Interaction between isoprenaline and aminophylline in asthma

I. A. CAMPBELL[1], W. G. MIDDLETON, G. J. R. McHARDY, MARGARET V. SHOTTER, ROBIN McKENZIE, AND A. B. KAY[2]

From the University Department of Respiratory Diseases, City Hospital, Edinburgh, the University Department of Statistics, Edinburgh, and the South-East Scotland Regional Blood Transfusion Service, Royal Infirmary, Edinburgh

Campbell, I. A., Middleton, W. G., McHardy, G. J. R., Shotter, Margaret V., McKenzie, R., and Kay, A. B. (1977). *Thorax*, **32**, 424–428. **Interaction between isoprenaline and aminophylline in asthma.** Using a factorially designed study, 38 patients with bronchial asthma received a single dose of either isoprenaline by inhalation (9), aminophylline intravenously (10), isoprenaline and aminophylline (11), or placebo (8). The maximum expiratory flow ($\dot{V}$ max), the maximum expiratory flow at 50% of vital capacity ($\dot{V}$ max$_{50}$), and the concentrations of plasma cyclic AMP were measured at time intervals up to two hours. The combination of isoprenaline and aminophylline acted synergistically in terms of the percent increase in $\dot{V}$ max$_{50}$. However, this was statistically significant only at 20 minutes. Plasma cyclic AMP concentration rose with a similar time course of response to the changes in small airways. The elevations in plasma cyclic AMP observed with the drug combination were higher than those for the individual drugs at 10, 20, 30, and 60 minutes but these differences were not statistically significant. These observations support the concept that changes in bronchial smooth muscle tone are mediated by concentrations of cyclic nucleotides and that combinations of isoprenaline and aminophylline, rather than the administration of each drug separately, may have therapeutic advantages in the treatment of bronchial asthma.

It is common practice to use beta-adrenergic stimulants and methyl xanthines in the treatment of bronchial asthma. The studies of Robison *et al.* (1971) suggest that the beta-adrenergic effects of catecholamines are mediated through 3'5' cyclic adenosine monophosphate (cyclic AMP), the intracellular 'second messenger'. Methyl xanthines inhibit phosphodiesterase, the enzyme which inactivates cyclic AMP (Butcher and Sutherland, 1962), and it is proposed (Leading article, 1970) that methyl xanthines and beta-adrenergic agents may produce bronchodilatation by increasing the level of cyclic AMP in bronchial and bronchiolar smooth muscle.

Drugs which individually act at different points in the metabolic pathway of a compound in such a way as to increase the amount of that compound may, when used together, produce an increase greater than the sum of the increases produced by each drug alone, that is, they may interact synergistically (Veldstra, 1956). There is evidence *in vitro* for synergy between beta-adrenergic agents and methyl xanthines (Rall and West, 1963; Lichtenstein and Margolis, 1968; Kaliner *et al.*, 1971). The present study was designed to establish whether such interaction could be demonstrated *in vivo* in man. We studied the effects of inhaled isoprenaline and intravenous aminophylline on the airways and on heart rate. The level of cyclic AMP in plasma was also measured as it has been described as a convenient index of effects of drugs on tissue levels of cyclic AMP (Ball *et al.*, 1972; Karlberg *et al.*, 1974; Wehman *et al.*, 1974).

Patients and methods

Thirty-eight patients were studied. All patients had airways obstruction and, at some time in the past, had shown a 20% or more increase in

[1]Present address: Respiratory Department, Bristol Royal Infirmary
[2]University Department of Pathology, Edinburgh

FEV_1 after inhaled isoprenaline or salbutamol. At the time of study they were symptomatically well and were therefore considered to be in a stable state. Informed consent was obtained from each patient, and the protocol was approved by the Ethical Committee of the City Hospital, Edinburgh.

DESIGN AND DOSAGE

A factorial design permitting the detection of drug interaction (Armitage, 1971) was used. Patients were allocated in random order to one of four treatments:

1 Placebo inhaler plus placebo injection (P), 8 patients;
2 Isoprenaline inhaler plus placebo injection (I), 9 patients;
3 Placebo inhaler plus aminophylline injection (A), 10 patients; or
4 Isoprenaline inhaler plus aminophylline injection (I+A), 11 patients.

The placebo inhaler delivered inactive propellant only and the placebo injection consisted of 10 ml of normal saline. The inhalers and ampoules were prepared by the hospital pharmacist in such a way that active and placebo preparations were indistinguishable. Two 'puffs' were taken from the isoprenaline inhaler (0·16–0·18 mg ex valve) and the intravenous dose of aminophylline was 250 mg in 10 ml. The treatments were given in a double-blind fashion.

