

Antigen-induced dyspnea in Sprague Dawley rats

Effects of local treatment with anti-asthmatic drugs

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*We wanted to study the effect of anti-asthma drugs on antigen-induced dyspnea in conscious Sprague Dawley (S.D.) rats. A line of dyspnea bred rats was produced, where all immunized animals respond with dyspnea when challenged with aerosolized ovalbumin (OA). The animals were immunized intraperitoneally (i.p.) with OA (10 µg) together with Al(OH)₃ (100 mg) and challenged 2 to 3 weeks later with OA aerosol. We examined the effects of terbutaline (TERB), disodium cromoglycate (DSCG), atropine (ATRO), theophylline (THEO), a 5-HT receptor antagonist methysergide (METH), and two glucocorticosteroids (GCS) budesonide (BUD) and dexamethasone (DEX), on this response. The drugs were given locally either by intratracheal (i.t.) instillation or by aerosol. The rats were placed one by one in an air tight box and the breathing pattern was recorded. Parameters such as time of onset of dyspnea after end of challenge (elapsed time), duration and occurrence of dyspnea, were used to quantify the response. In the vehicle-treated group nearly all animals responded to OA with signs of dyspnea. TERB was the only drug effective when given as an aerosol, whereas when given i.t., also DEX and METH inhibited the dyspnea. Dyspnea is a serious response and can only be alleviated by the most effective anti-asthma drugs.

Key words: aerosol; anti-asthma drugs; dyspnea; S.D. rats; intratracheally; ovalbumin; sensitized.

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Actively sensitized rats have often been used in experimental models of asthma (8, 9, 21, 22, 23). Most animals are immunized either by the intraperitoneal or the subcutaneous route, both of which appear effective. The natural way to be sensitized is by inhalation. Rats exposed to aerosolized antigen, either in combination with or without an adjuvant, showed an increased IgE antibody production (1, 21). Ahlstedt et al. (1) were also able to record a bronchial response in rats challenged with aerosolized antigen. Brown Norway rats, repeatedly exposed to antigen, showed increased levels of specific IgG serum antibodies but the IgE antibody response was transient (21). This study also provided

evidence that IgE isotype specific suppressor T cells control IgE responses to inhaled antigen in specific regions of the lung. This may explain the difficulties in sensitizing a rat by the inhaled route. Rats immunized either i.p. and/or s.c. responded with respiratory symptoms, such as apnea, dyspnea or with expiratory notching (7, 20).

We immunized outbred S.D. rats i.p. with OA together with alum and challenged them 2 to 3 weeks later with OA aerosol. To obtain a uniform group that all responded with dyspnea, a line of rats was produced by selective brother and sister matings in a way similar to that described by Holme & Piechuta (15).

Four different types of drugs are used clinically in the treatment of asthma (3). Intal (sodi-

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um cromoglycate) is used as a prophylactic drug given by repeated inhalation over long periods, often to children. Beta₂-agonists (e.g. terbutaline) are used in the treatment of mild to severe asthma. Glucocorticosteroids (e.g. budesonide) and xanthine derivatives (e.g. theophylline) are used in mild to severe asthma. Our aims in the present study were, firstly, to determine how effectively anti-asthma drugs prevented dyspnea, and secondly, to compare the hyperreactivity to 5-hydroxytryptamine (5-HT), acetylcholine (ACh) and methacholine (MCh) in sensitized and unsensitized outbred and dyspnea bred S.D. rats.

MATERIAL AND METHODS

Animals and immunization

Outbred female and male Sprague Dawley rats ($n = 24$) of specific pathogen free status (SPF) at a mean body weight of 165 g and 200 g, respectively, were obtained from Møllegaards Breeding Centre Ltd., Skensved, Denmark and reared under standard animal housing conditions. Five days after arrival at our laboratory, the animals were immunized with an i.p. injection of 10 µg OA (Sigma, grade III, crystallized and lyophilised) in 0.5 ml saline mixed with 100 mg Al(OH)₃ (F 2200, Reheis Chemical Company, USA) (9). This mixture was prepared 1 h before immunization.

