

TEA-sensitive Potassium Conductance Changes Induced by α_1 -adrenoceptor and ATP-receptor Activation in Guinea-Pig Taenia Coli

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Abstract. The actions of α - and β -adrenoceptor agonists and ATP were studied on the smooth muscle of the guinea-pig taenia coli using the double sucrose gap method. The membrane hyperpolarization, the decrease in membrane resistance and muscle relaxation induced by noradrenaline, adrenaline, phenylephrine and ATP were not inhibited by ouabain pretreatment. The suppression of the spontaneous spike generation induced by isoprenaline and adrenaline, however, was reduced by ouabain pretreatment. The actions of noradrenaline, phenylephrine and ATP on the smooth muscle membrane and tone were not abolished by lowering the temperature of the bathing fluid to 16°C. The actions of α -adrenoceptor agonists and ATP were not affected significantly during the first 15 min in the K^+ -free solution and were diminished only after 180 min. The conditioning depolarization increased and the hyperpolarization decreased the actions of noradrenaline, phenylephrine and ATP on the smooth muscle tone and membrane potential, with reversal at the level of potassium equilibrium potential. In the Ca^{++} -free solution the action of adrenoceptor agonists and ATP gradually decreased and were blocked in 30 min. 3,4-diaminopyridine and low concentrations of TEA, which influenced the innervation of the taenia coli increased the actions of the adrenoceptor agonists. The higher concentrations of TEA, however, increased the membrane resistance and abolished the actions of noradrenaline, phenylephrine and ATP. It is suggested that the sodium-pump did not participate markedly on the inhibitory actions of α_1 -adrenoceptor and ATP-receptor activations. The role of the sodium pump activity is more pronounced in the β_2 -adrenoceptor mediated inhibition of the spontaneous spike generation. The result of α_1 -adrenoceptor and ATP-receptor activations is proposed to be due to an increase in the Ca^{++} -dependent TEA-sensitive K^+ -conductance of the smooth muscle membrane of the taenia coli.

Key words: Taenia coli — α_1 -, β_2 -adrenoceptors — ATP-receptor — Sodium pump — Potassium conductance

Introduction

The inhibition of the spontaneous activity of the taenia coli induced by β -agonists is due to the activation of β_2 -adrenoceptors (Bauer 1982) and is characterized by abolition of the pacemaker activity, accompanied by a small membrane hyperpolarization and without any change in the membrane conductance (Bülbring and Tomita 1969b; Watanabe 1976; Bauer and Zakhari 1977; Bülbring and Den Hertog 1980). In contrast, the activation of α -adrenoceptors results in an increase in membrane conductance, pronounced hyperpolarization, cessation of action potential discharge and relaxation (Bülbring and Tomita 1969a, b, 1977a, b; Bauer and Zakhari 1977). The inhibitory action of α -adrenoceptor agonists is due to the activation of α_1 -adrenoceptors and is similar to the actions of ATP on the taenia coli (Bauer 1982). The increase in the membrane conductance induced by adrenaline, noradrenaline and ATP was suggested to be due to an increase in the potassium permeability (Bennett et al. 1963; Bülbring et al. 1966; Shuba and Klevetz 1967; Tomita 1972; Tomita et al. 1974; Bauer 1976). On the other hand, Burnstock (1958), Bülbring (1960), Török and Vizi (1980) reported that the α -adrenoceptor activation induced by adrenaline is due to an increase in the sodium pump activity. Watanabe (1976), however, suggested that the sodium pump is activated as a result of an increase in the intracellular c-AMP level induced by β -adrenoceptor agonists.

Therefore, the actions of α -, β -adrenoceptor agonists and ATP on the guinea-pig taenia coli warrants reinvestigation. For this purpose, the effects of α -, β -adrenoceptor agonists and ATP on the membrane activities and muscle tone were analysed under the conditions known to inhibit the sodium pump activity and potassium conductance, using the double sucrose gap method.

Some of the results have been communicated to the Czechoslovak Pharmacological Society (Bauer 1978).

