British Journal of Pharmacology (1999) 126, 312–316
http://www.stockton-press.co.uk/bjp

Anaphylactic bronchoconstriction in BP2 mice: interactions between serotonin and acetylcholine

[1]Seok-Yong Eum, [2]Xavier Norel, [1]Jean Lefort, [2]Carlos Labat, *,[1]B. Boris Vargaftig & [2]Charles Brink

[1]Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur-INSERM 485, Institut Pasteur 25, Rue du Dr. Roux, 75015 Paris, France; [2]CNRS ERS 566 Centre Chirurgical Marie-Lannelongue, 133 Avenue de la Résistance, 92350 Le Plessis-Robinson, France

1 Immunized BP2 mice developed an acute bronchoconstriction *in vivo* and airway muscle contraction *in vitro* in response to ovalbumin (OA) and these contractions were dose dependent.

2 Methysergide or atropine inhibited OA-induced bronchoconstriction *in vivo* and airway muscle contraction *in vitro*.

3 Neostigmine potentiated the OA-induced bronchoconstriction *in vivo* and airway muscle contraction *in vitro* of BP2 mice. This potentiation was markedly reduced by the administration of methysergide or atropine and when the two antagonists were administered together, the responses were completely inhibited.

4 Neostigmine also potentiated the serotonin (5-HT)- and acetylcholine (ACh)-induced bronchoconstriction and this potentiation was significantly reversed by atropine.

5 These results indicate that OA provokes a bronchoconstriction in immunized BP2 mice by stimulating the release of 5-HT, which in turn acts *via* the cholinergic mediator, ACh.

Keywords: Anaphylactic bronchoconstriction; mice; 5-HT; atropine; methysergide; ACh; neostigmine

Abbreviations: ACh, Acetylcholine; BP2 mice, Biozzi prepared hyperreactive mice; OA, Ovalbumin; 5-HT, Serotonin

Introduction

A number of studies have shown that lungs of rats (Church *et al*., 1972) and mice (Levitt & Mitzner, 1989) are responsive to 5-HT and ACh. In the rat, the response to 5-HT is mediated by a direct action of this agonist on airway smooth muscle (Pauwels *et al*., 1985). In contrast, in the mouse, the effect of 5-HT may be mediated by indirect mechanisms, such as the release of ACh. In fact, Levitt & Mitzner (1989) showed that in certain inbred strains of mice, which are very responsive to 5-HT, the bronchoconstriction induced by 5-HT was blocked by atropine. These data implicated an interaction of 5-HT with the nervous system and cholinergic mechanisms.

In sensitized mice, the ovalbumin dose-dependent contractions have been reported to be associated with the release of 5-HT (Iff & Vaz, 1966; Lima, 1967). Larsen and co-workers (1994) showed that repeated airway exposure to an allergen and the development of an IgE-responsive state lead to an altered neural control of airways with an increase in ACh release from nerve terminals. These *in vitro* data supported similar observations suggesting a link between elevated IgE titers in mice and airway hyper-responsiveness to contractile agents (Saloga *et al*., 1994; Weinmann *et al*., 1990). These results provided evidence for the hypothesis that an ovalbumin-induced bronchoconstriction in mice may be due to the direct contractile effect on airway muscle of ACh which is released by 5-HT. However, no studies have been performed which demonstrate that this series of physiological events occurred in any one of the proposed animal models of airway hyper-reactivity. Therefore, the aim of this investigation was to examine the ovalbumin induced bronchoconstriction in a mouse model where an elevated serum IgE titer and airway hyper-responsiveness have been reported (Eum *et al*., 1995).

*Author for correspondence; E-mail: <vargafti@pasteur.fr>

Methods

Mice and immunization procedure

Female BP2 mice were obtained from the 'Centre d'Elevage R. Janvier' (BP5, 53940 Le Genest Saint-Isle, France). Mice from 8–11 weeks were used in the experiments. These BP2 mice, a selection of Biozzi mice, produce high titers of antibodies (Biozzi *et al*., 1979) including IgE (Eum *et al*., 1995). These animals were derived from Swiss mice which were bred for the production of either high or low amounts of antibodies against selective antigens. After 16 generations the animals differed by 280-fold in mean agglutinin titers of antibodies produced against sheep erythrocytes (Biozzi *et al*., 1970).