METHODS

Maximum expiratory flow volume (MEFV) curves were recorded at the mouth with a Fleisch pneumotachograph and an integrator, displayed on a storage oscilloscope (Tektronix 5100), and photographed on Polaroid film. The system was calibrated for volume with a Gaensler spirometer, which had previously been calibrated by water displacement, and for flow using a rotameter and vacuum cleaner. Calibration was performed before, during, and at the end of each experiment. The maximum expiratory flow rate ($\dot{V}$ max) and the maximum expiratory flow rate at 50% of forced vital capacity ($\dot{V}$ max$_{50}$) were measured from two acceptable records of the MEFV curve and the greater value of each index was used for analysis. Obvious differences in the forced vital capacity or distortion by coughing were the usual reasons for considering the records unacceptable. Heart rate was measured from lead II of the electrocardiogram (ECG) recorded for 30 seconds on a Sharp MT23 machine. Plasma cyclic AMP was measured by the competitive protein binding assay of Gilman (1970) using the materials supplied by the Radiochemical Centre, Amersham. Blood was drawn into ethylenediaminetetracetate (EDTA) to a final concentration of 0·005 M and the plasma was separated and stored at −80° C. The samples were thawed once and the proteinaceous material removed by precipitation with 67% ethanol. In order to show that the drugs or their metabolites did not influence the assay 8 picomoles of cyclic AMP were added to 1 ml aliquots of the seven plasma samples from a patient who received the combination of isoprenaline and aminophylline. Values obtained gave the expected increase above aliquots to which no cyclic AMP had been added.

PROCEDURE

The patients were instructed not to use any bronchodilator preparations for at least six hours before the investigation. A Medicut cannula was inserted into a forearm vein. The patient remained at rest in an armchair for at least 15 minutes and then the ECG was recorded for 30 seconds. Five millilitres of blood were drawn and two MEFV curves recorded with the patient in the sitting position. Two puffs of isoprenaline or of a placebo inhaler were then taken by the patient during the first half minute of a five-minute infusion of either aminophylline or saline. During the last 30 seconds of the infusion the ECG was recorded. Blood was taken immediately after the end of infusion, that is, at time five minutes, and two MEFV curves were recorded. Measurements and samples were repeated 10, 20, 30, 60, and 120 minutes from the start of the infusion.

One patient in the placebo group, one in the A group, and two in the I+A group fainted during or immediately after the injection. All four insisted on continuing the investigation and their results are included in the analysis.

STATISTICAL ANALYSIS

The data obtained were subjected to an analysis of variance for a factorial experiment (Armitage, 1971). This analysis provided an assessment of the effects of isoprenaline, aminophylline, and of the combination of the two drugs. An interaction term was derived at each time and its standard error was calculated. If the interaction term was larger than twice its own standard error (that is, was significantly different from zero), the drugs had interacted synergistically.

Results

The mean pre-treatment values of $\dot{V}$ max$_{50}$,

Table *Mean pre-treatment values ($\pm SE$) of $\dot{V}$ max, $\dot{V}$ max$_{50}$, heart rate, and plasma cyclic AMP*

	Placebo	Isoprenaline	Aminophylline	Isoprenaline + aminophylline
No. of patients	8	9	10	11
$\dot{V}$ max$_{50}$ (l/sec)	1·32 ($\pm 0·25$)	1·02 ($\pm 0·27$)	1·53 ($\pm 0·22$)	1·13 ($\pm 0·11$)
$\dot{V}$ max (l/sec)	4·36 ($\pm 0·63$)	3·82 ($\pm 0·52$)	4·42 ($\pm 0·45$)	3·85 ($\pm 0·2$)
Heart rate per min	75 (± 4)	80 (± 3)	83 (± 5)	71 (± 4)
Cyclic AMP (nanomoles/l)	11·8 ($\pm 2·15$)	11·3 ($\pm 1·5$)	11·7 ($\pm 1·7$)	9·7 ($\pm 1·8$)

V max, heart rate, and plasma cyclic AMP for each group are shown in the Table. The differences between groups were not statistically significant.

