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Effects of relaxants on electrical and mechanical activities in the guinea-pig tracheal muscle

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1 In isolated tracheal muscles of the guinea-pig, effects of several relaxants were studied by simultaneously recording the membrane potential and mechanical response.

2 Intracellular recordings showed regular slow waves in most preparations. There was a close correlation between membrane potential and slow wave amplitude. The linear regression line of the slow wave amplitude (Y mV) on the membrane potential (X mV) could be expressed by $Y = -0.35X - 5.9$. The mean values of resting potential and slow wave amplitude were -50.6 ± 0.6 mV and 11.9 ± 0.5 mV, respectively.

3 Relaxant drugs used (isoprenaline, terbutaline, adrenaline, noradrenaline, theophylline, forskolin and dibutyryl cyclic AMP) all produced hyperpolarization of the membrane and abolished the slow wave. The degree of relaxation was closely related to these electrical responses, although the recovery of electrical responses was faster than the mechanical response.

4 It was concluded that the relaxation caused by the agents, which are known to increase the intracellular cyclic AMP level, was accompanied by a clear hyperpolarization and suppression of slow waves.

Introduction

The tracheal smooth muscles of some species have been shown to produce spontaneous oscillatory contractions accompanied by rhythmic changes in membrane potential, at least under some conditions (Kirkpatrick, 1981). The guinea-pig tracheal smooth muscle is probably the most prominent preparation having such an electrical activity (slow waves) (Clark & Small, 1979; Small, 1982). Although the rhythmicity does not necessarily always appear in tension recording, the electrical activity may be responsible for spontaneous development of relatively high muscle tone in the guinea-pig compared with other species. Thus, in guinea-pig tracheal muscle, relaxation caused by noradrenaline and isoprenaline has actually been reported to be accompanied by abolition of the slow wave (Clark & Small, 1979; Small 1982).

Since these observations were mainly based on recordings with extracelluar electrodes, we have attempted further studies on the relationship between the relaxation and electrical response using intracellular microelectrodes in the isolated tracheal muscle of the guinea-pig. Drugs that are supposed to increase intracellular cyclic AMP level were selected as the relaxants in the present experiments; i.e. β-adrenoceptor stimulants (isoprenaline, noradrenaline, terbutaline), a phosphodiesterase inhibitor (theophylline) and an adenylate cyclase stimulant (forskolin). It was confirmed that most of the preparations generate very regular slow waves and a close correlation was found between relaxation and suppression of slow waves.

Methods

The methods employed were essentially similar to those previously described by Dixon & Small (1983). Guinea-pigs (300–350 g) of either sex were killed by stunning and bleeding. The trachea was excised and two segments of the tracheal ring (about 2 mm in width) were isolated. The ring was opened by cutting longitudinally through the cartilaginous region opposite the muscle part, and the mucosa covering the muscle was carefully removed with fine forceps under the microscope. The muscle of one segment was appropriately stretched and fixed, mucosal side uppermost, by use of small pins in the cartilaginous parts in a recording chamber. The cartilage of the contiguous

segment was used for recording mechanical response by connecting to an isometric transducer, and it was slowly stretched to give tension of 0.25–0.5 g. The chamber (0.2 ml in capacity) was perfused with the solution kept at 36°C at a constant flow rate of 2.5 ml min^{-1}.

The membrane potential was recorded with intracellular microelectrodes having resistances of between 25 and 40 MΩ. Impalement was usually performed in the muscle of the segment connected to the transducer. When prolonged impalement was difficult, the muscle in the adjacent segment fixed in the chamber was used for electrical recordings. However, even with this arrangement, a good correlation was found between electrical and mechanical responses. Only successful continuous recordings are shown in the results.

The normal solution had the following composition (mM): NaCl 117, $NaHCO_3$ 5.9, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 11.8, Tris-HCl 20 (pH 7.4 at 36°C). The following drugs were used: isoprenaline (Sigma), terbutaline (Fujisawa), adrenaline (Sigma), noradrenaline (Sankyo), metoprolol (Ciba-Geigy), forskolin (Nippon Kayaku), theophylline (Sigma) and dibutyryl cyclic AMP (Daiichi). Stock solutions of (−)-isoprenaline, noradrenaline and adrenaline contained 0.1 N HCl, and forskolin (10 mM) was dissolved in ethanol.