Test

Two to three weeks after immunization each rat was sealed in an airtight tube (plethysmograph) and only the head protruded through a rubber collar into an inhalation chamber (made of perspex 73 × 10 × 9 cm). A pressure transducer (Statham P23 ID) was connected to the plethysmograph and the signal was amplified and traced on a Grass Polygraph 7D. Normal breathing was first recorded for 1 min. Six animals were exposed and recorded simultaneously. The animals were then challenged with a solution containing 3 g OA dissolved in 100 ml

0.9% (w/w) saline which also contained 100 µl of a wetting agent, dimethicone (Minifom, Tika, Sweden), to prevent foaming. The aerosol was generated by a Bird 890 nebulizer at a pressure of 0.3 bar. Changes in respiratory breathing pattern such as apnea and dyspnea were measured for up to 15 min. OA aerosol-challenged rats showing severe respiratory symptoms were removed from the restraining tubes and put back in their cages to recover. Eight of 24 outbred male and female rats (33%) responded with dyspnea, (4 males and 4 females).

Breeding

Four pairs of brothers and sisters (F0) were put together to breed the first generation (F1). The occurrence of dyspnea among the rats in F1 generation increased to 40%. New pairs showing dyspnea were put together producing a second generation (F2). When generation F4 was tested almost 100% of the animals responded with dyspnea after OA aerosol challenge. This line continued at our laboratory until the fifth generation (F5) and was then transferred to Møllegaards Breeding Centre. The present study is based on rats from the 8th generation (F8). All animals from that generation responded with dyspnea, if properly immunized, when challenged with OA aerosol.

Respiratory recordings in conscious animals

The plethysmograph used for early testing had a rubber collar that could strangle the animals at dyspnea. A new exposure and plethysmograph box (25 × 13 × 15 cm) was built to let the animals move more freely and to minimize stress (see Fig. 1). Each animal was challenged once by either an antigen aerosol in the range of 1–30 mg OA/ml or a bronchoconstrictor aerosol of either 5-HT, ACh or MCh, 2 to 3 weeks after immunization. The aerosol was generated from a MA2 nebulizer (Viasol, Malmö), at a flow rate of 4.5 l/min. The size distribution (MMD, Master-sizer, Malvern Instruments, Malvern,

UK) of the nebulized drug solution was $2.4\ \mu\text{m}$ with a geometric standard deviation of 1.9. For further output characteristics see Dahlbäck (12). The aerosol was led via a silicone tube to the box and evenly distributed through a grid at the top of the chamber. An outlet exhaust hole was at one side of the box. A pneumotachograph (Fleish 0000) was connected to a pressure transducer (Validyne MP-45, $2.5\ \text{cmH}_2\text{O}$) and the flow signal was recorded and amplified on a Grass polygraph 7D which also recorded the breathing pattern. All respiratory monitoring was done in conscious animals. Two rats could be observed at the same time; placed in two separate inhalation chambers and with separate recordings. Breathing was first recorded after sham or saline exposure. One minute after OA challenge the boxes were cleared of aerosol and sealed so that the respiratory pattern could be recorded. The elapsed time ($\blacktriangle\text{EL}$), defined as the time between end of challenge to start of dyspnea was measured and the duration of dyspnea ($\blacktriangle\text{DUR}$) was calculated. The frequency of dyspnea for each group and the ratio $\blacktriangle\text{EL}/\blacktriangle\text{DUR}$ was also calculated. When symptoms were severe the ratio $\blacktriangle\text{EL}/\blacktriangle\text{DUR}$ was small (< 1), whereas mild symptoms corresponded to a ratio above 1. None of the animals challenged with aerosolized saline and non-immunized animals challenged with aerosolized OA showed any airway responses (dyspnea). Animals challenged with the highest dose of OA without showing signs of dyspnea, were killed, the abdo-

men opened, and signs of encapsulated $\text{Al}(\text{OH})_3$ in the visceral connective tissue were sought. Animals not responding to OA aerosol challenge and not showing $\text{Al}(\text{OH})_3$ in abdomen ($< 2\%$ failed to be immunized) were excluded from the study.

Respiratory recordings in anesthetized animals

The method for respiratory measurements has been described earlier by Stotland & Share (22) and Dahlbäck (9) and is therefore only briefly described here. Immunized animals were anesthetized and the trachea cannulated and connected to a respiratory pump with a constant volume. The intratracheal pressure was measured with a pressure transducer connected to a side arm of the cannula. All recordings were made on a Grass polygraph 7D. The drugs were given intravenously (i.v.) through the jugular vein.

