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The Action of Catecholamines on Guinea-Pig Taenia Coli

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The action of catecholamines on guinea-pig taenia coli

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Observations of the effects of catecholamines on taenia coli indicate that the α and β actions are exerted on two different enzyme systems both involved in cellular mechanisms which regulate the intracellular Ca^{2+} concentration, one by translocation of Ca within the cell and the other by removing Ca out of the cell.

The β action reduces the tension response to membrane excitation, probably by increased Ca uptake at intracellular stores, in all smooth muscles.

The α effect is associated with a change in membrane conductance resulting in hyperpolarization in some, including taenia coli, but depolarization in other smooth muscles. Nevertheless, the underlying mechanism may be the same, i.e. Ca release from intracellular stores and stimulation of Ca extrusion.

This hypothesis is based on the assumption that the membrane permeability to K (and hence the membrane polarization and the degree of spontaneous activity) is largely determined by the amount of Ca bound at the inner surface of the plasma membrane. The change in membrane potential and membrane activity produced by the intracellular release of Ca caused by the α-adrenergic action will, therefore, depend on the ratio between the amount of Ca bound at the inside of the cell membrane and the rate of Ca pump activity in individual tissues.

The catecholamines are a particularly interesting group of substances because their action consists of two components, the α and β action (Ahlquist 1948) which, in the majority of smooth muscles, produce opposite effects so that they seem to have a built-in mechanism for limiting their own action.

As a rule, the response to the α action is contraction and that to the β action is relaxation. The exception is that in most intestinal smooth muscles, including the taenia coli, the two actions are synergistic: both cause relaxation.

The mechanical response is, of course, only the final manifestation of a sequence of changes in cellular functions, and the purpose of this paper is to describe some observations which have lead to the idea that, fundamentally, the action of catecholamines may be the same on all smooth muscles.

It is necessary, first, to describe briefly the phenomena which are observed in the taenia coli. In this tissue α- and β-adrenergic agents both block the spontaneous electrical activity of the cell membrane which is the main cause for relaxation, but the sequence of events which lead to the cessation of spike discharge is quite different (Bülbring & Tomita 1969*a*, *b*).

The α effect consists primarily of a change in membrane conductance (figure 1*a*). Since the conductance change is mainly an increase in K conductance it results in hyperpolarization. When the membrane is hyperpolarized beyond the firing threshold for an action potential, spontaneous activity stops.

The β action stabilizes the membrane potential, but it produces no change in membrane conductance. However, the slow depolarization or pacemaker potential which normally triggers the spike is abolished (figure 1*b*). It is this disappearance of the generator potential which stops activity.

The β action blocks only the spontaneous spike discharge. An action potential can always be

evoked by electrical stimulation. But the tension response to the spike is reduced. This interference with EC coupling (figure 1 *b*) indicates that part of the β action takes place in the interior of the cell.

Although the experimental evidence is as yet not conclusive (for references see Robison, Butcher & Sutherland 1971) many observations suggest that β-adrenergic effects in general are associated with an increased intracellular content of cyclic AMP which is believed to activate the process of Ca uptake at intracellular storage sites. Consequently, the cytoplasmic Ca^{2+} concentration is decreased and less Ca is available for contraction. The β-adrenergic effect on EC coupling is, to my knowledge, the same in all smooth muscles, without exception. It is in the response to the α action where intestinal smooth muscle differs.

