Br. J. Pharmac. (1980), **69**, 467–472

ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN ACTIVELY SENSITIZED GUINEA-PIGS: ANTI-ANAPHYLACTIC EFFECTS OF SODIUM CROMOGLYCATE AND AMINOPHYLLINE

PER ANDERSSON

AB Draco, Research and Development Laboratories, Fack, S-221 01 Lund, Sweden

1 The inhibitory effects of sodium cromoglycate (SCG) and aminophylline on antigen-induced bronchial anaphylaxis in guinea-pigs, actively sensitized according to different regimens, were examined.

2 SCG (1 mg/kg administered intravenously) reduced the anaphylactic response in animals sensitized with 1 μg ovalbumin (OA) together with $Al(OH)_3$ 100 mg, and challenged at 14 and 40 days after sensitization. If higher doses of antigen (10 μg OA together with $Al(OH)_3$ or 5 mg OA on day 0 plus 10 mg OA on day 2) were used for sensitization, the protective effect of SCG was found only in animals tested 14 days after sensitization.

3 A low dose of aminophylline (0.3 mg/kg) that was without a direct bronchodilator effect when tested against a histamine (4 μg/kg)-induced bronchospasm, produced an anti-anaphylactic effect. The anti-anaphylactic effect of aminophylline varied slightly with the way the animals were immunized and the time at which they were tested.

4 It is concluded that bronchial anaphylaxis in guinea-pigs sensitized with low doses of ovalbumin is a suitable model for the evaluation of anti-anaphylactic properties of drugs.

Introduction

In the pathogenesis of human asthma, reactions mediated by reaginic (IgE-like) antibodies seem to play a major role (Ishizaka, Ishizaka & Hornbrook, 1966; Kay & Austen, 1971; Orange, Austen & Austen, 1971). One of the most significant advances in recent years in the prophylactic treatment of asthma was the discovery of sodium cromoglycate (SCG) (Cox, 1967). Experimental results demonstrated that SCG inhibited immediate (Type I) hypersensitivity reactions, i.e. the reactions mediated by the heat-labile mast cell sensitizing IgE-antibody (Cox, 1971). Therefore the evaluation of SCG-like compounds has relied on tests which assess inhibition of such IgE-mediated immediate hypersensitivity reactions. Only recently, the guinea-pig was shown to produce IgE-like antibodies in response to a number of antigenic stimuli (Mota & Perini, 1970; 1975; Parish, 1970; Dobson, Marseth & Soulsby, 1971; Levine, Chang & Vaz, 1971; Catty & Fraser, 1972; Perini & Mota, 1972; 1973; Taylor & Roitt, 1973; Carney, 1976; Andersson, 1979).

The present study evaluates the inhibitory effect of the anti-allergic compound SCG, and describes an anti-anaphylactic effect of the theophylline salt, aminophylline, in guinea-pigs actively sensitized to ovalbumin by different regimens, including IgE-antibody promoting ones.

Methods

Guinea-pigs (Dunkin-Hartley) of either sex (250 to 300 g), bred by Sahlins, Malmö, Sweden, were used.

Sensitizing procedures

Two major sensitization procedures were used: (A) the animals were injected intraperitoneally with ovalbumin (OA) 5 mg on day 0 and 10 mg on day 2. OA was dissolved in 0.9% w/v NaCl solution (saline); injection volume, 0.1 ml. (B) The animals were sensitized by one intraperitoneal injection of 0.5 ml of saline containing 100 mg $Al(OH)_3$ and OA. The antigen dose was either 1 μg or 10 μg. The adjuvant was added to the antigen solution 1 h before the injection.

