DISCUSSION

Bronchodilator activity of five phenylalkane derivatives, PA-1, PA-2, PA-3, PA-4 and PA-5 (their structures are shown in Table 3), has been evaluated. The results obtained showed that all PAs exerted marked antagonistic effects on histamine-induced bronchoconstriction of the isolated guinea-pig tracheal preparation. Among the five derivatives, PA-2 was the most potent, and the order of potency from high to low was as follows:PA-2, PA-3, PA-1, PA-5 and PA-4. Two derivatives of phenylalkane i.e. PA-2 and PA-3 were found to be more potent than compound D, a 3, 4-dimethoxy phenyl butenol present in Zingiber cassumunar Roxb., the EC_{50} values for PA-2, PA-3 and compound D were 0.34 ug/ml, 1.47 ug/ml and 3.55 ug/ml, respectively.. As PA-2 cannot be dissolved in NSS or distilled water and it was hard to find suitable solvent, which can be used systemically, PA-3, which is soluble in NSS and about 2 times more potent than compound D, was therefore selected as a representative of the PAs for further study.

Drugs which are known to possess smooth muscle relaxant activity and can effectively antagonize experimental bronchoconstriction were used as reference drugs for the investigation of the bronchodilator effect of PA-3. These drugs include isoproterenol (a beta-adrenergic agonist), aminophylline (a xanthine compound), atropine (an antimuscarinic agent), verapamil (a calcium channel antagonist) and papaverine (an antispasmodic). Compound D, a proven bronchodilator (Kiatyingungsulee et al., 1979;

Soparat, 1984) was used to compare the antagonistic effect on the histamine-induced bronchoconstriction in this present study, as this compound is a derivative of phenylalkane as well.

Drugs with smooth muscle relaxant activity can cause bronchodilation, but anyhow by different mechanisms dependent on types of smooth muscle relaxants themselves. It has been proposed that the relaxation of smooth muscles and the increase in cAMP are causally related (Gogg, 1982). Isoproterenol, aminophylline and papaverine are known to cause an increase in cAMP. This increase is, however, mediated by different mechanisms. The stimulation of $beta_2$-adrenergic receptors by beta-adrenergic agonists leads to activation of the adenylate cyclase enzyme located in the cell membrane of the bronchial smooth muscle, which in turn stimulates the synthesis of cAMP. An increase in the cAMP level facilitates the sequestering and transport of calcium out of the cell, and the fall in calcium would relax the contractile proteins (Norn, Skov and Stahl, 1979).

An increase in cAMP by aminophylline and papaverine was originally thought to occur through an inhibition of phosphodiesterase (Tatham and Gellert, 1985) leading to an accumulation of cAMP. However, remarkable differences in the mechanisms of increasing cAMP between aminophylline and papaverine have been demonstrated. Relaxation of smooth muscle occurs when free or unbound calcium in the cytoplasm is reduced as a result of (1) inhibition of calcium influx, (2) re-uptake into intracellular stores, (3) active extrusion across the cell membrane,

or a combination of these three processes (Triggle, 1983). It has been proposed that papaverine acts mainly via the last two processes whereas aminophylline acts chiefly by the first process (Ferrari, 1974).

Verapamil, a calcium channel blocker, is believed to prevent calcium influx through voltage-operated channels in causing smooth muscle relaxation (George and Payne, 1984; Schwartz, 1983), whereas an anticholinergic agent produces bronchodilation by antagonizing the effect of released acetylcholine at muscarinic receptors, thereby blocking the bronchoconstrictor action of vagal efferent impulses.

In the histamine-induced contractions of isolated guinea-pig tracheal muscle, all tested drugs were found to exhibit a dose-related antagonistic effect. Comparison of the dose-response curves of these tested drugs (Fig. 10), it was found that the dose-response curve of PA-3 was parallel with those of aminophylline, verapamil, papaverine and compound D but not with that of isoproterenol. As suggestive evidence about the mechanism of action of any tested substance can be established by the determination of the parallelism of the dose-response curves of tested drug and reference drugs, the results obtained, therefore, suggest that PA-3 might share some common relaxant mechanisms with aminophylline, verapamil, papaverine and compound D. In other words, the bronchodilator effect of PA-3 might be due to its inhibitory action on a cyclic nucleotide phosphodiesterase leading to an increase of cAMP, or its action might be related to the reduction of intracellular calcium. Anyhow, its precise mechanism in increasing cAMP or on intracellular

calcium can not be postulated, as such experiments were not performed. Compound D was studied previously for the relaxant effect on the smooth muscle (Soparat, 1984). It was postulated that compound D caused uterine relaxation by inhibiting phosphodiesterase enzyme leading to the accumulation of cAMP in the uterus. PA-3, which is a compound in the same group as compound D, might exhibit similar mechanism on the smooth muscle as compound D does.

