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Impairment of pharmacological modulation of airways smooth muscle by a respiratory pathogen

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Impaired hormonal receptor mechanisms in the autonomic regulation of airways smooth muscle have been proposed as aetiological factors in the pathogenesis of bronchial asthma (Szentivanyi 1968). Certain microorganisms are prevalent in chronic asthmatic/bronchitic subjects (Hirschmann & Everett 1979) and recent clinical observations by Busse (1977) suggest that upper respiratory tract infection may cause decreased granulocyte responsiveness to isoprenaline. These findings have promoted great interest in the possibility of respiratory infection predisposing to the asthmatic state. Experimental evidence compatible with this hypothesis comes from *Bordetella pertussis* vaccinated rats and mice (Szentivanyi 1968) which show β-adrenergic hyporesponsiveness coupled with hyperreactivity to several pharmacological mediators of inflammation; both characteristic of asthma. However, *B. pertussis* infection is not usually observed in bronchial asthma. The human respiratory pathogen, *Haemophilus influenzae*, has been isolated from the deeper airways of asthmatic patients, and experiments by Nijkamp et al (1980) have shown that vaccination of guinea-pigs and rats with this microorganism also results in a β-adrenoreceptor 'lesion', thus providing a useful animal model. Since *H. influenzae* vaccine is not readily obtainable in North America, we investigated the effects of vaccination of small animals with the related bovine respiratory pathogen, *Haemophilus somnus*. This organism has been implicated in the aetiology of pulmonary disease in cattle (Saunders et al 1980). Although β-adrenoreceptor stimulation mediates bronchodilation, other pharmacological mechanisms such as histamine H_2-receptor stimulation have also been reported to relax airways of man (Dunlop & Smith 1977), guinea-pig (Okpako et al 1978) and rat (Eyre & Besner 1979). It is therefore now important to examine any potential animal model of bronchial asthma for possible impairment of H_2-receptors, as well as β-adrenoceptors in airways smooth muscle. This report examines such a possibility of dual receptor impairment of the *H. somnus*-treated rat and guinea-pig.

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Methods

Male, Wistar rats (450–600 g) and Hartley guinea-pigs (450–700 g), each in groups of 6, were vaccinated with 1 ml *Haemophilus somnus* vaccine (all taken from the same batch of Somnugen, Bio-Ceutic Labs. Inc.) i.p. At 3, 5 and 10 days, one group of animals was killed. One group of non-vaccinated rats and guinea-pigs served as controls. Helical strips of trachea were prepared as described previously (Eyre 1973) and mounted in 10 ml overflow organ baths containing oxygenated Krebs-Henseleit solution at 37 °C. Tissue responses were measured with isotonic myographs and trans-

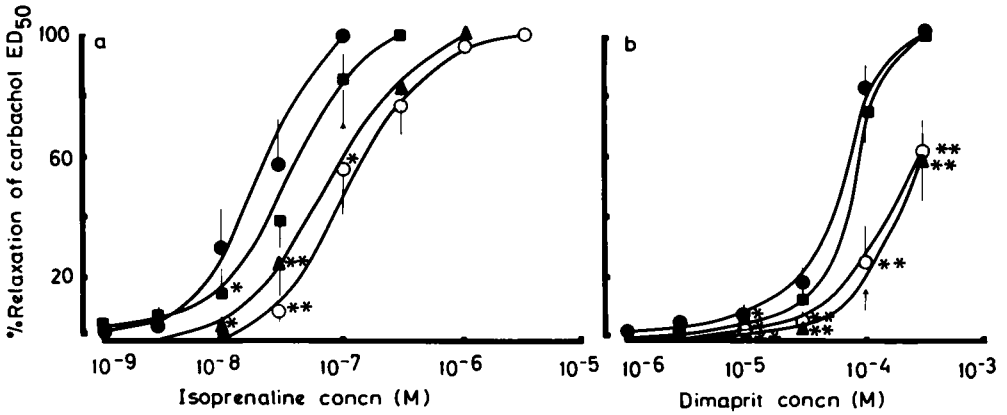

FIG. 1. Effects of *H. somnus* vaccination on log-dose response curves for (a) isoprenaline and (b) dimaprit in guinea-pig trachea of control non-vaccinated (●), 3 day (▲), 5 day (○) and 10 day (■) post-vaccinated animals. Points represent mean ± s.e.m. (n = 6). Values are significantly different from control as indicated: $P < 0.01$ (**); $P < 0.05$ (*), (Student's *t*-test).

