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ION FLUXES DURING THE INHIBITORY JUNCTION POTENTIAL IN THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Contribution of different ions to the inhibitory junction potential (i.j.p.) in the guinea-pig taenia coli was studied by measuring the ^{42}K, ^{24}Na and ^{36}Cl fluxes, the membrane resistance and the influence of various external ion concentrations.

2. The membrane resistance, as measured by the electrotonic potential, decreased transiently during the i.j.p. A maximal reduction of the electrotonic potential of about 50% was found at the top of the i.j.p.

3. The i.j.p. amplitude could be reduced by raising the external potassium concentration. Extrapolation of the relationship observed shows that the inhibitory response would be abolished at 115 mM potassium. Similar experiments were made in chloride-free medium, chloride being replaced by isethionate. Amplitude and time course of the response were not different in chloride containing Locke solution and in chloride-free medium.

4. The half-times of ^{42}K, ^{24}Na and ^{36}Cl effluxes during rest were 29, 10 and 9 min respectively. The ^{42}K-efflux from the preparation was markedly increased to about three times the resting efflux during field stimulation. In low-chloride solution a similar effect on ^{42}K-efflux was observed during field stimulation. Only a slight increase in the chloride efflux was observed but the sodium efflux was not affected during field stimulation.

5. From the results presented it is concluded that the inhibitory junction potential is caused by a selective increase in potassium permeability of the smooth-muscle cell membrane.

INTRODUCTION

Field stimulation of the guinea-pig taenia coli produces a transient hyperpolarization, resistant to agents which block cholinergic and adrenergic transmission (Bennett, Burnstock & Holman, 1966). From the observation that tetrodotoxin abolishes this hyperpolarization (Bülbring & Tomita,

1967), and from the temperature dependence of the response (Jager & Den Hertog, 1974), it is assumed that this response is caused by a transmitter, released from intramural nerves after field stimulation. The observation that the amplitude of the hyperpolarization, called the inhibitory junction potential (i.j.p.), depends on external potassium concentration (Bennett, Burnstock & Holman, 1963) suggests that the response is due to an increase in the potassium permeability of the smooth-muscle cell membrane. This suggestion is supported by the finding that the i.j.p. amplitude depends on the membrane potential (Tomita, 1972). If there is an increase in potassium permeability during the i.j.p., a rise in potassium efflux and a reduction in membrane resistance can be expected. The aim of this study was to investigate membrane resistance, and movement of potassium, chloride and sodium during the i.j.p. From these electrophysiological and flux measurements evidence is presented that the i.j.p. is due to a specific increase in potassium permeability of the smooth-muscle cell membrane.

METHODS

Guinea-pigs of either sex (250–300 g) were stunned and bled. A strip of the guinea-pig taenia coli of about 0·8 mm thick and 40 mm long was used throughout. The composition of the Locke solution used was (mM): NaCl, 157·6; KCl, 2; $CaCl_2$, 2·2; $NaHCO_3$, 1·8; glucose, 5·6, and Tris, 4·0. In experiments carried out at different external chloride concentrations a modified Locke solution was used in the control experiments containing (mM): NaCl, 157·6; K_2SO_4, 1·0; Ca-laevulinate, 2·2; $NaHCO_3$, 1·8; glucose, 5·6; and Tris, 4·0. In low-chloride medium, isethionate was substituted for chloride. The pH of the solutions was adjusted to 7·3 with sulphuric acid and the solution was bubbled with 100 % O_2. In order to vary the potassium concentration, this cation was substituted for sodium, keeping the sum of NaCl + KCl constant. Under these conditions the cell volume is assumed to be constant (Casteels, 1971). Atropine ($1{\cdot}4 \times 10^{-7}$ M) and guanethidine ($2{\cdot}5 \times 10^{-6}$ M) were added to the solutions to block the cholinergic and adrenergic nervous systems. To prevent spontaneous activity of the preparation, and to minimize rebound contraction, the experiments were carried out at room temperature (20–22° C). At this temperature the time course of the i.j.p. is slowed down, and electrotonic potentials can be studied during the response. Furthermore, the lower sodium efflux rate, at room temperature compared with that at 38° C (Casteels, 1969), simplifies the investigation of efflux changes.