Mice were immunized by injecting 0.4 ml of an OA solution (s.c. 250 μg ml^{-1}) mixed with A1(OH)$_3$ (4 mg ml^{-1}) twice at an interval of 7 days. Mice were utilized after 1 week following the second injection.

Evaluation of bronchoconstriction in vivo *and airway muscle contraction* in vitro

Bronchoconstriction *in vivo* was measured by the methods described by Eum *et al*. (1995) and the contraction of isolated trachea *in vitro* by Norel *et al*. (1993). Briefly, in the *in vivo* experiments, immunized mice were anaesthetized with ethyl carbamate (i.p., 1.5 mg g^{-1}). The trachea was cannulated and prepared for recording of dynamic compliance and airway resistance, by adapting the equipment of the computerized pulmonary analyser (Mumed PR800 system, U.K.) to mice airways at a tidal volume of 2.10^{-2} ml g^{-1} and a frequency of

100 breaths min^{-1}. The animals were paralysed with pancuronium bromide (Pavulon®, 10 $\mu g\ kg^{-1}$ i.v.) and airway resistance was calculated from the differential pressure between the airways and pleural cavity and the airflow. Control basal values of resistance were 500 ± 20 (cm water $(1\ sec^{-1})^{-1}$) for $n = 20$ animals (mean ± s.e.mean). Depending on the protocol used, the data were expressed either as per cent increase of resistance (% increase), or as the increase above basal resistance. The per cent increase was calculated as follows: ((resistance after treatment − basal resistance)/basal resistance) × 100%. In the protocols using neostigmine the data are shown as the resistance measured after treatment − basal resistance after neostigmine.

In the *in vitro* experiments, tracheal preparations were set up in the 10 ml organ baths containing Tyrode's solution (concentration in mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4 and glucose 5.5; pH 7.4; gassed with 95% O_2/5% CO_2 under initial loads (1 g). These loads ensured that responses to contractile agonists were optimal. Isometric force displacement transducers (Narco F-60) and physiographs (Linseis) were used to record the changes in force. The tissues were allowed to equilibrate for 90 min and the bath fluid was exchanged every 15 min with fresh Tyrode's solution. After this period, all tracheal preparations were contracted with carbachol (3 μM). The tissues were washed with fresh Tyrode's solution and allowed to return passively to their resting tone. When resting tone was established, the preparations were incubated for 30 min in Tyrode's solution or Tyrode's solution containing either neostigmine (0.1 μM), atropine (1 μM) or methysergide (1 μM) and the response to an individual dose of OA or the relationship to 5-HT was determined.

Drug treatment

Drugs used to study the mechanisms of anaphylactic bronchonconstriction were injected in 0.9% NaCl (saline) through the cannulated jugular vein before OA injection. After the animals were prepared on the Mumed system (see 'Evaluation of bronchoconstriction *in vivo*'), drugs were injected in the following order at 5 min intervals; ACh (80 $\mu g\ kg^{-1}$), 5-HT (40 $\mu g\ kg^{-1}$), saline or neostigmine (10 $\mu g\ kg^{-1}$), 5-HT (40 $\mu g\ kg^{-1}$), OA (50 mg kg^{-1}) and ACh (80 $\mu g\ kg^{-1}$). In order to determine the effects of each drug on OA-induced bronchoconstriction, either methysergide (200 $\mu g\ kg^{-1}$), atropine (10 $\mu g\ kg^{-1}$; 200 $\mu g\ kg^{-1}$), or methysergide (200 $\mu g\ kg^{-1}$) + atropine (10 $\mu g\ kg^{-1}$) were injected after the injection of saline or neostigmine and prior to the second injection of 5-HT.

