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ACTIVITIES OF CAFFEINE, THEOPHYLLINE, AND ENPROFYLLINE ANALOGS AS TRACHEAL RELAXANTS

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Abstract—A variety of xanthines cause tracheal relaxation, an activity predictive of antiasthmatic potential. Structural analogs of caffeine, theophylline, and enprofylline were examined for their abilities to relax carbamylcholine-stimulated guinea pig trachea *in vitro*. All caffeine analogs tested were more potent than caffeine (EC_{50} = 551 ± 81 μM) except the 8-*p*-sulfophenyl analog. 1,3,7-Tripropylxanthine and 1,3,7-tripropargylxanthine were among the more potent analogs with EC_{50} values of 12 ± 1.3 and 65 ± 11 μM respectively. Increasing the polarity at the 1- or 3-position by substituting a propargyl group for an *n*-propyl group decreased relaxant activity, an effect not observed at the 7-position. The 8-cyclohexyl-, 8-cyclopentyl- and 8-phenyl-derivatives of caffeine were relatively potent (EC_{50} = approximately 75 μM). The theophylline analog 1,3-di-*n*-propylxanthine was approximately two times more active than theophylline (EC_{50} = 162 ± 17 μM). 3-Isobutyl-1-methylxanthine (EC_{50} = 7.1 ±1.8 μM) and 1-isoamyl-3-isobutylxanthine (EC_{50} = 37 ± 4.2 μM) were among the most potent tracheal relaxants. The 8-substituted theophylline analogs were weak or inactive relaxants except for 8-cyclopentyl- and 8-cyclohexyltheophylline, which were more potent or as potent as theophylline. In contrast to enprofylline (EC_{50} = 56 ± 9 μM), 8-substituted enprofylline analogs were weak or inactive as relaxants with the exception of the 8-cyclohexyl analog. The potency of xanthines as tracheal relaxants was unrelated to potency as adenosine receptor antagonists and may reflect activity as phosphodiesterase inhibitors.

Theophylline (1,3-dimethylxanthine), a potent tracheal relaxant, is widely used in the treatment of asthma. However, theophylline has a very narrow therapeutic range for blood levels of 10–20 μg/mL. Toxic side-effects at higher levels include nausea, vomiting, tremor, seizure, tachycardia, and cardiac arrythmias. A variety of xanthine analogs have been screened as tracheal relaxants in search of a compound with a better clinical profile than theophylline. Caffeine (1,3,7-trimethylxanthine), a closely related xanthine, is a weak tracheal relaxant, whereas enprofylline (3-propylxanthine) is a very potent tracheal relaxant and has fewer CNS-stimulant and -diuretic effects than theophylline in animals and humans *in vitro* and *in vivo* [1–6]. The lack of side-effects has been proposed to be due to the low activity of enprofylline as an adenosine receptor antagonist [3–7].

A series of xanthines related to caffeine, theophylline, and enprofylline has been examined for relaxant effects on tracheal smooth muscle. The biochemical mechanism for relaxation of tracheal smooth muscle by xanthines remains unclear. Most of the xanthines in the present series have activity as adenosine receptor antagonists and as phosphodiesterase inhibitors [8–11]. The tracheal relaxant effect of xanthines from the present series is correlated with the activity as phosphodiesterase inhibitors, suggesting an important role for phosphodiesterase in tracheal function.

MATERIALS AND METHODS

Drugs. 3-Propargyl-1,7-dimethylxanthine, 8-cyclopentyl-1,3-dimethylxanthine, 8-*p*-sulfophenyl-1,3-dimethylxanthine, 8-phenyltheophylline, 1,7-dimethylxanthine and enprofylline were from Research Biochemicals Inc. (Natick, MA). 1-Propargyl-3,7-dimethylxanthine and 1,3,7-tripropargylxanthine were provided by Dr. J. Neumeyer of Research Biochemicals Inc. Caffeine and 1-methylxanthine were from the Sigma Chemical Co. (St. Louis, MO); and 3-isobutyl-1-methylxanthine and carbamylcholine chloride from the Aldrich Chemical Co., Inc. (Milwaukee, WI). 8-Bromo-1,3-dimethylxanthine was from the Fluka Chemical Corp. (Ronkonkoma, NY); theophylline (B-grade) from Calbiochem (La Jolla, CA); and *d,l*-isoproterenol hydrochloride from the Regis Chemical Co. (Morton Grove, IL). The remainder of the xanthines were synthesized as described [8, 11–13]. Stock solutions of drugs were dissolved in water, Tyrode's solution, dimethyl sulfoxide (DMSO), or in 10, 40, or 100 mM NaOH at concentrations of 3 mM or greater.

