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# DILTIAZEM-INDUCED VASODILATATION OF SMOOTH MUSCLE CELLS OF THE CANINE BASILAR ARTERY

SHIGERU FUJIWARA, YUSHI ITO, TAKEO ITOH, HIROSI KURIYAMA & HIKARU SUZUKI

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

**1** The effects of diltiazem on smooth muscle cells of the canine basilar artery were investigated by means of microelectrode, double sucrose gap and isometric tension recording methods.

**2** The mean membrane potential of the smooth muscle cells was −49.8 mV and they were electrically quiescent. Diltiazem (over $10^{-5}$ M) depolarized the membrane. After pretreatment with 5 and 10 mM tetraethylammonium (TEA), an outward current pulse (1 and 2 s in duration) produced a spike and this spike was abolished by application of $10^{-5}$ M diltiazem.

**3** The spike could also be generated by the excitatory junction potential (e.j.p.) evoked by perivascular nerve stimulation (0.05 ms in pulse duration) in the presence of 5 mM TEA. Diltiazem ($>10^{-6}$ M) suppressed both the spike and the e.j.ps, the suppression being more apparent for spike generation. The amplitude of the e.j.ps was reduced by diltiazem in concentrations greater than $10^{-6}$ M. The effects were dose-dependent: when the amplitude of e.j.ps was reduced by application of diltiazem, the resulting mechanical response was also proportionally smaller.

**4** The contractions evoked by 128 mM $[K]_o$, $10^{-3}$ M adenosine 5′-triphosphate (ATP) or, NaCl-free solution were abolished in Ca-free solutions containing 2 mM EGTA, but the amplitude of caffeine-induced contraction (10 mM) was only slightly reduced. Diltiazem, in concentrations above $3 \times 10^{-7}$ M suppressed the contraction evoked by excess $[K]_o$, ATP or caffeine, but the inhibitory action of diltiazem on the K-induced contraction was greatest.

**5** Following pretreatment with 2.5 mM $[Ca]_o$, a contraction was evoked by caffeine in Ca-free solution. The amplitude of the caffeine-induced contraction was increased by simultaneous application of 2.5 mM $[Ca]_o$ with 128 mM $[K]_o$ and to a lesser extent by simultaneous application of 2.5 mM $[Ca]_o$ with 5.9 mM $[K]_o$. The amplitude of the caffeine-induced contraction generated in the presence of 5.9 mM or 128 mM $[K]_o$ was suppressed to the same extent by application of diltiazem ($10^{-5}$ M) during preincubation in $[Ca]_o$. This result suggests that the Ca stored in the cell is replenished by Ca-influx from $[Ca]_o$ during the resting and active states of the membrane, and that diltiazem has no effect on the mobilization of Ca stored in the cell.

**6** Thus, diltiazem acts on the canine basilar artery suppressing the Ca-influx during the active condition as a Ca-spike suppressor and the voltage-dependent Ca-influx induced by excess $[K]_o$ or by chemical depolarization. Diltiazem has no effect on the Ca mobilization from the store site. This agent also suppresses the amplitude of e.j.ps due to inhibition of the release of chemical transmitter from nerve terminals following the suppression of the Ca-influx. Diltiazem appears to act as a vasodilator on the canine basilar artery.

## Introduction

Diltiazem suppresses Ca influx, that is it acts as a Ca channel blocker, on smooth muscles of the rabbit pulmonary artery (Ito, Kuriyama & Suzuki, 1978) and the guinea-pig mesenteric artery (Suzuki, Itoh & Kuriyama, 1981). This agent also acts on visceral smooth muscles such as the guinea-pig taenia coli (Magaribuchi, Nakajima & Kiyomoto, 1977a, b). In the guinea-pig mesenteric artery, it suppresses the spike evoked by outward current pulses in the presence of tetraethylammonium (TEA), and contractions evoked by excess $[K]_o$, noradrenaline or electrical depolarization, but does not suppress the contraction evoked by caffeine or Na-deficiency. Furthermore, diltiazem does not affect the Ca accumulation into and release of Ca by caffeine from the Ca store site in saponin-treated skinned muscle cells. Therefore, Suzuki *et al.* (1981) postulated that diltiazem suppresses nonselectively the Ca-influx across the myoplasmic membrane with no effect on the Ca store site.

In vascular smooth muscles, NaCl-free solution produces a contraction in the guinea-pig mesenteric

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artery but not in the porcine coronary artery (Suzuki *et al.*, 1981; Hirata, Itoh & Kuriyama, 1981). Furthermore, adenosine 5'-triphosphate (ATP) hyperpolarizes the membrane in the guinea-pig coronary artery, but this agent causes depolarization in the guinea-pig mesenteric and basilar arteries and portal vein (Kitamura & Kuriyama, 1979; Karashima & Takata, 1979; Takata, 1980; Karashima & Kuriyama, 1981). Thus, responses of vascular smooth muscles differ with the region and species. As diltiazem is an effective chemical agent for treatment of angina pectoris and because vasospasm in cerebral arteries occurs 1–2 weeks after subarachnoid haemorrhage, it was of interest to determine the effects of this drug on the electrical and mechanical responses of the basilar artery.

## Methods

Mongrel dogs of either sex (1.5–2 years old, 10–15 kg) were given pentobarbitone Ca (Tanabe) parenterally, and the brain and cerebral arteries were immediately excised and placed in Krebs solution (20°C).

The basilar artery (diameter of artery 0.5–1.0 mm) was carefully dissected with the aid of a binocular microscope. The vascular bed was dissected helically so that it was 0.2 mm in width and 5 mm in length for the microelectrode recordings and 0.2 mm in width and 0.5 mm in length for the tension recordings.

In the microelectrode experiments, the dissected artery was mounted in an organ bath with a capacity of about 2 ml and warmed Krebs solution (35°C) was superfused at a flow rate of about 3 ml/min. In the case of electrical stimulation of the muscle, the partition stimulating method (Abe & Tomita, 1968) was used, and current pulses were applied to the long axis of the vessel. Single cells were impaled from the outer surface by glass capillary microelectrodes filled with 3 M KCl and with a tip resistance of 40–80 MΩ; the electrical activities of the membrane were recorded. The fundamental procedures were much the same as those described by Ito *et al.* (1978).

To stimulate the perivascular nerve for recordings of excitatory junction potentials (e.j.ps), electrical stimuli 0.05–0.1 ms in duration were applied with different frequencies to the tissue through the stimulating plate of the partition stimulating electrode.

