EFFECTS OF ANAESTHETICS ON GUINEAPIG TRACHEAL SMOOTH MUSCLE

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SUMMARY

The effects of i.v. and local anaesthetics on guineapig tracheal smooth muscle have been investigated in vitro. Diazepam, propanidid and ketamine induced relaxation and antagonized histamine-, acetylcholine- and potassium chloride-induced contractures in the tracheal smooth muscle. The relaxing effects of the anaesthetics were not affected by propranolol. Thiopentone, at concentrations which would be used clinically, caused contracture of the tracheal smooth muscle. Morphine induced relaxation and antagonized histamine-induced contracture, but did not antagonize acetylcholine- or potassium chloride-induced contractures. Lignocaine and procaine, at clinical concentrations, induced contracture of tracheal smooth muscle. Large doses of lignocaine relaxed the tracheal muscle, but large doses of procaine induced spontaneous rhythmical movements. Lignocaine and procaine antagonized contractures induced by histamine, acetylcholine and potassium chloride.

Anaesthetic drugs may affect airways smooth muscle, and may be implicated in the onset of bronchoconstriction, particularly in patients who suffer from asthma.

This has prompted the present investigation in which the effects of commonly used i.v. and local anaesthetics on guineapig tracheal muscle have been compared in vitro.

METHODS

The tracheal smooth muscle was prepared by a modification of the method described by Akcasu (1959). One hundred and four tracheal preparations were made from 34 adult guineapigs of both sexes. Each guineapig was killed by stunning and bleeding, after which the trachea from the larynx to the carina was quickly removed and placed in a muscle buffer solution at 37 °C. After removing any attached tissue the trachea was cut transversely to leave a piece about 5 mm long (four cartilages long), and then cut longitudinally, leaving the tracheal smooth muscle intact. A metal ring was tied to each end of this preparation with cotton thread. One ring of the preparation was attached to a force transducer (FO202 SER, Watson Victor Ltd) via a thin stainless steel wire, the other to a fixed glass rod. The tracheal preparation was immersed in a 25-ml bath of buffer, bubbled with a mixture of 5% carbon dioxide in oxygen (carbogen) and maintained at 37 °C. The composition of the buffer (mmol litre⁻¹) was: NaCl 121.2, KCl 5.4, MgSO₄ 1.2, Na₂HPO₄ 1.2, NaHCO₃ 15.0, CaCl₂ 2.5 and glucose 11.5.

The isometric changes in tension of the tracheal preparations were recorded on a Perkin–Elmer Recorder 56 through a Sanei 6M51 Strain Amplifier. For examination of the direct effects of anaesthetics on the tracheal smooth muscle, the initial resting tension used in the muscle preparations was 2 g. The anaesthetics were added to the organ bath in cumulative doses. The results are expressed as a percentage relaxation or contracture in comparison with the amount of relaxation caused by isoprenaline 50 μmol litre⁻¹, which was found to induce maximal relaxation.

The antagonism between the anaesthetics and three bronchoconstrictors (histamine, acetylcholine and potassium chloride) was also studied. The contractures to histamine 10 μmol litre⁻¹ (1.78 g ± 0.56 SD, n = 5), acetylcholine 10 μmol litre⁻¹ (1.93 g ± 0.29 SD, n = 7) and potassium chloride 40 mmol litre⁻¹ (1.91 g ± 0.93 SD, n = 14) were of similar size. After establishing an initial resting tension of 1.5 g, each bronchoconstrictor was added to the bath to produce the concentrations given above. As soon as the contracture plateau induced by the bronchoconstrictor had been reached, successive doses of the...
anaesthetic were added. The results are expressed as a percentage relaxation or contracture in comparison with the contracture induced by the broncho-constrictor.

