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Correlation Between Hydrophobicity of *N*-Alkylxanthine Derivatives and their Biological Activities on Guinea-pig Isolated Tracheal Smooth Muscle

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Abstract—Cyclic (c) AMP phosphodiesterase (PDE) inhibitory activities of *N*-alkylxanthine derivatives (3-methyl-, 3-ethyl-, 3-propyl-, 3-butyl-, 1,3-dimethyl-, 1-methyl-3-ethyl-, 1-methyl-3-propyl- and 1-methyl-3-butyl xanthines) and their relaxant effects on carbachol-induced contraction and on resting tone guinea-pig isolated tracheal smooth muscle have been investigated. The PDE inhibition constant (K_i) and the concentration producing 50% tracheal smooth muscle relaxation in-vitro (EC50) were determined. Significant correlations between the -log K_i values and the -log EC50 values on the carbachol-induced contraction or on the resting tone were found ($r = 0{\cdot}902$ and $0{\cdot}892$). The apparent partition coefficient (P) between *n*-octanol and pH 7·4 phosphate-buffered saline (PBS) was measured as an index of hydrophobicity of the xanthine derivatives. There were significant correlations between log P and both -log EC50 values and between the log P and -log K_i values. These findings suggest that the cAMP PDE inhibitory activity of *N*-alkylxanthine derivatives contributes to the mechanism of bronchodilatory action, and that an increase in hydrophobicity of the xanthine molecule enhances the biological activity.

A number of hypotheses have been proposed to explain the mechanism of the bronchodilatory action of xanthines: cyclic (c)AMP phosphodiesterase (PDE) inhibitory effect (Katsuki & Murad 1977; Polson et al 1982), antagonism of endogenous adenosine (Persson et al 1982a; Fredholm & Persson 1982), control of intracellular calcium (Peach 1972), release of catecholamines from the adrenal medulla (Farmer & Chick 1967, and the inhibition of the production of prostaglandin (Horrobin et al 1977).

For many years, the mechanism of the bronchodilatory action of 1,3-dimethylxanthine (theophylline) was believed to be due to elevation of intracellular cAMP through inhibition of PDE. Thereafter, the hypothesis that the mechanism of action is related to its adenosine receptor antagonism became widely accepted. Recently, it has been found that a new synthetic alkylxanthine, enprofylline, which is 4 to 5 times more potent in its relaxant effects than theophylline both in-vitro and in-vivo (Persson & Kjellin 1981; Lunell et al 1981; Laursen et al 1983; Takagi et al 1988), does not possess a theophylline-like antagonism on the adenosine receptors (Persson et al 1982a, b). Therefore, the bronchodilatory effect of theophylline may not be related to the adenosine receptor antagonism. These findings suggest that the xanthine molecule operates as a bronchodilator by a mechanism other than adenosine receptor antagonism. The precise mechanism, however, is still not clearly understood.

In our earlier studies, the alkyl chain length at the N3 position of the xanthine molecule was shown to correlate with the inhibition of cAMP PDE on guinea-pig isolated tracheal smooth muscle (Takagi et al 1988). We also found that a new xanthine derivative, 1-methyl-3-propylxanthine, possesses a much stronger relaxant effect than theophylline and enprofylline, suggesting that the N3-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).

The present study evaluates the cAMP PDE inhibitory activity and the relaxant effects of eight *N*-alkylxanthine derivatives on carbachol-induced contraction and on the resting tone in guinea-pig isolated tracheal smooth muscle. This investigation should give some information whether alkyl groups at the N3 position of both xanthine and methylxanthine molecules can play roles in cAMP PDE inhibitory activity and bronchodilatory action. We also evaluated the relationship between apparent partition coefficients and the relaxant effects or cAMP PDE inhibitory effects on the tracheal preparations, and whether the parameter of hydrophobicity can be used as an indicator to predict the biological activities of *N*-alkylxanthine derivatives.

