Effects of Alkyl Substituents of Xanthine on Phosphodiesterase Isoenzymes

Ken-ichi Miyamoto,¹,² Ryosuke Sakai,³ Mariko Kurita,³ Shinji Ohmae,³ Fujiko Sanae,³ Hiroyuki Sawanishi,³ Takaaki Hasegawa,³ and Kenzo Takagi³

Research Laboratory for Development of Medicine, Faculty of Pharmaceutical Sciences, Hokuriku University,² Ho-3 Kanagawa-machi, Kanazawa 920–11, Japan, and Department of Hospital Pharmacy³ and Second Department of Internal Medicine,¹ Nagoya University School of Medicine, Tsurumai, Nagoya 466, Japan.

Received October 14, 1994; accepted December 1, 1994

The structure–activity relationships of a series of alklyxanthine derivatives were investigated. The partition coefficient of alklyxanthines enlarged with an elongation of the alkyl chain at the 1-, 3-, or 7-position of xanthine. There was a mild correlation between the apparent partition coefficient and the tracheal relaxant activity of the inhibitors on phosphodiesterase (PDE) IV isoenzyme, while the tracheal relaxant activity closely correlated with the PDE IV inhibitory activity. Regarding substituents at different positions, the alkylation at the 3-position increased the inhibitory activity on every PDE isoenzyme. The alkylation at the 1-position potentiated the inhibitory activity on PDE IV with the alkyl chain length, but decreased the activities on other PDE isoenzymes. The alkylation at the 7-position was characteristic in its decrease in inhibitory activity on PDE III. These results suggested that the potency of the inhibitory activity of xanthine derivatives on PDE isoenzymes is not dependent simply upon their hydrophobicity but upon change in the affinity for the active sites on PDE isoenzymes by the introduction of the alkyl group at particular positions of the xanthine skeleton.

Key words: structure–activity relationship; alklyxanthine; hydrophobicity; phosphodiesterase isoenzyme; tracheal relaxant activity

Theophylline is now frequently used for the treatment of bronchial asthma and other obstructive airway diseases, but its adverse reactions on the cardiovascular and central nervous systems are also well known. We reported that elongation of the alkyl chain length at the 3-position of the xanthine skeleton increased the potency for tracheal relaxant activity on the isolated trachea of guinea pigs, and that there were close correlations among the hydrophilicity, tracheal relaxant activity, and cyclic AMP phosphodiesterase (PDE) inhibitory activity of 3-alklyxanthines.¹⁻³ However, our further studies on the structure–activity relationships of xanthine derivatives indicated that some substituents at the 1- or 7-position of the xanthine skeleton are important for selective tracheal relaxation.⁴⁻⁵

Cyclic nucleotide PDE is now classified into seven isoenzymes, which differ in their substrate specificity, affinity for cyclic nucleotide, and regulatory properties, with a variable tissue distribution.⁶⁻⁷ Among them, PDE III and IV isoenzymes have been indicated to play important roles in the heart and tracheal smooth muscle, respectively.⁸⁻¹⁰ The PDE IV inhibitors, but not other PDE inhibitors, synergistically increased the effect of the β₂-adrenergic agonists, which relax the tracheal muscle through cyclic AMP production in bovine and guinea-pig tissues.¹¹⁻¹²

We have recently reported that the substitution of n-propyl, n-butyl, or 2-oxopropyl groups at the 1- or 7-position of 3-n-propylxanthine provides selective tracheal relaxant activity and PDE IV inhibitory activity.¹³ In this paper, we further studied the structure–activity relationships of a series of alklyxanthines and suggested that their potency and selectivity for inhibitory activity on PDE IV isoenzyme, which is closely concerned with the tracheal relaxant activity, may be dependent upon interactions between the macromolecule of isoenzymes and the substituents of xanthine.

MATERIALS AND METHODS

Materials The alklyxanthine derivatives used were synthesized in our laboratory,⁴⁰ and these structures are shown in Table I. Theophylline, cyclic AMP, cyclic GMP, calmodulin (Sigma Chemical Co., St. Louis, MO, U.S.A.), [³H]cyclic AMP (1.2 GBq/μmol), and [³H]cyclic GMP (1.2 GBq/μmol) (New England Nuclear, Boston, MA, U.S.A.) were purchased.

