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Sodium-dependence of the non-specific desensitization of the guinea-pig ileum induced by acetylcholine and histamine

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1 The isometric maximal responses of the guinea-pig ileum to acetylcholine and to histamine (but not those to prostaglandin E_2 and to high K^+) exhibited a secondary transient increase in tonus during the tonic component of the contraction.

2 After desensitizing treatment with acetylcholine or histamine, the isometric responses to either agonist showed decreased phasic and enhanced tonic components, whereas both components of the response to prostaglandin E_2 were markedly depressed.

3 During the desensitizing treatment the degree of desensitization went through a maximum that coincided with the occurrence of the secondary tonic increment.

4 In low-Na^+ medium, or in ouabain-treated tissues, the responses to the three agonists were similar to the respective responses in the desensitized state.

5 It is concluded that the non-specific desensitization is due to changes in Na^+ translocation and that the increased tonic component of the isometric response is due to a reduced Na^+ gradient across the cell membrane and consequent increase in Ca^{2+} loading.

Introduction

Prolonged or repeated exposure of smooth muscles to high concentrations of agents that induce contractions may lead to desensitization, usually characterized by a reduction of the peak response to subsequent treatments with these agents. The desentization may be specific, affecting only the responses to one group of chemically related agonists, probably reflecting alterations at the receptor level. On the other hand, it may be non-specific, when treatment with one drug depresses subsequent responses not only to that drug and its analogues, but also to other agonists acting at different receptors. Non-specific, as well as specific desensitization, is reversible, and is not due to fatigue of the contractile machinery, since tissues desensitized to one agonist can still respond fully to other types of stimulation.

A well known example of non-specific desensitization is that observed in the guinea-pig ileal muscle upon treatment with large doses of acetylcholine, histamine and other agonists (Cantoni & Eastman, 1946; Paton, 1961; Bown *et al.*, 1973; Siegel *et al.*, 1984). In the case of the non-specific desensitization induced by acetylcholine, there is evidence that it depends on a gain of Na^+ by the smooth muscle cells (Paton & Rothschild, 1965; Joiner, 1973). The increase in intracellular Na^+ would enhance Na^+/K^+-pump activity (Bolton, 1973), leading to hyperpolarization (Bülbring & Burnstock, 1960), which might explain the decrease in sensitivity to different contractile agonists. Acetylcholine desensitization in the guinea-pig ileum has also been attributed to K^+ loss by the smooth muscle cell (James-Kracke & Roufogalis, 1981).

Besides the quantitative loss of response that characterizes desensitization, a qualitative change in the time course of the response has been associated with specific desensitization to angiotensin II (Paiva *et al.*, 1976) and with non-specific desensitization to a cholinoceptor agonist (James-Kracke & Roufogalis, 1981). In both cases, the isometric response of the desensitized ileum of the guinea-pig was shown to be similarly altered: the phasic component of the response is depressed, whereas the tonic component is increased and occurs earlier than in control responses.

In a more detailed investigation of the time course of the isometric responses to acetylcholine, to histamine and to prostaglandin E_2, we have now observed that the desensitized state occurs mainly during a portion of the tonic phase of the response which appears to be associated with increased intracellular Na^+. We have

also observed that, of the three agonists that were studied, the responses to prostaglandin E_2 are the most sensitive to changes in Na^+ concentration in the medium and are also the most affected in desensitized tissues. This paper presents the results of these studies.