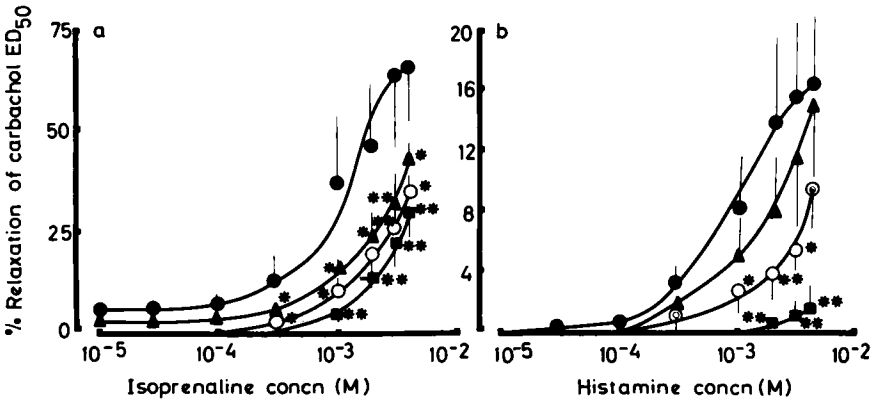

FIG. 2. Effects of *H. somnus* vaccination on log-dose response curves for (a) isoprenaline and (b) histamine in rat trachea of control non-vaccinated (●), 3 day (▲), 5 day (○) and 10 day (■) post-vaccinated animals. Points represent mean ± s.e.m. (n = 6). Values are significantly different from control as indicated: $P < 0.01$ (**); $P < 0.05$ (*), (Student's *t*-test).

ducers and recorded with a Physiograph recorder (Desk Model DMP-4B, Narco Biosystems Co. Inc. Houston, Texas). After a period of 1–2 h for equilibration, doses of agonists (expressed as final molar bath concentration) were added to the bath in a cumulative fashion. Relaxation responses to isoprenaline, histamine or dimaprit (H_2-receptor agonist) were taken from preparations partially contracted (50 ± 10% maximum tissue response) to carbachol. Since rat trachea has previously been shown (Eyre & Besner 1979) to contain only H_2-receptors, histamine was used as the agonist with the preparation, whereas in guinea-pig trachea, where both H_1 and H_2-receptors are present (Okpako et al 1978) it was necessary to use a specific H_2-receptor agonist (dimaprit).

Results

Haemophilus somnus vaccination induced an impairment of relaxation normally observed in rat and guinea-pig trachea to isoprenaline, to histamine (rats) and to dimaprit (guinea-pigs). The action appeared to be time-related, as shown in Figs 1 and 2.

Maximum impairment of relaxation in rats to both spasmolytic agents was observed at 10 days post-vaccination, while in guinea-pigs, a shorter time-period was necessary; 3 days and 5 days for dimaprit and isoprenaline, respectively.

Taken as a percentage of maximum control relaxation, the greatest impairment, at the most effective post-vaccination time studied for each species, occurred with H_2-receptors in rats. The highest spasmolytic agonist concentration used resulted in 15 and 48% relaxation of control values for histamine and isoprenaline, respectively, in rats, versus 61% (dimaprit) and 56% (isoprenaline) in guinea-pigs.

Carbachol concentrations responsible for partial contraction (50 ± 10%) of rat (10^{-6} to 10^{-5} M) and guinea-pig trachea (10^{-8} to 10^{-7} M) were not affected by vaccination.

Discussion

Vaccination of guinea-pigs and rats with *H. somnus* induced a functional impairment of homeostatic responses of the trachea to β-adrenoceptor and histamine H_2-receptor stimulation. This finding supports the work of Nijkamp et al (1980) on β-adrenoceptors, and now extends it to the discovery of a histamine H_2-receptor malfunction. The precise mechanism involved in the impairment of relaxation is unknown, although the possibility of a defect in a post-receptor pathway such as the cAMP system, rather than actual receptor blockade was proposed by Szentivanyi (1968) to explain the β-adrenoceptor 'lesion' observed after *B. pertussis* vaccination. Both β-adrenoceptor and H_2-receptor stimulation are effective through activation of cAMP (Lichtenstein & Gillespie 1975). Schreurs et al (1980) were able to show an impairment of isoprenaline-induced inhibition of anaphylactic histamine release from the lungs (mast cells) of *H. influenzae*-vaccinated guinea-pigs. The immunosuppressant effect of H_2-receptor stimulation (Lichtenstein & Gillespie 1975) was not however examined in that model. The pharmacological changes in the present study are also seen in human granulocytes following incubation in vitro with live, bivalent influenzae virus vaccine (Busse et al 1979).

