Bidder’s organ extract induced anaphylaxis in experimental animals

A Gomes, M Das, M I Alam & S C Dasgupta
Laboratory of Toxinology and Experimental Pharmacodynamics, Department of Physiology, University of Calcutta, 92, A P C Road, Calcutta 700 009, India

Received 5 February 1999; revised 16 September 1999

Bidder’s organ (BO, a vestigial organ), present in toad Bufo melanosticus (Schenider), is a characteristic feature of all male bufos. Its possible anaphylactic properties are investigated on experimental animals. BO extract produced both in vivo and in vitro anaphylactic reaction in guinea pig. Dyspnoea and bronchoconstriction was a major cause of anaphylactic death. Blood histamine level was significantly increased in the anaphylactic animals. BO extract significantly released histamine from chopped lung preparation, an action antagonised by disodium chromoglycate. BO extract degranulated peritoneal mast cells in vitro. Passive cutaneous anaphylactic reactions were enhanced by BO extract and were significantly inhibited by disodium chromoglycate. Anaphylotoxin (identity not known) present in bidder’s organ is probably involved in toad defence.

Bidder’s organ, a vestigial organ, is a predominant feature of male amphibian species of the genus Bufo. The functional status of this gland is still an open question. It has been pointed out that bidder’s organ is definitely involved in toad defence. Subsequently, a haemolytic protein toxin and a lethal cardiotoxin has been identified from the crude extract of this vestigial organ. The present investigation explored bidder’s organ extract induced anaphylaxis in experimental animals.

Chemicals—Chemicals used in the present investigation were agarose A (SRL, India), amido black 10B (Sigma, USA), compound 48/80 (Sigma, USA), disodium chromoglycate (DSCG; Unique Pharmaceutical Laboratory, India), disodium hydrogen orthophosphate (Merck, India), Freund’s complete adjuvant (Sigma, USA), histamine diphasphate (Sigma, USA), ovalbumin (Sigma, USA), potassium dihydrogen phosphate (Merek, India), sodium azide (Merck, India), thiomersal (Sigma, USA) and toluidine blue (Sigma, USA).

Collection and preparation of bidder’s organ (BO) extract—Experimental animals, adult male toad (40-50 g) Bufo melanosticus, (Schneider) were obtained commercially during March to September. The animals were pithed and bidder’s organ samples were collected and stored at −20°C until used. The frozen organ was then homogenized with chilled phosphate buffer (0.01 M, pH 7.2) and centrifuged at 15,000 rpm at 4°C for 20 min. The supernatant, crude BO extract, was stored at 4°C. Concentration of the extract has been expressed in terms of protein equivalent.

Determination of LD 50 —BO extract was administered in batches of male Swiss albino mice (20 g, iv) at different concentrations. Mortality was recorded after 24 hr and LD 50 was calculated.

Anaphylactic reaction (in vivo)—Male guinea pigs (250 ± 20 g, n=4 in each group) were divided into five groups and sensitized with BO extract (0.2; 0.4; 0.8; 1.2 and 1.6 mg/kg, sc) on day 1. On day 7th, the same groups of animals were challenged with BO extract (0.2; 0.4; 0.8; 1.2 and 1.6 mg/kg, sc) and mortality was recorded in each group within 24 hr. Effective anaphylactic dose (EAD) was defined as the minimum dose of BO extract, which produced 100% mortality within 24 hr after the second challenging dose of BO extract.

Anaphylactic reaction (in vivo)—Male guinea pig (250 ± 20 g) were sensitized with BO extract (250 μg, sc) on day 1. After one week, ileum was removed from the sensitized guinea pig and suspended in Tyrode’s solution at 37°C bubbled with carbogen (95%, O2 + 5%, CO2). Contraction was recorded on a smoked drum with a frontal writing lever. ED 50 of BO, was calculated in both BO-sensitized and non-sensitized control guinea pig ileum.

