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Effects of anterior hypothalamic lesions and sham-operations on bacterial endotoxin-induced non-specific airway hyperreactivity *in vivo* and *in vitro*

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1 In the present study the effects of anterior hypothalamic (AHA) lesions and sham-operations were investigated on the endotoxin-induced airway hyperreactivity in guinea-pigs. Unoperated, sham-operated and AHA-lesioned guinea-pigs were injected intraperitoneally with *Escherichia coli* endotoxin and the airway reactivity tested four days later in isolated tracheal spirals and in spontaneously breathing anaesthetized animals. Control animals were given sterile saline.

2 Sham-operated control animals demonstrated a diminished responsiveness of the tracheal spirals *in vitro* and of the lung resistance (ΔR_1) *in vivo* to histamine receptor and cholinoceptor-muscarinic agonists as compared to unoperated control animals.

3 AHA-lesioned control animals showed a responsiveness of the respiratory airways *in vitro* and *in vivo* between the values of unoperated and sham-operated control animals, suggesting that lesions partially restored the diminished responsiveness.

4 In unoperated and sham-operated guinea-pigs, endotoxin administration induced hyperreactivity of the tracheal spirals and ΔR_1 to histamine receptor and cholinoceptor-muscarinic agonists with respect to the control groups.

5 In AHA-lesioned animals, the endotoxin-induced airway hyperreactivity *in vitro* and *in vivo* to histamine receptor and cholinoceptor-muscarinic agonists was absent.

Introduction

A common characteristic feature of asthmatic patients is an increased responsiveness of the respiratory airways to physical, chemical and pharmacologic stimuli (Committee on diagnostic standards for non-tuberculous respiratory diseases, 1962). Despite much effort, the underlying mechanisms of airway hyperreactivity are still obscure and difficult to unravel. In part at least, this is due to the multifactorial cause of this phenomenon.

Intraperitoneal administration to rodents of killed Gram-negative bacteria like *Bordetella pertussis* and *Haemophilus influenzae* or the common cell-wall component endotoxin has been shown to be a valid animal model for atopy in which non-specific airway hyperreactivity occurs, together with other criteria for atopy like eosinophilia and enhanced IgE antibody production (Szentivanyi, 1968; Terpstra *et al.*, 1979; Nijkamp & Schreurs, 1984). Furthermore, we showed previously that an imbalance between the cholinergic bronchoconstrictor- and the β-adrenergic bronchodilator systems in guinea-pig isolated trachea occurred, four days after i.p. injection of *H. influenzae* (Schreurs *et al.*, 1980). Additionally, hyperreactivity to histamine *in vivo* developed which might be caused by this imbalance (Folkerts & Nijkamp, 1985). Endotoxin appeared to be the active moiety with respect to the reduction in the number of β-adrenoceptor binding sites (Schreurs *et al.*, 1983; Van Heuven-Nolsen *et al.*, 1986). It was also found that anterior hypothalamic (AHA) lesions could prevent the decrease in the number of β-adrenoceptor binding sites in peripheral lung tissue after *Escherichia coli* O_{111}:B_4 endotoxin administration to guinea-pigs (Van Oosterhout & Nijkamp, 1984).

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It has been shown that lesions in the AHA also modulate several other atopic criteria. They induce protection against lethal anaphylactic reactions in guinea-pigs and rats (Schiavi *et al.*, 1975; Luparello *et al.*, 1964), reduce IgE antibody production (Tyrey & Nalbandov, 1972) and decrease airway smooth muscle responsiveness to anaphylactic mediators (Schiavi *et al.*, 1966; Van Oosterhout & Nijkamp, 1986). Furthermore, lesions in the ventral posterior hypothalamus prevented the eosinophila which can be induced by injection of *B. pertussis* bacteria (Tjabbes & De Wied, 1962). In the present study we attempted to establish a role for the AHA in the development of non-specific hyperreactivity by investigating the influence of AHA lesions on the induction of histaminergic and cholinergic-muscarinic hyperreactivity *in vivo* and *in vitro* after endotoxin administration to guinea pigs.