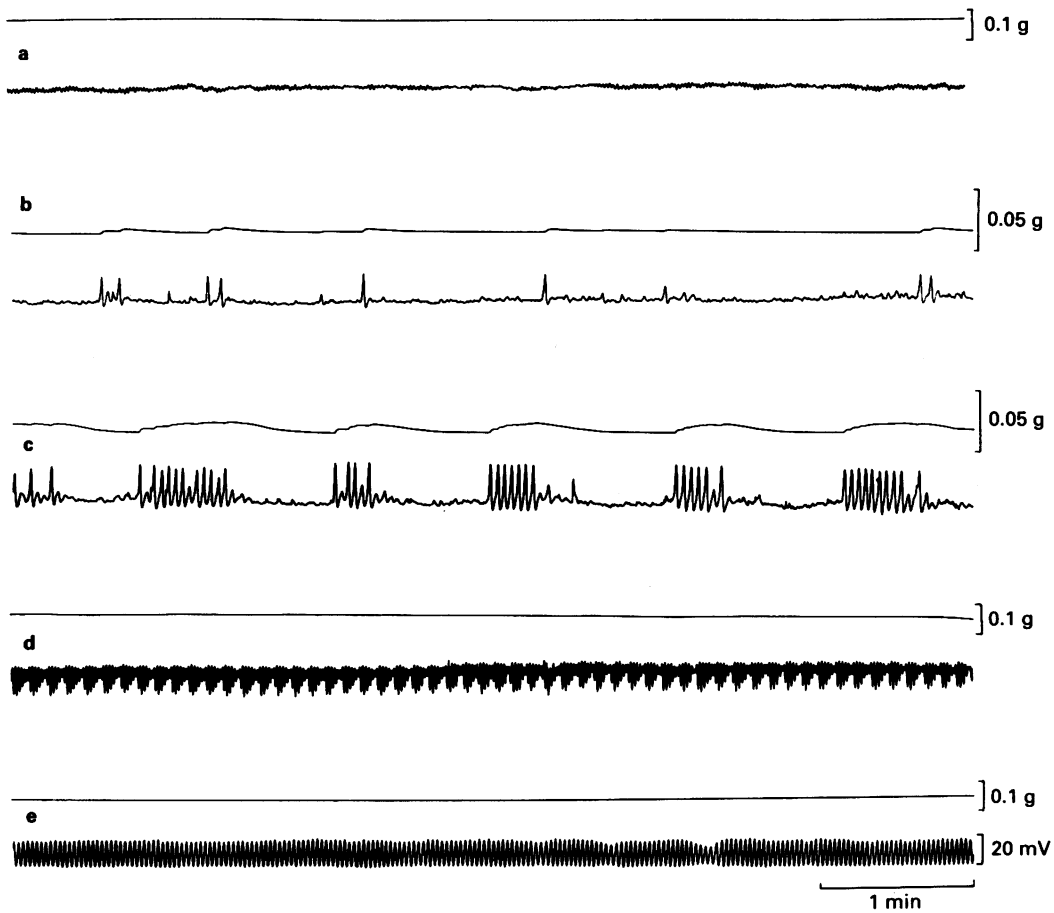

Figure 1 Different patterns of electrical activity recorded intracellularly from 5 different preparations of guinea-pig tracheal muscle. In each record, upper trace shows mechanical and lower trace electrical activities in the same tracheal ring. The pattern of continuous slow wave shown in (e) was most frequently observed. Note synchronous changes in electrical and mechanical activities in (b) and (c), tension recording being obtained with a high gain.