Methods

Male guinea-pigs (250–400 g) were sacrificed by a blow on the neck. Fine strips of the taenia coli were removed from the caecum. The preparations were then inserted into the double sucrose gap apparatus (Bauer and Zakhari 1977). Any contact between the isotonic sucrose solution (270 mmol.l⁻¹) and the isotonic K₂SO₄ solution (75.5 mmol.l⁻¹), or the modified Krebs solution (Na⁺ 136.6, K⁺ 5.9, Ca⁺⁺ 2.5, Mg⁺⁺ 1.2, Cl⁻ 133.3, HCO₃⁻ 15.4, H₂PO₄⁻ 1.2 and glucose 11.5 mmol.l⁻¹) flowing on either side was prevented by using latex membranes through which the taenia was threaded. Constant current pulses of alternating polarity were applied and the membrane potential, spontaneous and electrically induced action potentials, membrane resistance and isometric muscle activity were registered on an Elema Mingograph. The experiments were carried out at 30 °C under initial tension of 1.0 g.

The following drugs were used: adrenaline hydrochloride (Spofa), ATP (adenosine-5'-triphosphate disodium salt, Reanal), atropine sulphate (Spofa), 3,4-diaminopyridine (Fluka), isoprenaline hydrochloride (Spofa), noradrenaline hydrogentartrate (Spofa), ouabain (Merck), phenylephrine hydrochloride (Boehringer, Ingelheim), TEA (tetraethylammonium hydrochloride, Fluka).

Fresh drug solutions were prepared just before the experiments in the modified Krebs solution. The test solutions were gassed with a mixture of 95% O_2 and 5% CO_2 . All the drug concentrations are expressed as mol (base). l^{-1} . A period of 30 to 40 min was allowed to elapse after mounting the preparation in the sucrose gap chamber to stabilize the preparations. The adrenoceptor agonists and ATP were left in contact with the preparations for 90 to 120 s, respectively.

The results are expressed as an arithmetic mean (\pm SEM). Differences were tested by Student's t-test for paired observations (Delaunois 1973).

Results

The effect of the sodium pump activity on the action of adrenoceptor agonists
Ouabain (1 – $10 \mu\text{mol}.l^{-1}$) depolarized the smooth muscle membrane, increased the spontaneous spike frequency and muscle tone of the taenia coli by 5–10 mV, 60–170% and 200–500 mg, respectively. During the increased muscle tone induced by 15 min ouabain ($1 \mu\text{mol}.l^{-1}$) treatment the isoprenaline (0.5 – $5 \mu\text{mol}.l^{-1}$) induced relaxation was significantly enhanced, but its effect on the spontaneous spike generation was markedly depressed. The effects of noradrenaline (0.1 – $1 \mu\text{mol}.l^{-1}$) on the membrane potential, spontaneous and evoked spikes and the membrane resistance were not influenced by ouabain pretreatment. The noradrenaline induced muscle relaxation was, however, increased by ouabain pretreatment (Fig. 1). The membrane hyperpolarization and decrease in membrane resistance induced by noradrenaline ($1 \mu\text{mol}.l^{-1}$), adrenaline ($1 \mu\text{mol}.l^{-1}$), phenylephrine ($50 \mu\text{mol}.l^{-1}$) and ATP ($0.5 \text{ mmol}.l^{-1}$) were not significantly influenced even by the higher ouabain ($10 \mu\text{mol}.l^{-1}$) concentration.

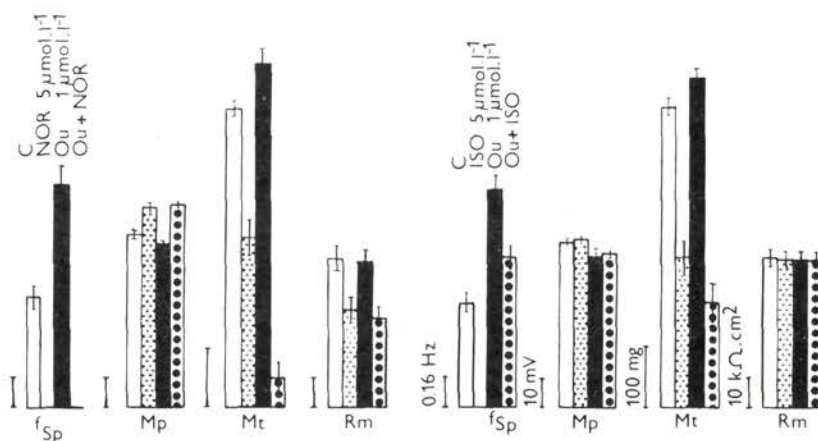


Fig. 1. Effects of ouabain (Ou), of isoprenaline (ISO) and noradrenaline (NOR) on the spontaneous spike frequency (f_{Sp}), membrane potential (Mp), muscle tone (Mt) and membrane resistance (Rm) of the guinea-pig taenia coli before and after 15 min lasting ouabain pretreatment. Each column represents the mean of at least seven trials, vertical lines show SEM. C — control.