Methods

Animals

The animals used in this study were male Dunkin Hartley guinea-pigs (Olac Ltd., Bicester, England) weighing 270–320 g at the start of the experiment. Three different groups were studied comprising unoperated, sham-operated and anterior hypothalamus (AHA)-lesioned guinea-pigs. Prior to the operations the animals were anaesthetized with fluanison ($30\,mg\,kg^{-1}$, s.c.) and fentanyl base ($0.6\,mg\,kg^{-1}$, s.c.) (Hypnorm) and pentobarbitone sodium ($60\,mg\,kg^{-1}$, i.p.) (Nembutal). Subsequently, bilateral electrolytic lesions were made under aseptic conditions in the AHA according to an atlas of the guinea-pig forebrain (Benson & Ward, 1971) with the aid of a stereotaxic instrument. The coordinates at the position of the bregma were 0.9 mm right and left of the sagittal sinus and 9.4 mm vertical from the dorsal brain surface. The lesions were produced by a current of 2 mA passed for 10 s through an insulated stainless steel electrode of 0.2 mm diameter with a 0.5 mm bare tip. The sham-operated animals were treated in the same way except that the electrode was lowered 7.0 mm and no current was administered. Unoperated guinea-pigs were also studied. At the end of the experiment, lesion size and location were determined histologically. In approximately 20% of the animals the localization appeared incorrect and these animals were excluded from the data. Ten days after the operation and four days before the experiment the animals were injected (i.p.) with endotoxin, $1\,mg\,kg^{-1}$ (L.P.S.; *E. coli* O_{111}:B_4) in a volume of $1\,ml\,kg^{-1}$. Control animals were injected with sterile saline, $1\,ml\,kg^{-1}$.

Tracheal spirals

The tracheae were removed, dissected free of connective tissue and blood vessels and cut in a spiral fashion (Constantine, 1965). One trachea was divided in two parts each of seven rings. The tracheal spirals were mounted in an organ bath filled with Krebs bicarbonate buffer (37°C) of the following composition ($mmol\,l^{-1}$): NaCl 118.1, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 8.3. Krebs was continuously gassed with a 5% CO_2 and 95% O_2 gas mixture. Changes in length were measured by an isotonic transducer (Harvard Bioscience) and displayed on a two-channel pen recorder (Servogor, type 220). The tracheal spirals were maintained under a load of 0.8 g throughout the experiment and allowed to equilibrate for at least 45 min. On one part of the tracheal spirals a cumulative histamine concentration-response relationship was established while the other part was used for the construction of a cumulative concentration-response curve for the cholinoceptor-muscarinic agonist, arecoline. For both drugs alternately the proximal or distal part of the tracheal spiral was used to avoid a possible difference between the parts. The individual concentration-response curves were analyzed by a computerized curve fitting technique based on the four-parameter logistic equation (De Lean *et al.*, 1978). The parameters thus obtained (i.e. maximal response, EC_{50} and slope factor) were averaged and the mean values used to construct the mean concentration-response curve.

Lung function parameters

The guinea-pigs were anaesthetized with urethane ($2.8\,g\,kg^{-1}$, i.p.) and able to breathe spontaneously. To avoid anaesthesia-induced fall in body temperature the animals were placed in a heated chamber of approximately 30°C. They were prepared for measurement of lung resistance (R_1) and dynamic compliance (C_{dyn}) as follows. To determine airflow ($\dot{V}$) and tidal volume (V_t) the trachea was cannulated and connected to a Gould Godart pneumotachograph with a Fleisch flow head (nr 000). A Validyne MP45-24 pressure transducer measured the pressure difference between the tracheal cannula and a cannula filled with saline inserted in the oesophagus which presented the transpulmonary pressure (TPP). R_1 and C_{dyn} were determined breath by breath by a modified method of Amdur & Mead (1958) using a computerized respiratory analyzer. Dividing ΔTPP by $\Delta\dot{V}$ at isovolume points (50%) yielded the R_1 and dividing V_t by ΔTPP between points of zero flow yielded the C_{dyn}. A small polyethylene catheter used for intravenous administration of drugs was placed

in the right jugular vein. First, a histamine dose-response curve was constructed, followed after a recovery period of 30 min by a dose-response curve for the cholinoceptor-muscarinic agonist, methacholine. Injections were given at time intervals of at least 5 min. In the figures, ΔR_1 is shown which represents the actual value minus the basal value. The ED_{50} values were estimated by a computerized curve fitting technique based on the four parameter logistic equation (De Lean *et al.*, 1978). This was only performed on the mean data points of the dose-response curves of unoperated guinea-pigs since only in these animals did the responses reach a maximal level.