Results

Membrane potential and slow wave

The experiments were started after equilibration for at least 1 h in normal solution, during which the muscle tone gradually developed and reached a steady level. In most preparations, some spontaneous electrical activity was observed, which consisted of very regular oscillation of the membrane potential (slow waves). The pattern of the slow wave was variable in different preparations. Examples are shown in Figure 1. In some preparations, the slow wave was very small and irregular (Figure 1a). Occasionally, electrical activities appeared sporadically as shown in Figure 1 (b) and (c). These activities were accompanied by contractions. In most preparations, slow waves of more than 5 mV were continuously observed (Figure 1d,e). Their amplitude was generally constant (Figure 1e), but periodical modulation was also seen (Figure 1d). When the pattern of the slow waves changed slowly with time, synchronous fluctuation of the muscle tone was observed, particularly with a high gain of recording, although the electrical event slightly preceded the mechanical change. The reason for the variation of electrical pattern was not clear, but the difference in degree of stretch seemed to be one of the factors. When the muscle became overstretched, there was a tendency for the membrane to be depolarized and for the slow wave to be smaller in amplitude and higher in frequency. The mean frequency of regular slow waves having an amplitude larger than 5 mV was $43.6 \pm 0.7\ \text{min}^{-1}$ ($n = 135$).

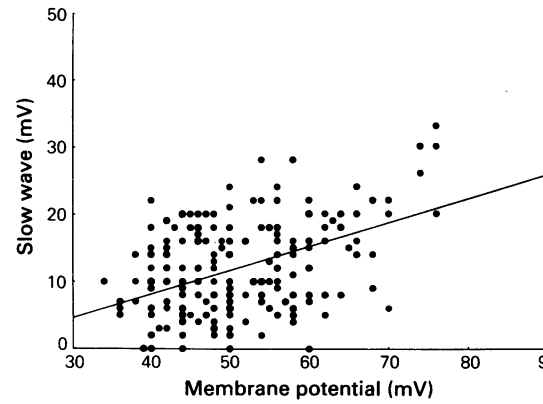

Figure 2 Relationship between the amplitude of slow waves (ordinate scale) and the membrane potential (abscissa scale) obtained from 200 impalements in 45 preparations. The line indicates the linear regression line of slow wave amplitude (Y mV) on membrane potential (X mV) in absolute values, expressed by $Y = 0.35X - 5.9$.

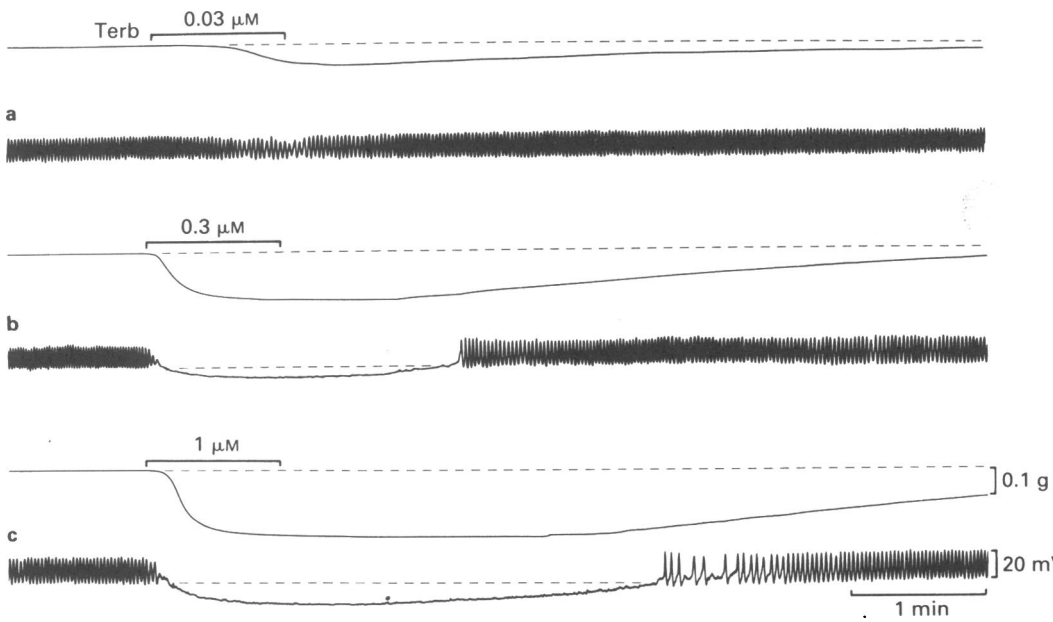

Figure 3 Effects of terbutaline (Terb) on the tension (upper) and membrane potential (lower trace) recorded simultaneously from the same segment. Continuous recording from the same cell. Terbutaline (0.03 – 1 μM) was applied for 1 min, as indicated above each record, at intervals of 20 min. See text for further explanation.