The means of the percentage increases in V max$_{50}$ produced by each treatment are shown in Figure 1. The placebo group remained much the same over the two hours. The combination I+A produced increases which were larger than those produced by either drug alone, but at 60 minutes and 120 minutes the differences between A and I+A were not significant at the 5% level. Although I+A was significantly different from the other three groups at all other times, it was only at 20 minutes that the interaction term was greater than 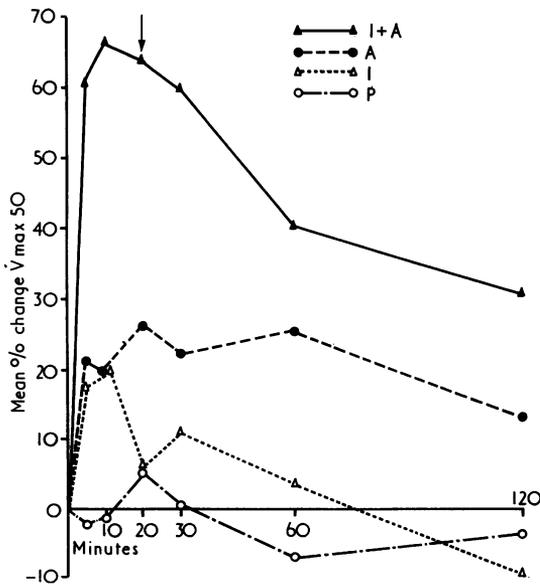twice its standard error. At 5, 10, and 30 minutes the suggestion that the combination was synergistic was not significant at the 5% level.

The same trend towards a synergistic effect on V max was apparent up to 30 minutes after treatment (Fig. 2), but this trend did not achieve statistical significance. At 60 and 120 minutes the effects of I+A were greater than those of I alone (p<0·05) but were much the same as A alone.
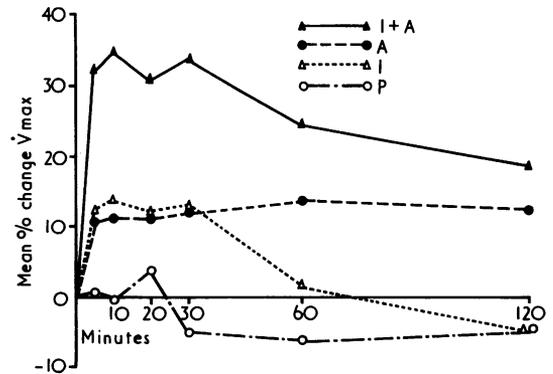
Fig. 2 *Mean percent change in $\dot{V}$ max*

The effects on heart rate are shown in Figure 3. Neither synergy nor addition occurred. I, A, and I+A produced increases which were not statistically distinguishable from each other. At five
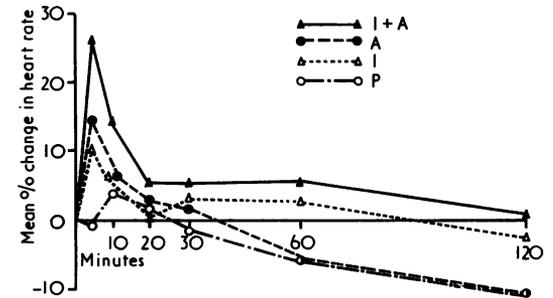
Fig. 1 *Mean percent change in $\dot{V}$ max$_{50}$. For the sake of clarity standard errors are not shown. Significant differences at each time are stated in the text, and the arrow indicates the time at which interaction between I and A was found.*

Fig. 3 *Mean percent change in heart rate.*

minutes A and I+A were different from placebo
(P<0·05) but the changes resulting from I alone
were at no time significantly different from
placebo.

I, A, and I+A produced similar changes in
plasma cyclic AMP (Fig. 4), and these changes
paralleled the time course of the changes in the
airways. At 10, 20, 30, and 60 minutes the com-
bination I+A produced changes greater than
those produced by each drug alone but the differ-
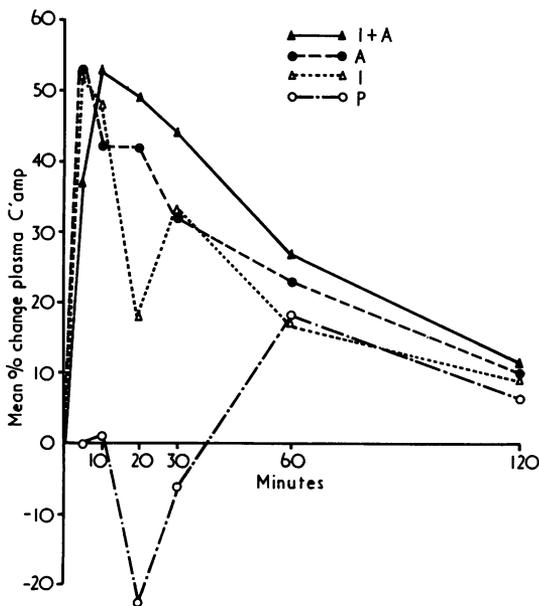ences were not statistically significant.