Statistics

Levels of significance between the concentration-response curves of the tracheal spirals from different groups were calculated by two-way analysis of variance (ANOVA). For comparing the parameters of the concentration-response curves of the tracheal spirals and the differences between the groups in lung function parameters after drug administration, the unpaired Student's t test was used. $P < 0.05$ was considered as significant. Responses are presented as mean ± s.e. mean.

Materials

Nembutal, containing pentobarbitone sodium 60 mg ml^{-1} was from Abbot Laboratories, North Chicago, IL, USA. Hypnorm, containing fluanison 10 mg ml^{-1}, and fentanyl base 0.2 mg ml^{-1} was from Duphar B.V., Amsterdam, The Netherlands. Urethane was from Brocades-ACF, Maarssen, The Netherlands. Histamine phosphate was obtained from the Onderlinge Pharmaceutische Groothandel, Utrecht, The Netherlands. Arecoline hydrobromide, methacholine chloride and endotoxin, *E. coli* O_{111}:B_4 lipopolysaccharide (Phenolic extraction) were from Sigma Chemical Company, St. Louis, USA.

Results

In vitro *hyperreactivity*

The mean histamine concentration-response curve of the tracheal spirals from unoperated animals treated four days earlier with endotoxin showed that there was a significant ($P < 0.01$, ANOVA) hyperreactivity as compared to the control saline-pretreated animals (Figure 1). This hyperreactivity was not specific for histamine since an increased responsiveness ($P < 0.01$, ANOVA) of the tracheal spirals to the cholinoceptor-muscarinic agonist, arecoline, also occurred (Figure 1). The extent of the hyperreactivity was approximately identical, amounting to 32% ($P < 0.01$) and 25% ($0.05 < P < 0.1$) increases in contraction at the maximal concentration of histamine and arecoline respectively (Table 1). The EC_{50} values and slope factors of the concentration-response curves of the two drugs did not differ between the saline-pretreated and endotoxin-pretreated groups (Table 1).

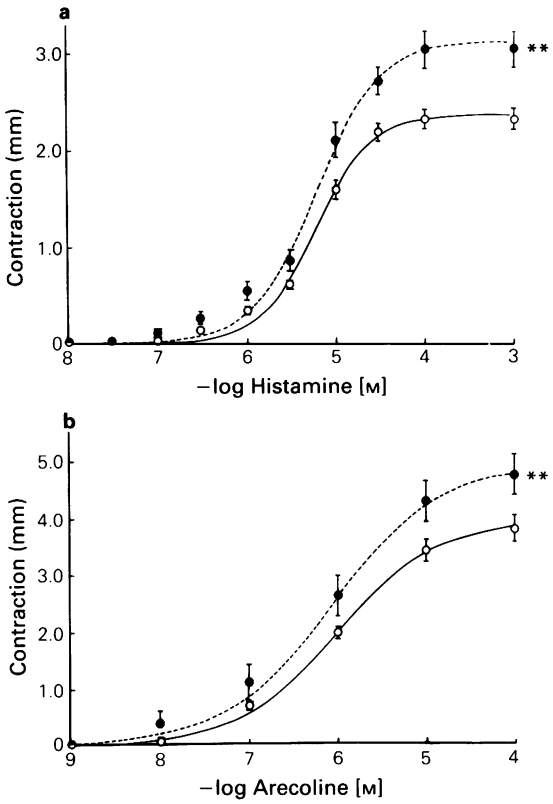

Figure 1 Mean concentration-response curve for histamine (a) and arecoline (b) on isolated tracheal spirals from unoperated guinea-pigs treated intraperitoneally with saline (○) or endotoxin (*E. coli* O_{111}:B_4, 1 mg kg^{-1}, ●) four days prior to the experiment. The responses are shown as mean with s.e. mean indicated by vertical lines. See Table 1 for the parameters: maximum response, EC_{50} and slope factor of these curves and for the number of experiments (n).
** $P < 0.01$ as compared to the curve from saline-treated unoperated guinea-pigs and determined with ANOVA.

