Preventive Effects of Theophylline on Anaphylactic Shock in Rats

Eiichiro OKABE

Department of Pharmacology, Kanagawa Dental College,
82 Inaoka-cho, Yokosuka 238, Japan

Accepted January 7, 1980

Abstract—Anaphylactic shock was induced by administration of ovalbumin to sensitized rats. Preventive effects of theophylline on anaphylactic shock were examined with regard to the relationship between cyclic AMP and prostaglandin (PG) E₂ in lung tissue, and plasma histamine. During anaphylactic shock, levels of cyclic AMP content and PGE₂ content in lung tissue decreased, while plasma histamine content increased. Theophylline increased levels of cyclic AMP content and PGE₂ content in lung tissue, in a dose dependent manner, and pretreatment of animals with theophylline prevented the onset of anaphylactic shock. Pretreatment with indomethacin abolished the preventive effects of theophylline on anaphylactic shock, and the effect of theophylline on the cyclic AMP content. Dibutyryl cyclic AMP (DBcyclic AMP) increased PGE₂ content in lung tissue, in a dose dependent manner, and also prevented the onset of anaphylactic shock. These effects of DBcyclic AMP were inhibited by the pretreatment with indomethacin, and here, cyclic AMP in lung tissue was maintained at a high level. In the group in which anaphylactic shock was prevented with these interventions, PGE₂ content in lung tissue was significantly high in all cases. In addition PGE₂ infusion prevented anaphylactic shock. These data suggest that theophylline increases cyclic AMP levels in lung tissue only in the presence of endogenous PG, that increased cyclic AMP content in lung tissue subsequently increases PGE₂ content in lung tissue, and that the preventive effects of theophylline on anaphylactic shock result from increased PGE₂ content in lung tissue.

Acute and specific pathophysiologic changes occur in both circulatory and cell functions during shock (1, 2). The onset of these changes is induced by interaction of various shock mediators (3–8). Histamine (3–6) is one of these shock mediators and is considered to play an important role in the onset of anaphylactic shock. In our previous experiments (9) theophylline was found to inhibit the increase in plasma histamine level during anaphylactic shock and therefore to prevent anaphylactic shock. These findings led to the assumption that theophylline prevented anaphylactic shock by increasing levels of adenosine 3′-5′-monophosphate (cyclic AMP), a substance which regulates (10–12) the production and release of shock mediators and maintains extensive cell functions (13), by inhibiting phosphodiesterase activity (13, 14). It was suggested that prostaglandin (PG) E₂ also inhibits the decomposition of cyclic AMP (15, 16), therefore, PGE₂ may be related to the preventive effects of theophylline on anaphylactic shock. As there is apparently no documentation on this subject, we attempted to elucidate the role of PGE₂ in the mechanism of preventive

General principles of this paper were presented at "The first annual conference on shock (Airlie, Virginia, 1978)" and "The second annual conference on shock (Williamsburg, Virginia, 1979)".
effects of theophylline on anaphylactic shock. We compared the action of theophylline with the action of indomethacin, a potent inhibitor of PG synthesis (17, 18).

MATERIALS AND METHODS

Male Wistar rats 4 weeks of age were purchased and after acclimatization for a week, 0.5 ml of a 2% ovalbumin-complete adjuvant emulsion per rat was given i.m. into the hind-leg twice a week for 5 weeks. After the sensitization the antibody titre of IgG was about 64-fold and that of IgE was 43-fold. Anaphylactic shock was induced by i.v. administration of 0.2 ml of 2% ovalbumin-physiologic saline per rat. Experiments of preventive effects of theophylline, indomethacin, dibutyryl cyclic AMP (DBcyclic AMP), PGE$_2$ and combination of these drugs on anaphylactic shock were conducted as follows: Sensitized rats were anesthetized by an i.p. administration of pentobarbital sodium 30 mg/kg per rat, and pretreated with chosen drugs; anaphylactic shock was then induced and 1 hr survivors were examined. The carotid pressure was measured by a pressure transducer (Nippon Koden MTU-0.5) and the heart rate was recorded with a polygraph (Nippon Koden RM-45) through a tachometer (Nippon Koden RT-5) in Lead II of ECG. Each drug was given i.p. or i.v. in a volume of 0.1 ml/100 g body weight.

