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The effect of noradrenaline on mucociliary activity in the rabbit maxillary sinus*

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SUMMARY

The effect of noradrenaline (NA) on mucociliary activity in the rabbit maxillary sinus was investigated in vivo *by injecting it at increasing dosages (10^{-11} to 10^{-4} mol/kg) into the maxillary artery, the mucociliary response being recorded photoelectrically. NA increased mucociliary activity at a dosage of 10^{-5} mol/kg, the maximal increase being 16.1±2.6%. The NA-induced stimulation of mucociliary activity had a latency of 20 s, and the activity returned to base-line level within 3 min. Pretreatment with the α-antagonist phentolamine (0.2 and 1.0 mg/kg) or the cholinergic antagonist atropine (1 mg/kg) did not alter mucociliary response to NA. Blockade with the β-antagonist propranolol did not significantly reduce the maximal response to NA, which was 16.1±2.6% before and 11.1±3.0% after pretreatment with propranolol (n=7; p=0.2). In contrast, pretreatment with the prostaglandin-synthesis inhibitor indomethacin reduced the response from 12.9±2.9% to 6.3±1.3% (n=6; p<0.05), suggesting that at high dosages NA stimulates mucociliary activity via the cyclo-oxygenase pathway.*

Key words: atropine, indomethacin, mucociliary activity, noradrenaline, phentolamine, propranolol

INTRODUCTION

Noradrenaline (NA) is the classic transmitter in postganglionic sympathetic nerve fibers and has both α- and β-agonistic properties. Morphological studies have shown a rich adrenergic supply to the nasal mucosa, predominantly around smaller arteries and veins (Dahlström and Fuxe, 1965; Änggård and Densert, 1974). In the rabbit maxillary sinus a rich network of adrenergic nerve fibers is seen around blood vessels in the lamina propria and also to some extent surrounding the exocrine glands, whereas no such fibers are seen close to the ciliated epithelium (Schindelmeiser et al., 1982). Besides NA, sympathetic nerve fibers also contain the putative transmitter neuropeptide Y (NPY), which coexists with NA (Lundberg et al., 1982). NPY is also found in the rabbit maxillary-sinus mucosa, where NPY-containing nerve fibers are located mostly around blood vessels in the lamina propria, although a few such nerve fibers are also seen subepithelially (Cervin et al., 1991).

A previous *in vivo* study in the rabbit has shown intra-arterial injections of NPY to reduce mucociliary activity (Cervin et al., 1991). Regarding the effects of NA on mucociliary activity conflicting results have been reported. In an *in vitro* study from the rabbit trachea, NA had no effect on the ciliary beat frequency (Burn, 1956) whereas, in the dose range 10^{-5} to 10^{-3} M NA depressed ciliary activity in the frog palate (Deshpande et al., 1970). In another *in vitro* study on the frog palate, NA was found to suppress ciliary activity at concentrations below 10^{-7} M, but to stimulate ciliary movements at concentrations exceeding 2×10^{-6} M. An accelerating effect that was blocked in the presence of the cyclo-oxygenase inhibitor indomethacin, which blocks the synthesis of prostaglandins (Maruyama, 1984). Apart from a pilot study on the rabbit maxillary sinus where no distinct responce could be recorded after intra-arterial bolus injections of NA (Hybbinette and Mercke, 1982a), no systematic *in vivo* studies on the effect of NA on mucociliary activity have been performed.

The purpose of the present investigation was to study the possible role of NA in the regulation of mucociliary activity *in vivo* in the rabbit maxillary sinus. Adrenergic and cholinergic antagonists, and an inhibitor of cyclo-oxgenase, were used to further clarify the mechanism by which NA may affect mucociliary activity.