The mean concentration-response curves of the tracheal spirals from sham-operated saline-pretreated guinea-pigs (Figure 2) showed a diminished responsiveness ($P < 0.01$, ANOVA) to

Table 1 The mean parameters of the histamine and arecoline concentration-response curves on the guinea-pig isolated tracheal spirals

Drugs	*Treatment*	n	*Maximum response* (mm)	*Slope factor*	$[EC_{50}]$ [μM]
Histamine	Unoperated control	6	2.40 ± 0.10	1.36 ± 0.05	6.00 ± 0.56
	Unoperated endotoxin	6	3.16 ± 0.21**	1.22 ± 0.10	6.00 ± 0.57
	Sham control	10	2.05 ± 0.15	1.36 ± 0.17	14.15 ± 0.99†
	Sham endotoxin	9	2.80 ± 0.21**	1.40 ± 0.21	13.25 ± 1.85
	Lesion control	8	2.52 ± 0.17	0.94 ± 0.09	11.35 ± 2.06*
	Lesion endotoxin	7	2.65 ± 0.31	0.95 ± 0.12	8.64 ± 1.36
Arecoline	Unoperated control	6	3.94 ± 0.25	0.77 ± 0.03	1.00 ± 0.16
	Unoperated endotoxin	6	4.93 ± 0.39	0.71 ± 0.05	0.89 ± 0.18
	Sham control	8	3.44 ± 0.15	0.75 ± 0.03	0.84 ± 0.07
	Sham endotoxin	7	4.08 ± 0.26	0.82 ± 0.03	0.52 ± 0.06**
	Lesion control	8	3.86 ± 0.35	0.91 ± 0.06	0.66 ± 0.14
	Lesion endotoxin	6	3.95 ± 0.38	0.90 ± 0.03	0.68 ± 0.09

The parameters were calculated by a computerized analysis of the individual curves based on the four parameter logistic equation. Results are expressed as mean ± s.e. mean.
** $P < 0.01$ as compared to the control value of the same group. * $P < 0.05$ and † $P < 0.01$ as compared to the unoperated control value.

histamine and arecoline as compared to those from unoperated saline-pretreated animals. The decreases in maximal contractions of the tracheal spirals from sham-operated versus unoperated saline pretreated animals to histamine and arecoline did not reach the level of significance ($0.05 < P < 0.1$ and $0.05 < P < 0.2$, respectively, Table 1). The sensitivity of the histamine concentration-response curve was significantly ($P < 0.01$) decreased in the sham-operated versus unoperated saline-pretreated animals. No differences in the slope factors of the concentration-response curves and the EC_{50} values for arecoline were observed between sham-operated and unoperated saline-pretreated animals.

Sham-operated and unoperated guinea-pigs responded similarly to endotoxin administration in that the tracheal spirals showed an increased responsiveness ($P < 0.01$, ANOVA) to histamine and arecoline in the endotoxin-pretreated group (Figure 2). Additionally, the sensitivity to arecoline was slightly increased in the endotoxin-pretreated group (Table 1). No differences in slope factors were observed between the saline-pretreated and endotoxin-pretreated sham-operated animals (Table 1).

The tracheal spirals from AHA-lesioned saline-pretreated guinea-pigs and from unoperated saline-pretreated animals showed similar responsiveness to histamine and arecoline, except for the sensitivity to histamine which was still significantly decreased ($P < 0.05$, Table 1). In the animals with AHA-lesions endotoxin was no longer capable of inducing hyperreactivity of the tracheae *in vitro* to histamine and arecoline (Figure 3).

Figure 4 shows two consecutive sections of the area in which the effective bilateral lesions were made. Animals with lesions outside the AHA were excluded from the results. The size of the lesioned tissue varied from 0.5 to 1.0 mm diameter.

In vivo *hyperreactivity*

Four days after endotoxin administration to unoperated guinea-pigs, intravenous injection of histamine and methacholine caused a larger increase in lung resistance, ΔR_1, than in saline-pretreated controls (Figure 5). The endotoxin-induced potentiation of the ΔR_1 after the administration of histamine and methacholine was larger at the lowest doses of these drugs, suggesting that a leftward shift of the dose-response relationship has to be considered. The estimated ED_{50} values decreased from approximately 5.5 to 3.3 μg histamine per 100 g and from 1.2 to 0.9 μg methacholine per 100 g. No hyperreactivity was observed regarding ΔC_{dyn} (results not shown). The basal values of R_1 were not different between both groups (Table 2).

