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# CONDUCTANCE CHANGE DURING THE INHIBITORY POTENTIAL IN THE GUINEA-PIG TAENIA COLI

By T. TOMITA

*From the Department of Physiology, Faculty of Medicine, Kyushu University, Fukuoka, Japan*

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SUMMARY

1. Effects of membrane polarization and of reduction in external K and Cl concentration on the inhibitory potential were investigated in the guinea-pig taenia coli.

2. Depolarization of the membrane increased the inhibitory potential while hyperpolarization decreased it. The relationship between the degree of membrane polarization and the amplitude of inhibitory potential was linear. The inhibitory potential was abolished or slightly reversed in polarity, when the membrane was hyperpolarized by 25–40 mV in different preparations.

3. Removal of external K ion depolarized the membrane for about 5 min and increased the inhibitory potential more than could be accounted for by the depolarization. Readmission of K transiently hyperpolarized the membrane, probably due to an activation of the Na-K pump, and reduced the inhibitory potential, but no reversal of polarity in the inhibitory potential was observed during this hyperpolarizing phase.

4. The membrane was transiently depolarized when the external Cl concentration was reduced by substituting with isethionate. Hyperpolarization was produced by restoring the external Cl concentration to normal. Changes in the amplitude of inhibitory potentials during alterations in Cl concentration occurred as expected from the shift of the membrane potential.

5. From the results, it is concluded that the membrane conductance is increased during the inhibitory potential, and that an increase in the K permeability is the main factor for hyperpolarization of the membrane.

## INTRODUCTION

It is known that in the smooth muscle of the guinea-pig taenia coli field stimulation produces an inhibitory potential which is resistant to cholinergic and adrenergic blocking agents (Bennett, Burnstock & Holman, 1966). The inhibitory potential is blocked by tetrodotoxin which has little effect on the smooth muscle membrane, and the electrophysiological evidence obtained with various stimulus parameters and with various electrode arrangements has shown that the inhibitory potential is the result of stimulation of nerve fibres rather than of direct stimulation of smooth muscle fibres (Bülbring & Tomita, 1967). Recently it has been suggested that the inhibitory transmitter released by field stimulation is ATP or a related nucleotide (Burnstock, Campbell, Satchell & Smythe, 1970).

The inhibitory potentials have also been demonstrated in smooth muscles of the jejunum (Hidaka & Kuriyama, 1969), the colon (Furness, 1969), the stomach of the guinea-pig (Beani, Bianchi & Crema, 1971) and the avian gizzard (Bennett, 1969). However, the mechanism of the inhibitory potential has not been fully investigated.

From the observations that the amplitude of inhibitory potentials depends on external K concentration, it is suggested that the inhibitory potentials is due to an increase in K conductance (Bennett, Burnstock & Holman, 1963; Bennett, 1966). If there is a conductance increase during the inhibitory potential, a linear relationship between the magnitude of membrane potential changes and the amplitude of inhibitory potentials would be expected when the membrane potential is shifted by means of applied currents, as observed in other inhibitory junctions (see Eccles, 1964). The aim of the present experiments is to demonstrate such a relationship in the inhibitory potential in the guinea-pig taenia coli. Some evidence is presented for an increase in K conductance during the inhibitory potential, from the effects of membrane polarization and also from effects of reduction of external K and Cl ion concentrations.

## METHODS

Guinea-pig of either sex was stunned and bled. The taenia coli of about 3 cm was dissected and mounted on the double sucrose-gap apparatus as previously described (Bülbring & Tomita, 1969).

In order to produce the inhibitory potential, field stimulation was applied by a pair of electrodes placed in the centre pool of the apparatus. The stimulating electrodes consisted of two silver wires (0·3 mm in diameter) separated by 2 mm, and they were placed so as to pass current pulse transversely across the taenia. A single stimulation was applied, at roughly one minute intervals, using a current pulse of 0·5 msec in duration and about 10 V in strength.

A few records were obtained by intracellular micro-electrode, in combination with the external stimulation. This method was the same as that described by Bülbring & Tomita (1967).