Fig. 4 *Mean percent change in concentration of
cyclic AMP in plasma.*

Discussion

Synergy between two drugs is said to occur if the
effect produced when they are given together is
greater than the sum of their effects when given
individually. It is generally accepted that a dis-
placement to the left of the dose-response curve of
one drug by another drug indicates synergistic
interaction. To be conclusive this shift should be
significantly greater than the response to the
second drug alone. An alternative method for
investigating interaction, which avoids the neces-
sity of establishing dose response curves, is to use
single, fixed doses of the drugs in a factorially
designed experiment (Armitage, 1971; Pearson
et al., 1976), and we have used this latter method.

Hume and Rhys Jones (1961) observed an addi-

tive effect of isoprenaline and aminophylline on
FEV_1 in six patients. Pihlajamäki *et al.* (1972)
examined the effects of glyphylline and isoprena-
line singly and in combination, and Cohen and
Elizabeth (1974) studied ephedrine and theophyl-
line, but none of these studies was designed in a
way which would allow the observed effects to be
analysed quantitatively for synergy. Using a
factorial design, we have now shown a synergistic
bronchodilator effect between isoprenaline and
aminophylline on $\dot{V}$ max$_{50}$ but not on $\dot{V}$ max.
While it is possible that reducing variation by
studying larger numbers of patients or by using a
within-patient comparison of the drugs might have
resulted in the demonstration of synergistic inter-
action on $\dot{V}$ max it is also possible that the differ-
ent findings for $\dot{V}$ max and $\dot{V}$ max$_{50}$ represent a
true difference of the effects of the drugs at differ-
ent sites in the airways, because it has been argued
that changes in maximum expiratory flow rate at
50% forced vital capacity reflect changes in calibre
of the smaller airways (McFadden and Linden,
1972).

It would seem that plasma levels of cyclic AMP
reflect only broadly changes occurring at tissue
level for although the time courses of the plasma
cyclic AMP changes were similar to those of the
airways we have found no difference between the
effect of I, A, and I+A on plasma cyclic AMP.
This is not entirely surprising when one considers
that the level of plasma cyclic AMP is only 1% of
the level in tissues (Karlberg *et al.*, 1974) and that
the plasma concentration of cyclic AMP is
thought to be the result of a leakage of cyclic
AMP from cells into the extracellular space
(Broadus *et al.*, 1971). Nevertheless the marked
response in plasma cyclic AMP 10 minutes after
the administration of drugs used in treating acute
asthma may have important therapeutic implica-
tions. In status asthmaticus the response to beta-
adrenergic stimulants is sometimes impaired or
absent, and it has been suggested that alpha-
adrenergic blocking drugs can restore beta-
responsiveness (Palmer *et al.*, 1974; Patel and
Kerr, 1975). Furthermore, some patients with
chronic asthma appear to have lost their ability
to respond to beta-adrenergic stimulants, and
Ellul-Micallef and Fenech (1975) have shown that
this responsiveness can be restored by administer-
ing corticosteroids. The measurement of the
responses of plasma cyclic AMP to beta-adrenergic
stimulants might therefore provide an objective
means of deciding when beta-responsiveness is no
longer present in a clinical situation in which tests
of ventilatory function are either too insensitive
or too impractical to be a useful index of response.

Neither synergy nor addition was found in the action of the drugs on heart rate. At rest heart rate is controlled predominantly by the parasympathetic nervous system (Robinson *et al.*, 1953; Robinson *et al.*, 1966) and reflex parasympathetic changes might be expected to redress changes induced by isoprenaline and aminophylline, thereby making it difficult to show interaction of these drugs on heart rate. However, both A and I+A produced a significant rise in heart rate five minutes after treatment. In status asthmaticus such a rise might be unacceptable unless oxygen was available to counteract the possible worsening of hypoxaemia that might result.

Our demonstration of the synergistic interaction between a beta-adrenergic stimulant and a methyl xanthine on the airways of man *in vivo* supports the concept that cyclic AMP may participate in the events leading to bronchodilatation and provides a rationale for their combined use in the treatment of asthma.

References

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 226–239. Blackwell, Oxford.