In sham-operated saline-pretreated guinea-pigs (Figure 6) a reduced ΔR_1 after methacholine and histamine administration occurred as compared to unoperated saline-pretreated controls. In endotoxin-pretreated sham-operated animals the increase in R_1 in response to histamine or methacholine injection was also significantly potentiated as compared to the saline-pretreated sham-operated group (Figure 6). In sham-operated animals the ΔR_1 to histamine and methacholine did not reach a plateau at the highest

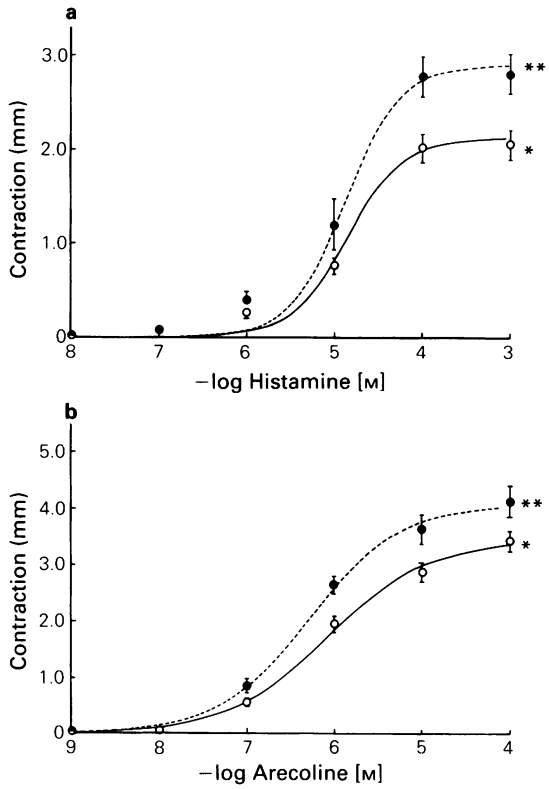

Figure 2 Mean concentration-response curve for histamine (a) and arecoline (b) on isolated tracheal spirals from sham-operated guinea-pigs treated intraperitoneally with saline (○) or endotoxin (*E. coli* O_{111}:B_4, 1 mg kg^{-1}, ●) four days prior to the experiment. The responses are shown as mean with s.e. mean indicated by vertical lines. See Table 1 for the parameters: maximum response, EC_{50} and slope factor of these curves and for the number of experiments (*n*).
** $P < 0.01$ as compared to the curve from saline-treated sham-operated guinea-pigs and determined with ANOVA. * $P < 0.01$ as compared to the curve from saline-treated unoperated guinea-pigs.

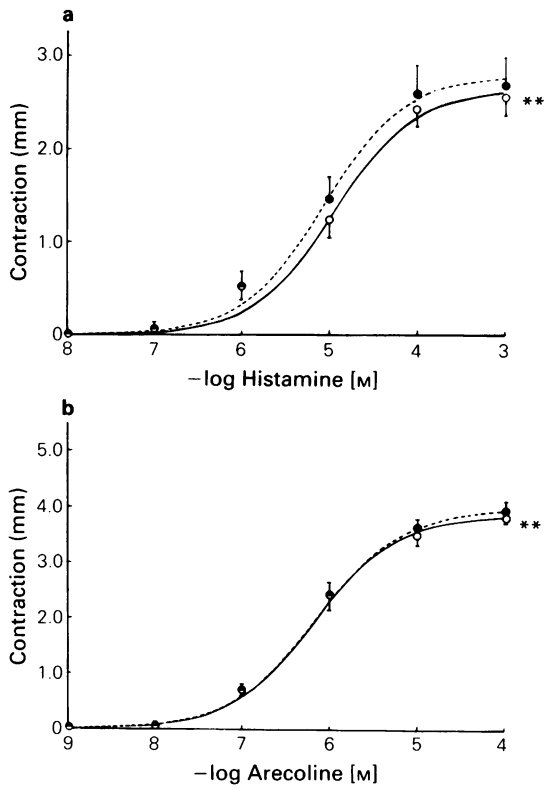

Figure 3 Mean concentration-response curve for histamine (a) and arecoline (b) on isolated tracheal spirals from AHA-lesioned guinea-pigs treated intraperitoneally with saline (○) or endotoxin (*E. coli* O_{111}:B_4, 1 mg kg^{-1}, ●) four days prior to the experiment. The responses are shown as mean with s.e. mean indicated by vertical lines. See Table 1 for the parameters: maximum response, EC_{50} and slope factor of these curves and for the number of experiments (*n*).
** $P < 0.01$ as compared to the curve from saline-treated sham-operated guinea-pigs.

