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Effects of noradrenaline and acetylcholine on electro-mechanical properties of the guinea-pig portal vein

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1 Effects of acetylcholine (ACh) and noradrenaline (NA) on mechanical properties of smooth muscle cells of the guinea-pig portal vein were investigated using intact and skinned muscle preparations. In some preparations, the electrical activity was also recorded. In addition to ACh and NA, the effects of caffeine, procaine, excess concentrations of $[K]_o$ and $MnCl_2$ were investigated.

2 NA enhanced the mechanical response due to increase in the spike generation, receptor activated depolarization and release of Ca stored in the cell.

3 ACh also enhanced the mechanical response, but this agent had little effect on the Ca release from the storage sites.

4 Caffeine and procaine (10 mM) depolarized the membrane and enhanced the electrical activity. Caffeine enhanced the mechanical activity due to an increase in the membrane activity and the release of Ca stored in the cell, while procaine inhibited the contraction. Procaine inhibited and caffeine accelerated the Ca-induced Ca-release mechanism in the cell. NA released the Ca from the storage sites to a greater extent than did caffeine or ACh.

5 $MnCl_2$ inhibited the spontaneous membrane activity and contraction; however, low concentrations of $MnCl_2$ increased the caffeine- or NA-induced contraction in Ca-free solution.

6 When the Ca-tension relationship was observed in saponin-treated skinned muscles, the minimum concentration of Ca required to produce the contraction was 10^{-7} M, as observed in other vascular tissues. Caffeine released the stored Ca, NA and ACh had no effect on the Ca release and procaine inhibited the caffeine-induced Ca release in skinned muscles.

7 It was concluded that the membrane properties of smooth muscle cells in the portal vein are much the same as observed in other spontaneously active visceral muscles. Differences observed in the actions of NA and ACh on mechanical properties seems to be mainly due to different receptor-operated Ca release mechanisms.

Introduction

When investigating the membrane properties of vascular smooth muscles in various species, regional differences become evident; for example spontaneous membrane activity is recordable only from smooth muscle of the portal vein. In the guinea-pig portal vein, Takata (1980) found that both acetylcholine (ACh) and noradrenaline (NA) depolarized the membrane, increased the ionic conductance and the number and frequency of spike generation; as a consequence, the mechanical response was enhanced. On the other hand, in the mesenteric vein, NA depolarized the membrane and triggered spike generation, yet ACh hyperpolarized the membrane and produced a contraction.

It was of interest to compare the contraction evoked by NA or ACh in the portal vein, because both agents produced excitation of the membrane and also contraction. Whether or not activation of the muscarinic receptor and α-adrenoceptor utilize the same sources of Ca stored in the cell should be clarified. In addition, it is necessary to determine whether the properties of Ca storage sites in the cells of spontaneously active vascular tissue are similar to those in electrically quiescent vascular muscle cells.

The present experiments were carried out to investigate the effects of ACh and NA on the mechanical properties of the portal vein. To compare the effects of the above agents, caffeine, procaine, $MnCl_2$ and

excess concentrations of $[K]_o$ were also observed. To investigate the nature of Ca storage sites and contractile proteins, the muscles were chemically skinned. The electrical activity of the smooth muscle membrane was also investigated. The results obtained were compared with findings in the vascular smooth muscles of other tissues.

Methods

Guinea-pig of either sex were stunned and bled. The portal vein was excised and connective tissue was carefully removed under a binocular microscope. For tension recording from the chemically skinned muscle cells, the vessel was teased apart using jeweller's forceps, cut along the longitudinal direction, and longitudinally cut strips of 0.1 mm in width and 0.3 mm in length were prepared. The preparation was set up in a small chamber with a capacity of 0.9 ml through which the test solution was superfused rapidly by means of a jet at one end and by siphoning off simultaneously with a water pump at the other end. Both ends of the preparation were fixed by Scotch double stick tape (3M Co., St. Paul, Minn.), and isometric tension was recorded by means of a strain gauge transducer (U-gauge, Shinko, Co.). For measurement of electrical properties the tissue prepared was 2 mm in width and 5 mm in length.

Procedures

Tension recording from skinned muscles: The experimental procedures were much the same as those described by Itoh *et al.* (1981b). The tissue was superfused with modified Krebs solution (Bülbring, 1954) of the following composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5, and then replaced with 128 mM $[K]_o$ containing solution in which the NaCl was replaced with KCl isoosmotically. After recording the 128 mM K-induced contraction, the solution was replaced with the relaxing solution containing 130 mM KCl, 20 mM Tris-maleate, 5 mM $MgCl_2$, 5 mM ATP and 4 mM EGTA at pH 6.8 (10 mM Na remained as ATP salt). The muscle was then skinned by leaving the preparation for 20 min in relaxing solution containing saponin 50 $\mu g\,ml^{-1}$. The preparation was washed again with the same solution and left until the tension became constant at about zero. To obtain the caffeine-induced contraction, the concentration of EGTA was reduced to 10^{-4} M throughout the experiment. The pH of the caffeine containing solution was kept at 6.8 by adding KOH instead of KCl to the relaxing solution. Various concentrations of Ca were prepared by adding an appropriate amount of $CaCl_2$ to EGTA, the apparent binding constant for the Ca-EGTA complex was considered to be maintained at $10^6\,M^{-1}$ and pH 6.8. For the binding constant of ATP for Mg at pH 6.8 ($4 \times 10^3\,M^{-1}$), we used the value calculated by Martell & Schwarzenbach (1956), as reported by Saida & Nonomura (1978) and Iino (1981), and the free Mg concentration was kept at 1 mM.

Tension recording from the intact muscle preparation The experimental set up was similar to that used for the skinned muscle. Preparations similar to those used for the skinned muscle tissue were superfused in Krebs solution, and excess concentrations of $[K]_o$, caffeine, NA, and ACh were applied. To estimate the amount of stored Ca, the amplitude of caffeine-induced contraction was measured in Ca-free 2 mM EGTA containing solution. The experimental protocol is shown in Figure 1, i.e. after depletion of stored Ca by repetitively applied caffeine or NA in Ca-free 2 mM EGTA containing solution, 2.5 mM Ca was applied for a certain time (stage 1), and subsequently caffeine or NA was applied (stage 3) following re-superfusion of Ca-free 2 mM EGTA containing solution (stage 2). To observe the effects of ACh or NA on the Ca store site, similar procedures to those employed for the caffeine-induced contraction were used.

To record the membrane potential and electrical activity, a microelectrode filled with 3 M KCl (50–80 MΩ d.c. resistance) was inserted into the cell from the outer side of the vessel (Ito & Kuriyama, 1971; Kuriyama *et al.*, 1971) The recording electrode was connected to cathode-ray oscilloscope through a preamplifier (Nihon Kohden, VC 10 and MZ-3) and displayed on the pen recorder (Nihon Kohden; RJG 4024) or camera (Nihon Kohden; PC 2B). Since the cell generated spikes spontaneous-

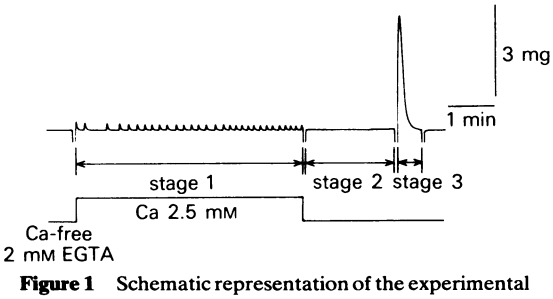

Figure 1 Schematic representation of the experimental procedure for estimation of the stored Ca in smooth muscle cells of the intact tissue from the mechanical response: stage 1, application of 2.5 mM Ca in Ca-free 2 mM EGTA containing solution, following pretreatment with Ca-free 2 mM EGTA solution for 10–15 min; stage 2, application of Ca-free 2 mM EGTA containing solution; stage 3, application of caffeine or noradrenaline in Ca-free 2 mM EGTA containing solution.

ly, the membrane potential was measured during the silent period between trains of discharges.