To measure the membrane activity and tension simultaneously, the double sucrose gap method was used. The apparatus for measurement of electrical and mechanical properties of vascular tissues (0.3 mm in width and 10 mm in length) was the same as that described by Ito *et al.* (1978). The resting tension was kept below 0.05 g by slightly stretching the tissue. The tension was recorded by means of a mechano-transducer (FD pick-up; Nihon Kohden: TB 612-T).

To record the tension development of the basilar artery, a force transducer (U-gauge, type UL; Shinkoh) with a carrier amplifier (RP-3; Nihon Kohden) was used. The preparation was placed in 0.9 ml organ bath; to change the bathing fluid about 8 ml of well oxygenated solution was changed within 5 s. The temperature of the organ bath was kept at 35 ± 0.5°C. The resting tension of the tissue was kept at approx. 1 mg.

The values of the membrane potential and tension were expressed as mean ± s.d.

For the control solution, a modified Krebs solution was used (Bülbring, 1954). The solution was bubbled with 97% $O_2$ and 3% $CO_2$, and the pH of the solution was maintained at 7.2–7.3. Excess $[K]_o$ solution was prepared by replacing NaCl with an equivalent amount of KCl up to 128 mM isotonically. NaCl-free solution was prepared by use of choline Cl (plus $10^{-6}$ M atropine) instead of NaCl. The pH of the solution was adjusted to 7.3.

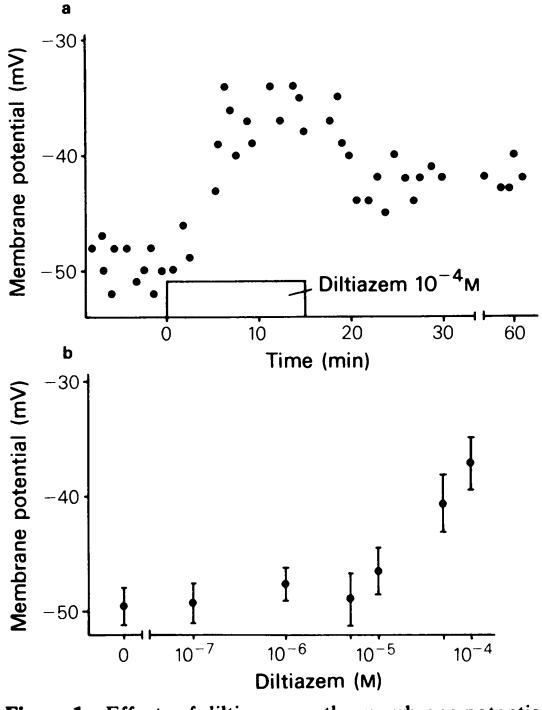


**Figure 1** Effects of diltiazem on the membrane potential of smooth muscle cells of the canine basilar artery. (a) Effects of $10^{-4}$ M diltiazem on the membrane potential. Different cells were impaled by a microelectrode before, during and after application of diltiazem. (b) Effects of various concentrations of diltiazem ($10^{-7}$ M–$10^{-4}$ M) on the membrane potential. Vertical bars: 2 × s.d.; ($n$ = 15–30).

The following drugs were used; caffeine (Daiichi), ATP-$Na_2$ (Wako), atropine sulphate (Daiichi), tetraethylammonium chloride (TEA); Tokyo Kasei) EGTA (Dozin) and diltiazem (Tanabe).

## Results

### *Effects of diltiazem on the membrane properties of smooth muscles of the basilar artery*

The membrane potential of smooth muscle cells of the canine basilar artery was $-49.8 \pm 1.5$ mV ($n = 30$) and it was electrically quiescent. Application of diltiazem in concentrations up to $5 \times 10^{-6}$ M did not modify the membrane potential. Superfusion of $10^{-4}$ M diltiazem depolarized the membrane to about $-35$ mV within 10 min and this depolarization remained in the presence of diltiazem (Figure 1a). When the tissue was rinsed, repolarization of the membrane to the control level required more than 60 min. In Figure 1a, after 45 min, the membrane potential was not completely restored to the control value. The dose-response curves for the membrane potential change produced by various concentrations of diltiazem are shown in Figure 1b. Diltiazem depolarized the membrane in concentrations above $10^{-5}$ M. In the presence of $10^{-4}$ M diltiazem, the mean membrane potential was $-38.1 \pm 1.6$ mV ($n = 20$, $P < 0.001$).

In the smooth muscle membrane of the basilar artery, a spike could be evoked by application of outward current pulses in the presence of TEA. The application of TEA (10 mM) produced a depolarization of the membrane ($-50.3 \pm 1.4$ to $-42.3 \pm 2.1$ mV, $n = 15$). Figure 2 shows the effects of $10^{-5}$ M diltiazem on the electronic potential (1 s in pulse duration) and spike evoked by applications of various intensities of outward current pulses in the presence of 10 mM TEA. The spike was accompanied by an after-hyperpolarization. Application of diltiazem ($> 10^{-5}$ M) in the presence of TEA (10 mM), further depolarized the membrane and reduced the membrane resistances measured from the amplitude of electronic potentials. Diltiazem ($10^{-5}$ M) consistently suppressed the spike generation (Figure 2b).

To observe the effects of diltiazem on the electrical and mechanical activities of the canine basilar artery, the double sucrose gap method was used. As shown in Figure 3A, after pretreatment with 5 mM TEA, outward current pulses (2 s in duration) generated the spike and contraction. Increased intensities of stimulation increased the amplitudes of both the spike and the contraction. The application of $10^{-5}$ M diltiazem suppressed the spike generation, as was observed with the microelectrode method. Furthermore, the mechanical response ceased with cessation of the spike generation (Figure 3A). In the presence of TEA, perivascular nerve stimulation (0.05 ms in pulse duration) increased the amplitude of the excitatory junction potentials (e.j.ps) and the spike was superimposed on the e.j.ps. Figure 3B shows the effects of diltiazem ($10^{-5}$ M) on the spike evoked by perivascular nerve stimulation by the double sucrose