Ball, J. H., Kaminsky, N. I., Hardman, J. G., Broadus, A. E., Sutherland, E. W., and Liddle, G. W. (1972). Effects of catecholamines and adrenergic-blocking agents on plasma and urinary cyclic nucleotides in man. *Journal of Clinical Investigation*, **51**, 2124–2129.

Broadus, A. E., Hardman, J. G., Kaminsky, N. I., Ball, J. H., Sutherland, E. W., and Liddle, G. W. (1971). Extracellular cyclic nucleotides. *Annals of the New York Academy of Sciences*, **185**, 50–66.

Butcher, R. W., and Sutherland, E. W. (1962). Adenosine 3'5' phosphate in biological materials. I. Purification and properties of cyclic 3'5' nucleotide phosphodiesterase and use of this enzyme to characterise adenosine 3'5' phosphate in human urine. *Journal of Biological Chemistry*, **237**, 1244–1250.

Cohen, B. M., and Elizabeth, N. J. (1974). Sympathomimetic/xanthine broncholysis in obstructive ventilatory disorders. *International Journal of Clinical Pharmacology*, **9**, 6–15.

Ellul-Micallef, R., and Fenech, F. F. (1975). Effect of intravenous prednisolone in asthmatics with diminished adrenergic responsiveness. *Lancet*, **2**, 1269–1271.

Gilman, A. G. (1970). A protein binding assay for adenosine 3'5'-cyclic monophosphate. *Proceedings of the National Academy of Sciences*, **67**, 305–312.

Hume, K. M., and Rhys Jones, E. (1961). The response to bronchodilators in intrinsic asthma. *Quarterly Journal of Medicine*, **30**, 189–199.

Kaliner, M. A., Orange, R. P., Koopman, W. J., Austen, K. F., and Laraia P. J. (1971). Cyclic adenosine 3'5'-monophosphate in human lung. *Biochimica et Biophysica Acta*, **252**, 160–164.

Karlberg, B. E., Henriksson, K. G., and Andersson, R. G. G. (1974). Cyclic adenosine 3'5'-monophosphate concentration in plasma, adipose tissue and skeletal muscle in normal subjects and in patients with hyper- and hypothyroidism. *Journal of Clinical Endocrinology and Metabolism*, **39**, 96–101.

Leading article (1970). Cyclic AMP: The second messenger. *Lancet*, **2**, 1119.

Lichtenstein, L. M., and Margolis, S. (1968). Histamine release in vitro: inhibition by catecholamines and methylxanthines. *Science*, **161**, 902–903.

McFadden, J. R., Jr., and Linden, D. A. (1972). A reduction in maximum mid-expiratory flow rate— A spirographic manifestation of small airway disease. *American Journal of Medicine*, **52**, 725–737.

Palmer, K. N. V., Gaddie, J., and Skinner, C. (1974). Alpha-adrenoceptor-blocking drugs in asthma (letter). *British Medical Journal*, **4**, 409.

Patel, K. R., and Kerr, J. W. (1975). Alpha-receptor-blocking drugs in bronchial asthma (letter). *Lancet*, **1**, 348–349.

Pearson, R. M., Bending, M. R., Bulpitt, C. J., George, C. F., Hole, D. R., Williams, F. M., and Breckenridge, A. M. (1976). Trial of combination of guanethidine and exprenolol in hypertension. *British Medical Journal*, **1**, 933–936.

Pihlajamäki, K., Kanto, J., and Iisalo, E. (1972). Human and animal studies on the interactions between glyphylline and isoprenaline. *Journal of Asthma Research*, **2**, 255–261.

Rall, T. W., and West, T. C. (1953). The potentiation of cardiac inotropic responses to norepinephrine by theophylline. *Journal of Pharmacology and Experimental Therapeutics*, **139**, 269–274.

Robinson, B. F., Epstein, S. E., Beiser, G. D., and Braunwald, E. (1966). Control of heart rate by the autonomic nervous system. *Circulation Research*, **19**, 400–411.

Robinson, S., Pearcy, M., Brueckman, F. R., Nicholas, J. R., and Miller, D. I. (1953). Effects of atropine on heart rate and oxygen intake in working man. *Journal of Applied Physiology*, **5**, 508–512.

Robison, G. A., Sutherland, E. W., and Butcher, R. W. (1971). *Cyclic AMP*, pp. 146–231. Academic Press, London and New York.

Veldstra, H. (1956). Synergism and potentiation. *Pharmacological Reviews*, **8**, 339–387.

Wehmann, R. E., Blonde, L., and Steiner, A. L. (1974). Sources of cyclic nucleotides in plasma. *Journal of Clinical Investiation*, **53**, 173–179.