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The effect of methacholine on nasal transmucosal potential difference in normal human subjects*

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SUMMARY

This study was proposed to test the hypothesis that the parasympathetic system might attribute to the transmucosal potential difference. In a double-blind, placebo-controlled study six volunteers had nasal transmucosal potential difference (NTPD) recorded at 4-min intervals during 12-min periods of rest, before and after treatment, as well as during exercise and recovery. Application of placebo did not significantly alter NTPD at rest. There was a significant rise during exercise ($p < 0.05$). The application of methacholine significantly increased NTPD at rest ($p < 0.01$); there was a further rise during exercise ($p < 0.02$). We conclude that parasympathetic stimulation can increase the transmucosal potential difference.

Key words: transmucosal potential difference, methacholine

INTRODUCTION

Transmucosal potential difference is caused by the transmembrane movement of ions, controlled by ion channels. Neurotransmitters are known to regulate the activity of ion channels, possibly through regulation of G-proteins (Dunlap et al., 1987). Nasal mucosal epithelium generates an electrical potential difference, the magnitude of which is known to vary in different regions of the nose as has been demonstrated by Knowles et al. (1981a). In cystic fibrosis the nasal transmucosal potential difference (NTPD) is significantly greater, being negative on the mucosal side (Knowles et al., 1981b). Exercise is also known to increase NTPD (Harris et al., 1990). The purpose of this study is to investigate the influence of cholinergic receptors on NTPD both at rest and during exercise.

MATERIAL AND METHODS

Six female volunteers aged between 21 and 29 years (mean age 23.5) were recruited. They had no history of nasal or respiratory diseases, and were free from respiratory infections for at least 6 weeks. All were non-smokers and were not receiving any medication. NTPD measurements were made using an exploring bridge consisting of a silastic Foley catheter size 8 (Simpla®), filled with a 1:1 (v/v) mixture of Ringer's solution and electrocardiograph electrode cream (Siemens®). This resulted in a mixture which ensured contact with the mucosal surface without perfusion of the nasal mucosa, via the aperture at the tip of the catheter. The reference bridge was a Calomel electrode placed over a 3-mm diameter area of lightly abraided skin on the forearm. Readings were made by advancing the tip of the catheter along the floor of the nose, between the inferior turbinate and septum, to the site of maximum stable recorded voltage (PD_{max}), which was usually reached at 3 cm. The catheter was marked at this distance to enable re-insertion to the same place, and readings were taken from this site throughout the study. Recordings from a high-impedance voltage meter were averaged by a computer. Once the site of PD_{max} was established at the beginning of the study, the time taken for each reading – including 5 s for computer averaging – was about 10 s, thus causing minimal interruption of the study. Readings were taken from the right naries in all cases. Subjects in whom there was difficulty placing the catheter due to hypertrophy of the turbinates or septal deflection, or who had difficulty tolerating catheter insertion, causing sneezing or rhinorrhoea, were excluded from the study.

NTPD readings were taken at 4-min intervals during

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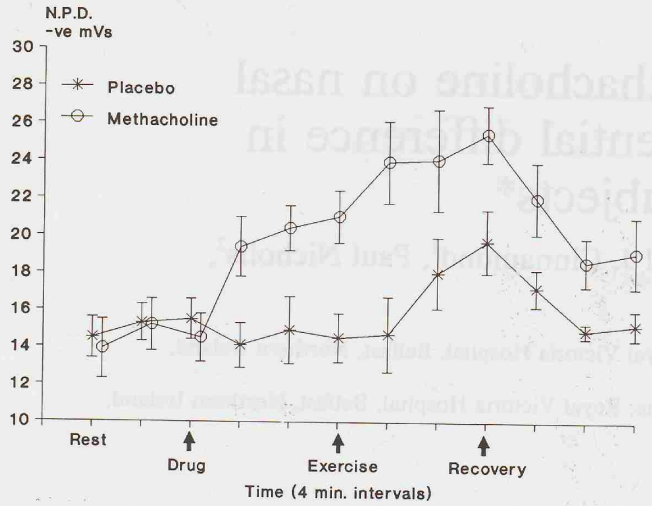

Figure 1. Change in nasal transmucosal potential difference at rest and during exercise, with either placebo or methacholine (error bars indicate SEM).

12 min of rest, before and after treatment, as well as during 12 min of exercise and recovery. The timing of the exercise after application of drug was chosen because preliminary experiments in two subjects demonstrated that the potential difference rise had started to wain by 16 min after drug application.

