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Relationship between hydrophobicity and biological activities of xanthine derivatives in guinea pig tracheal smooth muscle

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Summary

Relaxant effects and cyclic AMP phosphodiesterase (PDE) inhibitory activities of N^3-alkylxanthine and N^3-alkyl-1-methylxanthine derivatives on tracheal smooth muscle isolated from guinea pigs were investigated. The PDE inhibition constant (K_i) and the concentration producing 50% tracheal smooth muscle relaxation (EC_{50}) were estimated. A significant correlation between the $-\log K_i$ values and the $-\log EC_{50}$ values ($r = 0.922$, $P < 0.01$) was found. The apparent partition coefficient between chloroform or *n*-octanol and pH 7.4 phosphate-buffered saline (PBS) as an index of hydrophobicity of various xanthine derivatives was also measured. There was a positive correlation between the logarithmic values of partition coefficients in *n*-octanol and the $-\log EC_{50}$ values ($r = 0.982$, $P < 0.01$). A significant correlation was also found between the logarithmic values of partition coefficients in chloroform and the $-\log K_i$ values ($r = 0.961$, $P < 0.01$). These facts suggest that the increase of hydrophobicity of the xanthine molecule enhances the potency of biological activity such as the bronchodilating effect and PDE inhibitory activity. The partition coefficient, as a parameter of hydrophobicity, may be useful in predicting the activities of *N*-alkylxanthine derivatives.

Introduction

Studies on the relationship between the chemical structures and pharmacological actions of xanthine derivatives indicate that alkyl groups at the N^1 and N^3 positions of the xanthine molecule play important roles in adenosine receptor antagonism and bronchodilatory action, respectively (Persson et al., 1982a). Based on these observations, a new xanthine derivative, 3-propylxanthine (enprofylline), was recently synthesized. Enprofylline is 4–5 times more potent in the relaxant effect than theophylline and does not exert a theophylline-like antagonism on adenosine receptors, although the chemical structures are similar (Persson and Kjellin, 1981; Fredholm and Persson, 1982; Persson, 1983; Collis et al., 1984; Hedman and Andersson, 1984). Therefore, the bronchodilatory effect of theophylline may not be related to the adenosine receptor antagonism. However, the precise mechanism is not yet fully understood.

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As part of a program of research on bronchodilators, we were interested in developing compounds with a stronger relaxant effect by chemical modifications of the xanthine molecule. The role of the alkyl chain length at the N^3 position of the xanthine molecule in the inhibition of cyclic AMP phosphodiesterase (PDE) in isolated guinea pig tracheal smooth muscle has been previously reported (Takagi et al., 1988). A new xanthine derivative, 1-methyl-3-propylxanthine was synthesized in our laboratories and found to possess a much stronger relaxant effect than theophylline and enprofylline, suggesting that the N^3-alkyl chain length is significant for increasing the relaxant effect and for affecting the pharmacokinetic and physicochemical properties of xanthine derivatives (Apichartpichean et al., 1988).

In order to investigate the effect of hydrophobicity on the biological activities of various N^3-alkylxanthine and N^3-alkyl-1-methylxanthine derivatives, we evaluated the relationship between apparent partition coefficients and relaxant effects or cyclic AMP PDE inhibitory effects on guinea pig tracheal smooth muscle preparations.

Materials and Methods

Materials

The *N*-alkylxanthine derivatives, 3-ethyl-, 3-propyl-, 3-butyl-, 1-methyl-3-propyl- and 1-methyl-3-butylxanthines were synthesized in our laboratory according to the methods reported previously (Papesch and Schroeder, 1951; Wooldridge and Slack, 1962; Ohtsuka 1973; Sneddon, 1982). 3-Methylxanthine and 1,3-dimethylxanthine (theophylline) were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. Carbamylcholine chloride (carbachol, Sigma Chemical Co.) was used for evaluation of the relaxant effect. All other agents and reagents used in the experiment were obtained commercially and were used without further purification.

Subjects

Hartley-strain male guinea pigs (Shizuoka Laboratory Animal Center, Shizuoka, Japan), weighing 230–300 g each, were used in this study.

Biological activities

The relaxant effects of each xanthine derivative on the carbachol-contracted tracheal smooth muscle isolated from guinea pigs were evaluated. Methods were essentially the same as those of Baba and others (1985). In order to evaluate the relaxant effects of the xanthine derivatives on the carbachol-contracted tracheal preparations, each drug was administered after the preparation had been constricted by carbachol and had reached a constant tension. Carbachol was used at a concentration of 3×10^{-6} M, at which 90% muscle contraction was induced. Relaxation without external Ca^{2+} was defined as 100% and that induced by each compound was expressed as a percentage. The EC_{50} value was used as an index of potency of the relaxant effect.