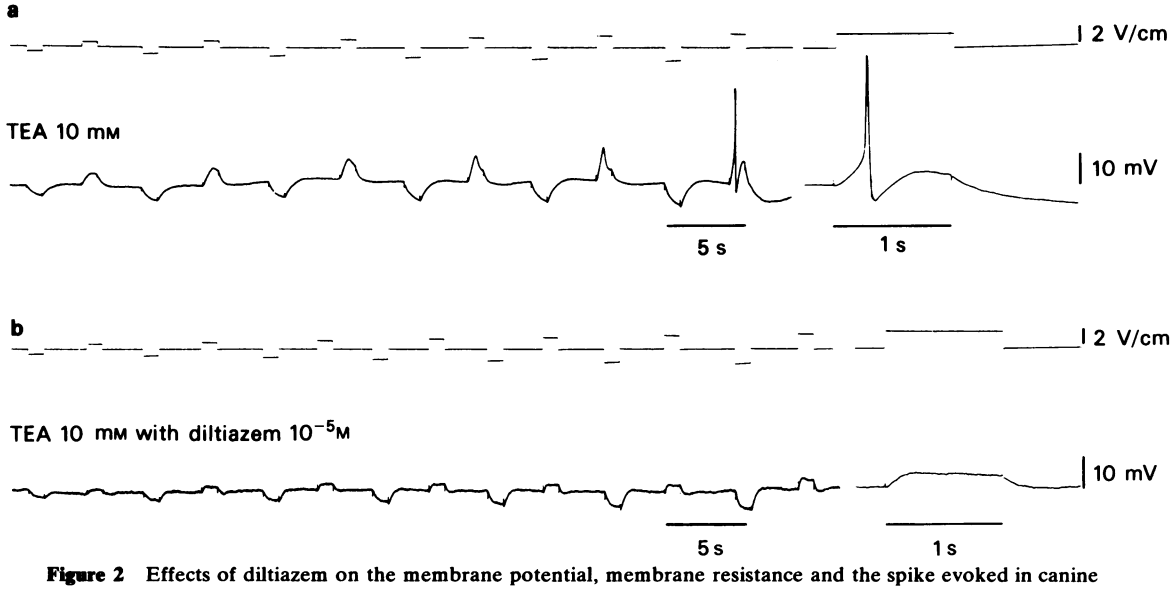


**Figure 2** Effects of diltiazem on the membrane potential, membrane resistance and the spike evoked in canine basilar artery by various intensities of outward current pulses (1 s in duration) in the presence of tetraethylammonium (TEA) 10 mM. The microelectrode method was used: (a) control, in the presence of 10 mM TEA; (b) effects of $10^{-5}$ M diltiazem after pretreatment with TEA (10 mM).

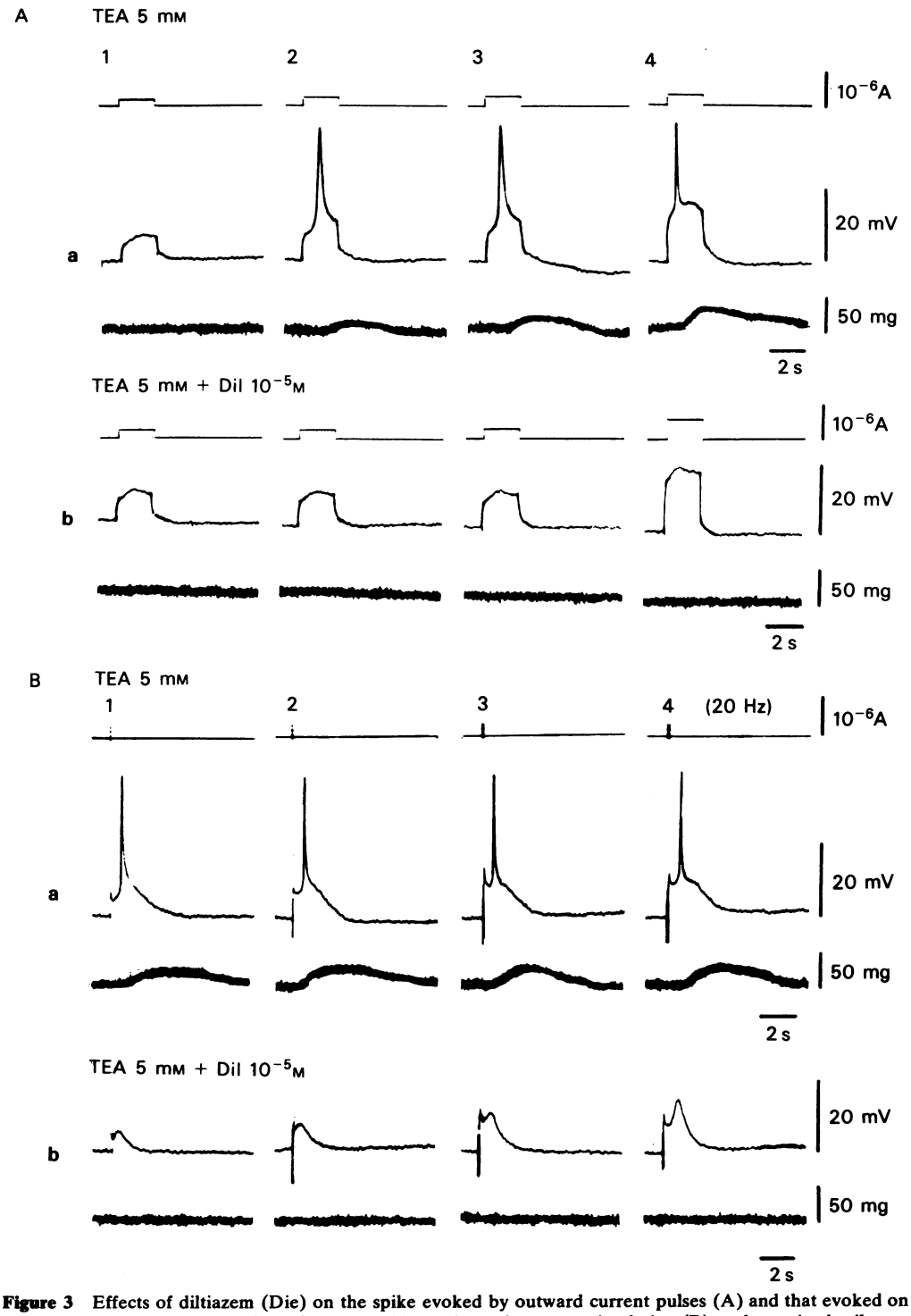


**Figure 3** Effects of diltiazem (Die) on the spike evoked by outward current pulses (A) and that evoked on the excitatory junction potentials (e.j.ps) generated by perivascular nerve stimulation (B) to the canine basilar artery. The stimulus conditions are described in the text. The double sucrose gap method was used. Top, current monitor, middle, potential changes, bottom; contraction recorded by the isometric tension recording method.

gap method. Application of $10^{-5}$ M diltiazem suppressed generation of the spike evoked by a single stimulus of the perivascular nerve after pretreatment with TEA (5 mM), it also reduced the amplitude of the e.j.ps so that diltiazem suppresses the spike generation more than it suppresses the e.j.ps. The cessation of the spike in the presence of diltiazem, caused the mechanical response to disappear (Figure 3B).