Materials

Ovalbumin (5× crystallized) was from Immunobiological (Costa Mesa, U.S.A.). Aluminium hydroxide was from Merck (Darmstadt, Germany). 5-Hydroxytryptamine (5-HT), ethyl carbamate, neostigmine, atropine, carbachol and acetylcholine were from Sigma (St. Louis, MO, U.S.A.). Pancuronium bromide (Pavulon) was from Organon Teknika (France). Methysergide was from Sandoz (Bâle, Swiss).

Statistical analysis

The results of each measurement are reported as means ± s.e.mean. Values significantly different between experimental and control groups were analysed by the Student's *t*-test (unpaired) and *P* values of less than 0.05 were considered to be significant.

Results

OA-induced bronchoconstriction and contractions of isolated trachea in immunized BP2 mice

Intravenous challenge with OA in immunized BP2 mice induced a dose-dependent augmentation of bronchial resistance, the maximal effect (approximately 160% augmentation) was reached at 50 mg kg^{-1} (Figure 1a). Saline-challenged mice (controls) showed no significant augmentation in bronchial resistance. The addition of OA to the organ bath containing trachea from immunized BP2 mice also induced dose-dependent contractions, which were approximately 40% of the carbachol effect (carbachol, 1 μM: 1.70 ± 0.11 g, $n = 45$: Figure 1b).

Drug modulation of anaphylactic bronchoconstriction in vivo *and on OA contractions of isolated trachea* in vitro

Anaphylactic bronchoconstriction was markedly attenuated by methysergide (200 $\mu g\ kg^{-1}$) and atropine (10 $\mu g\ kg^{-1}$: Figure 2a). In addition, a higher dose of atropine (200 $\mu g\ kg^{-1}$) completely abolished the OA response in these animals ($n = 3$; data not shown). Neostigmine significantly potentiated the OA-induced bronchoconstriction (Figure 3). This potentiation was blocked by methysergide or atropine (10 $\mu g\ kg^{-1}$) co-administered with neostigmine (Figure 3). When the drug combination of methysergide and atropine (10 $\mu g\ kg^{-1}$) was administered, the anaphylactic bronchoconstriction in the presence of neostigmine was suppressed (data not shown). In isolated trachea, the OA-induced contraction was also inhibited by either methysergide or atropine (Figure 2b). This contraction was potentiated by neostigmine (approximately 100%, $n = 8$) and the combined injection of methysergide and atropine inhibited this contraction ($n = 3$; data not shown).

Intravenous injection of neostigmine also potentiated the 5-HT-induced bronchoconstriction. This augmentation was blocked by either methysergide or atropine (Figure 3) and the simultaneous injection of both antagonists blocked the responses to 5-HT (data not shown). The ACh-induced bronchoconstriction was also increased by neostigmine and this was blocked by atropine, but not by methysergide (Figure 3). In isolated trachea, the 5-HT-induced contraction was also increased by neostigmine, but was not significantly inhibited by atropine (Figure 4).

Discussion

OA produced a dose-dependent contraction of airway muscle both *in vivo* and *in vitro* (present report) in immunized BP2 mice. These airway muscle responses were blocked by treating the animals or tissues with methysergide or atropine, suggesting that 5-HT and ACh were involved in the contraction. In addition, neostigmine potentiated the bronchoconstriction to OA, 5-HT and ACh, an effect markedly inhibited in each case by atropine. These data support the hypothesis that ovalbumin provoked a bronchoconstriction by stimulating the release of 5-HT and the direct contractile effect on airway smooth muscle was due to ACh. Thus the present study shows that in hyper-responsive BP2 mice, antigen-induced anaphylactic bronchoconstriction has a dual mediator release profile. While 5-HT and ACh measurements were not performed, the antagonist data indirectly support the involvement of both agents.