The following drugs were used; (±)-noradrenaline-HCl (NA; Sankyo), acetylcholine Cl (ACh; Daiichi), atropine sulphate (Daiichi), caffeine (Wako), procaine HCl (Sigma), ethylene glycol-bis-(β-aminoethylether)-N-N′-tetraacetic acid (EGTA; Dozin), and tetrodotoxin (Sankyo).

The stock solution of drugs was freshly prepared just before each experiment. The measured values were expressed as the mean ± s.d., and Student's *t* test was used for statistical analysis. A *P* value of less than 0.05 was considered statistically significant.

Results

Effects of chemical agents and $[K]_o$ on the smooth muscle cell membrane of portal vein

The resting membrane potential measured during the interval of spontaneous membrane activities was -46.7 ± 1.8 mV ($n = 30$). A burst spike discharge with a slow depolarization appeared between the silent period in many cells, while in others, spikes were generated continuously. In most cells, the amplitude of the spike rarely showed overshoot, only an undershoot, potential.

Figure 2 shows the effects of 5×10^{-6} M ACh, 5×10^{-6} M NA, 10^{-3} M and 10^{-2} M caffeine, 10^{-3} M procaine and 10^{-3} M $MnCl_2$ on the electrical activity of the membrane. The first four agents enhanced the number and frequency of spike discharges and when the depolarization was significant, a depolarization block of spike generation or an abortive spike appeared, as shown in the case of NA (a) or caffeine (d). With application of 10^{-3} M caffeine (c), the number of spikes in a train discharge was reduced. Applications of high concentrations of caffeine (10^{-2} M) increased the spike frequency with depolarization (d), ACh also increased the spike frequency (b) but the depolarization of the membrane was less than that evoked by NA. When 10^{-3} M procaine was applied, the membrane was depolarized and the spike fre-

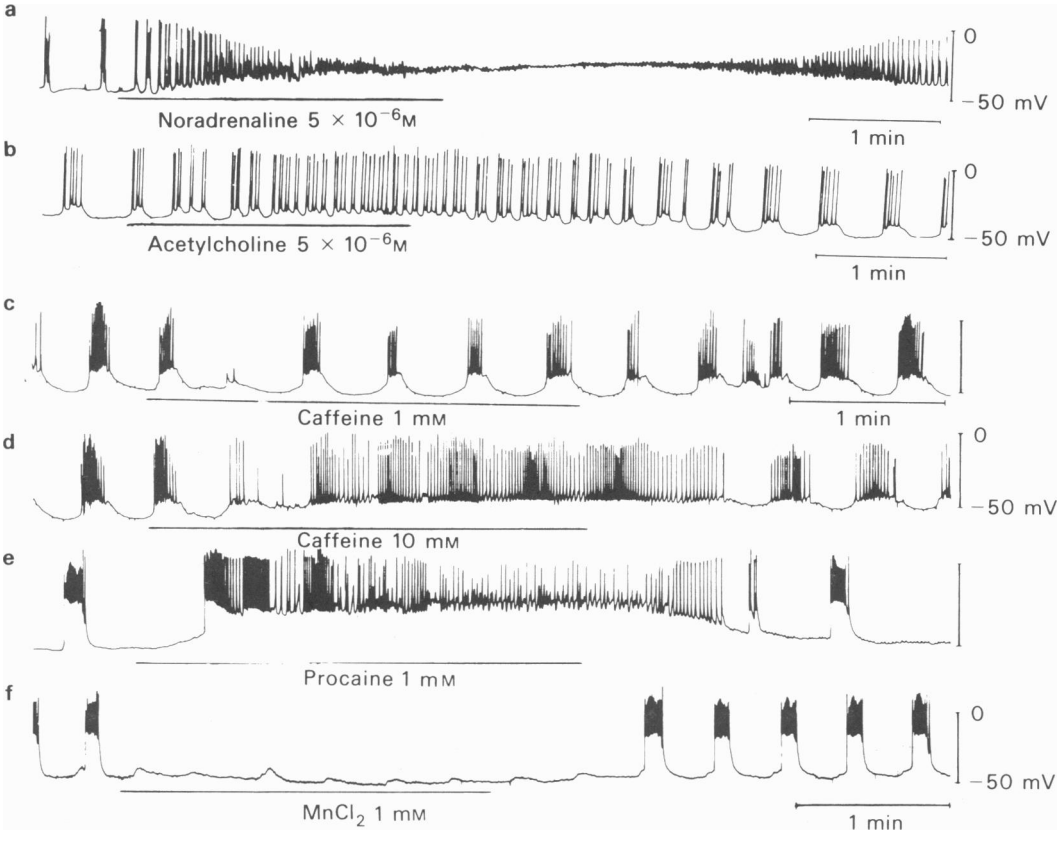

Figure 2 Effects of various chemical agents on spontaneously active smooth muscle cell membranes in the guinea-pig portal vein: (a) 5×10^{-6} M noradrenaline; (b) 5×10^{-6} M acetylcholine; (c) 10^{-3} M caffeine; (d) 10^{-2} M caffeine; (e) 10^{-3} M procaine; (f) 10^{-3} M $MnCl_2$. Bars indicate applications of chemical agents.

