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Calcium and the activation of the α_1-adrenoceptors in the guinea-pig taenia caeci

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1 The actions of phenylephrine (0.1–100 μmol l^{-1}) and methoxamine (0.1–100 μmol l^{-1}) were compared with that of adrenaline (0.01–10 μmol l^{-1}) using the single sucrose gap method and mechanical recording in the guinea-pig taenia caeci. Drugs were applied for variable periods of time.

2 The characteristics of the inhibitory effects of α-adrenoceptor agonists were the same when exposure time did not exceed 5 min. When the exposure was prolonged, in contrast to the sustained effects of adrenaline (0.1–3 μmol l^{-1}), phenylephrine and methoxamine (1–10 μmol l^{-1}) produced a transient inhibitory action.

3 During the delayed recovery phase of phenylephrine, adrenaline preserved its ability to suppress the spontaneous electrical and mechanical activities of the taenia both when phenylephrine was replaced by adrenaline or when adrenaline was applied in addition to phenylephrine. All the above effects were found in untreated preparations, as well as during blockade of muscarinic cholinoceptors by atropine (1.4 μmol l^{-1}), β-adrenoceptors by propranolol (3 μmol l^{-1}) and release of endogenous catecholamines by guanethidine (2.5 μmol l^{-1}).

4 In the presence of phorbol 12,13-dibutyrate adrenaline ceased to be effective, while the inhibitory action of phenylephrine was converted to a contraction.

5 In calcium-free conditions in the presence of EGTA (0.4 mmol l^{-1}) the initial hyperpolarization induced by adrenaline and phenylephrine was significantly reduced and with repeated applications of the agonists the inhibitory response disappeared. Similar results were obtained using tissues treated with nifedipine (1 and 10 μmol l^{-1}). When caffeine (30 mmol l^{-1}) was present in the calcium-free solution the α-agonists studied were unable to produce any membrane potential changes.

6 The present results imply that the inhibitory effect of α_1-adrenoceptor agonists is mediated by the opening of potassium channels, which are activated by calcium derived from an intercellular source supplied from the extracellular space via a nifedipine-sensitive mechanism.

Introduction

The role of calcium in producing stimulation or inhibition of different smooth muscles has been well documented. Many cellular processes are regulated by calcium mobilizing receptors which can use the hydrolysis of an inositol lipid as a part of transduction mechanism for generating second messengers i.e. inositol 1,4,5-triphosphate (IP_3) and diacyl-glycerol. IP_3 diffuses into the cytosol to release calcium from intracellular sources (for review see Berridge, 1985). In addition to the voltage-dependent influx, calcium also seems to enter the cell through ion channels associated with the receptors for stimulant and possibly some inhibitory substances (Bolton, 1979). It has also been demonstrated that the response of guinea-pig taenia caeci to adrenaline requires both extra- and intracellular calcium (Den Hertog, 1981; 1982; Bauer & Rusko, 1982). Increase in calcium entry and release of intracellularly bound calcium from a limited intracellular source increases the intracellular free calcium which activates tetraethylammonium (TEA)-sensitive potassium channels (Bülbring & Tomita, 1977; Bauer & Rusko, 1982).

Den Hertog (1981, 1982) suggested that the activation of α-receptors increases calcium entry which leads to a sustained hyperpolarization, and that in the absence of extracellular calcium the release of bound calcium leads to a transient hyperpolarization. The action of α-adrenoceptor agonists on the taenia of the guinea-pig caecum has been shown to

be due to the activation of the α_1-adrenoceptor subtype (Bauer, 1982).

In the present study with guinea-pig taenia caeci we found that adrenaline produced a sustained inhibitory response, while the effects of phenylephrine and methoxamine were transient. It was therefore of interest to study whether the adrenaline- and phenylephrine-induced hyperpolarization results from the activation of the same calcium-dependent process.

Methods

Male guinea-pigs (200 to 400 g) were stunned and bled. Fine strips of taenia caeci about 0.5 to 0.8 mm thick and 20 mm long were quickly removed from the caecum. The preparations were equilibrated in Krebs solution at room temperature for at least 60 min before the experimental procedure was started.