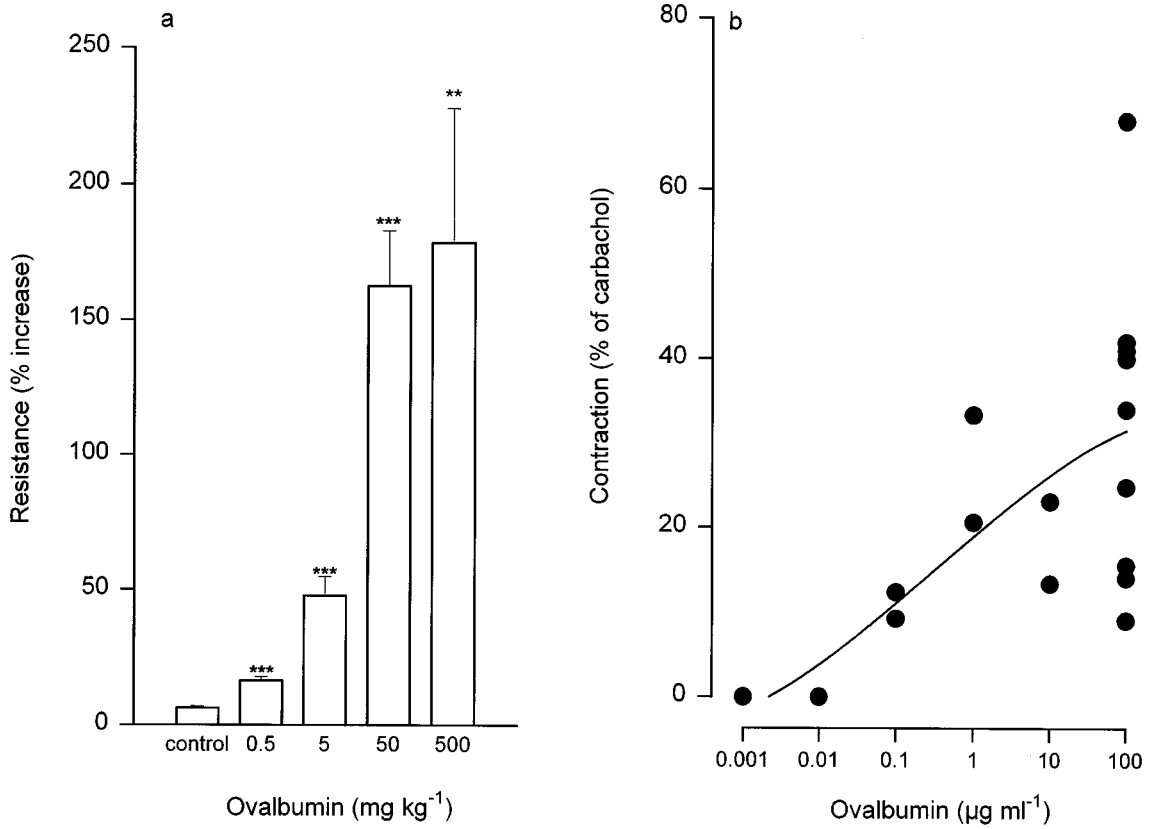

Figure 1 In immunized BP2 mice OA-induced bronchoconstriction *in vivo* (a) and airway muscle contraction *in vitro* (b). The values are in (a) means±s.e.mean derived from 4–20 mice whereas in (b) data were from individual animals. $^{**}P<0.01$, $^{***}P<0.001$ compared with the control group (saline challenged mice).