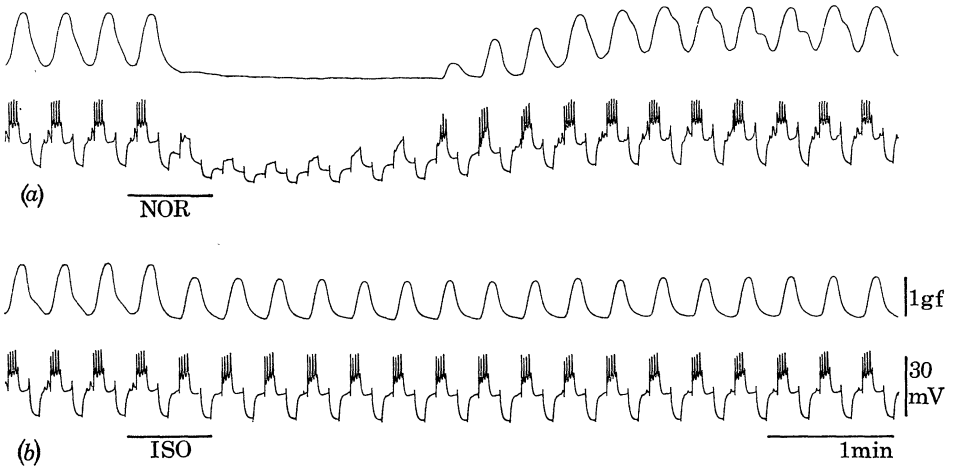

FIGURE 1. The α and β effects of catecholamines on taenia coli. Double sucrose gap method; Krebs solution; 35 °C. Upper trace: mechanical responses: lower trace; electrical potential changes evoked by constant current pulses of alternating polarity. (*a*) Effect of noradrenaline (NOR), 1.75×10^{-7} mol/l, applied for 40 s (bar). Note reduction of electrotonic potential, hyperpolarization, and abolition of spontaneous and evoked electrical and mechanical activity. (*b*) Effect of isoprenaline (ISO), 2.25×10^{-7} mol/l, applied for 40 s (bar). Note abolition of spontaneous electrical activity and reduction of tension, while the electrotonic potential and evoked electrical activity are unchanged.

Earlier papers in this volume draw attention to the considerable evidence indicating that part of the α action, at least in vascular smooth muscle, may also take place in the interior of the cell. However, in contrast to the β action, the α action appears to cause release of Ca from intracellular stores (Hinke 1965; van Breemen & Lesser 1971; Peiper, Griebel & Wende 1971; van Breemen, Farinas, Gerba & McNaughton 1972; Golenhofen, Hermstein & Lammel 1972), thus increasing the cytoplasmic Ca^{2+} concentration and producing contraction. Here, then, we have an antagonism between the α and β action at the intracellular level.

We do not know whether the Ca binding associated with the β component, and the Ca release associated with the α component take place at the same Ca store or at different storage sites. Moreover, little is known about the enzymes involved. Though the adenylcyclase system is probably involved in the β effect, nothing is known about the chain of events involved in the α effect which leads to the release of Ca from stores.

It is unlikely that the α effect is produced by a direct inhibitory action on the adenylcyclase system (Robison *et al.* 1971). The α action may, however, affect another enzyme system which competes with adenylcyclase for the same, limited, pool of ATP. This possibility has recently been envisaged by several workers (Robison *et al.* 1971; Born 1971).

