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Selective Bronchodilators from 1-(5′-Oxohexyl)xanthines

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Abstract—A series of twenty one 1-(5′-oxohexyl)xanthines substituted with alkyl chains at the *N*3 and *N*7 positions of the xanthine nucleus were prepared and their relaxant activity in guinea-pig isolated tracheal muscle and positive chronotropic activity in isolated right atrium of guinea-pig were compared. The tracheal relaxant activities were markedly increased with alkyl chain length at the *N*3 position, but decreased by the *N*7 alkylation. The positive chronotropic activities in the right atrium were increased by introduction of an n-propyl group at the *N*3 position but decreased by substitution of longer alkyl chains, and the action on the heart was diminished by *N*7 substitution. The activities of compounds on cAMP-phosphodiesterase (PDE) and binding of [^{3}H]8-cyclopentyl-1,3-dipropylxanthine were measured in the homogenate of tracheal muscle and the membrane preparation of cerebral cortex, respectively. No relationship among tracheal muscle relaxant activity, cAMP-PDE inhibitory activity and adenosine antagonism of these xanthines was observed, and other action mechanisms should be considered for their relaxant activities. This study indicated that *N*3 alkylation is important for the selectivity for tracheal muscle, while the introduction of long alkyl chains such as n-butyl and n-pentyl groups at the *N*3 and *N*7 positions diminished the potency for the right atrium in guinea-pigs. 3-n-Pentyl- and 7-methyl-3-n-pentyl-1-(5′-oxohexyl)xanthines showed much higher bronchoselectivity than oxpentifylline and theophylline.

Despite the introduction of new classes of anti-asthma drugs, theophylline is still frequently used for the treatment of pulmonary diseases. Adverse reactions of theophylline on cardiovascular and central nervous systems are also well known, because of its many pharmacological actions or low selectivity for bronchus muscle. We have reported a close relationship between smooth muscle relaxant activity and cAMP-phosphodiesterase (PDE) inhibitory activity of the *N*3-alkylxanthines, and found that 1-methyl-3-n-propylxanthine has a much stronger in-vitro relaxant effect than either theophylline or enprofylline (Apichartpichean et al 1988; Miyamoto et al 1989; Ogawa et al 1989). On the other hand, oxpentifylline (3,7-dimethyl-1-(5′-oxohexyl)xanthine) and propentofylline (3-methyl-7-n-propyl-1-(5′-oxohexyl)xanthine), which were developed by structural modification of theobromine, have been used for peripheral and central circulatory disturbances without apparent cardio-excitatory and central nervous system-stimulatory actions (Spagnoli & Tognoni 1983; Aviado & Porter 1984; Grome & Stefanovich 1985). Hoping to obtain selective bronchodilators, we prepared a series of 3,7-dialkyl substituents of 1-(5′-oxohexyl) xanthine and studied their pharmacological properties in guinea-pigs.

Materials and Methods

Materials

Twenty one 3,7-dialkyl-1-(5′-oxohexyl)xanthines were synthesized in our laboratory (Papesch & Schroeder 1951; Wooldridge & Slack 1962; Ohtsuka 1973; Sneddon 1982) and are shown in Table 1. Theophylline was purchased from the Sigma Chemical Co., St Louis, MO, USA). The purity of each compound was determined by elemental analysis and they were within 0·03% of the calculated value.

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Animals

Male Hartley guinea-pigs, 300–500 g (SLC, Shizuoka, Japan), were used in this study. The animals were killed by stunning and bleeding, and the tissues were rapidly removed.

Tracheal muscle relaxation

Isolated tracheal ring chains were placed in a 10 mL thermostatically controlled organ bath (37°C) containing Krebs-Henseleit solution (pH 7·4) of the following composition (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·6, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 24·9, and glucose 11·1, gassed with 95% O_2–5% CO_2. Isoprenaline (1·0 μM) was added to produce complete relaxation and washed out. A tension of 0·5 g was then applied to the preparation, and spontaneous tone was allowed to develop. After the tension had become constant, the preparation was treated with cumulative concentrations (10 nM–100 μM) of the test compound. The tension changes were recorded through an isotonic transducer. Relaxation by 1·0 μM isoprenaline was defined as 100%, and the concentration producing 50% tracheal muscle relaxation (EC50) was calculated from data of three to four experiments.