Tracheal smooth muscle relaxation. Male Hartley guinea pigs (275–500 g) were killed by decapitation or by stunning and exsanguination. The trachea was cut into four sections 4–8 mm long and used fresh or stored in oxygenated Tyrode's Balanced Salt Buffer at 4° for 2–3 hr until used. Each section was cut into a spiral and attached to a Grass FTO-3C or Gould force transducer in an organ bath chamber containing Tyrode's Balanced Salt Buffer at 37°. The buffer consists of the following (in mM): NaCl, 137; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 0.49; NaH_2PO_4, 0.36; dextrose, 5.55; and $NaHCO_3$, 11.9. The buffer was oxygenated continually with 95% O_2, 5% CO_2 and had a pH of 7.15 to 7.2 at 37°. The tracheal preparation was initially set on 2 g of tension and

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readjusted to 2 g within the first 15 min. It was then allowed to equilibrate for 1 to 2.5 hr, with 3–4 washes over that period. Responses at isometric tension were recorded on a Grass or Gould RS3600 physiograph. The tissue first was contracted with 0.1 μM carbamylcholine chloride (45 ± 7% of maximal contraction with carbamylcholine), and a cumulative relaxation concentration–response curve was determined for each xanthine. Maximal relaxation was measured at the end of each experiment using 0.2 μM *d,l*-isoproterenol. The EC_{50} values for relaxation were determined using the GRAPHPAD™ computer program (Graphpad Software, Inc., San Diego, CA). For compounds dissolved in dimethyl sulfoxide or NaOH, control experiments were performed with these solvents in parallel, using tissue from the same animal. Solvent effects were subtracted from experimental results. The concentrations of DMSO used ranged from 0.25% to 1.0% except in two experiments where the maximum was 2.0%. The lowest concentration caused 12 ± 2% maximal relaxation, while 1.0% caused 44 ± 6.6% maximal relaxation. Student's *t*-test was used to determine the statistical difference in potency of the compounds and was considered significant if $P < 0.05$. The method of least squares was used in regression and correlation analysis for Figs. 1–5 [14].

RESULTS

The EC_{50} (mean ± SE) values for relaxation of carbamylcholine-contracted spiral rings by caffeine, theophylline and enprofylline analogs are shown in Table 1. In the caffeine series, all substitutions of the caffeine molecule increased the potency as tracheal relaxants except for a *p*-sulfophenyl group in the 8-position. For substitution in the 1-position the potency for caffeine analogs was as follows: 1-propyl > 1-allyl = 1-propargyl > caffeine. The 3-propyl analog of caffeine was more potent than the 3-propargyl analog, which in turn was more potent than caffeine. Substitution at both the 1- and 3-positions of caffeine did not further increase potency, as the EC_{50} for 1,3-dipropyl-7-methylxanthine was equivalent to the EC_{50} values of either the 1- or 3-propyl analog. The 7-propyl, 7-allyl and 7-propargyl analogs of caffeine were all equipotent, and more potent than caffeine. 1,3,7-Tripropylxanthine was more potent than 1,3,7-tripropargylxanthine. Substitution at the 8-position of caffeine by a cyclohexyl, cyclopentyl, or phenyl group increased potency approximately 7-fold over the parent compound. 8-*p*-Sulfophenylcaffeine was the least potent of all substituted caffeine analogs in this series, and indeed was probably inactive as a tracheal relaxant.