Membrane potential measurements. The double sucrose-gap method was used to measure changes in membrane potential and electrotonic potential simultaneously (Den Hertog, 1973). The inhibitory junction potential was elicited by field stimulation for 1 sec (pulse rate 30/sec; pulse duration 0·3 msec). Electrotonic potentials were evoked by constant current pulses with an intensity of 10^{-7} to 10^{-6} A. According to the cable theory (Hodgkin & Rushton, 1946), which is applicable to the taenia coli (Abe & Tomita, 1968), there is a relation between electrotonic potential and membrane resistance. The mean time constant of the electrotonic potential was $56{\cdot}2 \pm 3{\cdot}6$ msec ($n = 7$) in our experiments, a value similar to that found by Tomita (1966). Potential changes across the sucrose-gap were measured by means of calomel electrodes making contact with the test solution and the reference solution (isotonic KCl). Potential changes were recorded, via a cathode follower, with a low-speed

recorder for continuous registration and with a high-resolution recorder to measure the time course of the i.j.p.

Flux measurements. After equilibration in Locke solution the preparation was transferred to a Locke solution containing ^{42}K, ^{36}Cl or ^{24}Na (The Radiochemical Centre, Amersham, England). To obtain maximal labelling, the preparations were loaded for 2 hr with ^{42}K, for 40 min with ^{36}Cl or for 30 min with ^{24}Na. Then the preparation was mounted in a single sucrose-gap apparatus to measure the efflux of radioactive ions and membrane potential simultaneously. Samples of the effluent (0·5 ml./min) were taken at 2 min intervals. The radioactivity of the effluent was counted in a liquid scintillation spectrometer (^{36}Cl) or in a gamma-spectrometer (^{42}K and ^{24}Na).

RESULTS

Electrophysiological measurements

Electrotonic potentials were evoked to investigate whether the membrane resistance varied during the i.j.p. A transient reduction of the membrane resistance was found (Fig. 1), which was most pronounced at the top of the i.j.p ($45{\cdot}4 \pm 4{\cdot}7\%$; $n = 6$). This reduction might be due to hyperpolarization of the membrane during the i.j.p. However, external hyperpolarization of the preparation to twice the i.j.p. amplitude caused an increase of the electrotonic potential (about 10%) as shown in Fig. 1. Consequently, it is unlikely that reduction of the electrotonic potential found during the i.j.p. is caused by the changed membrane potential. These observations confirm the interpretation, made by Tomita (1972), of the relationship between i.j.p. amplitude and external polarization, that the membrane resistance is reduced during the inhibitory response.

The observation that the i.j.p. amplitude varies with external potassium concentration (Bennett *et al.* 1963) suggests that the i.j.p. is caused by an increase in potassium permeability. Assuming that this mechanism underlies the i.j.p., the amplitude would be determined by the difference in potassium equilibrium potential and resting potential. Therefore, the relation between response amplitude and potassium equilibrium potential was determined. The inhibitory potential was elicited at different potassium concentrations after stabilization of the membrane potential for 10 min. As shown in Fig. 2, the amplitude of the inhibitory response decreased as the potassium concentration was increased, which is in agreement with previous studies (Bennett *et al.* 1963). The concentration range used was limited in order to prevent modification of the response by spontaneous activity of the preparation. It is unlikely that neural function is affected by potassium in the concentration range used, for the time course of the inhibitory response did not change.

The role of chloride in the development of the inhibitory response was studied by replacing this anion by isethionate in the modified Locke solution used (Methods). After a transient increase, the i.j.p. amplitude reached again (within 30 min) the value found in chloride containing

solution ($105 \pm 5\%$, $n = 60$). The transient increase in the response, also observed by Tomita (1972), might be due to the initial depolarization of the muscle cell membrane in low-chloride medium found by Kuriyama (1963) and Ohashi (1970). In this chloride-free medium the i.j.p. amplitude appeared to be dependent on external potassium concentration in the same manner as found in chloride containing medium (Fig. 2).