The single sucrose-gap method was used to measure simultaneously changes in membrane potential and mechanical activity at 32°C. Any contact between the isotonic sucrose solution (270 mmol l^{-1}) and isotonic K_2SO_4 solution (77.5 mmol l^{-1}) or Krebs solution was prevented by using latex membranes through which the taenia was threaded. A period of 40 to 60 min was allowed to elapse after mounting the preparation in an open type of sucrose-gap chamber to stabilize the preparations under a tension of 5 mN. The potential changes were measured by means of calomel electrodes making contact with the Krebs solution and the reference K_2SO_4 solution. One end of the muscle was fixed and the other end was attached to a strain gauge system for isometric measurement of the mechanical activity.

In a separate series of experiments the action of adrenoceptor agonists on the spontaneous mechanical activity of taenia caeci was also studied. Segments of the taenia, about 2.5 cm in length were placed in an organ bath containing Krebs solution. The isometric muscle activity was recorded using a strain gauge transducer at 37°C. One end of the tissue was tied to the bottom of a 20 ml tissue chamber and the other to the transducer. The tissues were equilibrated under 20 mN tension for 30 min and the actual experiments were carried out under a basal tension of about 10 mN.

Solutions and drugs

Modified Krebs solution contained (mmol l^{-1}): Na^+ 136.6, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 133.3, HCO_3^- 15.4, $H_2PO_4^-$ and glucose 11.5. The perfusion was kept constant at a rate of 0.5 ml min^{-1}. In experiments with calcium-free solution $CaCl_2$ was omitted from the superfusion solution and 0.4 mmol l^{-1} of EGTA was added. As shown previously (Den Hertog, 1981), superfusion with such calcium-free solution for 20 min is sufficient to remove residual extracellular calcium. In some experiments guanethidine (2.5 μmol l^{-1}), atropine (1.4 μmol l^{-1}) and propranolol (3 μmol l^{-1}) were added to the perfusion fluid to avoid stimulation of muscarinic receptors and β-adrenoceptors and to prevent the release of endogenous catecholamines from the adrenergic nerve terminals.

The following drugs were used: acetylcholine hydrochloride (Hofmann La Roche), (±)-adrenaline hydrochloride, atropine sulphate (Spofa), EGTA (ethylenglycol-*bis*-(β-aminoethyl ether) N,N,N',N'-'tetraacetic acid; Sigma), guanethidine sulphate (Tokyo Kasei), methoxamine hydrochloride (Wellcome), phorbol 12,13-dibutyrate (Sigma), prazosin hydrochloride (Pfizer) propranolol (Galenika) and phenylephrine hydrochloride (Boehringer Ingelheim). Fresh stock solutions were made in distilled water just before the experiments, except for nifedipine which was dissolved in propylene glycol and phorbol 12,13-dibutyrate which was dissolved in dimethyl sulphoxide. Propylene glycol and dimethylsulphoxide alone in their final concentrations did not affect spontaneous or evoked activity.

Data are presented as mean values ± s.e. mean. Values are considered to be significant when $P < 0.05$.

Results

Taenia caeci superfused with Krebs solution at 32°C possesses spontaneous mechanical and electrical activity. Adrenaline (0.01 to 10 μmol l^{-1}), methoxamine (0.1 to 100 μmol l^{-1}) and phenylephrine (0.1 to 100 μmol l^{-1}) applied for 5 min hyperpolarized the membrane, suppressed the spontaneous spikes and phasic contractions, and relaxed the taenia caeci (examples are shown in Figures 1, 2, 5 and 7). These data confirm previous observations (Bülbring & Tomita, 1969a,b; Bauer, 1982). Maximal hyperpolarization of the smooth muscle cells of taenia caeci was achieved by 0.5 to 5 μmol l^{-1} of adrenaline or 1 to 50 μmol l^{-1} of phenylephrine and methoxamine within 1.5 to 3 min. These effects were also observed in preparations pretreated with propranolol (3 μmol l^{-1}), but were blocked by prazosin (1 μmol l^{-1}) and remained unaffected 15 min after pretreatment with yohimbine (1 μmol l^{-1}). These results indicate the involvement of α_1-adrenoceptors in this effect of catecholamines, in accordance with our previous results (Bauer, 1982; Bauer & Rusko, 1982).

