associated with the carbonyl groups of the adjacent xanthine molecule, and consequently their nmr signals are shifted to higher fields. Disintegration of stacked aggregates of xanthines, including theophylline, caused by dilution, has been decribed by Thakkar et al (1971) to be accompanied by a downfield shift of *N*-methyl signals.

Therefore it is suggested that two opposing factors operate in alkaline solutions of theophylline, namely the inductive effect of the negative charge which causes the upfield shift, and the 'dispersing' effect of the negative charge which leads to a downfield shift. Of these, the second effect is predominant only when about a third of theophylline is ionized. This would suggest that initially theophylline aggregates ionize without disintegration. Only when about one in every three theophylline molecules is ionized do the electrical repulsion forces become strong enough to effect a gradual disintegration of the stacks, an effect that is completed at an amine : theophylline molar ratio of 1·2. When theophylline is partially ionized, neutral molecules are solubilized by the anions which are present in the same stacks.

In conclusion, it seems that the phenomenon of ethylenediamine reacting and crystallizing with two molecules of theophylline to give aminophylline, presumably the double salt, is limited to the solid phase only. In aqueous solution, calculations based on pK_a values suggest that the stoichiometry of the reaction is only 1 : 1, and so a solution of aminophylline contains about 50% of neutral theophylline. Nmr data also failed to show the existence of any specific structure at the ratio found in aminophylline. On the other hand, the nmr data strongly suggest that such partially neutralized solutions of theophylline are composed of mixed aggregates in which both neutral molecules and ions co-exist. This arrangement is probably the key factor in its enhanced solubility in the presence of partial amounts of ethylenediamine, and it seems to be related more to the effect of pH and less to any specific effect of the counter-ion.

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J. Pharm. Pharmacol. 1984, 36: 760–762
Communicated April 4, 1984

Anti-inflammatory action of drugs that raise adenosine-3′,5′-cyclic monophosphate and putrescine levels in-vivo

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Isoprenaline and salbutamol, two β-adrenoceptor agonist drugs, and 3-isobutyl-methyl xanthine and thioacetamide have been shown to be anti-inflammatory. Their mode of action is probably due to an increase in the levels of the three endogenous substances adenosine-3′,5′-cyclic monophosphate and putrescine and spermidine in-vivo.

Putrescine and, to a lesser extent, spermidine are anti-inflammatory against carregeenan-induced oedema in the rat footpad, and putrescine is anti-inflammatory against adjuvant-induced arthritis in the rat (Bird et al 1983). Putrescine, spermidine and spermine are also anti-inflammatory against 5-hydroxytryptamine pad oedema in mice as well as against carrageenan oedema in the rat (Oyanagui 1984). Since the levels of these endogenous oligoamines are controlled by ornithine decarboxylase (ODC), drugs that raise adenosine 3,5′-cyclic monophosphate (cAMP) may be anti-inflammatory since cAMP stimulates ODC activity (Beck et al 1972). cAMP is also anti-inflammatory in its own right (Zurier et al 1973). We have previously reported that theophylline, which raises both cAMP and putrescine levels, is anti-inflammatory (Bird et al 1983), and combinations of theophylline and prostaglandin, E_1 and aminophylline with salbutamol are also anti-inflammatory (Bonta et al 1978; Seo & Saeki 1980). We have now examined two β-adrenoceptor agonists, isoprenaline and salbutamol, for anti-inflammatory activity and related this effect to cAMP and putrecine levels. We have also examined the xanthine derivative 3-isobutyl-1-methyl xanthine for anti-inflammatory activity and examined the blocking action of propranolol on the anti-inflammatory action of salbutamol. Thioacetamide has also been reported to stimulate ODC activity and this compound was investigated for anti-inflammatory activity.

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Materials and methods

Drugs and chemicals. All drugs and chemicals were purchased from the Sigma Chemical Co., London, unless otherwise stated. Difluoromethylornithine (DFMO) was a kind gift from Dr J. Fozard, Centre de Recherche Merrell International, Strasbourg, France.

Assays. Plasma cAMP levels were determined by radioimmunoassay using a commercially available kit (Amersham International plc) Putrescine levels were determined by a dansylation procedure (Seiler & Askar 1971).