Methods

Guinea-pigs of either sex weighing 150–200 g were deprived of food for 24 h and killed by decapitation. A 15 cm portion of the terminal ileum was removed and washed with Tyrode solution at room temperature. Two or three 4 cm segments were cut from the distal end and suspended in identical chambers of 5.0 ml capacity, containing Tyrode solution maintained at 37°C and constantly bubbled with a stream of air. The composition of the nutritive solution was (in mM): NaCl 137, KCl 2.68, $CaCl_2$ 1.36, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.36 and D-glucose 5.1. The sodium-deficient (low-Na^+) solutions were obtained by isosmotic replacement of the NaCl with D-glucose. Glucose was chosen as a Na^+ substitute because, in contrast to sucrose, it did not affect the ileum's response to the addition of hyperosmotic KCl (Shimuta *et al.*, 1982). The pH of the solutions, when equilibrated with air, was 8.0 ± 0.1. The isotonic contractions of the ileum were recorded on a smoked drum through a frontal lever under 1 g load and six fold magnification. Isometric contractions were recorded with a Narco force transducer through a Hewlett-Packard amplifier (model 8805) and an ECB recorder (model RB-102). Resting tension was adjusted to 1 g. Unless otherwise noted, the agonists were left in contact with the preparation for 90 s. Supramaximal concentrations of the agonists were employed not only to induce desensitization, but also to detect the changes in response due to alterations occurring after the receptor agonist interaction. This implies the assumption of receptor saturation in the experimental conditions that were used. The results are presented as means ± s.e.mean, and the significance of the differences between the values was assessed by Student's *t* test. *P* values less than 0.05 were considered significant.

The drugs used were histamine dihydrochloride (California Corporation for Biochemical Research), acetylcholine chloride (Sigma Chemical Corporation), prostaglandin E_2 (Sigma Chemical Corporation), and ouabain octahydrate (Aldrich Chemical Company, Inc.).

Results

Isometric responses in normal medium

Maximum responses of the guinea-pig ileum to acetylcholine (5.5 μM), histamine (2.2 μM), prostaglandin E_2 (0.6 μM) or high K^+ (40 mM), were similar during the first 90 s of contact, i.e. a phasic component consisting of a sharp rise in tension followed by a 'fade' to a lower tonus, initiating the tonic component of the response. However, the time course of the tonic component differed. In the case of prostaglandin E_2 or high K^+, the tonus was maintained at a steady level during the period of observation (50 min), whereas the responses to acetylcholine and histamine presented a transient secondary increase in tonus during the tonic phase of the response (Figure 1). This transient increase in tonus, which lasted 20–30 min, occurred significantly earlier in the case of acetylcholine than in that of

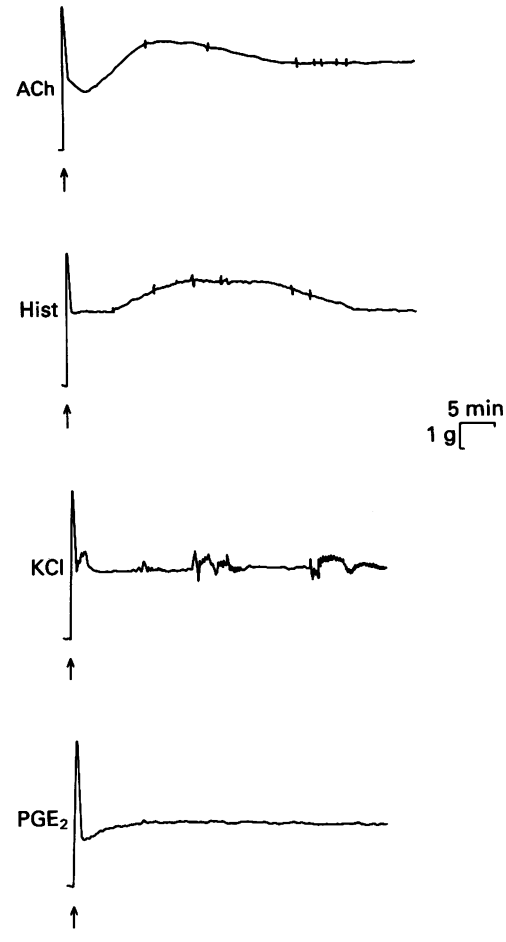

Figure 1 Isometric responses of the guinea-pig isolated ileum in normal Tyrode solution to 5.5 μM acetylcholine (ACh), 2.2 μM histamine (Hist), 40 mM hyperosmotic KCl and 0.6 μM prostaglandin E_2 (PGE_2).

histamine: the average time for the initiation of the secondary increase was found to be 2.5 ± 0.3 min for acetylcholine ($n = 16$) and 5.8 ± 0.5 min for histamine ($n = 26$).