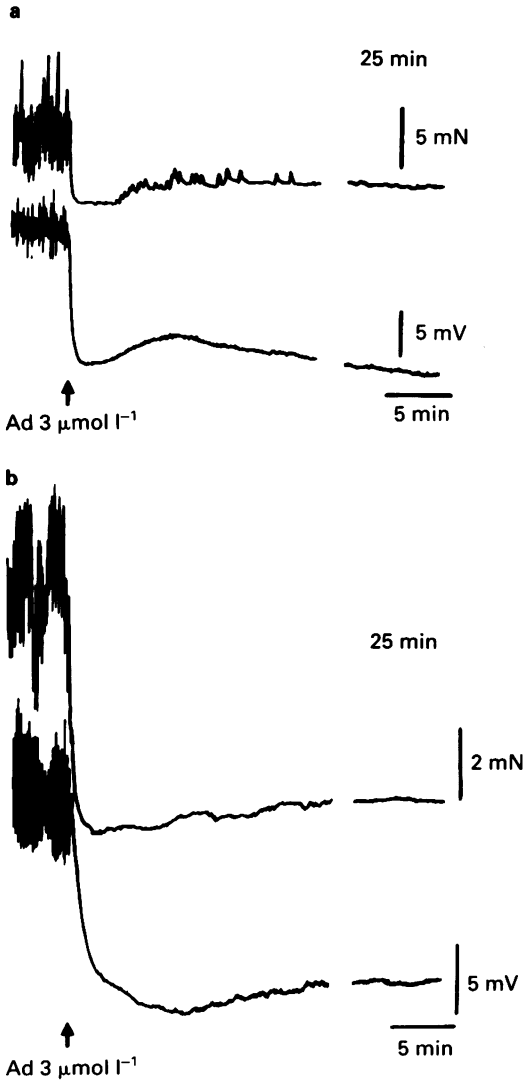

Figure 1 The effects of 30 min application of adrenaline (Ad) on mechanical (upper trace) and electrical (lower trace) activities of taenia caeci. (a) Untreated preparation, (b) after 15 min pretreatment with atropine (1.4 μmol l^{-1}), guanethidine (2.5 μmol l^{-1}) and propranolol (3 μmol l^{-1}). Recording was interrupted for 5 min.

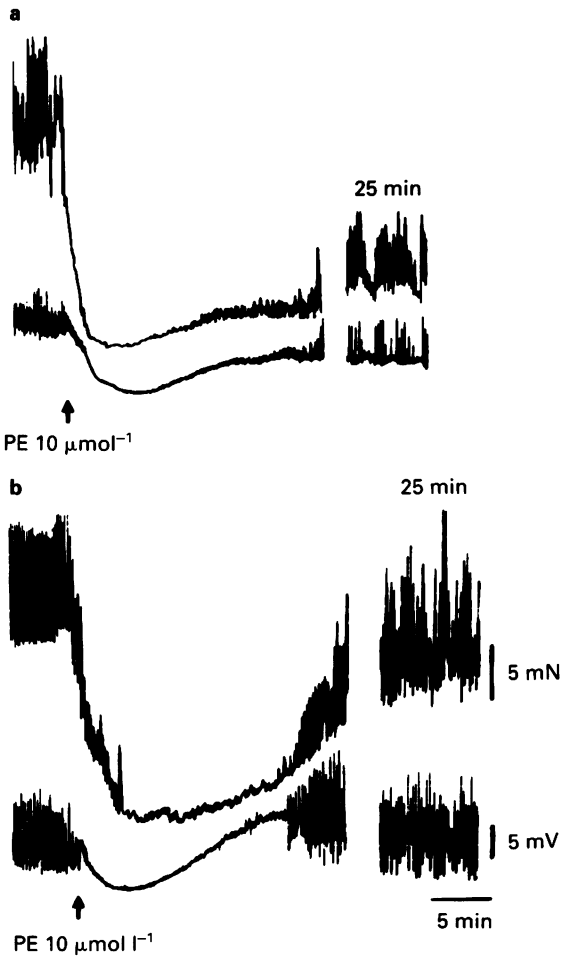

Figure 2 The effects of 30 min application of phenylephrine (PE) on the mechanical and electrical activity of taenia caeci. Recording was interrupted for 5 min. (a) and (b) as in Figure 1.