The membrane hyperpolarization, spike inhibition and muscle relaxation induced by adrenaline ($5 \mu\text{mol.l}^{-1}$) were gradually abolished during 60 min in the K^+ -free solution. The readmission of K^+ to the testing solution induced a gradual reappearance of adrenaline actions (Fig. 2). Exposure to the K^+ -free solution of longer than 180 min duration was needed to abolish the actions of phenylephrine ($50 \mu\text{mol.l}^{-1}$) and ATP (0.5 mmol.l^{-1}).

The membrane hyperpolarization, muscle relaxation and decrease in the membrane resistance induced by noradrenaline, phenylephrine and ATP were not affected significantly by lowering the bathing solution temperature to 16°C .

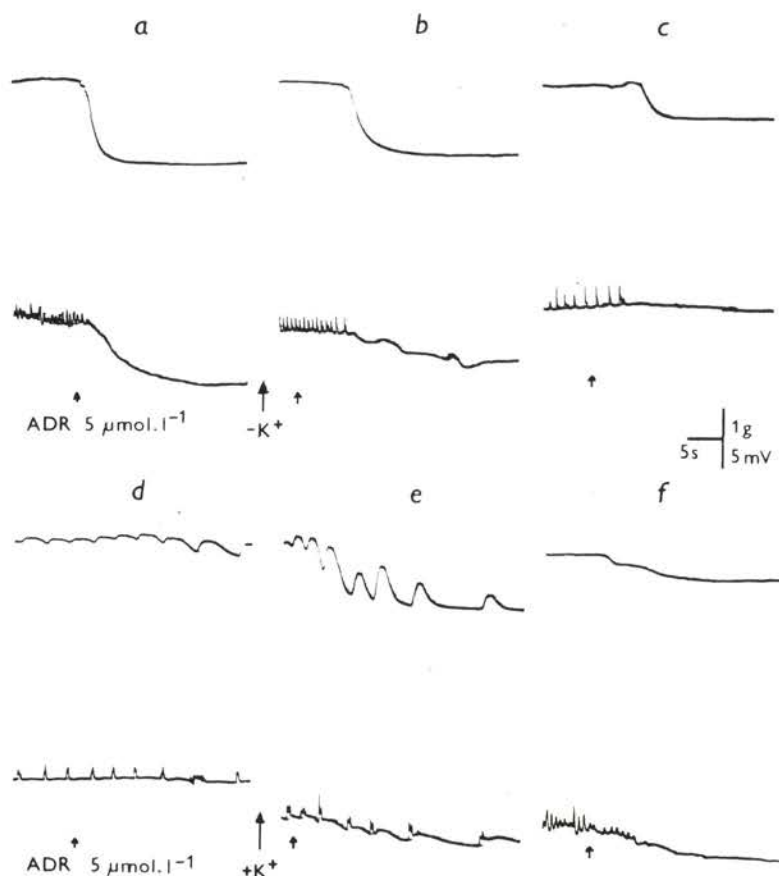


Fig. 2. Effects of adrenaline (\uparrow ADR) on the guinea-pig taenia coli. *a*-control response, *b*-15 min, *c*-30 min, *d*-60 min in the potassium-free solution, *e*-15 min and *f*-30 min after readmission of K^+ to the bathing solution. The upper trace is the muscle tone and the lower trace is the membrane potential.

The effect of conditioning polarization on the actions of α_1 -agonists and ATP
Phenylephrine ($50 \mu\text{mol.l}^{-1}$) actions on the membrane potential and muscle tone were increased by conditioning depolarization and decreased or reversed by conditioning hyperpolarization of the membrane. The effect of phenylephrine on the membrane resistance was, however, only slightly influenced by the conditioning polarization (Fig. 3). ATP (0.5 mmol.l^{-1}) induced similar effects as phenylephrine and these were influenced by the conditioning polarization in the same manner as those of phenylephrine (Fig. 4).