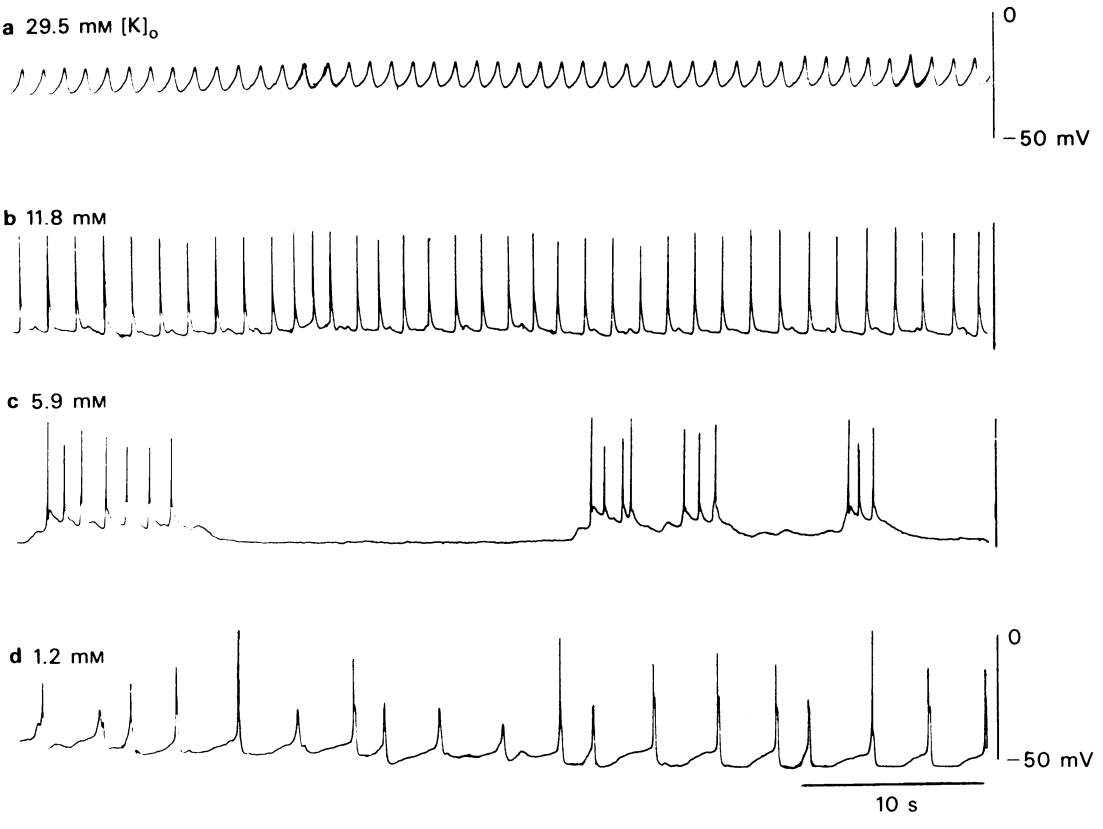

Figure 3 Effects of various concentrations of $[K]_o$ on the membrane potential and spontaneously generated spikes in smooth muscle cells of the guinea-pig portal vein; (a–d) 29.5 mM, 11.8 mM, 5.9 mM (control) and 1.2 mM $[K]_o$, respectively. Records were taken after 5–10 min superfusion in various concentrations of $[K]_o$.

quency increased. With application of 10^{-3} M $MnCl_2$, the spike activity ceased (f).

Application of various concentrations of $[K]_o$ depolarized the membrane to different levels (Figure 3). In Krebs solution (5.9 mM K), the spike appeared on a slow depolarization on which a few or sometimes several spikes were superimposed. With reduction in the concentration of $[K]_o$ to 1.2 mM, the membrane was hyperpolarized from, −48.1 mV to −53.4 mV ($n = 10-15$), and the pattern of spike generation changed from one of burst spike generation to continuous spike discharge with slow depolarization. Spike amplitude was irregular but on some occasions, overshoot potential was recorded (d). When the concentration of $[K]_o$ was increased to 11.8 mM (b), the membrane was depolarized, and the amplitude and the frequency of spike generation became regular with continuous discharge. When the concentration of $[K]_o$ was increased to 29.5 mM (Figure 3a), the membrane was further depolarized and only oscillatory slow potential changes appeared.

Effects of various agents on the membrane potential and mechanical response

Figure 4 shows the effects of 10^{-5} M NA (a), 128 mM $[K]_o$ (b), 10^{-2} M caffeine (c), 10^{-5} M ACh (d) and 10^{-3} M procaine (e) on the mechanical activity. Application of 10^{-5} M NA produced a large contraction composed of an initial phasic and prolonged tonic response. The amplitude of the tonic response gradually developed during application of NA, and in some tissues, the amplitude of the tonic response exceeded the phasic response. The NA-induced contraction was blocked by pretreatment with phentolamine (10^{-6} M). When the same concentration of NA was applied in the Ca-free 2×10^{-3} M EGTA containing solution, the phasic contraction was still generated, but not the tonic response (a). Application of 128 mM $[K]_o$ produced a marked phasic contraction but the amplitude gradually declined close to the resting tension (b). When 128 mM $[K]_o$ was applied during treatment with Ca-free 2×10^{-3} M EGTA containing

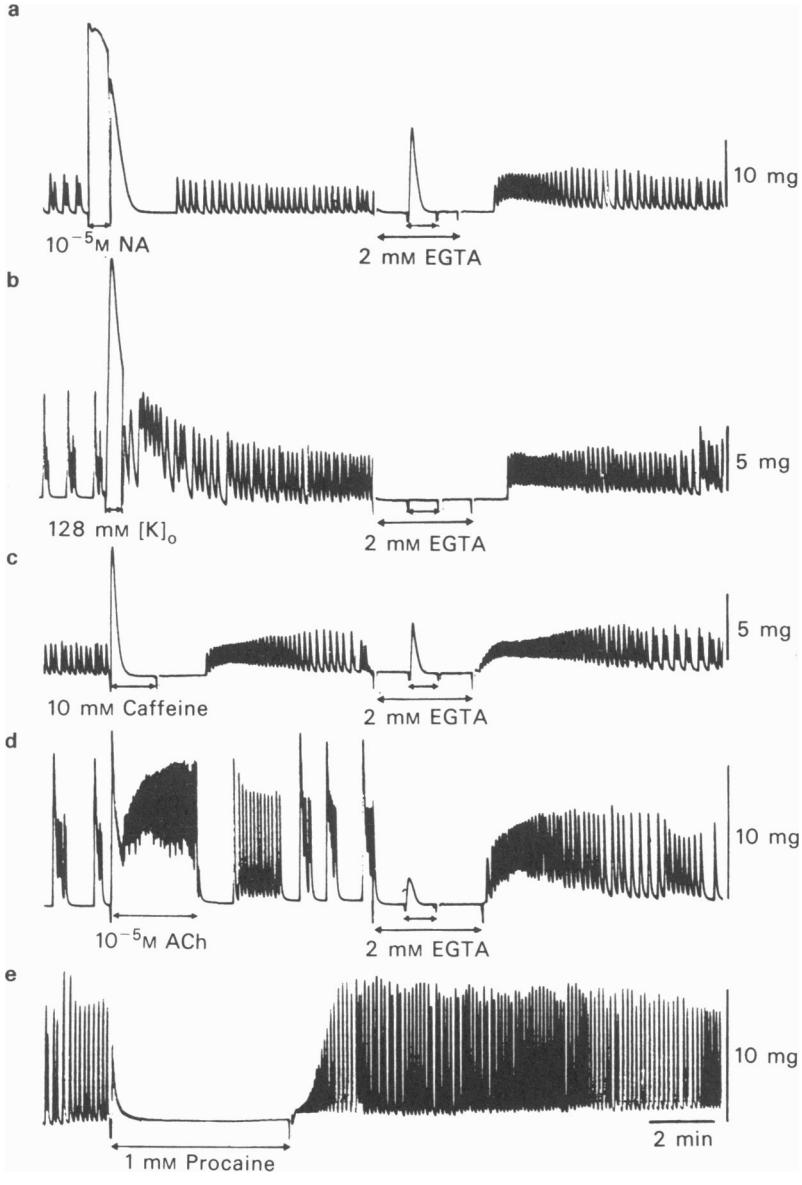

Figure 4 Effects of (a) 10^{-5} M noradrenaline (NA), (b) 128 mM $[K]_o$, (c) 10^{-2} M caffeine, (d) 10^{-5} M acetylcholine (ACh) and (e) 10^{-3} M procaine on the mechanical response in the presence (2.5 mM Ca) or absence of Ca. Bars indicate application of drug or Ca-free 2 mM EGTA containing solution; 2 mM EGTA in (a) – (d) indicates application of Ca-free 2 mM EGTA containing solution.

solution, a contraction was not evoked (b). With application of 10^{-2} M caffeine, a transient phasic contraction was evoked (c). The contraction was still present in the Ca-free EGTA containing solution when the amplitude was reduced (c). On application of 10^{-5} M ACh, the transient phasic contraction was followed by repetitive twitch contractions with a slightly elevated resting tone (d). The ACh-induced contraction was blocked by application of 10^{-6} M atropine. In the Ca-free 2×10^{-3} M EGTA containing solution, ACh evoked a small contraction. Procaine (1 mM) suppressed the spontaneously generated contraction (e). When the amplitude of the 128 mM $[K]_o$-induced phasic contraction was regis-

tered as a relative tension of 1.0, the relative amplitudes of phasic and tonic responses of the NA-induced contraction were 0.9 ± 0.1 and 0.8 ± 0.1 times (after 3 min) ($n = 5$), respectively and the caffeine-induced and ACh-induced contractions were 0.4 ± 0.1 times ($n = 5$) and 0.3 ± 0.1 times ($n = 5$) of the K-induced contraction, respectively.