METHOD

The experiments were performed on rabbits of either sex, weighing 2.0-3.5 kg (mean 2.7 kg). The animals were

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anaesthetized with urethane (2 g/kg being given i.m. initially, followed by an extra dose of 0.5 g/kg given i.v. during the operation). An intravenous cannula was inserted into one of the ear veins and perfused with saline (2 ml/h). The arterial inlet for test substances was a cannula, size 3 FG (Portex, UK) inserted into the facial or lingual artery with its tip close to the maxillary artery, and continuously perfused with saline (2 ml/h). The mucosa in the maxillary sinus was exposed through a trepanation of about 2×8 mm, which was immediately covered with a heated window. Mucociliary activity (visible as flickering light reflections) was observed through a binocular microscope. Once the criterion of a properly functioning preparation (visible transportation of small particles such as mucus clumps and shed cells) was obtained, one of the eyepieces was switched to a phototransducer and the mucociliary activity was recorded photoelectrically (Hybbinette and Mercke, 1982a). The mucociliary wave pattern was monitored continuously on an oscilloscope and recorded by an ink writer during challenges. The recordings were analyzed by a computerized frequency calculator, which computed the mucociliary wave frequency in waves/min every 10 s during challenge and intervals of 1–5 min otherwise. Induced frequency changes were expressed as percentages of the frequency zero-level (i.e. the basal mucociliary-wave frequency) immediately preceding drug administration. ECG and rectal temperature were monitored, and body temperature was maintained at 37.0–38.5°C by means of a heating pad. Respiratory rate was registered by a tocotransducer (No. 152 78B; Hewlett Packard, Germany), and blood pressure was measured in the femoral artery with a pressure transducer (Novatrans MX 807; Medex, England). Both respiratory rate and blood pressure were recorded simultanously with mucociliary activity on an ink writer.

The following drugs were used: (−)-arterenol hydrochloride, ((−)-Arterenol; Sigma, USA), phentolamine methansulphonate (Regitin®; Ciba-Geigy, Switzerland), propranolol hydrochloride (Inderal®; ICI, UK), atropine sulphate (ACO, Sweden) and indomethacin (Confortid®; Dumex, Denmark).

(−)-Arterenol hydrochloride (NA) was dissolved in sterile water containing 10^{-3} M citric acid, to a stock solution of 10^{-2} mol/ml. The stock solution was kept in a refrigerator at −26°C, further dilutions being made in 0.9% saline. Regitin® contains 10 mg phentolamine per ml in sterile water. NA and phentolamine were injected intra-arterially as bolus doses of 0.1 ml/kg. Saline injections of 0.1 ml/kg served as controls. All intra-arterial injections were given within 3 s, followed by 0.3 ml saline in order to flush the cannula.

Inderal®, which contains 1 mg/ml propranolol hydrochloride in sterile water, was given as an intravenous injection (into an ear vein) of 1 ml/kg during 3 min followed by 1 ml saline to flush the cannula. Indomethacin (50 mg/ml) in sterile water was further diluted in saline to 5 mg/ml and injected intravenously (1.2 ml/kg). Atropine sulphate (2 mg/ml) was dissolved in saline and injected intravenously (0.5 ml/kg). The intravenous injections of indomethacin and atropine were given over 30 s, followed by 1 ml saline to flush the cannula.

Experimental procedures

The effect of increasing dosages of NA from 10^{-11} to 10^{-5} mol/kg (equalling 2.0 ng/kg to 2.0 mg/kg) was investigated in seven rabbits. The effect of 10^{-4} mol/kg (or 20.5 mg/kg) NA was investigated in a separate series of nine animals. The occurrence of tachyphylaxis was investigated in six animals challenged with NA at 2 consecutive dosages of 10^{-5} mol/kg at a 15-min interval.

The effect of NA at dosages of 10^{-9}, 10^{-7}, and 10^{-5} mol/kg during α-receptor blockade by 0.2 mg/kg phentolamine (four rabbits) and 1 mg/kg phentolamine (3 rabbits) was investigated. Owing to the short duration of the α-blockade, phentolamine was injected 30 s before each challenge with NA.

The effect of NA at dosages of 10^{-9}, 10^{-7}, and 10^{-5} mol/kg during β-receptor blockade by 1 mg/kg propranolol was investigated in seven rabbits. The first challenge with NA was given approximately 10 min after the propranolol injection.

The effect of NA at a dosage of 10^{-5} mol/kg during blockade with atropine was investigated in six animals. Atropine was given as an intravenous bolus injection of 1 ml/kg, 5 min prior to challenge.