We also measured cyclic AMP PDE activity in the $10000 \times g$ supernatant of homogenate prepared from guinea pig tracheal smooth muscle by a method similar to that reported by Thompson and Appleman (1985). The reaction mixture of 0.2 ml contained 40 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 0.1–40 μM unlabeled cyclic AMP, 0.1 μCi cyclic [^{3}H]AMP (specific activity 34.5 Ci/mmol, New England Nuclear, Boston, MA), various concentrations of each xanthine and an appropriate concentration of enzyme preparation which was incubated for 10 min at 30 °C and boiled to stop the reaction. [^{3}H]Adenosine produced by the treatment with snake venom (*Crotalus atrox*, Sigma) and Dowex 1-X2 (Bio-Rad, Richmond, U.S.A.) was counted in a liquid scintillation counter. The K_i value against the low K_m (Michaelis constants) PDE calculated by the Dixon method (1953) was used to estimate the cyclic AMP PDE inhibition of each xanthine derivative.

Protein contents were measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Apparent partition coefficient

Each xanthine derivative was dissolved at a concentration of 10 μg/ml in pH 7.4 phosphate-buffered saline (PBS) solution. Five ml of the PBS solution was added to an equal volume of chloroform or *n*-octanol, and equilibrated at 25 °C by

continuous shaking for 2 h. The concentration of each compound in the aqueous phase or organic phase was determined by spectrophotometry at 278 nm. 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, and hydrophobicity was expressed as a logarithmic partition coefficient (log PC_{CH} for chloroform and log PC_{OC} for octanol).

Statistical analysis

The results were expressed as the mean ± standard error. The regressions were performed using linear regression analysis. A personal computer, FM-11 EX (Fujitsu, Tokyo) was used in this study.

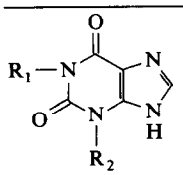
Results and Discussion

Table 1 shows the chemical constitution, biological activities (EC_{50} and K_i values) and physico-chemical parameter (log PC value) of each xanthine derivative. Both the cyclic AMP PDE inhibitory activity and the relaxant effect on isolated guinea pig tracheal smooth muscle increased with prolongation of the alkyl chain length of the xanthine molecule. Hydrophobicity of xanthines was found to be positively correlated with biological activities. There were highly significant relationships between log PC_{OC} and $-\log EC_{50}$ values ($r = 0.982$, $P < 0.01$; Fig. 1) and between log PC_{CH} and $-\log EC_{50}$ values ($r = 0.882$, $P < 0.01$; figure not shown). In the same way, a significant linear relationship was also obtained from the plot of the partition coefficient in chloroform (PC_{CH}) vs. the negative logarithmic value of cyclic AMP PDE inhibition activity ($r = 0.961$, $P < 0.01$) as shown in Fig. 2. A significant relationship between log PC_{OC} and $-\log K_i$ values was also found ($r = 0.882$, $P < 0.01$). These findings indicate that the hydrophobic property which increases with the alkyl chain length at the N^1 and N^3 positions of the xanthine derivatives is related to the potency of their biological activities. Figure 3 shows a significant positive correlation between $-\log K_i$ and $-\log EC_{50}$ values ($r = 0.922$, $P < 0.01$).

The Lineweaver–Burk plot of the hydrolysis of cyclic AMP by PDE enzyme activity obtained from guinea pig smooth muscle showed biphasic slopes which indicated two forms of enzyme reactions, i.e., high affinity with low K_m (0.61 ± 0.04 μM, $n = 3$) and low affinity with high K_m ($12.2 \pm$

TABLE 1

Cyclic AMP phosphodiesterase (PDE) inhibitory activity, relaxant effect and apparent partition coefficient for each xanthine derivative



R_1	R_2	PDE inhibition, K_i (μM)	Relaxant effect, EC_{50} (μM)	Partition coefficient	
				log PC_{CH}	log PC_{OC}
H	Me	122.0	2950	−1.431	−0.716
H	Et	63.0	395	−1.151	−0.106
H	Pr	42.0	129	−1.075	0.331
H	Bu	32.0	85	−0.514	0.839
Me	Me	56.3	395	−0.456	−0.042
Me	Pr	1.85	17.3	0.603	1.022
Me	Bu	1.20	12.3	1.230	1.286

Each value represents the mean of six measurements for relaxant effect and three measurements for PDE inhibitory activity and partition coefficient. Me, methyl; Et, ethyl; Pr, propyl; Bu, butyl. PC_{CH} and PC_{OC} represent the partition coefficient in chloroform and *n*-octanol, respectively.