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Materials and Methods

Materials

The *N*-alkylxanthine derivatives, 3-ethyl-, 3-propyl-, 3-butyl, 1-methyl-3-ethyl-, 1-methyl-3-propyl- and 1-methyl-3-butyl xanthines were synthesized in our laboratory according to the methods reported previously (Papesch & Schroeder 1951; Wooldridge & Slack 1962; Ohtsuka 1973; Sneddon 1982). 3-Methylxanthine and 1,3-dimethylxanthine (theophylline) were obtained from Sigma Chemical Co., St Louis, MO. Carbamylcholine chloride (carbachol, Sigma Chemical Co.) and isoprenaline (isoproterenol) (Sigma Chemical Co.) were used for evaluation of the relaxant effect. All other agents

and reagents were obtained commercially and used without further purification.

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

Biological activities

The relaxant effects of each xanthine derivative on carbachol-induced contraction and on the resting tone in the tracheal smooth muscle isolated from guinea-pigs were evaluated. Our methods were essentially the same as those of Baba et al (1985). The tracheal preparation was perfused at a constant flow of 1·5 mL min^{-1} at 37°C in Krebs solution (mM NaCl 137, $KHCO_3$ 5·9, $CaCl_2$ 2·4, $MgCl_2$ 1·2, and glucose 11·8) and a weight of 0·5 g was applied to the preparation which had been completely relaxed with isoprenaline (2×10^{-5} M).

To evaluate the relaxant effect of each xanthine derivative on the carbachol-induced contraction, the drugs were added after the preparations had responded to carbachol, 3×10^{-6} M, and had reached a constant tension. This concentration of carbachol induced a 90% contraction. To evaluate the relaxant effect of each drug on the resting tone, they were added to the preparation in the resting state.

The amount of relaxation induced by each drug was expressed as a percentage of that attained in Ca^{2+}-free state. The concentration producing 50% tracheal smooth muscle relaxation (EC50) was calculated from log concentration-response curves.

We also measured the effect of each xanthine derivative on cAMP PDE activity in the 10000 *g* supernatant of homogenate prepared from guinea-pig tracheal smooth muscle according to a method similar to that reported by Thompson & Appleman (1985). The reaction mixture of 0·2 mL contained mM Tris-HCl 40 (pH 8·0), $MgCl_2$ 10, 2-mercaptoethanol 4, unlabelled cAMP 0·1 to 40 μM, 0·1 μCi [^{3}H]cAMP (specific activity 34·5 Ci $mmol^{-1}$, New England Nuclear, Boston, MA), various concentrations of each xanthine and an appropriate concentration of the enzyme preparation was incubated for 10 min at 30°C and boiled to stop the reaction. [^{3}H]Adenosine produced by treatment of the homogenate with snake venom (*Crotalus atrox*, Sigma) and Dowex 1-X2 (Bio-Rad Lab., Richmond, VA.) was counted in a liquid scintillation counter. A Lineweaver-Burk plot of the hydrolysis of cAMP by PDE enzyme activity obtained from guinea-pig smooth muscle preparations showed biphasic slopes which indicated two forms of enzyme reactions, i.e., high affinity with low K_m (0·61 ± 0·04 μM, mean ± s.e. of three measurements) and low affinity with high K_m (12·2 ± 1·5 μM). In estimating the K_i value of each xanthine derivative, the inhibitory activity was exhibited against the low K_m form. The inhibition of the low K_m PDE by xanthine derivatives was analysed by Dixon plots (1953). The K_i value was used to estimate the cAMP 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^{-1} in pH 7·4 phosphate-buffered saline (PBS) solution. 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. The concentration of each drug in the aqueous phase was determined by spectrophotometry at 278 nm. The apparent partition coefficient (P) 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 P).

Statistical analysis

The regression lines were calculated by a non-linear least-squares method program, 'MULTI', written by Yamaoka et al (1981) using a personal computer, FM-11 EX (Fujitsu, Tokyo).

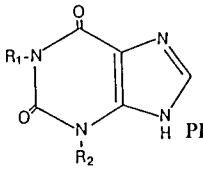
Results and Discussion

Each xanthine derivative caused a concentration-dependent relaxation on both carbachol-induced contraction and resting tone in tracheal smooth muscle preparations isolated from guinea-pigs. Each compound inhibited the PDE activity in tracheal smooth muscle, the effect increasing with concentration. The chemical constitution, biological activities (EC50 and K_i values) and apparent partition coefficients of each xanthine derivative are shown in Table 1.