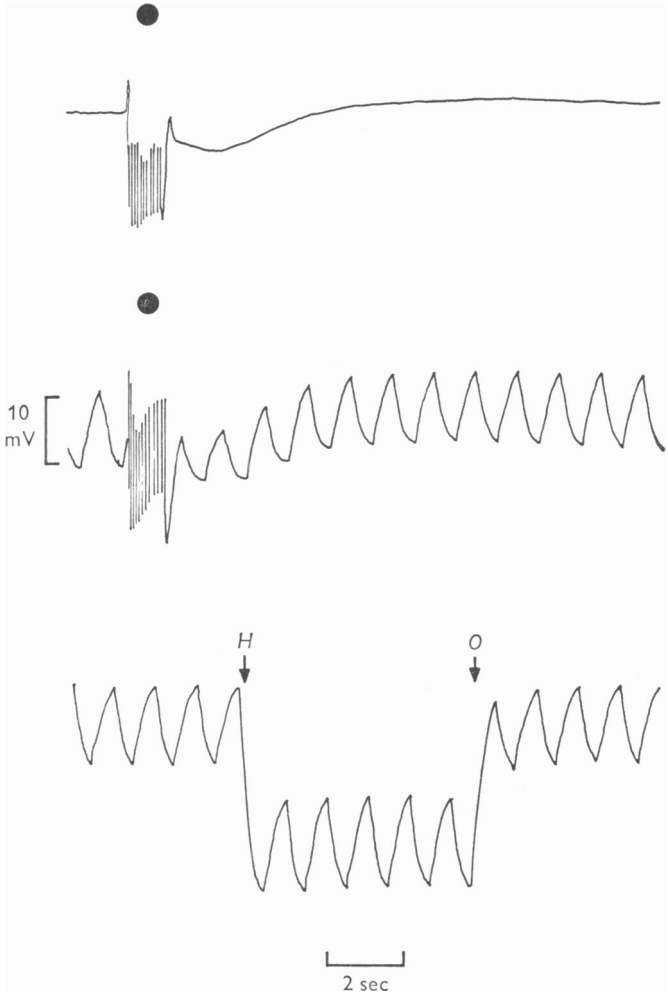

Fig. 1. Electrotonic potentials and the i.j.p. recorded simultaneously with double sucrose-gap method. The i.j.p. produced by field stimulation (dot) is shown in the upper trace. Decrease of the amplitude of the electrotonic potentials (pulse rate, 1/sec; intensity, 3×10^{-7} A) evoked during the inhibitory response is demonstrated in the middle trace. A small increase of the electrotonic potential amplitude was seen with conditioning hyperpolarization (*H* to *O*) of the preparation (lower trace).

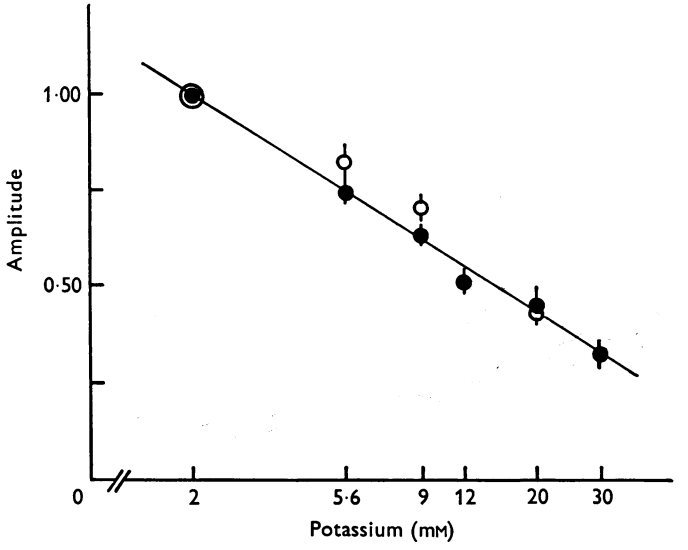

Fig. 2. The inhibitory potential at different external potassium concentrations. Decrease of the response amplitude (●) at higher potassium concentrations can be fitted by an exponential relation. The amplitude of the response at 2 mM potassium was taken as unity (1·00 = 8·8 ± 0·4 mV; n = 10). A similar relationship was found in the absence of external chloride (○), chloride being replaced by isethionate (1·00 = 8·7 ± 1·2 mV; n = 26).

From these electrophysiological measurements evidence is obtained that potassium is involved in the generation of the i.j.p., but that chloride is not.

Flux measurements

These measurements were carried out to obtain direct evidence for the contribution of different ions to the inhibitory response, as indicated by the results of electrophysiological measurements presented in previous paragraph. Preparations were loaded with the isotope ^{42}K, ^{36}Cl or ^{24}Na (Methods) and mounted in the single sucrose-gap apparatus in order to measure variations in efflux and membrane potential simultaneously. The loaded preparation was superfused with Locke solution and samples (1 ml.) were taken every 2 min. The isotope flux from the intramural nerves was assumed to be small compared with the efflux from the muscle cells.