Changes in the isometric responses in the desensitized state

In order to study the effect of pretreatment with maximally effective concentrations of the agonists or high K^+ on the subsequent isometric responses to the same or to a different agent, the following experimental protocol was adopted. Initially, a control response of the ileum to a maximum dose of the agent was recorded for 90 s. After a resting period of 10 min the tissue was again stimulated with the same or a different agent for 12 min (desensitizing treatment). After washing and 3.5 min rest, the response to the same dose of the first agent (test dose) was again recorded for 90 s.

Using this protocol, the treatment with high K^+ or with prostaglandin E_2 did not affect the subsequent responses to any of the agents studied, as is illustrated by the example in Figure 2. However, when the tissue was treated with the maximum dose of acetylcholine or histamine, the subsequent responses to all the agents were differently affected, as is illustrated by the typical examples given in Figure 3. After the desensitizing treatment with either acetylcholine or with histamine, the responses to test doses of either of these agonists showed a decrease of the phasic component and a much faster onset of the secondary increase in tonus, which reached its maximum much earlier than in the control response. In the case of prostaglandin

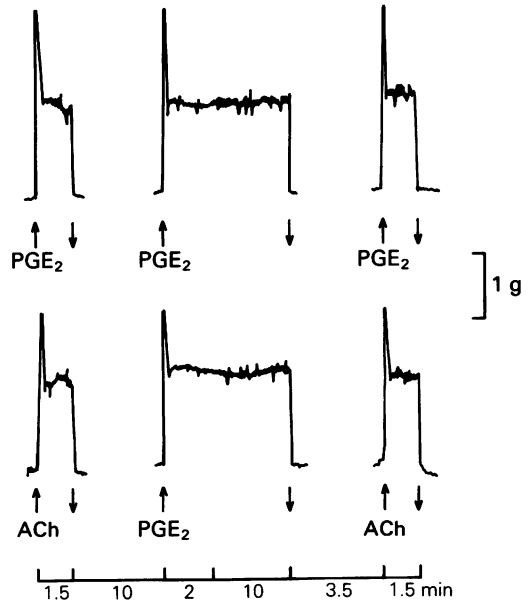

Figure 2 Effect of 0.6 μM prostaglandin E_2 (PGE_2) (12 min contact) upon subsequent responses induced by 0.6 μM PGE_2 or 5.5 μM acetylcholine (ACh). Upward and downward arrows indicate addition and removal of the agonist, respectively. Time intervals (in min) between events are indicated in horizontal scale (note change of recording speed during treatment with desensitizing agent).