The effects of 10^{-5} M ACh, 10^{-5} M NA and 10^{-2} M caffeine on the mechanical response during application of 128 mM $[K]_o$ were also observed. When 128 mM $[K]_o$ was applied, the phasic contraction appeared, and after the tissue relaxed close to the resting level (a tonic response), the above three agents were applied. During treatment with 128 mM $[K]_o$, the amplitude of the phasic contraction to ACh was 0.1 ± 0.1 times the 128 mM K-induced contraction ($n = 5$) and the tonic contraction was not evoked, while the amplitudes of phasic and tonic responses of the NA-induced contraction were 0.9 ± 0.2 and 0.7 ± 0.1 times the 128 mM K-induced contraction, respectively ($n = 5$). The amplitude of the caffeine-induced contraction was 0.6 ± 0.1 times the K-induced contraction ($n = 5$). These results indicate that in the presence of excess concentrations of $[K]_o$, the phasic contraction produced by caffeine is enhanced, the tonic contraction produced by ACh is reduced, and the contraction evoked by NA is only slightly modified.

Since the mechanical responses evoked by application of NA, ACh, excess concentration of $[K]_o$ or caffeine differed in Krebs and Ca-free solutions, the relationship between changes in the membrane potential and contraction were studied (Figure 5). As shown in Figure 5a, the minimum increase in $[K]_o$ required to produce the mechanical response was 18.1 mM $[K]_o$ at the membrane potential of -43.4 ± 2.4 mV ($n = 15$, 5.1 mV depolarization from -48.5 ± 1.6 mV, $n = 15$). The maximum slope of the depolarization induced by a 10 fold increase of $[K]_o$ plotted on a log scale was -45 mV. The maximum contraction was observed in 115.7 mM $[K]_o$ at the membrane potential of -6.7 ± 1.1 mV ($n = 15$). Further increased concentrations of $[K]_o$ to 128 mM, the depolarization and the amplitude of contraction were no longer enhanced (-7.4 ± 0.9 mV, $n = 10$). When the concentration of $[K]_o$ was increased to 54.7 mM, spontaneous electrical activity could not be observed as there was a depolarization block of the spike generation. As shown in Figure 5b, the NA-induced phasic contraction developed with concentrations over 5×10^{-8} M. Below this concentration, the spike frequency was increased with no appreciable depolarization of the membrane. The maximum amplitude of the contraction was evoked by application of 5×10^{-7} M NA and further increased concentrations did not enhance the amplitude of the phasic contraction. The membrane potential observed with

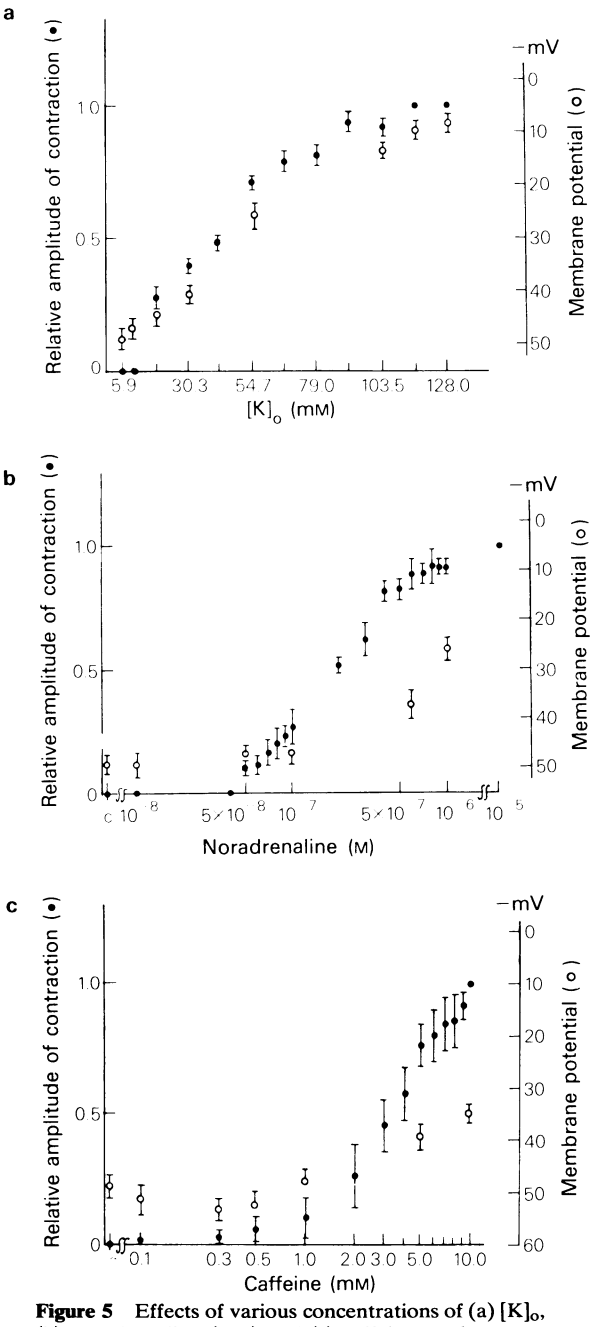

Figure 5 Effects of various concentrations of (a) $[K]_o$, (b) noradrenaline (NA) and (c) caffeine on the membrane potential (o) of single muscle cells and tension development (●). The amplitude of contraction evoked by 128 mM $[K]_o$, 10^{-5} M NA or 10^{-2} M caffeine was registered as a relative tension of 1.0. Vertical bars indicate 2 × s.d. The tension was recorded from 4–7 preparations and the membrane potential was measured from 15–30 impalements in 2–3 preparations.

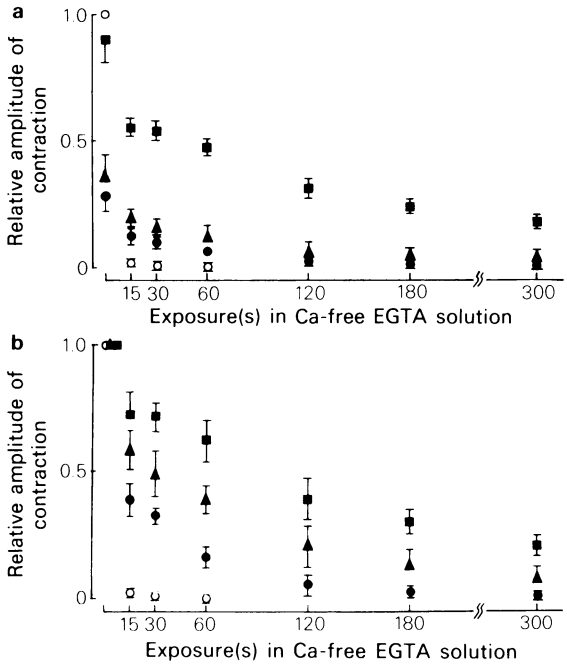

Figure 6 Effects of Ca-free 2 mM EGTA containing solution on the phasic contraction evoked by excess concentrations of $[K]_o$, acetylcholine (ACh), caffeine and noradrenaline (NA). (a) The amplitude of contraction evoked by 128 mM $[K]_o$ (○) was registered as a relative tension of 1.0; 10^{-5} M ACh (●); 10 mM caffeine (▲); 10^{-5} M NA (■). (b) The amplitude of phasic contraction evoked by 128 mM $[K]_o$, 10^{-5} M ACh, 10 mM caffeine and 10^{-5} M NA was registered as a relative tension of 1.0. Symbols as in (a).

application of 10^{-6} M NA was -25.4 ± 1.5 mV ($n = 10$).