## RESULTS

An inhibitory potential was produced by a field stimulation with a short current pulse (0·5 msec) which was applied to the tissue through external electrodes, as previously described (Bennett *et al.* 1966; Bülbring & Tomita, 1967). The maximum amplitude of the inhibitory potential

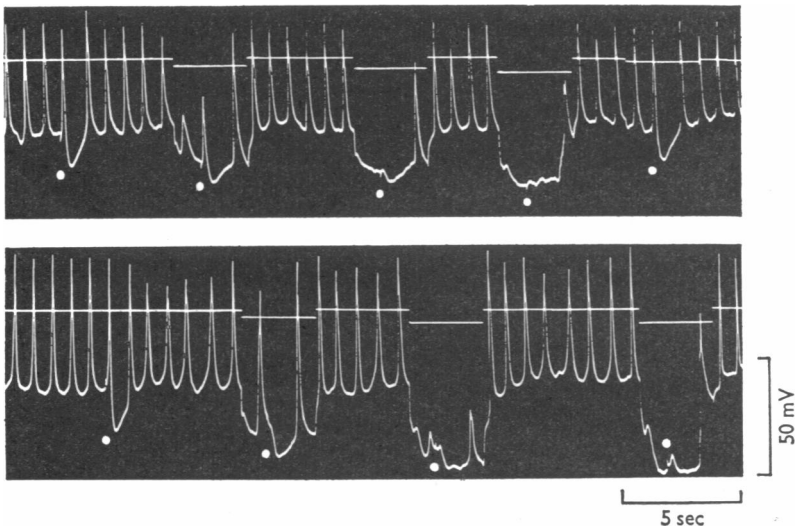


Fig. 1. Intracellular recording of spontaneous spikes, inhibitory potentials and effects of membrane hyperpolarization in guinea-pig taenia coli (top and bottom: two different preparations). Top tracing: relative current intensities for conditioning hyperpolarization. At dots, 0·5 msec field stimulation was applied to produce inhibitory potential. Note reduction of amplitude of inhibitory potential with conditioning hyperpolarization.

recorded intracellularly was between 10 and 20 mV. When a stimulation was applied immediately after a spike, only the inhibitory potential appeared, but when the stimulus was delayed in relation to the spike the inhibitory potential was often preceded by a spike as shown in Fig. 1.

The frequency of spontaneous spikes was reduced with hyperpolarization of the membrane caused by a long current pulse (3 sec). With a sufficiently strong current, spontaneous spikes disappeared, although irregular fluctuation of the membrane potential remained. The inhibitory potentials became smaller with increasing hyperpolarization, and were abolished when the membrane was hyperpolarized by about 25–40 mV in different preparations.

Since, in the experiment shown in Fig. 1, the whole tissue was immersed in Krebs solution, a large polarizing current pulse had to be applied to shift the membrane potential sufficiently. Therefore, it is possible that the polarizing current itself stimulates the intramural nerve fibres so that a short current pulse fails to produce the inhibitory potential during the conditioning hyperpolarization. However, this is unlikely because essentially similar results were obtained by using the double sucrose-gap method. In this method, the intramural nerve fibres were stimulated with a pair of electrodes immersed in the centre pool through which Krebs solution was flowing and weak polarizing currents (the order of $10^{-7}$ A) were applied through a sucrose-gap with different electrodes.

Fig. 2 shows an example of the results obtained with the double sucrose-gap method. The inhibitory potential was produced in response to a short current pulse (0·5 msec, 10 V). The amplitude of inhibitory potentials varied from about 5 to 15 mV in different preparations. Conditioning hyperpolarization blocked spontaneous activity and reduced the inhibitory potentials (*a*), while conditioning depolarization increased the frequency of spontaneous spikes and the amplitude of inhibitory potentials (*b*). Tetrodotoxin ($10^{-7}$ g/ml.) had no effect on the spontaneous spikes, but completely blocked the inhibitory potential, as previously reported (Bülbring & Tomita, 1967). After tetrodotoxin application no inhibitory potential was observed even during conditioning depolarization. Thus, the responses observed with the double sucrose-gap method were qualitatively the same as those recorded with intracellular micro-electrodes. Since experiments were much simpler with the double sucrose-gap method, the following results were all obtained with this method.

Occasionally, an excitatory potential of about 5 mV preceded the inhibitory potential. The excitatory potential was abolished by a treatment with atropine ($10^{-6}$ g/ml.) or hyoscine ($10^{-6}$ g/ml.) leaving only the inhibitory potential.