Table 2 The basal values of the computed lung function parameter lung resistance (R_1) in spontaneously breathing anaesthetized guinea-pigs

Treatment	n	R_1 ($cmH_2O\,ml^{-1}\,s^{-1}$)
Unoperated control	6	0.071 ± 0.019
Unoperated endotoxin	6	0.098 ± 0.038
Sham control	7	0.072 ± 0.020
Sham endotoxin	7	0.048 ± 0.007
Lesion control	6	0.100 ± 0.030
Lesion endotoxin	6	0.054 ± 0.052

Results are expressed as mean ± s.e. mean.

doses tested of the drugs, therefore no accurate value for the ED_{50} can be derived. The decrease in C_{dyn} after histamine or methacholine injection was not affected in endotoxin-pretreated versus saline-pretreated sham-operated animals (results not shown). The basal values of R_1 were not different between the two sham-operated groups (Table 2).

The ΔR_1 after histamine administration to AHA-lesioned saline-pretreated guinea-pigs was almost identical to the responsiveness of unoperated saline-pretreated animals, whereas the ΔR_1 to methacholine showed values between those from unoperated and sham-operated saline-pretreated animals (Figure 7). In the animal group with AHA-lesions, no *in vivo* hyperreactivity to histamine and

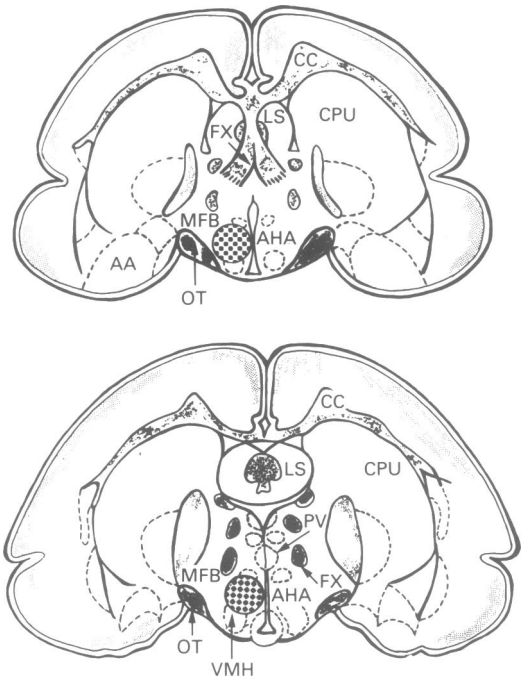

Figure 4 Consecutive coronal sections showing the area (checkered) in which the effective bilateral lesions in the anterior hypothalamic area (AHA) were made. The lesion size varied from 0.5 to 1.0 mm diameter. CC, corpus callosum; LS, nucleus lateralis septi; FX, fornix; CPU, nucleus caudatus putamen; MFB, median forebrain bundle; OT, tractus opticus; AA, anterior amygdaloid area; PV, nucleus paraventricularis; VMH, nucleus ventromedialis hypothalami.

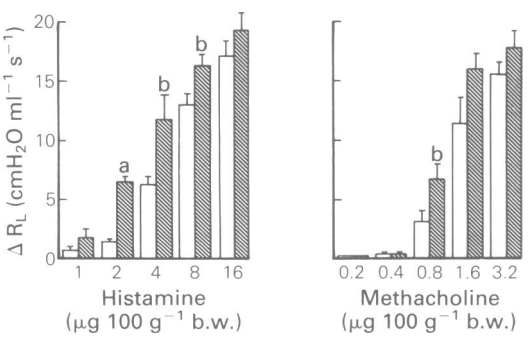

Figure 5 Changes in lung resistance (ΔR_1) in unoperated guinea-pigs treated intraperitoneally with saline (open columns, $n = 6$) or endotoxin (*E. coli* $O_{111}:B_4$, 1 mg kg^{-1}) four days prior to the experiment (shaded columns, $n = 6$) in response to histamine and methacholine. Values are presented as mean with s.e. mean indicated by vertical lines.
[a] $P < 0.01$ and [b] $P < 0.05$ as compared to the response of saline-treated unoperated guinea-pigs and determined with Student's t test.