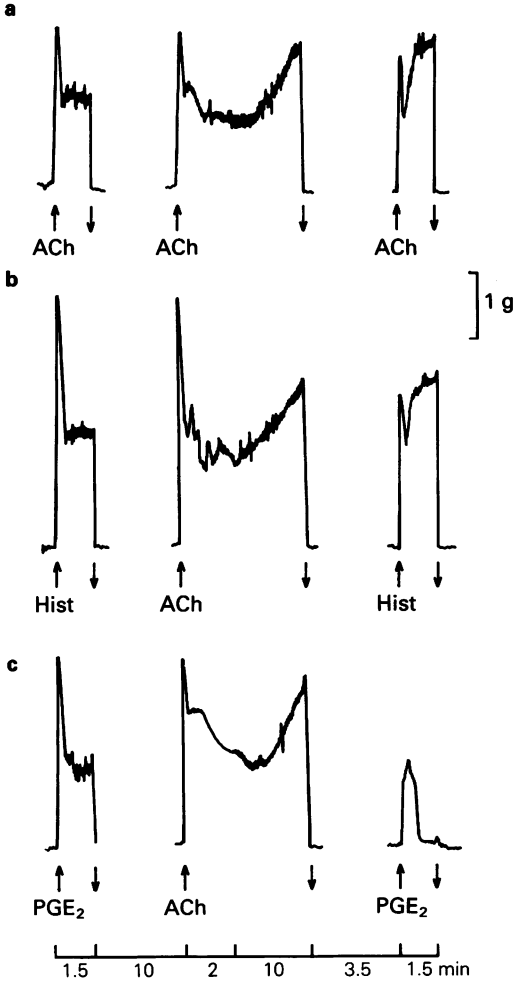

Figure 3 Effect of 5.5 μM acetylcholine (ACh) (12 min contact) upon subsequent responses induced by: (a) 5.5 μM ACh; (b) 2.2 μM histamine (Hist); (c) 0.6 μM prostaglandin E_2 (PGE_2). Upward and downward arrows indicate addition and removal of the agonist, respectively. Horizontal scale as in Figure 2.

E_2, pretreatment with either of the other two agonists caused a marked decrease in both the phasic and the tonic component of the responses (Figure 3c). Finally, the responses to high K^+ were the least affected by pretreatment with acetylcholine or histamine, which caused only a decrease in the phasic component without significantly altering the tonic component (not shown).

In some experiments, in which the responses were recorded isotonically, using the same protocol, the phasic and tonic components of the contractions could not be as clearly distinguished. The main change observed after the desensitizing treatment with acetylcholine or histamine was a decrease in the amplitude of the subsequent responses, which was much more pronounced in the case of prostaglandin E_2 than in those of the other two agonists. This indicates that the qualitative changes observed in the isometric responses (Figure 3) are associated with the desensitized state, which is usually identified only by the quantitative reduction in the amplitude of the responses.

In order to study the effect of the duration of the desensitizing treatment on the subsequent responses to the test doses, the above described protocol was modified to include variation in the time of contact of the preparation with the desensitizing agent. To quantitate the effect, the decrease in amplitude of the phasic component of the response to the test dose, given 3.5 min after washout of the desensitizing dose, was taken as a measure of the degree of desensitization. The results are summarized in Figure 4, which shows that the responses to prostaglandin E_2 were the most affected by pretreatment with either acetylcholine or histamine. It was also observed that the degree of desensitization was maximal 10–20 min after the addition of acetylcholine and 20–30 min after the addition of histamine. After longer treatments with the desensitizing agonist, the degree of desensitization tended to decrease. The maximal desensitization coincided with the maximum tension of the secondary increase in tonus observed during the tonic component of the responses to the desensitizing agonists (compare Figures 1 and 4).

No desensitization was observed to any of the agonists after pretreatment with either prostaglandin E_2, for up to 50 min (Figure 4c), or high K^+ (not shown).

Effect of Na^+ gradient upon the responses to agonists

Na^+ loading during the action of the agonists has been implicated in the origin of the desensitization phenomenon (Paton & Rothschild, 1965; Joiner, 1973). Hence, we have examined the sensitivity of the responses to changes in the Na^+ gradient produced either by exposing the tissues to low Na^+ medium or by treatment with ouabain.

The isotonic responses to histamine and acetylcholine were similarly affected by reduction of the Na^+ concentration in the medium, as is illustrated in Figure 5a for acetylcholine. A small reduction of the Na^+ concentration, from 149 to 137 mM, caused the concentration-response curve to shift to the left, indicating an increased sensitivity of the preparation. In the presence of 112 mM Na^+ a shift to the right was observed, which became more pronounced at 80 mM.