The reversal potential for membrane hyperpolarization and the smooth muscle relaxation induced by adrenaline ($1 \mu\text{mol.l}^{-1}$), noradrenaline ($1 \mu\text{mol.l}^{-1}$), phenylephrine ($50 \mu\text{mol.l}^{-1}$) and ATP (0.5 mmol.l^{-1}) were in the range of the membrane potential levels from 76 to 87 mV and from 74 to 82 mV, respectively (Fig. 5).

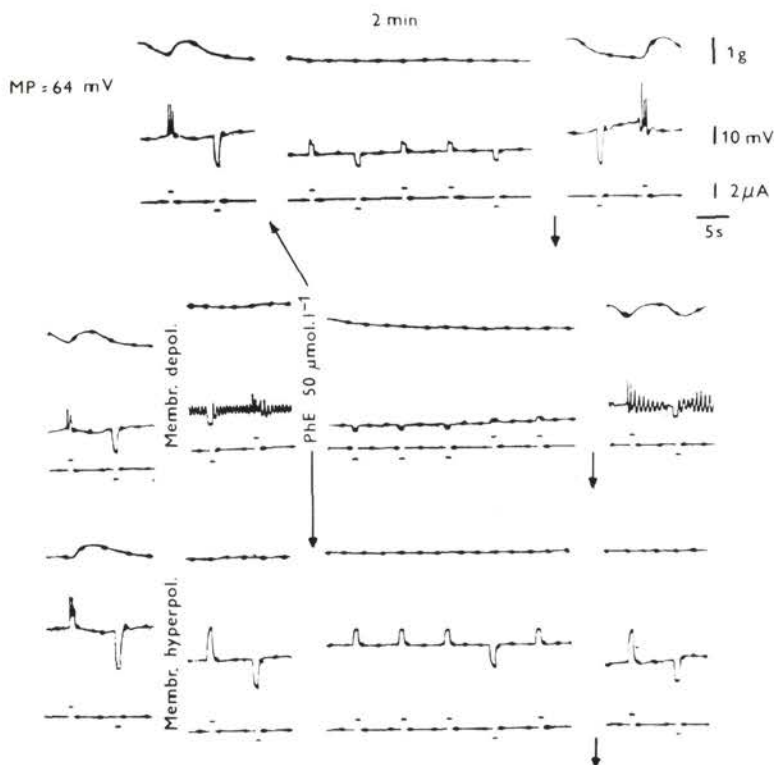


Fig. 3. Effects of phenylephrine (PhE) on the guinea-pig taenia coli under control conditions (membrane potential — MP) and during the conditioning depolarization and hyperpolarization. The upper trace is the muscle tone, the middle trace is the membrane potential and the lower trace is the applied current.