Application of 10^{-3} M caffeine produced a contraction and the maximum amplitude was evoked by application of 10^{-2} M caffeine. The caffeine-induced contraction showed a sigmoidal relation when the log concentration of caffeine was plotted against the amplitude of contraction. Caffeine produced biphasic responses of the membrane, i.e. low concentrations of caffeine (0.5–1 mM) slightly hyperpolarized the membrane and further increased concentrations (over 3 mM) depolarized the membrane (Figure 5c). This has been observed in the coronary artery of the pig and guinea-pig aorta (Itoh *et al.*, 1982 b; Kajiwara, 1982).

Effects of noradrenaline, acetylcholine and caffeine on mechanical responses in Ca-free solution

In Ca-free 2 mM EGTA containing solution, the 128mM $[K]_o$-induced contraction ceased within several seconds. In contrast, the NA- ACh- and caffeine-induced contractions decreased in amplitude and persisted longer. Figure 6 shows the time-dependent change in the amplitude of phasic contraction evoked by 10^{-5} M ACh, 10^{-5} M NA, 10^{-2} M caffeine and 128 mM $[K]_o$ in Ca-free 2×10^{-3} M EGTA containing solution. After replacing Ca-free solution, individual agents were applied, at various intervals. The amplitude of contraction is expressed against the exposure time, the amplitude of contraction evoked by individual agents in Krebs solution was registered as relative to control tension of 1.0 (Figure 6b), and that evoked by 128 mM $[K]_o$ as 1.0 (Figure 6a). The K-induced contraction ceased within 15–30 s, and the ACh-induced contraction ceased after 3 min; the caffeine- and NA -induced contractions persisted for a longer period.

The effects of ACh, NA and caffeine on release of Ca stored in the cell were investigated in detail, in Ca-free solution. Figure 7 shows the effects of NA

Table 1 Effects of 10^{-3} M procaine or $MnCl_2$ on the caffeine- or noradrenaline (NA) -induced contraction: procaine was applied during stages 1–3 in 5.9 mM $[K]_o$ or 128 mM $[K]_o$ containing solution

a Effects of 1 mM procaine on amplitude of 10 mM caffeine or 10^{-5} M NA-induced contractions in Ca-free 5.9 mM and 128 mM-K solutions ($n = 5$)

5.9 mM-K	*10 mM Caffeine*	*10^{-5} M NA*
Control	1.0	1.0
stage 1	1.1 ± 0.4	1.1 ± 0.1
stage 2	0.4 ± 0.1	0.0
stage 3	0.2 ± 0.1	0.0
128 mM-K		
Control	1.0	1.0
stage 1	0.5 ± 0.1	0.7 ± 0.1
stage 2	0.3 ± 0.1	0.0
stage 3	0.6 ± 0.1	0.1 ± 0.1

b Effects of 1 mM $MnCl_2$ on amplitude of 10 mM caffeine or 10^{-5} M NA-induced contractions in Ca-free 5.9 mM and 128 mM-K solutions ($n = 5$)

5.9 mM-K	*10 mM Caffeine*	*10^{-5} M NA*
Control	1.0	1.0
stage 1	0.1 ± 0.1	0.0
stage 2	1.3 ± 0.2	1.6 ± 0.3
stage 3	0.8 ± 0.1	0.6 ± 0.1
128 mM-K		
Control	1.0	1.0
stage 1	0.6 ± 0.1	0.4 ± 0.1
stage 2	1.2 ± 0.2	1.5 ± 0.3
stage 3	0.9 ± 0.1	0.6 ± 0.1

The amplitude of the caffeine- or NA -induced contraction was registered as a relative tension of 1.0 in the absence of procaine or $MnCl_2$ in polarized and depolarized muscles, respectively.

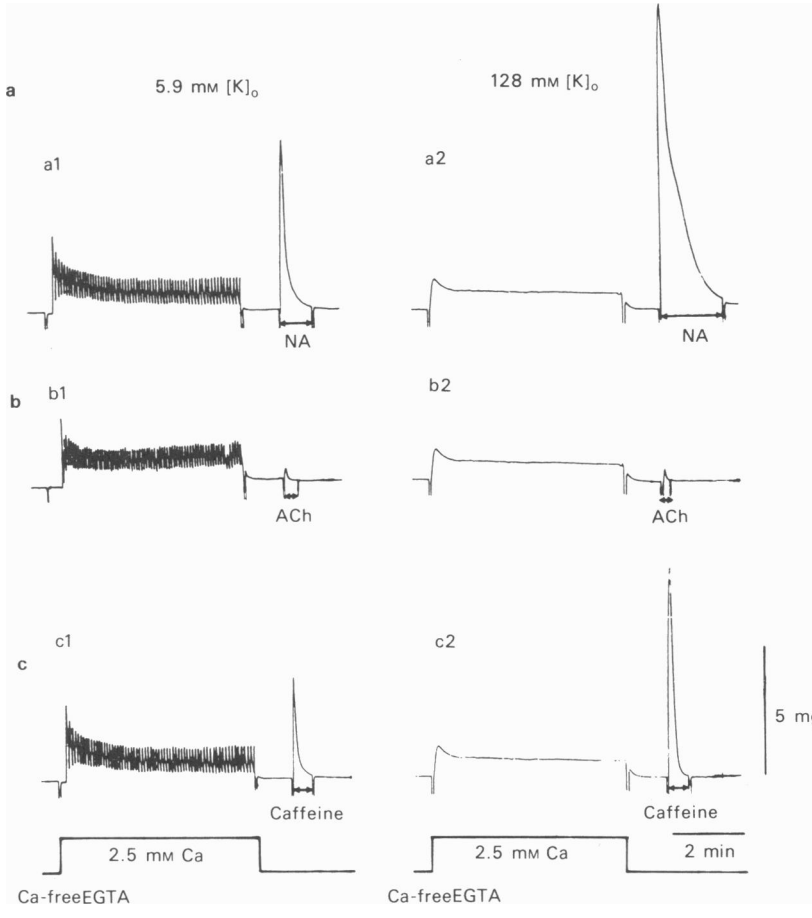

Figure 7 Effects of (a) 10^{-5} M noradrenaline (NA), (b) 10^{-5} M acetylcholine (ACh) and (c) 10^{-2} M caffeine on the mechanical response in Ca-free 5.9 mM $[K]_o$ (polarizing) or Ca-free 128 mM $[K]_o$ (depolarizing) containing solution. Ca 2.5 mM was added for 5 min during stage 1, the tissue was superfused with Ca-free 2 mM EGTA containing solution for 1 min (stage 2) and during stage 3, NA, ACh or caffeine was applied in 5.9 mM $[K]_o$ (a_1, b_1, c_1) or 128 mM $[K]_o$ (a_2, b_2, c_2).

(10^{-5} M), ACh (10^{-5} M), and caffeine (10^{-2} M) in Ca-free 2×10^{-3} M EGTA containing solution on polarized (5.9 mM K) and depolarized (128 mM K) muscles. After depletion of stored Ca by repetitive applications of NA or caffeine, 2.5 mM Ca was applied for 5 min (stage 1, Figure 7), the tissue was again washed with Ca-free 5.9 mM $[K]_o$ or 128 mM $[K]_o$ containing solution for 1 min (stage 2, Figure 7), after which the amplitude of the subsequently generated drug-induced contraction was measured (stage 3, Figure 7). During application of Ca, spontaneous twitch contractions were generated in some tissues in the presence of Krebs solution. The amplitude of the subsequently generated NA-induced contraction after 1 min treatment with Ca-free EGTA containing solution was 0.5 ± 0.1 times the 128 mM K-induced contraction in Krebs solution ($n = 5$). When the same experimental procedures were used for production of the ACh-induced or caffeine-induced contractions, the amplitudes were 0.1 ± 0.1 and 0.3 ± 0.1 times the 128 mM K-induced contraction ($n = 5-7$), respectively.