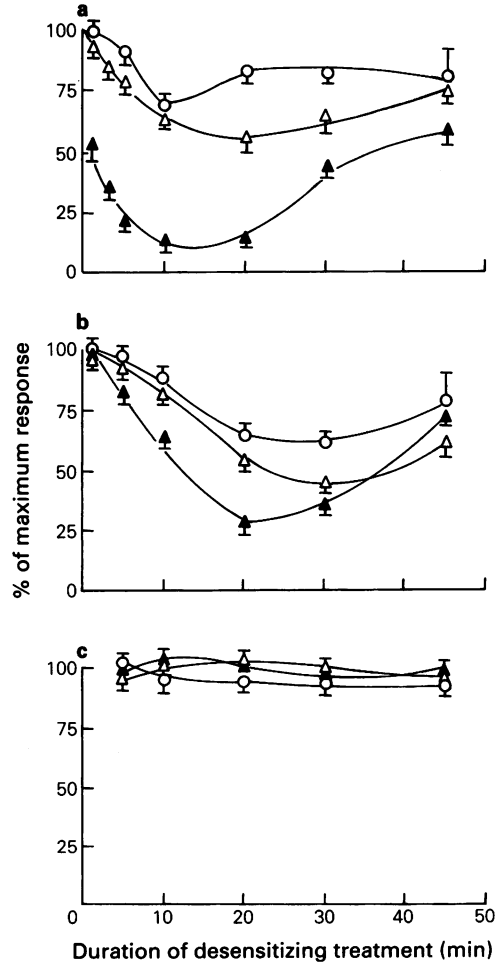

Figure 4 Effect of the duration of pretreatment with 5.5 μM acetylcholine (a), 2.2 μM histamine (b) and 0.6 μM prostaglandin E_2 (c) on the amplitude of the phasic component of the responses to the same concentrations of acetylcholine (○), histamine (△) and prostaglandin E_2 (▲). Each point represents the mean of 8–12 measurements from individual preparations and the s.e.mean are indicated by vertical lines.

At these Na^+ concentrations the maximum responses were not not affected, whereas at 12 mM external Na^+ the responses were almost completely blocked (Figure 5a).

The responses to prostaglandin E_2 were much more affected in low Na^+ medium than those to acetylcholine and histamine. Reduction from 149 to 137 mM already shifted the dose-response curve significantly to the right (Figure 5b) and at 80 mM the responses to this agonist were practically abolished.

Isometric recordings also evidenced a different effect of the low Na^+ medium on the responses to prostaglandin E_2 in comparison with those to the other two agonists. At diminished Na^+ concentrations, in the range 137 to 80 mM, the responses to acetylcholine (Figure 6b) and to histamine (not shown) had their tonic component increased without a significant change in the phasic component. However, the phasic component was significantly decreased at Na^+ concentrations below 80 mM. In contrast, both the phasic and tonic components of the responses to prostaglandin E_2 were markedly decreased by diminishing the Na^+ concentration in the range 137 to 80 mM (Figure 6a).

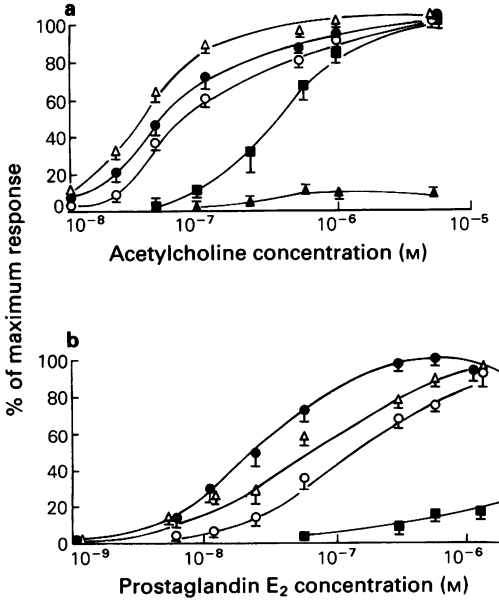

Figure 5 Concentration-response (isotonic) curves for acetylcholine (a) and for prostaglandin E_2 (b) in the presence of different Na^+ concentrations in the medium: 12 mM (▲), 80 mM (■), 112 mM (○), 137 mM (△), 149 mM (●). Each point represents the mean of 8–10 experiments and the s.e.means are indicated by vertical lines.