Drug pretreatment

The animals were pretreated locally either by i.t. instillation ($0.5\ \text{ml/kg}$) or by aerosol (MA2) with anti-asthma drugs or vehicle at 30 min, 4 or 18–24 h before challenge. When the animals were pretreated with aerosol, they were restrained in polycarbonate holders connected to an inhalation chamber (Battelle, (6)) and exposed during a period of 10 min. After aerosol pretreatment the animals were returned to their cages to settle. The dose deposited in the rat after aerosol treatment has been calculated previously for some of the tested drugs and since animals in this study were treated with comparable doses a similar amount was expected to be deposited (11). The sources of drugs were: Apoteksbolaget, Sweden (atropine sulphate (ATRO), disodium cromoglycate (DSCG), Lomudal); Sandoz, Switzerland (methysergide hydrogen maleate (METH); Sigma (acetylcholine (ACh), 5-hydroxy-tryptamine (5-HT), methacholine (MCh); Draco, Sweden, (terbutaline (TERB), budesonide (BUD), 3,7-dihydro-1, 8-dimethyl-3-phenyl-1H-purine-2,6-dione,

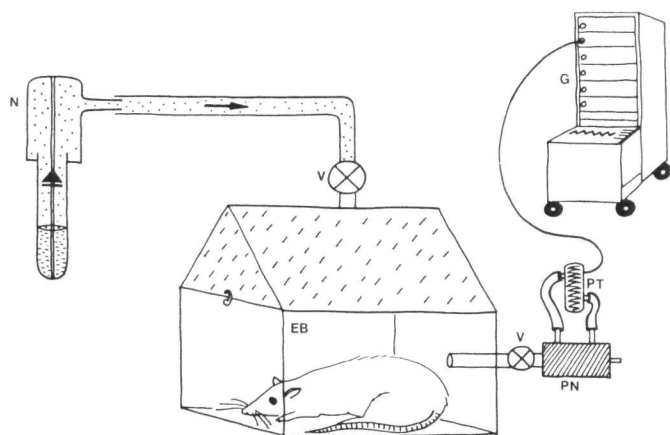


Fig. 1. A schematic drawing of the exposure box and breathing recording system. N, nebulizer; V, valve; EB, exposure and breathing recording box; PN, pneumotachograph; PT, pressure transducer; G, Grass polygraph.

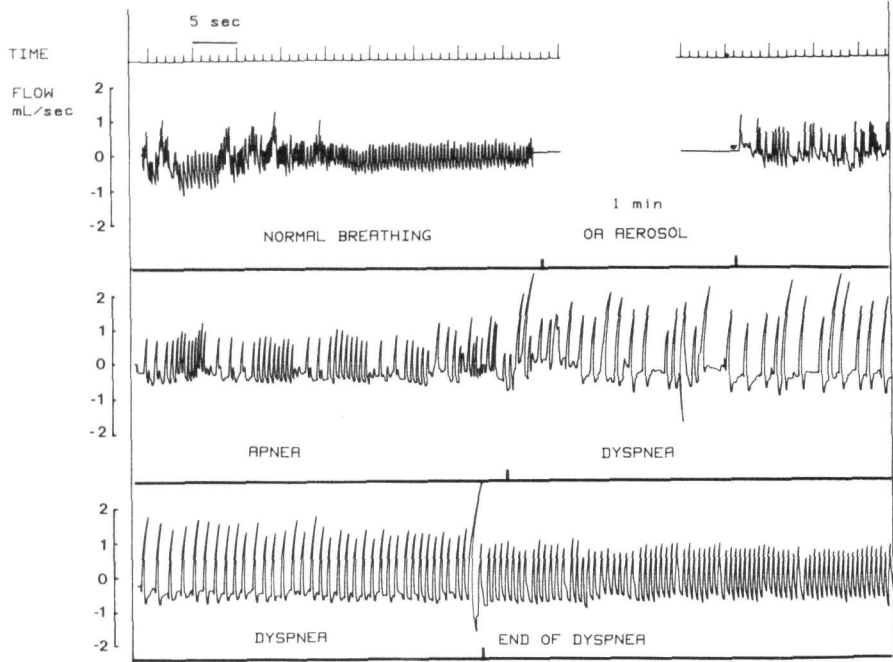


Fig. 2. The breathing pattern of an OA (10 µg) and Al(OH)₃ (100 mg) sensitized dyspnea bred rat, challenged with OA (5 mg/ml) aerosol showing symptoms of apnea and dyspnea.