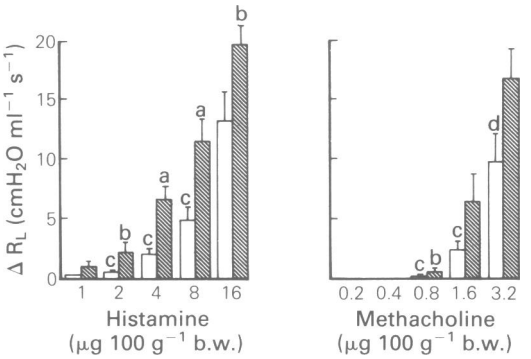

Figure 6 Changes in lung resistance (ΔR_1) in sham-operated guinea-pigs treated intraperitoneally with saline (open column, $n = 7$) or endotoxin (*E. coli* $O_{111}:B_4$, 1 mg kg^{-1}) four days prior to the experiment (shaded columns, $n = 7$) in response to histamine and methacholine. Values are presented as mean with s.e. mean indicated by vertical lines.
[a] $P < 0.01$ and [b] $P < 0.05$ as compared to the response of saline-treated sham-operated guinea-pigs and determined with Student's t test. [c] $P < 0.01$ and [d] $P < 0.05$ as compared to the response of saline-treated unoperated guinea-pigs.

methacholine could be observed four days after endotoxin administration (Figure 7). The basal values of R_1 in the lesioned guinea-pigs were not different from other groups (Table 2).

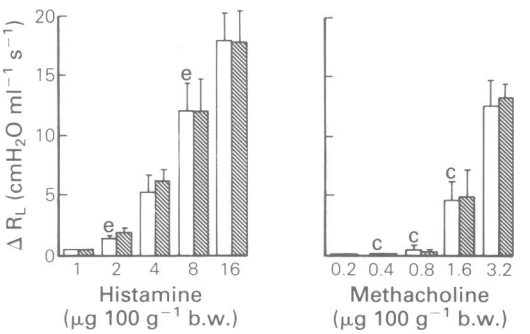

Figure 7 Changes in lung resistance (ΔR_1) in AHA-lesioned guinea-pigs treated intraperitoneally with saline (open columns, $n = 6$) or endotoxin (*E. coli* $O_{111}:B_4$, 1 mg kg^{-1}) four days prior to the experiment (shaded columns, $n = 6$) in response to histamine and methacholine. Values are presented as mean with s.e. mean indicated by vertical lines.
[c] $P < 0.01$ as compared to the response of saline-treated unoperated guinea-pigs and determined with Student's t test. [e] $P < 0.05$ as compared to the response of saline-treated sham-operated guinea-pigs.

Discussion

In the present study, we have attempted to investigate the effect of AHA-lesions on the development of non-specific respiratory airway hyperreactivity after endotoxin administration to guinea-pigs. In evaluating the data, it has to be considered that the sham-operations diminished the responsiveness of the airways to histamine receptor and cholinoceptor-muscarinic stimulation *in vitro* and *in vivo* as compared to unoperated animals. An elevation of plasma glucocorticoid level due to operation stress might offer an explanation for this diminished reactivity. The partial restoration of the responsiveness by AHA lesions might be due to destruction of the hypothalamus-pituitary-adrenal axis. However, the operations were carried out 14 days before the experiment which makes a stress-effect less likely. Another explanation might be that in sham-operated animals the electrode has damaged structures which result in neuroendocrine alterations. These suggestions are strengthened by recent results of an identical experiment which showed that plasma cortisol levels in sham-operated saline-pretreated guinea-pigs were higher than the levels in AHA-lesioned saline-pretreated animals (unpublished results).

Four days after intraperitoneal endotoxin administration to unoperated or sham-operated guinea-pigs, hyperreactivity of the tracheal spirals to histamine and arecoline developed. The increase in R_1 *in vivo* after intravenous histamine and methacholine injection was also larger in endotoxin-pretreated unoperated and sham-operated guinea-pigs. Since the changes in dynamic lung compliance to histamine and methacholine were not potentiated after endotoxin pretreatment, it can be concluded that the airway hyperreactivity was limited to an effect in the major conducting airways. The endotoxin-induced potentiation of the ΔR_1 after the administration of histamine and methacholine to unoperated guinea-pigs was larger at the lowest doses of these drugs, suggesting that leftward shifts of the dose-response relationships are occurring. However, endotoxin pretreatment of unoperated guinea-pigs only increased the maximal responses and not the sensitivities of the tracheal spirals *in vitro* as indicated by the concentration-response curves to histamine and arecoline. Since the maximal increase in R_1 *in vivo* might be limited by other factors than smooth muscle reactivity *per se*, such as the elasticity of the cartilage rings and circulating adrenaline, the leftward shifts of the dose-response curves *in vivo* might be due to increased reactivities of smooth muscle to histamine and methacholine.

