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THE EFFECTS OF α- AND β-ADRENOCEPTOR ACTIVATION ON TENSION AND MEMBRANE PROPERTIES OF THE LONGITUDINAL SMOOTH MUSCLE OF THE CHICKEN RECTUM

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1 Isolated longitudinal muscle strips from the chicken rectum responded to isoprenaline, adrenaline and noradrenaline with a prolonged relaxation. The concentrations required to produce 50% of the maximum relaxation were 1.3×10^{-8} M for isoprenaline, 1.7×10^{-8} M for adrenaline and 10^{-6} M for noradrenaline. The relaxing potency of isoprenaline is about equal to that of adrenaline, but more than 50 times that of noradrenaline.

2 Propranolol, 3.4×10^{-6} M, blocked the isoprenaline-induced relaxation, and in the presence of this drug the responses to adrenaline and noradrenaline were converted into small, transient relaxations. The residual relaxation was blocked by phentolamine, 2.6×10^{-6} M.

3 These catecholamines suppressed spontaneous spike discharge and produced membrane hyperpolarization. Propranolol, 3.4×10^{-6} M, prevented the inhibitory effects of isoprenaline, and reduced but did not completely abolish those of adrenaline and noradrenaline.

4 Adrenaline and noradrenaline, but not isoprenaline, reduced membrane resistance in some preparations.

5 In the rectal muscle of the chicken, the β-adrenoceptor mediates a prolonged relaxation and the α-adrenoceptor a fast and short-lasting relaxation which is usually obscured by the β-response and unmasked only after blockade of the β-adrenoceptors. The α- and β-mediated relaxations are each associated with the suppression of spontaneous spike activity.

Introduction

The chicken rectum receives a dense adrenergic innervation from Remak's nerve and from periarterial nerves (Bennett & Malmfors, 1970; Takewaki, Ohashi & Okada, 1977). High levels of adrenaline are present in the chicken rectum (Konaka, Ohashi, Okada & Takewaki, 1979) associated with Remak's nerve (Komori, Ohashi, Okada & Takewaki, 1979a). However, information about the pharmacological characteristics of the adrenoceptors in the effector cells which would promote an understanding of the function of the adrenergic nerves in the chicken rectum, is limited.

Both α- and β-adrenoceptors are present on the rectal muscles of the young chick (Bartlet & Hassan, 1970). Phenylephrine, a relatively selective stimulant of α-adrenoceptors (Ahlquist, 1948; Levy & Ahlquist, 1967), relaxes the chick rectum, an effect antagonized by the β-adrenoceptor antagonist, propranolol. In the presence of propranolol, phenylephrine produces a small relaxation which is blocked by phentolamine. The adrenoceptors present in the chick rectum might not be similar therefore to those generally present in other intestinal muscles (Levy & Ahlquist, 1967).

In the present experiments, an attempt has been made to characterize the adrenoceptors of the chicken rectum and to gain information on the underlying changes in the membrane properties of smooth muscle cells. The longitudinal muscle layer was chosen because of its importance in determining the mechanical responses along the longitudinal axis of the organ (Takewaki *et al.*, 1977) and, in addition, the smooth muscle cells could be impaled with a microelectrode (Takewaki & Ohashi, 1977).

Methods

Tissue preparation

Adult White Leghorn cocks were stunned and bled. The rectal region of the intestine was removed and strips excised longitudinally. The longitudinal layer was carefully dissected from each strip.

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Measurement of mechanical responses

The longitudinal muscle strips (about 1 mm in width and 10 mm in length) were mounted in a 5 ml organ bath containing Tyrode solution of the following composition (mM): NaCl 137.0, KCl 2.7, NaH_2PO_4 0.4, $NaHCO_3$ 12.0, $MgCl_2$ 1.0, $CaCl_2$ 1.8 and glucose 5.6, kept at 30°C and bubbled with air. These conditions allowed constant responses to be obtained for many hours. Changes in tension were recorded isotonically on smoked paper. The resting tension was adjusted by loading the recording lever (magnification approximately 8 times) with a 0.5 g weight, and the strips equilibrated for at least 30 min. During this period, tone of the muscle developed naturally. Agonists (adrenaline, noradrenaline and isoprenaline) were added to the organ bath in a volume of 0.5 ml with a fine syringe to give the desired concentration and allowed to act for 3 min. The action was terminated by washing four times with fresh Tyrode solution. The interval between successive doses was more than 10 min. Both α- and β-adrenoceptor antagonists were applied in a way similar to the agonists and allowed to remain in the bathing solution for 30 min to produce their maximum blocking effect before the application of agonists.