As shown in Fig. 1, highly significant correlations between the -log K_i value and -log EC50 values on the carbachol-induced contraction and on the resting tone of the tracheal muscle were obtained ($r = 0{\cdot}902$ and 0·892; $P < 0{\cdot}01$), suggesting that cAMP PDE inhibitory activity of these xanthine derivatives at least contributes to their mechanism of bronchodilatory action. A positive correlation of the relaxant effects of these xanthine derivatives between the carbachol-induced contraction and the resting tone in the tracheal preparations was also obtained ($r = 0{\cdot}971$, $P < 0{\cdot}01$). However, a higher concentration of the xanthine derivatives was required to inhibit carbachol-induced contractions compared with those required to reduce spontaneous tone. The molar ratio of the concentration required for relaxation activity to the concentration required for cAMP PDE inhibition activity (EC50/K_i ratio) was 0·628 ± 0·153 for the resting tone and 8·215 ± 2·503 for the carbachol-induced contraction, respectively. These results indicate that the EC50 value for the resting tone in the tracheal preparations clearly reflects the concentration required for the cAMP PDE inhibitory activity compared with that for the carbachol-induced contraction.

A number of experiments describing a correlation between tracheal relaxation and the inhibition of PDE have been published. However, cAMP PDE inhibitors used in those reports included compounds unrelated to xanthine derivatives, such as papaverine and dipyridamole (Lugnier et al 1972; Newman et al 1978; Polson et al 1978; Fredholm et al 1979). Polson et al (1982) reported the strong relationship between high affinity cAMP PDE inhibitory activity and the dynamic relaxant effect of six different methylxanthines (1-methylxanthine, 3-methylxanthine, 7-methylxanthine, caffeine, theophylline and 3-isobutyl-methylxanthine) on isolated canine tracheal smooth muscle contracted by methacholine. They also reported that the K_m value of cAMP PDE

Table 1. cAMP 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 EC50 (μM) Resting tone	Relaxant effect EC50 (μM) Carbachol-induced contraction	Partition coefficient log P
H	Me	122·0	141·2	2950	−0·716
H	Et	63·0	69·2	395·0	−0·106
H	Pr	42·0	7·24	129·0	0·331
H	Bu	32·0	8·32	85·0	0·839
Me	Me	56·3	28·2	395·0	−0·042
Me	Et	26·0	4·68	76·0	0·516
Me	Pr	1·85	0·98	17·3	1·022
Me	Bu	1·20	1·35	12·3	1·286

Each value represents the mean of six measurements for relaxant effect and three measurements for PDE inhibitory activity and partition coefficient.
Log P represents the partition coefficient in n-octanol.

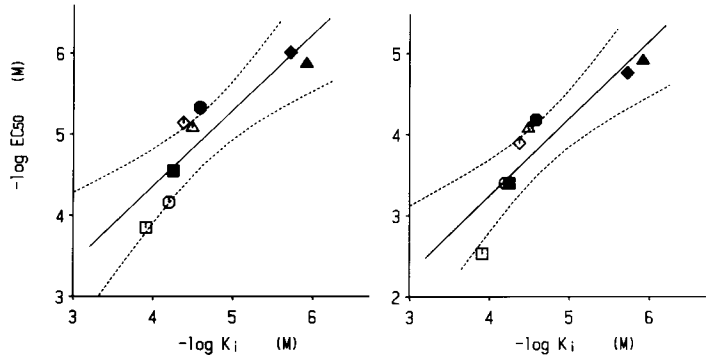


FIG. 1. Correlation of the cAMP phosphodiesterase inhibitory activities (-log K_i) of eight xanthine derivatives and their relaxant effects (-log EC50) on the resting tone (left) and on carbachol (3×10^{-6} M)-induced contraction (right) in guinea-pig isolated tracheal smooth muscle. The regression line (solid line) was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. □ 3-methylxanthine; ○ 3-ethylxanthine; ◇ 3-propylxanthine; △ 3-butylxanthine; ■ 1,3-dimethylxanthine; ● 1-methyl-3-ethylxanthine; ◆ 1-methyl-3-propylxanthine; ▲ 1-methyl-3-butylxanthine.

in the canine tracheal smooth muscle was $0{\cdot}63 \pm 0{\cdot}09$ μM (n = 3), which is nearly equal to the value obtained in the present study using the guinea-pig, suggesting that there may be no difference among animal species. These results and other evidence suggest that the high affinity cAMP PDE inhibitory activity of the xanthine molecule is closely related to its relaxant effect.