Carrageenan-induced oedema in the rat. The method of Winter et al (1962) was used and foot volumes were measured by an electronic modification of a plethysmographic method (Harris & Spencer 1962). Male wistar rats (150–200 g) were used. Drugs were administered by i.p. injection 30 min before the carrageenan unless otherwise stated.

Results and discussion

Putrescine and cAMP induction. The results in Table 1 show that both isoprenaline and salbutamol elevated cAMP and putrescine levels. The stimulatory effect of isoprenaline on cAMP levels in plasma, although at a maximum 3 h after the administration of the drug, had returned to normal 2 h later.

Table 1. Plasma cAMP and liver putrescine levels in rats treated with β-adrenoreceptor agonists.

	Plasma cAMP p mol ml^{-1}			Liver putrescine μg mg^{-1} dry wt
	Time after dosing (h)			
	0·5	3·5	5·5	3·5
None (0·9% NaCl)	14·5 ± 1·1			3·9 ± 0·2
Isoprenaline (8 mg kg^{-1})	*25·5 ± 0·3	*47·5 ± 0·3	12·0 ± 0·9	*14·9 ± 0·5
Salbutamol (15 mg kg^{-1})	*36·0 ± 2·0			*19·2 ± 0·7

*$P < 0·05$ v control. Four animals in each group.

Action of drugs on carrageenan-induced oedema in the rat. The maximum anti-inflammatory effect of isoprenaline was achieved 3·5 h after dosing (Fig. 1) i.e. 3 h after the administration of the carrageenan. However, 5 h after the administration of carrageenan the anti-inflammatory action of the drug had been lost. This loss of activity coincided with the return of the plasma cAMP levels to normal (Table 1). When a second dose of isoprenaline was administered 3 h after the injection of carrageenan, the anti-inflammatory action of the drug was sustained for 5 h after the injection of carrageenan. In one experiment when isoprenaline was administered to rats at 8 mg kg^{-1} (i.p.), 0·5 h before the carrageenan, the ODC inhibitor DFMO was also

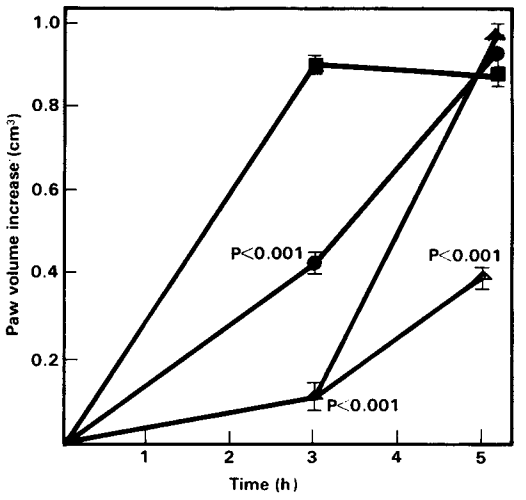

FIG. 1. The effect of isoprenaline on carrageenan-induced oedema in the rat. The drug was administered at 4 mg kg^{-1} (●) and 8 mg kg^{-1} (▲). The drug was anti-inflammatory at both doses at 3 h but not at 5 h. When a second dose of the drug was administered at 8 mg kg^{-1} immediately after the 3 h measurement the anti-inflammatory effect was still present at 5 h after the administration of carrageenan (△). Six rats were used in each experimental group. Full details are given in the text.