Measurement of electrical responses

The effects of catecholamines on membrane potential, spontaneous and evoked spike activity and membrane resistance were recorded intracellularly. Glass microelectrodes filled with 3 M KCl, with resistances of 40 to 80 MΩ were used. To record spontaneous spike activity, strips (3 × 10 mm) were pinned out, serosal surface up, on rubber (20 × 20 × 5 mm) and immersed in a 3 ml organ bath perfused at a rate of 5 ml/min with aerated Tyrode solution. The bathing solution was kept at 37°C to maintain spontaneous discharge of spikes. The evoked spikes and electrotonic potentials were recorded as described by Abe & Tomita (1968). In this series of experiments, Tyrode solution with tonicity increased to about 1.6 to 1.8 times normal by the addition of sucrose (175 to 214 mM) was used to prevent dislodgment of the microelectrode. Changes in the membrane potential were displayed on an oscilloscope and photographed.

Drugs

Drugs used were: (−)-adrenaline base (Merck), (−)-noradrenaline bitartrate (Merck), (−)-isoprenaline sulphate (Merck), (−)-propranolol hydrochloride (Sumitomokagaku) and phentolamine mesylate (Ciba-Geigy). The concentrations referred to are those of the base in the bathing solution. The stock solutions of all drugs were dissolved in distilled water, made up at 1000 or more times higher concentrations than those used for the experiments, and stored at −20°C. Final dilutions were made in Tyrode solution just before use.

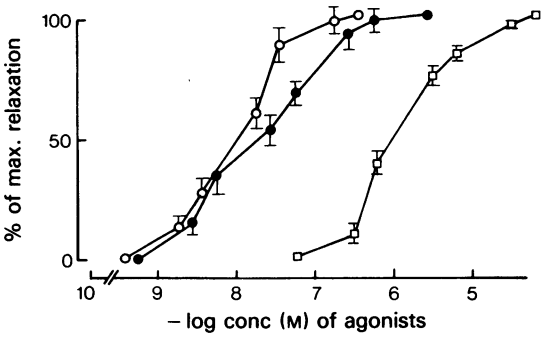

Figure 1 Log dose-response curves to isoprenaline (○), adrenaline (●) and noradrenaline (□) in the chicken rectum. Each point presents the mean of six to nine separate determinations. Vertical lines show s.e. mean.

The significance of the results was evaluated by Student's t test. Statistical significance was taken to be when $P < 0.05$.

Results

Effects of isoprenaline, adrenaline and noradrenaline on mechanical activity

Isoprenaline, adrenaline and noradrenaline, each produced a concentration-dependent relaxation of the muscle strips, but the effective concentration differed for each catecholamine. Relaxations started 2 to 5 s after application of each catecholamine, there was no appreciable difference in the time of onset of each. The development of the relaxation varied with different catecholamines. Isoprenaline-induced relaxation developed progressively to reach a maximum, usually within 2 min, which was maintained throughout the presence of the drug. Sometimes, the relaxation continued to develop throughout the application period (3 min). The effect subsided so slowly after removal of the drug that the time taken for recovery to the initial tone varied between 5 and 15 min depending on the concentration. Adrenaline-induced relaxation was usually as long-lasting as that of isoprenaline, but the time taken to reach its maximum was slightly less. In some experiments, adrenaline, particularly with the smaller doses, caused a fast and brief relaxation, which reached a maximum within 20 s and which was followed by a more prolonged one. Noradrenaline behaved similarly to adrenaline but required higher concentrations.