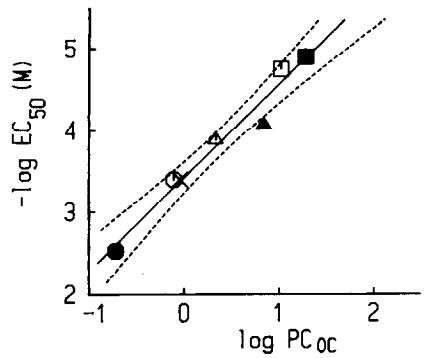


Fig. 1. Correlation between apparent partition coefficients in *n*-octanol (log PC_{OC}) of various xanthine derivatives and their relaxant effects ($-\log EC_{50}$) on isolated guinea pig tracheal smooth muscle. The regression line (solid line) was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. Combined compounds: ●, 3-methylxanthine; ○, 3-ethylxanthine; △, 3-propylxanthine (enprofylline); ▲, 3-butylxanthine; ×, 1,3-dimethylxanthine (theophylline); □, 1-methyl-3-propylxanthine; ■, 1-methyl-3-butylxanthine.

1.5 μM, $n = 3$). In estimating the K_i value of each xanthine derivative, the inhibitory activity was exhibited in the low K_m form. A number of experiments describing a correlation between tracheal relaxation and inhibition of PDE have been published. However, cyclic AMP PDE inhibitors used in published reports included compounds unrelated to xanthine derivatives such as

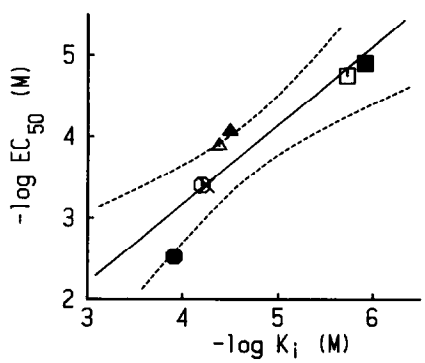


Fig. 2. Correlation between apparent partition coefficients in chloroform (log PC_{CH}) of various xanthine derivatives and their cyclic AMP phosphodiesterase inhibitory activities ($-\log K_i$) on isolated guinea pig tracheal smooth muscle. The regression line (solid line) was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. Symbols as in Fig. 1.

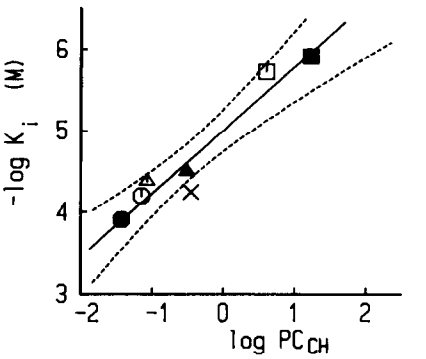


Fig. 3. Correlation between cyclic AMP phosphodiesterase inhibitory activities ($-\log K_i$) and relaxant effects ($-\log EC_{50}$) of various xanthine derivatives on isolated guinea pig tracheal smooth muscle. The regression line (solid line) was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. Symbols as in Fig. 1.

papaverine and dipyridamol, etc. (Lugnier et al., 1972; Newman et al., 1978; Polson et al., 1978; Fredholm et al., 1979). Polson et al. (1982) reported the significant relationship between high affinity cyclic AMP PDE inhibitory activity and the dynamic relaxant effect of methylxanthine (1-methylxanthine, 3-methylxanthine, 7-methylxanthine, caffeine, theophylline and 3-isobutylmethylxanthine) on isolated canine tracheal smooth muscle. The K_m value was 0.63 ± 0.09 μM ($n = 3$) and the K_m value reported in that study is nearly equal to the values obtained in the present study (0.61 ± 0.04 μM), suggesting that there may be no difference among animal species. Based on these observations, it may be concluded that the high affinity cyclic AMP PDE inhibitory activity of the xanthine molecule is closely related to its relaxant effect, although no evidence for low affinity (high K_m) enzyme activity was obtained in this study.