Potassium. The ^{42}K activity of the effluent as a function of time was plotted as a semi-logarithmic graph (Fig. 3). The points representing the effluent activity after perfusing the preparation for more than 10 min could be fitted by a straight line. The initial deviation from linearity is assumed to be due to loss of extracellular localized isotopes, while the

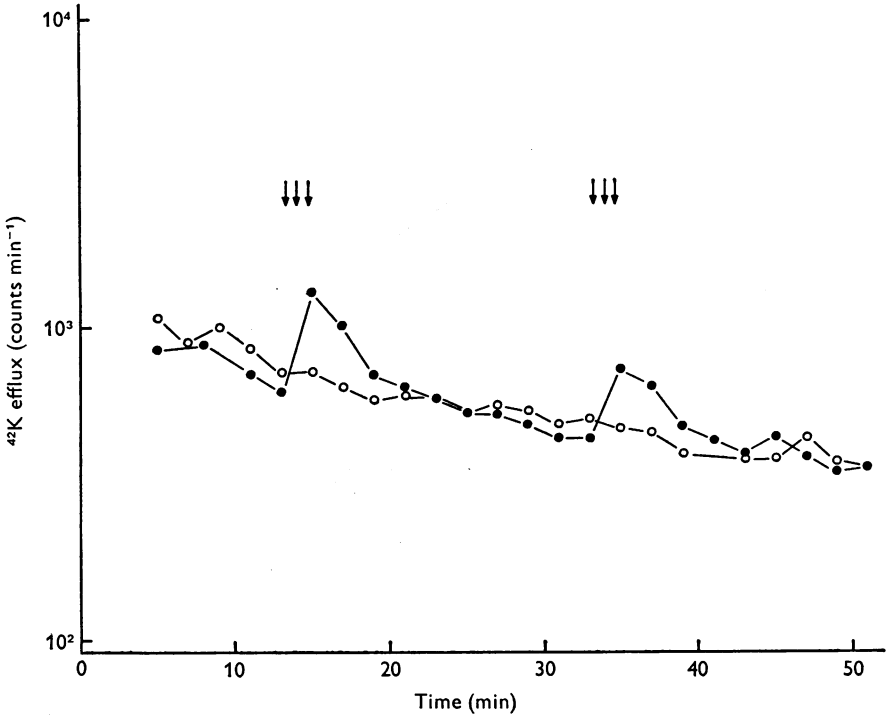

Fig. 3. Efflux of ^{42}K from taenia coli during rest (○) and field stimulation (●). Field stimulation of the preparation (arrows) produces an increase in ^{42}K efflux. The activity of the effluent was counted during 4 min.

TABLE 1. The ^{42}K, ^{24}Na and ^{36}Cl effluxes during the inhibitory junction potential (i.j.p.) and the half-time ($t_{\frac{1}{2}}$)

	Locke solution			Low-chloride solution (2 mM)		
Isotope	Efflux during the i.j.p.* (%)	$t_{\frac{1}{2}}$ during rest (min)	$t_{\frac{1}{2}}$ with tetracaine (min)	Efflux during the i.j.p.* (%)	$t_{\frac{1}{2}}$ during rest (min)	$t_{\frac{1}{2}}$ with tetracaine (min)
^{42}K	11·2	26	26	21·7	26	29
	27·0	32	24	14·9	27	29
	13·7	—	—	21·3	—	—
^{36}Cl	0·5	10·6	9·6	—	—	—
	0·6	9·4	8·6	—	—	—
	0·6	8·2	—	—	—	—
^{24}Na	0	11	12	0	9	9

* Excess efflux after field stimulation expressed as a percentage of the muscle load.

linear phase is thought to be determined by the flux from the intracellular compartment (Casteels, 1969). From the linear phase, the ^{42}K efflux half-time was estimated and appeared to be about 29 min (Table 1). In spite of the lower temperature the efflux half-time presented is shorter than that reported by Casteels (1969). This might be due to the superfusion procedure and to the preparation technique of the taenia coli used by us. Similar differences were observed for the ^{36}Cl half-time (next paragraph). However, these differences do not invalidate the efflux changes observed by us.