Figure 1 shows dose-response curves to the three

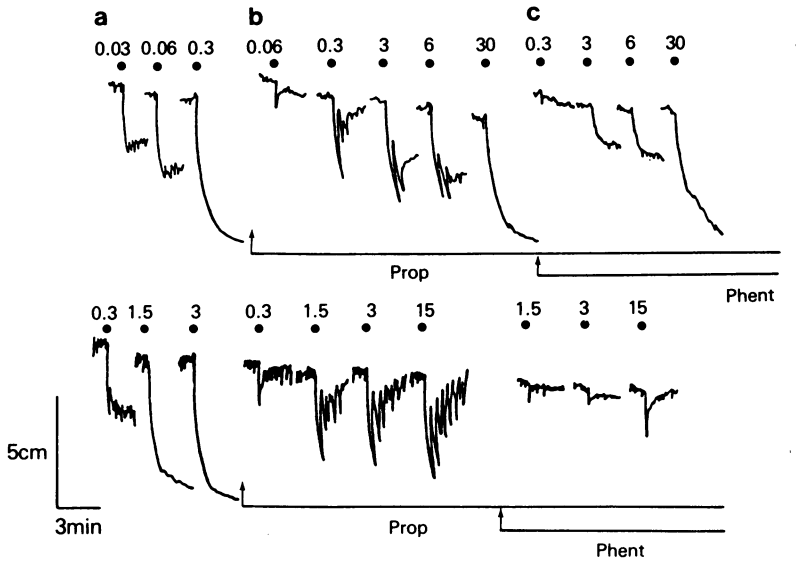

Figure 2 Mechanical responses of the longitudinal muscle strips of the chicken rectum to adrenaline (upper series) and noradrenaline (lower series) before and after treatment with propranolol alone and propranolol with phentolamine. Control responses to adrenaline (a, upper trace) (0.03, 0.06 and 0.3 μM) and to noradrenaline (a, lower trace) (0.3, 1.5 and 3 μM); (b, upper trace) responses to adrenaline (0.06, 0.3, 3, 6 and 30 μM) after pretreatment with propranolol (3.4×10^{-7} M) for 30 min; (b, lower trace) responses to noradrenaline (0.3, 1.5, 3 and 15 μM) after pretreatment with propranolol (3.4×10^{-6} M) for 30 min; (c, upper trace) responses to adrenaline (0.3, 3, 6 and 30) and (c, lower trace) to noradrenaline (1.5, 3 and 15 μM) after additional pretreatment with phentolamine (2.6×10^{-6} M) for 30 min. About 30 min elapsed between (a) and (b) and between (b) and (c) in each row.

catecholamines. The responses were determined by single additions at intervals of more than 10 min, and expressed as percentages of the maximum relaxations obtained to each drug. The ED_{50} values were $1.3 \pm 0.3 \times 10^{-8}$ M (mean ± s.e. mean of 6 experiments) for isoprenaline, $1.7 \pm 0.3 \times 10^{-8}$ M (mean ± s.e. mean of 9 experiments) for adrenaline and $1.0 \pm 0.4 \times 10^{-6}$ M (mean ± s.e. mean of 6 experiments) for noradrenaline, and the pD_2 values were 7.9 ± 0.1, 7.8 ± 0.1 and 6.0 ± 0.1, respectively. The potency of isoprenaline was not significantly different from that of adrenaline, but it was above 50 times that of noradrenaline.

Effects of an α, or β-adrenoceptor blocking agent on catecholamine-induced relaxations

The relative potencies of the catecholamines suggested that the relaxation could be due to activation of β-adrenoceptors. This was tested by the use of propranolol. Propranolol antagonized the effect of isoprenaline in a competitive manner and its pA_2 value was 8.79 ± 0.11 ($n = 5$). Relaxations were produced by adrenaline and noradrenaline first in the absence and then after pretreatment with propranolol (3.4×10^{-7} or 3.4×10^{-6} M) for 30 min. Propranolol reduced the relaxations produced by adrenaline and noradrenaline in magnitude and duration (Figure 2). In the presence of propranolol, as the concentration of noradrenaline and adrenaline was increased, a transient phase of relaxation followed by a long-lasting relaxation (a biphasic effect) was observed. The transient effect was masked by the prolonged relaxation. These findings suggest that relaxations produced by adrenaline and noradrenaline may consist of a transient and a long-lasting component, mediated by different mechanisms and that the latter component may be mediated by β-adrenoceptors. The initial, transient relaxations produced by both catecholamines in the presence of propranolol were markedly reduced or abolished by additional treatment with phentolamine (2.6×10^{-6} M) for 30 min (Figure 2c). The dose-transient relaxation curves to both catecholamines in the presence of propranolol (3.4×10^{-6} M), each lay near to the right of the dose-response curve before propranolol, the slope of the curve was slightly steeper, and the maximum relaxation was 61.7 ± 4.6% ($n = 10$) of the maximum relaxation in the absence of propranolol. The pA_2 value for phentolamine was found to be 6.85 ± 0.13 ($n = 3$). Phentolamine alone blocked the initial, fast component, but not the prolonged component of the biphasic relaxation produced by both adrenaline and noradrenaline. As a result these catecholamines in-