Substitution at the 3-position of theophylline with an isobutyl group resulted in 3-isobutyl-1-methylxanthine, the most potent compound tested. Increasing the bulk at the 1-position of this xanthine by substitution with an isoamyl group decreased potency slightly. Substitution of both the 1- and 3-positions of theophylline with *n*-propyl increased potency by 2-fold. Addition of an 8-cyclopentyl group to theophylline increased potency, whereas addition of an 8-cyclohexyl group to theophylline had no effect on potency. 8-Phenyltheophylline had little effect on trachea near the limit of water solubility (50 μM). Substitution with *p*-sulfophenyl or bromo groups in the 8-position of theophylline eliminated or markedly decreased activity as tracheal relaxants.

The effect of 8-substitution was examined in the enprofylline series. An 8-cyclohexyl group had little effect on potency. The 8-phenyl-, 8-(*p*-carboxyphenyl)- and 8-(*p*-sulfophenyl) derivatives of enprofylline had either low or no activity.

Two additional xanthines, namely paraxanthine (1,7-dimethylxanthine) and 1-methylxanthine, had low potency as tracheal relaxants.

Determinations of the EC_{50} values were, in some cases, limited by the solubility of the compounds, in particular for 8-cyclohexyl-1,3-dipropylxanthine, 8-phenyltheophylline, 8-cyclohexylenprofylline, and 8-phenylenprofylline (see footnotes, Table 1).

DISCUSSION

Due to the widespread use of theophylline as an antiasthmatic, the structure–activity relationships of xanthines in tracheal relaxation are of great interest. In the present study, xanthine analogs of the caffeine, theophylline, and enprofylline class were examined for their potencies as tracheal smooth muscle relaxants.

The order of potency for enprofylline > theophylline > caffeine is in agreement with prior studies [15–18]. The potencies of enprofylline, aminophylline, and 8-phenyltheophylline on the relaxation of acetyl-β-methylcholine-stimulated guinea pig trachea as reported by Farmer *et al.* [19] are all greater than in the present study. The precontraction of the tissue with 2 μM acetyl-β-methylcholine, followed by washing before each experiment, may explain the differences between that study and our own.

The structure–activity relationships indicate that substitution of a group larger than a methyl in the 3-position of xanthines is important for potency. 3-Propylxanthine was a potent tracheal relaxant, as were the 1,3-dialkylxanthines (3-isobutyl-1-methylxanthine and 1-isoamyl-3-isobutylxanthine) with an isobutyl group in the 3-position. Increasing the polarity at the 1- or 3-position by substituting a propargyl group for an *n*-propyl group decreased relaxant activity, an effect not observed for similar alteration at the 7-position.

The 8-cyclohexyl and 8-cyclopentyl analogs were relatively potent tracheal relaxants. The activity was dependent on the nature and position of other substituents on the xanthine ring. Thus, the 8-cyclohexylcaffeine, which has a 7-methyl group, was more potent than 8-cyclohexyltheophylline, which lacks the additional methyl. 8-Cyclopentyltheophylline was more potent than 8-cyclohexyltheophylline and equipotent to 8-cyclopentyl- and 8-cyclohexylcaffeine.

The low solubility of many of the xanthines may limit their usefulness, particularly the 8-substituted enprofyllines. Attempts to improve solubility with the addition of *p*-sulfo- or *p*-carboxy-groups to the 8-phenyl ring resulted in very weak tracheal relaxants.