For many years, the mechanism of the bronchodilatory action of 1,3-dimethylxanthine (theophylline) has been believed to be due to elevation of the intracellular cyclic AMP by inhibition of PDE. Thereafter, the hypothesis that the mechanism of action is related to adenosine receptor antagonism (theophylline antagonizes the activity of adenosine) has become widely accepted. Recently, it has been found that a new synthetic alkylxanthine called enprofylline, which is 4–5

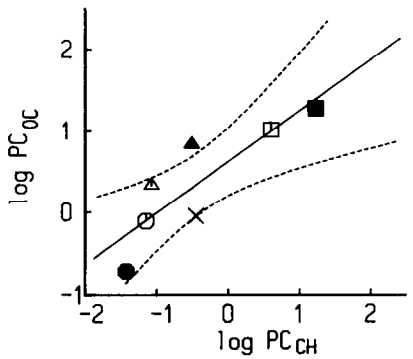


Fig. 4. Correlation between chloroform/pH 7.4 phosphate-buffered saline (PBS) and octanol/PBS partition coefficients of various xanthine derivatives. The regression line (solid line) was calculated by the least-squares method. Symbols as in Fig. 1.

times more potent in its relaxant effects than theophylline both in vitro and in vivo (Lunell et al., 1981; Persson and Kjellin, 1981; Laursen et al., 1983; Takagi et al., 1988), does not possess theophylline-like antagonism on adenosine receptors (Persson et al., 1982a, b). These findings suggest that the xanthine molecule operates as a bronchodilator by a mechanism other than the adenosine receptor antagonism. The precise mechanism, however, is still not clearly understood. The present study at least suggests that cyclic AMP PDE inhibitory activity of alkylxanthine derivatives contributes to their mechanism of bronchodilatory action.

Furthermore, in the course of studies on bronchodilatory agents in our laboratories, it was found that the relaxant effect of a newly synthesized xanthine derivative, 1-methyl-3-propylxanthine, was nearly equal to that of 1-methyl-3-butylxanthine and was about 20-fold and 7-fold as potent in its relaxant effect as theophylline and enprofylline, respectively, on isolated guinea pig tracheal smooth muscle. The drug is now undergoing further pharmacological evaluation. We postulate that the alkyl group (methyl) in the N^1 position of the 3-alkylxanthine molecule has not only an important role in the adenosine receptor antagonism, but also exhibits additive bronchodilatory action, which is mainly attributable to the alkyl groups in the N^3 position of the 1-methylxanthine molecule.

It is generally thought that the apparent partition coefficient, a physicochemical parameter expressed as the ratio of drug concentration in the organic phase to that in the aqueous phase, may be useful for predicting drug disposition in the body. It is also well-known that hydrophobicity increases with the length of the alkyl chain. In our previous studies, a significant correlation between the chain length of the alkyl groups at the N^3 position of the xanthine molecule and cyclic AMP PDE inhibitory activity was found (Takagi et al., 1988). The present study also indicated the relationship between the degree of the potency of the relaxant effect, cyclic AMP PDE inhibitory activity and hydrophobicity of xanthine derivatives. This may indicate that the potency of the relaxant effect of the xanthine molecule depends on the cell membrane permeability and affinity for cyclic AMP PDE based on its hydrophobic property.

Fig. 4 shows a significant correlation between chloroform/PBS and *n*-octanol/PBS partition coefficients of these xanthine derivatives ($r = 0.862$, $P < 0.05$). It is commonly accepted that the *n*-octanol/water partition coefficient under physiological conditions (pH 7.4, temperature 37 °C) is the index of hydrophobicity of the compound and that *n*-octanol is the best organic solvent to model the properties of biological membranes. In this study, different correlation coefficients between partition coefficients in different organic solvents and their biological activities were obtained which suggest the importance of selecting an appropriate organic solvent.

Conclusion

This report is the first to discuss the structure–activity relationship of N^1- and N^3-alkylxanthine derivatives from the standpoint of physicochemical property and biological activities. The finding in this study at least supports the role of cyclic AMP PDE inhibitory activity on bronchodilatory effects. The hydrophobicity of these xanthine derivatives is an important determinant of biological activities such as relaxation and cyclic AMP PDE inhibitory activity. The hydrophobicity may be useful for chemical modifications of more

potent xanthine derivatives as smooth muscle relaxant.

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