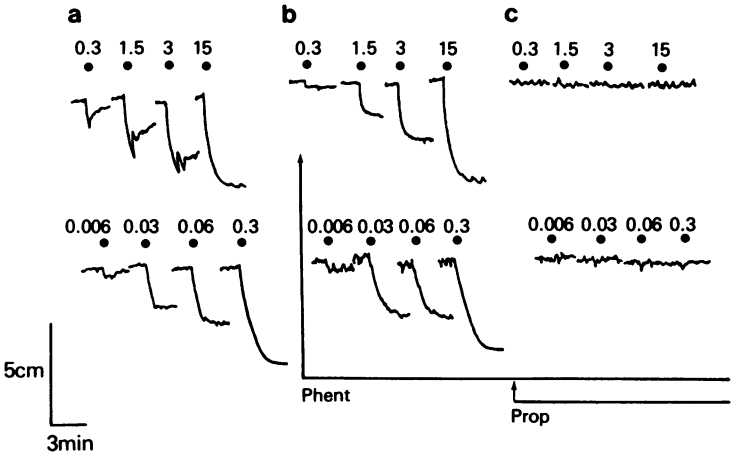

Figure 3 Mechanical responses of the longitudinal muscle strips of the chicken rectum to noradrenaline (upper series) and adrenaline (lower series) before and after treatment with phentolamine alone and phentolamine with propranolol. (a, upper trace) Control responses to noradrenaline (0.3, 1.5, 3 and 15 μM) and to adrenaline (a, lower trace) (0.006, 0.03, 0.06 and 0.3 μM); (b) after treatment with phentolamine (2.6×10^{-6} M) for 30 min; (c) after additional treatment with propranolol (3.4×10^{-6} M) for 30 min respectively. About 30 min elapsed between (a) and (b), and between (b) and (c) in each row. Note specific blockade with phentolamine of the initial, transient component of the biphasic relaxations produced by noradrenaline.

variably produced a prolonged relaxation in the presence of phentolamine (Figure 3). The prolonged relaxation was reduced substantially or blocked completely by the higher concentration of propranolol (3.4×10^{-6} M). The pA_2 values for propranolol for the adrenaline and noradrenaline-induced relaxations in the presence of phentolamine (2.6×10^{-6} M) were 8.65 ± 0.14 and 8.77 ± 0.11 ($n = 3$ for each drug) respectively and were not significantly different from that of the isoprenaline-induced relaxation.

These findings suggest that the longitudinal muscle of the chicken rectum possesses both α- and β-adrenoceptors; the α-adrenoceptor serves to mediate a transient and the β-adrenoceptor a prolonged relaxation.

Effects on electrical activity

The longitudinal muscle cells have a resting membrane potential ranging between −45 and −55 mV and discharged spikes with or without overshoot spontaneously. Stable impalements were maintained for periods from 10 min up to 2 h. Frequencies of the spontaneous spike discharges ranged from 0.4/s to

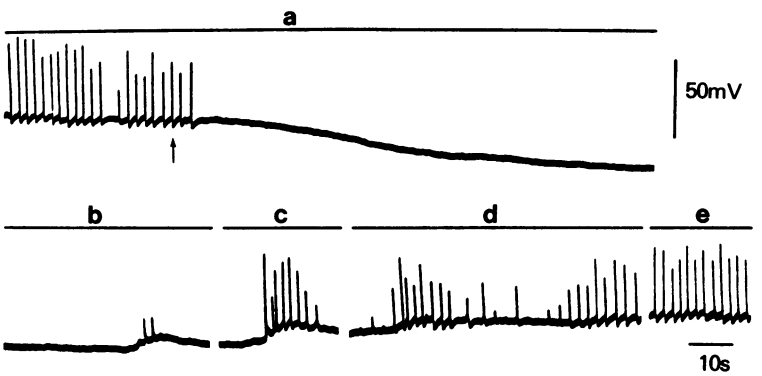

Figure 4 The effect of isoprenaline on spontaneous spike activity and on membrane potential of the chicken rectum. (a) Response to isoprenaline, 3.6×10^{-7} M, applied at the arrow and allowed to act for 2 min; (b), (c), (d) and (e) 7, 9, 10 and 15 min after removal of the drug, respectively. The upper horizontal line corresponds to the zero potential of the cell. Times between panels in sequence are 450, 75, 35 and 240 s. Note an increase in the membrane potential during exposure to isoprenaline.