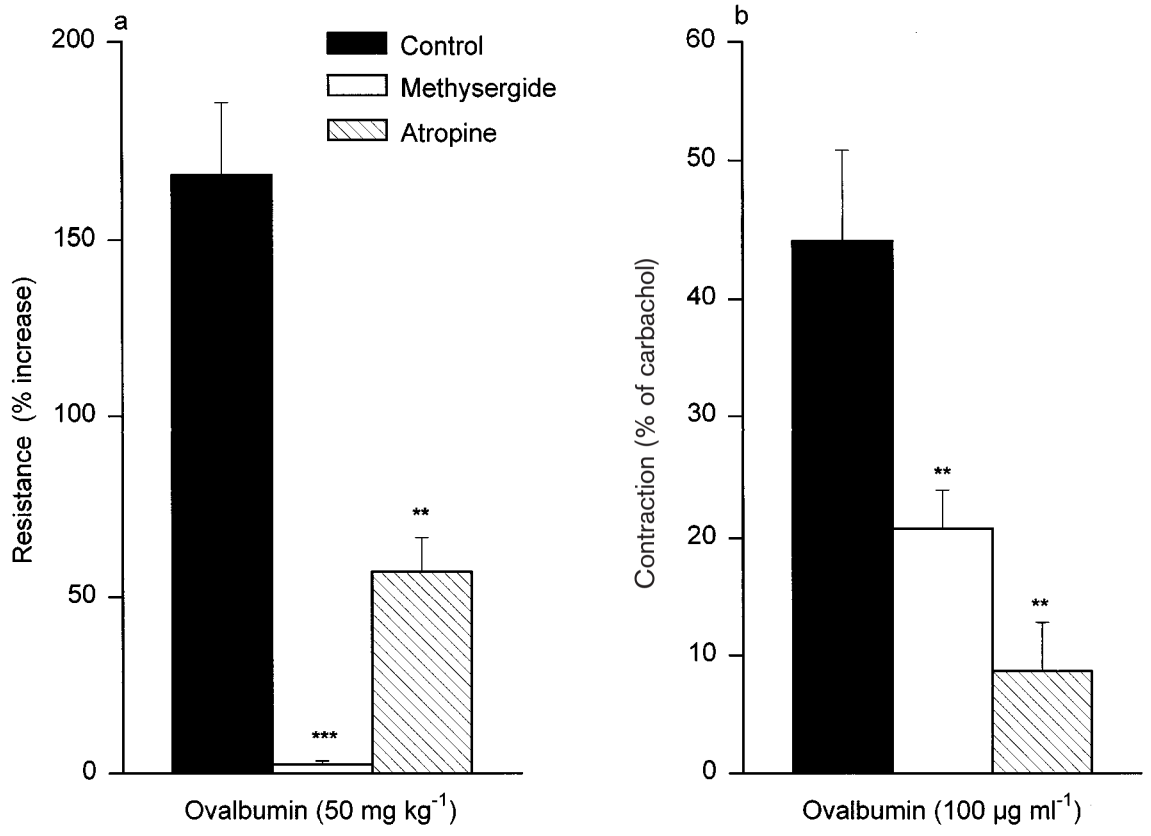

Figure 2 The effects of methysergide (200 μg kg^{-1}) and atropine (10 μg kg^{-1}) on ovalbumin induced bronchoconstriction *in vivo* (a) and on airway muscle contraction *in vitro* (b) in immunized BP2 mice. The values are means±s.e.mean derived from 5–10 mice. $^{**}P<0.01$, $^{***}P<0.001$ compared with controls (OA challenged).