PA-3, like the other drugs, i.e. aminophylline, verapamil and papaverine does not act via beta-adrenergic receptor stimulation in causing bronchodilation, as it still exhibited antagonistic effect on the histamine-induced contraction of the guinea-pig tracheal muscle which was pretreated with propranolol, a beta-adrenergic antagonist. The bronchodilator effect of isoproterenol, on the contrary, was completely blocked by propranolol. These results suggest the different mechanisms of bronchodilator effect of PA-3 and isoproterenol and also support the previous results that the dose-response curve of PA-3 was not parallel with that of isoproterenol.

Although the initiating mechanism of the asthmatic response is probably the release of various chemical mediators, alterations in the autonomic control of the airways seem to play an important role in asthma. The airways of most mammals are richly innervated by the parasympathetic nervous system (Richardson, 1979). Vagal nervous activity maintains a resting tone in the smooth muscle of normal and asthmatic airways and vagal reflex seems to be involved in the

bronchoconstriction of asthmatics induced by various nonspecific stimuli, e.g. dust, sulphur dioxide, histamine and cold air (Widdicombe, 1979; Boushey et al., 1980). Asthmatics have also shown a striking bronchial hyperreactivity to some exogenous pharmacological agents, e.g. the muscarinic receptor agonist (methacholine) and histamine, when these agents have been given either parenterally (Curry, 1947) or by inhalation (Parker et al., 1965; Muittari, 1968, Laitnen, 1974). Nowadays a standardized methacholine challenge test is commonly used in the diagnosis of asthma and laboratory evaluation of bronchodilator drugs (Townley et al., 1979).

In the rat tracheal strip preparation of the present study, methacholine was used as the bronchoconstrictive inducer. As isoproterenol is found to be ineffective on the rat tracheal muscle, atropine, an antimuscarinic agent,was then used as reference drug in this study, together with others i.e. aminophylline, verapamil and papaverine. Bronchodilator activity of compound D was also compared with PA-3 and those reference drugs. Comparison of the parallelism of the dose-response curves of all tested drugs (Fig. 13), it was found that the linear regression line of PA-3 was parallel to those of aminophylline, verapamil, papaverine and compound D. The finding supports the results obtained from the experiment on guinea-pig tracheal chain preparation that PA-3 might share a common mechanism of bronchodilator action with these drugs. The dose-response curve of PA-3 was not parallel to that of atropine which suggests that PA-3 does not act by blocking the muscarinic cholinergic

receptor. The other evidence, which supports the above suggestion is the observation of the effect of PA-3 in conscious rats using the Hippocratic screening test, which was previously studied by Pichipalakorn (1983), i.e. a mydriatic effect caused by the administration of antimuscarinic drug such as atropine, was not observed with all dosage levels of PA-3 (0.25, 0.5, 1 , 2 and 3 g/kg body weight, intraperitoneally).

Since the processes involved in asthma are complex and still uncompletely defined and there are many chemical mediators released locally during these complex responses, the bronchoconstriction induced in the whole animal models should bear the closest mechanical similarity to human asthma (Salonen, 1985). In the present study, the bronchodilator effects of PA-3 and reference drugs were investigated in two different animal species, guinea-pigs and rats, in order to increase the relevance of the results. The experiments were performed in anesthetized and artificially ventilated animals, whose spontaneous breathing was prevented by anesthesia and muscle relaxation (pancuronium bromide). Two respiratory measurements i.e. intratracheal pressure response (PIPR) and response area (RA) values, representing the peak airway response and overall severity of obstruction, respectively (Salonen, 1985), were assessed. A reproducible obstruction of airways was induced by an intravenous administration of two different pharmacological agents, histamine in guinea-pigs and methacholine in rats. Both bronchoconstrictive inducers were selected in order to simulate some important pathophysiological mechanism of asthma in small animals, viz. vagal

reflex bronchoconstriction (Widdicombe, 1979; Bouschey et al., 1980). Preliminary experiments in rats carried out by Salonen and Matilla (1981) showed that methacholine was better than acetylcholine in this respect, because acetylcholine induced airway obstruction only at relatively large doses which often caused irreversible bradycardia. In addition, the action of methacholine is a simple muscarinic stimulation whilst acetylcholine acts in a more complex way on muscarinic and nicotinic receptors (Salonen and Mattilla, 1981).