Much the same experimental procedures were used to evoke the contraction with applications of NA, ACh or caffeine in 128 mM $[K]_o$ (depolarized muscles; Figure 7). When 2.5 mM Ca was added in Ca-free 128 mM $[K]_o$ solution, contraction occurred. The subsequently generated NA-, ACh- and caffeine-induced contractions after treatment with Ca-free solution (1 min) were 0.7 ± 0.1 times, 0.1 ± 0.1 times and 0.4 ± 0.1 times the 128 mM K-induced contraction, respectively ($n = 5-7$). The amplitudes of evoked contractions were consistently enhanced to a greater extent in 128 mM $[K]_o$ than those induced in 5.9 mM $[K]_o$, except for the ACh-induced contraction. These results indicate that the

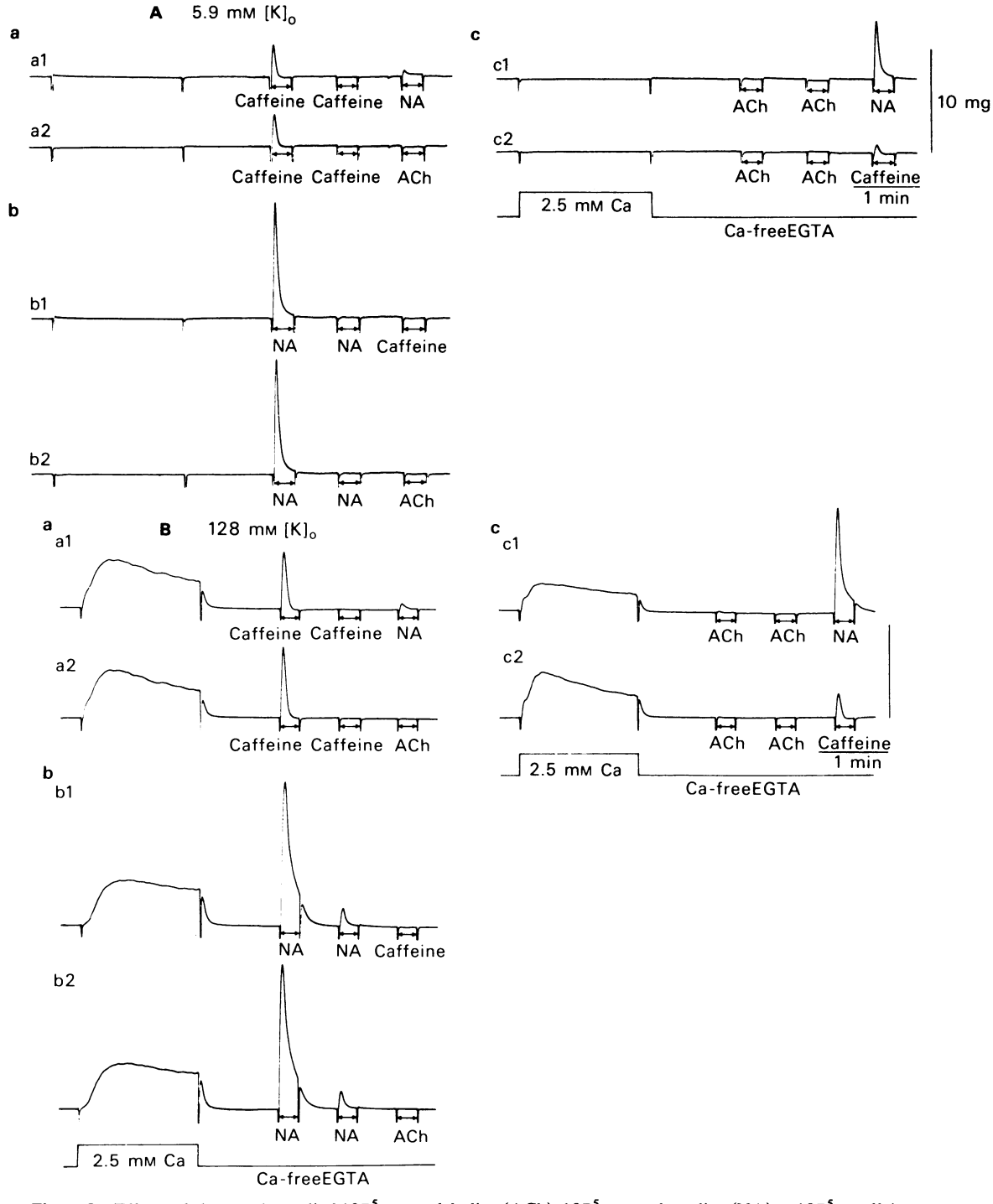

Figure 8 Effects of alternately applied 10^{-5} M acetylcholine (ACh), 10^{-5} M noradrenaline (NA) or 10^{-5} M caffeine after loading the Ca in Ca-free 5.9 mM $[K]_o$ and Ca-free 128 mM $[K]_o$. Procedures for the Ca loading and generation of contraction were as described in Methods, but caffeine, ACh or NA were successively applied after generation of contraction by caffeine, ACh or NA. (A) (a–c): polarized muscles in 5.9 mM $[K]_o$; (B) (a–c): depolarized muscles in 128 mM $[K]_o$. Ca-free EGTA solution contains 2 mM EGTA.

amount of Ca stored in the depolarized muscle is larger, and the extent of Ca release from store sites seems to be the same in the presence of NA and caffeine, but not ACh.

Table 1 shows the effects of procaine on the mechanical response. The experimental protocol is described in Methods. Application of 1 mM procaine during stage 1 enlarged the amplitude of the subsequent caffeine-induced contraction. When procaine was applied during stage 2, the caffeine-induced contraction was reduced to 0.4 ± 0.1 times, and when procaine and caffeine were applied simultaneously during stage 3, the amplitude was further reduced (Table 1a). With similar experimental procedures in the presence of 128 mM $[K]_o$, application of procaine during stage 1 inhibited the amplitudes of Ca-induced and resulting caffeine-induced contractions. When procaine was applied during stage 2 or stage 3, the amplitude of the Ca- and caffeine-induced contractions was reduced (Table 1a). When NA (10^{-5} M) instead of caffeine was used to release the stored Ca, the inhibitory effects of procaine were also observed in polarized and depolarized muscles (except for application of procaine during stage 1). With application of 10^{-3} M procaine during stage 2 or stage 3 in polarized muscles and during stage 2 in depolarized muscles, generation of the NA-induced contraction ceased (Table 1a). These results indicate that procaine suppresses the influx of Ca at the depolarized muscle (voltage-dependent Ca-influx) But not the passive Ca influx at the polarized muscle. Furthermore, this agent suppresses the release of Ca from store sites activated by NA or caffeine.

In smooth muscles of the portal vein, $MnCl_2$ modified the Ca influx and Ca sensitivity of the contractile proteins. During treatment with 1 mM $MnCl_2$ in stage 1, the resulting caffeine-induced contraction was markedly inhibited, or in the case of NA, ceased completely. When $MnCl_2$ was applied during stage 2, the resulting caffeine-induced and NA-induced contractions were enhanced. These effects of $MnCl_2$ on the contraction were also observed in depolarized muscles (Table 1b). When 2.5 mM Ca was applied at the same time as $MnCl_2$, the Ca-induced contraction ceased and the resulting caffeine-induced or NA-induced contraction was suppressed. When $MnCl_2$ was applied during stage 2, the amplitude of the resulting caffeine- or NA-induced contraction was enhanced. The results indicate that procaine and $MnCl_2$ act differently with regard to release of stored Ca and that the action on the contractile proteins also differs.