Determination of cyclic AMP: Samples extracted from lung tissue were immediately fixed by focussed microwave irradiation (600W, 2,450 MHz) for 5 sec (19). The blood was washed out by physiologic saline and the physiologic saline was removed by filter paper. Each sample was weighed out by 100 mg wet weight. Then 1.0 ml of 6% trichloroacetic acid (TCA) was added to the samples, and the mixture was homogenized. After centrifugation at 3,000 g for 20 min and removal of the protein fraction, 0.1 ml of 1N-HCl was added. Extraction with a 2-fold volume of ethyl ether was repeated five times to remove TCA, then the remaining supernatant was warmed to 80°C in a warm bath in a draft, to completely evaporate the remaining ether. The liquid phase was lyophilized and was redissolved in 2.0 ml of a 50 mM acetate buffer solution at pH 4.0, and this sample was preserved at -20°C. The quantitative determination of cyclic AMP was carried out by the protein binding method of Gilman (20). The materials used for assay were cyclic AMP standard test drug and ³H-cyclic AMP obtained from Boehringer Mannheim Co.; cellulose ester filters of HAWP-02500 purchased from the Millipore Co. All determinations were conducted in duplicate.

Determination of plasma histamine: Plasma histamine levels were measured by a modified method of Shore et al. (21). Blood was taken from the pulmonary artery and immediately centrifuged at 10,000 r.p.m. at 4°C for 10 min and plasma was obtained; a 9-fold volume of 0.4N-HCIO$_4$ was added to the obtained plasma, and the mixture was centrifuged at 3,000 r.p.m. at 4°C for 15 min, then a protein fraction was removed and histamine was extracted with butanol from the supernatants. After the extraction, histamine was coupled with O-phthalaldehyde (OPT) at a highly alkaline pH, and the fluorescent assay was conducted. The histamine standard test drug was obtained from the Merck Co., the OPT from commercial sources, and OPT was purified for the assay. The fluorescence
at 450 m\textmu{} resulting from activation at 360 m\textmu{} was measured using a spectrofluorometer (Hitachi 204).

**Determination of PGE\textsubscript{2}**: For the determination of PGE\textsubscript{2}, 200 mg wet weight of lung tissue was weighed and homogenized at 0°C after adding 10-fold volumes of 10\textsuperscript{-3} M indomethacin/L-95% ethanol. Precipitates were allowed to remain overnight at 4°C and were then centrifuged at 3,000 g for 15 min. Each precipitate was washed with absolute ethanol three times and supernatants were combined and evaporated to near dryness. The residues were redissolved in ethanol-water (2 : 1, by volume) and washed three times with petroleum ether (boiling point : 40–60°C). After a removal of the petroleum ether phase, the ethanol was removed with a concentrator (Taiyo TC-8) before acidification to pH 3.0 with 1N-HCl. The aqueous phase was then extracted three times with equal volumes of ethyl ether. The organic phases were combined, evaporated to dryness, and redissolved in acetate-methanol (3 : 1, by volume) for thin-layer chromatography to isolate PGE\textsubscript{2}.

The determination of PGE\textsubscript{2} was performed by the radioimmunoassay of a double antibody method according to Levine et al. (22). PGE\textsubscript{2} was a gift from Japan Upjohn Co. and the PG radioimmunoassay kit (Clinical Assay Co.) was used for the determination.

**RESULTS**

Relationship between cyclic AMP content in lung tissue and plasma histamine content during anaphylactic shock: After ovalbumin challenge, cyclic AMP content in lung tissue was decreased gradually and a significant decrease was reached in 60 sec (\textit{p}<0.05), whereas plasma histamine content was increased significantly also in 60 sec (\textit{p}<0.05) (Fig. 1). The 1 hr survivors of anaphylactic rats was zero, and the survival time was 163.3±8 sec (mean±SE) (Table 1). Cyclic AMP, histamine and PGE\textsubscript{2} levels determined 60 sec after ovalbumin challenge are shown in Table 2 and Figs. 3–5.

![Fig. 1. Relationship between cyclic AMP content in lung tissue and plasma histamine content during anaphylactic shock. The amount of cyclic AMP or histamine at zero time represents the control value. ( )=number of rats.](image_url)
TABLE 1. Effects of pretreatment with theophylline, indomethacin, DBcyclic AMP and PGE₂ on survival rate of ovalbumin-induced anaphylactic shock in rats