Blood histamine level—Male guinea pig (250 ± 20 g) was sensitized with BO extract (250 μg, sc). After one week same guinea pig was challenged with BO extract (250 μg, ip). After 12 hr, blood was collected from fasting guinea pigs and histamine level was measured fluorometrically.
Histamine release from chopped lung—Lung perfusion was carried out as described by Brocklehurst. Lung of guinea pig was removed, suspended in a double-walled chamber at 37°C and perfused through the pulmonary artery with oxygenated (95%, O₂ + 5%, CO₂) Tyrode solution. Chopped perfused guinea pig lung was prepared. About 100 mg of lung tissue was suspended in 3 ml oxygenated Tyrode at 37°C and incubated with BO extract (20 μg/ml), saline control and compound 48/80 (1 μg/ml) for 30 min. The slides were then stained with 0.1% toluidine blue and observed under microscope to determine the percentage of degranulated and intact mast cells.

Passive cutaneous anaphylaxis (PCA): Antiserum production—Male albino rabbit, New Zealand strain (2.5 ± 0.2 kg) were immunized with ovalbumin (1 mg) and Freund’s complete adjuvant (mixed v/v, 1:1) and injected sc in 1st, 2nd and 3rd week. After one week rest, booster injection of ovalbumin (1 mg; iv) was given for another 3 weeks (one injection in each week). 2 mg of ovalbumin was used in the whole schedule. The rabbits were finally bled by cardiac puncture, one week after the final booster dose of ovalbumin. Serum was obtained by centrifugation at 900 g for 15 min. Thiomersal was added as a preservative and the serum was stored at 4°C until further use.

Testing of antibody—Immunoglobulin diffusion was carried out after ouchterlony by using agarose A (1%) in normal saline containing sodium azide (0.01%). Indirect haemagglutination test was performed to assess the potency of antiserum titre. Serum was diluted until cessation of visible reaction with antigen (ovalbumin) by agglutination of sheep RBC, which indirectly gives the titre of antibody.

PCA testing—Swiss albino male mice (20 ± 2 g) were passively sensitized by intradermal injection of ovalbumin antiserum (1:20 dilution) at two separate sites near the dorsal midline. After 48 hr, BO extract (20 μg, ip) / DSCG (5 mg, ip) was injected. After 1 hr of treatment, ovalbumin (0.1 mg) dissolved in saline (0.9%) with Evan’s blue (1%, 0.1 ml) dye was injected into the tail vein of each animal. After 3 hr, mice were killed by cervical dislocation and the dorsal skin reflected. Diameter of blue spots at each site of antiserum injected was measured. Diameter size for control and BO extract/DSCG treated group was compared.

All results are expressed as Mean ± SEM. The significance of the difference between mean was determined by Student’s t test.

LD₅₀ of BO extract was found to be 10 mg/kg (iv) in male albino mice. BO extract induced mortality due to anaphylactic shock was established in guinea pig. Anaphylactic in vivo lethal dose of BO extract was found to be 2.0 mg/kg (ip) in guinea pig.

Anaphylaxis in vitro—BO extract sensitized guinea pig ileum produced strong contractile response in presence of BO extract (0.1 μg/ml). ED₅₀ of BO extract on sensitized guinea pig ileum was 0.2 μg/ml, whereas ED₅₀ of nonsensitized control guinea pig ileum was 5 μg/ml. BO extract (2.5 mg/kg, ip) significantly increased the blood histamine level in anaphylactic guinea pigs (21.52 ± 1.29 μg/ml) as compared to control guinea pigs (7.97 ± 0.25 μg/ml). Both BO extract (20 μg/ml) and compound 48/80 (1 μg/ml) significantly released histamine from guinea pig chopped lung preparation. Disodium chromoglycate (0.1 mg/ml) significantly antagonised histamine release from chopped lung preparation. DSCG exhibited about 33.99 ± 5.22% protection against BO induced histamine release. BO extract (50 μg/ml) degranulated (78.35 ± 1.73%) rat peritoneal mesentery cell in vitro, as compared with compound 48/80 (88.83 ± 2.53%). DSCG (0.1 mg/ml) effectively antagonised BO extract induced mast cell degranulation. About 36.83 ± 3.7% protection fold was achieved with DSCG. Rabbit antiserum raised against ovalbumin showed identical precipitation bands in immunoglobulin diffusion. Indirect haemagglutination titre of the antiserum was found to be 1:4096. Rabbit antiserum (0.1 ml, 1:20 dilution, id) injected in male albino mice produced a diameter of 10 ± 2 mm, due to antigen ovalbumin and Evan’s blue challenge. BO extract (35 μg in 0.1 ml, sc) significantly
increased the diameter of blueing as compared to control. DSCG (2 mg /20 g) significantly antagonised (62.71 ± 4.96%) BO extract induced anaphylactic reaction in mice (Table 1).