The effect of NA at a dosage of 10^{-5} mol/kg during blockade with indomethacin was investigated in six animals. Indomethacin (6 mg/kg) was injected into an ear vein approximately 2 h before challenge. A further bolus dose of 2 mg/kg was given 5 min before each challenge.

Control experiments with NA alone were run in all rabbits at least 30 min before the experiments with the various antagonists; thus, all rabbits served as their own controls. The time interval between injections of NA was at least 15 min. The dosages of phentolamine, propranolol, atropine, and indomethacin were those previously used in experiments with the same animal model (Cervin et al., 1991; Dolata et al., 1989; Hybbinette and Mercke, 1982b).

Dose-response curves were plotted from the maximum change of mucociliary activity during the first 3 min after each challenge. The results were expressed as means and standard error of the means (SEM), with the exception of frequency zero-levels which were expressed as means and standard deviations (SD). Peak response and the area under the curve (AUC) were used for statistical evaluations. Data were analyzed with Student's t-test for paired data; p-values of <0.05 were considered significant.

RESULTS

Mucociliary activity remained unaffected by injections of either saline or citric acid in saline (the vehicle for the stock solution of NA), or of the various antagonists (atropine, indomethacin, phentolamine, propranolol).

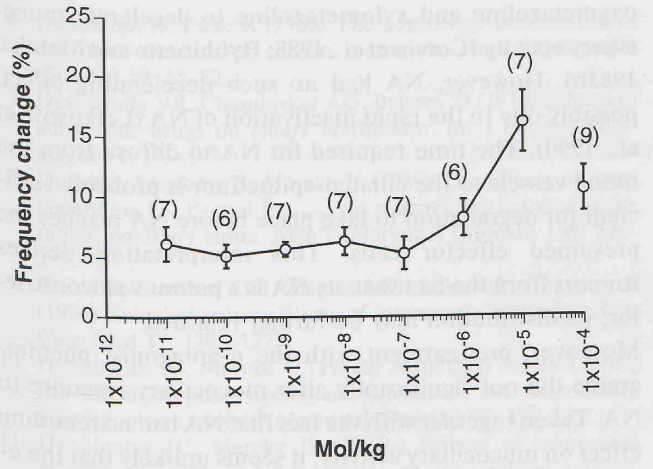

Figure 1. The effect of NA on mucociliary activity in the dosage range 10^{-11} to 10^{-4} mol/kg (equalling 2.0 ng/kg to 20.5 mg/kg). Figures in brackets refer to the number of animals examined at each dosage. Frequency zero level (10^{-11} mol/kg) was 1307±127 waves/min. The correlation coefficient for raw data in the interval 10^{-7} to 10^{-5} was r=0.67. Note that the experiments with NA at a dosage of 10^{-4} mol/kg were performed in a separate series of animals.

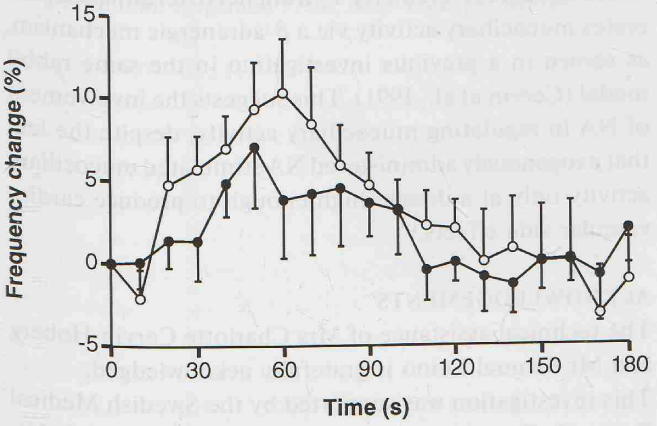

Figure 2. The time-course curve (n=7) for the effect of NA (10^{-5} mol/kg) before (open circles) and after blockade with the β-antagonist propranolol (closed circles). The respective frequency zero-levels were 1,252±90 and 1,261±56 waves/min.