Each experiment used from four to 10 tracheal preparations. The results obtained with different drugs were reproducible. The standard deviations for the results from individual doses of different drugs were of the order of ±5-10 per cent. The drugs used were: thiopentone sodium (Pentothal); ketamine hydrochloride (Ketalar); morphine sulphate; propanidid (Eptonol); diazepam (Valium); lignocaine hydrochloride; procaine hydrochloride; histamine dihydrochloride; acetylcholine chloride; isoprenaline hydrochloride (Isuprel); propranolol hydrochloride (Inderal). All drugs except propanidid, diazepam, isoprenaline and propranolol were dissolved in distilled water. Commercial preparations for injections were used in the case of these four drugs.

RESULTS

The direct effects of the anaesthetics on the intrinsic tone of guineapig tracheal muscle are shown in figure 1. Diazepam, propanidid, morphine and ketamine produced dose-dependent relaxation. Thiopentone caused contracture at concentrations less than 0.4 mmol litre⁻¹, but relaxation at concentrations greater than this.

Lignocaine induced large contractures, the maximal occurring at a concentration of 950 µmol litre⁻¹. Procaine also produced contracture, and at concentrations greater than 1.4 mmol litre⁻¹ the tracheal muscle preparation exhibited spontaneous rhythmical contraction and relaxation.

The effects of the anaesthetics on the contractures induced by histamine and acetylcholine are shown in figures 2 and 3. All the anaesthetics antagonized histamine-induced contracture (fig. 2) except for lignocaine at a concentration of less than 1.5 mmol litre⁻¹, and morphine sulphate at a concentration of less than 50 µmol litre⁻¹. Procaine produced relaxation of histamine-induced contracture, although procaine by itself caused contracture.

All the anaesthetics except morphine and ketamine (at a concentration less than 1.5 mmol litre⁻¹) reversed acetylcholine-induced contracture (fig. 3).

The effects of anaesthetics on contractile responses to potassium chloride are shown in figure 4. All anaesthetics except morphine and lignocaine (at concentrations less than 1.5 mmol litre⁻¹), antagonized potassium chloride-induced contractures.

Propranolol 13.5 µmol litre⁻¹ completely blocked the effect of isoprenaline 50 µmol litre⁻¹, but had no effect on the relaxing ability of the anaesthetics tested (fig. 5). The concentrations of anaesthetics required to produce a 50% antagonism of the contractures induced by histamine 10 µmol litre⁻¹, acetylcholine 10 µmol litre⁻¹ and potassium chloride 40 mmol litre⁻¹ were obtained from figures 2, 3 and 4 (table I).

Practical difficulties prevented the calculation of EC₅₀ values for each of the anaesthetic drugs.

Fig. 1. The effects of anaesthetics on guineapig tracheal smooth muscle. The results are expressed as a percentage relaxation or contracture in comparison with the amount of relaxation induced by isoprenaline 50 µmol litre⁻¹. Per cent relaxation or contracture is shown on the vertical axis and the concentrations of the anaesthetic drugs are shown on the horizontal axis.
TABLE I. Concentrations of anaesthetics required to produce 50% relaxation of the contractures induced in guineapig tracheal smooth muscle by histamine 10 μmol litre⁻¹, acetylcholine 10 μmol litre⁻¹ and potassium chloride 40 mmol litre⁻¹

<table>
<thead>
<tr>
<th>Anaesthetic (mol litre⁻¹)</th>
<th>Histamine (10 μmol litre⁻¹)</th>
<th>Acetylcholine (10 μmol litre⁻¹)</th>
<th>Potassium chloride (40 mmol litre⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam</td>
<td>1.3 x 10⁻³</td>
<td>3.6 x 10⁻⁴</td>
<td>4.5 x 10⁻⁵</td>
</tr>
<tr>
<td>Thiopentone</td>
<td>3.0 x 10⁻⁴</td>
<td>2.1 x 10⁻⁴</td>
<td>1.5 x 10⁻⁴</td>
</tr>
<tr>
<td>Ketamine</td>
<td>1.3 x 10⁻³</td>
<td>2.9 x 10⁻⁴</td>
<td>4.7 x 10⁻⁴</td>
</tr>
<tr>
<td>Morphine</td>
<td>3.4 x 10⁻⁴</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Propanidid</td>
<td>3.5 x 10⁻⁴</td>
<td>4.1 x 10⁻⁴</td>
<td>1.6 x 10⁻⁴</td>
</tr>
<tr>
<td>Lignocaine</td>
<td>4.0 x 10⁻⁴</td>
<td>3.5 x 10⁻⁴</td>
<td>1.4 x 10⁻³</td>
</tr>
<tr>
<td>Procaine</td>
<td>1.5 x 10⁻⁴</td>
<td>3.7 x 10⁻⁴</td>
<td>4.4 x 10⁻³</td>
</tr>
</tbody>
</table>

