly passed through the lumen to flush
91 out any contents. The rectum was mounted in a
92 thermostatically controlled organ bath and aerated. The
93 tissue response was isotonicly recorded (tension
94 weight 1 gm, magnification 10 times) in non-cumulative
95 and cumulative manner that this tissue invariably had
96 spontaneous motility. The rectum was exposed to
97 salbutamol in log doses starting from 100 nanogram for
98 1 min each to record the tissue responses, until tissue
99 stopped responding which is said to be desensitized.
100 Continuing further, tissue responses with prazosin
101 (PRA) in different microgram concentrations were
102 observed for 5-10 minutes. Finally, once the tissue
103 regained the original baseline and motility which can be
104 resensitized, salbutamol in microgram concentrations
105 produced responses.

Drug Solutions

107 Tyrodes solution (composition: sodium chloride 8.0
108 gm, potassium chloride 0.2 gm, magnesium chloride 0.1
109 gm, calcium chloride 0.2 gm, sodium bicarbonate 1.0
110 gm, dextrose 1.0 gm, distilled water 1 litre).

111 Krebs solution (composition: sodium chloride 6.9
112 gm, potassium chloride 0.35 gm, calcium chloride 0.28
113 gm, sodium bicarbonate 2.1 gm, magnesium sulphate
114 0.29 gm, potassium/sodium di-hydrogen phosphate 0.15
115 gm, dextrose 2.0 gm, distilled water 1 litre).

116 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
119 and 1mg using distilled water. Prazosin tablets
120 purchased from local drug store, dissolved in distilled
121 water, filtered and prepared different concentrations in
122 micrograms.

RESULTS

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

130 As shown in Fig 2, salbutamol (10 µg) produced a
131 brief contraction followed by relaxation; with washings
132 the tone of the tissue went up to half the original
133 baseline. salbutamol (30 µg) produced relaxation, with
134 washings the tone did not regain its baseline.
135 Salbutamol (100 µg) did not produce any response
136 showing desensitization. Prazocin (10 µg) produced
137 tone and motility, then 10 µg salbutamol produced some
138 relaxation, with washings the tone regained its baseline
139 and 10 µg salbutamol produced prominent relaxation.
140 With washing, the tone did not rise and finally
141 salbutamol 10 µg produced slight relaxation. Second
142 dose of prazosin (10 µg) reproduced tone and motility,
143 and subsequent doses of 10 µg, 30 µg and 100 µg
144 salbutamol produced relaxations. Prazocin (30 µg) did
145 not produce any response, with wash, the tone went up

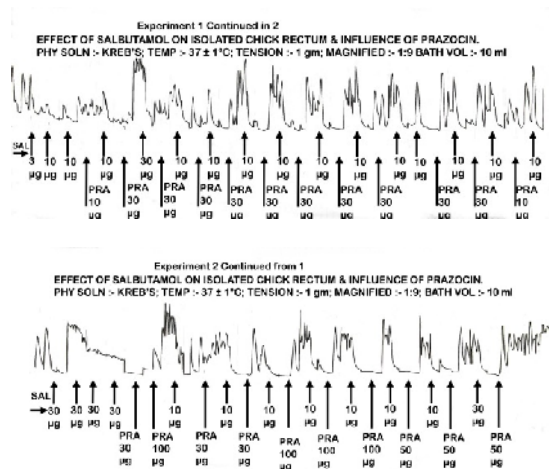


Fig 3. Effect of salbutamol (SAL) on isolated chick rectum and influence of various concentration of prazosin (PRA) in vitro

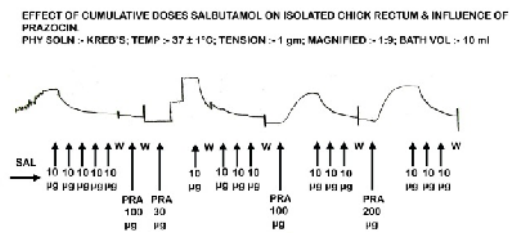


Fig 4. Effect of cumulative doses of salbutamol (SAL) on isolated chick rectum and influence of various concentration of prazosin (PRA) in vitro

DISCUSSIONS

146 and finally prazosin 100 μg did not produce any
147 response.

148 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 μg) produced slight relaxations.
152 Prazocin (10 μg) produced tone and motility and in the
153 presence of prazosin, SAL (10 μg) produced relaxation.
154 Similarly prazosin 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
157 μg). Continuing in second tracing in the Fig 3, four
158 doses of salbutamol 30 μg were added with intermittent
159 washings, the first dose did not produce any response,
160 the second dose produced some relaxation, third and
161 fourth doses did not produce any response. Prazocin (30
162 μg) did not produce any response, a second higher dose
163 of prazosin (100 μg) produced tone and motility and in
164 the presence of 10 μg salbutamol-produced relaxation.
165 Similarly prazosin in different doses was added and
166 produced tone and motility and in its presence
167 salbutamol-produced relaxations.

168 As shown in Fig 4, first dose of 10 μg salbutamol
169 produced relaxation; second dose of salbutamol (10 μg)
170 produced slight relaxation. Subsequent three cumulative
171 doses of 10 μg salbutamol did not produce any response
172 could be due to desensitization. Prazocin (100 μg) did
173 not produce any response, second dose of 30 μg
174 prazosin produced contraction. Salbutamol (10 μg)
175 produced relaxation. Subsequent addition of three
176 cumulative fixed doses of 10 μg salbutamol did not
177 produce any response but the tone fell down. Two sets
178 of prazosin and cumulative doses of salbutamol
179 produced contractions followed by relaxations
180 respectively.

81

182 Salbutamol produced desensitization at beta-2
183 receptor in Fig 1. Many of our experiments showed that
184 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].
189 Salbutamol produced response by acting on alpha-1 and
190 beta-2 receptors till receptor saturation, Prazosin per se
191 produced tone and motility, and it seems to facilitate
192 relaxation. Combination of salbutamol-prazosin by
193 alternate administration showed beneficial effects. This
194 is fairly satisfactory combination which might help in
195 preventing the desensitization. The numerous
196 experiments are quite supportive that salbutamol and
197 prazosin combination could be a suitable combination in
198 the therapy of asthma. The actual mechanism involved
199 in tissue resensitization is subject of further research.

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

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