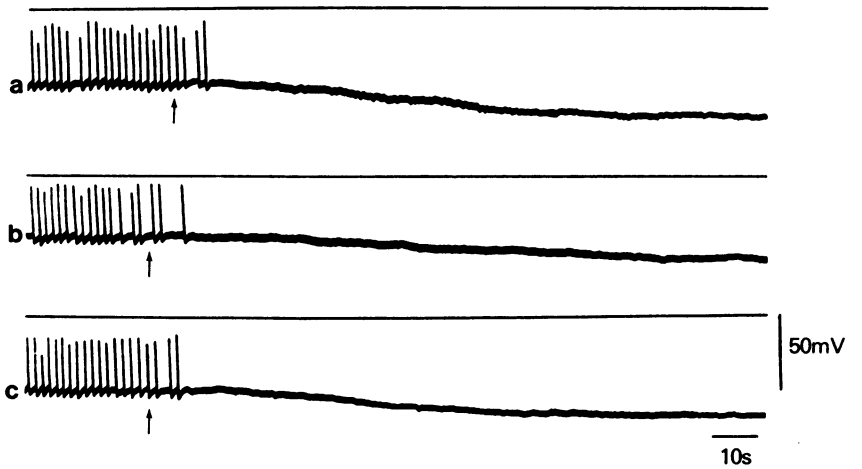

Figure 5 The effects of noradrenaline, adrenaline and isoprenaline on the intracellularly recorded electrical activity in the same cell of the longitudinal muscle of the chicken rectum. (a) Noradrenaline, 15 μM; (b) adrenaline, 0.12 μM; (c) isoprenaline, 0.07 μM. Each drug was applied at the arrow. These concentrations suppressed spontaneous spike activity for about the same period. Relative potencies of noradrenaline, adrenaline and isoprenaline are estimated to be 1, 125 and 214. About 40 min elapsed between (a) and (b), and 20 min between (b) and (c). In each case the upper horizontal line corresponds the zero potential of the cell.

11/s in different tissues and spike sizes varied from less than 10 mV up to 70 mV in one tissue and varied within the same cell depending mainly on the membrane potential. Higher frequencies and smaller size spikes were observed on tissues with lower membrane potentials.

Isoprenaline hyperpolarized the membrane by up to 25 mV and suppressed the spontaneous spike discharge. The effects increased with increasing isoprenaline concentration. The suppression of spike activity occurred several seconds before membrane hyperpolarization in most cells and in the others both effects developed simultaneously. Spikes which were discharged at the increased membrane potential in the presence of isoprenaline were usually larger in amplitude and faster in rate of rise. Isoprenaline at a concentration of 3.6×10^{-7} M (the highest concentration tested) hyperpolarized the membrane by about 25 mV (Figure 4). Removal of the drug led to the gradual restoration of the membrane potential and of a sustained discharge of spontaneous spikes.

Adrenaline and noradrenaline also had inhibitory effects on the membrane activity. Figure 5 shows the comparable effects of noradrenaline (1.5×10^{-5} M), adrenaline (1.2×10^{-7} M) and isoprenaline (7.2×10^{-8} M) on the discharge of spontaneous spike activity in the same cell. The order of potency was again, isoprenaline $\geqq$ adrenaline $\gg$ noradrenaline. If spikes were discharged during membrane hyperpolarization produced by noradrenaline, they were larger in amplitude and faster in their rate of rise as illustrated in Figure 6. This reflects increased action current during the membrane hyperpolarization and indirectly suggests that the hyperpolarization was not accompanied by a marked decrease in membrane resistance.

Effects of propranolol on the action of catecholamines

Propranolol (3.4×10^{-7} or 3.4×10^{-6} M) itself sometimes produced a slight depolarization of 5 to 10 mV which resulted in an increase in the spontaneous spike

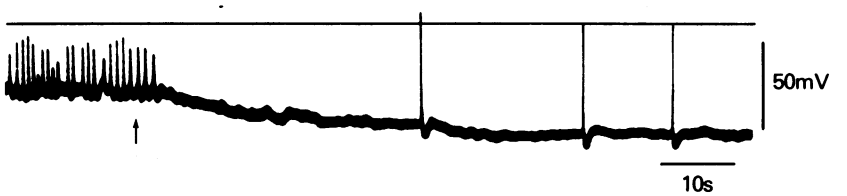

Figure 6 The effect of noradrenaline on electrical activity in the chicken rectum. Noradrenaline, 15 μM, was applied at the arrow. Note spikes with an increased amplitude and rate of rise during the membrane hyperpolarization. The upper horizontal line corresponds to the zero potential of the cell.

Figure 7 The effect of propranolol on the response to adrenaline in the chicken rectum. (a) Control response to adrenaline, 0.6 μM, applied at the arrow and allowed to act for 2 min; (b) 30 min after treatment with propranolol, 0.34 μM. The upper horizontal line indicates the zero potential of the cell. The residual effect of adrenaline remained unaltered even after increasing the antagonist concentrations up to 3.4 μM.

activity. Both the membrane hyperpolarization and the suppression of spike discharges produced by isoprenaline in concentrations up to 3.6×10^{-7} M were blocked. Propranolol, up to 3.4×10^{-6} M, markedly reduced, but did not prevent, the inhibitory effects of adrenaline and noradrenaline (Figure 7). The residual response to the catecholamines in the presence of propranolol was a slight, transient decrease in the frequency of the spike discharge with little change in membrane potential. These remaining effects may be mediated through stimulation of α-adrenoceptors. However, this could not be demonstrated by the use of phentolamine as this antagonist itself had an excitatory effect on the membrane activity, which made it impossible to maintain impalement of microelectrodes.

