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THE EFFECTS OF SODIUM CROMOGLYCATE ON LUNG IRRITANT RECEPTORS AND LEFT VENTRICULAR CARDIAC RECEPTORS IN THE ANAESTHETIZED DOG

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1 The time from the injection of sodium cromoglycate 10 to 50 μg/kg into a saphenous vein, the cervical carotid arteries, the left ventricle and the aortic arch, to the onset of reflex hypotension has been measured in anaesthetized dogs. The shortest latency was 16.9 s on injection of sodium cromoglycate into the left ventricle.

2 Instillation of 2% lignocaine into the pericardium of an anaesthetized dog blocked the reflex hypotensive response to sodium cromoglycate (10 to 50 μg/kg i.v.), and also prevented sodium cromoglycate (100 μg/kg) from reversing reflex bronchoconstriction induced by inhalation of an aerosol of histamine.

3 The effect of sodium cromoglycate (100 μg/kg i.v.) on resting discharge and histamine-induced discharge (20 μg/kg i.v.) of five lung irritant receptors in five anaesthetized dogs has been studied. Sodium cromoglycate (100 μg/kg i.v.) did not affect the resting discharge of these receptors or their ability to respond to histamine.

4 Sodium cromoglycate (100 μg/kg i.v.) increased the rate of discharge of three receptors found in the endocardium of the left ventricle of the canine heart. A solution of sodium cromoglycate (0.1%) was applied topically to one receptor and its rate of discharge was increased.

5 It is suggested that in the dog, sodium cromoglycate produces reflex hypotension and reverses histamine-induced reflex bronchoconstriction by activating receptors in the left ventricle of the heart.

Introduction

Sodium cromoglycate, a drug used in the treatment of asthma, can reverse reflex bronchoconstriction in the anaesthetized dog, and it has been suggested that this reversal is the result of an action on lung irritant receptors (Jackson & Richards, 1977). Cox, Beach, Blair, Clarke, King, Lee, Loveday, Moss, Orr, Ritchie & Sheard (1970) and Jackson & Richards (1977) have also reported that sodium cromoglycate, given intravenously in bolus doses, to the anaesthetized dog produces a reflex hypotension and bradycardia which is mediated via the vagus nerves. The purpose of this study was to elucidate these responses by investigating the nervous receptors involved. The results of four different types of experimental study are described in this paper. They are: the location of the receptors responsible for initiating the hypotension and bradycardia by measurement of response onset times; the effects of instillation of the local anaesthetic, lignocaine, into a pericardial sac on both the cardiovascular and respiratory responses to sodium cromoglycate; the effect of sodium cromoglycate on lung irritant receptor activity; and the effect of sodium cromoglycate on receptors found in the endocardium of the left ventricle of the heart. The results allow an explanation of how sodium cromoglycate reverses reflex bronchoconstriction and of the cardiovascular actions of the drug in the anaesthetized dog.

Methods

Measurement of latencies to injections of sodium cromoglycate

Beagle dogs (9 to 12 kg) of either sex were anaesthetized with pentobarbitone sodium (30 mg/kg i.v.) and intubated. The right and left saphenous veins were catheterized for drug administration and for infusion of pentobarbitone sodium (0.1 mg kg^{-1} min^{-1}). The muscularis branch of the right femoral artery was catheterized for recording blood pressure with a Statham P23Db pressure transducer, and heart rate

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was derived from this signal with a Devices instantaneous rate meter. Arterial blood pressure and heart rate were recorded on a M19 Devices multichannel recorder.

Catheters were passed via the left femoral artery into the left ventricle and the aortic arch. Catheters were also placed in both cervical carotid arteries by direct puncture. In some dogs the chest was opened and a branch of the left coronary artery was catheterized. The exact position of the catheter tips was judged by pressure patterns and confirmed *post mortem*.

A dose–response relationship for the hypotensive response to intravenous sodium cromoglycate was obtained in each dog. Each dose volume was 0.5 ml and was washed in with 2.0 ml isotonic saline, with a total injection time of 3 to 5 s. The dose range of sodium cromoglycate used was 10 to 50 μg/kg and doses were given every 15 min to minimize tachyphylaxis. An intravenous ED_{50} dose was then selected and was given in turn intravenously, into the left ventricle, into the coronary bed, into the aortic arch and into the carotid arteries. The time from the end of the injection to the onset of the hypotensive response was measured from the paper trace running at a speed of 1 cm/s.