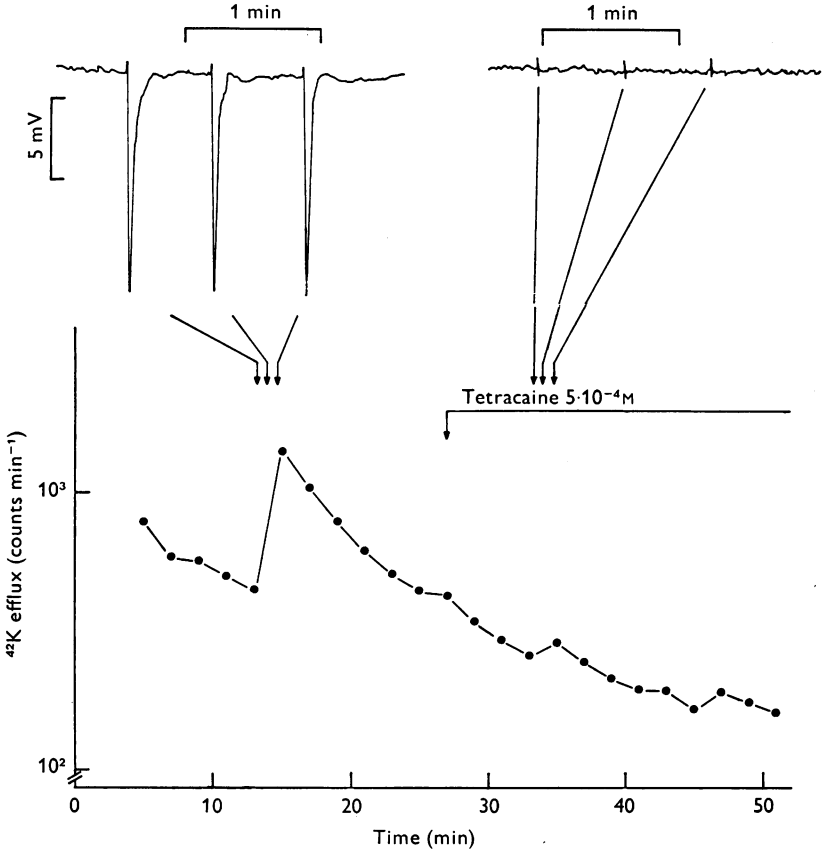

Fig. 4. Inhibitory potentials evoked by field stimulation (upper trace) and efflux of ^{42}K from taenia coli measured simultaneously (lower graph). The inhibitory potentials and increase of ^{42}K loss from the preparation produced by field stimulation (arrows) is abolished completely in the presence of tetracaine. The activity of the effluent was counted during 4 min.

Inhibitory potentials were evoked by field stimulation, after perfusing the preparation for 13 min to be sure that any change in ^{42}K effluent

activity represents variations in loss from the intracellular compartment. A remarkable rise of the ^{42}K efflux was seen to occur simultaneously with the i.j.p. (Figs. 3, 4). The increase in effluent activity lasted for about 10 min, which period is apparently necessary to clear the extracellular compartment from excess ^{42}K, as discussed above. The extra potassium lost from the preparation after field stimulation is about 17% of the muscle load (Table 1), the resting efflux being calculated by interpolation.

Field stimulation of the preparation after blocking the nervous system with a local anaesthetic (tetracaine) abolished both the increase in ^{42}K efflux and the i.j.p. (Fig. 4). The half-time of the ^{42}K efflux found in the presence of tetracaine was calculated to be about the same as in the absence of this drug (Table 1).

