Br. J. Pharmac. (1980), **70**, 419–423

A MODEL OF REFLEX TRACHEAL CONSTRICTION IN THE DOG

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1 A tracheal pouch with its nerve and blood supply intact has been prepared *in situ* in dogs.

2 Mechanical stimulation of the upper airways in dogs anaesthetized with chloralose induced a consistent increase in pouch pressure which was abolished by bilateral vagal section.

3 The response of the pouch following mechanical stimulation of the airways was abolished by intravenous pentobarbitone, atropine, administered systemically or when present in the pouch, and tetracaine, applied to the stimulus area or when present in the pouch.

4 Salbutamol had no inhibitory effects on the response regardless of its route of administration.

5 These results suggest that the increase in pouch pressure following mechanical stimulation of the upper airways is mediated by a vagal reflex arc.

6 The technique may distinguish between drugs the site of action of which is at the afferent or efferent end of this reflex arc.

Introduction

Following the characterization by Widdicombe (1954) of rapidly adapting stretch, or irritant, receptors in the airways, there has been a growing interest in the role of the reflex activated by these receptors in the pathogenesis of reversible airways obstruction. Animal models used to study parasympathetic reflex bronchoconstriction have produced conflicting results. For example, Gold, Kessler & Yu (1972) showed that a vagal reflex was largely responsible for the increase in airways resistance following inhalation of antigen in allergic dogs, whereas Krell, Chakrin & Wardell (1975) concluded that reflex mechanisms played only a minor role. Other models, which measure action potentials in nerves arising from irritant receptors during stimulation (Sellick & Widdicombe, 1971), establish the existence of the reflex but do not allow analysis of its effects on lung function to be made. Another method of studying airways diameter in dogs by fluoroscopy (Benson & Graf, 1977) is a complicated procedure which might not allow large studies to be undertaken. In this publication we report the development of a model in the dog where constriction of an *in situ* tracheal pouch is measured following mechanical stimulation of the upper airways.

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0007-1188/80/110419-05 $01.00

Methods

Adult beagle dogs (11 to 15 kg) of either sex were used throughout the study.

Preparation of the tracheal pouch

Each dog was anaesthetized with etorphine (10 μg/kg) and acepromazine (40 μg/kg) intramuscularly, intubated and anaesthesia was then maintained with 1% halothane in oxygen (3 l/min). The trachea was exposed and a segment of 8 to 10 cartilage rings isolated whilst taking care to retain an intact nerve and blood supplies. A Silastic patch with a tube (i.d. 1.5 mm) of the same material attached through its centre, was sewn to each end of the tracheal segment. The pouch thus formed was located in the surrounding tissue of the neck and the tube from each end exteriorized. The trachea was anastamosed and the wound sutured.

Measurement of reflex tracheal constriction

A minimum of two weeks after surgery the dog was premedicated with acetylchlorpromazine (0.15 mg/kg i.m.), anaesthetized with chloralose (80 mg/kg i.v.) and intubated with a 9 mm cuffed endotracheal tube. The animal was placed on its side in a natural sleeping position. The tracheal pouch was flushed through and then filled with sterile saline (0.9% w/v NaCl solution). One tube was then closed to the atmosphere and the other connected to a saline-filled pressure

transducer (EM 750, Elcomatic). A fine nylon cannula (external diameter 1.5 mm) was then introduced into the airways via the endotracheal tube. Rotation of this cannula at a fixed depth, usually below the carina but predetermined in each animal (see Results), resulted in a rise in pouch pressure. This stimulus was repeated at 5 min intervals.

For the vagotomy experiments only, a femoral artery was cannulated and blood-pressure measured by a saline-filled pressure transducer (EM 750, Elcomatic). Heart rate was electronically derived from this signal. All signals were displayed on a heat-sensitive chart recorder (M19, Devices).

At the end of an experiment (excepting the vagotomy experiments) the dog was allowed to recover from the anaesthesia. The animal could then be re-anaesthetized a minimum of one week later for studies with a different drug. In two animals which had been anesthetized seven times, at weekly intervals, blood chemistry and haematological values were found to be within the normal range for such dogs.

Administration of drugs

Drugs were applied topically to the stimulus area of the airways through the nylon cannula used for stimulation. All drugs were given in a volume of 0.5 ml. Drugs were administered to the tracheal pouch by flushing through and filling with the drug solution. Intravenous administration was via a brachial vein. All drugs were disolved in sterile saline. Salbutamol (Allen and Hanbury) and tetracaine (Sigma) were used as the free bases, atropine (Sigma) as the sulphate. Anaesthetics used were α-chloralose (Aldrich), pentobarbitone sodium ('Sagatal', May & Baker) and etorphine hydrochloride (Small Animal Immobilon, Reckitt and Colman).