(D4026)); Knoll, West Germany (anhydrous theophylline (THEO)) and Lark, S.P.A., Milano, Italy (dexamethasone (DEX)). Aerosolized THEO and D4026 were dissolved in equimolar quantities of 0.5 mol/l NaOH/saline and further diluted in saline. THEO, D4026, BUD and DEX were suspended in a kenacort vehicle (0.04 % Tween80, 0.65 % NaCl and 75 % carboxymethyl cellulose in water) when given i.t. BUD and DEX were dissolved in 95 % ethanol when nebulized. All other drugs were dissolved and diluted in saline. The vehicles themselves did not influence the response to antigen.

Statistics

In tables, mean and SEM values are given for ▲EL and ▲DUR. The ratio ▲EL/▲DUR is

calculated individually and mean values are shown for each group. The mean value of per cent increase in intratracheal pressure was calculated for each group. The differences in inhibition as well as increase in intratracheal pressure between drug-treated and vehicle-treated groups or between drug-treated groups were assessed by unpaired Student's t-test or chi-square analysis.

RESULTS

Characteristics of the response

Immediately after OA challenge, episodes of shallow breathing were noticed which were referred to as apnea (Fig.2). Apnea could also

Table 1
Effects of aerosolized OA in conscious immunized S.D. rats

Nebulized OA-solution (mg/ml)	Occurrence of dyspnea		▲EL (sec)	▲DUR (sec)	Ratio ▲EL/▲DUR
	n	%			
1.0	3/6	50	204±36	186±30	1.10
2.5	5/6	83	192±12	294±78	0.65
5.0	11/11	100	168±12	270±36	0.62

been seen in saline-challenged animals and was therefore not further evaluated. Dyspnea occurred only in immunized rats, with symptoms of heavy breathing. The start of dyspnea was characterized by a suddenly marked increase in breathing amplitude (flow) and also a reduction in breathing frequency. The criterion for the end of dyspnea was a return to regular steady breathing at a rate of 90 breaths/min or more. After dyspnea there was an increase in tidal flow, compared with the starting value. The period of dyspnea usually persisted for between 0.5 and 12 min. To establish a challenge dose that produced a reproducible response, a group of immunized rats was exposed to nebulized OA at a dose between 1 and 5 mg OA/ml. This produced dyspnea in 50 % of the animals at 1 mg OA/ml, 83 % at 2.5 mg OA/ml and 100 % at 5 mg OA/ml (Table 1). In some experiments the responsiveness to OA had decreased so the challenge dose had to be raised to 10 mg OA/ml.

Effects of locally administered TERB

TERB was administered i.t. to groups of rats in a dose between 0.05 and 5 mg/kg 30 min before challenge. A dose-related decrease in the occurrence of dyspnea and a significant increase in ratio of ▲EL/▲DUR values were noticed (Table 2). When the time between i.t. adminis-

tration and challenge was increased to 1 h, the occurrence of dyspnea was still low (12 %) but after 2 h it had returned to 88 % or more. The ratio of ▲EL/▲DUR was significantly increased, both at 2 and 3 h (data not shown).

When TERB was administered as an aerosol in doses between 0.1 and 10 mg/ml, the dyspnea decreased significantly in relation to dose (Table 2). The ratio of ▲EL/▲DUR was lower in these animals compared with intratracheally treated, especially in animals treated with 1 mg/ml.

Effects of other locally administered anti-asthma drugs

Anti-asthma drugs such as THEO, a xanthine derivative D4026, ATRO, DSCG, the glucocorticosteroids (GCS) BUD and DEX and a 5-HT receptor antagonist METH were administered either by i.t. instillation or by aerosol. The only drugs that could significantly decrease dyspnea were DEX (10 mg/kg at 18–24 h) and METH (1 and 10 mg/kg) given intratracheally (Table 3). However, THEO, D4026, and BUD produced a significant increase in ratio ▲EL/▲DUR but did not affect the occurrence of dyspnea. These drugs when given by the inhaled route were less effective, except for BUD (18–24 h) which showed a much higher ratio ▲EL/▲DUR than DEX (18–24 h).