Measurement of the effects of lignocaine infiltration into the pericardial sac on the cardiovascular and respiratory effects of sodium cromoglycate

Beagle dogs (9 to 14 kg) of either sex were anaesthetized with chloralose (80 mg/kg i.v. initially followed by 10 to 12 mg/kg i.v. every 15 min) and prepared for the measurement of total lung resistance (R_L), dynamic lung compliance (C_{dyn}) and also arterial blood pressure. R_L and C_{dyn} were measured by a manual graphic method using the displayed signals of flow, volume, and transpulmonary pressure (P_{TP}) (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 for C_{dyn}. The dogs were artificially ventilated at constant pressure (0.98 kPa) and a thoracotomy was routinely performed (for complete experimental details see Jackson & Richards, 1977). Aerosols were generated on inspiration only, using a Vaponefrin inhalajet nebuliser modified to deliver an aerosol containing mainly large particles (12.8 μm).

An incision was made in the pericardium over the left atrium and an edge of this incision was retracted with a thread to form a pouch.

Two to four lung inflations of histamine aerosol generated from 0.125 or 0.25% solutions were given to produce an increase in R_L greater than 0.98 kPa l^{-1}s. The reflex basis of this resistance change was checked by rapidly cooling the cervical vagi to 0.5°C. 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, sodium cromoglycate (100 μg/kg) was given intravenously as a bolus injection.

Twenty-five min later a 2% solution of lignocaine was infiltrated into the pericardial sac and 5 min after this a reflex bronchoconstriction was produced with histamine aerosol. Sodium cromoglycate (100 μg/kg) was again given intravenously as a bolus injection during the sustained reflex bronchoconstriction. The lignocaine was then washed from the pericardial sac with isotonic saline and 30 min later sodium cromoglycate was tested against another histamine-induced reflex bronchoconstriction.

On two occasions a 1 mg/ml solution of sodium cromoglycate was infiltrated into the pericardial sac during a reflex bronchoconstriction produced by histamine aerosol.

Measurement of the effects of sodium cromoglycate on lung irritant receptors and left ventricular cardiac receptors

Beagle dogs of either sex (9 to 14 kg) were initially prepared as in the preceding section above. The dogs were then paralysed with succinylcholine (150 μg/kg i.v. every 15 min) and the cervical vagi were cut. The distal end of the left vagus was placed in a trough filled with liquid paraffin. 'Single fibres' were teased from the nerve trunk and placed on platinum electrodes. The nerve action potentials were amplified by a high gain RC amplifier and together with P_{TP}, tidal volume and blood pressure were recorded on magnetic tape using a seven channel recorder (Ampex SP300). Five dogs were used to study lung irritant receptors and eight dogs to study left ventricular receptors.

Lung irritant receptors Two criteria for selection of fibres from lung irritant receptors were used. First, the receptor was stimulated by and adapted to an inflation of 1 kPa and a deflation of −1 kPa. Any fibre having an inflation adaptation index of less than 70% was discarded.

$$\text{Adaptation index} = \frac{\text{peak frequency} - \text{average frequency during 2nd second of inflation}}{\text{peak frequency}} \times 100\%$$

(Widdicombe, 1954). The adaptation index provides a mathematical definition of 'rapid adaptation' but it can only be used reliably when the receptors receive constant stimulation during its measurement. Care was therefore taken to ensure that the lungs received a constant inflation of 1 kPa when the receptors were tested for their rate of adaption. Secondly, each receptor was located in the airways by touching the lung gently with the fingers and finally locating the receptor with a cotton wool bud. All receptors selected fired irregularly during a respiratory cycle. Irritant receptor activity was measured by electronically counting action potentials over consecutive 15 s periods throughout the experiment.

When a suitable fibre had been selected the effect of intravenous histamine 20 μg/kg was tested. The receptor discharge was then allowed to return to control values and sodium cromoglycate (100 μg/kg i.v.) given. Two min later the response to histamine (20 μg/kg i.v.) was retested.

Generally irritant receptor discharge was recorded for a control period of 1 min, the drug was given and the change occurring over the following 2 min recorded. The maximal drug-induced change in the rate of discharge is given in this paper.