Results

Integrity of the pouch

In all the animals prepared, the pouch remained viable and functional for at least 2 months. The dogs were used for these experiments routinely at weekly intervals during this time. No gross infection of the pouches was seen in any animal.

Nature of responses

In all the animals studied, mechanical stimulation of the airways resulted in a rapid rise in pouch pressure which reached a maximum and then started to fall, in spite of continued stimulation. Rotation of the cannula was stopped at this time, and all subsequent stimuli were given at this depth. The response was often associated with coughing, but in some experiments constriction of the pouch occurred in the absence of cough. Conversely, some dogs could be made to cough without an accompanying rise in pouch pressure, demonstrating that coughing was not causing constriction of the pouch. At no time did stimulation of the airways result in swallowing.

Consistency of responses and expression of results

In any one animal the rises in pouch pressure following stimulation were very reproducible with a variability of ±5%. In one dog, ten consecutive stimuli gave a mean rise in pouch pressure ±s.e. mean of 1.54 ± 0.05 kPa.

Although the responses were consistent for each dog from week to week, the rises in pouch pressure varied from 0.4 to 3.0 kPa between animals. Consequently, in any one dog, three control responses were first obtained and the mean of these calculated. All the responses in this dog (including each control) were expressed as percentages of this mean. Thereafter data from different animals could be pooled and analysed statistically.

Vagotomy

Two dogs were anaesthetized as before and restrained in a supine position. Both vagi were isolated and three control responses subsequently obtained, to ensure that the reflex had not been affected by re-opening of the neck. The vagi were then sectioned below the nodose ganglion resulting in a rise in heart rate and blood pressure. The respiratory pattern changed to one of slow, deep breathing which, because of the position of the animal, results in exaggerated movement of the neck tissue surrounding the pouch. This caused the small rhythmic changes in pouch pressure seen in Figure 1. After vagotomy, there was no constriction of the tracheal pouch in either dog following three mechanical stimuli.

Chloralose and pentobarbitone

Following the report by Jackson & Richards (1977) that the reflex component of bronchoconstriction was reduced by pentobarbitone anaesthesia, we studied the effects of a low dose of this anaesthetic. Control responses were obtained in four animals anaesthetized with chloralose, and pentobarbitone (10 mg/kg i.v.) was then injected. Subsequent stimulation of the airways failed to elicit a response from the tracheal pouch in any dog.

Atropine

Atropine (500 μg) failed to inhibit the reflex when applied to the stiumlus area; 5 min after its appli-