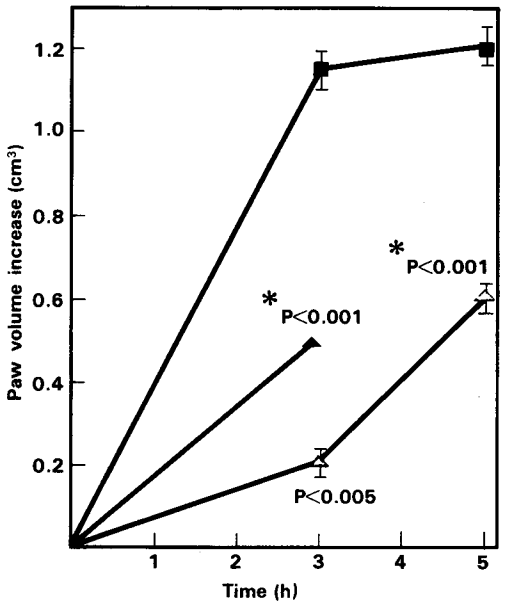

FIG. 2. The effect of salbutamol on carrageenan-induced oedema in the rat. The drug was administered at a dose of 10 and 12 mg kg^{-1}. Experimental details are in the text. ■ represent control values, ▲ salbutamol at 10 mg kg^{-1} and △ salbutamol at 12 mg kg^{-1}. Each value is the mean ± s.e.m. from 7 animals.

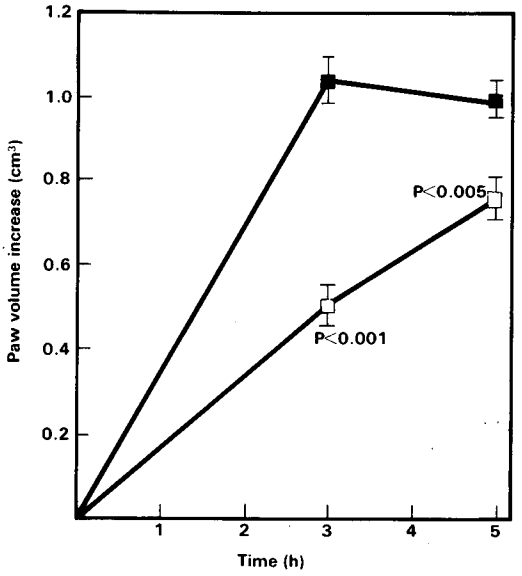

FIG. 3. The effect of 3-isobutyl-1-methyl xanthine on carrageenan-induced oedema in the rat. The drug was given at a dose level of 14 mg kg^{-1}. Experimental details are in the text. Values for the 5 animals quoted as mean ± s.e.m. ■ represent control values and □ 3-iso-butyl-1-methyl-xanthine-treated animals.

administered at the same time at 400 mg kg^{-1}. The mixed dose still produced an anti-inflammatory effect but DFMO suppressed the anti-inflammatory action of isoprenaline by 11% ($P < 0.05$). DFMO itself was not anti-inflammatory. This result suggests that the anti-inflammatory action of isoprenaline is due to both cAMP and putrescine.

Salbutamol was anti-inflammatory (Fig. 2) at 10–12 mg kg^{-1}. When propranolol at 15 mg kg^{-1} was administered with salbutamol (0·5 h before the carrageenan) the anti-inflammatory activity of salbutamol was still present at 3 h but was abolished at 5 h after the adminstration of carrageenan. However, when propranolol was administered 0·5 h before salbutamol the anti-inflammatory action of salbutamol was totally abolished. Propranolol itself was neither pro- or anti-inflammatory. In addition to the two β-adrenoceptor agonists, the xanthine derivative 3-isobutyl-1-methyl xanthine was also anti-inflammatory (Fig. 3). A single dose of thioacetamide (150 mg kg^{-1}) given by i.p. injection 24 h before the carrageenan was also anti-inflammatory (80% inhibition at 5 h) against carrageenan-induced oedema.

β-Adrenoceptor agonists raise cAMP by stimulating adenyl cyclase and the xanthines raise cAMP by inhibiting its breakdown by the catabolic enzyme phosphodiesterase. Previous work (Bonta et al 1978; Seo & Saeki 1980) has shown that combinations of drugs using both the above mechanisms to raise cAMP have a synergistic anti-inflammatory action.

In conclusion, we have shown that isoprenaline, salbutamol, 3-isobutyl-1-methyl xanthine and thioacetamide are anti-inflammatory and that the anti-inflammatory action is due to elevated cAMP and putrescine levels in-vivo. The β-adrenoceptor blocking drug propranolol abolished the anti-inflammatory action of salbutamol.

One of us (S. M. H.) wishes to thank the University of Malaya for a scholarship and we both wish to thank the Arthritis and Rheumatism Council for a project grant.

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