Each of the six volunteers received in a randomly allocated Latin-square design, double-blind, cross-over study either placebo (0.24 ml of normal saline to each nostril) or methacholine (10 mg in 0.24 ml solution to each nostril). The drugs were supplied by the University Department of Pharmacology in coded containers. The code was broken at the end of the study. The solutions were applied via a pump-action atomizer which delivered 0.12 ml/puff of solution in a mist form. The nozzle was placed past the nasal vestibule and the subject sniffed during delivery of the spray. Exercise was performed on a cycle ergometer, each volunteer achieving a pulse rate of 80% maximum, predicted for age.

The study was approved by the Research Ethical Committee, Faculty of Medicine, The Queens University of Belfast. Statistics were calculated using Student's paired t-test.

RESULTS

The resting values of potential difference on placebo and active drugs were not significantly different ($p>0.05$; Figure 1). Application of placebo did not alter the potential difference ($p>0.05$), but methacholine increased it significantly ($p<0.01$). Exercise significantly increased nasal potential difference in the placebo group ($p<0.05$). The methacholine group showed a further significant rise ($p<0.02$) with exercise, and this peak value was significantly greater from the peak value of the placebo group ($p<0.02$). After exercise both returned to the resting value, neither being significantly different from the pretreatment resting values ($p>0.05$).

DISCUSSION

These results demonstrate that methacholine increases NTPD at rest in normal subjects. A previous study by Boucher et al. (1980) using canine tracheal mucosa had failed to demonstrate an effect of muscarinic stimulation upon transmucosal potential difference. The agonist used in that experiment was acetylcholine which is rapidly hydrolysed by acetylcholinesterase. The different results reported here may be due to intraspecies differences or to the slower metabolism due to a methyl group instead of hydrogen in the methacholine molecule. Muscarinic receptors have been identified in the glandular acini of human nasal mucosa but not on blood vessels (Van Megen et al., 1991). Physiologically, this is confirmed by the profuse nasal secretion caused by the application of methacholine (Borum, 1979). It is possible that the change in potential difference noted here has been related to the increase in secretion either from the glands or the goblet cells lining the mucosa. It is known that exercise increases nasal secretion production (Stanford and Stanford, 1983), transmucosal potential difference (Harris et al., 1990), and sympathetic activity as indicated by nasal decongestion (Richerson and Seebohm, 1967). Muscarinic receptor stimulation is known to regulate transmembrane movement of calcium, potassium and sodium ions, possibly by an action on the G-proteins (North, 1986), but it is not possible in the present experiment to know if the potential difference changes are related to increase of chloride secretion or relatively less absorption. The increased potential difference with exercise is enhanced by prior stimulation with methacholine in the present experiment, suggesting a priming effect, but does not necessarily imply that the exercise effect is itself a straightforward parasympathetic response. Excess secretion from the respiratory tract is a symptom in most respiratory diseases (e.g. rhinitis, chronic bronchitis, asthma, cystic fibrosis). The present observation that parasympathetic stimulation alters the transmucosal potential difference as well as increasing secretion will provide a better understanding of the mucosa and may lead to the development of better therapy.

REFERENCES

1. Alton EW, Hay JG, Munro C, Geddes DM (1987) Measurement of nasal potential difference in adult cystic fibrosis, Young's syndrome and bronchiectasis. Thorax 423: 815–817.
2. Borum P (1979) Nasal methacholine challenge. J Allergy Clin Immunol 63: 253–257.
3. Boucher RC, Bromberg PA, Gatzy JT (1980) Airway transepithelial electric potential difference *in vivo:* Species and regional differences. J Appl Physiol 48: 169–176.
4. Dunlap K, Holz GG, Rane SG (1987) G-proteins as regulators of ion channel function. Trends Neurosci 10: 241.
5. Harris W, Giebaly K, Aluswaidan S, Stanford CF (1990) Alteration in nasal transepithelial potential difference during exercise. Otolaryngol Head Neck Surg 103: 2194.

6. Knowles MR, Carson JL, Collier AM, Gatzy JT, Boucher RC (1981a) Measurements of nasal transepithelial electric potential differences in normal human subjects *in vivo*. Am Rev Resp Dis 124: 484–490.
7. Knowles MR, Gatzy JT, Boucher RC (1981b) Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. New Engl J Med 305: 1489–1495.
8. North RA (1986) Muscarinic receptors and membrane ion conductances. Trends Pharmacol Sci Supplement 2: 19.
9. Richerson HB, Seebohm PM (1968) Nasal airway response to exercise. J Allergy Clin Immunol 41: 269–284.
10. Stanford CF, Stanford RL (1983) Exercise-induced rhinorrhoea (athlete's nose). Brit Med J 21: 6–60.
11. Van Megen YJB, Klassen ABM, Rodrigues de Miranda JF, Van Ginneken AM, Wentes RTR (1991) Alterations of muscarinic acetylcholine receptors in the nasal mucosa of alleregic patients in comparison with non-allergic individuals. J Allergy Clin Immunol 87: 521–519.

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