Table 1. Xanthines as guinea pig tracheal relaxants

Compounds	EC_{50} (μM)	N
1. Caffeine (1,3,7-Trimethylxanthine)	551 ± 81	6
2. 3,7-Dimethyl-1-propylxanthine	110 ± 19	6
3. 1-Allyl-3,7-dimethylxanthine	208 ± 29	4
4. 3,7-Dimethyl-1.propargylxanthine	226 ± 43	5
5. 1,7-Dimethyl-3-propylxanthine	176 ± 23	4
6. 1,7-Dimethyl-3-propargylxanthine	261 ± 25	6
7. 1,3-Dimethyl-7-propylxanthine	164 ± 23	4
8. 7-Allyl-1,3-dimethylxanthine	162 ± 23	8
9. 1,3-Dimethyl-7-propargylxanthine	153 ± 17	6
10. 7-Methyl-1,3-dipropylxanthine	152 ± 43	5
11. 1,3-Diallyl-7-methylxanthine	142 ± 23	4
12. 1,3,7-Tripropylxanthine	12 ± 1.3	3
13. 1,3,7-Tripropargylxanthine	65 ± 11	4
14. 8-Cyclopentylcaffeine	68 ± 18	5
15. 8-Cyclohexylcaffeine	74 ± 15	3
16. 8-Phenylcaffeine	75 ± 10	3
17. 8-(*p*-Sulfophenyl)caffeine	⩾500 (0 ± 4; 500 μM)*	2
18. Theophylline (1,3-Dimethylxanthine)	162 ± 17	4
19. 3-Isobutyl-1-methylxanthine	7.1 ± 1.8	4
20. 1-Isoamyl-3-isobutylxanthine	37 ± 4.2	4
21. 8-Cyclopentyltheophylline	68 ± 8	3
22. 8-Cyclohexyltheophylline	163 ± 32	4
23. 8-Phenyltheophylline	>50† (18 ± 7; 50 μM)	4
24. 8-(*p*-Sulfophenyl)theophylline	⩾250 (5 ± 5; 250 μM)	2
25. 8-Bromotheophylline	279 ± 55	4
26. 1,3-Dipropylxanthine	88 ± 23	6
27. 8-Cyclopentyl-1,3-dipropylxanthine	⩾250 (47 ± 3; 250 μM)	2
28. 8-Cyclohexyl-1,3-dipropylxanthine	>50† (22 ± 3; 50 μM)	2
29. 1,3-Dipropyl-8-(*p*-sulfophenyl)xanthine	>500 (23 ± 5; 500 μM)	3
30. Enprofylline (3-Propylxanthine)	56 ± 9	5
31. 8-Cyclohexylenprofylline	>25‡ (34 ± 3; 25 μM)	2
32. 8-Phenylenprofylline	>50† (0 ± 5; 50 μM)	2
33. 8-(*p*-Carboxyphenyl)enprofylline	>250 (31 ± 2; 250 μM)	2
34. 8-(*p*-Sulfophenyl)enprofylline	>500 (25 ± 5; 500 μM)	2
35. Paraxanthine (1,7-Dimethylxanthine)	>250 (38 ± 9; 250 μM)	3
36. 1-Methylxanthine	>250 (31 ± 16; 250 μM)	2

Data are presented as the micromolar concentration of the xanthine analog required to elicit 50% of the maximum relaxation to isoproterenol (0.2 μM). Values are means ± SE; the number of observations (N) is indicated.

* Percent maximal relaxation at indicated concentrations.

† Precipitate formation at 100 μM.

‡ Precipitate formation at 50 μM.

The decreased relaxant activity with the polar 8-*p*-sulfophenyl analogs may reflect the inability of such compounds to cross the cell membrane [20].

Related studies on the structure–activity relationship of various methylxanthines and tracheal relaxation have shown that methyl groups at the 1- and 3-positions are important for potency and that di- and trimethylxanthines possessing an 8-methyl group are more potent than di- and trimethylxanthines possessing a 7-methyl group [16]. A study on 3-substituted xanthines showed potency in the order of: 3-isobutylxanthine = 3-propylxanthine = 3-butylxanthine > theophylline > 3-ethylxanthine = 3-isopropylxanthine [18]. 1-Methyl-3-propylxanthine and 1-methyl-3-butylxanthine have been shown to be about 30- and 20-fold more potent than theophylline in relaxation of guinea pig tracheal smooth muscle [21]. 6-Thiotheophylline and 6-thiocaffeine are 6-fold more potent than theophylline [22]. In canine trachea the relaxant potencies of several xanthines are as follows: 3-isobutyl-1-methylxanthine > theophylline > 3-methylxanthine > 7-methylxanthine = 1-methylxanthine > caffeine [17].

The mechanism by which xanthines cause tracheal relaxation is unclear. Putative mechanisms for xanthine action are as follows: (1) adenosine receptor antagonism; (2) inhibition of phosphodiesterases; (3) direct effects on release or sequestration of intracellular calcium; (4) direct inhibitory effects of contractile elements to calcium; and (5) membrane hyperpolarization with consequent decrease in calcium influx [23].