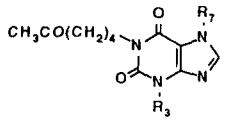
Beating rate of isolated right atrium

The right atrium was placed in an organ bath (25°C) filled with Krebs-Henseleit solution aerated with a gas of 95% O_2–5% CO_2. The preparation was allowed to equilibrate under a tension of 0·5 g and then treated with cumulative concentrations (1·0–100 μM) of test compound. The beating rate was recorded through a cardiotachometer triggered by the developed force. Data were expressed as the concentration required to increase the unstimulated beating rate (120–140 beats min^{-1}) by 15% (EC15).

cAMP-phosphodiesterase assay

Trachealis muscle was homogenized in Tris-HCl buffer (mM: Tris-HCl 40, $MgCl_2$ 10, 2-mercaptoethanol 4·0, pH 8·0) and

Table 1. Pharmacological activities of 3, 7-dialkyl-1-(5′-oxohexyl)xanthines.



R_3	R_7	Relaxant activity in tracheal muscle EC50 (μM)	Stimulatory activity in right atrium EC15 (μM)	Ratio[a]	cAMP-PDE inhibitory activity K_i (μM)	Affinity for adenosine receptor K_i (μM)
Me	H	19±8	72±12	3·8	67±7	64±8
Et		0·39±0·09	39±5	100	104±3	36±4
n-Pro	H	0·058±0·017	3·6±0·7	62	16±2	23±5
n-But	H	0·064±0·004	29±3	445	27±16	18±5
n-Pen	H	0·17±0·04	>100	558	16±8	8·3±1·7
Me	Me	28±1	52±12	1·9	38±4	180±2
(Oxpentifylline)						
Et	Me	3·6±0·9	45±9	12	53±16	81±12
n-Pro	Me	0·43±0·04	7·2±2·2	17	42±5	56±10
n-But	Me	0·24±0·02	>100	417	24±9	39±4
n-Pen	Me	0·20±0·04	>100	500	40±4	26±7
Me	Et	11±4	39±2	3·6	34±5	47±4
Et	Et	6·7±0·6	35±8	5·3	31±6	24±3
n-Pro	Et	0·76±0·10	12±3	15	38±5	23±9
Me	n-Pro	5·7±4·0	53±6	9·3	20±3	60±2
(Propentofylline)						
Et	n-Pro	1·7±0·3	48±11	28	22±8	40±5
n-Pro	n-Pro	0·70±0·34	35±12	49	25±10	14±3
Me	n-But	5·3±0·6	>100	19	14±3	29±1
Et	n-But	1·3±0·2	66±12	51	10±1	19±2
n-Pro	n-But	0·52±0·16	>100	192	10±2	7·1±1·1
Me	n-Pen	2·5±0·9	>100	40	15±2	8·1±1·9
n-Pro	n-Pen	2·1±0·1	>100	48	19±5	9·6±2·2
Theophylline		28±2	62±4	2·2	57±9	9·8±0·4

Each value represents the mean±s.e. of three to four measurments. [a] The stimulatory activity in the right atrium (EC15, μM) vs the relaxant activity in the tracheal muscle (EC50, μM). The value over 100 μM was calculated as 100 μM.

centrifuged at 10000 *g*. cAMP-phosphodiesterase (PDE) activity with a low K_m in the supernatant was measured by the two-step assay system of Thompson & Appleman (1971). The reaction mixture, of 0·2 mL, containing 40 mM Tris-buffer, unlabelled cAMP (0·05–0·1 μM), 3·7 kBq [^{3}H]cAMP (sp. act. 1·24 GBq μmol^{-1}, New England Nuclear, Boston, MA, USA), various concentrations (1·0–100 μM) of test compound and about 5 μg protein of the enzyme preparation, was incubated at 30°C for 10 min and boiled to terminate the reaction. [^{3}H]Adenosine produced by treatment with snake venom (*Crotalus atrox*, Sigma) and Dowex 1-X2 (Bio-Rad Lab., Richmond, VA, USA) was counted in a Beckman LS5801 liquid scintillation counter. In this assay system, the PDE inhibitory activity of compounds used was not influenced by their action on 5′-nucleotidase in snake venom. The inhibition constant (K_i) was calculated by the method of Dixon (1953).