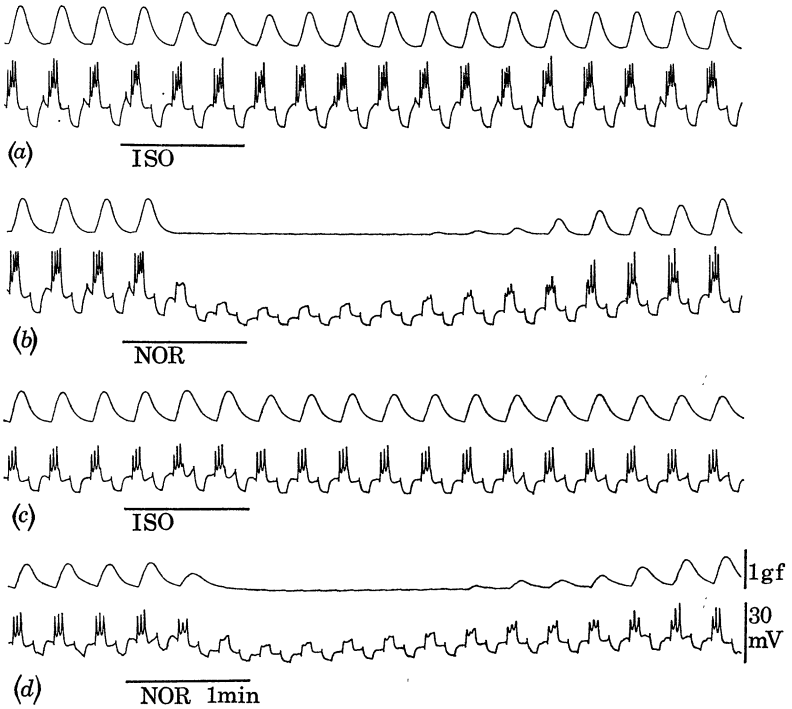

FIGURE 2. The effect of theophylline on taenia coli. Records as in figure 1. Responses to isoprenaline (ISO), 2.25×10^{-7} mol/l and noradrenaline (NOR), 1.75×10^{-7} mol/l, applied for 1 min (bars). (*a*) and (*b*): controls; (*c*) and (*d*) in the presence of theophylline (3×10^{-4} mol/l). Note prolonged reduction of tension by isoprenaline in (*c*), while the effect of noradrenaline on membrane conductance (*d*) is unchanged.

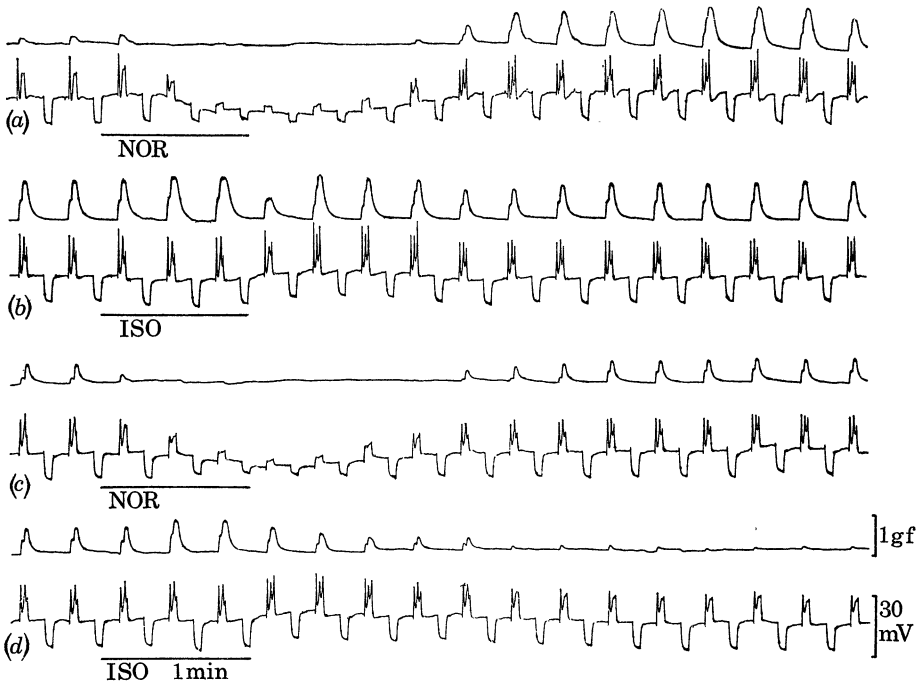

FIGURE 3. The effect of a high concentration of theophylline on taenia coli. Records as in figure 1. Responses to noradrenaline (NOR), 3.5×10^{-7} mol/l, and isoprenaline (ISO), 1×10^{-6} mol/l, applied for 1 min (bars). (*a*) and (*b*): controls; (*c*) and (*d*) in the presence of theophylline (5×10^{-4} mol/l). Note that isoprenaline caused slight depolarization and transiently a larger tension response to the depolarizing current pulse (*b*) before reducing the tension. In (*d*) the tension is nearly abolished and repetitive discharge suppressed. The effect of noradrenaline (*c*) is not changed.

The two components of the action of catecholamines can, of course, be distinguished by observing the response of the tissue after treatment with specific α or β antagonists. The two components can also be separated in the presence of substances which are known to affect some factors believed to be essential in the mechanism of action of either component. For example, theophylline is known to inhibit phosphodiesterase thus preventing the breakdown of cyclic AMP which, as mentioned above, is thought to be involved in the β action. Figures 2 and 3 show that the action of isoprenaline, i.e. the reduction of the tension response to membrane excitation, is enhanced and prolonged in the presence of theophylline, but the α action of noradrenaline, i.e. the membrane conductance change, is not affected.