In Fig. 3, field stimulations were applied to produce the inhibitory potential during conditioning depolarization or hyperpolarization produced by three different current intensities (1, 2, and $4 \times 10^{-7}$ A). As observed with intracellular recordings (Fig. 1), the amplitude of inhibitory potentials became smaller with increasing conditioning hyperpolarization, and larger with increasing conditioning depolarization. It was, however, difficult to reverse the inhibitory potential to depolarization by conditioning hyperpolarization, probably due to an increase in the chord conductance which was observed when the membrane was hyperpolarized by more than 10 mV, and due to the fact that the maximum current intensity was limited by a high series resistance of the tissue in the sucrose channel.

Spikes and inhibitory potentials had a slower time course and the

frequency of spontaneous activity was lower in the sucrose-gap method compared with intracellular recording method. This may partly be due to a lower temperature in the sucrose-gap method. Desynchronization of spikes may also be another factor for broader and irregular spikes.

In Fig. 4 the amplitude of inhibitory potential (IP) on the ordinate was plotted against the displacement of the membrane potential (RP) on the

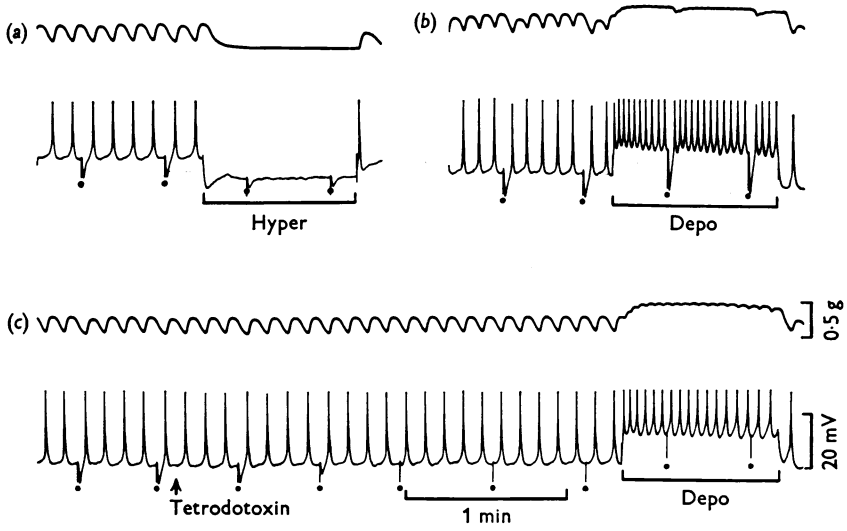


Fig. 2. Recording with double sucrose-gap method. Inhibitory potentials evoked by field stimulation at dots in bottom trace. Conditioning hyperpolarization (*a*) and depolarization (*b*) and (*c*) are indicated by horizontal bars. In (*c*), tetrodotoxin ($5 \times 10^{-7}$ g/ml.) was infused. Top tracings in each record show mechanical responses. Note changes in amplitude of inhibitory potential with conditioning polarization, and also note abolition of inhibitory potential by tetrodotoxin.

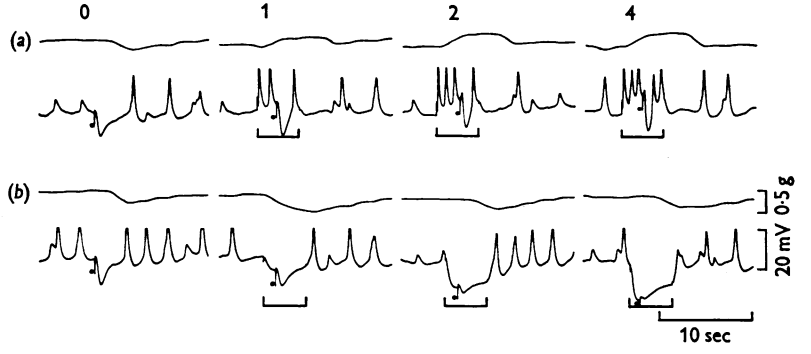


Fig. 3. Effects of conditioning depolarization (upper) and hyperpolarization (lower) (shown by bars) on amplitude of inhibitory potentials. Numbers indicate intensity of conditioning current ($\times 10^{-7}$ A).

abscissa at which the inhibitory potential was recorded. In this Figure, hyperpolarization of the membrane and hyperpolarizing inhibitory potential have a negative sign.