Apparent Partition Coefficient Each xanthine derivative was dissolved at a concentration of 10 μg/ml in pH 7.4 phosphate-buffered saline (PBS). Five ml of the PBS solution was added to an equal volume of n-octanol and equilibrated at 25°C by continuous shaking for 2 h.

| Table I. Structures of Alklyxanthine Derivatives |
|-----------------|---------|---------|---------|
| R¹ | R²   | R³   |
| 1-Methylxanthine | Me     | H      | H       |
| Theophylline     | Me     | Me     | H       |
| 3-Ethyl-1-methylxanthine | Me     | Et     | H       |
| 1-Methyl-3-n-propylxanthine | Me     | n-Pro  | H       |
| 3-n-Butyl-1-methylxanthine | Me     | n-Bu   | H       |
| Enprofylline     | H      | n-Pro  | H       |
| 1-Ethyl-3-n-propylxanthine | Et     | n-Pro  | H       |
| 1,3-Di-n-propylxanthine | n-Pro | n-Pro  | H       |
| 1-n-Butyl-3-n-propylxanthine | n-Bu   | n-Pro  | H       |
| 1,7-Dimethyl-3-n-propylxanthine | Me     | n-Pro  | Me      |
| 7-Ethyl-1-methyl-3-n-propylxanthine | Me     | n-Pro  | Et      |
| 1-Methyl-3,7-di-n-propylxanthine | Me     | n-Pro  | n-Pro   |
| 7-n-Butyl-1-methyl-3-n-propylxanthine | Me     | n-Pro  | n-Bu    |

© 1995 Pharmaceutical Society of Japan
concentration of each compound in both aqueous and octanol phases was determined by a procedure using high-performance liquid chromatography. The apparent partition coefficient (PC) of each compound was estimated as the ratio of the concentration in the organic phase to that in the aqueous phase.

**Cyclic Nucleotide PDE Isoenzymes and Inhibition Assay**

According to the methods reported by Reeves et al. and Nicholson et al., PDE isoenzymes were separated from the cerebral cortex and cardiac ventricle of guinea pigs by DEAE-Sepharose CL-6B chromatography, and these activities were measured by the two-step assay system of Thompson and Applemen at a substrate concentration of 1.0 μM cyclic AMP or cyclic GMP in a reaction mixture containing 40 mM Tris, 10 mM MgCl₂, and 4 mM 2-mercaptoethanol, pH 8.0. Calmodulin-stimulated cyclic AMP hydrolysis and cyclic GMP-inhibited cyclic AMP hydrolysis were assessed by the addition of 50 U/ml calmodulin plus 1 mM CaCl₂ and 10 μM cold cyclic GMP. Calcium/calmodulin-stimulated cyclic AMP PDE (PDE I, Kₘ = 5.4 μM, Vₘₐₓ = 2.4 nmol/min per mg protein) and cyclic AMP-specific PDE (PDE IV, Kₘ = 2.9 μM, Vₘₐₓ = 0.85 nmol/min per mg protein) from the cerebral cortex and cyclic GMP-inhibited cyclic AMP PDE (PDE III, Kₘ = 0.67 μM, Vₘₐₓ = 1.2 nmol/min per mg protein) from the ventricle were used in the experiments.

Various concentrations of a test compound were added to the cyclic AMP PDE assay mixture and incubated at 30°C for 10 min, and the concentration producing 50% inhibition of cyclic AMP hydrolysis (IC₅₀) for each PDE isoenzyme was calculated by the nonlinear least-squares method.

**Statistics**

The regression lines were calculated with the program for the nonlinear least-squares method (MULTI) of Yamaoka and co-workers.

**RESULTS AND DISCUSSION**

Figure 1 shows the PC of alkylxanthines. The PC of compounds generally depends on the number of substituents and the length of the alkyl chain. The lipophilicity of 1-substituted 3-n-propylxanthines was higher than that of 3-substituted 1-methylxanthines, and the lipophilicity of both types of xanthine derivatives increased with the alkyl chain length. On the other hand, although 7-substituted 1-methyl-3-n-propylxanthine derivatives have more

![Graph](image)

**Fig. 1.** Relationships between the Alkyl Chain Length of Xanthine Derivatives and Their Apparent Partition Coefficient

- □, 3-substituted 1-methylxanthines; □, 1-substituted 3-n-propylxanthines; △, 7-substituted 1-methyl-3-n-propylxanthines. Data are the mean (standard errors within the range of symbols) of three experiments.

**Table II.** Correlation Constants (r²) among the Apparent Partition Coefficient, the Tracheal Relaxant Activity, and the Inhibitory Activity on PDE Isoenzymes of Alkylxanthine Derivatives

<table>
<thead>
<tr>
<th>Inhibitory activity on</th>
<th>log PC</th>
<th>PDE I</th>
<th>PDE III</th>
<th>PDE IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>log EC₅₀</td>
<td>-0.295</td>
<td>0.033</td>
<td>0.686</td>
<td>0.615</td>
</tr>
<tr>
<td>Tracheal relaxation</td>
<td>-0.043</td>
<td>0.038</td>
<td>0.000</td>
<td>0.865</td>
</tr>
</tbody>
</table>

a) Data are from reference 4.