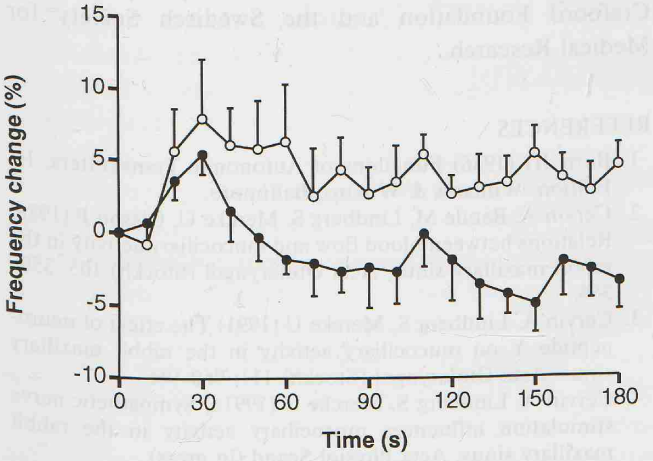

Figure 3. The time-course curve (n=6) for the effect of NA (10^{-5} mol/kg) before (open circles) and after blockade with the cyclooxygenase inhibitor indomethacin (closed circles). The respective frequency zero levels were 1,264±127 and 1,291±70 waves/min.

In the dosage range 10^{-11} to 10^{-6} mol/kg, NA did not affect mucociliary activity. At a dosage of 10^{-5} mol/kg, NA accelerated mucociliary activity by 16.1±2.6% as compared to saline (n=7; $p<0.01$; see Figure 1). The increase appeared within 20 s, peaked at about 60 s, and lasted approximately 3 min (Figure 2). Two consecutive injections of NA at 10^{-5} mol/kg and at a 15-min interval did not result in any tachyphylaxis, the increases of mucociliary activity being 12.9±2.8% and 12.8±2.2%, respectively (n=6). Further increase of the dosage of NA (10^{-4} mol/kg) did not significantly affect mucociliary activity.

Blockade by phentolamine at a dosage of 0.2 or 1.0 mg/kg did not alter the response to NA. Blockade with the β-antagonist propranolol did not significantly reduce the maximal response to NA, which was 16.1±2.6% before and 11.1±3.0% after pretreatment with propranolol (n=7; p=0.2; see Figure 2). The accelerating effect of NA (10^{-5} mol/kg) on mucociliary activity was not blocked by atropine, the maximal response being 12.8±1.4% before and 13.4±2.3% after pretreatment (n=5; $p>0.5$).

On the other hand, pretreatment with indomethacin reduced the accelerating effect of NA (10^{-5} mol/kg) from 12.9±2.9% to 6.3±1.3% (n=7; $p<0.05$; AUC: p=0.07). A weak initial response remained after pretreatment with indomethacin (Figure 3).

Injection of NA or either of the two antagonists, phentolamine and propranolol, resulted in cardiovascular side effects but no change in respiratory rate. At a dosage of 10^{-5} mol/kg, NA increased blood pressure by 30.3±6.6% (n=16; $p<0.01$), and at a dosage of 10^{-4} mol/kg NA increased blood pressure by 108.5±18.3% (n=9; $p<0.001$). The duration of the blood pressure elevation did not exceed 3 min. At dosages of 10^{-6} mol/kg and below, NA did not affect blood pressure significantly. At a dosage of 10^{-4} mol/kg NA decreased the pulse rate from 266±6 to 198±16 beats/min (n=9; $p<0.01$), but lower dosages of NA had no effect on pulse rate. Propranolol (1 mg/kg i.v.) decreased the heart rate by 27% (from 305±19 to 222±13 beats/min; n=8; $p<0.001$), and lowered blood pressure by a maximum of approximately 20%, the decrease lasting 3–5 min. Phentolamine (0.2 and 1.0 mg/kg i.a.) had no effect on pulse, but lowered blood pressure by approximately 20%, the effect lasting about 2 min.

Pretreatment with atropine or indomethacin had no cardiovascular or respiratory effects.