The relationship between the amplitude of inhibitory potential and the membrane polarization was fairly linear as shown in Fig. 4. Similar results were obtained from two other different preparations. The inhibitory potential nearly disappeared, or occasionally a small reversal was observed

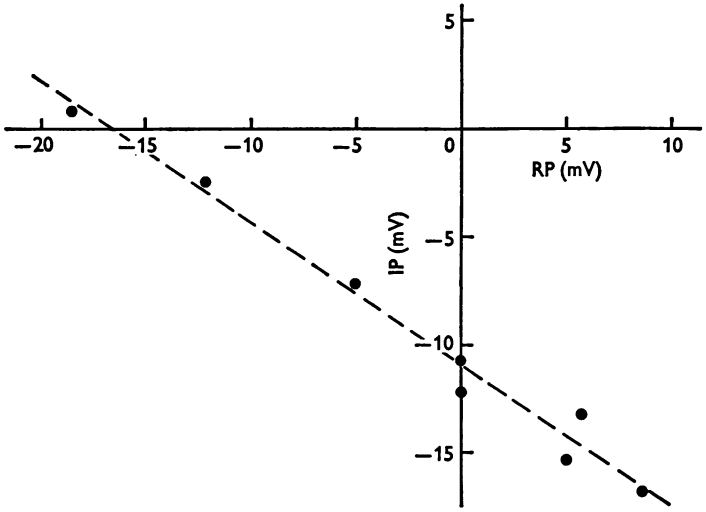


Fig. 4. Relationship between amplitude of inhibitory potential (IP) and membrane polarization (RP) plotted from the results shown in Fig. 3. Negative sign indicates hyperpolarization.

when the membrane was hyperpolarized by 15–20 mV. The potential change recorded with the sucrose-gap method is probably about a half of the real change in membrane potential due to a shunting effect of sucrose solution (Bennett & Burnstock, 1966). Therefore, the true reversal potential of the inhibitory potential would be 30–40 mV negative than the resting potential, which is in agreement with those recorded with intracellular electrodes.

Effects of K-free solution were studied on four preparations. An example of the results is shown in Fig. 5. When K was removed from the external medium, the membrane was transiently depolarized by about 5 mV and then gradually hyperpolarized. A transient hyperpolarization of about 10 mV and gradual recovery of the membrane potential were produced when the external potassium was readmitted. Changes in spike activity paralleled changes in the membrane potential in that the frequency of spontaneous and evoked spikes was increased during depolarization of the

membrane and decreased during hyperpolarization. These results were the same as those obtained with intracellular recordings (Casteels, Droogmans & Hendrickx, 1971; Tomita & Yamamoto, 1971), although the effect appeared more quickly and was shorter-lasting in the present experiment, probably due to a faster change in the test solutions.

As shown in Fig. 5, the amplitude of inhibitory potential was dependent on a shift of the membrane potential caused by a change in the external K concentration. The inhibitory potentials were increased during depolarization produced by K removal, and they were gradually decreased in

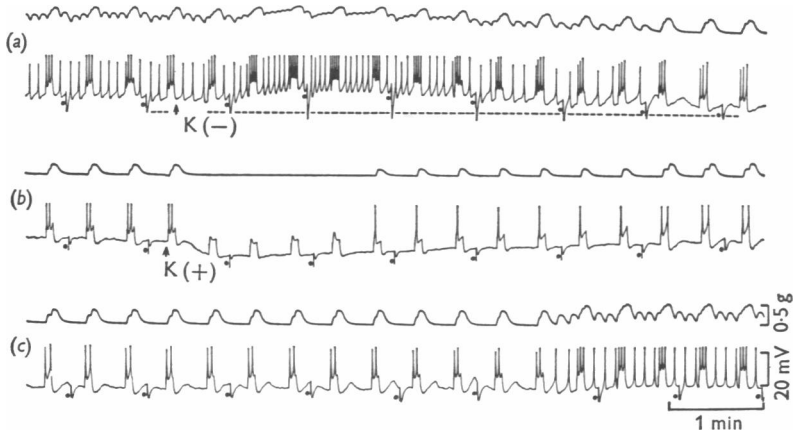


Fig. 5. Effects of K removal (*a*) on inhibitory potentials (field stimulation shown by dot), and on spontaneous and evoked spikes (*a*). (*b*) and (*c*): recovery from the effects. Dashed line indicates potential level of peak of inhibitory potentials in normal solution. At every 30 sec, 5 sec depolarizing current of constant intensity ($2 \times 10^{-7}$ A) was applied. For further description see text.