α-Adrenoceptor mediated response to long-lasting application of agonists

Long-lasting (30 min) exposure of the taenia caeci to adrenaline both in high (3 μmol l^{-1}) and low (0.1 to 0.5 μmol l^{-1}) concentrations produced an initial peak response followed by a transient but partial (about 20%) recovery of the membrane potential and muscle tension, before a steady state was attained which was sustained for a long period of time (Figure 1a). The sustained effect of adrenaline was well preserved in the presence of atropine, guanethidine and propranolol (Figure 1b). In contrast, the effects of the α_1-adrenoceptor agonists phenylephrine (Figure 2a, b) and methoxamine (1 and 10 μmol l^{-1}) were not sustained either in normal preparations or during blockade of muscarinic receptors, β-adrenoceptors or in the presence of guanethidine. In the continued presence of phenylephrine and methoxamine the membrane potential, basal tension, spontaneous spikes and phasic contractions partially recovered. This recovery was even

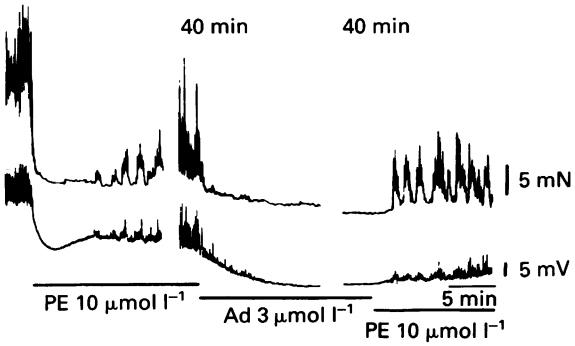

Figure 3 The effect of substitution of adrenaline (Ad) during tachyphylaxis to phenylephrine (PE). See text for further details. The interruptions represent 15 min.

more pronounced in the presence of atropine, propranolol and guanethidine, virtually reaching the initial control levels.

Substitution of adrenaline (0.5 or 3 μmol l^{-1}) for phenylephrine (1 or 10 μmol l^{-1}) during the recovery phase of the phenylephrine-induced response resulted in muscle relaxation and membrane hyperpolarization and suppression of spontaneous mechanical and electrical activity. This result was also observed in the presence of atropine, propranolol and guanethidine. When phenylephrine was re-added to the bath in place of adrenaline, recovery from the inhibitory response again occurred (Figure 3). It should be noted that adrenaline suppressed recovery from inhibition when adrenaline was added to the phenylephrine-containing solution. This effect of adrenaline was not altered by atropine, propranolol or guanethidine ($n = 6$).

In order to find out whether there are differences in the involvement of calcium in the hyperpolarizing actions of adrenaline and phenylephrine in taenia caeci, their initial effects were studied in calcium-free conditions, and in the presence of caffeine, nifedipine or phorbol dibutyrate.

Extracellular calcium and the α_1-adrenoceptor-mediated response

During the course of a 20 to 60 min superfusion of preparations with calcium-free solution, containing EGTA (0.4 mmol l^{-1}) to bind the residual extracellular calcium, both the spontaneous phasic contractions and spikes ceased and the α_1-adrenoceptor-mediated response was markedly reduced. Inhibition of the α-adrenoceptor-mediated response reached a steady state level within 25 min. In zero calcium solution the membrane hyperpolarization elicited by phenylephrine (10 μmol l^{-1}) or adrenaline (3 μmol l^{-1}) diminished to 40–60% of controls (Figure 4a, b). In agreement with previous observations (Den Hertog 1981; 1982), the persistence of a substantial part of the membrane hyperpolarization on the first exposure of the tissue to adrenoceptor agonists observed, even after 40 min superfusion with calcium-free, EGTA-containing solution, suggests that calcium released from an intracellular compartment could also participate in the α_1-adrenoceptor-mediated action of catecholamines.

Effect of nifedipine on the α_1-adrenoceptor-mediated response

Exposure of the taenia to nifedipine (1 to 10 μmol l^{-1}) caused disappearance of the spontaneous spikes and phasic contractions and relaxed the smooth muscle without significant alterations in the membrane potential ($n = 9$). Pretreatment of the taenia caeci with nifedipine (1 μmol l^{-1}) for 20 to 40 min resulted in a reduction of the phenylephrine-induced hyperpolarization by 50–60%. The magnitude of this inhibition could not be enhanced either by extending the pretreatment of preparations with nifedipine or by increasing its concentration (Figure 4c). Repeated exposure of the tissues to phenylephrine in the presence of nifedipine (1 μmol l^{-1}) resulted in further reduction of its hyperpolarizing effect. As seen in calcium-free conditions, the adrenaline-induced hyperpolarization was less sensitive to nifedipine pretreatment than that of phenylephrine, and the reduction reached only about 40% (Figure 4d). With repeated application of adrenaline and phenylephrine to nifedipine-pretreated preparations the effects of the drugs on membrane potential gradually disappeared and had ceased by the 4th to 5th application ($n = 4$).