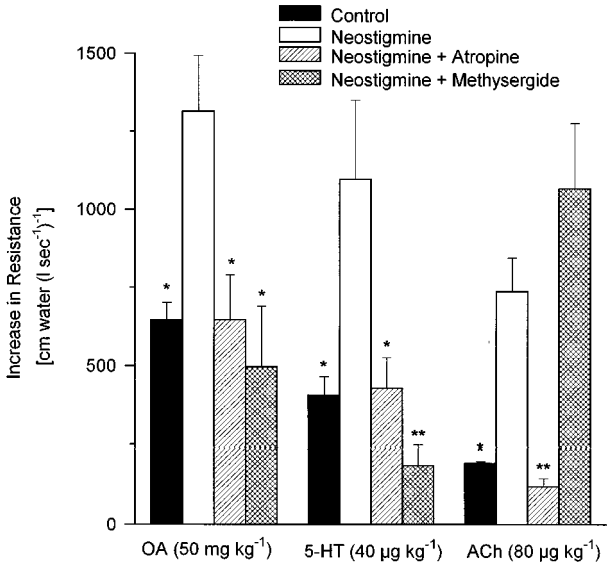

Figure 3 The effect of atropine and methysergide in combination with neostigmine on bronchoconstriction *in vivo* induced by different agents in immunized BP2 mice. Bronchoconstriction (control: agonist in absence of drugs) and in presence of neostigmine (10 μg kg^{-1}) or neostigmine (10 μg kg^{-1}) + atropine (10 μg kg^{-1}) or neostigmine (10 μg kg^{-1}) + methysergide (200 μg kg^{-1}) are shown. The agonist challenges were: acetylcholine (ACh), serotonin (5-HT) and ovalbumin (OA). Values are means ± s.e.mean derived from 4–6 mice. *$P<0.05$, **$P<0.01$ compared with neostigmine group.

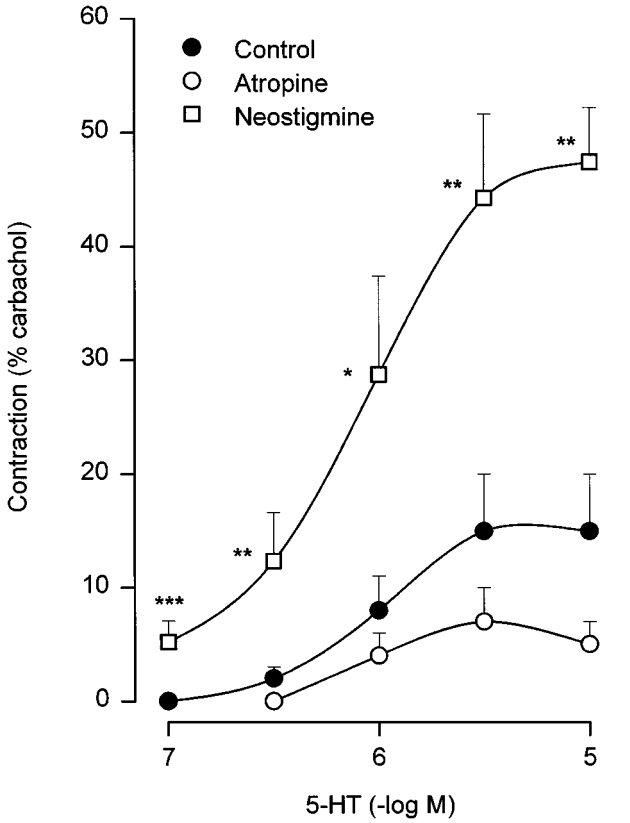

Figure 4 The 5-HT contractions in presence of either Tyrode's solution (Control), neostigmine (0.1 μM) or atropine (1 μM) in airway muscle derived from immunized BP2 mice. The values are means ± s.e.mean derived from 4–10 tracheal strips. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with control group.

5-HT is released from mast cells (Kitamura, 1989) upon activation by antigen-specific IgE or by antigen binding T cell factors in mice. In rats 5-HT is the major mediator of antigen-induced anaphylactic bronchoconstriction (Church *et al.*, 1972) and airway smooth muscle contraction (Nagase *et al.*, 1995). In this study, methysergide suppressed the OA-induced bronchoconstriction, indicating that 5-HT is also a major mediator of OA-induced anaphylactic bronchoconstriction in BP2 mice. This observation is in agreement with that of Church *et al.* (1972) who also showed that antigen-induced bronchoconstriction is suppressed by methysergide in the rat. In BP2 mice (present report) methysergide also inhibited the OA-induced tracheal contractions *in vitro*. Church *et al.* (1972) initially demonstrated that atropine is inactive against the antigen-induced bronchoconstriction in the rat suggesting a direct action of this amine on the airway smooth muscle. OA-induced bronchoconstriction *in vivo* (present report) was significantly inhibited by atropine. Furthermore, atropine at a higher dose completely abolished the OA-induced constriction *in vivo*. These data suggest a cholinergic participation in the airway anaphylactic responses in BP2 mice. Previous reports have demonstrated that 5-HT facilitates cholinergic nerve-mediated contraction of the airways in a number of species including the rat (Aas, 1983; Szarek *et al.*, 1993), mouse (Van-Oosterhout *et al.*, 1991), the guinea-pig (Rizzo *et al.*, 1993), and man (Takahashi *et al.*, 1995). In mice, atropine inhibited the airway response to 5-HT (Levitt & Mitzner, 1989). These data suggest that 5-HT acts *via* a cholinergic pathway.