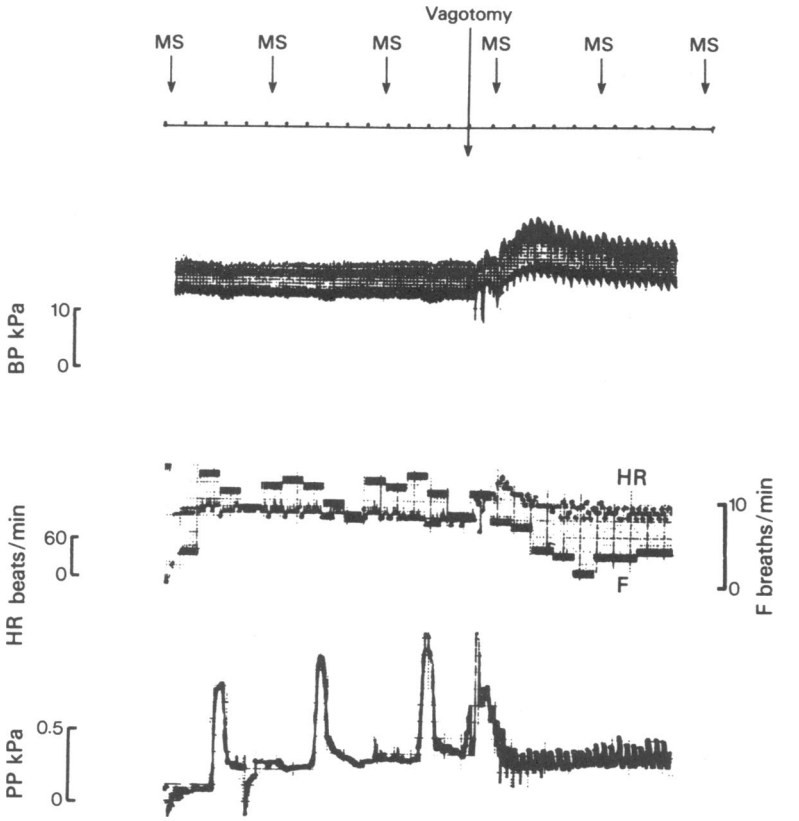

Figure 1 Trace from one dog showing the effects of bilateral vagotomy on constriction of the tracheal pouch. Following section of the nerves the blood pressure (BP) rose, as did the heart rate (HR, second trace, thinner line). The breathing frequency (F) fell (second trace, thicker line). The rises in pouch pressure (PP) to mechanical stimuli, (MS) are abolished following vagal section.

cation into the airways, stimulation caused an increase in pouch pressure of 118 ± 10% of the control value (mean ± s.e. mean $n = 4$). Intravenous administration (0.2 mg/kg) completely abolished the rise in pouch pressure in four animals. When atropine was present in the tracheal pouch at 10 mg/ml the response was progressively significantly reduced ($P < 0.01$) over a 15 min period. While at 1 mg/ml there was a smaller effect over the same time scale, which by 15 min was statistically significant ($P < 0.05$), see Figure 2.

Tetracaine

The local anaesthetic tetracaine, applied directly to the stimulus area at 50 μg and 500 μg, reduced the responses to 84 ± 13% and 17 ± 6% respectively of the control rise in pouch pressure (mean ± s.e. mean $n = 4$). The effect of the higher dose was more rapid in onset (Figure 3). When present in the tracheal pouch at 1 mg/ml and 10 mg/ml tetracaine also reduced the tracheal constriction.

Salbutamol

The β-adrenoceptor stimulant, salbutamol, had no inhibitory effect on the reflex when administered intravenously (0.1 mg/kg), into the airways (5 μg) or into the pouch (1 mg/ml). Four animals were used to study each dose route and responses were monitored up to 15 min after administration of the drug.

Discussion

The tracheal pouch preparation in the dog was first described by Wardell Chakrin & Payne (1970) for the collection of normal tracheal mucus. They demonstrated that innervation was retained after surgery as the pouch was constricted by electrical stimulation of

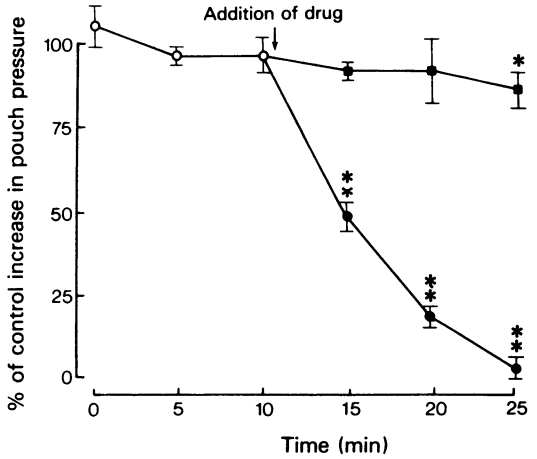

Figure 2 Inhibition by atropine of reflex tracheal constriction. Three control responses (○) were obtained before addition into the pouch of atropine 1 mg/ml (■) or 10 mg/ml (●) on separate occasions. Each point represents the mean ($n = 4$): vertical lines show s.e. mean. The control responses are combined results from all the atropine experiments. Asterisk indicate significantly different from controls (*$P < 0.05$) or (**$P < 0.01$) by Student's t-test.