Since it has been demonstrated that endotoxin administration affects the β-adrenoceptor system of the respiratory airways in the guinea-pig (Schreurs *et al.*, 1983), the non-specific airway hyperreactivity *in vivo* could, at least partially, be secondary to this β-adrenoceptor down-regulation. A β-adrenoceptor down-regulation, however, cannot explain the *in vitro* hyperreactivity of tracheal smooth muscle after endotoxin treatment since there is no valid evidence for an inverse relation with the β-adrenoceptor function. It has been shown that contraction of tracheal airway smooth muscle to both histamine receptor and cholinoceptor-muscarinic agonists is mediated by stimulation of the inositol-phosphate-cycle and concomitant elevation of intracellular calcium which increases the activity of myosin light chain-kinase (Grandordy *et al.*, 1986; Barnes *et al.*, 1986). A direct or indirect potentiating effect of endotoxin on these post-receptor signal transduction mechanisms has to be taken into account. If endotoxin itself would affect one of these mechanisms, an immediate influence on contractility could be expected. Although it has been shown that endotoxin induces airway hyperreactivity within a few hours after i.v. administration to sheep (Hutchinson *et al.*, 1983) and inhalation in some rat strains, in another rat strain no hyperreactivity occurred (Pauwels *et al.*, 1986). Also in guinea-pigs no airway hyperreactivity *in vivo* and *in vitro* could be observed at 4 and 24 h after endotoxin inhalation (Folkerts *et al.*, 1988). Thus, a direct effect of endotoxin on post-receptor mechanisms seems unlikely. In the present study, the airway hyperreactivity was demonstrated four days after intraperitoneal endotoxin administration. Therefore, a more likely explanation of the non-specific airway hyperreactivity might be an endotoxin-elicited factor which affects the stimulus-response coupling. Since endotoxin is a well-known stimulus for the immune-system (see Morrison & Ryan, 1979) this factor might be derived from immunocytes. Interestingly, it has been shown that immunization which induces the production of IgE antibodies coincided with an increased cholinergic-muscarinic- (Patterson & Harris, 1985; Halonen, 1984) and histaminergic reactivity (Antonissen *et al.*, 1980) in respiratory smooth muscle of laboratory animals. Thus, induction of hyperreactivity of the respiratory airways is not specific for endotoxin but might be a general phenomenon coinciding with a particular stimulation of the immune system.

In AHA-lesioned guinea-pigs, endotoxin could not induce airway hyperreactivity *in vivo* and *in vitro*. Several possible explanations can be postulated for the absence of airway hyperreactivity. Firstly, endotoxin, either directly or through an endotoxin-induced factor, might activate the AHA which leads to the increased airway reactivity *in vivo* and *in vitro*. In fact, interleukin-1, which can be synthesized by macrophages after endotoxin administration, activates the preoptic-anterior hypothalamic area

resulting in fever and the hepatic synthesis of acute-phase proteins (Blatteis *et al.*, 1984). Moreover, endotoxin administration stimulates release of hormones from the brain such as thyrotropin (Kasting & Martin, 1965) which elevates thyroxine release from the thyroid gland. It has been shown that thyroxine potentiates anaphylactic reactions (Filipp & Mess, 1969). The activation of the AHA after endotoxin administration would be abrogated by its lesioning and consequently this prevents the induction of airway hyperreactivity. Secondly, the protection against the development of airway hyperreactivity might be related to the immunosuppressive nature of these lesions. It has been shown that AHA-lesions suppress several immunobiological parameters such as anaphylactic reactions, antibody production, delayed type hypersensitivity reactions and lymphocyte proliferation (see Stein *et al.*, 1981) and endotoxin activates immunocytes (see Morrison & Ryan, 1979). Recently it was suggested that AHA-lesions prevented the induction of hyperreactivity of tracheal spirals to histamine after immunization of guinea-pigs with ovalbumin (Van Oosterhout & Nijkamp, 1986), which supports the latter hypothesis. However, more studies are needed to unravel a putative role for the AHA in the development of airway hyperreactivity.

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