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The role of neurokinin A and calcitonin gene-related peptide in the mucociliary defence of the rabbit maxillary sinus

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SUMMARY

Substance P (SP) released from sensory C-fibres in the airways increases the mucociliary (m.c.) activity in the rabbit maxillary sinus. The purpose of the present study was to investigate the m.c. effects of two other neuropeptides, coexisting with SP in sensory neurones, neurokinin A (NKA) and calcitonin gene-related peptide (CGRP). NKA increased the m.c. activity dose-dependently (dose range 0.1-5.0 µg/kg) the maximum increase being 33.6 ± 6.0%. The effect was inhibited by pretreatment with the tachykinin antagonist (D-Pro^2, D-Trp^{7,9})SP, but not with atropine or hexamethonium. Thus NKA released from sensory C-fibres may contribute to the non-cholinergic increase of m.c. activity observed after C-fibre stimulation.

In contrast CGRP did not influence the m.c. activity. Neither did it influence the responses to NKA or SP. It is concluded that CGRP is unlikely to be involved in the control of m.c. function.

A mucociliary defence reflex has been demonstrated in the mucous membrane of the rabbit maxillary sinus. In this reflex capsaicin-sensitive C-fibres constitute the afferent arm and cholinergic effector neurones the efferent arm. Activation of the reflex results in an acceleration of the mucociliary activity due partly to substance P (SP), partly to acetylcholine (Lindberg and Mercke, 1986).

However, during recent years it has become evident that substance P is not the only putative transmitter in C-fibres. Besides substance P, another tachykinin called neurokinin A (NKA) has been demonstrated in the sensory nerve fibres in the upper respiratory tract (Sundler et al., 1985). It has also been reported that another neuropeptide, calcitonin gene-related peptide (CGRP), coexists with substance P in some capsaicin-sensitive sensory neurones and is distributed

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abundantly in the respiratory tract of mammals, including man (Uddman et al., 1985).

It has therefore been regarded as important to elucidate whether these two transmitters play any role in the regulation of mucociliary activity in conjunction with C-fibre stimulation.

An experimental series was performed on rabbits in which, after anesthesia with urethane, a catheter was placed in the artery feeding the mucous membrane of the maxillary sinus on the one side. The mucosa in the maxillary sinus on the same side was then exposed through a trepanation hole. To prevent desiccation of the mucosa, the hole was immediately covered with an antimist window. By aiming a cold light beam obliquely to the mucous membrane through the window, a light reflection is created. The mucociliary activity of the maxillary sinus mucosa makes this reflection flicker. This continuously changing light intensity, being an expression of the mucociliary activity of the sinus mucosa, is recorded in the form of a curve via a microscope, a phototransducer, a filter and an ink writer (Figure 1). Substances to be tested are administered through the catheter into the artery feeding the sinus mucosa and the resulting change in mucociliary activity can be recorded directly. Any change in activity is expressed as a percentage of the mucociliary activity (= frequency zero level) recorded immediately before the administration of the substance tested (Hybbinette and Mercke, 1982).

Neurokinin A has been tested in the dose interval 0.001–5.0 µg/kg and a log dose response curve has been plotted. It is shown that NKA accelerates mucociliary activity, the maximum increase being 33.6 $\pm$ 6.0%, and this increase is statistically significant. For comparison, substance P has also been administered and it is clearly seen that the increased mucociliary activity induced by substance P is more pronounced than after NKA, here the maximum increase is about 50% (Figure 2).

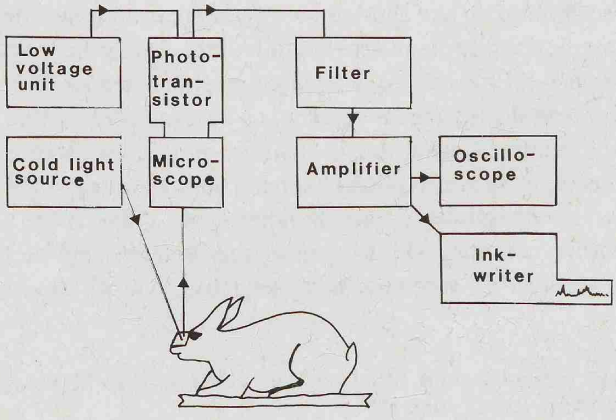

Figure 1.
Block diagram of the experimental set-up.

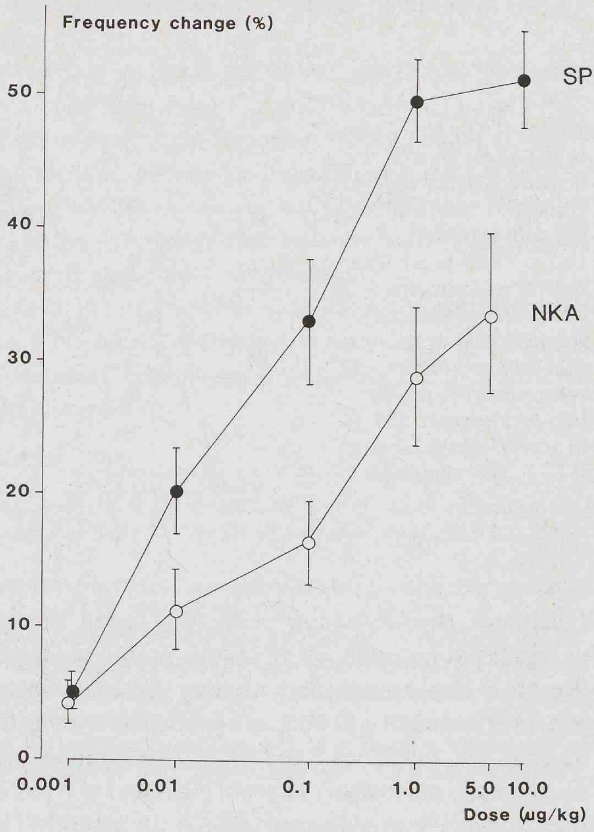

Figure 2.
The effect of NKA on m.c. wave frequency (0-0), (n=6, $P<0.001$ for the whole curve) compared to the effect of SP (•-•), (n=6, $P<0.001$). Frequency zero levels were 1221 ± 37 (NKA) and 1289 ± 72 (SP). The differences between the maximum m.c. responses to 0.1 and 1.0 µg/kg of the two tachykinins were significant ($P<0.05$ for 0.1 µg/kg and $P<0.01$ for 1.0 µg/kg.