Respiratory measurements

The animals were anaesthetized with pentobarbitone (30 mg/kg i.p.), tracheotomized and ventilated by a Braun constant respirator (frequency 60/min, volume 7 ml/kg). Pulmonary mechanics, i.e. lung resistance (R_L) and dynamic lung compliance (C_{Dyn}), were estimated by the method of Amdur & Mead (1958) modified for anaesthetized guinea-pigs. Airflow ($\dot{V}$) was measured by a mesh screen pneumotachograph (Fleisch no. 000) connected to a Statham differential

0007-1188/80/070467-06$01.00

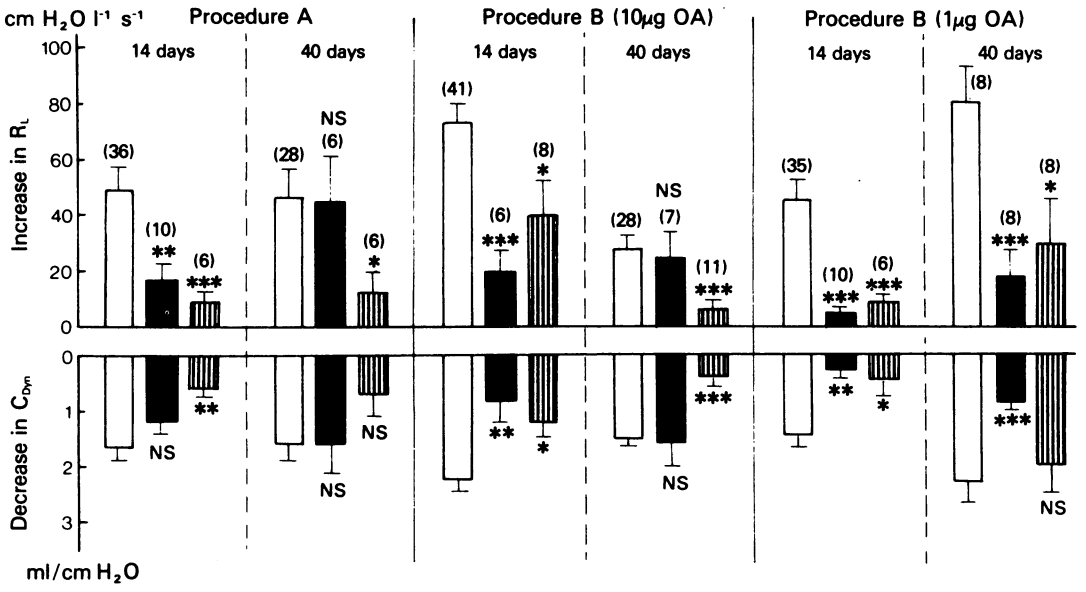

Figure 1 Anti-anaphylactic effects of sodium cromoglycate (SCG) and aminophylline in actively sensitized guinea-pigs. The doses of the drugs used were: 1 mg/kg SCG and 0.3 mg/kg aminophylline. They were administered intravenously 2 min before the challenging doses of ovalbumin (OA) 40 μg/kg (procedure A) and 5 μg/kg (procedure B). Sensitization regimen used: procedure A: 5 mg OA (day 0) + 10 mg OA (day 2); procedure B: 1 μg or 10 μg OA together with $Al(OH)_3$, 100 mg. The anti-anaphylactic effects of the compounds were examined 14 or 40 days after sensitization. Open columns = control; solid columns = SCG-treated animals; striped columns = aminophylline-treated animals. Columns show mean results; vertical lines indicate s.e. mean. Figures in parentheses indicate number of animals. NS = not significant. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

pressure transducer (PM 15). Tidal volume (V_T) was determined as the electrical integral of airflow. The transpulmonary pressure (P_{TP}) was determined by connecting one inlet of a differential pressure transducer (Statham PM 5) to a needle inserted through the 5th or 6th intercostal space to measure intrapleural pressure; and the other inlet to a small piece of rubber tubing placed between the endotracheal tube and the pneumotachograph to measure the intratracheal pressure. Output signals representing P_{TP}, $\dot{V}$ and V_T were registered simultaneously on a Grass polygraph model 7. R_L and D_{Dyn} were determined by manual calculations according to the method of Amdur & Mead (1958). The animals were challenged with various doses of OA (5 and 40 μg/kg) injected intravenously through the left jugular vein.