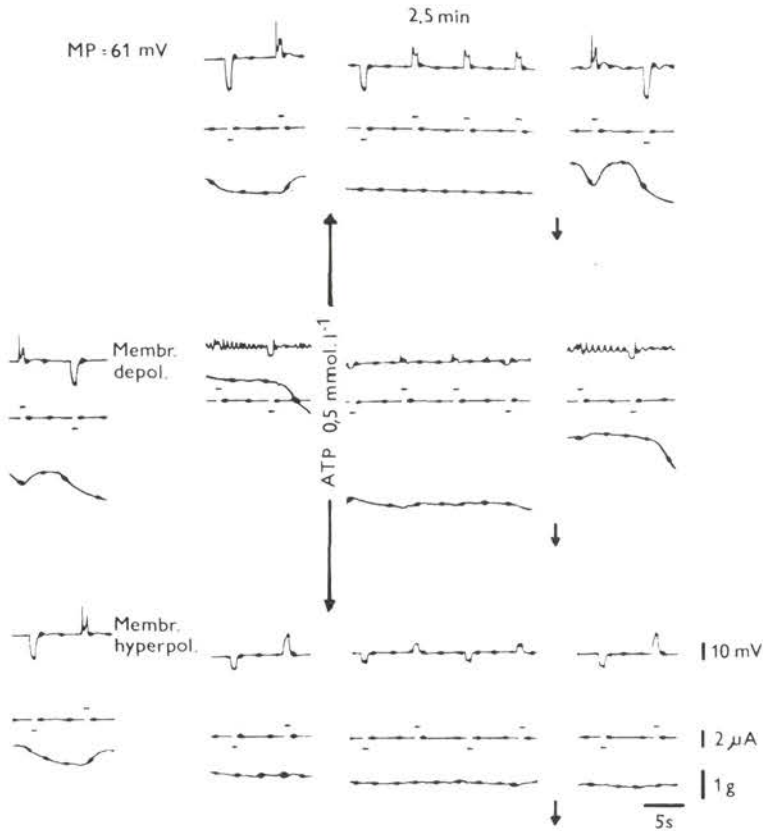


Fig. 4. Effects of ATP on the guinea-pig taenia coli under control conditions (membrane potential — MP) and during the conditioning depolarization and hyperpolarization. The upper trace is the membrane potential, the middle trace is the applied current and the lower trace is the muscle tone.

The effect of TEA and 3,4-diaminopyridine on the actions of α_1 -agonists and ATP
 TEA (5–30 mmol.l⁻¹) depolarized the membrane by 5 to 12 mV, increased the smooth muscle tone by 300 to 900 mg, increased the spontaneous spike frequency by 80 to 350% and the amplitude of evoked action potentials by 30 to 95%, as well as the membrane resistance by 10 to 60%. The actions of TEA in concentration of 5 mmol.l⁻¹ were partly antagonized by 5 min atropine (1 μmol.l⁻¹) pretreatment, whereas the actions of higher TEA concentrations were not influenced significantly by atropine. The effects of noradrenaline (1 μmol.l⁻¹), phenylephrine (50 μmol.l⁻¹) and ATP (0.5 mmol.l⁻¹) on the membrane potential and muscle tone were increased in the presence of TEA in the concentration of 5 mmol.l⁻¹. In contrast, however, TEA in the concentration of 30 mmol.l⁻¹, which significantly

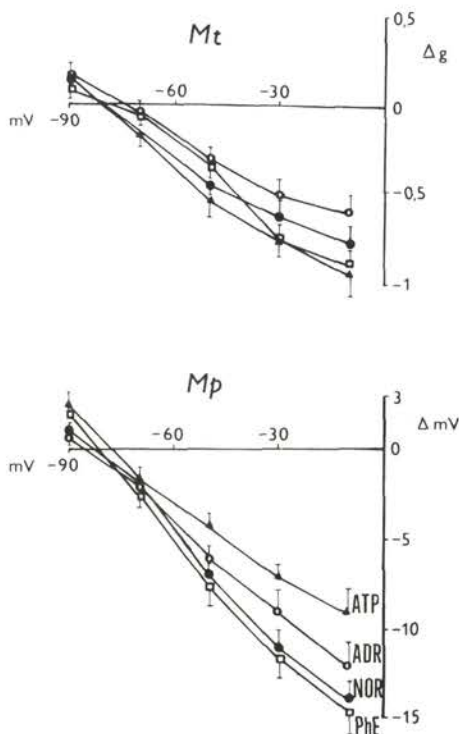


Fig. 5. Effects of adrenaline (\circ ADR, $1 \mu\text{mol.l}^{-1}$), noradrenaline (\bullet NOR $1 \mu\text{mol.l}^{-1}$), phenylephrine (\square PhE $50 \mu\text{mol.l}^{-1}$) and ATP (\blacktriangle 0.5 mmol.l^{-1}) on the muscle tone (*Mt*) and membrane potential (*Mp*) of the taenia coli at different membrane potentials induced by conditioning polarization. Each point represents the mean of at least five determinations, vertical lines show SEM.

increased the smooth muscle membrane resistance markedly diminished the actions of noradrenaline, phenylephrine and ATP (Fig. 6, 7).

3,4-diaminopyridine (0.5 and 5 mmol.l^{-1}) depolarized the membrane (by 2.65 ± 0.65 and $3.01 \pm 0.83 \text{ mV}$, respectively, $n=6$), increased the smooth muscle tone (by 152.1 ± 25.8 and $370.4 \pm 61.2 \text{ mg}$, respectively, $n=6$), increased the spontaneous spike frequency and the amplitude of evoked spikes up to 130% without any change in the membrane resistance. These effects were sensitive to the action of atropine ($1 \mu\text{mol.l}^{-1}$). During the action of 3,4-diaminopyridine the effects of noradrenaline ($1 \mu\text{mol.l}^{-1}$), phenylephrine ($50 \mu\text{mol.l}^{-1}$) and ATP (0.5 mmol.l^{-1}) were, however, not inhibited, but slightly increased.