Left ventricular receptors Filaments were teased from the cervical vagus and placed on a pair of platinum recording electrodes. Possible activity in the filaments was tested by palpation of the left ventricular wall followed by intravenous injection of sodium cromoglycate (100 μg/kg). To minimize tachyphylaxis, sodium cromoglycate was not given more frequently than every 15 min. Nerve filaments which responded to these stimuli, where necessary, were further subdivided to obtain 'single fibre' preparations or preparations which gave records from two or three fibres. The effects of sodium cromoglycate (100 μg/kg i.v.) on the rate of discharge of the receptor were tested.

The receptors used in the study were confirmed to be in the left ventricle *post mortem*. This confirmation was obtained by carefully opening the left ventricle at its apex and gently probing the endocardial surface until the receptor was seen to discharge. After location the ventricle was cut away until receptor activity ceased. On one occasion a solution of sodium cromoglycate (1 mg/ml) was applied on a cotton wool bud to the receptor immediately after the ventricle had been opened and the receptor located.

Results

(For R_L $cmH_2O/l/s \times 0.1 \equiv kPa\ l^{-1}s$; for C_{dyn} $ml/cmH_2O \times 10 \equiv ml/kPa$; for $mmHg \times 0.133 \equiv kPa$).

Response-onset times of sodium cromoglycate

Sodium cromoglycate (10 to 50 μg/kg i.v.) produced dose-related falls in mean arterial blood pressure (1.5 to 9.5 kPa; ED_{50} response being approximately 4.0 kPa). The times from completion of the injection of an ED_{50} dose of sodium cromoglycate into the left ventricle, intravenously, or into the coronary vascular bed and the start of the hypotension were 16.9 ± 0.6 s ($n = 24$); 22.6 ± 0.6 s ($n = 32$) and 21.1 ± 2.1 s ($n = 11$) respectively (means ± s.e., 8 dogs). Thus injection directly into the left ventricle produced the shortest response-onset time ($P < 0.01$).

Injection of an intravenous ED_{50} dose of sodium cromoglycate into the carotid arteries and aortic arch produced only very small falls in blood pressure (approximately one tenth of that seen after giving the same dose i.v.) and the latencies were greater than 25 s suggesting that the drug had circulated once before an effect was produced.

The falls in blood pressure produced by sodium cromoglycate given by any of the selected routes were accompanied by bradycardia (5 to 80 beats/min) and the responses were abolished by bilateral cervical vagotomy.

The effects of infiltration of lignocaine into the pericardial sac on the respiratory and cardiovascular responses of sodium cromoglycate

In five dogs reflex bronchoconstriction produced by 2 to 4 inhalations of an aerosol of histamine generated from a 0.125 or 0.25% solution was completely reversed by intravenous sodium cromoglycate 100 μg/kg. Subsequent instillation of 2% lignocaine into the pericardial sac 5 min before the next administration of histamine aerosol did not affect the ability of histamine to produce a reflex bronchoconstriction but it did prevent sodium cromoglycate 100 μg/kg given intravenously from reversing the bronchoconstriction. The hypotension and bradycardia seen when sodium cromoglycate was given intravenously was also abolished. Lignocaine instillation lowered heart rate by 20 to 40 beats/min but did not affect mean arterial blood pressure. Thirty min after washing lignocaine from the pericardial sac the cardiovascular response to and reversal of reflex bronchoconstriction by sodium cromoglycate was recovered.

The effects of sodium cromoglycate on lung irritant receptors

Before sodium cromoglycate, histamine (20 μg/kg i.v.) increased the rate of discharge of 5 irritant receptors from 5 dogs from 39 ± 31 impulses/15 s to 229 ± 84 impulses/15 s; 2 min after sodium cromoglycate (100 μg/kg i.v.) histamine (20 μg/kg i.v.) increased the rate