In Figure 2, the slow wave amplitude (ordinate scale) has been plotted against the membrane potential (abscissa scale) for 200 successful impalements in 45 preparations. There was a close correlation between the slow wave amplitude and the membrane potential. The linear regression line of the slow wave amplitude (Y mV) on the membrane potential (X mV) could be expressed by $Y = -0.35X - 5.9$. The maximum values for slow wave amplitude and membrane potential were 33 mV and -76 mV, respectively, and their mean values were 11.9 ± 0.5 mV (mean ± s.e.) and -50.6 ± 0.6 mV, respectively.

Effects of relaxant drugs

In the following experiments, the results obtained from preparations showing typical large slow waves were selected for the figures, but similar results were also obtained from the preparations which had small slow waves.

When terbutaline, an agonist selective for the β_2-adrenoceptor, was applied at 0.03 μM for 1 min, the amplitude and frequency of slow waves were slightly reduced and slow relaxation appeared (Figure 3a). At this concentration, there was no hyperpolarization. As the concentration of terbutaline was increased, the membrane was hyperpolarized and the slow wave was abolished (Figure 3b,c). Complete relaxation was produced and the maximum hyperpolarization of more than 15 mV was obtained at 1 μM. Although the time course of the mechanical response was slower than the electrical response, there was a close correla-

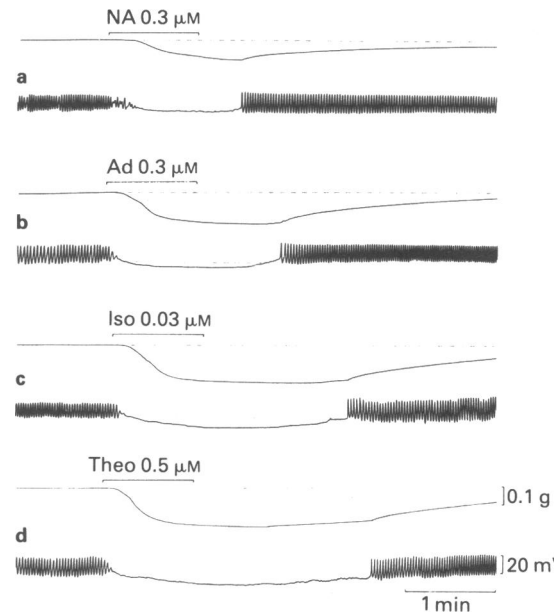

Figure 4 Effects of various relaxants (noradrenaline, NA (a), adrenaline (Ad) (b) at 0.3 μM, isoprenaline, (Iso) (c) at 0.03 μM, and theophylline (Theo) (d) at 0.5 mM). Continuous record from the same cell located in the same segment from which the mechanical recording was obtained. The drugs were applied for 1 min at 20 min intervals.