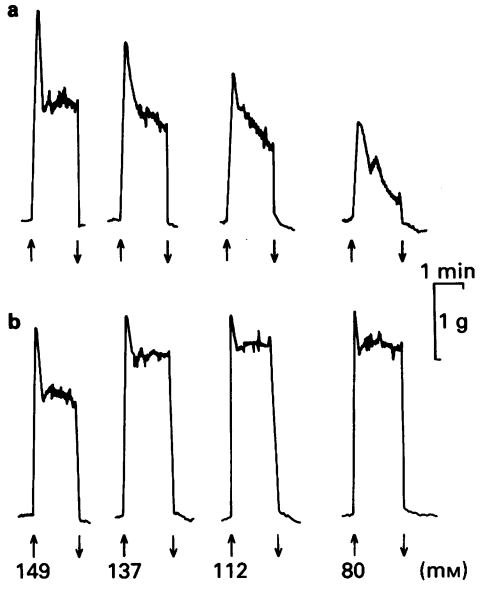

Figure 6 Responses to 0.6 μM prostaglandin E_2 (a) and 2.2 μM histamine (b) in the presence of the indicated Na^+ concentration in the medium. Upward arrows indicate addition of the agonist, and downward arrows, replacement of the medium with a fresh solution containing a different Na^+ concentration, followed by a 30 min equilibration period.

We also studied the effect of ouabain, an agent commonly used to reduce the Na^+ gradient across the cell membrane (Aaronson & van Breemen, 1981; Kishimoto & Urakawa, 1982). The preparation was initially stimulated by an agonist for 90 s (control) and, after a 10 min resting period, 5 μM ouabain was added. After the transient contraction induced by this drug, and still in its presence, the preparation was again stimulated with the same agonist. The responses to histamine (Figure 7a) and to acetylcholine (not shown) exhibited an unaltered phasic component and an increased tonic component. However, under the same conditions, both components of the response to prostaglandin E_2 were greatly reduced (Figure 7c) whereas the response to high K^+ was not affected (Figure 7b).

Discussion

The response of smooth muscle to spasmogenic agents is usually composed of a phasic component, thought to be due to Ca^{2+} release from intracellular stores, and a tonic component, due to influx of extracellular Ca^{2+}

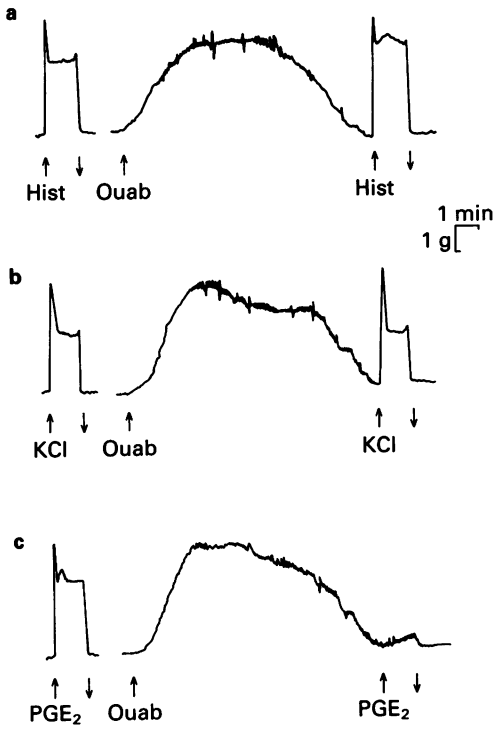

Figure 7 Effect of 5 μM ouabain (Ouab) upon the isometric response induced by 2.2 μM histamine (a), 40 mM KCl (b) and 0.6 μM prostaglandin E_2 (c). Upward and downward arrows indicate addition and removal of the agents.

(for a review see Bolton, 1979). In the guinea-pig ileum, we have observed that during the tonic component of the response to some agonists, a secondary transient increase in tension may occur. This secondary tonic increase is not observed in the response to high K^+ or to prostaglandin E_2, but is present in contractions elicited by acetylcholine or histamine (Figure 1). Furthermore, it occurs earlier in the case of the former than in that of the latter.

The previous finding that the secondary increase in tonus is enhanced and occurs earlier in low-Na^+ than in normal medium (Nouailhetas *et al.*, 1985) suggests that this component of the response reflects a Na^+-dependent mechanism. This suggestion is also supported by the observation of Joiner (1973) that the intracellular Na^+ content in the guinea-pig ileum goes through a maximum during the tonic phase of the response. We have found that, in conditions similar to those employed by Joiner, that maximum coincides with the maximum tension attained during the secondary tonic increase in tension.