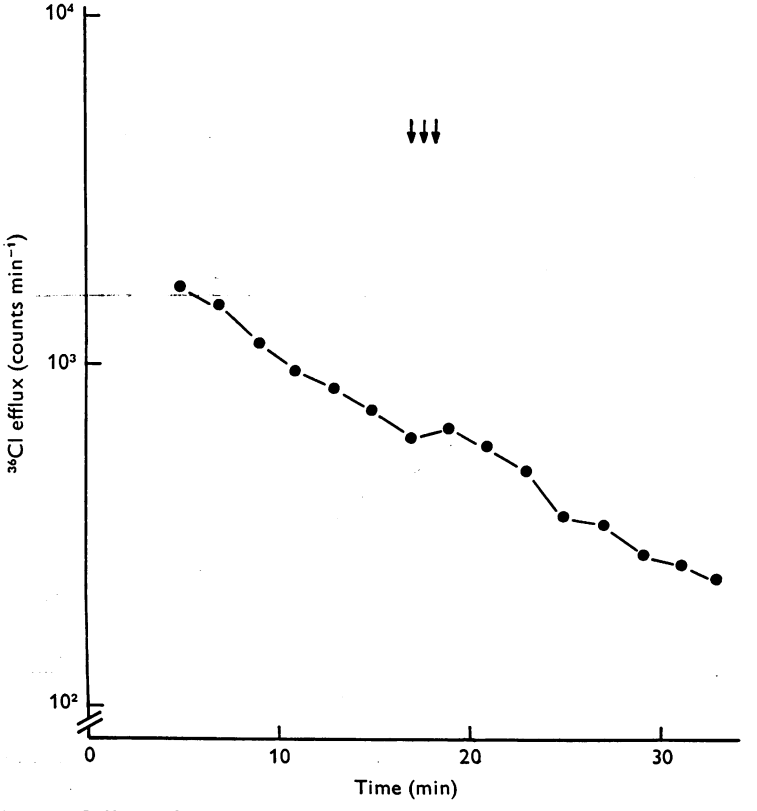

Fig. 5. Efflux of ^{36}Cl from taenia coli during field stimulation at arrows. A slight increase in ^{36}Cl loss is produced by field stimulation. The counting time was 10 min.

In low-chloride medium (2 mM, ^{42}K being added as chloride) the efflux half-time was about 27 min, which is comparable with the half-time found

in chloride Locke solution (Table 1). The total increase in efflux after field stimulation, also found under these conditions, was 19% of the muscle load at that time (Table 1). The time courses of the ^{42}K efflux during rest and after field stimulation were similar in the presence of a local anaesthetic and not different from the graphs found in chloride containing medium (Table 1).

From these results it can be concluded that the i.j.p. is accompanied by an increase in potassium efflux, independent of external chloride.

Chloride. Although the inhibitory response and the ^{42}K efflux were not modified by changing the external chloride concentration, movement of this anion during the response was also measured. Therefore, ^{36}Cl efflux was measured following the same experimental procedure as described above. The half-time of the ^{36}Cl resting efflux was calculated to be about 9 min. The efflux graph of ^{36}Cl shows that field stimulation increases the chloride effluent activity by about 0·5% (Fig. 5, Table 1). This increase is not due to stimulus artifacts as shown by the normal exponential decay of the efflux graph in the presence of tetracaine (Table 1). It seems likely that the small increase in efflux observed is due to hyperpolarization of the membrane during the i.j.p.

Sodium. It is unlikely that sodium contributes to the inhibitory potential in view of the sodium equilibrium potential. To test this assumption, movement of sodium ions during the i.j.p. was investigated. The half-time of the ^{24}Na efflux during rest was calculated from the graph, representing the effluent activity and appeared to be about 10 min (Table 1). The efflux was not affected by field stimulation or modified by tetracaine.

DISCUSSION

The flux experiments presented confirm our interpretation of the electrophysiological measurements, namely that potassium is certainly involved in the generation of the inhibitory potential.

The reduced membrane resistance during the inhibitory junction potential found by us, suggests an increase in membrane permeability to one or more ions during the inhibitory response. The fact that the i.j.p. amplitude depends on external potassium concentration makes it likely that this cation is involved in the development of the response. Extrapolation of the relationship between i.j.p. amplitude and potassium concentration shows that the response would be abolished at 115 mM potassium. At this concentration the potassium equilibrium potential equals the membrane potential (Casteels & Kuriyama, 1965; Casteels, 1971). The assumption that the i.j.p. is due to movement of this cation is supported by the increase in ^{42}K efflux from the smooth muscle cells during the response.

The contribution of chloride to the inhibitory response seems to be negligible, because the relation between response amplitude and external potassium was not modified by removing chloride from the medium. It was reported by Casteels (1971) that the potassium equilibrium potential and the resting potential found in chloride-containing solution hardly changed in the absence of this anion. Thus, if the inhibitory response were determined only by the differences between these potentials, as discussed above, it would be expected that the response amplitude would be independent of the extracellular chloride concentration, which is in agreement with our observations. Furthermore, the increase in ^{42}K efflux during the i.j.p. is independent of external chloride. Hyperpolarization of the membrane during the inhibitory response might account for the small change in ^{36}Cl efflux found.

The reduction in membrane resistance during the i.j.p., the dependence of this response on external potassium concentration and the concomitant increase in potassium efflux provide strong evidence that the inhibitory response is due to a specific increase in potassium permeability of the smooth muscle cells.

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