These findings are consistent with a β-adrenoceptor and histamine H_2-receptor impairment in bronchial hyperreactivity and have therefore prompted us to further evaluate the *H. somnus* vaccinated guinea-pig and rat for their response to clinically effective anti-asthmatic and anti-inflammatory agents.

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Subsensitivity to 5-hydroxytryptamine in agonists occurs in streptozocin-diabetic rats with no change in [^{3}H]-5-HT receptor binding

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Diabetic rats display large decreases in brain tryptophan concentrations, but no significant changes in the concentrations of 5-hydroxytryptamine (5-HT) or its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Curzon & Fernando 1977; MacKenzie & Trulson 1978a,b). This outcome may be attributable, at least in part, to a compensatory increase in tryptophan hydroxylase activity in diabetic animals (Trulson & MacKenzie 1980). Diabetes reduces brain tryptophan by producing large increases in plasma branched chain amino acids which compete with tryptophan for entry into the brain and by decreasing the plasma tryptophan concentration (Clark et al 1968; Bloxam 1972; MacKenzie & Trulson 1978c). As a result of the reduced tryptophan uptake, brain 5-HT and 5-HIAA accumulation is attenuated following systemic tryptophan loading in diabetic rats (MacKenzie & Trulson 1978b). This attenuation is manifested in behavioural subsensitivity to tryptophan after monoamine oxidase inhibition, as assessed with a behavioural syndrome that specifically reflects the activity in central 5-HT-mediated synapses (MacKenzie & Trulson 1978d). Diabetic rats were also subsensitive to *p*-chloroamphetamine, a 5-HT-releasing agent (Gallager & Sanders-Bush 1973; Wong et al 1973; Trulson & Jacobs 1976), even though the endogenous stores of 5-HT are not changed in the diabetic state. To more fully explore this phenomenon, we have examined the behavioural responsiveness of diabetic rats to L-5-hydroxytryptophan, which bypasses the rate-limiting tryptophan hydroxylase step (Gal 1975), 5-methoxy-*NN*-dimethyltryptamine, a direct-acting 5-HT receptor agonist (Fuxe et al 1972; Trulson & Jacobs 1976), and amphetamine, a 5-HT releaser (Sloviter et al 1978) for which the uptake into the brains of diabetic rats has been measured. We also measured specific [^{3}H]-5-HT receptor binding in diabetic animals to determine whether the behavioural subsensitivity to 5-HT agonists is attributable to an alteration in 5-HT receptors.

Behavioural observations were made with rats placed in pairs in round plastic buckets (20 cm high × 35 cm in diameter) with metal screen lids and wood shavings covering the floor. After drug administration, the rats were examined for signs of the behavioural syndrome consisting of resting tremor, rigidity, Straub tail, hindlimb abduction, lateral head weaving and reciprocal forepaw treading (Jacobs 1976). If at least four of these six signs were observed the syndrome was rated as present.

Female Sprague-Dawley rats (250–320 g) were made diabetic by injections of streptozocin (75 mg kg^{-1} i.p.) dissolved in citrate buffer pH 4·5 to 75 mg ml^{-1}. Controls received equivolume injections of buffer alone. Diabetes was verified by polydipsia, polyuria, and glucosuria. In addition, blood glucose concentrations (measured on blood from the tail vein) were determined (using a YSI Model 23A glucose analyzer) on a random sample of 6 diabetic and 4 control rats. Four to six weeks after the injections of streptozocin or buffer, the rats were administered L-5 hydroxytryptophan (5-HTP, 50, 100, 150, 200, 250, 300, 350 or 400 mg kg^{-1}, i.p.), 5-methoxy-*NN*-dimethyltryptamine (5-MeODMT, 0·50, 1·0, 1·5, 2·0, 2·5 or 3·0 mg kg^{-1}, i.p.) or (+)-amphetamine sulphate (10, 20, 40, 60, or 80 mg kg^{-1}, i.p.). The rats were observed for signs of the syndrome for 1 h after drug administration. Estimates of the ED50 for each drug were obtained by probit analysis (Bliss 1952). Differences between control and diabetic groups were

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