The effect of the Ca^{++} -free solution

The Ca^{++} -free solution induced initially an increase in the spike frequency (up to

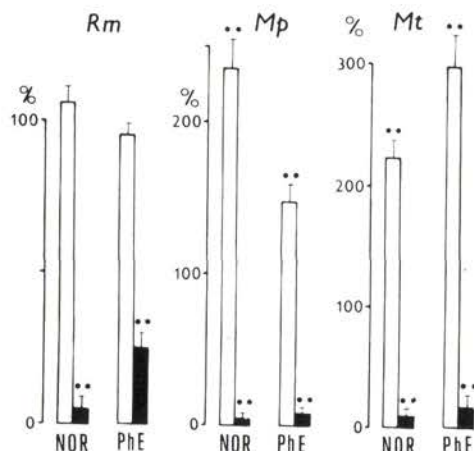


Fig. 6. Effects of noradrenaline (NOR 1 $\mu\text{mol.l}^{-1}$) and phenylephrine (PhE 50 $\mu\text{mol.l}^{-1}$) on the membrane resistance (Rm), membrane potential (Mp) and muscle tone (Mt) after 15 min TEA pretreatment. The open columns represent 5 mmol.l^{-1} TEA and the filled columns represent 30 mmol.l^{-1} TEA pretreatment. The control responses were taken as 100%. Each column represents the mean of at least six trials, vertical lines show SEM. ** — $p < 0.01$.

150%) and membrane depolarization (12.5 ± 2.4 mV, $n=7$). After 30 min the Ca^{++} -free solution treatment, the membrane depolarization and spike activity gradually disappeared. The responses of the taenia coli induced by isoprenaline (5 $\mu\text{mol.l}^{-1}$), phenylephrine (50 $\mu\text{mol.l}^{-1}$) and ATP (1 mmol.l^{-1}) were either inhibited, or slightly increased during the first 5 min of the Ca^{++} -free solution treatment when the membrane was depolarized and the spike activity was increased. In contrast, however, during the decreased spike activity and gradual repolarization of the membrane the tested agonists were unable to influence significantly the membrane potential and muscle tone.

Discussion

The inhibitory action of catecholamines in the taenia coli was suggested to be mediated via α_1 - and β_2 -adrenoceptors and the sequence of events induced by α_1 -adrenoceptor and ATP-receptor activations was supposed to be the same (Bauer 1982).

The effect of catecholamines, due to the β -adrenoceptor activation, is mediated by an increase in c-AMP (Anderson and Mohme Lundholm 1970). Watanabe (1976) proposed that c-AMP may lead to metabolic changes, thus the sodium pump may be activated and could participate in the inhibitory mechanism of isoprenaline in the taenia coli. Iwayama et al. (1979) showed, however, that phenylephrine did not change the c-AMP and c-GMP levels in the taenia coli. They