In the isolated trachea from Swiss mice, contractions were potentiated during electrical field stimulation, after the addition of 5-HT and this potentiation was suppressed by atropine (Van-Oosterhout *et al.*, 1991). These results suggested that ACh was released by stimulation of 5-HT receptors in trachea from these mice, specifically since 5-HT failed to affect the response to exogenously applied ACh. A similar phenomenon has also been observed in human and guinea-pig airways by Takahashi *et al.* (1995). Such data strengthen the observation (present report) that OA-induced contractions of trachea were inhibited by atropine. Two observations further support a cholinergic pathway. First, neostigmine, a cholinesterase inhibitor, potentiated the anaphylactic bronchoconstriction of BP2 mice *in vivo* and *in vitro*, and this potentiation was markedly reduced by atropine. Secondly, neostigmine also potentiated 5-HT-induced bronchoconstriction and tracheal contraction in these mice, supporting ACh release upon stimulation of 5-HT.

Increased release of ACh induced by antigen challenge from airway parasympathetic nerve endings can contribute to increases in airway responsiveness (Larsen *et al.*, 1994). These investigators measured the release of ACh in electrically stimulated tracheal preparation derived from sensitized mice. The quantities detected were markedly different than results observed in unsensitized mice. This increased release of ACh may be due either to the absence of inhibitory prejunctional M_2 receptors or the presence of a 5-HT prejunctional facilitory receptor on the parasympatic neuronal fibres. Another possible explanation may be a decrease in cholinesterase activity. Previous studies have shown a modification of cholinesterase activity in dog (Mitchell *et al.*, 1991) and pig (Taisne *et al.*, 1997) airways related to the anaphylactic release of mediators. The findings (present report) suggest that ovalbumin-induced bronchoconstriction in BP2 mice may be due to the direct contractile effect on airway smooth muscle of ACh which is released by 5-HT.

References

AAS, P. (1983). Serotonin induced release of acetylcholine from neurons in the bronchial smooth muscle of the rat. *Acta. Physiol. Scand.,* **117,** 477–480.

BIOZZI, G., ASOFSKY, R., LIEBERMAN, R., STIFFEL, C., MOUTON, D. & BENACERRAF, B. (1970). Serum concentrations and allotypes of immunoglobulins in two lines of mice genetically selected for 'high' or 'low' antibody synthesis. *J. Exp. Med.,* **132,** 752–764.

BIOZZI, G., MOUTON, D., SAINT'ANNA, O.A., PASSOS, H.C., GENNARI, M., REIS, M.H., FERREIRA, V.C.A., HEUMANN, A.M., BOUTHILLIER, Y., IBANEZ, O.M., STIFFEL, C. & SIQUEIRA, M. (1979). Genetics of immunoresponsiveness to natural antigens in the mouse. *Curr. Top. Microbiol. Immunol.,* **85,** 31–98.

CHURCH, M.K., COLLIER, H.O.J. & JAMES, W.L. (1972). The inhibition by dexamethasone and disodium cromoglycate of anaphylactic bronchoconstriction in the rat. *Br. J. Pharmacol.,* **46,** 56–65.

EUM, S.Y., HAILE, S., LEFORT, J., HUERRE, M. & VARGAFTIG, B.B. (1995). Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin-5-dependent bronchial hyperresponsiveness. *Proc. Natl. Acad. Sci., U.S.A.,* **92,** 12290–12294.

IFF, E.T. & VAZ, N.M. (1966). Mechanisms of anaphylaxis in the mouse. *Int. Arch. Allergy,* **30,** 313–322.