Na^+ and K^+ movements are known to be associated with the increase in intracellular Ca^{2+} that causes the smooth muscle's contractile response. Thus, the initiation of the response to acetylcholine is associated with a depolarization due to an increase in permeability to Na^+ and K^+ which also causes a gain of Na^+ and loss of K^+ in the smooth muscle cells, and consequently a decrease in the concentration gradients of these ions across the cell membrane (Bolton, 1979). A similar effect occurs in the action of histamine, but with less intensity than in the case of acetylcholine (Bolton, 1973). There are no data available on the effect of prostaglandin E_2 on ion movements in intestinal smooth muscle in comparison with acetylcholine or histamine, but the response to K^+ is accompanied by an increase in the intracellular concentration of K^+ (James-Kracke & Roufogalis, 1981), in contrast to the decrease provoked by the agonists. Thus, with the exception of prostaglandin E_2, for which the data are not available, there appears to be a correlation between the occurrence of the secondary tonic increase and the decreased ionic gradients across the smooth muscle cell membrane. Particularly, the Na^+ gradient appears to exert a fundamental control over tone, and a decrease in that gradient has been shown to promote Ca^{2+} influx and force development in several intestinal preparations (for a review see van Breemen *et al.*, 1979). The reduction in Na^+ gradient also could decrease Na^+-Ca^{2+} exchange and the consequent decrease in Ca^{2+} extrusion might be responsible for the increased tension. Although the role of the Na^+-Ca^{2+} exchange mechanism in smooth muscle cells is controversial (Brading, 1981), it appears to occur in guinea-pig ileum (Morel & Godfraind, 1984).

We have found that the two agonists capable of eliciting the secondary increase in tonus are similarly affected by two different conditions in which the Na^+ gradient is reduced, namely, the reduction of $[Na^+]_e$ (Figure 6b) and the increase in $[Na^+]_i$ by blocking of the Na^+/K^+ pump (Figure 7a). In both cases, the tonic component of the response is enhanced. This appears to be due to a premature occurrence of the 'secondary tonic increase' since, at least in the case of $[Na^+]_e$ reduction, it has been shown that a gradual decrease in $[Na^+]_e$ causes a gradual decrease in the time for onset of the tonic increase until the fade is no longer observed (see Figure 4 in Nouailhetas *et al.*, 1985).

Prostaglandin E_2, in contrast to the other two agonists, appears to be unable to generate the decreased Na^+ gradient that would give rise to the secondary increase in tonus (Figure 1b) and was also unable to induce desensitization (Figure 2). The responses to prostaglandin E_2 were particularly sensitive to changes in the Na^+ gradient (Figures 5, 6a and 7c) and were severely impaired in preparations previously submitted to desensitizing treatment with

either histamine or acetylcholine (Figure 3c).

The shapes of the responses of the desensitized preparations to the three agonists were similar to the shapes of the respective responses in low-Na^+ medium (compare Figures 3 and 6) and in the presence of ouabain (compare Figures 3 and 7). This suggests that the changes in the responsiveness of the desensitized tissue may be due to the observed decrease in Na^+ gradient.

Further evidence in favour of this conclusion is the finding that these changes are maximal (Figure 4) during the secondary tonic increase, which appears to be associated with the transient decrease in the Na^+ gradient across the smooth muscle cell membrane.

In conclusion, our results show that the responses of the desensitized guinea-pig ileum to different agonists correlate with their dependence on the Na^+ gradient, suggesting that the non-specific desensitization elicited by treatment with maximal concentrations of histamine or acetylcholine is due to a decreased Na^+ gradient across the cell membrane. Prostaglandin E_2 appears to be particularly sensitive to this change, and this property should be further explored, as it might become a useful pharmacological probe for indirectly assessing the state of the Na^+ gradient across the smooth muscle cell membrane.

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