Throughout the entire experiment, the blood pressure was recorded via a catheter inserted in the right carotid artery.

Drugs

The following drugs were used: ovalbumin, (OA, Sigma, grade, III) $Al(OH)_3$ (obtained as dry powder from a local dealer), pentobarbitone (Mebumal, ACO, Sweden); sodium cromoglycate (SCG, Fisons) and aminophylline (ACO, Sweden). The compounds were dissolved and diluted in saline.

Statistics

The results were statistically evaluated by Student's *t* test.

Results

The anti-anaphylactic effects of SCG and aminophylline were examined at different times after sensitization according to procedure (A) or procedure (B) (1 or 10 μg OA). The drugs were administered intravenously 2 min before the OA challenge. Animals sensitized according to procedure (A) were challenged with 40 μg/kg OA and the protective effects of SCG and aminophylline were examined. An earlier study (Andersson, 1979) investigated the effects of various challenging doses and the temporal development of the sensitized state in guinea-pigs sensitized according to the regimens used in the present paper. The earlier study showed that guinea-pigs sensitized with small

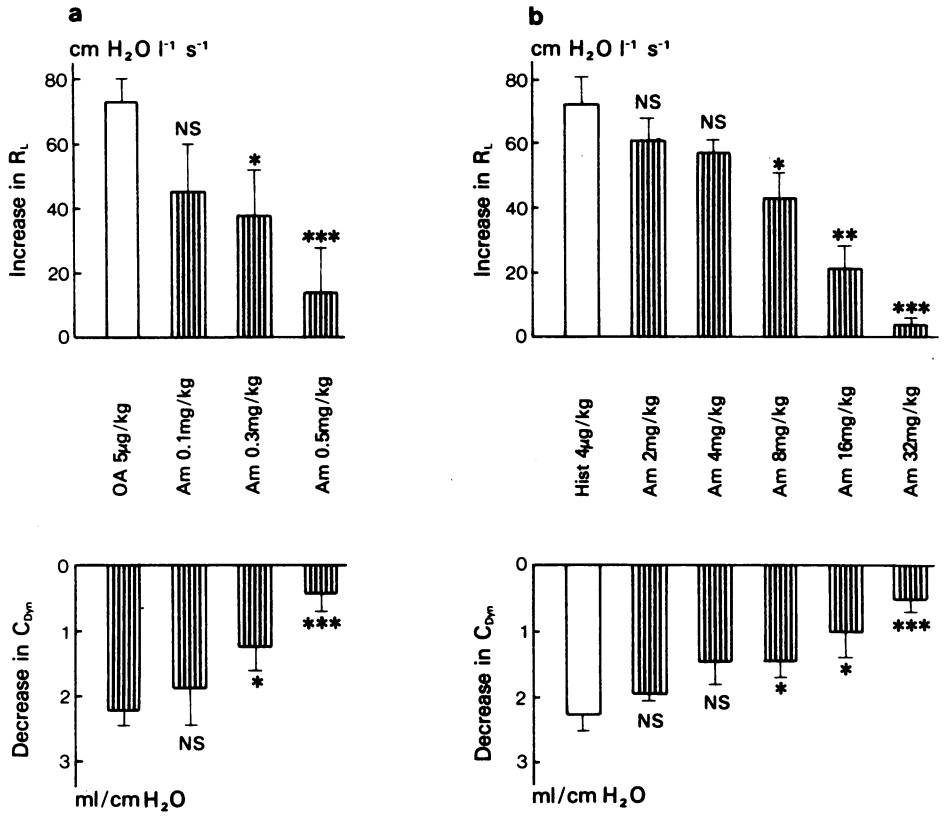

Figure 2 Anti-anaphylactic and bronchospasmolytic effects of aminophylline (Am). (a) The anti-anaphylactic effect of aminophylline was tested 14 days after sensitization with 10 µg of ovalbumin (OA) together with $Al(OH)_3$ 100 mg. Aminophylline was given intravenously 2 min before an intravenous challenge with 5 µg/kg of OA. (b) The bronchospasmolytic effect of aminophylline was examined on histamine-induced bronchoconstriction. Aminophylline was given as an intravenous injection 2 min before histamine (4 µg/k) given by the same route. Columns show mean results; vertical lines indicate s.e. mean (n = 6–8). NS = not significant. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