<table>
<thead>
<tr>
<th>Group and pretreatment</th>
<th>1 hr survivors (number of animals)</th>
<th>Mean survival time±SE (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovalbumin (ALB)</td>
<td>0/28</td>
<td>163.32±7.90</td>
</tr>
<tr>
<td>Theophylline (Theo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 mg/kg i.p. + ALB</td>
<td>8/10</td>
<td></td>
</tr>
<tr>
<td>60 mg/kg i.p. + ALB</td>
<td>27/28</td>
<td></td>
</tr>
<tr>
<td>90 mg/kg i.p. + ALB</td>
<td>12/13</td>
<td></td>
</tr>
<tr>
<td>Indomethacin (IDM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/kg i.v. + ALB</td>
<td>0/9</td>
<td>174.11±32.53</td>
</tr>
<tr>
<td>10 mg/kg i.v. + ALB</td>
<td>0/13</td>
<td>185.92±22.36</td>
</tr>
<tr>
<td>15 mg/kg i.v. + ALB</td>
<td>0/16</td>
<td>158.50±18.51</td>
</tr>
<tr>
<td>DBcyclic AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg/kg i.v. + ALB</td>
<td>9/10</td>
<td></td>
</tr>
<tr>
<td>IDM + Theo + ALB</td>
<td>3/25</td>
<td></td>
</tr>
<tr>
<td>IDM + DBcyclic AMP + ALB</td>
<td>3/18</td>
<td></td>
</tr>
<tr>
<td>PGE₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 µg/50 µl/kg/min infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 min + ALB</td>
<td>1/9</td>
<td></td>
</tr>
<tr>
<td>5 min + ALB</td>
<td>5/8</td>
<td></td>
</tr>
<tr>
<td>10 min + ALB</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>15 min + ALB</td>
<td>7/9</td>
<td></td>
</tr>
<tr>
<td>20 min + ALB</td>
<td>6/8</td>
<td></td>
</tr>
</tbody>
</table>

ALB=2% ovalbumin 0.2 ml/rat i.v.. Theophylline was injected 15 min before an injection of ALB. Indomethacin or DBcyclic AMP was injected 10 min before ALB. Indomethacin was injected 10 min before an injection of theophylline or DBcyclic AMP. PGE₂ infusion was continued until ALB injection.

*Effects of theophylline and indomethacin on anaphylactic shock:* Cyclic AMP content in lung tissue of sensitized rats 15 min after the i.p. administration of theophylline was increased in a dose dependent manner; a significant increase was observed after injection of 30 mg/kg i.p. \( p<0.001 \).

Half maximum (30 mg/kg) and maximum (60 mg/kg) doses of theophylline to increase the cyclic AMP content in lung tissue were estimated from the dose-response curve (Fig. 2). Pretreatments with these doses of theophylline 15 min prior to ovalbumin challenge prevented the onset of anaphylactic shock (Table 1); in theophylline-pretreated group, the cyclic AMP content in lung tissue was significantly higher \( p<0.01 \) while plasma histamine content was significantly lower \( p<0.01 \) than values in anaphylactic induced-shock group (ALB) (Fig. 3). Effects of theophylline on the cyclic AMP content were inhibited by pretreatment of animals with indomethacin (10 mg/kg i.v.), as shown in Fig. 2.

Pretreatment with indomethacin 10 min before ovalbumin challenge did not prevent the onset of anaphylactic shock (Table 1); the cyclic AMP and PGE₂ levels were decreased significantly (Fig. 4 and Table 2).
FIG. 2. Effect of indomethacin on relationship between dose of theophylline and cyclic AMP content in lung tissue. Theophylline was injected 15 min before an extraction of lung tissue. Indomethacin was injected (10 mg/kg i.v.) 10 min before an injection of theophylline. The amount of cyclic AMP at zero dose represents the control value without theophylline treatment. IDM=indomethacin.

FIG. 3. Effects of pretreatment with theophylline on cyclic AMP content in lung tissue and plasma histamine content of ovalbumin-induced anaphylactic rats. ALB=2% ovalbumin 0.2 ml/rat i.v.. Theophylline was injected 15 min before ALB and lung tissue was extracted 1 min after the ALB injection. ( )=number of rats.

Effects of indomethacin on preventive effects of theophylline on anaphylactic shock:
Pretreatment with theophylline (60 mg/kg i.p.) 15 min before ovalbumin challenge prevented the onset of anaphylactic shock. Pretreatment with DBcyclic AMP (5 mg/kg i.v.) 10 min before ovalbumin challenge also prevented the onset of anaphylactic shock (Table 1). However, when animals were treated with indomethacin (10 mg/kg i.v.) 10 min before the administration of theophylline or DBcyclic AMP, the preventive effects of theophylline and DBcyclic AMP were abolished and 1 hr survivors were remarkably decreased (Table 1). In the group pretreated with theophylline, the levels of cyclic AMP and PGE₂ in lung tissue remained substantially the same or higher, in comparison with the sensitized group, but administration of indomethacin in addition to theophylline decreased these levels to the levels seen in the anaphylactic induced-shock group (Table 2). In these experimental groups, there was a significant correlation (r=0.885) between the levels of cyclic AMP and PGE₂ in lung tissue (Fig. 5). Concomitant pretreatment with indomethacin and DBcyclic AMP did not prevent anaphylactic shock. In this case, cyclic AMP content was increased
significantly \((p<0.001)\), while \(\text{PGE}_2\) content was decreased markedly \((p<0.001)\) (Table 2).