Adenosine receptor binding assay

Preparations of membrane from the cerebral cortex and the ligand binding assay were done essentially as described by Jacobson et al (1986). [^{3}H]8-Cyclopentyl-1,3-dipropylxanthine ([^{3}H]CPX, sp. act. 4·04 GBq μmol^{-1}, New England Nuclear), a selective antagonist of A_1 receptors (Bruns et al 1987), was used in this study. The Scatchard plot of the CPX binding to the brain membrane preparation was linear with a K_d of 2·33 nM and a B_{max} of 388 fmol (mg protein)$^{-1}$. For examination of ligand binding replacement, a mixture of 2·0 nM [^{3}H]CPX, various concentrations (1·0–100 μM) of test compound, 0·2 units of adenosine deaminase, and about 100 μg of protein of a membrane preparation in 50 mM Tris-HCl (pH 7·4) was incubated at 37°C for 120 min. Bound and free radioligand were separated by rapid filtration through Whatman GF/B glass fibre filters using a cell harvester (Model 2000 PHD, Cambridge Technology, Cambridge, MA, USA). The filters were treated with 0·3% polyethyleneimine before use. Non-specific binding of [^{3}H]CPX to the membranes was measured in the presence of 10 mM theophylline. The dose-inhibition data for each xanthine was analysed using a nonlinear least-squares fit to a competitive inhibition model. The inhibition constant (K_i) was calculated from the Cheng-Prusoff equation (1973).

Protein was measured by the method of Lowry et al (1951).

Results and Discussion

The pharmacological activities of twenty one 3,7-dialkyl-1-(5′-oxohexyl)xanthines were studied and compared with those of theophylline in several tissues of guinea-pigs, and are shown in Table 1. The activities of oxpentifylline in tracheal muscle relaxation and atrial beat stimulation were almost the same as those of theophylline. The relaxant activity of propentofylline was about 5-fold that of theophylline, having similar heart-stimulating activity. Here, the ratio of the positive chronotropic action in the right atrium (EC15, μM) to the relaxant action in the tracheal smooth muscle (EC50, μM), of these compounds was only 2–10. Therefore, when these drugs were used for bronchodilation as in a case of asthma, extrapulmonary adverse reactions such as arrhythmia may occur. We designed 3,7-dialkyl-1-(5′-