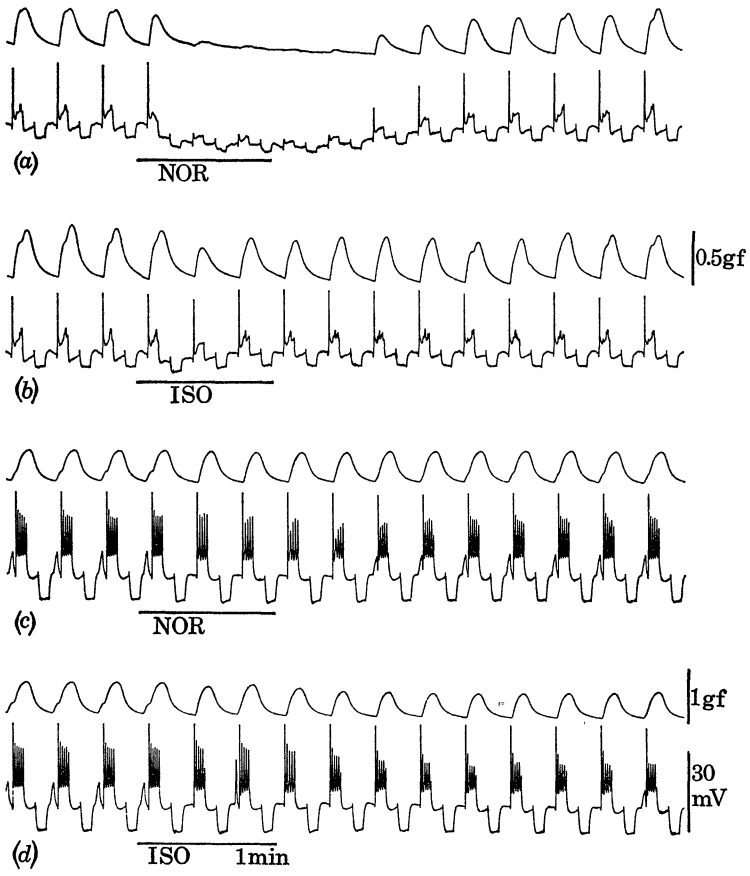

FIGURE 4. The effect of procaine on taenia coli. Records as in figure 1. Responses to noradrenaline (NOR), 1.75×10^{-7} mol/l, and isoprenaline (ISO), 2.25×10^{-7} mol/l, applied for 1 min (bars). (*a*) and (*b*): controls; (*c*) and (*d*) in the presence of procaine (5×10^{-3} mol/l). Note that in (*c*) noradrenaline caused no change in membrane conductance but suppressed transiently the spontaneous spike.

Local anaesthetics, including procaine, are believed to interfere with the release of Ca from binding sites and to reduce the Ca permeability of the cell membrane (Feinstein 1963). Figure 4 shows that, in the presence of procaine, the β effect of isoprenaline is unchanged (Bowman & Hall 1970), but the α effect of noradrenaline is abolished. (In fact, the β component of the noradrenaline effect can now be detected in figure 4*c*.) This observation could be interpreted by assuming that procaine, though not acting on specific α receptors (Fleisch & Titus 1972) acts like an α antagonist by interfering with an essential factor required for the action of noradrenaline, namely the release of Ca.

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The next question would then be whether the sequestered Ca which may be released by noradrenaline is bound intracellularly. It was found that, in conditions in which the participation of extracellular Ca was excluded by treatment with La (van Breemen *et al.* 1972), adrenaline still increased membrane conductance and caused hyperpolarization for a considerable time after spikes and contraction had been abolished (figure 5).