The effects of diltiazem (from $10^{-7}$ M to $10^{-4}$ M) on the e.j.ps evoked by perivascular nerve stimulation in the absence of TEA were investigated in detail by the double sucrose gap method. In the absence of TEA perivascular stimulation did not evoke a spike on the e.j.ps, allowing the amplitude of the e.j.ps to be observed more clearly than with TEA. As shown in Figure 4, in some tissues, a single stimulus to the perivascular nerves (0.05 ms in duration) evoked the e.j.p. When the stimulus frequency was increased to 20 Hz, the amplitude of the e.j.ps was proportionately enhanced. In this tissue, a contraction was evoked by generation of the e.j.p. without the presence of a spike. The e.j.ps produced by perivascular nerve stimulation were blocked by treatment with tetrodotoxin (TTX) $10^{-7}$ M. The amplitudes of the e.j.ps were reduced in the presence of $10^{-6}$ M or higher concentration of diltiazem, and in the presence of $10^{-5}$ M diltiazem, the amplitude was reduced to 0.65 times the control. With $10^{-4}$ M diltiazem, the amplitude of e.j.ps was reduced even further but this reduction was also due, in part, to postsynaptic changes, because the membrane was depolarized and the membrane resistance of smooth muscles was decreased by $10^{-4}$ M diltiazem. The amplitude of the contraction was proportionally reduced with the reduction in the amplitude of e.j.ps. These results indicate that under physiological conditions, the e.j.p. is able to generate a contraction with no spike generation, and that diltiazem suppresses the generation of e.j.p. in concentrations below those that affect the postsynaptic muscle membrane.

### *Effects of diltiazem on the mechanical response evoked in the basilar artery*

Several properties of the mechanical responses evoked in the basilar artery differ slightly from these of other vascular muscles, e.g. in this tissue, noradrenaline $10^{-4}$ M did not depolarize the membrane, and the minimum concentration of noradrenaline required to produce the contraction was $10^{-4}$ M (S.

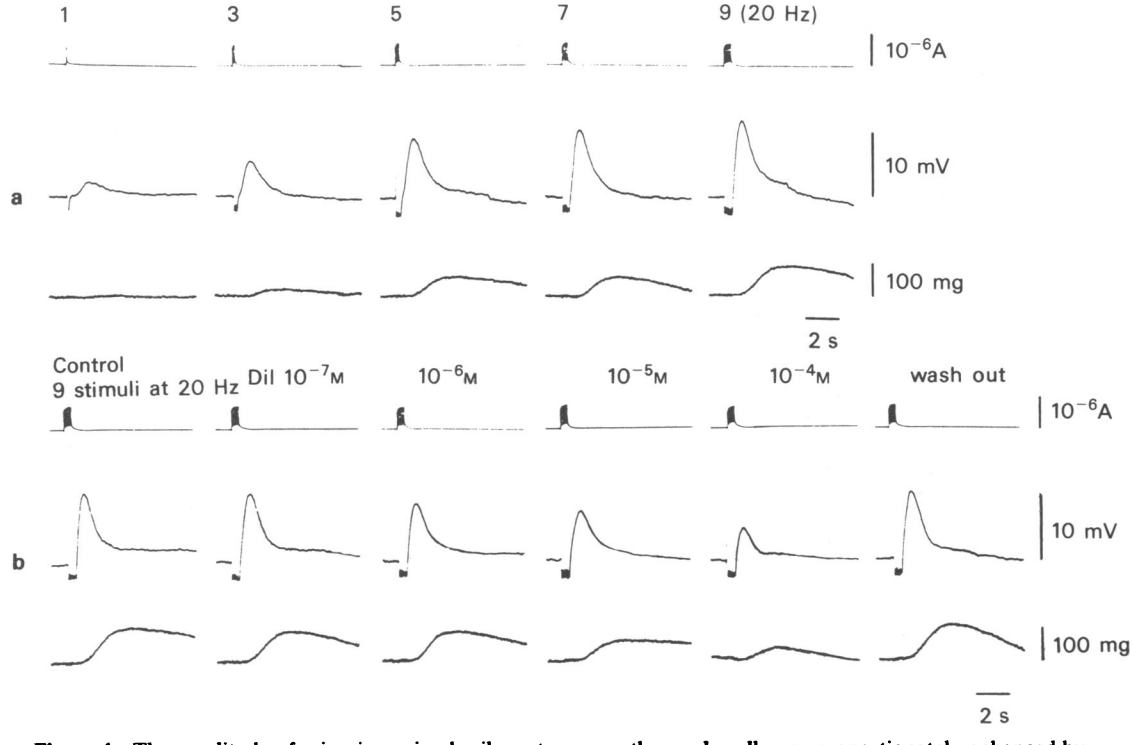


**Figure 4** The amplitude of e.j.p. in canine basilar artery smooth muscle cells was proportionately enhanced by increasing the number of stimuli at the frequency of 20 Hz to the perivascular nerves (a). Effects of diltiazem (Dil) on the e.j.ps evoked by perivascular nerve stimulation (b). The double sucrose gap method was used. Stimulus conditions are described in the text. Top: current monitor; middle: potential change; bottom: contraction. The electrical activity was recorded in the absence of TEA.