Table 2

Intratracheally and aerosolized terbutaline; effects on dyspnea, ▲EL, ▲DUR and ratio ▲EL/▲DUR

Drug	Dose i.t. mg/kg	Occurrence of dyspnea		▲EL (sec)	▲DUR (sec)	Ratio ▲EL/▲DUR
		n	%			
Vehicle	–	77/84	92	185±5	297±16	0.89
TERB	0.05	10/11	91	197±20	250±44	1.06
TERB	0.5	13/30	43*	221±13	195±27	1.54*
TERB	1	14/37	38***	297±13	111±14	3.16***
TERB	5	3/26	12**	248±39	127±31	2.30**
	Aerosol mg/ml					
Vehicle	–	38/44	86	182±7	307±18	0.68
TERB	0.1	13/13	100	214±14	218±21	1.15***
TERB	1	16/24	67	196±10	293±25	0.77
TERB	10	12/26	46***	224±9	218±20	1.14***

*P < 0.05, **P < 0.01, ***P < 0.001.

Table 3

Treatment with anti-asthma drugs administered i.t. or by aerosol; effects on dyspnea, ▲EL, ▲DUR and ratio ▲EL/▲DUR

Drug	Dose mg/kg	Time after treatment	Occurrence of dyspnea		▲EL (sec)	▲DUR (sec)	Ratio ▲EL/▲DUR
			n	%			
Vehicle	–	30 min	27/27	100	178±9	340±38	0.78
THEO	50	30 min	7/7	100	227±10	153±25	1.82**
D4026	10	30 min	6/7	86	206±34	142±26	2.07**
ATRO	10	30 min	11/11	100	217±9	474±81	0.88
DSCG	10	30 min	8/8	100	179±12	250±44	0.84
BUD	10	4 h	12/12	100	268±16	222±32	1.73**
BUD	10	18–24 h	7/8	88	285±19	160±31	2.21***
BUD Ae	15 mg/ml	18–24 h	7/8	88	311±31	143±24	2.94***
DEX	10	4 h	10/11	91	236±11	304±47	1.01
DEX	10	18–24 h	2/9	22***	317	90	3.82***
DEX Ae	15 mg/ml	18–24 h	10/10	100	241±16	188±29	1.76*
METH	0.1	30 min	5/6	86	229±13	195±24	1.24
METH	1	30 min	4/6	67***	258±18	125±31	2.62***
METH	10	30 min	0/5	0			
METH Ae	1 mg/ml	30 min	14/15	93	206±14	169±32	2.72**
METH Ae	10 mg/ml	30 min	11/15	73	179±12	79±23	1.61**

Ae = aerosol, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Effects of aerosolized mediators/bronchoconstrictors on immunized and saline-treated conscious outbred and dyspnea bred rats

Outbred and dyspnea bred rats were treated with either OA or saline and then challenged 2 to 3 weeks later with aerosolized 5-HT, ACh or MCh (Fig. 3). Dyspnea bred rats responded more to 5-HT and ACh, regardless of whether the rat was treated with OA or saline. In contrast, outbred rats reacted more to MCh.

Effects of bronchoconstrictors given i.v. to immunized or saline-treated anesthetized outbred and dyspnea bred rats

When 5-HT, ACh and MCh were given i.v., outbred rats responded significantly more than dyspnea bred rats (Fig. 4). No significant difference in per cent increase in intratracheal pressure was seen between OA-immunized and saline-treated rats, challenged with 50 µg/kg of 5-HT, ACh or MCh i.v. The one exception

was OA-immunized outbred rats which responded more to MCh than saline-treated.