To investigate further the properties of the NA-induced and caffeine-induced contractions, the following experiments were done. Figure 8A shows the effects of successively applied 10^{-5} M NA, 10^{-2} M caffeine or 10^{-5} M ACh in the Ca-free solution on the polarized muscle after Ca loading. When 10^{-2} M caffeine was applied during stage 3, the contraction could be evoked but successively applied caffeine did not produce the contraction, although application of NA but not ACh produced a small contraction (Figure 8A). When 10^{-5} M NA was applied during stage 3, successively applied NA, caffeine or ACh did not produce the contraction (Figure 8A b). On the other hand, when 10^{-5} M ACh was applied, NA and caffeine produced contraction with a small amplitude (Figure 8A c).

When similar experiments were done using depolarized tissues (in 128 mM $[K]_o$), application of 2.5 mM Ca (stage 1) produced a contraction (Figure 8B). Regardless of polarization or depolarization of the membrane, the response of the tissue to ACh, NA or caffeine was the same, i.e. when NA was applied successively, ACh or caffeine did not produce a contraction, while after application of caffeine, NA but not ACh produced a contraction and after application of ACh, NA and caffeine produced a contraction (Figure 8B a-c).

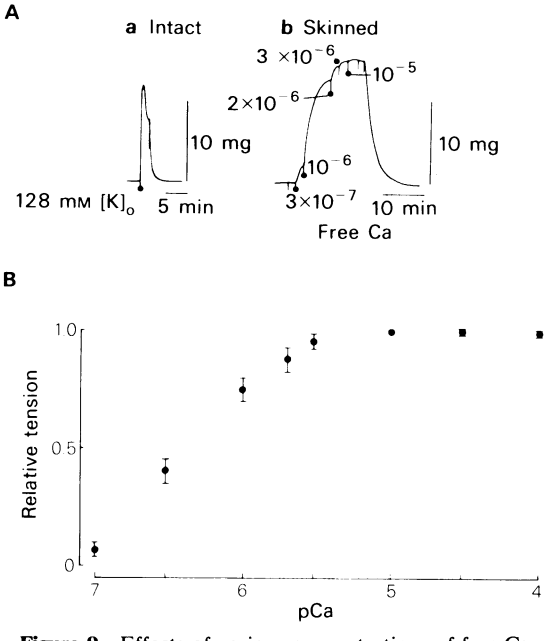

Figure 9 Effects of various concentrations of free Ca on the saponin-treated skinned muscles. (A)(a) 128 mM $[K]_o$-induced contraction in intact muscles; (b) after skinning the tissue (see Methods), various concentrations of Ca were applied cumulatively. (B) The pCa-tension relationship. The contraction evoked by 10^{-5} M Ca was registered as a relative tension of 1.0, $n = 3-5$.

Effects of noradrenaline, acetylcholine and caffeine on the contractile proteins and Ca storage sites

Figure 9 shows the pCa-tension relationship obtained from smooth muscle tissues of the portal vein after skinning with saponin (50 $\mu g\,ml^{-1}$ for 20 min). Before preparing the skinned muscles, the amplitude of 128 mM $[K]_o$ -induced contraction was registered (a). The tissue was then rinsed with the relaxing solution and saponin (50 $\mu g\,ml^{-1}$) was applied for 20 min, as described in Methods. One criterion for the completion of skinning was that the maximum amplitude of contraction produced by application of 10^{-5} M Ca was larger than that of the 128 mM $[K]_o$ -induced contraction of the intact tissue. To produce the contraction, various concentrations of Ca were cumulatively applied to the tissue (b). The pCa-tension relationship was plotted using the results obtained from 5 different preparations. The minimum concentration of Ca required to produce the contraction was 10^{-7} M (pCa = 7) and the amplitude of contraction increased in proportion to increased concentrations of Ca up to 10^{-5} M (pCa = 5). Further increased concentrations of Ca (over 10^{-5} M) did not enhance the amplitude, however, the amplitude was consistently larger than the 128 mM $[K]_o$ -induced contraction. This relationship was the same as that observed in the guinea-pig mesenteric artery and the pig coronary artery (Itoh *et al.*, 1981a; 1982a; 1983). Application of NA (10^{-5} M), ACh (10^{-5} M) and caffeine (10^{-2} M) did not significantly modify the pCa-tension relationship. These results indicate that NA, ACh and caffeine affect neither the Ca regulator protein of contractile proteins (calmodulin) nor the contractile proteins.

To estimate the amount of Ca stored in the skinned muscles, 10^{-2} M caffeine was applied after application of 10^{-6} M Ca with 10^{-4} M EGTA in the Ca-free relaxing solution, using the procedures shown in Figure 10, i.e. after skinning the tissue, 10^{-6} M Ca with 10^{-4} M EGTA were applied for 2 min (stage 1), then the tissue was rinsed with Ca-free 10^{-4} M EGTA containing solution for 2 min (stage 2) with 10^{-5} M ACh or 10^{-5} M NA and 10^{-2} M caffeine being subsequently applied (stage 3). The contraction was recordable with application of 10^{-2} M caffeine but application of 10^{-5} M NA or 10^{-5} M ACh neither generated a contraction nor modified the caffeine-induced contraction.

Amplitude of the caffeine-induced contraction in skinned muscle depended on the concentration and duration of pretreated Ca and also on the duration of treatment with Ca-free solution (stage 2) before application of caffeine. When 10^{-2} M procaine was applied during stage 2 and stage 3, application of caffeine did not produce contraction (Figure 10b2).