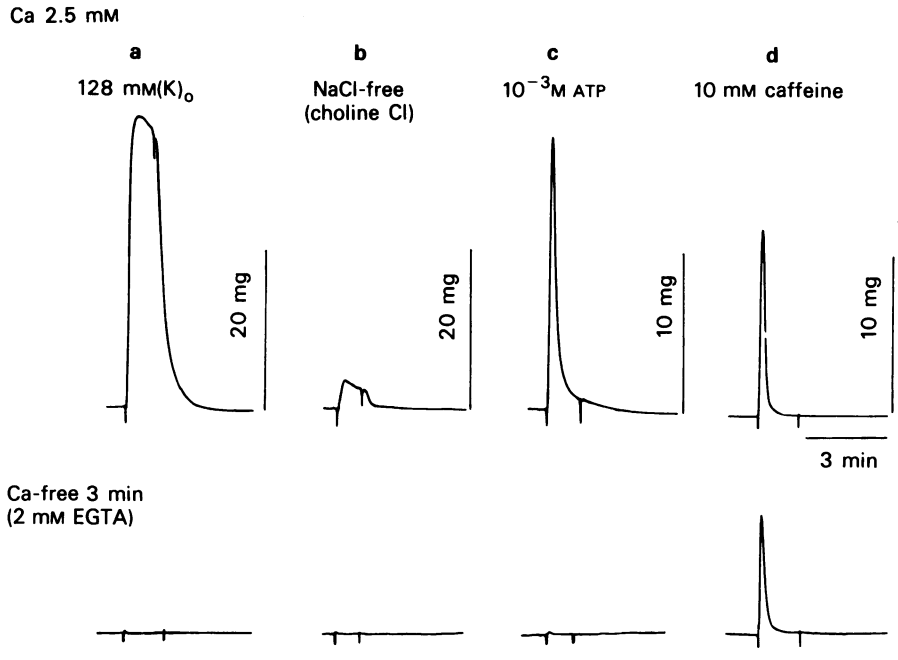


**Figure 5** Contractions of canine basilar artery smooth muscle cells evoked by application of 128 mM $[K]_o$ (a), NaCl-free solution (choline substituted and $10^{-6}$ M atropine containing solution) (b), $10^{-3}$ M ATP (c) and 10 mM caffeine (d) in the presence or absence of 2.5 mM Ca. Ca-free solution containing 2 mM EGTA was superfused before application of various agents.

Fujiwara, personal communication), while in the porcine coronary artery $10^{-5}$ M noradrenaline hyperpolarizes the membrane (Ito, Kitamura & Kuriyama, 1979). ATP ($10^{-4}$ M) depolarized the membrane (from $-49.3 \pm 0.6$ mV to $-44.1 \pm 0.7$ mV, $n = 15$) and generated a contraction, while in the guinea-pig coronary artery, ATP ($10^{-4}$ M) produced a hyperpolarization and relaxed the tissue (Takata & Kuriyama, 1980).

To study further the mechanism of action of diltiazem on the mechanical response, its effects on the contractions evoked by application of 128 mM $[K]_o$ solution, $10^{-3}$ M ATP, NaCl-free solution or 10 mM caffeine were examined. Figure 5 shows the effects of the above agents on the mechanical response in the presence of 2.5 mM $[Ca]_o$. Application of 128 mM $[K]_o$, evoked a maximal contraction in the basilar artery. A contraction was also evoked in NaCl-free solution (NaCl was replaced with choline Cl and $10^{-6}$ M atropine was added), but the amplitude was much smaller than that evoked by 128 mM $[K]_o$. Applications of $10^{-3}$ M ATP or 10 mM caffeine produced a contraction, but these contractions were only transient and in the presence of these agents the tissue relaxed almost to the resting tension level. In the Ca-free solution containing 2 mM EGTA, the contractions produced by 128 mM $[K]_o$, NaCl-free solution or $10^{-3}$ M ATP were abolished, but the amplitude of the caffeine-induced contraction was slightly reduced by the removal of Ca. These results indicate that the main source of Ca in the production

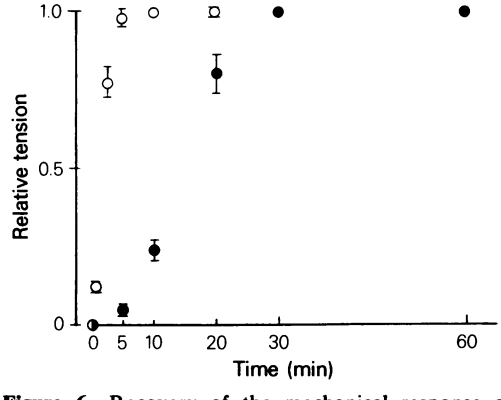


**Figure 6** Recovery of the mechanical response of canine basilar artery smooth muscle cell after application of caffeine (○) or ATP (●). The amplitude of contraction evoked by the first application of $10^{-3}$ M ATP or 10 mM caffeine was registered as a relative tension of 1.0, and the subsequent contractions at various intervals (horizontal axis) are shown. Vertical bars indicate 2 × s.d. ($n = 5$).

of the contraction in excess $[K]_o$, NaCl-free solution or ATP is an influx of Ca rather than a release of Ca stored within the cell.

The shapes of the caffeine-induced and ATP-induced contractions were much the same but the sources of Ca required to activate the contraction differed. The difference in the properties of the mechanical responses was also seen in the recovery process after application of these agents. Responses to two exposures of either caffeine or ATP at various intervals were compared. The caffeine-induced contraction was found to be completely restored within 5 min but the restoration of the ATP-induced contraction required more than 30 min (Figure 6).

Figure 7a shows the effects of various concentrations of diltiazem on the 128 mM $[K]_o$-induced con-