Effects on the membrane resistance

To see whether the inhibition of spontaneous electrical activity produced by the catecholamines arose from an increased ionic permeability of the membrane, the effects on the membrane resistance were examined. Electrotonic potentials were evoked and recorded as described by Abe & Tomita (1968). To prevent the microelectrode from being dislodged, the tonicity of the bathing fluid was increased by sucrose (see Methods). Electrotonic potentials, recorded at a given distance from the partition dividing the stimulating and recording compartments, increased in amplitude proportionally to the intensity of hyperpolarizing current pulses (see Figure 8). This indicates that the V–I relationship is almost linear. An electrotonic potential decayed along the tissue exponentially and the space constant was found to be 1.67 ± 0.11 ($n = 10$). In some cells, the membrane potential decreased below the initial level following the end of the current pulse (anodal break excitation).

Isoprenaline invariably caused no detectable change in electrotonic potentials (Figure 8) and no apparent change in membrane resistance. This supposition is tested by comparing the slope of the curves of the V–I relationship before and during exposure to isoprenaline (Figure 8c). The slope of the V–I curve was not reduced by isoprenaline, indicating there was no decrease in membrane resistance. Sometimes, a very small but appreciable increase in the electrotonic potential was observed for a short period during the course of removal of the drug (after-effect). The hyperpolarizing effect was smaller in hypertonic than in normal media. Isoprenaline (3.6×10^{-7} M) increased the membrane potential by 6.8 ± 0.7 mV ($n = 13$) in the hypertonic media. This value is about one-third that in the normal medium. Adrenaline or noradrenaline hyperpolarized the membrane potential and in so doing slightly reduced the size and time course of the electrotonic potential indicating a fall in membrane resistance; the effects were completely reversible after removal of these drugs (Figure 9). With a ten fold higher concentration of adrenaline, the effect was no greater. In other preparations, the electrotonic potentials remained unaffected during exposure to either drug whether or not it hyperpolarized the membrane. In some of these preparations, the electrotonic potentials increased slightly and briefly during the course of removal of the drug. Even after β-adrenoceptors had been blocked by propranolol (3.4×10^{-6} M), no change in the electrotonic potential was observed during or after exposure to these catecholamines. This suggests that the presence of functional β-adrenoceptors itself did not prevent these drugs from acting directly on the membrane resistance, but that these receptors might be involved in the transient, small increase in membrane resistance during the washing out (after-effect). The magnitude of hyperpolarization produced by adrenaline and noradrenaline was also reduced in the hypertonic media.

Isoprenaline-induced suppression of spontaneous spike activity prior to membrane hyperpolarization, with no accompanying effect on membrane resistance,

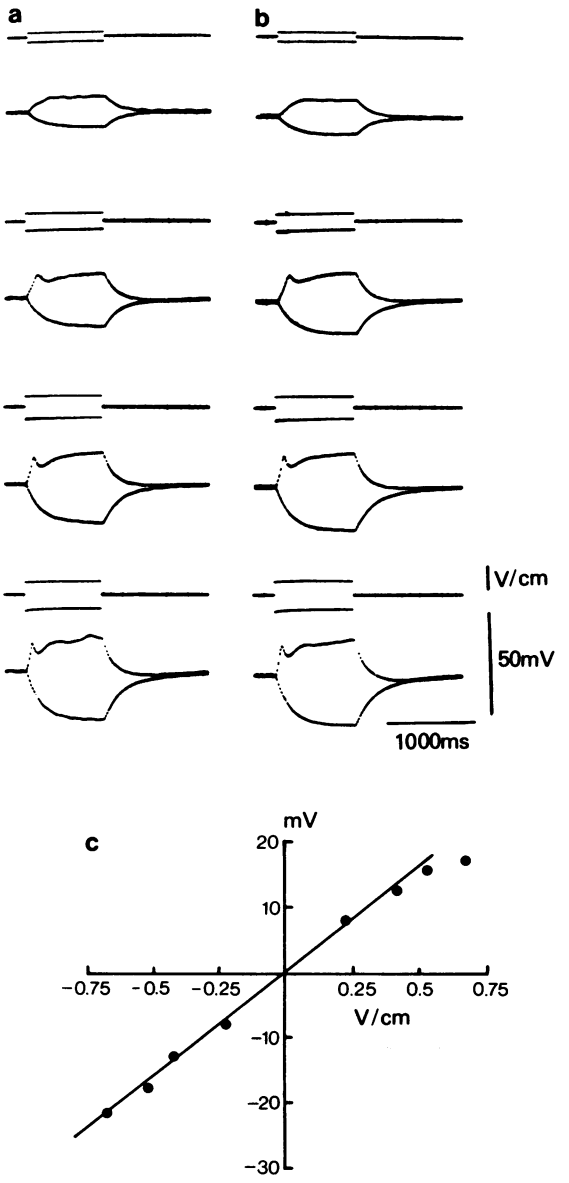

Figure 8 The effect of isoprenaline on the electrotonic potentials produced by current pulses of 800 ms in duration at four different intensities in the chicken rectum. Upper trace: potential field (V/cm). Lower trace: electrotonic potentials evoked by anodal (downwards) and cathodal (upwards) current pulses. (a) Control; (b) during exposure to a solution containing isoprenaline, 0.18 μM; (c) graph showing the relationship between the strength of the potential field (abscissa scale) and the size of the electrotonic potential (ordinate scale) before application of isoprenaline. The relationship is little altered by isoprenaline and has been omitted. These experiments were performed in hypertonic media.