![Graphs](image)

**Fig. 2.** Correlations between the Apparent Partition Coefficient and the Tracheal Relaxant Activity (Data from ref. 4) (A), the Tracheal Relaxant Activity and the PDE IV Inhibitory Activity (B), and the Apparent Partition Coefficient and the PDE IV Inhibitory Activity (C) of Alkylxanthines

□, 3-substituted 1-methylxanthines; □, 1-substituted 3-n-propylxanthines; △, 7-substituted 1-methyl-3-n-propylxanthines.
substituents than 1-substituted 3-n-propylxanthines, the PC of 1,7-dimethyl-3-n-propylxanthine was similar or less than that of 1-methyl-3-n-propylxanthine. Moreover, the lipophilicity increased with the alkyl chain length at the 7-position, but was low in comparison to 1-substituted 3-n-propylxanthines. This seems to result because 7-unsubstituted xanthines may partially exist as the N7H form in the N7H—N9H tautomerism, while 7-substituted xanthines are fixed in only the N7H form.19,20

We previously reported that the tracheal relaxant activity increased with the chain length of the alkyl groups substituted at the 3-position of xanthine and with their hydrophobicity.1–3 However, in this study using a series of alkylxanthine derivatives, the relationship between the hydrophobicity and the relaxant activity on the spontaneous tone of isolated trachea from guinea pigs (data from ref. 4) was poor (Fig. 2A, Table II) because of the low relaxant activity of the 7-alkylxanthines. Recently, we have indicated that the tracheal relaxant activity of 1-n-butyl-3-n-propylxanthine is closely related to its inhibitory activity on PDE IV isoenzyme in the tracheal smooth muscle of guinea pigs.12 This study confirmed a good correlation between tracheal relaxant activity and the inhibitory activity on PDE IV isoenzyme, but not on PDE I and III, in a series of alkylxanthines (Fig. 2B, Table II). Then, we examined the relationships between the hydrophobicity of these alkylxanthine derivatives and their inhibitory activities on PDE isoenzymes. As a result, there was no correlation between the hydrophobicity and the inhibitory activities on PDE I and III (Table II). A mild correlation between hydrophobicity and PDE IV inhibition was observed (Fig. 2C, Table II), which appeared to be lowered by the substitution of long alkyl groups at the 7-position, as was the case with tracheal relaxation (Fig. 2A). Figure 3 shows the relationship between the chain length of the alkyl groups substituted at the 1-, 3-, and 7-positions of xanthine and their inhibitory activities on PDE isoenzymes. It was clear that elongation of the alkyl chain at the 3-position increased the inhibitory activity on every PDE isoenzyme. This agrees with our previous reports that there were correlations between the tracheal relaxant activity, inhibitory activity on crude cyclic AMP PDE, and hydrophobicity in 3-alkylxanthines.2,3) Regarding 1-substituted 3-n-propylxanthines, elongation of the alkyl chain increased PDE IV inhibitory activity but decreased the activities on PDE I and III isoenzymes, indicating an increase in selectivity for PDE IV. In 7-substituted 1-methyl-3-n-propylxanthines, the most pronounced effect of the substituents was a loss of activity on PDE III, and elongation of the alkyl chain increased PDE I inhibitory activity. Introduction of the alkyl groups at the 7-position almost did not change the PDE IV inhibitory activity. This is one reason the N3-substituents lowered the correlation between the hydrophobicity and the PDE IV inhibitory activity, while there was a good correlation between their tracheal relaxant activity and PDE IV inhibitory activity (Fig. 2).

From these results, it was revealed that each substituent at the 1-, 3-, or 7-position of xanthine provides a particular effect on PDE isoenzymes, resulting in selective inhibitory activity on PDE IV and strong and highly selective tracheal relaxant activity of xanthines having long alkyl chains at both the 1- and 3-positions. Mechanisms for these effects of the substituents on PDE isoenzymes are thought to be as follows; 1) differences in bulk tolerance, long distance, and hydrophobicity modify the interaction between the xanthine molecule and its binding regions of enzyme molecule; 2) long alkyl groups bind to the N3 region in the macromolecule of every PDE isoenzyme; 3) bulk tolerance limits the molecular interaction at the N1 region in PDE I isoenzyme and at both the N1 and N7 regions in PDE III isoenzyme; 4) long alkyl groups increase the affinity for the N1 and N7 regions in PDE IV and I isoenzymes, respectively. On the other hand, actions of xanthines unsubstituted at the 1- and 7-positions on PDE I and III isoenzymes showed quite different behaviors from the corresponding alkyl-substituted xanthines (Fig. 3). The N1-proton of 3-n-propylxanthine may decrease the affinity only for the PDE I isoenzyme. The difference between actions of 1-methyl-3-n-propylxanthine and its 7-alkyl isomer on PDE I and III may be related to the N7H—N9H tautomerism, as mentioned above. Thus, the ionic interaction between the xanthine molecules and their binding regions on the enzyme macromolecule is also

![Graph](https://example.com/graph.png)

**Fig. 3.** Relationships between the Alkyl Chain Length at the 1-, 3-, and 7-Positions of Xanthine Derivatives and Their Inhibitory Activity on PDE Isoenzymes

- a, 1-substituted 3-n-propylxanthines; b, 3-substituted 1-methylxanthines; c, 7-substituted 1-methyl-3-n-propylxanthines. ○, PDE I; △, PDE III; ●, PDE IV. Data are the mean (standard errors within the range of symbols) of three to five experiments.
an important factor for the activity of xanthine derivatives.

REFERENCES