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THE EFFECTS OF SODIUM CROMOGLYCATE ON HISTAMINE AEROSOL-INDUCED REFLEX BRONCHOCONSTRICTION IN THE ANAESTHETIZED DOG

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1 The effects have been studied of sodium cromoglycate (SCG), given by aerosol or intravenously, on reflex bronchoconstriction induced by histamine aerosol in the anaesthetized dog.

2 Four breaths of an aerosol generated from a 2% solution of SCG significantly inhibited the vagally mediated increase in total lung resistance (R_L) produced by histamine.

3 SCG given intravenously as bolus injections (5–500 μg/kg) produced a dose-dependent reversal of a sustained reflex bronchoconstriction induced by histamine aerosol. Propranolol (500 μg/kg) did not prevent this reversal.

4 SCG did not inhibit the increase in R_L produced by supramaximal electrical stimulation of a vagus nerve.

5 The possibility is discussed that SCG may reduce the activity of lung irritant receptors in the anaesthetized dog.

Introduction

Sodium cromoglycate (SCG) is a drug of proven worth in the treatment of certain types of asthma (Editorial, British Medical Journal, 1972). Evidence obtained from *in vitro* and *in vivo* experiments has led to the proposal that SCG acts by the temporary stabilization of the mast cell so preventing the release of mediators (Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard, 1970). We have investigated another possible explanation for the action of SCG in the lung. Using a canine model of reflex bronchoconstriction we have assessed the ability of this drug, given by aerosol or intravenously, to inhibit or reverse bronchoconstriction mediated via the vagus nerves. Some of the results of this study have been communicated to the British Pharmacological Society (Jackson & Richards 1976).

Methods

Beagle dogs (9–12 kg) of either sex were initially sedated with thiopentone sodium (5–10 mg/kg i.v.) and then anaesthetized with chloralose (80 mg/kg i.v.). The animals were maintained at a light level of surgical anaesthesia by giving supplementary doses of chloralose 10–15 mg/kg every 15 min via a cannula inserted in the right saphenous vein.

The trachea was cannulated just below the cricoid cartilage with a plastic cannula (1 cm internal diameter and 3 cm long with a resistance of 0.04 kPa l^{-1} s) and the animals were ventilated with air at constant pressure (0.98 kPa) using a Bird Mark VII ventilator. The lungs were inflated to 1.96 kPa transpulmonary pressure (TPP) after severe histamine-induced falls in lung compliance and also periodically to reverse the gradual decrease in lung volume, which occurs during positive pressure ventilation. A thoracotomy was routinely performed.

A catheter was inserted in the muscularis branch of the right femoral artery for recording blood pressure with a Statham P23 Db pressure transducer. Heart rate was derived from the blood pressure signal using a Devices ratemeter. Catheters were placed in the left saphenous vein and the muscularis branch of the left femoral artery for the injection of drugs and the removal of arterial blood for blood-gas analysis respectively.

Both cervical vagosympathetic nerves were carefully exposed and placed on silver thermodes, 2.5 cm long, through which water at 0.5°C could be circulated. When the nerves were cooled, chilled saline (0.9% w/v NaCl solution) swabs were placed on the nerves which passed over the thermodes.

In three dogs both cervical vagosympathetic nerves were cut and the sheath removed from the peripheral cut end of the right vagosympathetic nerve with the

aid of a dissecting microscope. The sympathetic fibres were split away and the remaining parasympathetic nerve placed on bipolar electrodes under warm liquid paraffin. The electrodes were connected to a square wave stimulator (Devices) which was set to deliver pulses of 1 ms at a frequency of 20 Hz. The voltage was adjusted to obtain a maximal response on R_L.

The body temperature of the dogs was maintained at 38.5°C with a thermostatically controlled heating blanket in conjunction with a rectal thermocouple.

The partial pressures of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}) in the blood and the blood pH were monitored regularly with a Radiometer ABL 1 acid-base analyser. By adjusting the ventilation of the animal at the beginning of the experiment, or during the control periods, it was possible to maintain $P_{O_2} > 13.3$ kPa, P_{CO_2} between 3.32 kPa and 5.32 kPa and the pH between 7.36 and 7.46 Pa.