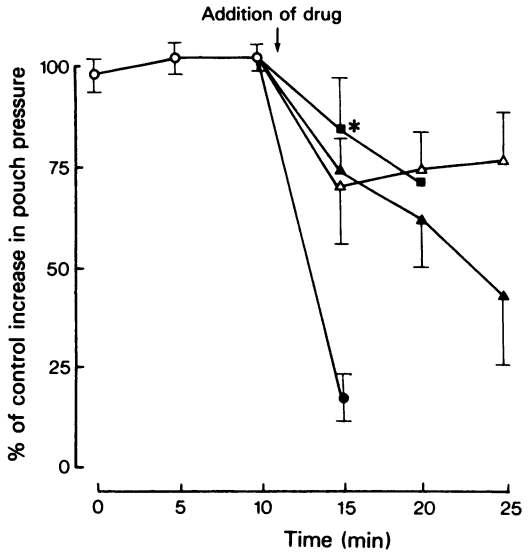

Figure 3 Inhibition by tetracaine of reflex tracheal constriction. Three control responses (○) were obtained before treatment with tetracaine: in the pouch (△ 1 mg/ml or ▲ 10 mg/ml) or at the site of stimulation in the airways (■ 50 μg or ● 500 μg) on separate occasions. Each point represents the mean ($n = 4$). Vertical lines show s.e. mean. The control responses are combined results from all the tetracaine experiments ($n = 8$). All points were significantly different from controls; $P < 0.01$ except points marked with a single asterisk ($P < 0.05$) by Student's t test.

the vagi. This response was inhibited by intravenous atropine.

The model has been developed to allow repeated studies of reflex tracheal constriction. Tactile stimulation of the airways resulted in a vagally mediated parasympathetic reflex constriction of the pouch (Figure 1) as judged by the absence of a rise in pouch pressure following bilateral vagotomy or the intravenous injection of atropine (0.2 mg/kg).

One advantage of this model of reflex airways constriction over others previously described, e.g. Jackson & Richards (1977) is that the effects of pharmacological agents at the afferent or efferent ends of the reflex can be studied in isolation. Thus, atropine applied to the afferent limb (in the airways) had no inhibitory effect whereas, when present in the pouch at the motor end it completely inhibited the response (Figure 2). The time taken for the inhibitory effect to develop probably reflects the rate of movement of the drug from the lumen of the pouch through the tissue to the underlying smooth muscle and may account for the large doses of drug required.

To demonstrate that inhibition of the sensory end of the reflex could be achieved, tetracaine was applied topically to the stimulus area in the airways. Five minutes after the application of 500 μg of the drug, stimulation of the treated area failed to elicit a response from the pouch (figure 3). Similar results in the dog were described by Dain, Boushey & Gold (1975) using the local anaesthetic bupivacaine. When tetracaine was present in the pouch (10 mg/ml) it caused a 50% reduction of the response presumably due to the stabilization of the muscle cell membrane or the efferent nerves themselves.

An unexpected result was that the β-adrenoceptor agonist, salbutamol, had no effect on tracheal constriction when administered intravenously or directly into the pouch. Because the technique is non-invasive, no measurements of blood pressure or heart rate were made; nonetheless, salbutamol was assumed to have been given in an effective dose (0.1 mg/kg) because it was much higher than has been reported to cause bronchodilation in dogs (Daly, Farmer & Levy, 1971). Kneussel & Richards (1977) showed that α-adrenoceptors were present in the tracheal and bronchial smooth muscle of tissue only from patients with chronic obstructive pulmonary disease, but normal human or canine airway muscle did not respond to noradrenaline, suggesting that there is usually an absence of α-adrenoceptors in this tissue. The same might be true of β-adrenoceptors in the trachea and that in the normal dogs in the present investigation there were no receptors on which salbutamol could act. This phenomenon certainly warrants further investigation.

Many of the differences in results, leading to controversy concerning the degree of involvement of a parasympathetic reflex in bronchoconstriction, stem from the use of animal models and, particularly, the choice of anaesthetic. Jackson & Richards (1977) showed the reflex component of airway narrowing to be greatly reduced in dogs anaesthetized with pentobarbitone when compared with dogs anaesthetized with chloralose. Our own results confirm that a low dose of pentobarbitone sodium (10 mg/kg i.v.) is sufficient to abrogate the reflex. This might account for the discrepancy between the work of Gold *et al.* (1972) who used chloralose and found the reflex to play a significant role in bronchoconstriction, and Krell *et al.* (1975) who used pentobarbitone and showed the opposite.

By using mechanical stimulation of the sensory endings problems associated with the use of pharmacologically active irritants such as histamine have been overcome. Histamine is known to have complex actions on the irritant receptors (Mills, Sellick & Widdicombe, 1969) being able to stimulate the receptors directly as well as constricting airways smooth muscle. When measurements of airways resistance are used to assess constriction, it is difficult to differentiate the reflex from the spasmogenic effects of a pharmacologically active irritant.

In conclusion, this model of reflex tracheal constriction in the dog enables studies to be made on the effects of drugs on the afferent or efferent ends of the reflex in isolation. The responses of the tracheal pouch to mechanical stimulation of the airways are highly reproducible and the technique allows repeated experiments to be carried out in the same dog.

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(Received August 22, 1979. Revised November 28, 1979.)