amplitude with progressive hyperpolarization. The peak of inhibitory potential reached more hyperpolarized level than the control (dashed line in Fig. 5), particularly during depolarization of the membrane. When the membrane was hyperpolarized by increasing the external K concentration to normal (5·9 mM), the inhibitory potential became very small, but a reversal of polarity was never observed. These observations are in agreement with previous studies in which intracellular recordings were employed (Tomita & Yamamoto, 1971).

Contribution of Cl conductance in producing the inhibitory potential was investigated by observing effects of replacement of Cl with a foreign anion, isethionate. Figs. 6 and 7 show an example of effects of replacement of Cl with isethionate and recovery from the effect obtained from the same preparation. The membrane was depolarized and spike activity increased

for about 10 min by a substitution of Cl with isethionate, as observed intracellularly with sulphate substitution (Kuriyama, 1963). During this depolarizing phase, the inhibitory potential was increased, but the peak of inhibitory potentials was shifted toward more depolarized level than the

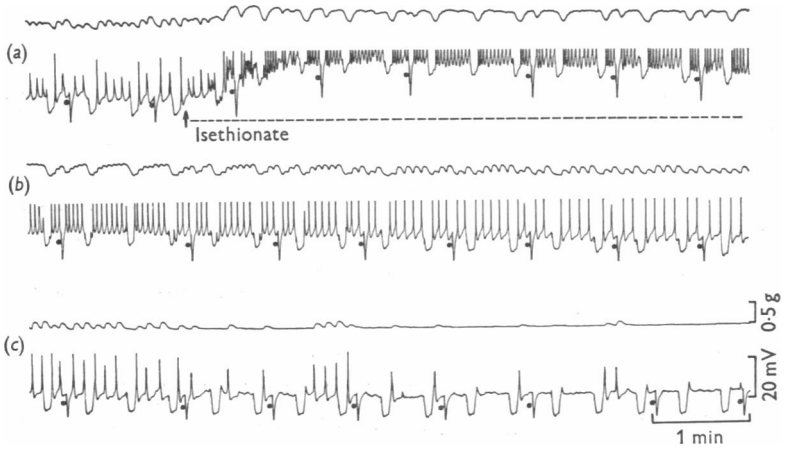


Fig. 6. Effects of isethionate substitution for Cl on inhibitory potential and electrical activities. Dotted line indicates potential level of peak of inhibitory potential in normal solution. Field stimulations at dots. At every 30 sec, 5 sec hyperpolarizing current of constant intensity ($2 \times 10^{-7}$ A) was applied.

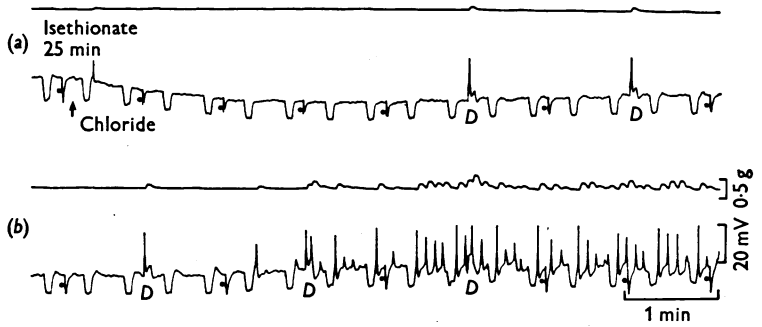


Fig. 7. Recovery from isethionate substitution (for 25 min) for Cl, continuous from Fig. 6. At *D*, depolarizing current (5 sec, $2 \times 10^{-7}$ A) was applied.

control (dashed line in Fig. 6). These effects were followed by repolarization of the membrane, reduction of spike activity and recovery in amplitude of the inhibitory potential.