Air flow rate was measured with a Fleisch pneumotachograph (Type 0; 9.8 Pa = 43.16 ml/s) and a Furness Controls micromanometer (100–0–100 Pa) and tidal volume obtained by electrical integration of the flow signal with a Devices integrator. TPP was measured by a Furness Controls micromanometer (1–0–1 kPa) one side of which was connected to the tracheal cannula and the other side of which was left open to the atmosphere. All recordings were displayed on a Devices M19 recorder. Total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were measured by a manual graphic method using the displayed signs of flow, volume and TPP (Amdur & Mead, 1958). The respiratory computer described by Carney, Pugh & Sheard (1972) was also used for the calculations of R_L and C_{dyn}, its displayed output being calibrated and checked for accuracy by comparison with simultaneous manual determinations of R_L and C_{dyn}. The computer was accurate to ± 0.02 kPa l^{-1} s for R_L and ± 6.2 ml kPa^{-1} for C_{dyn}.

Aerosols were generated on inspiration only using a Vaponefrin inhalajet nebuliser modified to deliver an aerosol containing mainly large particles (12.8 μm).

Experimental procedure

Aerosol study. Bronchoconstriction was produced by allowing the dogs to inhale 4 breaths of histamine aerosol. The concentration of histamine solution was selected from a range of 0.0625 to 0.25% such that a change in R_L of 0.98–1.96 kPa l^{-1} s was produced. At the peak of the change in R_L both cervical vagi were cooled to 0.5°C to determine the reflex component of the bronchoconstriction.

Histamine challenges were given every 30 min until 4 consistent bronchoconstrictions had been produced. Four breaths of an aerosol of SCG generated from either 1% or 2% solutions were then given 10 min before the next histamine challenge. The SCG aerosol was generated by a nebuliser similar to that used to generate the histamine aerosols. Further control histamine challenges were given every 30 min for at least 2 hours.

Solutions of SCG greater than 2% concentration are viscous and were not used in this study since difficulty was encountered in the generation of aerosols.

Intravenous study. Two to four breaths of histamine aerosol of appropriate concentration were given to produce an increase in R_L greater than 0.98 kPa l^{-1} s.

The reflex nature of this resistance change was checked by rapidly cooling the cervical vagi. The nerves were then quickly rewarmed to 38.5°C to re-establish the bronchoconstriction and when the change in R_L was seen to be stable, SCG was given intravenously as a bolus injection. At least 30 min were allowed between histamine challenges.

The effects of sodium cromoglycate given by aerosol and intravenously on the increase in R_L produced by stimulation of the right vagus nerve. The right cervical vagus nerve was stimulated every 15 min for 20 s until 3 consistent increases in R_L were seen. Ten minutes before the next stimulation 4 breaths of an aerosol of SCG generated from a 2% solution were given. Intermittent nerve stimulation was continued for at least a further 30 minutes.

In the same dogs, at least 2 h later, the right cervical vagus nerve was again stimulated until three consistent increases in R_L were seen; SCG 100 μg was then given intravenously 70 s before the next stimulation.

Drugs

The drugs used were: thiopentone sodium (Intraval, May and Baker Limited); α-chloralose (Koch-Light Laboratories); sodium cromoglycate (Fisons Limited); histamine acid phosphate (BDH); isoprenaline hydrochloride (Suscardia, Pharmax); propranolol hydrochloride (Inderal, ICI).

Drug solutions were freshly prepared in isotonic saline and concentrations are given in terms of the bases.