In the guinea-pig model, the bronchodilator activity of PA-3 and reference drugs was compared. Chlorpheniramine, an H_1-receptor antagonist, was also used to show the blocking effect against histamine-induced bronchoconstriction in guinea-pigs (Fig. 14 B). Although histamine was the first allergic mediator to be implicated in asthma, H_1-receptor antagonists are little use for the treatment of symptomatic asthma and have no place in the treatment of acute or chronic severe asthma. The reason is that antihistamines have no effect on other mediators and it might be difficult to achieve high enough lung concentrations by the oral or parenteral route (Paterson et al., 1979). Besides this, high doses of antihistamines produce various undesirable side effects.

In the present study all tested drugs exhibited the protective effect against bronchospasm induced by histamine in dose-related manner in terms of both PIPR and RA. The complete protective effect against histamine-induced bronchoconstriction was seen with the high doses of tested drugs. Comparison of the potency of PA-3 with reference drugs, it

was found that the percent inhibition of bronchoconstriction exhibited by PA-3 in terms of both PIPR and RA was not different from that of aminophylline (Fig. 16 and Fig. 17). The results obtained suggest that PA-3 might be beneficial in the prophylactic treatment or in the management of asthma likewise aminophylline. The dose-response curve of PA-3 in the term of PIPR was found to be parallel to those of aminophylline, verapamil and papaverine but not to that of isoproterenol. These findings suggest similar mechanisms of bronchodilator action of PA-3 and aminophylline,verapamil as well as papaverine and also support the suggestive mechanism of PA-3 in the in vitro experiments. Anyhow, when comparison was made in term of RA, it was found that the dose-response curve of PA-3 was parallel only to that of aminophylline. This result may give the suggestion of bronchodilator activity of PA-3 that, its mechanism of action is rather inclined to imitate aminophylline than to verapamil or papaverine.

In the experiment in rats, Salonen and Mattila (1981) found that the methacholine-induced bronchoconstriction and bradycardia were totally antagonized with atropine thus suggesting that the bronchoconstrictor action of methacholine was mainly due to the activation of muscarinic receptors in the bronchial tree. The present study supports this finding that atropine could completely prevent the methacholine-induced bronchoconstriction as shown in Fig. 22 B. Methacholine was given in a cumulative manner in order to prevent the marked depressor effect on the cardiovascular system, by injecting two sets of the drug intravenously

(the first set : 1.5, 3 and 4.5 ug; and the second set : 3, 6 and 9 ug). These cumulative doses caused a mild, moderate and submaximal rise in PIPR and RA values, respectively, and these parameters of the bronchoconstrictive response were found to be increased in dose-related manner in each set of the drug (Salonen and Mattila, 1981). Tested drugs were administered 2 min prior to the second set of methacholine doses. The protective effect of PA-3 and all reference drugs against the second set of a dose-related cumulative methacholine-induced bronchoconstriction was found to be moderately effective. No complete protection was seen even with the highest doses of tested drugs used in this study. Nevertheless, the inhibitory effect of all tested drugs on the methacholine-induced bronchoconstriction in the term of PIPR was found to be dose-related. The reason for a noncomplete protection of the bronchoconstriction by tested drugs might be due to an insufficiency of the doses used. Anyhow, larger doses of these drugs could not be administered because of the cardiovascular effects. Tachycardia or hypotension usually occurred in concomitant with the use of bronchodilators such as isoproterenol, aminophylline (Norn, Skov and Stahl, 1979). Tachycardia comes from either a direct action of drugs on cardiac muscle or a reflex occurring from hypotension, which is usually caused by drugs with smooth muscle relaxant activity. These effects of bronchodilators is further increased by adding the second set of methacholine-doses. The animals, therefore, could die easily if the larger doses of these tested drugs were intravenously administered.

The main difficulty when quantitating bronchoconstriction was the rise of the basal pressure level during the experiments both in guinea-pigs and in rats. The mechanism of this phenomenon is not clear. It may not be a simple long-lasting histaminic or cholinergic activation because both antihistamine and atropine failed to cancel already existing rise of the basal level occurring after the first dose of histamine or the first set of methacholine-doses, and since it rose with time even without histamine or methacholine stimulation. On the other hand, PA-3 and all reference drugs could not counteract this elevation when given after the first dose of histamine or the first set of methacholine-challenges. Salonen and Mattila (1981) postulated that this elevation is due to a leakage of fluid from the vessels to mucosal intercellular space.