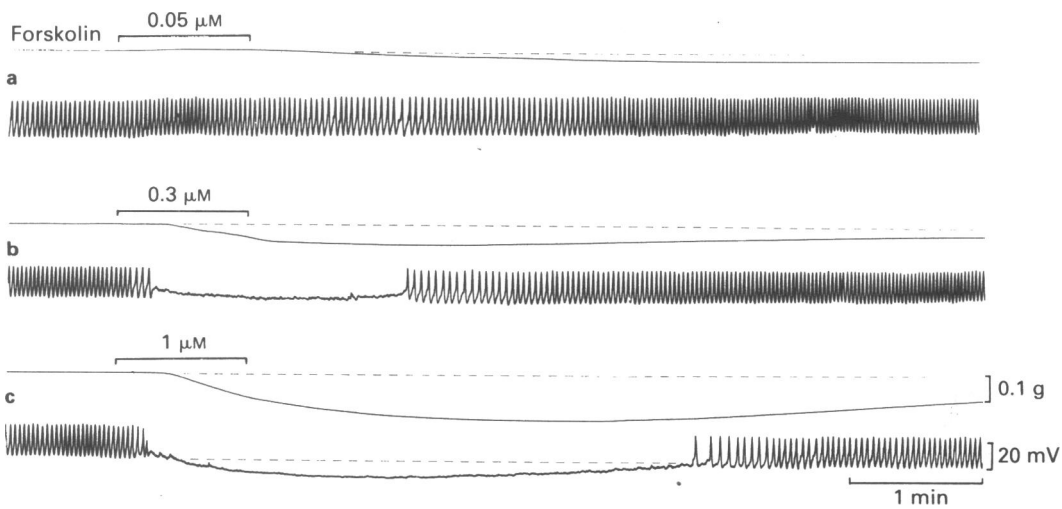

Figure 5 Effects of forskolin (0.05–1 μM). Electrical recording was from the same cell, but tension recording was made from the adjacent segment.

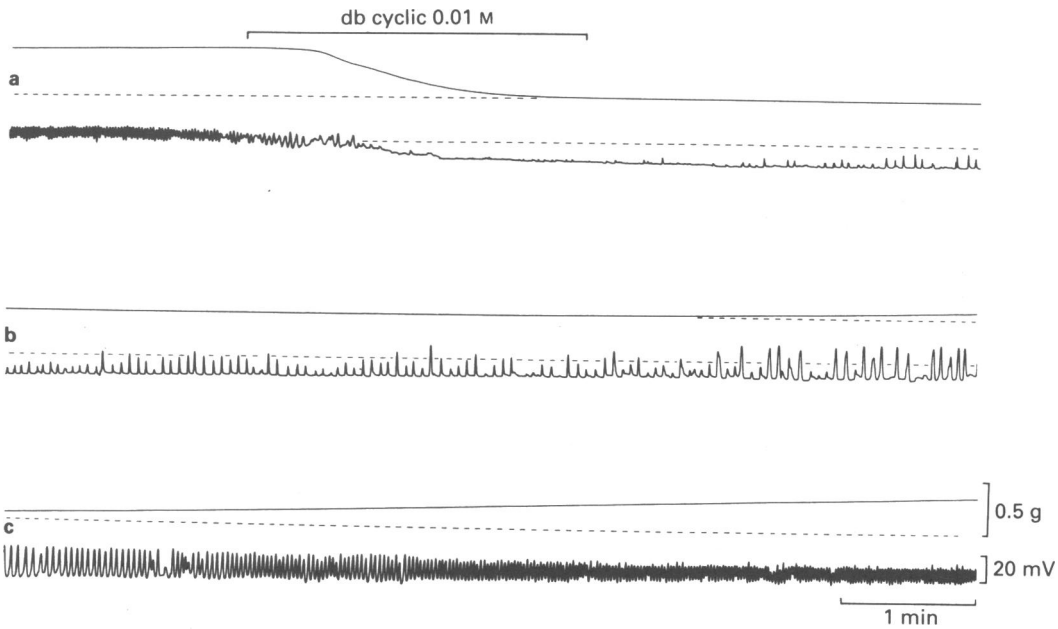

Figure 6 Effects of dibutyryl cyclic AMP (db cyclic AMP) (10 mM). Continuous record of mechanical and electrical responses obtained from the same segment. Dibutyryl cyclic AMP was applied for 2.5 min as indicated by a horizontal bar in (a).

tion between their magnitude. The recovery of electrical activity, particularly in amplitude, was faster than the mechanical response. The effects of terbutaline (0.3 μM) were not significantly affected by 0.1 μM metoprolol (an antagonist selective for β_1-receptors), although they were reduced by more than 50% at 1 μM.

The effects of isoprenaline were almost identical to those of terbutaline, except for a higher potency (about 10 times) of isoprenaline. The effect of isoprenaline (0.1 μM) was blocked by propranolol (0.1 μM), but not by phentolamine (0.1 μM).