Results

Aerosol study

The results of this study are shown in Figure 1. An aerosol of SCG generated from a 1% solution produced some reduction in the vagally mediated increase in R_L induced by histamine aerosol, although this was not statistically significant ($P > 0.05$). However, an aerosol of SCG generated from a 2% solution produced a significant reduction ($P < 0.05$) in

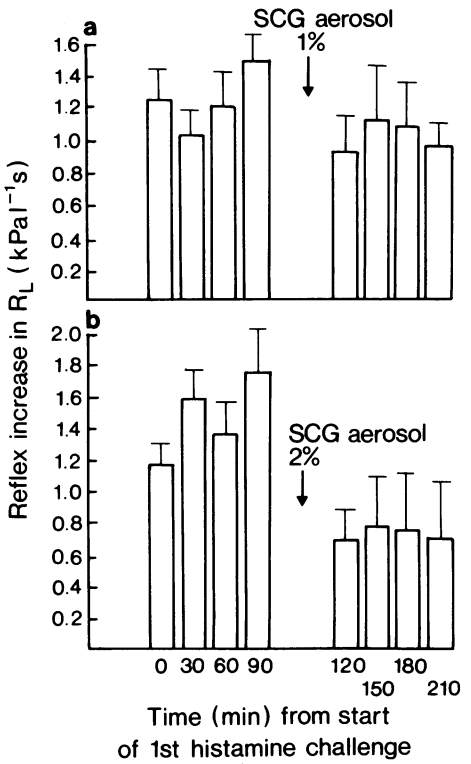

Figure 1 The effect of 4 breaths of an aerosol of sodium cromoglycate (SCG) generated in (a), from a 1% w/v solution and in (b), from a 2% w/v solution on the reflex increases in total lung resistance (R_L) induced by histamine aerosol. Bars are s.e. mean; $n=6$ for 1% aerosol and 4 for 2% aerosol.

the vagally mediated change in R_L and this reduction lasted for at least 2 hours. In one dog the response to histamine aerosol reverted to control values after 3 hours. Statistical comparison was made with the response prior to SCG.

SCG given by aerosol did not produce any cardiovascular changes.

In 5 dogs, 4 breaths of a saline aerosol were given as a control. This did not affect the resting values of R_L or C_{dyn}, nor did it affect the response of the airways to subsequent histamine challenges.

Intravenous study

The effect of 500 μg/kg SCG intravenously on a sustained bronchoconstriction is shown in Figure 2. The effects of 100, 50, 25, 10 and 5 μg/kg SCG (i.v.) on similar reflex bronchoconstrictions have also been investigated and the results are shown in Table 1, together with the changes in mean arterial pressure. SCG 5–50 μg/kg produced dose-related reversals of the reflex bronchoconstriction; 100% reversal occurring with 50, 100, and 500 μg/kg. The maximal effect of SCG occurred 70 s after injection and lasted for at least 5 minutes. In these experiments the duration of action of SCG could not be accurately determined because the falls in C_{dyn} induced by histamine necessitated reversal by hyperinflation.

SCG 5 μg/kg did not affect mean arterial blood pressure, but higher doses caused significant falls.

To eliminate the possibility that SCG was reversing the reflex bronchoconstriction by releasing endogenous sympathomimetic amines, we investigated the action of SCG in the presence of the β-adrenoceptor blocking drug, propranolol. In 3 dogs a reflex bronchoconstriction was produced and a partial reversal obtained by giving SCG 25 μg/kg intravenously. The remaining increase in R_L was then reversed with isoprenaline (500 ng/kg). Propranolol (500 μg/kg i.v.) was then given and 30 min later a reflex bronchoconstriction established. When SCG 25 μg/kg was given (i.v.) a partial reversal of the bronchoconstriction was again seen. However, the remaining increase in R_L was not reversed by isoprenaline. The records of one such investigation are shown in Figure 3.

The effects of sodium cromoglycate on the increases in R_L produced by stimulation of the right vagus nerve

The results of this study are shown in Table 2. Neither four breaths of an aerosol of SCG generated from a 2% solution nor 100 μg/kg SCG given intravenously affected the increases in R_L produced by electrical stimulation of the right vagus nerve; the resting values of R_L were similarly unaffected.

Electrical stimulation of the right cervical vagus caused cardiac arrest with subsequent 'vagal escape'.