Both the cAMP PDE inhibitory activity and the relaxant effects in the tracheal preparations increased with alkyl chain length at the N3 position of the xanthine nucleus. In addition, methylation of the N1 position of the N3-alkylxanthine molecule resulted in elevation of cAMP PDE inhibitory activity and an increase in the relaxant effect.

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 n-octanol is the best organic solvent to model the properties of biological membranes. It is also well-known that hydrophobicity increases with the length of the alkyl chain. As shown in Fig. 2, a significant correlation between the log P and -log K_i values was obtained ($r = 0{\cdot}872$, $P < 0{\cdot}01$). In the same way, positive linear correlations

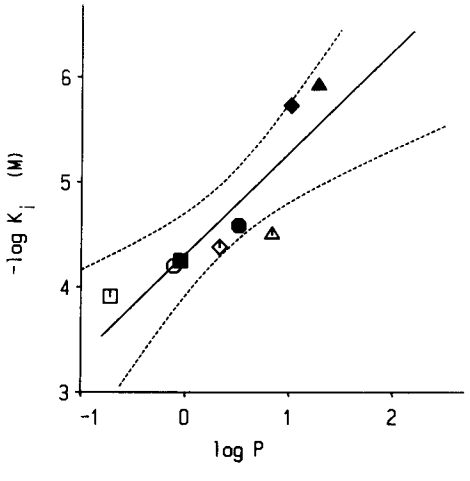


FIG. 2. Correlation of the apparent partition coefficients (log P) of xanthine derivatives and their cAMP phosphodiesterase inhibitory activities (-log K_i) in the tracheal preparations. The regression line was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. Symbols are the same as for Fig. 1.

between the log P value and both -log EC50 values ($r = 0.979$ on carbachol-induced contraction and $r = 0.944$ for the resting tone) were also obtained. These findings also indicate that the hydrophobic property which increases with the alkyl chain length at the N3 position of the xanthine derivatives is inevitably related to the potency of their biological activities, such as cAMP PDE activity and relaxant effects.

From the results from this study we postulate that the potency of the relaxant effect of the xanthine molecule depends on the cell membrane permeability and the affinity for cAMP PDE based on its hydrophobic property. This report is the first to discuss the structure-activity relationship of *N*1- and *N*3-alkylxanthine derivatives in the interpretation of the bronchodilation mechanism of theophylline from the standpoint of physicochemical properties. The parameter of hydrophobicity may be useful in designing chemical modifications of the xanthine molecule with a strong bronchodilatory action.

References

Apichartpichean, R., Takagi, K., Nadai, M., Kuzuya, T., Ogawa, K., Miyamoto, K., Hasegawa, T. (1988) Studies on alkyl-xanthine derivatives. II. Pharmacokinetic and pharmacodynamic studies of a new bronchodilator, 1-methyl-3-propylxanthine (MPX). Japan. J. Pharmacol. 48: 341–347

Baba, K., Kawanishi, T., Satake, T., Tomita, T. (1985) Effects of verapamil on the contractions of guinea-pig tracheal muscle induced by Ca, Sr, and Ba. Br. J. Pharmacol. 84: 203–211

Dixon, M. (1953) The determination of enzyme inhibitor constants. Biochem. J. 55: 170–171

Farmer, J. B., Chick, E. A. (1967) Bronchodilator actions of papaverine in the anesthetized guinea-pig. J. Pharm. Pharmacol. 19: 124

Fredholm, B. B., Persson, C. G. A. (1982) Xanthine derivatives as adenosine receptor antagonists. Eur. J. Pharmacol. 81: 673–676

Fredholm, B. B., Brodin, K., Strandberg, K. (1979) On the mechanism of relaxation of tracheal muscle by theophylline and other cyclic nucleotide phosphodiesterase inhibitors. Acta Pharmacol. Toxicol. 45: 336–344

