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The effect of nedocromil sodium and sodium cromoglycate on antigen-induced bronchoconstriction in the *Ascaris*-sensitive monkey

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Nedocromil sodium inhibited the bronchoconstriction caused by antigen challenge in *Ascaris*-sensitive monkeys and in addition it prevented the release of histamine from mast cells lavaged from sensitive monkeys. Sodium cromoglycate was relatively inactive in both these systems. It is suggested that nedocromil sodium can stabilize both mucosal and connective tissue mast cells and may represent a new type of drug.

Introduction The infestation of laboratory bred *Macaca arctoides* monkeys with the nematode parasite *Ascaris suum* induces an IgE mediated reactivity similar to that found in naturally sensitive animals (Pritchard *et al.*, 1983). The ability of nedocromil sodium (disodium 6,9,dihydro-9-ethyl-4,6,-dioxo-10-propyl - 4H-pyrano[3,2-g]-quinoline-2,8-dicarboxylate) to prevent the bronchoconstriction caused by antigen challenge in sensitive monkeys has been assessed. In addition the effects of nedocromil sodium on the mast cells lavaged from the lungs of sensitive moneys is described together with the results of a histological examination of these cells. A comparison with sodium cromoglycate has been made throughout the study and it is proposed that nedocromil sodium represents a new type of drug because of its ability to affect mucosal as well as connective tissue mast cells.

Methods Four male and one female *Macaca arctoides* monkeys (8–18 kg) were used in this study. All five monkeys had been infected previously with *Ascaris suum* on two or three occasions over a period of 18 months (Pritchard, *et al.*, 1983).

Approximately five weeks after the second or third infection the monkeys were challenged each week with an aerosol of *Ascaris suum*. The monkeys were sedated with ketamine (10 mg kg^{-1}) and subsequently anaesthetized with sodium pentobarbitone (10 mg kg^{-1} i.v.). After intubation with a 6–7 mm endotracheal tube the animals were ventilated at constant volume (13 ml kg^{-1}). Body temperature was maintained at 37–38°C.

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Respiratory air flow rate, tidal volume, transpulmonary pressure, total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were recorded as previously described (Jackson & Richards, 1977).

The *Ascaris* antigen was prepared from adult *Ascaris* psuedocoelomic fluid by gel filtration according to the method of Ambler *et al.* (1972). The antigen was administered by means of a Vaponefrin nebuliser connected to a Bird Mk VII respirator. A dose of antigen was selected for each monkey so that an increase in R_L of 3–4 cm H_2O l^{-1} s^{-1} and a fall in C_{dyn} of 10–12 ml cmH_2O^{-1} was produced. Once the dose had been selected it was not changed for subsequent challenges.

The antigen dose varied from four breaths of an aerosol generated from a 1 μg ml^{-1} solution to three breaths of an aerosol generated from a 10 μg ml^{-1} solution. Doses of antigen are quoted as weight of protein ml^{-1}.

When two or three consistent bronchoconstrictions had been produced, approximately 4 mg of sodium cromoglycate or nedocromil sodium (50 breaths of an aerosol generated from a 2% solution) was administered 5 min before antigen challenge. Several weeks of weekly challenges followed before the second drug was tested.

Bronchial lavage was performed on monkeys infected at the same time as those used in the study described above. The monkeys were anaesthetized (ketamine 10 mg kg^{-1} i.m., followed by methohexitone sodium 16 mg kg^{-1}, i.v.) intubated and lavaged using 3 × 30 ml aliquots of Hanks balanced salt solution. The fluid was recovered into heparinised tubes and after filtration through a 175 μm nylon mesh the cells were harvested by centrifugation (200 *g* for 5 min at 4°C). The cells were then washed and resuspended in HEPES-buffered Tyrode, pH 7.4 containing gelatin (1 mg ml^{-1}). These cells (approximately 10^4 mast cells in a final volume of 0.1 ml) were challenged with sheep anti-human IgE (Miles) to release 10–20% of the total histamine. Drugs were added at the same time as the challenge. Release was allowed to proceed for 20 min at 37°C then quenched with 0.25 ml ice cold Ca^{2+}-free buffer containing 1 mM

EDTA. After centrifugation histamine released into the supernatant was assayed by an adaptation of the double isotope method of Beaven *et al.* (1972).

In some cases the mast cells recovered from the lavage were stained with alcian blue/safranin followed by berberine sulphate and examined by conventional histological techniques (Enerback, 1966).

Results Pretreatment with sodium cromoglycate produced some inhibition of the changes in R_L and C_{dyn} caused by antigen challenge but this inhibition was not statistically significant. Nedocromil sodium inhibited the increase in R_L by approximately 85% and the decrease in C_{dyn} by approximately 55%. These inhibitions were significant (Figure 1).