Effects of pretreatment with theophylline, \(\text{DBcyclic AMP}\) and indomethacin on cyclic AMP and \(\text{PGE}_2\) contents in lung tissue of ovalbumin-induced anaphylactic rats

![Graph showing cyclic AMP content in lung tissue of ovalbumin-induced anaphylactic rats with effects of pretreatment with indomethacin.](image.png)

**FIG. 4.** Effect of pretreatment with indomethacin on cyclic AMP content in lung tissue of ovalbumin-induced anaphylactic rats. ALB=2% ovalbumin 0.2 ml/rat i.v. Indomethacin was injected 10 min before ALB and lung tissue was extracted 1 min after the ALB injection. sens=sensitized group. ( )=number of rats.

**TABLE 2.** Effects of pretreatment with theophylline, \(\text{DBcyclic AMP}\) and indomethacin on cyclic AMP and \(\text{PGE}_2\) contents in lung tissue of ovalbumin-induced anaphylactic rats

<table>
<thead>
<tr>
<th>Group and pretreatment</th>
<th>(n)</th>
<th>cyclic AMP content ((\text{pmol/mg lung wet wt}))</th>
<th>(\text{PGE}_2) content ((\text{ng/g lung wet wt}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitized</td>
<td>(7)</td>
<td>1.064±0.040</td>
<td>14.29±0.65</td>
</tr>
<tr>
<td>Ovalbumin (ALB)</td>
<td>(5)</td>
<td>0.864±0.032&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.72±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Theophylline (Theo)</td>
<td>(4)</td>
<td>1.378±0.048&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.95±1.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 mg/kg i.p. + ALB</td>
<td>(6)</td>
<td>2.549±0.033&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.38±0.43&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(\text{DBcyclic AMP}) 5 mg/kg i.v. + ALB</td>
<td>(4)</td>
<td>0.673±0.079&lt;sup&gt;o&lt;/sup&gt;</td>
<td>3.32±0.26</td>
</tr>
<tr>
<td>Indomethacin (IDM)</td>
<td>(4)</td>
<td>0.737±0.212&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.56±0.58&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>IDM + Theo + ALB</td>
<td>(4)</td>
<td>2.250±0.030&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.08±0.53&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a: \(p<0.01\) (vs. line 1), b: \(p<0.001\) (vs. line 1), c: \(p<0.01\) (vs. line 2), d: \(p<0.001\) (vs. line 2), e: \(p<0.05\) (vs. line 2), f: \(p<0.05\) (vs. line 3), g: \(p<0.001\) (vs. line 3), h: \(p<0.001\) (vs. line 4). ALB=2% ovalbumin 0.2 ml/rat i.v. Theophylline was injected 15 min before ALB and lung tissue was extracted 1 min after the ALB injection. \(\text{DBcyclic AMP}\) or indomethacin was injected 10 min before ALB and lung tissue was extracted 1 min after the ALB injection. Indomethacin was injected 10 min before theophylline or \(\text{DBcyclic AMP}\). Results are the means±SE.

**Effects of theophylline on \(\text{PGE}_2\) content in lung tissue:** \(\text{PGE}_2\) content in lung tissue of sensitized rats 15 min after the i.p. administration of theophylline was increased in a dose dependent manner; a significant increase \((p<0.001)\) was observed with a dose of 30 mg/kg i.p. (Fig. 6). The half maximum and maximum doses of theophylline required to increase the \(\text{PGE}_2\) content were much the same as those which elevated the cyclic AMP level (Fig. 2).

\(\text{PGE}_2\) content 10 min after the i.v. administration of \(\text{DBcyclic AMP}\) was also increased
in a dose dependent manner; a significant increase ($p<0.001$) was observed with a dose of 5 mg/kg (Fig. 6). When PGE$_2$ (0.5 $\mu$g/50 $\mu$l/kg/min) was continuously infused via the femoral vein, cyclic AMP content in lung tissue was increased in a manner dependent on the period of infusion from zero time to 15 min; a significant increase ($p<0.05$) was

![Fig. 5. Relationship between variations in PGE$_2$ content and cyclic AMP content in lung tissue; the group with the onset of anaphylactic shock (○, △), the sensitized group (●), the group preventing anaphylactic shock with the pretreatment with theophylline (▲). ALB=2% ovalbumin 0.2 ml/rat i.v.. Theophylline was injected (60 mg/kg i.p.) 15 min before ALB and lung tissue was extracted 1 min after the ALB injection. Indomethacin was injected (10 mg/kg i.v.) 10 min before an injection of theophylline. Theo=theophylline. IDM=indomethacin.](image)