Fig. 2. The effects of anaesthetics on contractures induced in guineapig tracheal smooth muscle by histamine 10 μmol litre⁻¹. The results are expressed as a percentage relaxation or contracture in comparison with the contractures induced by the histamine. Per cent relaxation or contracture is shown on the vertical axis and the concentrations of the anaesthetic drugs are shown on the horizontal axis.

Fig. 3. The effects of anaesthetics on contractures induced in guineapig tracheal smooth muscle by acetylcholine 10 μmol litre⁻¹. The results are expressed as a percentage relaxation or contracture in comparison with the contractures induced by the acetylcholine. Per cent relaxation or contracture is shown on the vertical axis and the concentrations of the anaesthetic drugs are shown on the horizontal axis.

Fig. 4. The effects of anaesthetics on contractures induced in guineapig tracheal smooth muscle by potassium chloride 40 mmol litre⁻¹. The results are expressed as a percentage relaxation or contracture in comparison with the contractures induced by the potassium chloride. Per cent relaxation or contracture is shown on the vertical axis and the concentrations of the anaesthetic drugs are shown on the horizontal axis.

Fig. 5. Experiments showing that propranolol 13.5 μmol litre⁻¹ blocks the relaxing effect of isoprenaline 50 μmol litre⁻¹ in guineapig tracheal smooth muscle, but has no effect on the relaxation induced by thiopentone 800 μmol litre⁻¹, ketamine 1.46 mmol litre⁻¹, morphine 500 μmol litre⁻¹, diazepam 35 mmol litre⁻¹ and propanidid 300 μmol litre⁻¹. Contracture and relaxation (g) are shown on the vertical axis and time (min) on the horizontal axis.

DISCUSSION

More is known about the effects of inhalation anaesthetics on airways smooth muscle than about the effects of anaesthetic drugs which are given i.v.

Halothane produces bronchodilatation in man (Meloche et al., 1969) and in the dog (Colgan, 1965). Methoxyflurane induces relaxation of the guineapig tracheal chain (Caujolle and Pham-Huu-Chanh, 1966). On the other hand, cyclopropane causes broncho-constriction in dog lung (Colgan, 1965) and also...
when applied directly to bronchial slices from the rat, dog and man (Adriani and Rovenstine, 1943).

The present study has shown that, like most anaesthetics given by inhalation, most anaesthetic drugs which are given i.v. also induce relaxation of airways smooth muscle.

Clinically, the administration of diazepam may increase arterial $P_{CO_2}$ in patients with chronic obstructive airways disease (Catchlove and Kafer, 1971), but does not cause an increase in $P_{CO_2}$ in anaesthetized patients when ventilation is controlled (Heinonen and Muituri, 1972).

In the present investigation, diazepam relaxed guineapig tracheal smooth muscle and antagonized contractures induced by histamine, acetylcholine and potassium chloride. The amounts of diazepam needed to produce 50% antagonism of contractures were similar for each of these bronchoconstricting agents, and the effects of diazepam were not influenced by propranolol. The commercial preparation of diazepam used in these experiments contains propylene glycol, ethanol, benzyl alcohol, sodium benzoate and benzoic acid, and, although these agents may have influenced the results, it is clear that diazepam, in the form in which it is used clinically, causes relaxation of guineapig airways smooth muscle.