led us to investigate its effect on spike generation. Isoprenaline (3.6×10^{-7} M, Figure 10) was applied to the bath after recording control responses to depolarizing current pulses. During development of the hyperpolarization to isoprenaline three current pulses of different intensities failed to evoke a spike, although the strongest pulse elicited an abortive-spike like depolarization (Figure 10c). This inhibitory effect on spike generation tended to subside thereafter, whereas the hyperpolarization was sustained. This reduction in excitability persisted for several minutes after higher concentrations of isoprenaline. In some cells, in which the membrane potential was little changed by isoprenaline, no spikes were evoked for up to 6 min in the presence of the drug. Thus the effect may be independent of the membrane potential.

Discussion

The present results show the presence of α- and β-inhibitory adrenoceptors in the longitudinal muscle of the chicken rectum. The β-adrenoceptor is presumably of the β_2-type (Lands, Arnold, McAuliff, Luduena & Brown, 1967), and shows the following order of potency: isoprenaline ≧ adrenaline ≫ noradrenaline. In these respects, the muscle is similar to intestinal muscles. The difference is that relaxations produced by adrenaline and even noradrenaline are due to a preferential stimulation of β-adrenoceptors; the α-adrenoceptor-mediated component is small and usually unmasked only after β-adrenoceptor blockade. This can be explained simply by assuming that the rectal muscle possesses only a small number of α-adrenoceptors. Alternatively, this could result from a much lower affinity of the α-adrenoceptor for these catecholamines or a much smaller unit stimulus produced by the agonist-receptor complexes. The absence of any significant change of the concentration-axis and the slope of the dose-response curves to noradrenaline and adrenaline after blockade of adrenoceptors does not favour the latter idea. The view that the muscle contains only a small number of α-receptors is consistent with the difficulties in demonstrating functional α-adrenoceptors which mediate a decrease in membrane resistance. The slight decrease in the membrane resistance which was observed appears to be exclusively mediated through α-adrenoceptor activation, since only adrenaline and noradrenaline, but not isoprenaline, were effective. A similar α-mediated effect on membrane resistance also occurs in the taenia coli of the guinea-pig (Bülbring & Tomita, 1969a, b; Ohashi, 1971). As can be seen in Figure 6, spikes occurred spontaneously with a larger amplitude and a higher rate of rise during membrane hyperpolarization in the presence of noradrenaline. This suggests there is normally no marked increase in

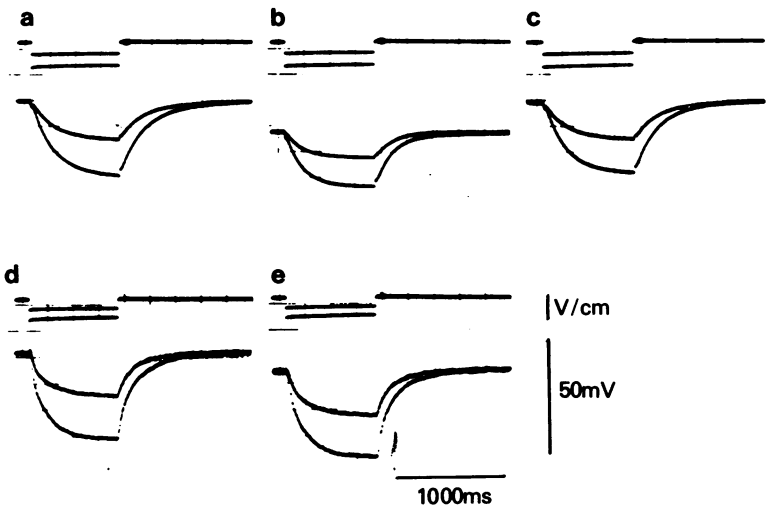

Figure 9 Comparison of the effects of adrenaline and isoprenaline on the electrotonic potentials produced by current pulses of 800 ms in duration at two different intensities in the chicken rectum. (a–c) Records from one preparation; (d) and (e) records from another preparation. Upper trace: potential field (V/cm). Lower trace: electrotonic potentials evoked by anodal current pulses. (a and d) Control; (b) during exposure to adrenaline, 3 μM; (c) 15 min after removal of adrenaline; (e) during exposure to isoprenaline, 1.8 μM. Adrenaline hyperpolarized the membrane potential and in so doing decreased the size and the time course of the electrotonic potential, isoprenaline hyperpolarized the membrane potential with no detectable change in the electrotonic potential. These experiments were performed in hypertonic media.