When normal Krebs solution was returned after an exposure to isethionate solution for about 25 min, the membrane was hyperpolarized and the inhibitory potential was reduced (Fig. 7). The spike activity and the

inhibitory potential were gradually recovered within about 10 min in the recovery solution. Similar effects of variation in external Cl concentrations were observed in three other preparations.

The change in membrane potential produced by alteration of the external Cl concentration was as would be expected from shifts in the Cl equilibrium potential. An increase in inhibitory potential by removing Cl and a decrease by returning Cl can be explained as a secondary effect of changes in membrane potential.

## DISCUSSION

Since the amplitude of the inhibitory potential depends on the external K concentration, the amplitude being larger the lower the K concentration, it has been proposed that the inhibitory potential is mainly due to an increase in K conductance (Bennett *et al.* 1963; Hidaka & Kuriyama, 1969). If there is a conductance change, polarization of the membrane by applying current, thus moving the membrane potential either closer to or further from the transmitter equilibrium potential, should reduce or increase the amplitude of the inhibitory potential, as demonstrated in many other junctions (see Eccles, 1964).

In smooth muscle, as in cardiac muscle, the fibres are electrically interconnected, and thus the responses to current pulse differ greatly depending on the method of current application (Noble, 1966; Tomita, 1970). When current pulses are applied through an intracellular micro-electrode, the spatial decay of the potential developed across the cell membrane is very sharp due to a large increase in the membrane area with distance from the electrode. For this reason, the input resistance is scarcely affected by a change in the membrane resistance, and measurements of conductance change with this method become meaningless since the size of the potential developed is virtually independent of the membrane resistance (George, 1961).

It has been reported that intracellular polarization failed to affect the amplitude of the inhibitory potential in smooth muscles (Bennett & Rogers, 1967; Hidaka & Kuriyama, 1969). This is not surprising for the reasons given above and does not prove that there is no increase in the membrane conductance during the inhibitory potential. However, when current is applied by external large electrodes the spatial decay of the electrotonic potential developed across the membrane is much slower and obeys cable equations (Tomita, 1970), its size being dependent on the membrane resistance. In the present experiments, polarizing current was applied to the whole tissue placed in Krebs solution, and a linear relationship was obtained between the amplitude of the inhibitory potential and

the membrane polarization. The simplest explanation for the change in amplitude is that there is an increase in membrane conductance during the inhibitory potential.

Various types of inhibitory potential in other tissues are produced by an increased permeability to either K or Cl or their combination in varying degree (see Eccles, 1964). According to ionic analysis in the taenia, the Cl equilibrium potential is calculated to be $-24$ mV and the K equilibrium potential, $-89$ mV (Casteels, 1969). Therefore, K ion may be only a candidate for the conductance increase to produce hyperpolarization, but a possibility of a concomitant increase in Cl permeability cannot be discarded.

When external K ions are removed the membrane is transiently depolarized probably due to a suppression of the electrogenic Na pump (Casteels *et al.* 1971; Tomita & Yamamoto, 1971). During this depolarization, the inhibitory potential is increased and its peak reaches more hyperpolarized level than the control. On the other hand, readmission of external K produces a transient hyperpolarization probably due to a potentiation of the Na pump, and reduces the inhibitory potential. These changes in the inhibitory potential are in accordance with the idea that an increase in K conductance is mainly responsible for the inhibitory potential. Since no reversal of polarity is observed in the inhibitory potential by K readmission, it is doubtful that the membrane potential becomes more negative than the K equilibrium potential during hyperpolarization caused by an activation of the Na pump.

Reduction of the external Cl concentration by substituting with isethionate first increases the inhibitory potential. However, this effect is probably mainly due to depolarization of the membrane, because inhibitory potentials recover their amplitude to the control size in parallel with repolarization of the membrane within about 10 min in Cl-deficient solution. Similar results have been reported for the guinea-pig taenia coli (Bennett *et al.* 1963) and for the guinea-pig jejunum (Hidaka & Kuriyama, 1969). If there were a large increase in the Cl conductance during the inhibitory potential, a reduction in amplitude or a reversal of polarity would be expected by Cl removal, as observed in the crayfish muscle (Boistel & Fatt, 1958) and stretch receptor (Hagiwara, Kusano & Saito, 1960). Therefore, Cl contribution to the inhibitory potential is, if any, quite small in the smooth muscle of the guinea-pig taenia coli.

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