amounts of OA together with alum (procedure B) produced the most pronounced bronchospasm when challenged with antigen. In these animals, 40 µg/kg OA was a supramaximal challenge dose: therefore 5 µg/kg OA, which produced between 40 and 80% of the maximum obtainable response, was used in the present experiments in which the anti-anaphylactic effects of SCG and aminophylline were examined.

A 1 mg/kg dose of SCG was used to assess its anti-anaphylactic effects as preliminary experiments had shown that 0.1 mg/kg SCG had only a borderline inhibitory effect. SCG inhibited the bronchial response in animals sensitized for 14 days according to procedure (A) and procedure (B) (10 µg OA). Animals immunized according to these two procedures for 40 days did not respond to SCG. However, in animals sensitized according to procedure (B) (1 µg OA) SCG showed a clear protective effect, not only in animals sensitized for 14 days before test, but also when the immunization period was extended to 40 days (Figure 1).

Aminophylline (2.0 to 32.0 mg/kg) given intravenously inhibited a submaximal bronchospasm to histamine (4 µg/kg) in a dose-dependent way. A nearly maximum protective effect of aminophylline on an antigen-induced bronchoconstriction was attained by 0.5 mg/kg, a dose that exerted no inhibitory effect when tested on a histamine-induced bronchospasm (Figure 2). The dose of aminophylline used in the present investigation (0.3 mg/kg) exerted a pure anti-allergic effect, which was not due to the bronchodilator properties of the compound. The effect of aminophylline varied slightly with the immunisation regimen and the time of testing (Figure 1). Some differ-

ences in the inhibitory effect of aminophylline compared with SCG were noted. Aminophylline showed a highly significant, protective effect in guinea-pigs sensitized for 40 days according to procedures (A) and (B) (10 μg OA) whereas SCG did not.