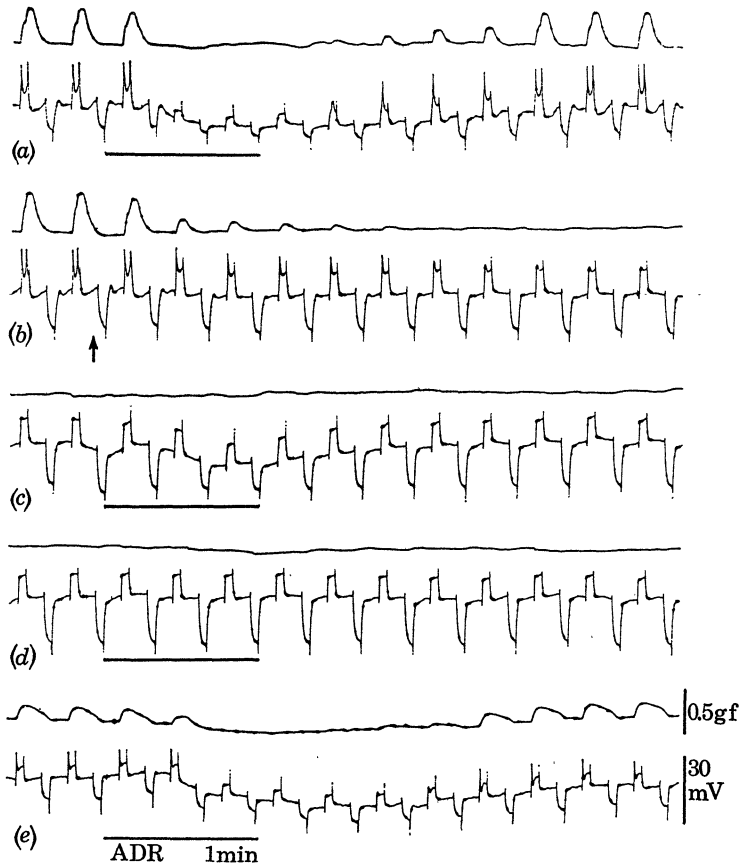

FIGURE 5. The effect of lanthanum on taenia coli. Records as in figure 1. Tris-buffered solution. Bars indicate the application of adrenaline (ADR), 1×10^{-6} mol/l, for 1 min. (*a*): control. (*b*): at arrow 1 mmol/l Lanthanum was added to the bathing solution. Spikes and tension response were abolished in 4 min. (*c*): after 17 min exposure to La the change in membrane conductance by adrenaline was still observed. 10 min later adrenaline had no effect (not shown). Total exposure to La was 30 min. (*d*): after 17 min washing with tris-buffered solution containing no La the effect of adrenaline was still absent. (*e*): 10 min exposure to bicarbonate-buffered Krebs solution restored the response to adrenaline.

Now, if part of the α effect is due to the release of Ca, causing an increase in the intracellular Ca^{2+} concentration, why does the taenia relax while other smooth muscles contract?

It may be possible to understand this phenomenon by considering various mechanisms by which the cell controls its internal Ca^{2+} concentration. One such mechanism which comes to mind may be concerned primarily with the uptake of Ca from the cytoplasm into stores, i.e. with the binding of Ca at the sarcoplasmic reticulum and other organelles, mitochondria, etc. Presumably, this mechanism involves cyclic AMP and is stimulated by the β component of the catecholamine action. It would cause an intracellular translocation of Ca but would only be indirectly concerned with the role of calcium at the plasma membrane.

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A second control mechanism, presumably influenced by the α action of catecholamines, may be closely integrated with the functions of Ca at the plasma membrane and, therefore, would be associated with changes in membrane conductance (Brading, Bülbring & Tomita 1969*a*; Bülbring & Tomita 1969*c*; 1970*a*, *b*). Recent evidence has shown that the relative contribution of the various ions to the total increase in membrane conductance produced by catecholamines varies widely (for references see Bülbring 1972). These differences could be due to differences not only in the amount of bound Ca, which is determined by the degree of active Ca extrusion, but also to differences in the location of Ca binding sites at the inside of the plasma membrane.