Discussion

Four breaths of an aerosol of SCG generated from a 2% solution significantly reduced the reflex bronchoconstriction to histamine aerosol, and SCG, 5–500 μg/kg intravenously produced dose-dependent reversals of sustained reflex bronchoconstrictions. SCG did not affect the bronchoconstriction produced by the direct electrical stimulation of the right vagus nerve, nor did it affect the basal total lung resistance. Although it is reasonable to propose that the modes of action of the drug, given by aerosol and by the intravenous route were the same, the results of this study cannot establish this. One significant difference between the intravenous route and aerosol routes of administration is that in the anaesthetized dog SCG given intravenously, as a bolus injection in doses of 10 μg/kg or more, produces a fall in arterial

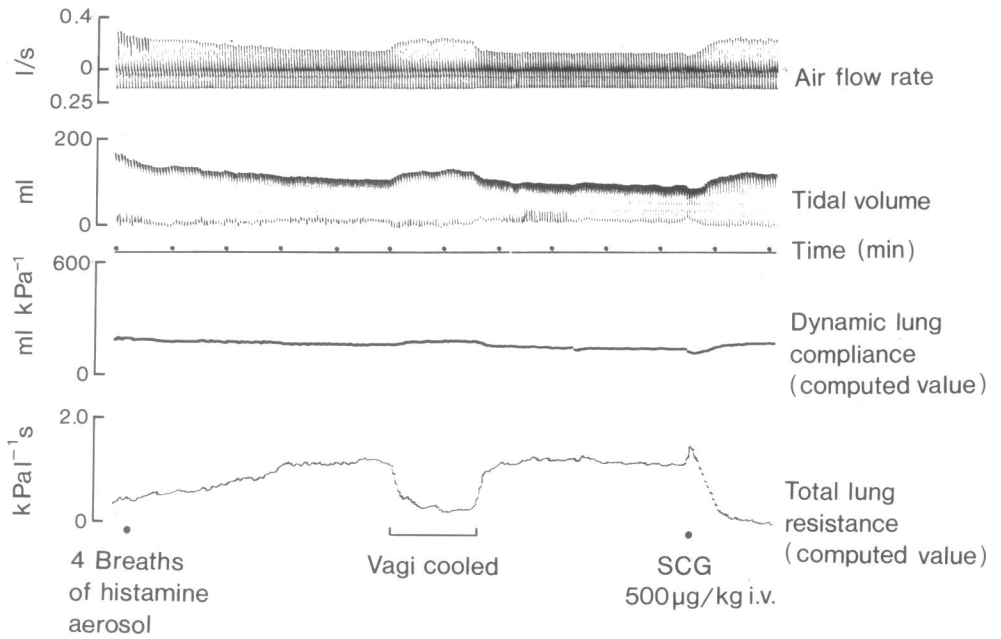

Figure 2 The effects of sodium cromoglycate (SCG) 500 µg/kg intravenously on a sustained reflex bronchoconstriction induced in a dog anaesthetized with chloralose.

blood pressure and heart rate (Cox *et al.*, 1970). This response depends on the vagus nerves and is similar to the response to the veratrum alkaloids described by Bezold & Hirt (1867) and Jarisch & Richter (1939). It was thought possible that the fall in blood pressure produced by SCG in the present experiments, when it was given intravenously, may have caused the release of endogenous sympathomimetic amines and that these could have been responsible for the reversal of the bronchoconstriction. However, SCG was still active in dogs which had received 500 µg/kg propranolol and in which the airways did not respond to isoprenaline (Figure 3).

These observations coupled with the result that SCG was active when given by aerosol, when a reflex fall in blood pressure and heart rate did not occur, make it unlikely that the cardiovascular changes produced by SCG are in any way connected with the reversal of the reflex bronchoconstriction. It therefore seems most likely that the mechanisms of the inhibition or reversal of the bronchoconstriction by SCG were the same whichever route of administration was employed.