Thiopentone appears to have differing effects on airways smooth muscle from different species. It causes bronchoconstriction in excised lung tissue preparations from the rat, dog and man (Adriani and Rovenstine, 1943). This bronchoconstrictor effect is augmented by physostigmine and prevented by atropine. Previous studies in guineapig trachea have shown that thiopentone causes relaxation and antagonizes acetylcholine-induced contractures (Fletcher, Flacke, and Alper, 1968). In rabbit trachea, thiopentone appears to have no effect (Edney and Downs, 1975).

In the present study thiopentone, in concentrations similar to those used in human anaesthesia (250 $\mu$mol litre$^{-1}$), caused contracture of guineapig tracheal smooth muscle, but at concentrations greater than 400 $\mu$mol litre$^{-1}$ it caused relaxation. Thiopentone antagonized contractures induced by histamine, acetylcholine and potassium chloride. The amounts of thiopentone needed to produce 50% relaxation of contractures were similar for each of these bronchoconstricting agents. The effect of thiopentone was not blocked by propranolol. The reason why thiopentone causes contraction at small concentrations and relaxation at large concentrations is not known. It is not because of a change in pH. Concentrations of barbiturate less than 1 mmol litre$^{-1}$ do not effect the pH in Krebs solution (Edney and Downes, 1975). In the present study, the concentrations of thiopentone used were all less than 1 mmol litre$^{-1}$, and there was no change in pH in the Krebs solution.

From the practical point of view it is important to remember that, in the present investigation, clinical concentrations of thiopentone caused contracture in guineapig tracheal muscle. It has also been shown that thiopentone releases histamine in man (Lorenz et al., 1972).

Ketamine has been used safely in patients with asthma (Betts and Parking, 1971; Corssen et al., 1972), and airways resistance is reduced by the administration of ketamine to patients with asthma, although it does not have this effect in non-asthmatic patients (Huber et al., 1972). Using guineapig tracheal smooth muscle preparations, Lundy, Gowdey and Colboun (1974) found that ketamine had a direct relaxant effect, that it antagonized contractures induced by carbachol and potentiated the relaxing effect of adrenaline. Similar observations were made in the present investigation.

Morphine has long been known to have a bronchoconstricting effect (Higgins and Meann, 1915; Jackson, 1916). In the present investigation morphine, at concentrations about 10 times greater than the clinical dose, induced relaxation of guineapig tracheal smooth muscle. It also relaxed histamine-induced contractures of tracheal smooth muscle, but had no effect on acetylcholine or potassium chloride-induced contractures. It seems that morphine exerts its bronchoconstrictor effect by histamine release, by histamine-induced axon reflex which increases vaso tone, and by a direct action on the medullary bronchoconstrictor centre (Aviado, 1975).

A commercial preparation of propanidid (Epontol) induced relaxation of guineapig tracheal smooth muscle and antagonized contractures induced by histamine, acetylcholine and potassium chloride in the present investigation, although i.v. injection of propanidid for anaesthesia increases the blood histamine concentration (Lorenz et al., 1972). Epontol contains 16% of a butyl alcohol extract of polyethoxylated castor oil, and the effect of this on the responses obtained is not known.

Lignocaine, at concentrations less than 1 mmol litre$^{-1}$, induced contractures in guineapig tracheal preparations, but produced relaxation at concentrations greater than this. Lignocaine, at concentrations greater than 1 mmol litre$^{-1}$, antagonized con-
tracts induced by histamine, acetylcholine and potassium chloride. Lignocaine 1 mmol litre⁻¹ is about 10 times more concentrated than would be reached by the usual clinical dose. Similar observations have been made by Weiss, Anderson and O'Brien (1975) and Downes and Loehning (1977).

Clinically, it has been suggested that lignocaine applied topically to the airways is effective in preventing bronchospasm (Pelton et al., 1970), and that the i.v. administration of lignocaine can abolish bronchospasm (Brandus et al., 1970). Ultrasonically nebulized lignocaine has also been reported to antagonize the increased respiratory resistance induced by nebulized water (Loehning, Waltemath and Bergman, 1976). It is not known whether the action of lignocaine in preventing bronchospasm in humans is a direct action on smooth muscle, or a central action as inhibition of the vagal reflex or respiratory cough reflex.