![Fig. 6. Relationship between dose of DBcyclic AMP or theophylline and PGE$_2$ content in lung tissue. DBcyclic AMP was injected 10 min before an extraction of lung tissue. Theophylline was injected 15 min before an extraction of lung tissue. The amount of PGE$_2$ at zero dose represents the control value without DBcyclic AMP or theophylline treatment.](image)

![Fig. 7. Relationship between period of infusion of PGE$_2$ and cyclic AMP content in lung tissue. PGE$_2$ infusion was continued until extraction of lung tissue, via the femoral vein. The amount of cyclic AMP at zero period represents the control value without PGE$_2$ infusion.](image)
observed in the infusion for 5 min (Fig. 7). Infusion of PGE₂ longer than 5 min prevented the onset of anaphylactic shock (Table 1).

**DISCUSSION**

Theophylline is a xanthine derivative which increases cyclic AMP levels by inhibiting phosphodiesterase activity (13, 14, 23). In the present experiments, theophylline increased cyclic AMP and PGE₂ contents in lung tissue, a target organ (24–26) of systemic anaphylactic reactions, in a dose-dependent manner (Figs. 2 and 6); the onset of anaphylactic shock was prevented by theophylline (Table 1). Levels of cyclic AMP and PGE₂ in the group pretreated with theophylline were significantly high in comparison with those in the anaphylactic induced-shock group; these levels were the same as those of the sensitized group. An increase in plasma histamine content induced by ovalbumin challenge was inhibited by pretreatment with theophylline. These results suggest two possibilities: (1) that the preventive effects of theophylline on anaphylactic shock are due to an increase in cyclic AMP levels which regulate (10–12) the release of histamine, and (2) that the effects are induced by an increase in PGE₂ content which has the same action (27, 28) as cyclic AMP.

Since the preventive effects of theophylline on anaphylactic shock were abolished by pretreatment with indomethacin, a potent inhibitor of PG synthesis (17, 18) (Table 1), and the increases in cyclic AMP and PGE₂ levels induced by theophylline in lung tissue were diminished significantly at the same time, endogenous PG may be closely related to the preventive effects of theophylline on anaphylactic shock.

DBcyclic AMP which directly increases cellular cyclic AMP levels (29–31) also prevented the onset of anaphylactic shock. When the endogenous PGE₂ level was decreased by pretreatment with indomethacin, these preventive effects of DBcyclic AMP disappeared, regardless of the high level of cyclic AMP in lung tissue. Furthermore, continuous infusion of PGE₂ prevented anaphylactic shock. When the onset of anaphylactic shock was prevented, the PGE₂ content was always maintained at a high level (Fig. 5). These results support the second possibility stated above.

Since the half maximum and maximum doses of theophylline required to increase the PGE₂ levels were much the same as those which elevated the cyclic AMP levels and the fact that DBcyclic AMP increased PGE₂ levels in a dose-dependent manner (Figs. 2 and 6), it was suggested that an increase in PGE₂ content induced by theophylline was a reaction mediated by cyclic AMP.

PGE₂ may increase cyclic AMP levels by activating adenylate cyclase (32, 33). It was also found that PGE₂ increased cyclic AMP levels in lung tissue, in a dose-dependent manner (Fig. 7). Accordingly, it may be that the increased PGE₂ content induced by theophylline also increases the levels of cyclic AMP, and that this action assists in the maintenance of the preventive effects of theophylline on anaphylactic shock.

In summary, the mechanisms of preventive effects of theophylline on anaphylactic shock are suggested to be as follows: Theophylline increases cyclic AMP levels in lung tissue in the presence of endogenous PG and the increased cyclic AMP content elevates
PGE₂ levels in lung tissue. The preventive effects of theophylline on anaphylactic shock results from the action of theophylline which indirectly increases PGE₂ levels in lung tissue. It is also assumed that the increased PGE₂ content elevates cyclic AMP levels in a feedback manner, and that this system contributes to the maintenance of the effects of theophylline.

Thus, the preservation of high PGE₂ levels is required to prevent anaphylactic shock.

Acknowledgements: I am grateful to Prof. H. Ito Department of Pharmacology, Kanagawa Dental College, for reviewing the manuscript and for pertinent advice.

REFERENCES

5) KAHLESON, G. AND ROSENBERG, E.: New approaches to the physiology of histamine. Physiol. Rev. 48, 155–196 (1968)