The availability of releasable calcium for the α_1-adrenoceptor-mediated response

Adrenaline (3 μmol l^{-1}) and phenylephrine (10 μmol l^{-1}) were applied for 5 min before and during the superfusion of preparations with calcium-free solution. The first exposure to adrenaline and phenylephrine following 35 min superfusion with calcium-free, EGTA-containing solution resulted in a reduced hyperpolarization with an onset of slower time course. This reduction was even more marked on the second and subsequent exposures, as shown with phenylephrine in Figure 5. In calcium-free conditions the hyperpolarizing effect of adrenaline and phenylephrine were completely abolished on their 4th application ($n = 5$). The effect of adrenaline and phenylephrine in control and calcium-free solutions was independent of the presence of atropine, guanethidine and propranolol ($n = 9$).

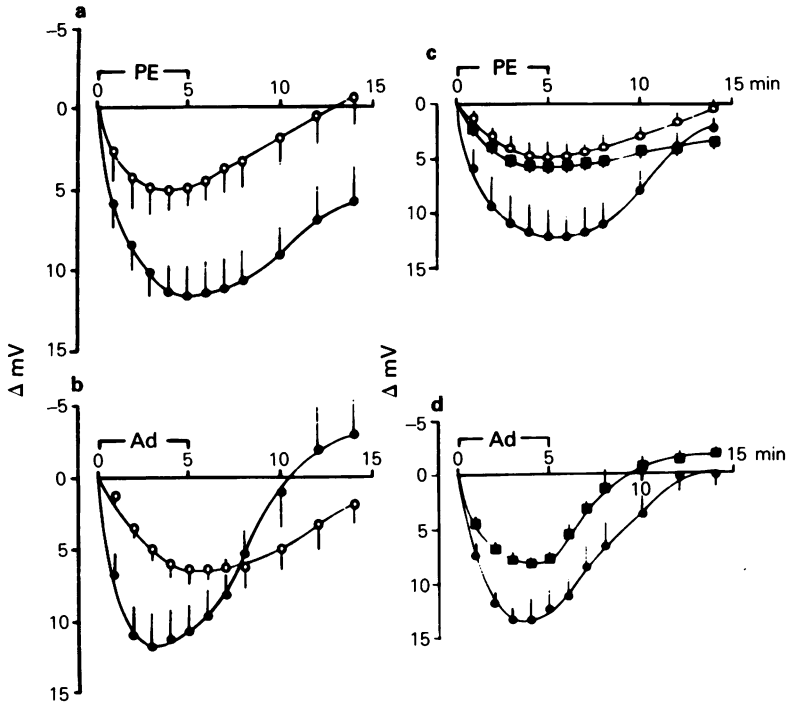

Figure 4 The effect of calcium-free solution (a,b) and nifedipine (c,d.) on the phenylephrine (PE, 10 μmol l^{-1})- and adrenaline (Ad, 3 μmol l^{-1})-evoked membrane potential changes. The positive values represent mean hyperpolarization and the negative values depolarization of the smooth muscle membrane. PE and Ad were applied as indicated from zero to 5 min. In (a) and (b): (●) control response and (○) response in calcium-free solution. In (c) and (d): (●) control response, (■) response in the presence of 1 μmol l^{-1} and (○) in the presence of 10 μmol l^{-1} nifedipine.

In taenia caeci superfused with calcium-free, EGTA-containing solution in the presence of caffeine (30 mmol l^{-1}), adrenaline (3 μmol l^{-1}) and phenylephrine (10 μmol l^{-1}) were unable to induce any membrane potential changes of the smooth muscle cells (n = 6).

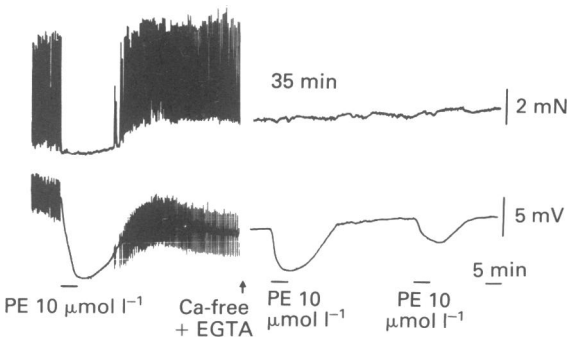

Figure 5 The effect of repeated exposures of taenia caeci to phenylephrine (PE) before and during perfusion with calcium-free solution. Before the second application of PE the tissue was perfused for 35 min with calcium-free solution containing EGTA 0.4 mmol l^{-1}.