DISCUSSION

Bolus injections of NA increased mucociliary activity in the rabbit maxillary sinus, a finding in accordance with previous *in vitro* findings in the frog palate (Maruyama, 1984). The effect of NA is dependent on the distribution of the adrenergic receptors of the target organ. In the vasculature, NA acts predominantly on α-receptors, except in the heart where it acts predominantly on β-receptors (Innes and Nickerson, 1975). The distribution of α- and β-receptors in the airway epithelium is not known, although

a study in the present animal model has shown the β_2-adrenoceptor agonist salbutamol to stimulate mucociliary activity, whereas the β_1-agonist prenalterol was without effect, suggesting the existence of β_2-receptors on the airway epithelium (Hybbinette and Mercke, 1982b). In a previous investigation in the same rabbit model, the acceleration of mucociliary activity by sympathetic nerve stimulation was reversed by pretreatment with propranolol, indicating the mucociliary response to be mediated via β-receptors (Cervin et al., 1991). In the present study, however, we were unable to confirm that the acceleration of mucociliary activity after administration of NA was mediated by activation of β-receptors, as the response was not blocked by propranolol. The presence of a cholinergic reflex mechanism could be ruled out, as pretreatment with atropine had no effect on mucociliary response to NA (10^{-5} mol/kg).

The bronchoconstrictive effect of NA in the guinea-pig trachea has been reported to be blocked by indomethacin, suggesting a release of prostaglandins (Takayanagi et al., 1990). Stimulatory effects of the prostaglandins E_1 and $F_{2\ alpha}$ on mucociliary activity in the rabbit maxillary sinus were found in previous *in vivo* experiments (Dolata et al., 1989). In the frog palate mucosa, exposure to 10^{-5} M NA produced an eight-fold increase in the prostaglandin E_1 concentration concomitant with an acceleration of ciliary activity, an acceleration that could also be blocked by indomethacin (Maruyama, 1984). Our study supports these findings, as pretreatment with indomethacin reduced the response to NA, suggesting that the cyclo-oxygenase pathway may be involved in the stimulation of the mucociliary activity by high dosages of NA.

Stress in such forms as surgery, general anesthesia, bleeding, and hyper- or hypothermia is readily reflected in increased plasma concentrations of NA (Hart et al., 1989). In the present study, care was taken to control bleeding during the operation and to substitute fluids when necessary. Body temperature was regulated to avoid hypothermia. Surgical trauma close to the ciliated epithelium (i.e. the trepanation) has been reported to result in increased mucociliary activity, possibly due to the release of inflammatory mediators (Wong and Yeates, 1990). That such an effect of released inflammatory mediators and circulating catecholamines might have been a confounding factor in the present result would seem unlikely as neither indomethacin nor propranolol affected basal mucociliary activity.

In *in vitro* studies mucociliary activity has been reported to be suppresed by NA at dosages below 10^{-7} M, but to be accelerated at dosages above 2×10^{-6} M (Maruyama, 1984), which suggest that the α-agonistic effects of NA predominantly occur in the lower dose range. It might be expected that after pretreatment with propranolol the α-agonistic properties of NA would predominate, resulting in reduced mucociliary activity. Previous studies using the same animal model have shown the α-agonists phenylephrine, oxymetazoline and xylometazoline to decelerate mucociliary activity (Cervin et al., 1988; Hybbinette and Mercke, 1982b). However, NA had no such decelerating effect, possibly due to the rapid inactivation of NA (Lefkowitz et al., 1990). The time required for NA to diffuse from the blood vessels to the ciliated epithelium is probably sufficient for degradation to take place before NA reaches the presumed effector cells. This interpretation derives support from the fact that, as NA is a potent vasoconstrictor, its distribution may be further retarded.

Moreover, pretreatment with the α-antagonist phentolamine did not significantly alter mucociliary response to NA. Taken together with the fact that NA had no retarding effect on mucociliary activity, it seems unlikely that the α-agonistic properties of NA are of any importance in regulating mucociliary activity. In contrast, the other sympathetic transmitter NPY, deccelerates mucociliary activity *in vivo* (Cervin et al., 1991).

Bolus injections of NA affected mucociliary activity only at a high dose level an effect that may be attributed to the release of prostaglandins. Sympathetic nerve stimulation, which releases NA (and NPY) from nerve terminals, accelerates mucociliary activity via a β-adrenergic mechanism, as shown in a previous investigation in the same rabbit model (Cervin et al., 1991). This suggests the involvement of NA in regulating mucociliary activity, despite the fact that exogenously administered NA stimulated mucociliary activity only at a dosage high enough to produce cardiovascular side effects.

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