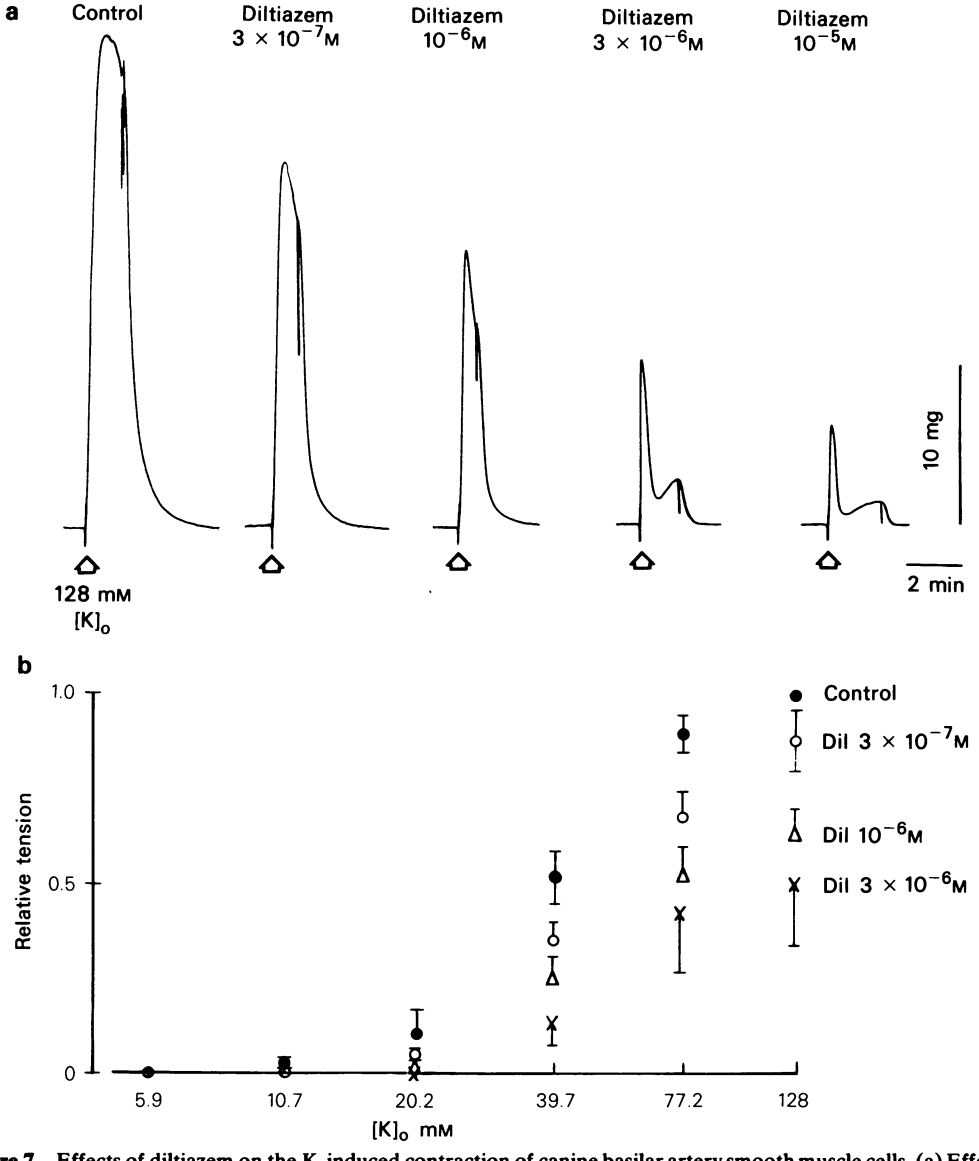


**Figure 7** Effects of diltiazem on the K-induced contraction of canine basilar artery smooth muscle cells. (a) Effects of various concentrations of diltiazem ($3 \times 10^{-7}$–$10^{-5}$ M) on the 128 mM $[K]_o$-induced contraction. Arrows indicate the application of 128 mM $[K]_o$; removal indicated by artifacts (application is about 2 min). (b) Relationship between the amplitude of K-induced contractions and concentrations of diltiazem ($[K]_o$ is varied from 5.9 mM to 128 mM). Three different concentrations of diltiazem (Dil) were used. The amplitude of the K-induced contraction evoked by 128 mM $[K]_o$ was registered as a relative tension of 1.0.

tractions. The amplitude of the contraction was suppressed by $3 \times 10^{-7}$ M diltiazem. This concentration of diltiazem was much lower than that which suppressed the K-induced contraction in the porcine coronary artery or the guinea-pig mesenteric artery (Tajima, Kanda, Kitamura, Ito & Kuriyama, 1980; Itoh, Kajiwara, Kitamura & Kuriyama, 1981; Suzuki *et al.*, 1981). Increased contractions of diltiazem proportionally decreased the amplitude of the K-induced contraction. The tension-$[K]_o$ concentration relationship of the K-induced contraction was shifted to the right by diltiazem, and the extent of the shift was concentration-dependent (Figure 7b).

In the guinea-pig mesenteric artery, a reduction of the $[Na]_o$ or an increase in the $[K]_o$ concentration produces a contraction (Suzuki *et al.*, 1981). The K-induced contraction was attributed to a Ca-influx due to activation of the voltage-dependent Ca channel, and the Na-free or Na-deficient-induced contraction was assumed to have a similar basis. To learn more about such effects in the basilar artery, the effects of diltiazem on the contraction evoked by NaCl-free solution were examined. Figure 8 shows the effects of diltiazem on the contraction evoked by (a) NaCl-free solution, (b) 128 mM $[K]_o$ solution and (c) by 128 mM $[K]_o$ after pretreatment with NaCl-free solution. NaCl-free solution evoked the contractions showing an initial phasic component as well as a subsequent tonic component. The application of NaCl-free solutions for 1 min generated only the phasic contraction (Figure 8a). Application of $10^{-5}$ M diltiazem reduced the amplitude of contraction but concentrations less than $10^{-5}$ M had no effect on the mechanical response. The K-induced contraction was reduced to a greater extent than the NaCl-free-induced contraction by the application of $10^{-5}$ M diltiazem (Figure 8b). When 128 mM $[K]_o$ was applied to the tissue after pretreatment with NaCl-free solution, the phasic contraction was smaller than that evoked by 128 mM $[K]_o$ with no pretreatment. However, the amplitude of the contraction measured from the basal level was much the same, because of in-

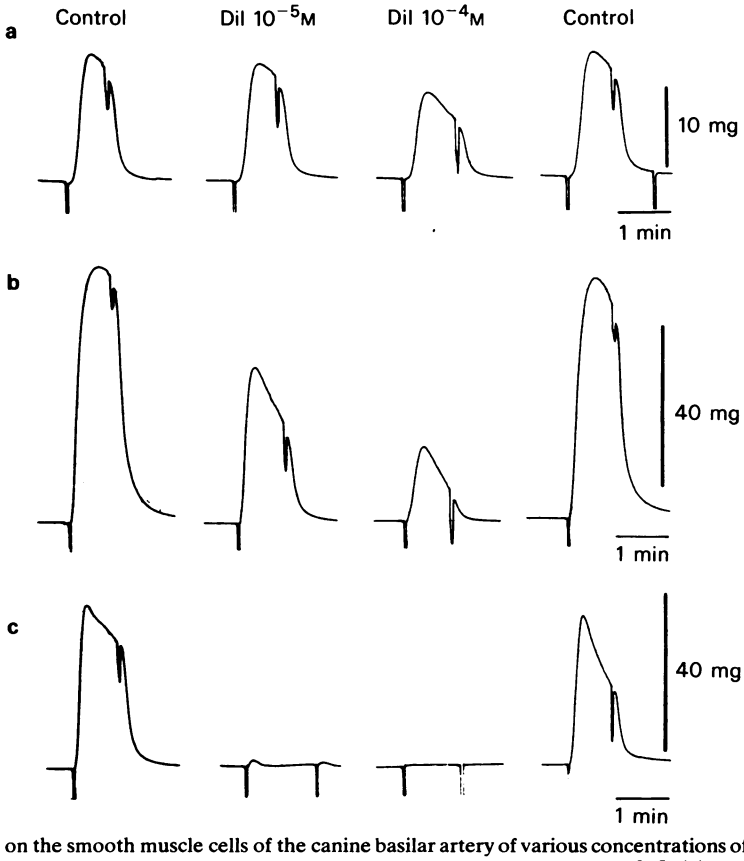


**Figure 8** Effects on the smooth muscle cells of the canine basilar artery of various concentrations of diltiazem (Dil) on the contraction evoked by NaCl-free solution (a), the contraction evoked by 128 mM $[K]_o$ (b) and the contraction evoked by 128 mM $[K]_o$ after pretreatment with NaCl-free solution (c). To prepare the NaCl-free solution, NaCl in Krebs solution was replaced with choline Cl and $10^{-6}$ M atropine was added.