Effect of phorbol dibutyrate on the α_1-adrenoceptor-mediated response

Phorbol 12,13-dibutyrate (1 μmol l^{-1}) produced a transient muscle contraction, reduced the frequency but enhanced the amplitude of spontaneous rhythmic contractions when only the mechanical response was recorded from larger segments of the tissue (Figure 6). With sucrose gap recording conditions phorbol dibutyrate produced muscle contraction associated with a transient mild increase in spike frequency without any significant changes in the basal (n = 5) membrane potential (Figure 7). Pretreatment of the taenia caeci with phorbol dibutyrate (1 μmol l^{-1}) for 20 min resulted in augmentation (by 74.6 ± 4.3%, n = 5) of the acetylcholine (1 μmol l^{-1})-induced contraction and complete inhibition of the adrenaline (1 μmol l^{-1})-induced relaxation (n = 4).

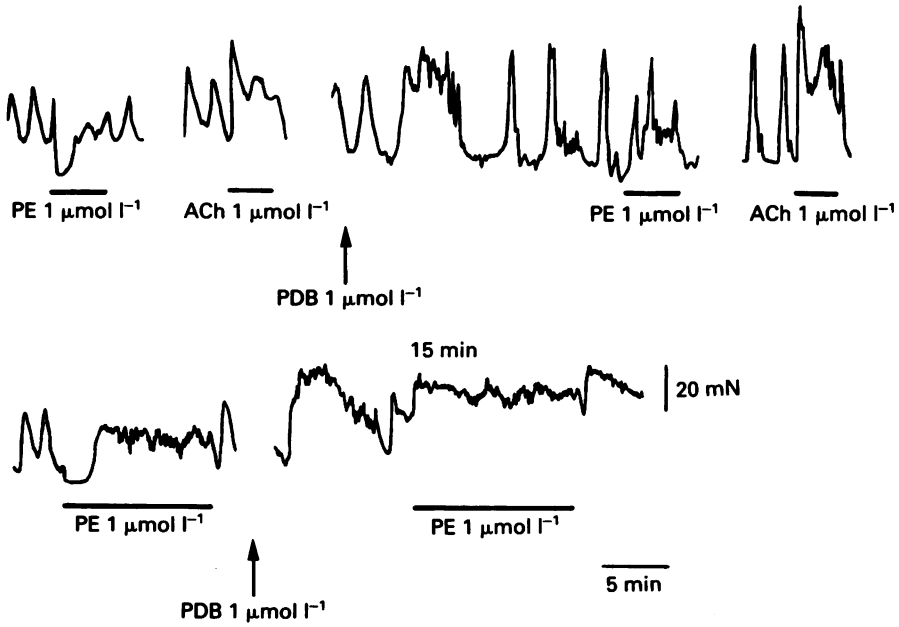

Figure 6 The effects of phenylephrine (PE) and acetylcholine (ACh) before and after 20 min pretreatment of taenia caeci with phorbol 12,13-dibutyrate (PDB).

The relaxing and hyperpolarizing effects of phenylephrine (1 μmol l^{-1}) were blocked by phorbol dibutyrate pretreatment and in its presence phenylephrine elicited muscle contraction (Figure 6). With sucrose gap recording conditions, no significant membrane potential changes were observed during the contractile effect of phenylephrine in the presence of phorbol dibutyrate ($n = 6$) (Figure 7).

Discussion

In agreement with our previous observations (Bauer, 1982; Bauer & Rusko, 1982) the present results show that the characteristics of the effect of phenylephrine and methoxamine on taenia caeci were in every respect the same as those of adrenaline when the exposure time to the agonist did not exceed 5 min.