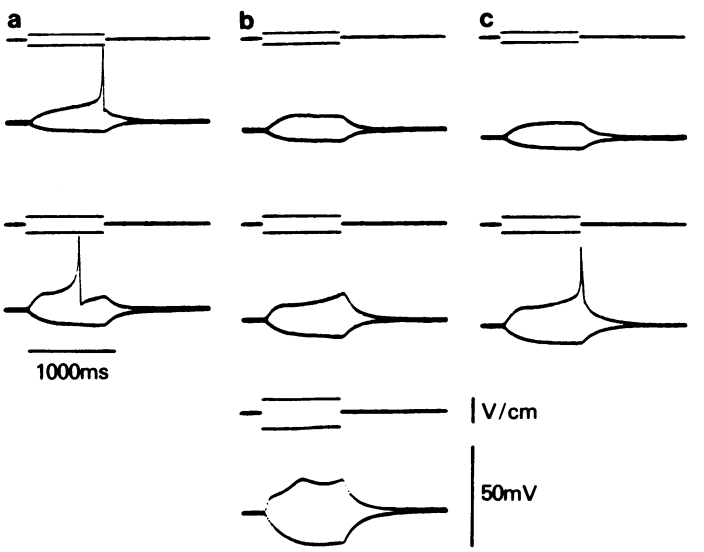

Figure 10 The effect of isoprenaline on spike potentials evoked by cathodal current pulses of 800 ms in duration in the chicken rectum. Upper trace: potential field (V/cm). Lower trace: electrotonic and spike potentials evoked by anodal (downwards) and cathodal (upwards) current pulses. (a) Electrotonic and spike potentials evoked by current pulses with different polarities at different intensities; (b) immediately and (c) 10 min after exposure to isoprenaline, 0.36 μM. Note transience of the inhibitory effect on spike generation.

the membrane conductance, although the hyperpolarization itself may modify the membrane resistance and the rate of rise of spikes. The experimental conditions (hypertonic condition) might also have a non-specific inhibitory effect on the α-action as well as the β-action, making it even more difficult to demonstrate.

The higher sensitivity of the muscle to adrenaline than to noradrenaline (50 times) may reflect the large contribution of β-adrenoceptors and their higher affinity for adrenaline (Lands *et al.*, 1967). Alternatively, noradrenaline could be taken up preferentially by the neuronal tissue and subsequently metabolized. In the rat heart, the uptake process has only about a two fold greater affinity for noradrenaline than adrenaline (Iversen, 1967). In the chicken rectum there is a 50 fold greater sensitivity to adrenaline than noradrenaline, yet blockade of uptake by phenoxybenzamine following stimulation of Remak's nerve increases the mean output of noradrenaline by only seven fold and that of adrenaline by three fold (Komori *et al.*, 1979a). It appears unlikely therefore that the high sensitivity to adrenaline arises from an increased uptake and subsequent metabolism of noradrenaline. The high sensitivity to adrenaline makes the chicken rectum a suitable tissue for discriminating adrenaline from noradrenaline in fluids containing both amines (see Vane, 1964).

The α-induced relaxation is accompanied by a transient inhibition of spontaneous spike discharge with or without membrane hyperpolarization. The α-action may produce, as in guinea-pig taenia coli, an ionic permeability change of the membrane and a partial hyperpolarization, which in turn inhibits spike discharge by reducing pace-maker potentials or by preventing propagation of spike potentials. The β-relaxation may be due to prolonged cessation of spontaneous spike discharge with marked membrane hyperpolarization. Membrane hyperpolarization by activation of β-adrenoceptors is not common in the intestine, but occurs in other smooth muscle (rat uterine muscle, Marshall, 1968; rabbit main pulmonary artery, Somlyo, Haeusler & Somlyo, 1970). This increase in membrane potential has been attributed to a stimulation of an electrogenic sodium pump (Somlyo *et al.*, 1970) or to an increased potassium permeability (Marshall, 1968). The hyperpolarization occurred without change in membrane resistance in the chicken rectum. This supports the former view as do the results from experiments where the effects of ouabain and the removal of the external sodium on isoprenaline-induced relaxation have been examined (Komori, Ohashi, Okada & Takewaki, 1979b). Ouabain in concentrations of more than 10^{-5} M caused a marked inhibition of the isoprenaline-induced relaxation. Removal of external sodium by substitution with Li, Tris, K or sucrose resulted in a gradual reduction of isoprenaline-induced relaxation.

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