The clinical effects of xanthines in the treatment of asthma were suggested as possibly due to adenosine receptor antagonism [24]. Certainly, asthmatic patients respond to inhaled adenosine with bronchoconstriction, and theophylline antagonizes this

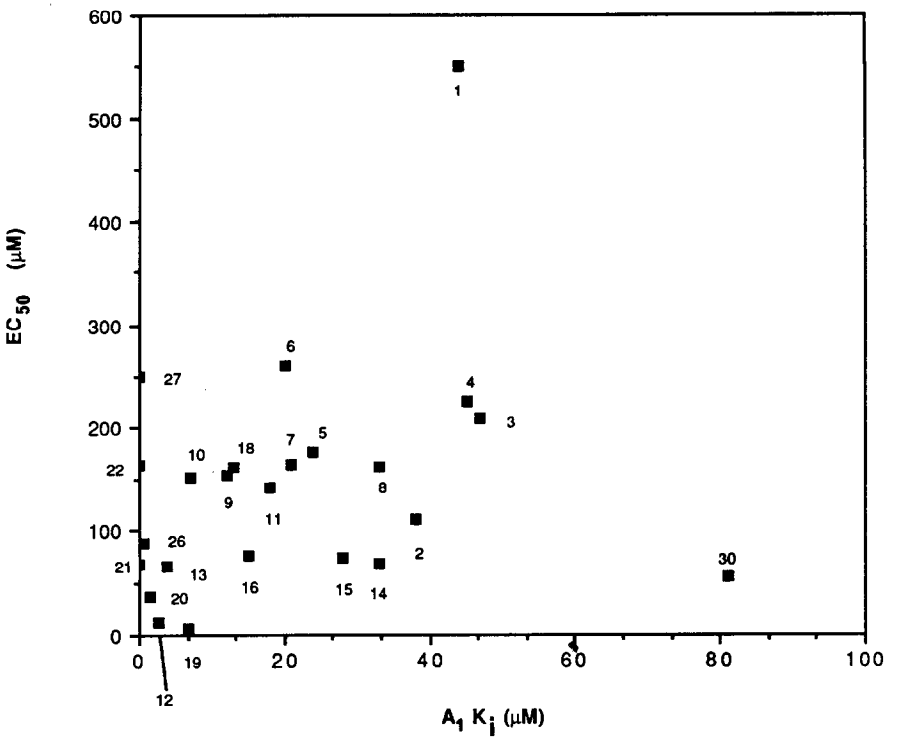

Fig. 1. A_1-adenosine receptor activity versus tracheal relaxation. A comparison of EC_{50} values for tracheal relaxation of xanthines and K_i values for blockade of A_1-adenosine receptors ([9–11, 28, 29], and unpublished results) is shown. See Table 1 for key to compound numbers. Regression and correlation analysis showed $r = 0.071$. Compounds not shown are: 8-bromotheophylline, 8-*p*-sulfophenylcaffeine, 8-phenyltheophylline, 8-*p*-sulfophenyltheophylline, 8-cyclohexyl-1,3-dipropylxanthine, 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine, 8-cyclohexylenprofylline, 8-phenylenprofylline, 8-(*p*-carboxyphenyl)enprofylline, 8-(*p*-sulfophenyl)enprofylline, paraxanthine, and 1-methylxanthine.

effect [25]. Bronchoconstriction could be due to inhibition of cyclic AMP production through an activation of inhibitory A_1 adenosine receptors by endogenously released adenosine [19, 26, 27]. However, the present and other studies indicate that relative potencies of xanthines as tracheal relaxants do not correlate with potencies of xanthines as adenosine receptor antagonists (Figs 1 and 2). For example, theophylline, with a K_i of 13 μM at A_1 receptors is a weaker relaxant than enprofylline with a K_i of 81 μM at A_1 receptors [11]. 8-*p*-Sulfophenyl-1,3-dipropylxanthine is a potent and selective A_1-receptor antagonist with a K_i at A_1 receptors of 0.14 μM [11], yet it was virtually inactive as a tracheal relaxant. Also, 8-cyclopentyl-1,3-dipropylxanthine with a $K_i = 0.9$ nM at A_1 receptors [11] was very weak as a tracheal relaxant. The relaxant effects of selected xanthines from the present series were not affected by the presence of adenosine deaminase in the medium (results not shown). Thus, the relaxant effects of xanthines on guinea pig trachea appear to be independent of adenosine receptor blockade.