Cox *et al.* (1970) reported that SCG does not act directly on airways smooth muscle and is not a pharmacological antagonist of the actions of histamine or acetylcholine. Although an action on the CNS cannot be entirely ruled out, SCG has been administered by a variety of routes (i.v., i.m., inhalation, conjunctival sac, intranasal) to the dog in single and multiple doses ranging from 1–20 mg/kg and the highest amount of SCG ever found in the total brain tissue in all these studies was only 0.09% of the dose. Even a proportion of this was probably due to a certain amount of contamination of the brain tissue by blood (personal communication from Dr B. Clark, the Department of Metabolic Studies, Fisons Limited). Further indirect evidence for lack of CNS penetration by SCG is given by Cox *et al.* (1970) and Ashton, Clark, Jones, Moss, Neale & Ritchie (1973). Our experiments further showed that SCG given by aerosol or intravenously did not modify the effects of direct electrical stimulation of the efferent vagus nerves to the lung. It is likely, therefore, that SCG reduced the reflex bronchoconstriction to histamine by an action on the afferent arm of the reflex. Mills, Sellick & Widdicombe (1969), Gold, Kessler & Yu (1972) and Sampson & Vidruk (1975) have suggested that the lung irritant receptors could participate in the afferent limb of histamine-induced reflex bronchoconstriction. It is possible that SCG might reduce the activity of lung irritant receptors; such an action would explain the inhibition or reversal of a reflex bronchoconstriction seen in these experiments. Only by measuring the effect of SCG on action potentials from single vagal fibres originating from lung irritant receptors could this hypothesis be confirmed.

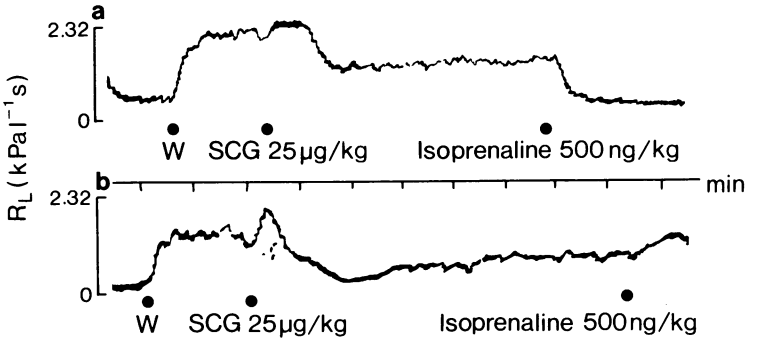

Figure 3 The effects of sodium cromoglycate (SCG) 25 μg/kg intravenously and isoprenaline 500 ng/kg intravenously on a sustained reflex bronchoconstriction; (a) in the absence of propranolol and (b) in the presence of 500 μg/kg propranolol. The records were taken from the same dog. Histamine was administered by aerosol prior to this record. The vagi were cooled to confirm the reflex component of the bronchoconstriction and were rewarmed at W after which bronchoconstriction became re-established.

Table 1 The effects of intravenous sodium cromoglycate (SCG) on histamine-induced reflex bronchoconstriction and on mean arterial blood pressure

Dose of SCG (μg/kg i.v.)	*% reversal of histamine induced bronchoconstriction mean ± s.e. mean*	*Fall in mean arterial blood pressure (mmHg) mean ± s.e. mean*	n
5	17 ± 2.1	No change	4
10	26 ± 4.0	36.6 ± 6.4	4
25	51.3 ± 9.7	48.8 ± 13.4	4
50	100	28.3 ± 14.8	3
100	100	70.0 ± 5.8	3
500	100	66.0 ± 13.0	3

Table 2 The effects of sodium cromoglycate (SCG) given by aerosol or intravenously on the increase in R_L produced by electrical stimulation of the right vagus

Time (min) of event	*Resting R_L (kPa l^{-1} s)*	*Increase in R_L (kPa l^{-1} s) produced by *stimulation of R. cervical vagus*
0	0.17 ± 0.03	1.24 ± 0.28
15	0.17 ± 0.02	1.23 ± 0.29
20	*4 breaths of an aerosol of SCG generated from a 2% solution*	
30	0.18 ± 0.03	1.28 ± 0.29
45	0.17 ± 0.03	1.13 ± 0.33
	Intravenous SCG	
0	0.15 ± 0.02	0.63 ± 0.07
13 min 50 s	100 μg/kg SCG i.v.	
15	0.15 ± 0.02	0.63 ± 0.08

Values are mean ± s.e. mean. $n=3$ (aerosol); $n=5$ (i.v.). * Stimulus parameters were 20 Hz, 1 ms, supramaximal voltage for 20 seconds.