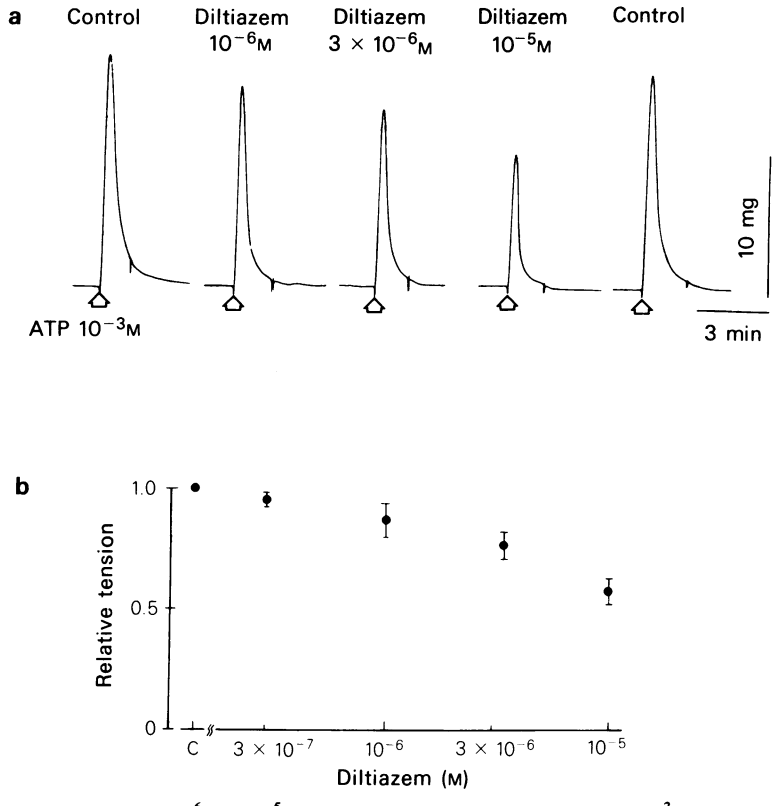


**Figure 9** Effects of diltiazem ($10^{-6}$M–$10^{-5}$M) on the ATP-induced contraction ($10^{-3}$M ATP) of smooth muscle cells of canine basilar artery. Drug was applied for 1 min. Interval between the drug applications was about 30 min. (a) Actual records of the ATP-induced contraction in the presence of three different concentrations of diltiazem. The control experiments were done before and after application of diltiazem. (b) Dose-response relationship observed on the ATP-induced contraction ($10^{-3}$M) with various concentrations of diltiazem. The amplitude of the ATP-induced contraction, in the presence of the drug, was normalized to the amplitude of contraction that occurred in Krebs solution.

creased muscle tone as a consequence of the tonic contraction produced by the Na-free solution. Application of $10^{-5}$M diltiazem almost abolished the 128 mM $[K]_o$-induced contraction (Figure 8c). These effects were similar to those observed in the case of the guinea-pig mesenteric artery, although, the amplitude of the NaCl-free induced contraction was much smaller in the basilar artery, and inhibitory actions of diltiazem on the NaCl-free induced contraction were small but apparent in the basilar artery.

Figure 9a shows the effects of diltiazem ($10^{-6}$M–$10^{-5}$M) on the contraction induced by $10^{-3}$M ATP. The contraction was evoked at 30 min intervals. Figure 9b shows the effects of various concentrations of diltiazem on the ATP-induced contraction. Diltiazem $3 \times 10^{-7}$M suppressed the contraction (0.95 times the control, $n = 5$). The action of diltiazem was less than that observed on the K-induced contraction; in the presence of $3 \times 10^{-6}$M diltiazem, the K-induced contraction was reduced to $0.50 \pm 0.16$ of the control and the ATP-induced contraction was reduced to $0.76 \pm 0.05$ of the control ($n = 5$). The ATP-induced and the K-induced contractions were generated by an influx of Ca, yet the action of diltiazem on the mechanical responses differed.

The effects of diltiazem on the caffeine-induced contraction were also observed. The tissues were superfused in Ca-free solution containing 2 mM EGTA for 10 min, then 2.5 mM Ca was added for 4 min and the tissue was again superfused with a Ca-free 2 mM EGTA containing solution for 2 min. Subsequently, 10 mM caffeine was applied for 1 min (Figure 10). When $10^{-5}$M diltiazem was applied in Ca-free solution for 2 min, the amplitude of the caffeine-induced contraction was slightly reduced ($0.93 \pm 0.05$ times the control, $n = 5$, Figure 10b). When diltiazem was applied with 2.5 mM Ca for 4 min, the contraction was further reduced ($0.70 \pm 0.02$ times the control, $n = 5$, Figure 10c).