Although an earlier study found that procaine caused relaxation of an isolated tracheal preparation (Macht and Ting, 1921), in the present investigation procaine consistently induced contracture of guinea-pig tracheal smooth muscle and produced rhythmical contracture and relaxation. Different concentrations of procaine were required to produce 50% relaxation of contractures induced by histamine, acetylcholine and potassium chloride. Lignocaine 1 mmol litre⁻¹ is about 10 times more concentrated than would be reached by the usual clinical dose. Similar observations have been made by Weiss, Anderson and O'Brien (1975) and Downes and Loehning (1977).

Although guinea-pig tracheal smooth muscle and human tracheal smooth muscle are pharmacologically similar in vitro, caution should be exercised in applying the results of the present investigations to man. It is possible also that reactions in tracheal smooth muscle may differ from those in bronchial smooth muscle (Fleisch and Calkins, 1976; Lulich, Mitchell and Sparrow, 1976).

REFERENCES


EFFETS DES AGENTS ANESTHESIANTS SUR LE MUSCLE LISSE TRACHEAL DU COBAYE

RESUME
On a fait des recherches in vitro sur les effets qu'ont les anesthesiants administrés par voie intraveineuse et les anesthesiants locaux sur le muscle lisse trachéal du cobaye. Le diazépam, le propanidid et la ketamine entraînent la relaxation et contrarient les contractions du muscle lisse trachéal provoquées par l'histamine, l'acétylcholine et le chlorure de potassium. Les effets relaxants des agents anesthesiants n'ont pas été affectés par le propranolol. Le thiopentone, aux concentrations que l'on utilise cliniquement, entraîne une contraction du muscle lisse trachéal. La morphine provoque la relaxation et contrarie la contraction provoquée par l'histamine, mais ne contrarie pas les contractions provoquées par l'acétylcholine ou le chlorure de potassium. La lignocaine et la procaine, aux concentrations cliniques, provoquent une contraction du muscle lisse trachéal. Les fortes doses de lignocaine relaxent le muscle trachéal, mais les fortes doses de procaine provoquent des mouvements rythmiques spontanés. La lignocaine et la procaine contrarient les contractions provoquées par l'histamine, l'acétylcholine et le chlorure de potassium.

WIRKUNGEN VON ANÄSTHESIEMITTEL AUF DIE GLATTE TRACHEALMUSKULATUR VON MEERSCHWEINCHEN

ZUSAMMENFASSUNG

EFFECTOS DE LOS ANESTETICOS EN EL MUSCULO LISO TRAQUEAL DEL COBAYO

SUMARIO
Se averiguó in vitro los efectos de anestésicos locales e i.v. en el músculo liso traqueal del cobayo. El diazépam, el propanidido y la quetamina indujeron la relajación y antagonizaron las contracciones del músculo liso traqueal inducidas por histamina, la acetilcolina y el cloruro de potasio. Los efectos relajantes de los anestésicos no fueron afectados por el propranolol. La tiopentona, en concentraciones en que se usaria clínicamente, causó contractura del músculo liso traqueal. La morfina indujo relajación y antagonizó la contractura inducida por la histamina, pero no antagonizó las contracturas inducidas por la acetilcolina o el cloruro de potasio. Los efectos relajantes de los anestésicos no fueron afectados por el propranolol. La tiopentona, en concentraciones clínicas, indujeron contractura del músculo liso traqueal. Grandes dosis de lignocaina relajan el músculo traqueal, pero grandes dosis de procaina indujeron movimientos rítmicos espontáneos. La lignocaina y la procaina, en concentraciones clínicas, indujeron contractura del músculo liso traqueal. Grandes dosis de lignocaina relajan el músculo traqueal, pero grandes dosis de procaina indujeron movimientos rítmicos espontáneos. La lignocaina y la procaina antagonizaron las contracturas inducidas por histamina, la acetilcolina y el cloruro de potasio.