Sodium cromoglycate and nedocromil sodium inhibited histamine release in a dose-dependent manner. The IC_{30} values (± s.e.mean) were 5.2×10^{-6}M ($\pm 0.91 \times 10^{-6}$M) for nedocromil sodium and 9.9×10^{-4}M ($\pm 2.2 \times 10^{-4}$M) for sodium cromoglycate. The maximal inhibition for nedocromil (10^{-4}M) and sodium cromoglycate (10^{-3}M) was 60% and 30% respectively of the total releaseable histamine.

The granules of the mast cells recovered from the lavage did not stain brown with alcian blue/safranin nor did they take up the fluorescent stain berberine sulphate, which is a characteristic of heparin containing connective tissue mast cells (Wingren & Enerback, 1983). Approximately 22% of the total cells recovered in 21 lavages were observed to be mast cells containing no heparin.

Discussion Sodium cromoglycate did not significantly inhibit the changes in lung mechanics produced by antigen challenge in *Ascaris*-sensitive monkeys, whereas nedocromil sodium did. These results were consistent with the *in vitro* findings where sodium cromoglycate was significantly less potent than nedocromil sodium in preventing the release of histamine from mast cells. The lack of an effect by sodium cromoglycate given intravenously and by aerosol over

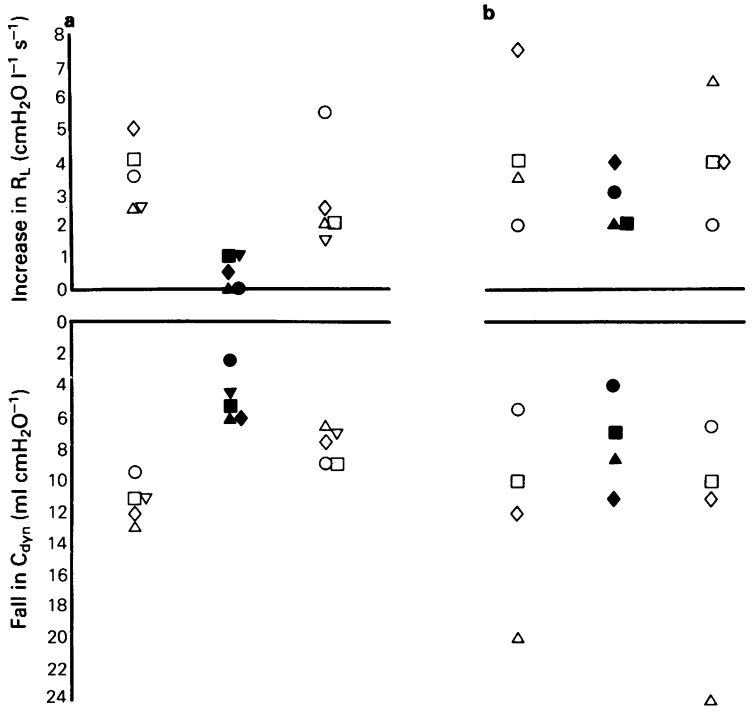

Figure 1 The effects of 4 mg of sodium cromoglycate and nedocromil sodium given by aerosol (50 breaths from a 2% solution) on the changes in total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) caused by antigen challenge. Each monkey received the same antigen challenge weekly, although the level of antigen challenge varied between monkeys. The effects of nedocromil sodium are shown in (a) and the effects of sodium cromoglycate in (b). Each symbol represents an individual monkey. The closed symbols are the responses on the day of drug treatment.

a wide dose range to *Ascaris*-sensitive monkeys has already been described (Richards *et al.*, 1983).

The histological examination of the mast cells obtained by lung lavage showed that they did not stain for heparin. This suggests that the mast cells in monkey lung are mucosal mast cells rather than connective tissue mast cells, since the latter contain heparin while the former do not (Wingren & Enerback, 1983). In other experiments, not described here, both sodium cromoglycate and nedocromil sodium inhibited mediator release from mast cells obtained from the rat peritoneal cavity with similar potencies ($IC_{30} = 0.86 \times 10^{-6}$M $\pm 0.27 \times 10^{-6}$M for sodium cromoglycate and $IC_{30} = 1.4 \times 10^{-6}$M $\pm 0.3 \times 10^{-6}$ for nedocromil sodium, values are means $\pm$ s.e.mean, $n = 3$). It is commonly believed that mast cells obtained from the rat peritoneal cavity are typical connective tissue mast cells (Enerback, 1966).

It may be suggested from this study that the bronchoconstriction produced by antigen challenge in the *Ascaris*-sensitive monkey resulted from mediator release from mucosal mast cells and that nedocromil sodium, unlike sodium cromoglycate, prevented the bronchoconstriction because it effectively stabilized this type of mast cell.

Preliminary studies in man have shown that nedocromil sodium reduces the symptoms of asthma and in addition it helps to relieve asthma in patients maintained on steroids (Lal *et al.*, 1984). Further clinical work is needed to investigate the full potential of this new class of drug. Nedocromil sodium may also represent a pharmacological tool for the investigation of mucosal mast cells.

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