From previous experiments, we know that neither atropin, hexamethonium, nor the SP antagonist (D-Pro^2, Trp^{7,9})SP has any mucociliary effect per se (Lindberg and Mercke, 1986).

If we now pretreat rabbits with atropin we find this does not influence the mucociliary response induced by NKA and this is also the case after pretreatment with hexamethonium.

Contrary to this, the SP antagonist inhibits the response after NKA. This inhibitory effect of the SP antagonist on the NKA response is illustrated in Figure 3, showing the time course of the mucociliary effect of 0.1 µg/kg NKA, the upper curve showing NKA only, the lower curve after pretreatment with the SP antagonist. The difference between these two curves is statistically significant.

These experiments speak in favour of NKA contributing to the non-cholinergic response of the above mentioned mucociliary defence reflex after C-fibre stimulation.

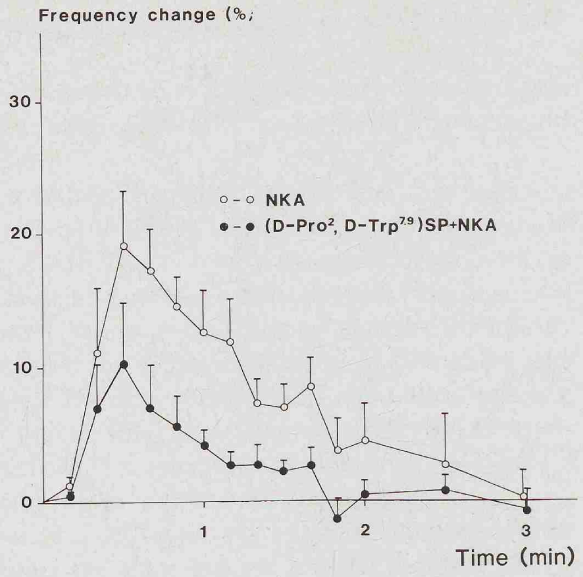

Figure 3. The time-course of the effect on m.c. wave frequency of 0.1 µg/kg NKA in 6 rabbits pretreated with (D-Pro^2, D-Trp^{7,9}) SP (●–●). The control experiments (○–○) were run in the same animals prior to the experiments with the SP antagonist. Frequency zero levels were 1389 ± 78 waves/min (●–●) and 1327 ± 31 waves/min (○–○).

Calcitonin gene-related peptide was tested in the dose interval 0.001–5.0 µg/kg. It did not induce any change on the mucociliary activity.

We have also investigated whether CGRP, although it lacks a mucociliary effect per se, might still work as a factor potentiating the effect of the other known transmitters, substance P and NKA. This turned out not to be the case. The time course of the mucociliary effect of NKA and of NKA + CGRP is almost identical. If substance P is used instead of NKA the result will be the same, i.e., CGRP has no potentiating effect on neither NKA nor substance P. If curves are plotted no statistical difference between the curves with and without CGRP is found.

Based on these experiments the following conclusions may be drawn:

1. NKA accelerates mucociliary activity.
2. This effect is non-cholinergic and is inhibited by an SP antagonist.
3. Release of NKA may contribute to the non-cholinergic increase of mucociliary activity seen after C-fibre stimulation.
4. CGRP has no effect on mucociliary activity neither per se nor as a potentiating factor.

ZUSAMMENFASSUNG

Die Substanz P (SP), die von den sensorischen C-Fibern der Atemwege befreit wurde, steigert die mukoziliäre Aktivität in der Kieferhöhle des Kaninchens. Die vorliegende Untersuchung hatte zur Absicht, den mukoziliären Effekt von zwei weiteren Neuropeptiden zu untersuchen, Neurokinin A (NKA)

und "calcitonin gene-related peptide" (CGRP), die zusammen mit SP in sensorischen Neuronen vorkommen.

Das NKA steigerte die mukoziliäre Aktivität auf Grund der Dosis, Dosierung 0,1–5,0 µg/kg, wobei die maximale Steigerung 33,6 ± 6,0% betrug. Dieser Effekt wurde durch eine Vorbehandlung mit dem Tachykininantagonisten (D-Pro^2, D-Trp ^{7,9}) SP gehemmt, doch nicht durch eine Vorbehandlung mit Atropin oder Hexamethonium. Das von sensorischen C-Fibern befreite NKA kann infolgedessen zu der nicht-kolinergen Steigerung der mukoziliären Aktivität beitragen, die nach Anregung mit C-Fibern beobachtet werden kann.

Dagegen beeinflusste das CGRP die mukoziliäre Aktivität nicht. Noch hatte es irgendeinen Effekt auf die nach NKA und SP erhaltenen Antworten. Die Schlussfolgerung lautet demnach, dass das CGRP an der Regulierung der mukoziliären Funktion scheinbar nicht teilnimmt.

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