In Figure 4, noradrenaline (0.3 μM), adrenaline (0.3 μM), isoprenaline (0.03 μM), and theophylline (0.5 mM) were applied for 1 min to the same preparations at intervals of 20 min. At these concentrations, the effect of the relaxants was stronger in this order, both on mechanical and electrical responses. The threshold concentrations for mechanical and electrical responses were almost the same for each of these drugs. When slow waves started during the recovery process, tension development was well synchronized with the electrical activity.

Forskolin is known to increase intracelluar levels of cyclic AMP by direct activation of adenylate cyclase. As shown in Figure 5, forskolin also produced essentially similar effects to isoprenaline. However, its potency was about one tenth of isoprenaline, and the onset and recovery of the effect were slower than with isoprenaline. In some preparations, a low concentration (0.05–0.3 μM) of forskolin produced a weak transient excitatory effect in mechanical and electrical activities, before the inhibitory effect appeared.

Relaxation produced by dibutyryl cyclic AMP (1–10 mM) was also accompaned by hyperpolarization of the membrane and suppression of slow waves. An example of the effects of dibutyryl cyclic AMP (10 mM) is shown in Figure 6. In this experiment, dibutyryl cyclic AMP was applied for 2.5 min. The effect appeared gradually and recovery was also very slow. Nevertheless, recovery of mechanical activity was more or less in parallel with recovery of the membrane potential and electrical activity.

Discussion

In guinea-pig tracheal muscle, the pattern of electrical activity differs greatly in different preparations, although very regular slow waves were seen in most preparations, confirming previous observations (Clark & Small, 1979; 1982). Statistical analysis showed that the maximal amplitude of the slow wave is linearly related to the membrane potential in these preparations. The difference in stretch of the preparations is probably one of the reasons for the variation in

amplitude and in the pattern of slow wave, although this relation was not carefully studied in the present experiments. The tension development, at least its main component, evidently results from the electrical activity. This can be seen when the slow wave appears sporadically or when the slow wave started after complete suppression by the relaxants. However, the time course of contraction corresponding to each slow wave is usually too slow to be discernible for continuous generation of slow wave, except for those of a low frequency.

All the β-adrenoceptor agonists used caused suppression of the slow wave and hyperpolarization of the membrane simultaneously with relaxation, as reported for noradrenaline and isoprenaline (Clark & Small, 1979; Small, 1982). Although the type of receptor involved was not properly analysed in the present experiments, the order of potency in producing inhibition (isoprenaline > terbutaline > adrenaline > noradrenaline) suggests that the effects were mainly mediated by β_2-adrenoceptors. The weak antagonistic action of metoprolol also supports this conclusion. However, since the guinea-pig tracheal muscle is known to contain both β_1- and β_2-receptors (O'Donnell & Wanstall, 1979; Zaagsma *et al.*, 1983), some contribution of β_1-receptors cannot be excluded. The threshold concentration is almost the same for relaxation and suppression of slow wave and, furthermore, electrical events always precede the mechanical response. It is, therefore, likely that the relaxants exert their effect on the plasma membrane to produce relaxation, although, in addition to this, intracellular actions may also be involved.

The relaxant effect of β-receptor agonists is generally believed to be due to intracellular Ca sequestration and/or Ca extrusion from the cell, effected by an increase in cyclic AMP (Hardman, 1981; Hisayama & Takayanagi, 1983). The fact that forskolin which activates adenylate cyclase and theophylline which inhibits phosphodiesterase produce relaxation also suggests an important role of cyclic AMP in relaxation (Seamon & Daly, 1981; Muller & Baer, 1983), although the degree of relaxation is not quantitatively correlated with an increase in cyclic AMP concentrations (Fredholm *et al.*, 1979; Kolbeck *et al.*, 1979; Vegesna & Diamond, 1983; 1984).

The results obtained in the present experiments using forskolin and dibutyryl cyclic AMP suggest the possibility that intracellular cyclic AMP may also mediate the electrical events in the plasma membrane. The underlying mechanism is not well analysed yet, but preliminary results are in favour of the idea that activation of the Na-pump may be involved in the inhibitory action in the plasma membrane, as has been suggested for vascular smooth muscles of the rat tail artery (Webb & Bohr, 1981) and frog stomach muscle (Scheid *et al.*, 1979).

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