Discussion

The reaginic antibody which contributes to the pathogenesis of atopical disease is immunoglobulin E (Ishizaka *et al.*, 1966; Johansson & Foucard 1978). Sufferers from extrinsic asthma, as a group, show elevated concentrations of IgE in their sera compared with non-allergic asthma patients (Bennich & Johansson, 1971). The symptomatic relief during SCG treatment of some patients suffering from allergic diseases has been clearly demonstrated (for references, see Church, 1978; Kingsley & Cox, 1978). Andersson (1979) described the temporal development of acute anaphylactic bronchoconstriction in guinea-pigs sensitized to ovalbumin by different regimens, including IgE-antibody promoting ones. In guinea-pigs sensitized with 1 μg ovalbumin together with alum, a strong and persistent bronchial anaphylactic reactivity was found whereas animals given 10 μg ovalbumin plus alum showed a transient bronchial hyper-reactivity. Guinea-pigs sensitized with two high doses of ovalbumin (5 mg on day 0 plus 10 mg on day 2) without alum developed a low degree of bronchial hyper-reactivity. Examination of the antibody classes by PCA technique showed that guinea-pigs sensitized with small amounts of antigen and alum produce both IgE and IgG_1 antibodies, whereas in sera from animals sensitized with large amounts of antigen, only IgG_1 antibodies could be detected. Attempts to reproduce the inhibitory effect of SCG in antigen-induced anaphylactic models in guinea-pigs have met with mixed success. It has been suggested that anaphylactic reactions in animals mediated by the heat-labile mercaptoethanol-sensitive reagin type antibody are more readily inhibited by SCG than anaphylactic reactions mediated by non-reaginic antibodies (Cox, 1967; Lopez & Bloch, 1969; Assem & Mongar, 1970; Church, 1978). Lack of inhibition of guinea-pig PCA by SCG was reported by Cox (1967) and Martin (1971). Martin showed that the failure of SCG to inhibit guinea-pig PCA is not only limited to reactions mediated by the non-reaginic IgG_1 antibody, but also occurs with the newly-recognized guinea-pig reagin-like antibody. The failure of SCG to inhibit PCA reactions mediated by IgG_1-antibodies was also reported by Lopez & Bloch (1969), Evans & Thomson (1975), and Taylor & Roitt (1973). Assem & Richter (1971) found that SCG inhibited both IgG_1 and reagin-mediated histamine release from guinea-pig lung. Experiments with chopped lung produced variable results (Cox, 1967; Evans & Thomson 1975). Taylor & Roitt (1973), Carney (1976), and Catty & Fraser (1972), in experiments with guinea-pigs actively or passively sensitized, showed that suppression of IgE-mediated responses, either with SCG or by previous heat treatment of the antibody, potentiates cutaneous and pulmonary responses to antigen. Thus SCG reduces the anaphylactic response in animals sensitized 12 days before challenge. At longer sensitization periods, SCG was either inactive or even increased the reactivity of the animals when challenged with antigen. The mechanism suggested for these effects was an increased production of IgG_1 antibodies late in the temporal development of the sensitized state, and an IgG_1-mediated anaphylactic reaction which was not inhibited but rather potentiated by SCG. Despite rather high IgG_{1a} and IgG_{1b} antibody titres in guinea-pigs sensitized for 40 days according to procedure (A) and procedure (B) (10 μg OA) (Andersson, 1979), no such potentiating effect of SCG was seen in the present investigation.

The results in the present paper indicate that bronchial anaphylactic reactions differ, depending on the way that the animals are sensitized and the time after sensitization that they are tested. The data in the present report also indicate the importance of choosing appropriately sensitized animals when the anti-anaphylactic effects of drugs are examined. Thus SCG was an effective inhibitor of the acute bronchial reaction in guinea-pigs sensitized with regimens shown to induce IgE antibody production. On the other hand, SCG inhibited the anaphylactic response in animals sensitized for 14 days according to procedure (A), which produces no IgE antibodies, but high amounts of IgG_1 antibodies. This effect could be explained by a transient low production of IgE antibodies when this sensitization regimen is used, although such antibodies could not be detected by PCA-tests. At this point, it is worth noting that the titre of antibodies with IgG-like properties, in sera from guinea-pigs sensitized according to procedure (A) 40 days before challenge is still high (Andersson, 1979). However the inhibitory effect of SCG seen in guinea-pigs sensitized for 14 days, disappeared in the guinea-pigs sensitized for 40 days. These findings agree with previous observations that SCG, if effective at all in guinea-pigs, inhibits IgE- but not IgG-antibody-mediated anaphylactic reactions (Carney, 1976; Church, 1978).

Theophylline has been shown to inhibit the release of pharmacological mediators of the immediate-type allergic reaction probably by increasing the mast cell cyclic AMP level (Kaliner & Austen, 1974; Holroyde, Burka & Eyre, 1977). Theophylline also blocks the potentiating effect of adenosine on mast cell histamine release (Marquardt, Parker & Sullivan, 1978). Theophylline has become useful not only as an acute bronchodilator but also as a major prophylactic agent for

the suppression of symptoms of chronic asthma. In recent years, theophylline has been found to control chronic asthma when administered regularly (Weinberger & Bronsky, 1974; 1975; Hambleton, Weinberger, Taylor, Cavanaugh, Ginchansky, Godfrey, Tooley, Bell & Greenberg, 1977; Weinberger, 1978). The present experiments strongly suggest that aminophylline, a theophylline salt, possesses anti-allergic properties that differ in character from those of SCG. Thus aminophylline was shown to inhibit antigen-induced bronchial anaphylaxis independent of mode of immunization and time of testing, at a concentration that did not show broncho dilator properties when tested against a histamine-induced contraction. The results with aminophylline obtained in the present experiments may be relevant to the beneficial effects of theophylline in bronchial asthma although the mechanism of action needs further investigation.