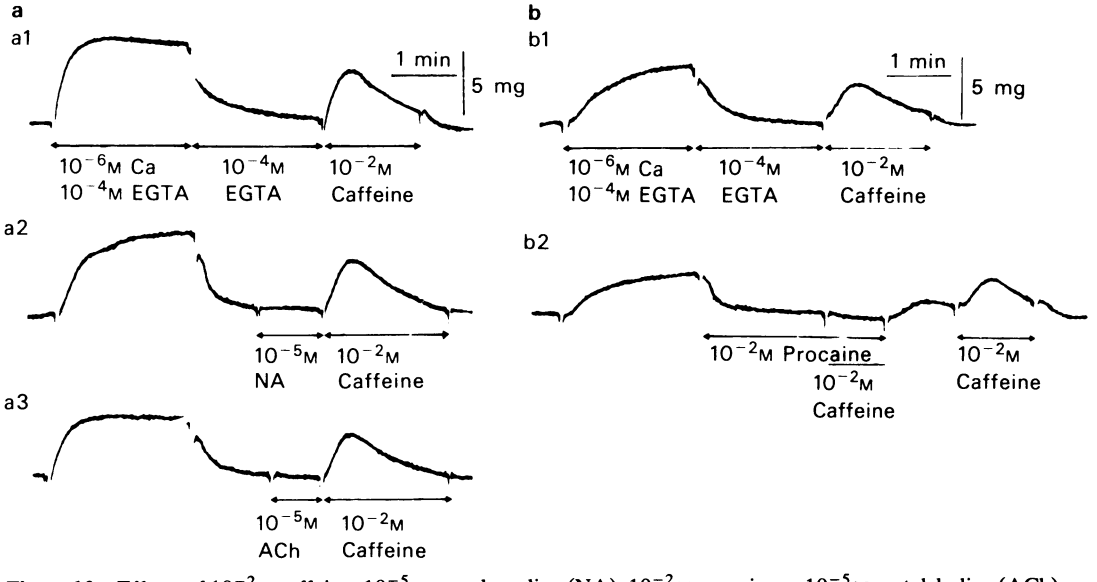

Figure 10 Effects of 10^{-2} M caffeine, 10^{-5} M noradrenaline (NA), 10^{-2} M procaine or 10^{-5} M acetylcholine (ACh) on skinned muscles after loading the Ca into the storage sites. (10^{-6} M Ca for 2 min). The experimental procedures were the same as those described in Methods for intact muscles but the experiments were carried out on skinned muscle. After Ca application for 2 min (stage 1), the tissue was rinsed with the relaxing solution (see Methods) for 2 min with NA, ACh or procaine (stage 2) and caffeine was applied (stage 3). (a_1 and b_2) Control; (a_2 and a_3) during stage 2, 10^{-5} M NA or 10^{-5} M ACh was applied; (b_2) during stages 2 and 3, 10^{-2} M procaine was applied. Caffeine was again applied after removal of procaine. (a) and (b) are different preparations.

Discussion

The spike generated in smooth muscle cells of the portal vein is due to influx of Ca (a Ca spike) since the spike persists in tetrodotoxin containing and Na-free solution (Ito & Kuriyama, 1971; Kuriyama *et al.*, 1971). The influx of Ca during generation of the spike triggers the contraction. In the presence of procaine, generation of the spike was continuous, yet the contraction ceased. Therefore, the spike did not directly increase the free Ca in the cell, as Ca would be sequestered in the store sites. With total occupation of the Ca store, Ca may activate a Ca-induced Ca-release mechanism, as postulated in the case of guinea-pig mesenteric artery (Itoh *et al.*, 1981b).

In intact muscles, the electrical and mechanical responses evoked by application of NA or ACh were blocked by phentolamine or atropine, respectively, and in skinned muscles, NA and ACh did not induce the contraction, therefore, the α-adrenoceptor and muscarinic receptors are probably located at the surface membrane of these muscle cells, and activation of α-adrenoceptors would release the Ca stored in the cell and activate the surface membrane, while activation of muscarinic receptors would mainly activate the surface membrane.

When caffeine was applied, there was a change in the electrical activity and a release of the Ca stored in the cell. Therefore, caffeine seems to activate the surface membrane as well as the Ca storage site in the cell, as in the case with NA.

The Ca located just beneath the cell membrane plays an important role in regulating the ionic permeability of the membrane (Tomita, 1970; 1975; 1981). An increase in the free Ca just beneath the cell membrane increases the K-conductance of the membrane (Ca-dependent I_k; Meech, 1978). Application of a low concentration of caffeine hyperpolarized the membrane and increased concentrations of caffeine depolarized the membrane. If a low concentration of caffeine increases the free Ca just beneath the cell membrane, the K-conductance may increase, and further increases of Ca in the presence of a high concentration of caffeine may suppress the K-conductance, as postulated in the case of the guinea-pig mesenteric artery (Itoh *et al.*, 1981b).

Procaine enhanced the spike activity with depolarization, and suppressed the contraction. These actions of caffeine and procaine on the muscle membrane were the same as those observed in the guinea-pig mesenteric artery (Itoh *et al.*, 1981a,b), basilar artery (Fujiwara *et al.*, 1982) and aorta (Kajiwara,1982), therefore both caffeine and procaine seem to penetrate the surface membrane of intact muscles and act on the Ca store sites.

$MnCl_2$ inhibits the spike generation and produces either a suppression (Keene *et al.*, 1972; Katase & Tomita, 1972; Osa, 1974; Yoshida *et al.*, 1977) or an acceleration of the mechanical responses (Shibata, 1969; Ogasawara *et al.*, 1980). In the guinea-pig portal vein, $MnCl_2$ inhibited the Ca-spike and Ca influxes in polarized and depolarized membranes, thus suppressing the contraction, while $MnCl_2$ may suppress the leakage of Ca from storage sites after re-addition of Ca to the Ca-free solution and enhance the contraction evoked by either caffeine or NA. These results are the same as those observed in the case of the smooth muscle cells of the stomach (Itoh *et al.*, 1982c) and mesenteric artery (Itoh, T. personal communication) of the guinea-pig. Presumably $MnCl_2$ penetrates the cell membrane and as a consequence, the actions on smooth muscles of the portal vein are biphasic.

In the coronary artery of the pig, the ACh-induced contraction was much greater than that evoked by 128 mM $[K]_o$. This contraction appeared with no change in the membrane potential and persisted in the Ca-free solution. The nature of the ACh-induced contraction in the coronary artery was therefore, postulated to be due to the release of Ca stored in the cell by activation of the muscarinic receptors distributed on the surface membrane (Itoh *et al.*, 1982a). In the guinea-pig portal vein, the membrane activity was accelerated but the amplitude of contraction was small and release of Ca from the storage site in the Ca-free solution was minute. It is plausible that the sensitivity of the muscarinic receptor to ACh is lower, or that the density of the receptor distribution is lower than that on the coronary artery of the pig.

The muscarinic receptor distributed on the smooth muscle cell membrane of the portal vein also has different properties from those distributed on the main mesenteric vein, as ACh depolarized the membrane of the portal vein but hyperpolarized the membrane in the main mesenteric vein (Takata, 1980; Suzuki, 1981). Regardless of whether ACh depolarized of hyperpolarized the membrane, a contraction was consistently evoked. Although, the muscarinic receptors distributed on the mesenteric system (main mesenteric vein and portal vein) have different ionophore subunits, they seem to possess a functional connection between the receptors and Ca releasing sites.

NA produced phasic and tonic contractions, the latter ceased but the former were retained in the Ca-free solution. The amplitude of the NA-induced phasic contraction was much larger than that evoked by caffeine in the Ca-free solution. Furthermore, after application of caffeine, NA continued to evoke the contraction. Application of caffeine after treatment with NA did not evoke the contraction in the Ca-free solution. Such effects of NA in the portal vein are the same as the effects of ACh observed in the coronary artery of the pig, with regard to the

action of caffeine (Itoh *et al.*, 1982a). If caffeine mainly releases Ca from the sarcoplasmic reticulum (SR), NA would release Ca not only from the SR but also from other areas in the cell. Intracellular Ca is sequestered in SR, mitochondria and just beneath the cell membrane. However, the Ca distributed in the mitochondria probably does not relate to the contraction generating mechanism in vascular smooth muscle (Johansson & Somlyo, 1980; Jones, 1981). Presumably, the Ca stored in the SR and the Ca distributed just beneath the cell membrane may also contribute toward increase in the free Ca in the myoplasm seen with application of NA as discussed for the rabbit mesenteric artery (Itoh *et al.*, 1982c).

When properties of smooth muscles of the portal vein were compared with those of other vascular smooth muscle tissues, differences were noted in the generation of spontaneous spikes. Furthermore, the capacity for Ca storage in these cells seems to be less than in other tissues as the action of procaine was less dominant than in the mesenteric and coronary arteries (Itoh *et al.*, 1981b; 1982a,b). Moreover, activation of ionophore subunits of both receptors increases the Na-permeability, but the muscarinic receptor-activated Ca release mechanism is less developed in comparison to that of the adrenoceptor. In the guinea-pig portal vein, the adrenoceptors are probably more densely distributed than the muscarinic receptors, and properties and distribution of muscarinic receptors on the portal vein also differ from those of the main mesenteric vein (Takata, 1980; Suzuki, 1981).

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