When comparing the heights of the PIPRs and the RAs as to their suitability to quantitate histamine or methacholine response, no major differences between PIPRs or RAs were found. When giving doses of histamine or methacholine alone the rise of the baseline level modified both parameters in the same direction because the peak heights were also measured from the initial baseline level. But when assessing the efficacy of bronchodilator drugs, it was found that in experiment with rats the RAs failed to reveal a statistically significant bronchodilation. The reason is that the RAs in some groups of rats showed different responses to the doses of 3 and 4.5 ug of the first set of methacholine-doses as shown in Table 10. Therefore in the experimental model using rats the PIPR values are more reliable than the RA values. The results obtained

from methacholine-induced bronchoconstriction in rats showed that only PA-3 and aminophylline inhibited the methacholine-induced bronchoconstriction in both terms, PIPR and RA, relatedly. Other reference drugs (isoproterenol, verapamil and papaverine) exhibited unrelated inhibition on PIPR and RA values. Anyhow, in histamine-induced bronchoconstriction in guinea-pigs, PA-3 and all reference drugs showed related inhibitory activity on PIPR and RA. The results obtained from both animal species, however, suggests the prophylactic effect of PA-3 and reference drugs in bronchoconstriction caused by allergy or by an increase of the parasympathetic tone.

In addition to the study of the bronchodilator effect, one derivative of PAs i.e. PA-1 was also studied in conscious animals to investigate the general behavioral changes by employing the Hippocratic screen. The results obtained showed that intraperitoneal injections of PA-1 caused CNS depression which was evidenced by the decrease of motor activity, loss of righting reflex, decrease of respiratory rate and loss of screen grip. Marked CNS depression leading to respiratory arrest was seen with the dose of 1000 mg/kg body weight. These results support the previous study by Pichipalakorn (1983) in which PA-3, one of the phenylalkane derivatives, was used. The loss of screen grip might be due to either the peripheral or central effects of PAs. Peripherally, the PAs can probably act at the neuromuscular junction similarly to compound D, which was proven to exhibit an inhibitory effect on the contraction of diaphragm in the rat phrenic nerve-diaphragm

experiment (Kiatiyingunsulee et al., 1979). Enophthalmus was observed with all doses of PA-1 similar to that found with PA-3 (Pichipalakorn, 1983). This symptom is the suggestive effect indicating hypotension which occurred in response to the administration of PA-1 and PA-3. It is noteworthy to notify that this hypotensive effect of PA-3 is confirmed in the rats under pentobarbital anesthesia experiment (Pichipalakorn, 1983).

In conclusion, the present investigation revealed the bronchodilator effect of PA-3, which was the representative of the five phenylalkane derivatives on some experimental animal models which were suggested to be good models for evaluation of bronchodilators (Muccitelli et al., 1987; Burns and Doe, 1978; Lulich and Paterson, 1980; Salonen, 1985). PA-3 was found to exhibit bronchodilator activity similar to other smooth muscle relaxants such as isoproterenol, aminophylline, atropine, verapamil and papaverine. It has been proposed that the relaxant effect of PA-3 on tracheo-bronchial muscle was not mediated via the beta-adrenergic stimulation, since in the presence of propranolol, a beta-adrenergic antagonist, it still possessed the antagonistic effect on the histamine-induced tracheal contraction. The possible mechanism of the bronchodilator action of PA-3 could be postulated to share a common mechanism of action with aminophylline, verapamil and papaverine. These reference drugs cause bronchodilation by causing a decrease of intracellular calcium either via an inhibition of a cyclic nucleotide phosphodiesterase which in turn leads to an increase in intracellular cAMP or by blocking entry of calcium ions

through voltage-dependent channels. The reason for this postulation comes from the parallel of the dose-response curves of PA-3, aminophylline, verapamil and papaverine. In the study to measure the protective effect of PA-3 against experimental bronchospasm in the in vivo, it was found that PA-3 exhibited effective protection against both histamine-and methacholine-induced bronchoconstriction. Anyhow, it is necessary to note that this study was carried out only under a few experimental models and in two species of animals. The use of other inducers known to participate in inflammatory reactions of asthma such as leukotrienes (Dahlen et al., 1980; Hedgvist et al., 1982), PAF (Morley et al., 1985) and the application of other experimental models which have been suggested to resemble human allergic asthma are nervertheless still nescessary for further evaluation of the bronchodilator activity of PA-3 and other PAs.