KITAMURA, Y. (1989). Heterogeneity of mast cells and phenotypic change between subpopulations. *Ann. Rev. Immunol.,* **7,** 59–66.

LARSEN, G.L., FAME, T.M., RENZ, H., LOADER, J.E., GRAVES, J., HILL, M. & GELFAND, E.W. (1994). Increased acetylcholine release in tracheas from allergen-exposed IgE-immune mice. *Am. J. Physiol.,* **266,** L263–L270.

LEVITT, R.C. & MITZNER, W. (1989). Autosomal recessive inheritance of airway hyperreactivity to 5-hydroxytryptamine. *J. Appl. Physiol.,* **67,** 1125–1132.

LIMA, A.O. (1967). Pharmacologically active substances released during anaphylactic shock in the mouse. *Int. Arch. Allergy,* **32,** 46–54.

MITCHELL, R.W., KELLY, E. & LEFF, A.R. (1991). Reduced activity of acetylcholine-esterase in canine tracheal smooth muscle homogenates after active immune-sensitization. *Am. J. Respir. Cell Mol. Biol.,* **5,** 56–62.

NAGASE, T., FUKUCHI, Y., DALLAIRE, M.J., MARTIN, J.G. & LUDWIG, M.S. (1995). In vitro airway and tissue response to antigen in sensitized rats: role of serotonin and leukotriene D4. *Am. J. Respir. Crit. Care Med.,* **152,** 81–86.

NOREL, X., ANGRISANI, M., LABAT, C., GORENNE, I., DULMET, E., ROSSI, F. & BRINK, C. (1993). Degradation of acetylcholine in human airways: role of butyrylcholinesterase. *Br. J. Pharmacol.,* **108,** 914–919.

PAUWELS, R., VAN DER STRAETEN, M., WEYNE, J. & BAZIN, H. (1985). Genetics factors in non-specific bronchial reactivity in rats. *Eur. J. Respir. Dis.,* **66,** 98–104.

RIZZO, C.A., KREUTNER, W. & CHAPMAN, R.W. (1993). 5-HT3 receptors augments neuronal, cholinergic contractions in guinea-pig trachea. *Eur. J. Pharmacol.,* **234,** 109–112.

SALOGA, J., RENZ, H., LARSEN, G.L. & GELFAND, E.W. (1994). Increased airways responsiveness in mice depends on local challenge with antigen. *Am. J. Respir. Crit. Care Med.,* **149,** 65–70.

SZAREK, J.L., ZHANG, J.Z. & GRUETTER, C.A. (1993). 5-HT2 receptors augment nerve-mediated contraction of rat bronchi. *Eur. J. Pharmacol.* **231,** 339–346.

TAISNE, C., NOREL, X., WALCH, L., LABAT, C., VERRIEST, C., MAZMANIAN, G.M. & BRINK, C. (1997). Cholinesterase activity in pig airways and epithelial cells. *Fundam. Clin. Pharmacol.,* **11,** 201–205.

TAKAHASHI, T., WARD, J.K., TADJKARIMI, S., YACOUB, M.H., BARNES, P.J. & BELVISI, M.G. (1995). 5-Hydroxytryptamine facilitates cholinergic bronchoconstriction in human and guinea-pig airways. *Am. J. Crit. Care Med.,* **152,** 377–380.

VAN OOSTERHOUT, A.J.M., HOFMANN, G., WOUTERSEN-VAN NIJNANTEN, F.M.A. & NIJKAMP, F.P. (1991). 5-HT1-like receptors mediate potentiation of cholinergic nerve-mediated contraction of isolated mouse trachea. *Eur. J. Pharmacol.,* **209,** 237–244.

WEINMANN, G.G., BLACK, C.M., LEVITT, R.C. & HIRSHMAN, C.A. (1990). In vitro tracheal responses from mice chosen for in vivo lung cholinergic sensitivity. *J. Appl. Physiol.,* **69,** 274–280.

(Received July 6, 1998
Revised October 15, 1998
Accepted October 20, 1998)