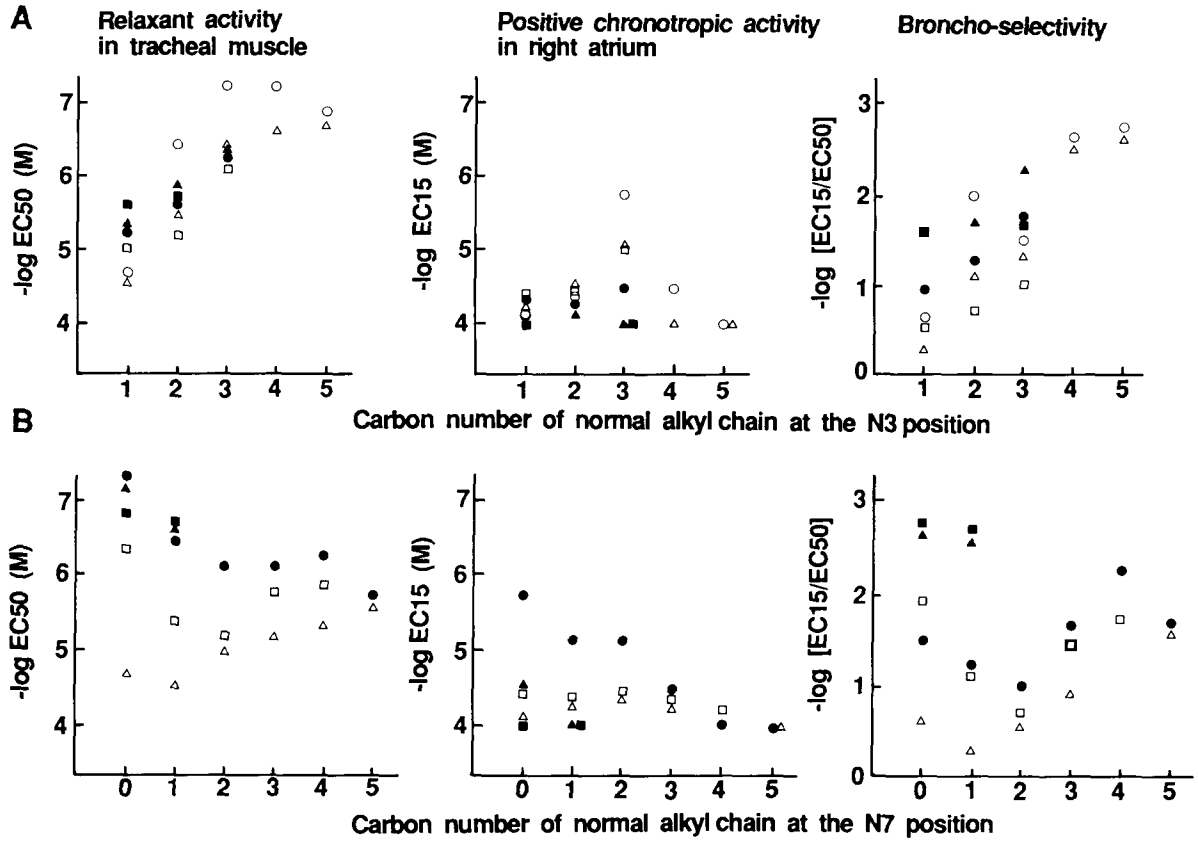


FIG. 1. Relationship between relaxant activity in the tracheal muscle, positive chronotropic activity in the right atrium or broncho-selectivity and alkyl chain length of 3,7-dialkyl-1-(5'-oxohexyl)xanthines. A. Activities of *N*3-alkyl substituents with 7-H (○), 7-methyl (△), 7-ethyl (□), 7-n-propyl (●), 7-n-butyl (▲) and 7-n-pentyl (■) groups. B. Activities of *N*7-alkyl substituents with 3-methyl (△), 3-ethyl (□), 3-n-propyl (●), 3-n-butyl (▲) and 3-n-pentyl (■) groups.

oxohexyl)xanthines to obtain broncho-selective compounds. As shown in Fig. 1, when the *N*3-alkyl chain was prolonged, the relaxant activity became much stronger. The atrial beat stimulatory activity was increased by introduction of an n-propyl group at the *N*3 position, but decreased by substitution of longer alkyl chains. In other words, *N*3-alkylation evoked broncho-selectivity. Regarding the *N*7-substituents, the tracheal relaxant activity tended to be lowered by alkylation, except in the case of *N*3-methyl derivatives of oxpentifylline and propentofylline, which moderately increased with the chain length, and the activity on the heart was lost by addition of n-butyl and n-pentyl groups as was the case for *N*3 substitution. The effects of *N*7-substituents on such broncho-selectivity showed a biphasic pattern, decreased by short chains and increased by longer chains. Consequently, 3-n-pentyl- and 7-methyl-3-n-pentyl-1-(5'-oxohexyl)xanthines exerted no stimulatory action on the heart and had about a 150-fold stronger relaxant effect on the tracheal smooth muscle than did the parent compounds, oxpentifylline and theophylline.

Several hypotheses may explain the mechanism of the bronchodilatory action of xanthines by cAMP-PDE inhibition (Katsuki & Murad 1977; Polson et al 1982). Our studies also indicated that the tracheal muscle relaxant effect of xanthine derivatives was closely correlated with the inhibitory activity of cAMP-PDE in the alkyl chain prolongation at the *N*3 position (Apichartpichean et al 1988; Takagi et al 1988; Miyamoto et al 1989; Ogawa et al 1989). We measured the cAMP-PDE inhibitory activity of 3,7-dialkyl-1-(5'-oxohexyl)xanthines. The inhibitory activity on the enzyme was not significantly changed by either substitution at the *N*3 or the *N*7 positions (Fig. 2) and was not correlated with their relaxant activity on the isolated trachea. Another well-documented activity of xanthines is adenosine antagonism. Xanthines exert many pharmacological actions on various tissues through antagonizing endogeneous adenosine on its particular receptors (Daly 1985; Persson et al 1986; Hamilton 1988). In the tracheal muscle, adenosine causes contraction through stimulation of the adenosine A_1 receptor and at higher concentrations elicits relaxation via the A_2 receptor; xanthines antagonize the A_1-mediated contractile reaction and relax the smooth muscle, while inhibiting the A_2-mediated relaxation (Farmer et al 1988). In this study, the adenosine antagonistic action, defined by displacement potency for [^{3}H]CPX binding to the brain membrane, was only slightly increased with alkyl chain length at the *N*3 position of 1-(5'-oxohexyl)xanthine and hardly changed by *N*7-alkylation (Fig. 2). This activity was not related to the bronchodilatory action of these xanthine derivatives. From these results, it is difficult to explain completely the pharmacological actions of 3,7-dialkyl-1-(5'-oxohexyl)xanthines by their cAMP-PDE inhibitory activity