DISCUSSION

In the present study sensitized dyspnea bred rats were pretreated locally with anti-asthma drugs. TERB and DEX were the anti-asthma drugs most effective in preventing dyspnea. We have shown in an earlier study that TERB, D4026, THEO, BUD or DEX administered as aerosols are able to significantly inhibit the bronchial anaphylactic response in anesthetized S.D. rats (11). In other studies DSCG and ATRO administered i.v. inhibited this anaphylaxis, but DSCG given as an aerosol could not prevent anaphylaxis or dyspnea (9, 10, 11). THEO administered by aerosol or i.t. could halve the anaphylactic response independent of dose given (11). Another study using isolated perfused guinea-pig lungs, showed that xanthines administered i.t. cleared rapidly from the lung (17). There is a great difference in the distribution of drugs given by i.t. installation or by aerosol. By i.t. installation an exact dose is distributed unevenly to the lung

lobes and most of the drug is absorbed from the central airways. On the other hand, aerosols deliver a dose to the lung that can only be estimated (20% to the lung and 80% absorbed from the gastrointestinal tract) from deposition studies (13). In the rat more than 90% of the total lung dose is deposited in the peripheral airways. Both

the dose and the site of deposition may explain the difference in results between the two routes of administration.

Holroyde et al. (16) demonstrated in an inbred rat strain that salbutamol and aminophylline were both able to reverse pulmonary airway obstruction when administered i.v.

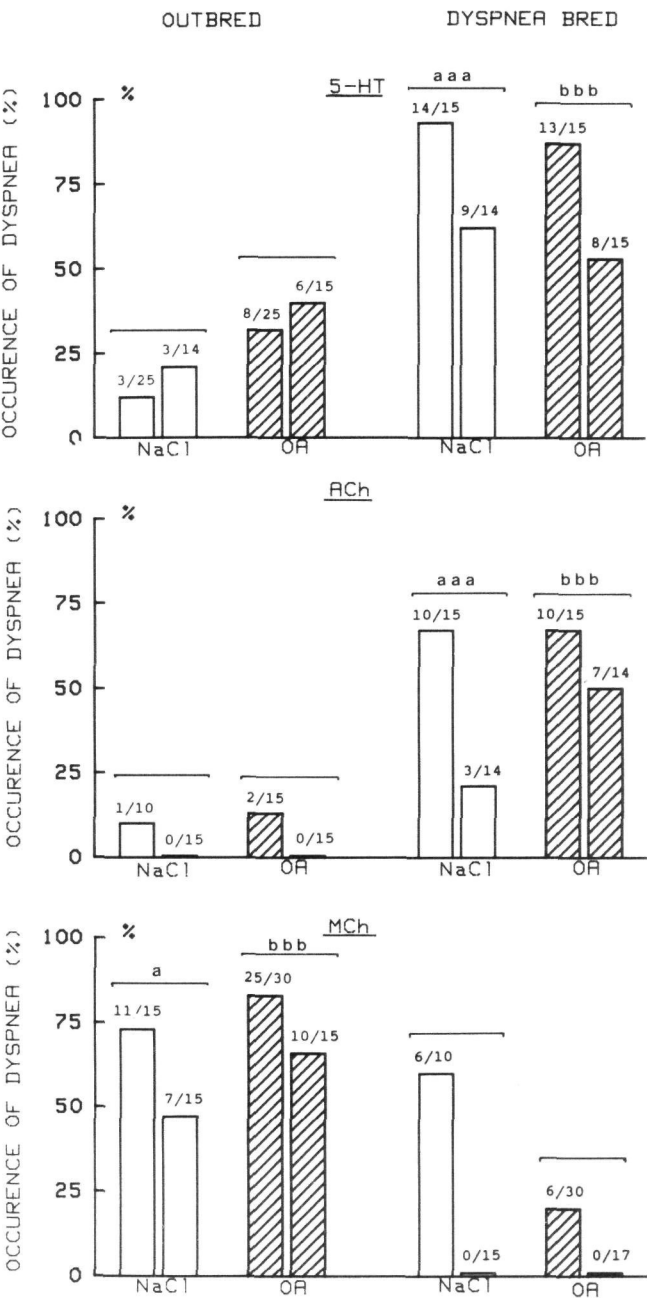


Fig. 3. Outbred and dyspnea bred SD rats treated with either OA or saline were challenged 2 to 3 weeks later with an aerosol (5×10^{-3} M) of either 5-hydroxytryptamine (5-HT), acetylcholine (ACh) or methacholine (MCh). Figures above columns indicate the total number of animals tested and those which responded. Column one and column two indicate the occurrence of dyspnea in per cent, from experiments performed with an old and a new exposure box of similar size; a (saline) and b (OA) indicate the difference between outbred and dyspnea bred animals, respectively.