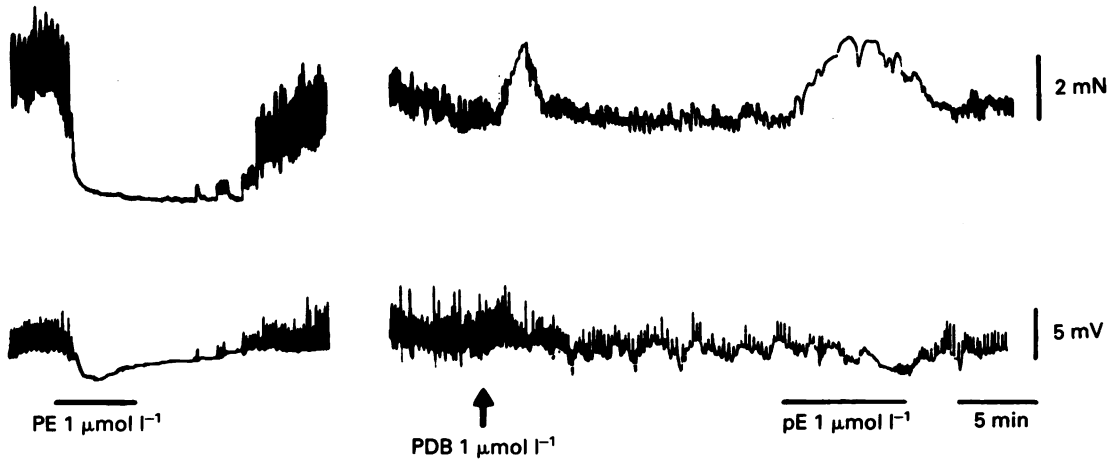

Figure 7 The effect of phenylephrine (PE) on the mechanical (upper trace) and electrical (lower trace) activities of taenia caeci before and in the presence of phorbol 12,13-dibutyrate (PDB).

Increasing the exposure time, however, resulted in substantial differences. The action of adrenaline with sucrose gap recording conditions was sustained even with prolonged superfusion (up to 30 min). In contrast, the membrane hyperpolarization, relaxation and suppression of spontaneous mechanical and electrical activity, to equieffective concentrations of phenylephrine and methoxamine, gradually declined on prolonged exposure of the taenia caeci. The recovery of the electrical and mechanical responses was not due to tachyphylaxis because phenylephrine induced the same degree of inhibition on repeated administration, even with the minimal time intervals necessary for partial or complete renewal of tension after the preceeding application (Rusko & Bauer, 1985). Moreover, during the delayed recovery adrenaline preserved its ability to inhibit taenia caeci even in the presence of propranolol, atropine, guanethidine and phenylephrine. Thus the differences between the actions of adrenaline and the selective α_1-adrenoceptor agonists on long-lasting application are unlikely to be accounted for by desensitization of α-adrenoceptors. The differences can be understood by assuming the existence of different mechanisms in their initial inhibitory action, or the existence of a component in the action of one of the studied agonists, which is lacking in the case of the others. Phenylephrine may also have a higher efficacy than adrenaline and during the early stages of the response it may cause so great a release of calcium that the stores become exhausted. However, the contractile effect of phenylephrine, both on short and long-lasting application, in the presence of phorbol dibutyrate suggests that this is not the case. Nelemans & Den Hertog (1987) recently showed that inositol metabolism could be involved in the mechanisms activated by adrenaline acting on α_1-adrenoceptors in taenia caeci. Phorbol esters, which have been reported to be selective activators of protein kinase C (Allgaier & Hertting, 1986), increased not only the spontaneous activity and the contractile effect of acetylcholine but also unmasked the excitatory effect of phenylephrine. This excitatory effect was not seen with adrenaline and was independent of the membrane potential changes, suggesting that it utilized either bound calcium or, less likely, calcium entering the cell via a potential-independent mechanism, because this excitation did not develop either in calcium-free solution or in barium substituted calcium-free solution (Rusko & Bauer, 1988).