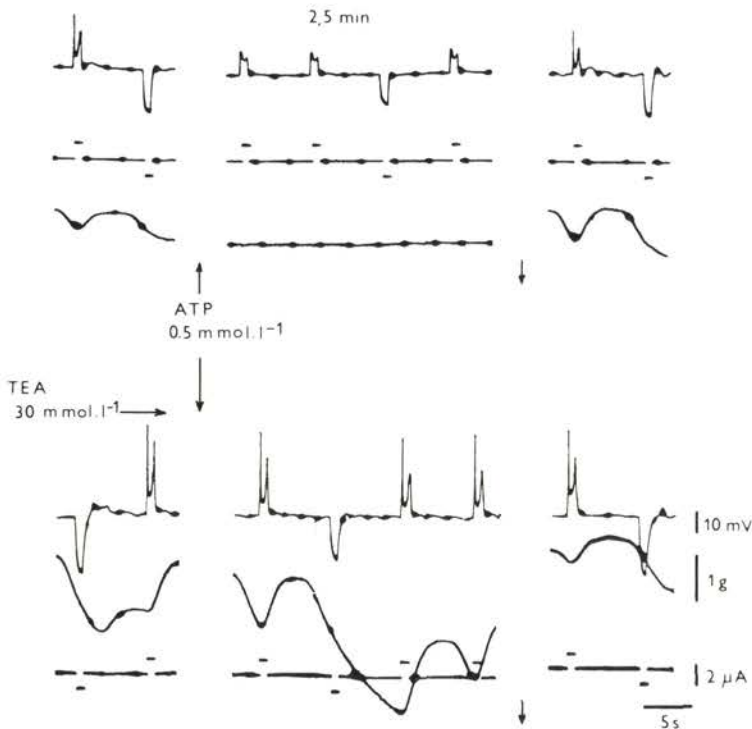


Fig. 7. Effects of ATP before (upper part) and 15 min after TEA pretreatment (lower part). The upper trace is the membrane potential, the middle trace is the applied current and the lower trace is the muscle tone.

suggested that the β -adrenergic action in the intestinal smooth muscle is not mediated through a change in the levels of intracellular cyclic nucleotides. Nilsson (1973), Takayanagi et al. (1977) and Crankshaw et al. (1977) reported that adrenaline, papaverine and isoprenaline, which increase the intracellular level of c-AMP, increased the Ca^{++} uptake by a microsomal fraction of the smooth muscle. Phenylephrine, however, was without any effect on the Ca^{++} movements across the membrane (Iwayama et al. 1979).

It has been reported that in the taenia coli as in other tissues lowering the intracellular Na^+ content, treatment with ouabain (Thomas 1972; Brading and Widdicombe 1974) and removal of extracellular K^+ suppresses the sodium pump activity and the readmission of K^+ activates the pump and inhibits the spontaneous activity (Taylor et al. 1969; Casteels et al. 1971). Török and Vizi (1980) suggested that the electrogenic Na^+ extrusion might be responsible for the hyperpolarization of the cell membrane and inhibition of the spontaneous spike activity induced by

α -adrenoceptor agonists, as it was hypothesized for adrenaline by Burnstock (1959) and Bülbring (1960). Brading and Widdicombe (1974) found that the uptake of ouabain in the taenia coli consists of at least two components. One at lower ouabain concentrations (up to $1\text{--}2\text{ }\mu\text{mol.l}^{-1}$) is saturable and dependent on the extracellular K^+ concentration and the other at higher concentrations of ouabain, similar to the non-specific binding in other tissues. They showed, moreover, that the saturable binding sites and sodium pumping sites are the same. In spite of the fact that in the presence of K^+ in the bathing fluid the concentrations of ouabain used in the present experiments and lowering the temperature to 16°C are unable to block completely the sodium pump activity, the involvement of the sodium pump in the α_1 -adrenergic and ATP induced actions is unlikely, since a) Noradrenaline, phenylephrine, adrenaline and ATP were still able to hyperpolarize the membrane and decrease the membrane resistance to the same degree in the presence of ouabain as under control conditions, in agreement with the results of Axelson and Holmberg (1969), who did not find any changes in ATP actions after ouabain pretreatment. b) The actions of phenylephrine, noradrenaline, adrenaline and ATP were not changed significantly during the first 15 min in the K^+ -free solution and gradually disappeared at the time when the intracellular ionic milieu was markedly changed by the intracellular accumulation of Na^+ and the loss of K^+ (Casteels and Muewissen 1976). c) The effect of adrenaline (Bülbring and Tomita 1977a) and, as we have shown, noradrenaline, phenylephrine and ATP on the membrane potential and muscle tone were not blocked by lowering the temperature of the bathing fluid to 16°C . d) A seven day cold storage did not diminish the actions of isoprenaline and phenylephrine (Shibata et al. 1970) and noradrenaline (Bauer, unpublished observation) and only a long lasting cold storage (14 days), when the intracellular K^+ content was decreased by 65% and Na^+ was increased by 100%, was able to diminish the action of isoprenaline and phenylephrine (Fukuda and Shibata 1972). e) The hyperpolarization and relaxation induced by adrenaline (Bülbring and Tomita 1969a; Den Hertog 1973; Bauer 1976), according to the present results using noradrenaline, phenylephrine and ATP, can be increased by the conditioning depolarization and reversed into depolarization and contraction by a conditioning hyperpolarization of the membrane. f) Adrenaline was able to hyperpolarize the membrane when it had already been hyperpolarized by the replacement of a chloride ion by a less permeable propionate anion (El Sharkawy and Daniel 1976). The inhibition of catecholamine actions in the action potential generation suggests, however, that the sodium pump should participate in the β_2 -adrenergic effect of catecholamines in the taenia coli. The changes in magnitude of β -responses during the exposure to ouabain may also be due to the gradual accumulation of Ca^{++} inside the cell (Van Breemen et al. 1973, (Van Breemen et al. 1973; Brading and Widdicombe 1976), when Na^+ pumping is blocked. Initial increase followed by gradual decrease in the action of