There has been some controversy over the role of the vagus nerves in allergic bronchoconstriction (Gold, 1973). Widdicombe and his colleagues have studied the role of vagally mediated reflexes in the response to systemically administered antigen in guinea-pigs and rabbits. Using single fibre recordings from lung irritant receptors and the analysis of lung dynamics during anaphylaxis, they demonstrated conclusively that the vagus plays an important role in the production of anaphylactic bronchoconstriction (Karczewski & Widdicombe, 1969; Mills, Sellick & Widdicombe, 1969; Mills & Widdicombe, 1970). It is likely, therefore, that a drug which reduced the sensitivity of lung irritant receptors in man would go some way to reliving an asthmatic attack. The results of our experiments suggest that SCG may reduce the activity of lung irritant receptors in the anaesthetized dog. This action may be relevant to the way in which the drug acts in asthma.

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References

AMDUR, M.O. & MEAD, J. (1958). Mechanics of respiration in unanaesthetized guinea-pigs. *Am. J. Physiol.*, **192**, 364–368.

ASHTON, M.J., CLARK, B., JONES, K.M., MOSS, G.F., NEALE, M.G. & RITCHIE, J.T. (1973). The absorption, metabolism and excretion of Disodium Cromoglycate in nine animal species. *Toxic. appl. Pharmac.*, **26**, 319–328.

BEZOLD, A. VON & HIRT, L. (1867). Über die physiologischen Wirkungen des essigsäuren Veratrins. *Unter. Physiol. Lab. Wurzburg.*, **1**, 73–156.

CARNEY, I., PUGH, I. & SHEARD, P. (1972). Application of an inexpensive analogue computer for the continuous determination of airways dynamic compliance and resistance. *Br. J. Pharmac.*, **46**, 578–579P.

COX, J.S.G., BEACH, J.E., BLAIR, A.M.J.N., CLARKE, A.J., KING, J., LEE, T.B., LOVEDAY, D.E.E., MOSS, G.F., ORR, T.S.C., RITCHIE, J.T. & SHEARD, P. (1970). Disodium Cromoglycate (Intal). *Adv. Drug Res.*, **5**, 115–193.

Editorial (1972). Disodium Cromoglycate in Allergic Respiratory Disease. *Br. med. J.*, **2**, 159–161.

GOLD, W.M. (1973). In *Asthma: Physiology, Immunopharmacology and Treatment*, ed. Austein, K.F. & LICHENSTEIN, L.M., pp. 169–184. New York: Academic Press.

GOLD, W.M., KESSLER, G.F. & YU, D.Y.C. (1972). Role of vagus nerves in experimental asthma in allergic dogs. *J. appl. Physiol.*, **33**, 719–725.

JACKSON, D.M. & RICHARDS, I.M. (1976). A further action of sodium cromoglycate. *Br. J. Pharmac.*, **58**, 301P.

JARISCH, A. & RICHTER, H. (1939). Der Bezold-Effect, eine vergessene Kreislangreaktion. *Klin. Wschr.*, **1**, 185–187.

KARCZEWSKI, W. & WIDDICOMBE, J.G. (1969). The role of the vagus nerves in the respiratory and circulatory reactions to anaphylaxis in rabbits. *J. Physiol., Lond.*, **201**, 292–304.

MILLS, J.E., SELLICK, H. & WIDDICOMBE, J.G. (1969). Activity of lung irritant receptors in pulmonary microembolism, anaphylaxis and drug-induced bronchoconstrictions. *J. Physiol., Lond.*, **203**, 337–357.

MILLS, J.E. & WIDDICOMBE, J.G. (1970). Role of the vagus nerves in anaphylaxis and histamine-induced bronchoconstrictions in guinea-pigs. *Br. J. Pharmac.*, **39**, 724–731.

SAMPSON, S.R. & VIDRUK, E.H. (1975). Properties of irritant receptors in canine lung. *Resp. Physiol.*, **25**, 9–22.

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