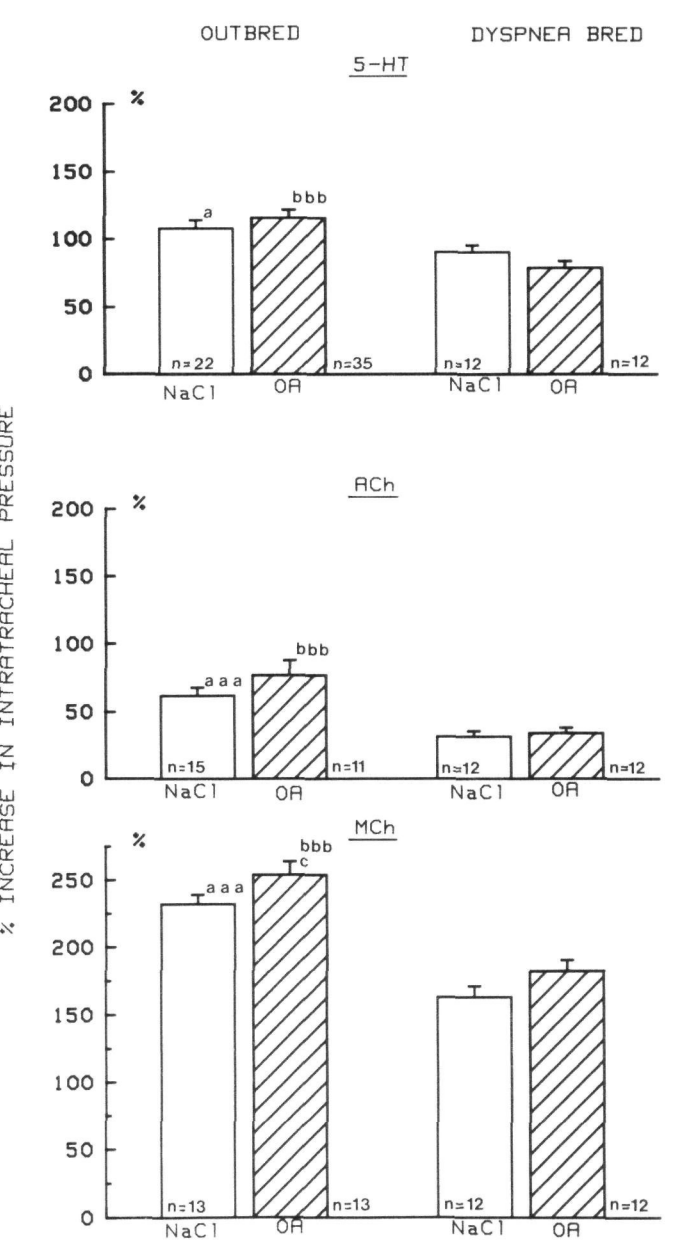


Fig. 4. Outbred and dyspnea bred SD rats treated with either OA or saline were anesthetized and challenged i.v. 2 to 3 weeks later with 50 μ g/kg of either 5-hydroxytryptamine (5-HT), acetylcholine (ACh) or methacholine (MCh). Columns indicate per cent increase in intratracheal pressure after challenge (8, 9); a (saline) and b (OA) indicate the difference between outbred and dyspnea bred rats; c indicates the difference between saline and OA in outbred rats.

either before or after aerosol challenge. Church et al. (8) showed that i.v. DEX and DSCG, but not ATRO, inhibited bronchial anaphylaxis. The main mediator involved in the IgE-mediated anaphylactic response in the rat airways is 5-HT (8, 9). A 5-HT receptor blocker METH administered either by aerosol or i.t. installation was an effective inhibitor of dyspnea. If a sufficient amount of METH was given, dyspnea could be completely prevented (Table 3), which indicates that other mediators may play only a minor role.