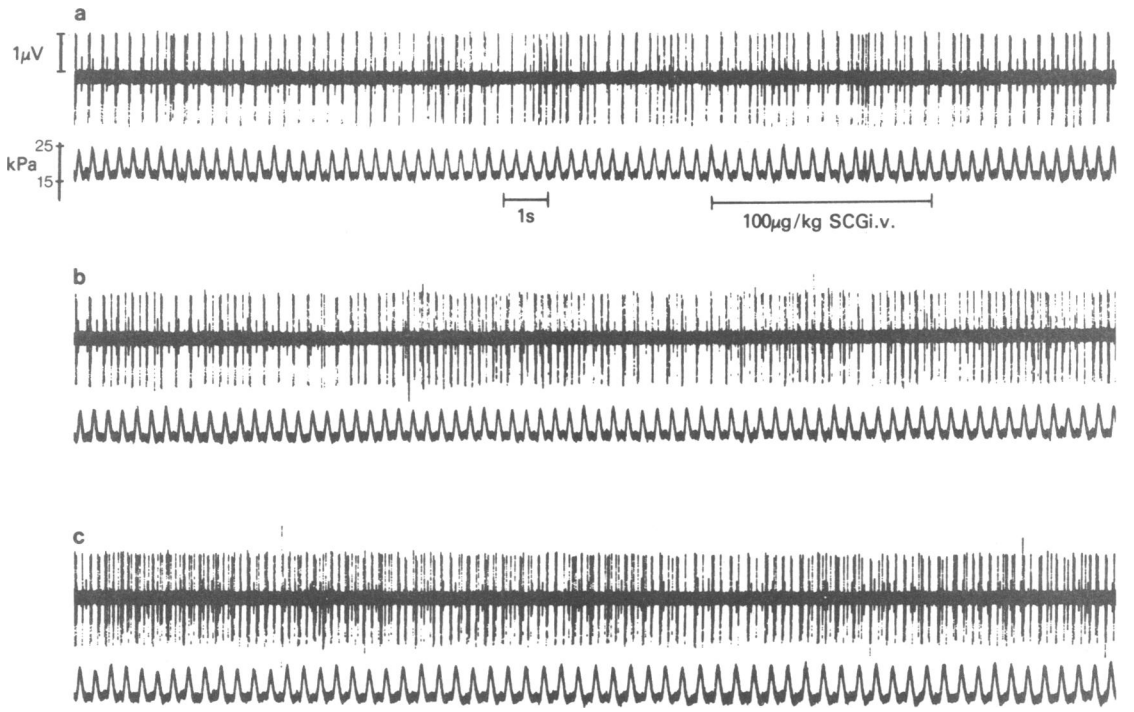

Figure 1 Upper trace: action potentials from a nerve bunch containing a fibre originating from a left ventricular receptor (confirmed *post mortem*). Lower trace: femoral arterial pulse. The ventricular receptor increased its rate of discharge after 100 μg/kg sodium cromoglycate (SCG) given intravenously; (a), (b) and (c) are consecutive.

of discharge of the same receptors from 34 ± 28 impulses/15 s to 238 ± 91 impulses/15 s. There is no significant difference between these two changes ($P > 0.05$).

Sodium cromoglycate (100 μg/kg i.v.) did not significantly ($P > 0.05$) affect the resting discharge of these receptors (38 ± 31 impulses/15 s before sodium cromoglycate, 39 ± 32 impulses/15 s after sodium cromoglycate. All values given are mean ± s.e., $n = 5$.

The effects of sodium cromoglycate on left ventricular cardiac receptors

Three left ventricular receptors were studied after recording from several hundred cardiac fibres from 8 dogs. The receptors had a cardiac rhythm and were excited by intravenous sodium cromoglycate 100 μg/kg (Figure 1). The three receptors were located in the endocardium of the left ventricle near the aortic orifice. In one case where the receptor remained active for a sufficient time after the 'death' of the dog it was activated by dropping a 0.1% solution of sodium cromoglycate onto the area of endocardium where mechanical distortion had produced an increased discharge.

Discussion

Sodium cromoglycate given intravenously and by aerosol can reverse and prevent reflex bronchoconstriction in the anaesthetized dog and it has been suggested that suppression of lung irritant receptor activity is responsible for this response (Jackson & Richards, 1977). Our present study shows sodium cromoglycate to have no effect on the resting discharge of lung irritant receptors, or on the ability of these receptors to respond to intravenous histamine.

In the anaesthetized dog, sodium cromoglycate given intravenously as a bolus dose (> 10 μg/kg) produces transient hypotension and bradycardia which is mediated via the vagus nerves (Cox *et al.*, 1970; Jackson & Richards, 1977). Measurements of latencies for the onset of hypotension, after injections of sodium cromoglycate at different sites in the cardiovascular system of anaesthetized dogs showed that delivery of the drug to the left ventricle produced the shortest latency (16.9 s). When sodium cromoglycate was injected directly into the coronary vascular bed there was a significantly longer latency period (21.1 s) suggesting that the sodium cromoglycate-sensitive nerve endings are not supplied directly by the coron-

ary circulation. In the dog only a very small portion of the coronary circulation drains into the left ventricle by the small cardiac veins (Miller, 1964) and if the sodium cromoglycate-sensitive receptors were located on the surface of the endocardium of the left ventricle, it is consistent that injection of the drug directly into this chamber produced a shorter latency for the response than injection into the left coronary artery. An electrophysiological investigation confirmed the presence of sodium cromoglycate-sensitive nerve endings in the endocardium of the left ventricle. The rather long time of 16.9 s for the onset of the hypotension even when the drug was injected into the left ventricle, the receptor site, is not unusual. Paintal (1955) observed that some sensory receptors in the left atria of cats had a delay of onset of activity of some 60 to 70 s when stimulated by veriloid.