Evidence that xanthines act by inhibition of phosphodiesterase with a resultant elevation of intracellular cyclic nucleotides and a subsequent relaxation of smooth muscle results from various studies [15, 17, 30, 31]. Enprofylline is three times more potent than theophylline as an inhibitor of cyclic AMP-specific phosphodiesterase in guinea pig lung, which correlates well with its relative potency as a tracheal relaxant [32]. Polson *et al.* [17] found a positive correlation of inhibition of a calcium-independent phosphodiesterase with the relaxant effect of four xanthines in canine trachea. Comparison of values reported for inhibition of rat brain phosphodiesterases [10] for a number of xanthines from the present study with potency as tracheal relaxants showed a positive correlation (Figs. 3–5). These results further suggest a role for phosphodiesterase inhibition in smooth muscle relaxation by xanthines.

This study has demonstrated the potencies of xanthines as tracheal smooth muscle relaxants. The association between phosphodiesterase inhibition and relaxant activity is suggestive; more information of xanthine effects on tracheal phosphodiesterase isozymes is needed. The tracheal relaxant effects are shown to be independent of adenosine receptor antagonism. The most potent tracheal relaxants of the present study were: 3-isobutyl-1-methylxanthine $\geq$ 1,3,7-tripropylxanthine > 1-isoamyl-3-isobutylxanthine (Table 1). The first two were several-fold more potent than enprofylline. 3-Isobutyl-1-methylxanthine is a potent phosphodiesterase inhibitor [10]. 1-Isoamyl-3-isobutylxanthine is also a potent phosphodiesterase inhibitor, although somewhat less potent than 3-isobutyl-1-methylxanthine for brain

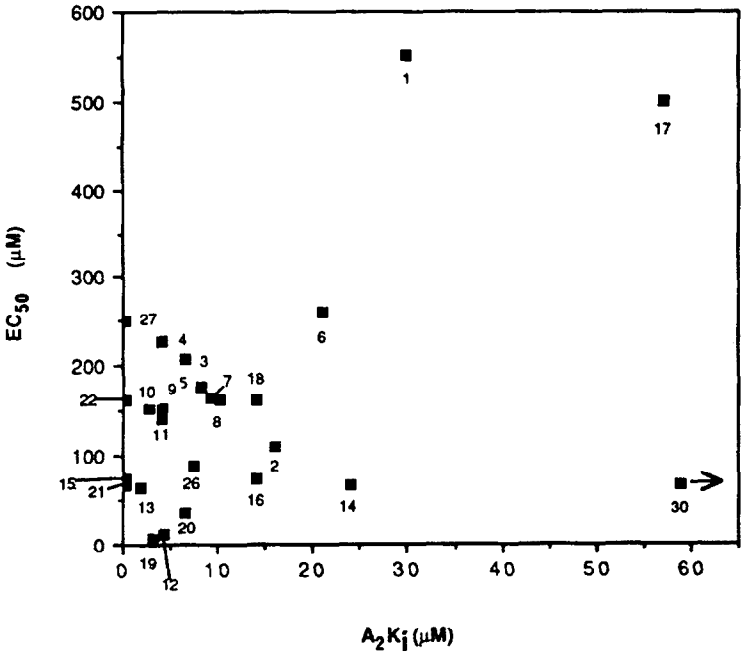

Fig. 2. A_2-adenosine receptor activity versus tracheal relaxation. A comparison of EC_{50} values for tracheal relaxation of xanthines and K_i values for blockade of A_2-adenosine receptors ([9–11, 28, 29], and unpublished data) is shown. See Table 1 for key to compound numbers. Regression and correlation analysis showed $r = 0.029$. The arrow indicates a K_i value greater than 100 μM. Compounds not shown are: 8-bromotheophylline, 8-phenyltheophylline, 8-*p*-sulfophenyltheophylline, 8-cyclohexyl-1,3-dipropylxanthine, 1,3-dipropyl-8-(*p*-sulfophenyl)xanthine, 8-cyclohexylenprofylline, 8-phenylenprofylline, 8-(*p*-carboxyphenyl)enprofylline, 8-(*p*-sulfophenyl)enprofylline, paraxanthine, and 1-methylxanthine.