isoprenaline on the generation of spikes may result from an increase in $[Ca^{++}]_i$. Saturation of Ca^{++} extrusion and storage could result in the abolition of β -effects (Bülbring and Den Hertog 1980) as well.

Since the proposed role of the sodium pump in the activation of α -adrenoceptors of the taenia coli was based predominantly on the results, when adrenaline was used as an agonist (Burnstock 1958; Bülbring 1960; Török and Vizi 1980) the β -adrenergic effect was ascribed to the activation of α -adrenoceptors. A more correct answer to the problem of the role of the sodium pump in the catecholamine actions could be obtained, however, only after direct measurements of ouabain sensitive K^+ -fluxes.

Bülbring and Tomita (1969 b, 1977 b) found that an increase in $[Ca^{++}]_o$ potentiates and a reduction in $[Ca^{++}]_o$ abolishes the action of adrenaline, whereas Shuba and Klevetz (1967) have shown that the effect of adrenaline and noradrenaline was unaltered in the Ca^{++} -free solution. As shown in the present study, the omission of Ca^{++} from the bathing solution for 30 min decreased the actions of phenylephrine, ATP and isoprenaline, indicating the possible participation of Ca^{++} in the α_1 -, β_2 -adrenergic and ATP induced responses.

Shuba (1961), Shuba et al. (1976) and Shuba and Klevetz (1967) proposed two mechanisms for the inhibitory effect of adrenaline and noradrenaline, namely the interaction with mechanism responsible for the action potential generation and the rise of membrane permeability for intracellular K^+ leading to hyperpolarization of the membrane and reduction of the membrane resistance. Jenkinson and Morton (1967), Bülbring et al. (1966) described an increase in the rate of K^+ -efflux due to α -adrenergic actions in the taenia coli depolarized by K^+ . Moreover, Iwayama et al. (1979) demonstrated an increase in K^+ -efflux by phenylephrine under untreated conditions. Based on the above-mentioned results and the actions of adrenaline at different membrane potential levels the hyperpolarization and relaxation induced by α -adrenoceptor stimulation was suggested to be due to an increase in the membrane permeability for K^+ (Shuba and Klevetz 1967; Setekliev 1967, 1970; Bülbring and Tomita 1969a, 1977a; Bauer 1976, 1978), as it has been suggested also for the guinea pig liver cells (Haylett and Jenkinson 1972).

TEA (Hille 1967) and aminopyridines (Johns et al. 1976; Pelhate and Pichon 1974) block the delayed potassium current. 3,4-diaminopyridine a potent potassium channel blocker in the neuronal membrane (Kirsch and Narahashi 1978) induced depolarization as a result of its action on the postganglionic nerve endings, like the low TEA concentrations, since they were blocked by atropine. During these depolarizations the effects of noradrenaline, phenylephrine and ATP were increased as during the conditioning depolarization.

The present data show that the membrane hyperpolarization and muscle relaxation induced by the α_1 -adrenoceptor agonists and ATP have the reversal

potential at the level of potassium equilibrium potential, as it was found previously for adrenaline and noradrenaline (Shuba et al. 1976). The actions of the α_1 -adrenoceptor agonists and ATP were blocked by TEA in concentrations which block the potassium conductance (Amstrong and Birnstock 1965; Suzuki et al. 1976). It seems probable that the observed changes are due to an increase in K^+ permeability across the TEA sensitive potassium channels. This suggestion is in good agreement with the results of Tomita et al. (1974) that adrenaline induces an increase in the outward current in the taenia coli. The increased TEA sensitive K^+ conductance, dependent on Ca^{++} elicited by the α_1 -adrenoceptor and ATP-receptor activations should be the result of an increase in the intracellular Ca^{++} concentration, as it was suggested for the parotid gland (Putney 1979), liver cells (Egashira 1980) and myenteric neurons (Grafe et al. 1980).

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