During screening of toxic profile of BO extract, it was observed that the extract was lethal in several species including rat, mice and guinea pig. Guinea pigs were insensitive towards the first low dose exposure of the extract but subsequent second exposure was fatal. This observation leads to the detailed study of the extract in the development of anaphylactic reactions in animals. The anaphylactic shock induced lethality in guinea pig was established. The major sign of death was dyspnea, bronchoconstriction and respiratory failure. Among several bronchoconstrictor agents, histamine was found to be one of the major contributing factor as evident by (1) rise of blood histamine in the anaphylactic animals, and (2) antagonism of anaphylactic death by disodium chromoglycate, a potent antihistaminic agent. It has been recognised clinically that DSCG is able to attenuate airway hyperreactivity of asthmatics to histamine. Involvement of histamine was identified and its level increased significantly in the anaphylactic animals. In vitro experiment confirmed that BO extract induced histamine release from mast cell antagonised by DSCG. Among the anaphylaxis test used, passive cutaneous anaphylaxis in mice is the most relevant test as immediate hypersensitivity reaction which is antagonised by DSCG. Histamine involvement was further demonstrated in BO-induced mast cell degranulation and histamine release from chopped lung preparation. Here also DSCG significantly interfere BO-induced histamine release phenomenon. Along with the histamine, other bioactive modulators, like slow reacting substances (SRS), thromboxane, leukotriene may be involved in this anaphylactic reaction. Present investigation confirmed the presence of anaphylatoxin in BO-extract. Further experiments are required to confirm the nature and mechanism of action of this anaphylatoxin present in bidder’s organ. Anaphylatoxin present in bidder’s organ was also responsible for defence mechanism in toad.

The authors thank Unique pharmaceutical Laboratory, Bombay, for providing disodium chromoglycate (DSCG) as gift.

### References

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control (µg/ml blood)</th>
<th>Compound (µg/g tissue)</th>
<th>Bidder’s organ extract (BO)</th>
<th>Disodium chromoglycate (DSCG) + Bidder’s organ (BO) extract</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood histamine</td>
<td>7.97 ± 0.25</td>
<td>—</td>
<td>21.52 ± 1.27*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Histamine release</td>
<td>1.23 ± 0.19</td>
<td>22.02 ± 1.99*</td>
<td>20.86 ± 0.81*</td>
<td>7.09 ± 0.68</td>
<td>33.99 ± 5.22</td>
</tr>
<tr>
<td>Mast cell degranulation (%)</td>
<td>7.83 ± 0.88</td>
<td>88.83 ± 2.53*</td>
<td>78.35 ± 1.73*</td>
<td>28.86 ± 2.23</td>
<td>36.83 ± 3.70</td>
</tr>
<tr>
<td>Passive cutaneous anaphylaxis (mm diam)</td>
<td>8.83 ± 0.41</td>
<td>—</td>
<td>19.51 ± 0.56*</td>
<td>12.23 ± 0.78</td>
<td>62.71 ± 4.96</td>
</tr>
</tbody>
</table>

* p<0.01

Table 1—Effect of bidder’s organ extract on blood histamine level, histamine release, mast cell degranulation and passive cutaneous anaphylaxis

[Values are mean ± SE of four individuals in each experiment]