What type of pathophysiologic situation does the dyspnea represent? Conscious guinea pigs and rats have both been used previously to study airway irritation and bronchoconstriction induced by of airborne materials or vapors (2). Respiratory pressure or flow changes from an unrestrained animal in a sealed box and/or directly from the nose have been recorded. However, air flow resistance measurements in rodents should be interpreted with caution, because in the rat the upper airways (nose and larynx) contribute with as much as 80% of the total airway resistance and only a minor part represents the lower airways (4). This means that changes in the lower respiratory tract can be obscured by larger changes in the upper airways. We therefore chose to record the breathing pattern instead of doing resistance measurements in conscious animals. When the animals were challenged with antigen or a bronchoconstrictor agent the breathing pattern changed significantly, frequency decreased and the amplitude increased compared with normal breathing. The breathing pattern during dyspnea differs between guinea pigs and rats (20, 24). The guinea pig has two breathing stages (18): the first characterized by an increase in frequency followed by a reduction, and the second by a marked decrease in breathing frequency and an increased amplitude (bronchospasm) and the animal "kneels", showing laboured breathing. In a sensitized rat, challenged with aerosolized OA, a similar change in respiratory pattern was noted. The guinea pig responds more to allergen and mediators than the rat, which usually tolerates much larger provocation doses.

We have a reproducible dyspnea model in rats but it was hard to moderate the dyspnea response by giving lower challenge doses as only some of the animals responded. The challenge dose was increased (10 mg/ml) in some experiments during the summer period. These experiments were repeated when the response was restored, and the same results were found. This slight change in response may be due to seasonal changes in the animals. Apnea usually preceded each episode of dyspnea, and these symptoms could also occur after saline challenge. The occurrence and shape were irregular, which made it hard to quantify. We therefore excluded these symptoms from further analysis. To gain more information from the dyspnea response and not only the occurrence, the $\blacktriangle EL$ and $\blacktriangle DUR$ were calculated. Vehicle-treated animals showed reproducible mean values between 168–185 s for $\blacktriangle EL$, between 270–340 s for $\blacktriangle DUR$ and between 0.62–0.89 for ratio $\blacktriangle EL/\blacktriangle DUR$.

What factors could influence the bronchial hyperactivity in rats? In our study, immunization *per se* did not affect the bronchial hyperreactivity. This was also seen in S.D. rats challenged i.v. with 5-HT or MCh 25 and 56 days after immunization (9). Genetic factors found in different rat strains seem to be of greater importance. Our dyspnea bred rats responded more to aerosolized 5-HT and ACh, whereas outbred S.D. rats showed a higher occurrence of dyspnea when challenged with MCh. Brunet et al. (5) challenged three different strains of rats (inbred and outbred S.D. and Fischer) with 5-HT, MCh or leukotriene D₄ (LTD₄). In *in vivo* tests, inbred rats responded more than outbred S.D. or Fischer rats when challenged with aerosolized bronchoconstrictors. On the other hand, *in vitro* tests demonstrated no difference between lung parenchyma from different rat strains when challenged with MCh, 5-HT or LTD₄, except Fischer rats, which responded less to LTD₄. The potency order between the bronchoconstriction *in vitro* was LTD₄ > MCh > 5-HT. When we administered 5-HT, ACh and MCh i.v. to anesthetized rats, the bronchial response in outbred S.D. rats was more pronounced than

in dyspnea bred rats. MCh produced the most potent bronchial response.

Pauwels et al. (19) also studied the bronchial reactivity in different rat strains challenged i.v. with either 5-HT or carbachol. They found similar responses to the two bronchoconstrictors in all rat strains tested, except for the OM/N strain which responded more to 5-HT. The bronchial reactivity to 5-HT and carbachol differed significantly between inbred strains. This reaction may be genetically dependent and not related to IgE antibody-producing capacity for each strain. They also concluded that challenge by the i.v. route correlated significantly to the inhaled. Di Maria et al. (14) also demonstrated that airway responsiveness to MCh correlated with rat strain *in vivo* but not *in vitro*.

In our animal model, the animals were conscious with intact nerve reflexes and this may lead to a bronchial response which more closely resembles the clinical situation than with anesthetized animals. The hyperreactivity in inbred and outbred S.D. rats to exogenously given mediators was more genetically determined. This was mainly due to a reduced inflammatory state in these rats and therefore the hyperreactivity is of less significance. In the clinical situation different degrees of inflammation persist which indicate an increased hyperreactivity to mediators and cold air.

We conclude that only the most potent anti-asthma drugs (TERB and DEX) at high local concentrations could protect the animals against dyspnea. To obtain a more sensitive model for selection of anti-asthma drugs, our model must be further developed so that the rat will respond with a consistent bronchial response that is milder and can be inhibited by anti-asthma drugs given locally at moderate concentrations.

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