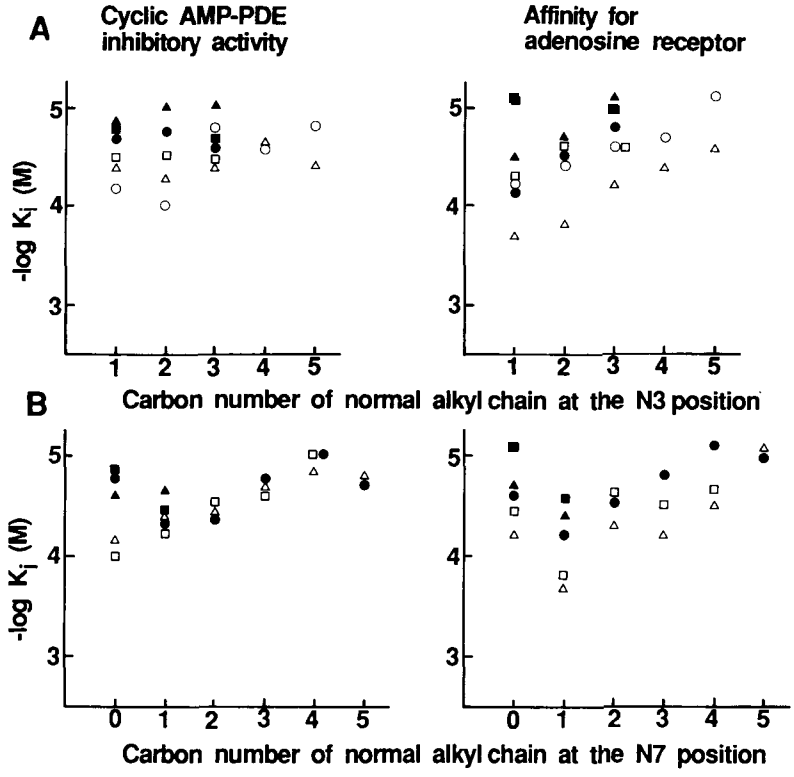


FIG. 2. Relationship between cAMP-PDE inhibitory activity or affinity for adenosine receptor and alkyl chain length at the *N*3 (A) and *N*7 (B) positions of the 1-(5′-oxohexyl)xanthine molecule. Symbols are the same as for Fig. 1.

and adenosine antagonism. Oxpentifylline and propentofylline have been reported to produce increases in peripheral and cerebral blood flow, but their primary mechanism of action is still unknown. Fredholm & Lindstrom (1986) have tried to explain their actions by the potentiation of endogenous adenosine by adenosine uptake inhibition. The tracheal relaxant effect found in this study remains a possibility related to their adenosine uptake inhibition, although there is negative evidence that dipyridamol, a potent inhibitor of adenosine uptake and cyclic nucleotide-PDE, does not have the expected relaxant effect on the trachea (Fredholm et al 1979; Ruffin & Newhouse 1981). The bronchodilatory effect of these xanthines may occur through multiple molecular mechanisms including unknown mechanisms, that cannot be attributed to PDE inhibition or adenosine receptor antagonism, as described by Howell (1990).

In conclusion, this study indicates that potent and selective bronchodilators are developed by alkylation at the *N*3 and *N*7 positions of the 1-(5′-oxohexyl)xanthine molecule. It seems that *N*3-alkylation increases the affinity for the tracheal muscle and *N*7-alkylation reduces the potency for the heart. 3-n-Pentyl- and 7-methyl-3-n-pentyl-1-(5′-oxohexyl)xanthines exerted about 150-fold stronger tracheal relaxant effect without atrial stimulation than oxpentifylline and theophylline.

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