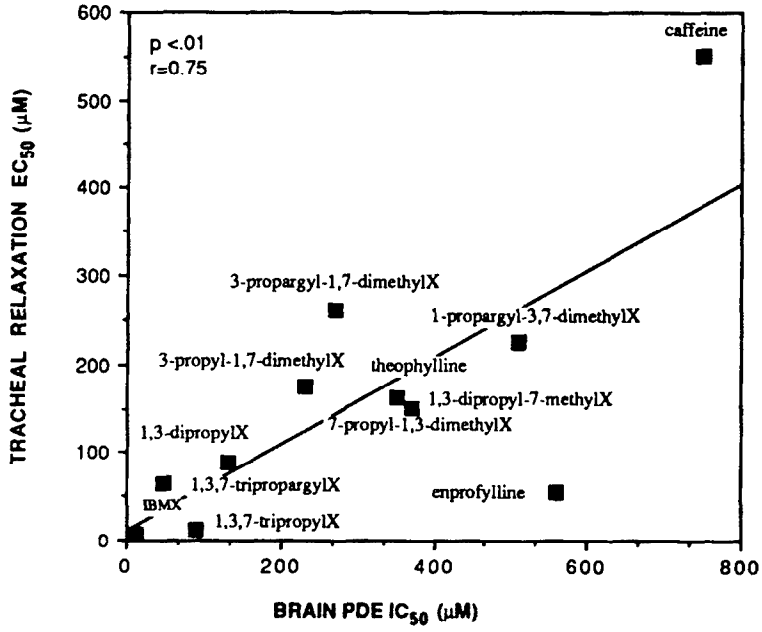

Fig. 3. PDE (soluble, Ca^{2+}-dependent) versus tracheal relaxation. A comparison of EC_{50} values for tracheal relaxation of xanthines and IC_{50} values for inhibition of soluble, calcium-dependent phosphodiesterase (PDE) from rat brain [10] is shown. Regression and correlation analysis showed $P < 0.01$, $r = 0.75$. X = xanthine.