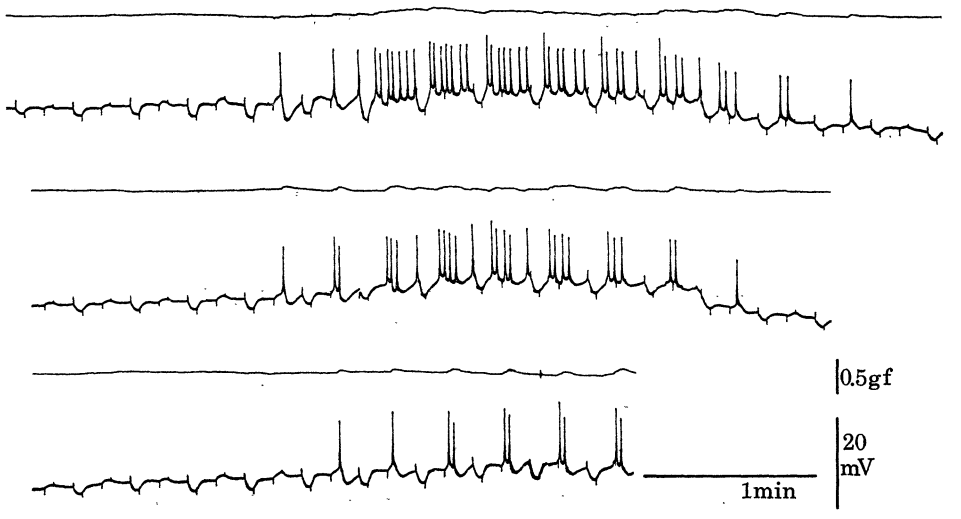

FIGURE 6. Continuous records of spontaneous activity of guinea-pig uterus (oestrogen and progesterone dominated). Records as in figure 1. Note gradual increase in size of the electrotonic potential when membrane becomes depolarized.

Tomita (this volume, p. 83) has described how the binding of Ca at negative sites on the inner surface of the plasma membrane may increase the membrane permeability to K and thereby increase the membrane potential. He also explained the metabolically driven pacemaker potential as being possibly due to a decrease in K conductance which is brought about by the removal of Ca from the inner binding sites of the membrane by a Ca pump. An observation which lends support to this hypothesis is shown in figure 6. The record is taken from guinea-pig uterus after treatment with oestrogen and progesterone. In this tissue the membrane potential is about 20 mV higher than that in uterus from untreated animals, mainly as a result of an increase in K permeability (Casteels & Kuriyama 1965; Bülbring, Casteels & Kuriyama 1968; Jones 1970), probably brought about by a high Ca binding capacity of the membrane (Coutinho & Csapo 1959). The oestrogen and progesterone dominated uterus has also a higher and more stable membrane potential than the taenia. Periodically, however (figure 6) a slow depolarization occurs spontaneously and this is associated with a gradual increase in membrane resistance indicating a decrease in K conductance. On the basis of the working hypothesis proposed by Tomita (this volume, p. 84) this may be interpreted as being due to the activity of the Ca pump removing Ca from the inside of the cell membrane.

In the taenia the resting state is seldom reached. The cause may be a very active Ca pump which, in balancing the continuous Ca entry due to continuous spontaneous spike activity, keeps the amount of Ca bound at the inside of the membrane very low. Of course, during an

action potential, when the increase in the intracellular Ca^{2+} concentration is associated with membrane depolarization, the cells of the taenia contract like those of any other smooth muscles, since Ca cannot be bound at the depolarized membrane. However, when the increase in the intracellular Ca^{2+} concentration is the result of Ca release from intracellular storage sites caused by the α action, Ca can be bound at the polarized membrane where the amount of bound Ca is low, i.e. at those sites which control K permeability. The resulting hyperpolarization blocks spike activity and therefore, since no contraction is evoked, no effect on the contractile force can be demonstrated.

The membrane stabilization by the β action is probably a secondary effect. Since the internal Ca^{2+} concentration is reduced, the stimulus for Ca pump activity is removed and, hence, the pacemaker potential is suppressed.

In conclusion, I would like to put forward the hypothesis that the mode of action of catecholamines on the taenia coli is fundamentally the same as that on other smooth muscles. The β component, promoting Ca uptake into stores, affects a process which controls the Ca^{2+} concentration in the cytoplasm by internal redistribution. The α component, promoting Ca release, affects another process which controls the Ca^{2+} concentration in the cytoplasm by pumping Ca out of the cell. In the taenia, a delicate balance is maintained at the cell membrane between low Ca binding at the inside of the membrane and a high rate of Ca extrusion. The ratio between Ca binding and Ca extrusion probably varies from tissue to tissue and may be one factor which determines the final α response which is observed.

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