Horrobin, D. F., Manku, M. S., Franks, D. J. (1977) Methylxanthine phosphodiesterase inhibitors behave as prostaglandin antagonists in a perfused mesenteric preparation. Prostaglandins 13: 33–40

Katsuki, S., Murad, F. (1977) Regulation of cyclic 3′, 5′-adenosine monophosphate and cyclic 3′, 5′-guanosine monophosphate levels and contractility in bovine tracheal smooth muscle. Mol. Pharmacol. 13: 330–341

Laursen, L. C., Johannesson, N., Fagerstrom, P. O., Weeke, B. (1983) Intravenous administration of enprofylline to asthmatic patients. Eur. J. Clin. Pharmacol. 24: 323–327

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275

Lugnier, C., Bertrand, Y., Stoclet, J. C. (1972) Cyclic nucleotide phosphodiesterase inhibition and vascular smooth muscle relaxation. Eur. J. Pharmacol. 19: 134–136

Lunell, E., Andersson, K.-E., Persson, C. G. A., Svedmyr, N. (1981) Intravenous enprofylline in asthma patients. Eur. J. Res. Dis. 65: 28–34

Newman, D. J., Colella, D. F., Spainhour, C. G., Brann Jr., E. G., Zabko-Potapovich, B., Wardell Jr., J. R. (1978) cAMP-phosphodiesterase inhibitors and tracheal smooth muscle relaxation. Biochem. Pharmacol. 27: 729–732

Ohtsuka, Y. (1973) Study on oxazolopyrimidines. VI. Formation of 3-substituted xanthine via 7(6H)-iminooxazolopyrimidines. Bull. Chem. Soc. Japan 46: 506–509

Papesch, V., Schroeder, E. F. (1951) Synthesis of 1-mono- and 1,3-di-substituted 6-amino-uracils. Diuretic activity. J. Org. Chem. 16: 1879–1890

Peach, M. J. (1972) Stimulation of release of adrenal catecholamine by adenosine 3′, 5′-cyclic monophosphate and theophylline in the absence of extracellular Ca^{2+}. Proc. Natl. Acad. Sci. U.S.A. 69: 834–836

Persson, C. G. A., Kjellin, G. (1981) Enprofylline, a principally new antiasthmatic xanthine. Acta Pharmacol. Toxicol. 49: 313–316

Persson, C. G. A., Karlsson, J.-A., Erjefalt, I. (1982a) Differentiation between bronchodilation and universal adenosine antagonism among xanthine derivatives. Life Sci. 30: 2181–2189

Persson, C. G. A., Erjefalt, I., Edholm, L.-K., Karlsson, J.-A., Lamm, C.-J. (1982b) Tracheal relaxant and cardiostimulant actions of xanthines can be differentiated from diuretic and CNS-stimulant effects. Role of adenosine antagonism. Ibid. 31: 2673–2681

Polson, J. B., Krzanowski, J. J., Fitzpatrick, D. F., Szentivanyi, A. (1978) Studies on the inhibition of phosphodiesterase-catalyzed cyclic AMP and cyclic GMP breakdown and relaxation of canine tracheal smooth muscle. Biochem. Pharmacol. 27: 254–256

Polson, J. B., Krzanowski, J. J., Szentivanyi, A. (1982) Inhibition of high affinity cyclic AMP phosphodiesterase and relaxation of canine tracheal smooth muscle. Ibid. 31: 3403–3406

Sneddon, J. M. (1982) Enprofylline. Drug Future 7: 623–625

Takagi, K., Hasegawa, T., Kuzuya, T., Ogawa, K., Watanabe, T., Satake, T., Miyamoto, K., Wakusawa, S., Koshiura, R. (1988) Structure-activity relationship in N3-alkyl-xanthine derivatives. Japan. J. Pharmacol. 46: 373–378

Thompson, W. J., Appleman, M. M. (1985) Multiple cyclic nucleotide phosphodiesterase activities from rat brain. Biochemistry 10: 311–316

Wooldridge, K. R. H., Slack, R. (1962) The synthesis of some 6-thioxanthines. J. Chem. Soc. 1863–1868

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885