As shown earlier (Bauer, 1982) and confirmed by the use of propranolol, prazosin and yohimbine in the present study, the initial inhibitory action of both adrenaline and phenylephrine was due to selective activation of α_1-adrenoceptors. Evidence has also been obtained that the effect of different α-adrenoceptor agonists (adrenaline, phenylephrine, noradrenaline and methoxamine) both in taenia caeci (Bauer & Rusko, 1982) and ileum (Bauer, 1985) is due to activation of a postjunctional TEA-sensitive potassium conductance. Regulation of potassium conductance by intracellular free calcium has been proposed in different tissues (Meech, 1974; Putney, 1976; Grafe *et al.*, 1980; Egashira, 1980; Yellen, 1984) and is suggested to be responsible for the α-adrenoceptor-mediated action of catecholamines in taenia caeci (Bülbring & Tomita, 1969a,c; 1977; Den Hertog, 1981; 1982; Bauer & Rusko, 1982). In the present study an attempt was made to examine whether the same calcium source is utilized in the hyperpolarizing effect of both adrenaline and phenylephrine. Therefore the effect of these agonists was analysed under conditions that modify calcium availability. Recently Den Hertog (1981, 1982) demonstrated that the adrenaline-induced α-adrenoceptor-mediated response of the guinea-pig taenia caeci in calcium-free conditions was transient even during a short-lasting (2 to 9 min) application. The amplitude of the phenylephrine- and adrenaline-induced hyperpolarization was reduced in zero calcium solution in the present experiments but, in contrast to the observations of Den Hertog (1981, 1982), 5 min exposure of the tissues to α-agonists in calcium-free solution produced sustained responses even when muscarinic cholinoceptors, α-adrenoceptors and the release of endogenous catecholamines were blocked. In addition, when agonists were applied repeatedly their action was sustained. This discrepancy is probably due to different experimental conditions, e.g. tissue size, superfusion rate, temperature, etc.

The initial hyperpolarization evoked by activation of α_1-adrenoceptors in taenia caeci is thought to result from activation of the calcium-dependent potassium conductance (Bülbring & Tomita, 1977; Den Hertog, 1981; Bauer & Rusko, 1982). Hence it may reflect the availability of free cytosolic calcium close to the inner surface of the plasma membrane essential for the opening of potassium channels. Two mechanisms have been proposed by which α-adrenoceptor stimulation can raise cytosolic free calcium; viz. stimulation of calcium entry from the extracellular space and/or mobilization of calcium from intracellular stores (Den Hertog, 1981; 1982). The preservation of roughly one half of the hyperpolarization upon activation of α_1-adrenoceptors, even after long-lasting superfusion with calcium-free, EGTA-containing solution or in the presence of nifedipine, favours the contention that a cellular calcium pool with tightly bound calcium may play a principal role in the action of α_1-adrenoceptor agonists. However, the amount of calcium in this cellular store is limited and is caffeine-sensitive, as indicated by the deterioration of α_1-adrenoceptor-mediated

effects on repeated application of agonists and their cessation in caffeine-treated preparations in calcium-free solution. Replenishment of this calcium store can be brought about only when extracellular calcium is available.

Stimulus-evoked plasmalemmal calcium entry is generally considered to occur through potential-sensitive channels, activated by depolarization, or through receptor operated channels, activated by agonists (for review see Bolton, 1979). Calcium channel agonists, such as nifedipine or D-600, appear to act primarily at potential-sensitive calcium channels (for review see Godfraind, 1985). Presumably in tissues such as taenia caeci which possesses spontaneous spike activity the potential-sensitive channels flicker between closed and open states. Inhibition of the potential-sensitive calcium influx due to the action of nifedipine resulted in spike suppression and relaxation. Prolonged treatment with D-600 was previously found to reduce the action of adrenaline (Den Hertog, 1981). This might be due to the interaction of D-600 with α-adrenoceptors as demonstrated in nervous tissues (Fairhurst *et al.*, 1980). In order to analyse further the possible involvement of potential-sensitive calcium channels, nifedipine was used as it does not interact with α-adrenoceptors (for review see Janis & Scriabine, 1983). Despite this, prolonged exposure of preparations to nifedipine also resulted in attenuation of the adrenaline- and phenylephrine-induced hyperpolarization. Reduction of the effects of the α-agonists in the presence of nifedipine thus might be due to suppression of calcium entry or its cellular mobilization. Upon blocking potential-sensitive channels, calcium could enter the cell via receptor operated channels or passive 'leak' channels (Cauvin *et al.*, 1983). The α_1-adrenoceptor-mediated hyperpolarization evoked in the presence of nifedipine might, therefore, reflect the calcium influx through these nifedipine-insensitive mechanisms or might be due to its cellular mobilization. Gradual deterioration of the hyperpolarizing action of α_1-agonists on their repeated application in the presence of nifedipine favours the latter mechanism. This conclusion suggests that cellularly bound calcium, supplied from the extracellular space via a nifedipine-sensitive mechanism, would be the principal source for the α_1-adrenoceptor-mediated action of catecholamines.

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