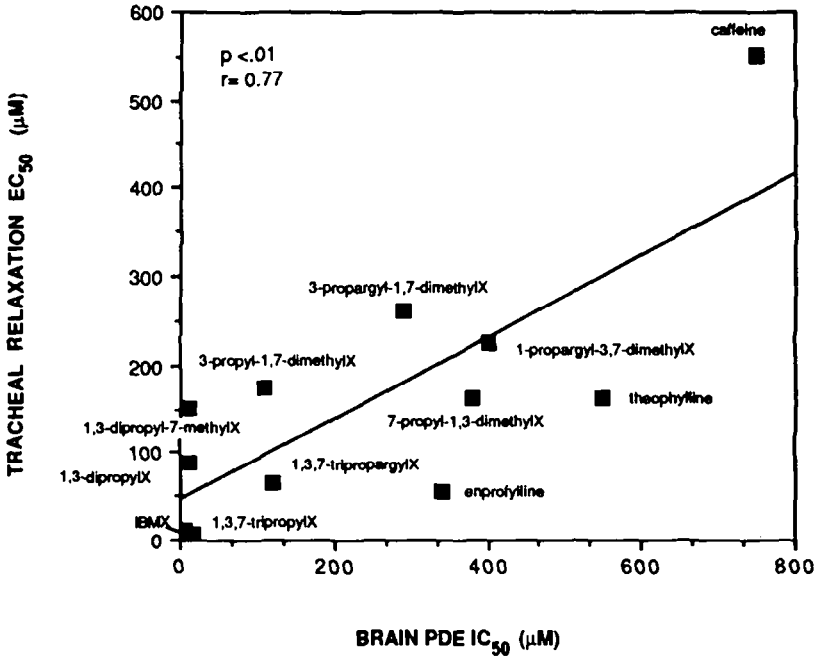

Fig. 4. PDE (membrane, Ca^{2+} independent) versus tracheal relaxation. A comparison of EC_{50} values for tracheal relaxation of xanthines and IC_{50} values for inhibition of membrane, calcium-independent phosphodiesterase (PDE) from rat brain [10] is shown. Regression and correlation analysis showed $P < 0.01$, $r = 0.77$. X = xanthine.

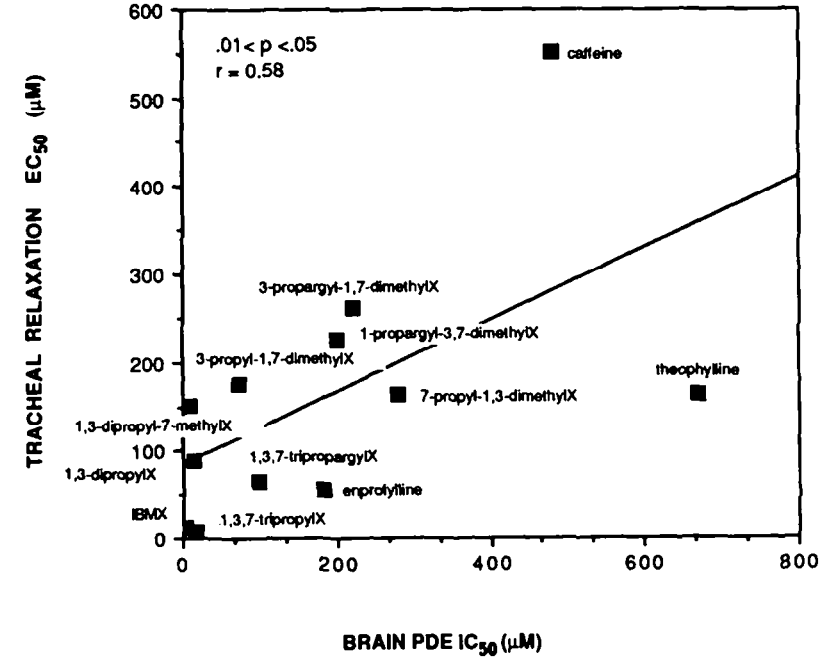

Fig. 5. PDE (soluble, Ca^{2+}-independent) versus tracheal relaxation. A comparison of EC_{50} values for tracheal relaxation of xanthines and IC_{50} values for inhibition of soluble, calcium-independent phosphodiesterase (PDE) from rat brain [10] is shown. Regression and correlation analysis showed $0.01 < P < 0.05$, $r = 0.58$. X = xanthine.

enzymes [33]. Certain of the 8-cycloalkylxanthines were relatively potent tracheal relaxants (Table 1), as are certain 8-methylxanthines [16], while 8-bromotheophylline was nearly inactive. Little is known of the activity of such 8-cycloalkylxanthines or of 8-phenylxanthines as phosphodiesterase inhibitors. 8-Cyclohexyl- and 8-cyclopentyl-1,3-dipropylxanthine were inactive at 1 μM, while at 6 μM 8-phenyl-1,3-dipropylxanthine causes a 20–30% inhibition of phosphodiesterases from coronary artery [34]. 8-Phenyltheophylline and certain 8-aryl analogs have been reported to be weak inhibitors of phosphodiesterases [33, 35]. The low activity of the 8-*p*-sulfophenylxanthines is consonant with an important role for inhibition of phosphodiesterases in tracheal relaxation by xanthines. The 8-*p*-sulfophenyl xanthines do not penetrate cells [20] and thus will not have access to intracellular phosphodiesterases. The structure–activity relationships suggest that further investigation of caffeine analogs with variations of alkyl substituents at the 3-, 7-, and 8-positions may lead to the discovery of even more potent tracheal relaxants.

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