In conclusion, the present results show that, when a low antigen dose is used at sensitization, a sustained anaphylactic state of reactivity can be induced in guinea-pigs. The antigen-induced bronchial response in such animals can be reduced by treatment with SCG even in animals tested 40 days after sensitization. If higher doses of antigen are used for immunization, the inhibitory effect of SCG is seen only in animals tested 14 days after sensitization. The experiments also indicate that aminophylline possesses anti-allergic activity with a profile of action that is different from that of SCG.

The skilful technical assistance of Miss G. Stahre and the secretarial work done by Mrs I. Källén and Mrs U. Tapper are gratefully acknowledged. I am most grateful to Fisons Ltd for the gift of SCG.

References

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

ANDERSSON, P. (1979). Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Pattern of response in relation to immunization regimen. *Allergy*, (in press).

ASSEM, E.S.K. & MONGAR, J.L. (1970). Inhibition of allergic reactions in man and other species by cromoglycate. *Int. Archs Allergy*, **38**, 68–77.

ASSEM, E.S.K. & RICHTER, A.W. (1971). Comparison of *in vivo* and *in vitro* inhibition of the anaphylactic mechanism by β-adrenergic stimulants and disodium cromoglycate. *Immunology*, **21**, 729–739.

BENNICH, H. & JOHANSSON, S.G.O. (1971). Structure and function of human immunoglobulin E. *Advances in Immunology*, **13**, 1–55.

CARNEY, I.F. (1976). IgE-mediated anaphylactic bronchoconstriction in the guinea-pig and the effect of disodium cromoglycate. *Int. Archs. Allergy appl. Immun.*, **50**, 322–328.

CATTY, D. & FRASER, K.F. (1972). Homocytotropic antibodies in the guinea-pig. *Int. Archs Allergy*, **43**, 371–382.

CHURCH, M.K. (1978) Cromoglycate-like anti-allergic drugs: A review. *Med. Act./Drugs of today*, **14**, 281–341.

COX, J.S.G. (1967). Disodium cromoglycate (FPL 670) (Intal®): A specific inhibitor of reaginic antibody-antigen mechanisms. *Nature*, **216**, 1328–1329.

COX, J.S.G. (1971). Disodium cromoglycate. Mode of action and its possible relevance to the clinical use of the drug. *Br. J. Dis. Chest.*, **65**, 189–204.

DOBSON, C., MARSETH, D.J. & SOULSBY, E.J.L. (1971). Immunoglobulin E-type antibodies induced by Ascaris Suum infections in guinea-pigs. *J. Immunol.*, **106**, 128–133.

EVANS, D.P. & THOMSON, D.S. (1975). Inhibition of immediate hypersensitivity reactions in laboratory animals by a phenanthroline salt (ICI 74, 917). *Br. J. Pharmac.*, **53**, 409–418.

HAMBLETON, G., WEINBERGER, M., TAYLOR, J., CAVANAUGH, M., GINCHANSKY, E., GODFREY, S., TOOLEY, M., BELL, T. & GREENBERG, S. (1977). Comparison of cromoglycate (Cromolyn) and theophylline in controlling symptoms of chronic asthma. *Lancet*, **i**, 381–385.

HOLROYDE, M.C., BURKA, J.F. & EYRE, P. (1977). Automodulation of release of pharmacological mediators of immediate (Type 1) hypersensitivity. A Review. *Agents and Actions*, **7**, 421–430.

ISHIZAKA, K., ISHIZAKA, T. & HORNBROOK, M.M. (1966). Physicochemical properties of human reaginic antibody IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J. Immunol.*, **97**, 75–85.