There are at least two types of sensory receptors in the walls of the cardiac ventricles, the ventricular pressure receptors with medullated fibres and epicardial receptors with non-medullated fibres (Coleridge, Coleridge & Kidd, 1964; Sleight & Widdicombe, 1965). The ventricular receptors used in this study had similarities with ventricular pressure receptors; they fired rhythmically with heart rate, and they were located in the endocardium of the left ventricle. (Coleridge *et al.*, 1964, when studying ventricular pressure receptors found two in the endocardium). They differ in that they were found only in the left ventricle (Coleridge *et al.*, 1964, found ventricular pressure receptors in the right and left ventricles) and when activated by sodium cromoglycate they produced a Bezold-Jarisch like effect (Krayer, 1961). Coleridge *et al.* (1964) found that ventricular receptors (3 were studied) were unaffected by veratridine and Paintal (1972) concluded that ventricular receptors in the dog, unlike those in the cat, are not responsible for producing the Bezold-Jarisch effect. It is possible that the sensory receptors in the endocardium of the left ventricle, sensitive to sodium cromoglycate are a specialised group of ventricular pressure receptors.

The instillation of a local anaesthetic into the pericardial sac of the dog has been shown to suppress the electrical activity (measured in a thoracic vagal fibre) originating from ventricular receptors (Sleight & Widdicombe, 1965). This may be because of a direct action of the local anaesthetic on the receptors or more probably by an action on the afferent nerves as they leave the heart. In our study, a 2% solution of lignocaine instilled into the pericardial sac prevented sodium cromoglycate from producing hypotension and reversing histamine-induced reflex bronchoconstriction. It is reasonable to assume, therefore, that the afferent receptors involved in both these responses are the same and, from the results discussed previously, are situated in the endocardium of the left ventricle. It is unlikely that the hypotension, *per se*, is responsible for the relief of reflex bronchoconstriction since the two effects do not appear to be related in magnitude. For example, a 17% reversal in reflex bronchoconstriction is seen with 5 μg/kg sodium cromoglycate intravenously but there is no hypotension (Jackson & Richards, 1977).

Aerosol administration of sodium cromoglycate has been shown to prevent histamine-induced reflex bronchoconstriction without cardiovascular changes (Jackson & Richards, 1977), but there is no evidence from our present study to confirm that sodium cromoglycate aerosol can prevent reflex bronchoconstriction by activating left ventricular receptors. Sodium cromoglycate is absorbed from the lung (Cox *et al.*, 1972) and after aerosol administration it could theoretically reach the left ventricle in sufficient quantity to activate the receptors in the endocardium. Evidence in support of this proposed mode of action could be obtained by testing other aerosol-administered drugs for their activity against reflex bronchoconstriction.

Because of the difficulty encountered in finding sodium cromoglycate-sensitive receptors in the left ventricle for this study (Coleridge *et al.*, 1964, had similar difficulties searching for ventricular pressure receptors) we did not test the effects of other drugs on them. There is therefore not yet an alternative drug to sodium cromoglycate which could be used and tested by aerosol administration.

The mechanism by which left ventricular receptor activation prevents or reverses reflex bronchoconstriction is difficult to explain. One may speculate that increased nerve activity from the receptors in the left ventricle suppresses central nervous activity associated with reflex bronchoconstrictor mechanisms in the brain stem. The ability of sensory receptors primarily associated with one organ significantly to affect another is common and the role drugs may play in this interaction has been reviewed by Ginzel (1975).

The results of this study provide evidence for the existence of receptors in the endocardium of the left ventricle of the heart of dogs which can be activated by sodium cromoglycate and produce reflex hypotension and reverse reflex bronchoconstriction. The possibility exists that there are receptors in the heart which control, or modify the control, of airway calibre and hence lung function. This may be a normal physiological control mechanism or a mechanism by which certain drugs can affect the airways.

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