JOHANSSON, S.G.O. & FOUCARD, T. (1978). IgE in immunity and disease. In *Allergy: Principles and Practice.* ed. Middleton, Jr. Elliott, Read, Charles E. & Ellis, Elliot F. pp. 551–562. Saint Louis, U.S.A.: C.V. Mosby Co.

KALINER, M. & AUSTEN, K.F. (1974). Hormonal control of the immunologic release of histamine and slow-contracting substance of anaphylaxis from human lung. In *Cyclic AMP, Cell Growth and the Immune Responses.* ed. Braun, W., Lichtenstein, L.M. & Parker, C.W. pp. 163–175. New York: Springer-Verlag.

KAY, A.B. & AUSTEN, K.F. (1971). The IgE-mediated release of an eosinophil leococytes chemotactic factor from human lung. *J. Immunol.*, **107**, 899–902.

KINGSLEY, P.J. & COX, J.S.G. (1978). Cromolyn sodium (sodium cromoglycate) and drugs with similar activities. In *Allergy: Principles and Practice.* ed. Middleton Jr., Elliott, Read, Charles E. & Ellis, Elliott F. pp. 481–498. Saint Louis, U.S.A.: C.V. Mosby Co.

LEVINE, B.B., CHANG, H. & VAZ, N.M. (1971). The production of hapten-specific reaginic antibodies in the guinea-pig. *J. Immunol.*, **106**, 29–33.

LOPEZ, M. & BLOCH, K.J. (1969). Effect of disodium cro-

moglycate on certain passive cutaneous anaphylactic reactions. *J. Immunol.*, **103**, 1428–1430.

MARQUARDT, D.L., PARKER, C.W. & SULLIVAN, T.J. (1978). Potentiation of mast cell mediator release by adenosine. *J. Immunol.*, **120**, 871–878.

MARTIN, L.E. (1971). Inhibition of cellular anaphylaxis by beta-stimulants. *Postgrad. med. J.*, **47** (Supplement March, 26–30).

MOTA, I. & PERINI, A. (1970). A heat labile mercaptoethanol susceptible homocytotropic antibody in the guinea-pig. *Life Sci., Oxford*, **9**, 923–930.

MOTA, I. & PERINI, A. (1975). The effect of a synthetic doublestranded RNA on IgG_1 and IgE production by guinea-pigs. *Immunology*, **29**, 319–326.

ORANGE, R.P., AUSTEN, W.G. & AUSTEN, K.F. (1971). Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. I. Modulation by agents influencing cellular levels of cyclic 3',5'-adenosine monophosphate. *J. exp. Med.* **134**, 136S–148S.

PARISH, W.E. (1970). Homologous serum passive cutaneous anaphylaxis in guinea-pigs mediated by two $\gamma 1$ or $\gamma 1$-type heat-stable globulins and a non-$\gamma 1$ heat-labile reagin. *J. Immunology*, **105**, 1296–1298.

PERINI, A. & MOTA, I. (1972). Heterogeneity of guinea-pig homocytotropic antibodies. *Immunology*, **22**, 915–923.

Perini, A. & Mota, I. (1973). The production of IgE and IgG_1 antibodies in guinea-pigs immunized with antigen and bacterial lipopolysaccharides. *Immunology*, **25**, 297–305.

TAYLOR, W.A. & ROITT, I.M. (1973). Effect of disodium cromoglycate on various types of anaphylactic reaction in the guinea-pig. *Int. Archs Allergy*, **45**, 795–807.

WEINBERGER, M.M. & BRONSKY, E.A. (1974). Evaluation of oral bronchodilator therapy in asthmatic children. *J. Pediatr.* **84**, 421–427.

WEINBERGER, M.M. & BRONSKY, E.A. (1975). Interaction of ephedrine and theophylline. *Clin. Pharmac. Ther.*, **17**, 585–592.

WEINBERGER, M. (1978). Theophylline for treatment of asthma. *J. Pediatr.*, **92**, 1–7.

(Received July 5, 1979.
Revised October 11, 1979.)