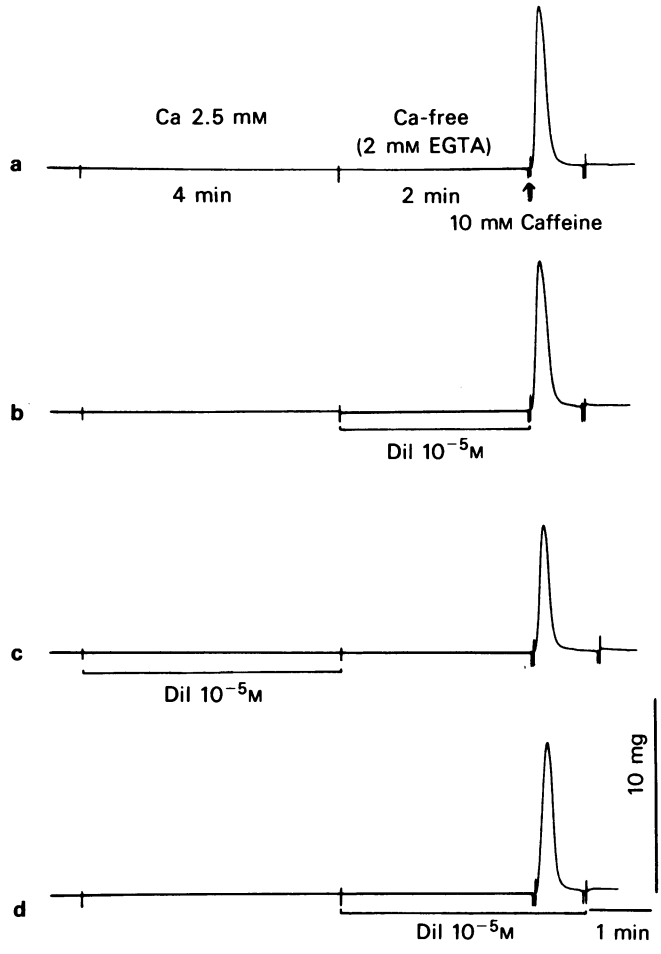


**Figure 10** Effects of diltiazem (Dil) on the caffeine-induced contraction of smooth muscle cells of canine basilar artery. (a) Control: to evoke the caffeine-induced contraction (10 mM), the tissue was superfused with Ca-free EGTA (2 mM) containing solution for 10 min then pretreated with 2.5 mM Ca for 4 min; the tissue was again superfused with Ca-free EGTA containing solution for 2 min and subsequently 10 mM caffeine was applied. Throughout the experiment, $[K]_o$ was kept at 5.9 mM. (b–d) Diltiazem ($10^{-5}$ M) was added to the perfusing solution during the different periods. (b) Diltiazem was applied in Ca-free solution for 2 min just before application of caffeine; (c) diltiazem was applied with 2.5 mM Ca; (d) diltiazem was applied in Ca-free solution and during application of caffeine.

The amplitude of the caffeine-induced contraction was little affected by simultaneous application of caffeine with diltiazem ($0.94 \pm 0.05$, $n = 5$, Figure 10d). In similar experiments done in the presence of high $[K]_o$, the application of 128 mM $[K]_o$ with 2.5 mM $[Ca]_o$ evoked a K-induced contraction (Figure 11). The caffeine-induced contraction evoked after 2 min application of Ca-free solution containing 2 mM EGTA was much larger than that evoked in the previous protocol (Figure 10a), i.e. pretreatment with Ca and 128 mM $[K]_o$ accumulated more Ca than pretreatment with 2.5 mM $[Ca]_o$ and 5.9 mM $[K]_o$. When $10^{-5}$ M diltiazem was applied in the Ca-free solution for 2 min, the amplitude of contraction was reduced to $0.93 \pm 0.01$ times the control ($n = 5$, Figure 11b) and when $10^{-5}$ M diltiazem was applied simultaneously with 128 mM $[K]_o$ and 2.5 mM $[Ca]_o$, the caffeine-induced contraction was reduced to $0.36 \pm 0.05$ times the control ($n = 5$, Figure 11c). The high K-induced contraction was not generated in the presence of diltiazem in concentrations over $10^{-5}$ M. When the amplitude of the caffeine-induced contraction was compared with that observed in the experiment shown in Figure 10a, the caffeine-induced contraction in the procedure described in Figure 11b was $1.71 \pm 0.12$ times ($n = 5$), and in Figure 11c, it was $0.66 \pm 0.10$ times ($n = 5$) the control. This means that the accumulation of Ca into the store site after appli-

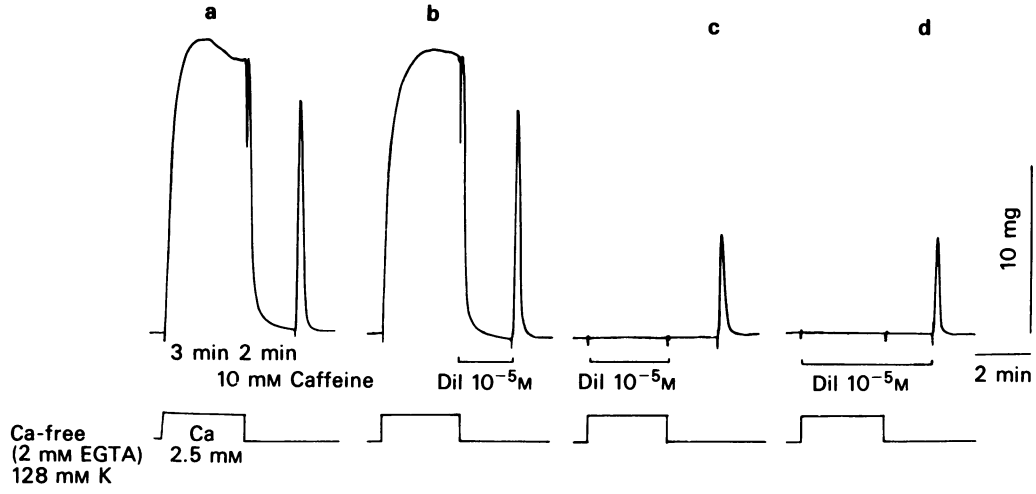


**Figure 11** Effects of diltiazem on the caffeine-induced contraction of smooth muscle cells in canine basilar artery after pretreatment with 2.5 mM Ca together with 128 mM $[K]_o$. The experimental procedures were the same as those described in the Figure 10, but 128 mM $[K]_o$ was used instead of 5.9 mM $[K]_o$. (b–d) Diltiazem (Dil) ($10^{-5}$ M) was applied during the various procedures. (a–d). Throughout the experiment, the concentration of $[K]_o$ was kept at 128 mM.

cation of 128 mM $[K]_o$ was much greater than that in the control Krebs solution, and as a consequence, the caffeine-induced contraction was less affected by diltiazem.

## Discussion

The smooth muscle cell of the canine basilar artery was electrically quiescent and the spike was evoked by outward current pulses or by the e.j.ps after pretreatment with TEA. ATP and 5-hydroxytryptamine depolarized the membrane but a spike was not evoked in the absence of TEA (S. Fujiwara, personal communication). In the guinea-pig basilar artery, Karashima & Kuriyama (1981) found that the spike could be evoked by outward current pulses, and also on the e.j.p. in the presence of TEA. Furthermore, the spike could be generated by treatment with ATP during application of outward current pulses. Harder (1980) showed that in the cat cerebral artery, the depolarization induced by excess $[K]_o$ generated the spike. However, such spike generation was not observed in the guinea-pig basilar artery (Karashima & Kuriyama, 1981) or in the canine basilar artery (S. Fujiwara, personal communication).

Diltiazem suppressed the spike generation produced by outward current pulses as well as that evoked by the e.j.ps. Diltiazem acts as a Ca-channel blocker (Fleckenstein, 1977; Golenhofen, 1981). The amplitude of the e.j.ps evoked by perivascular nerve stimulation was also suppressed by application of diltiazem. In the present experiments, the mechanism of the inhibitory actions of diltiazem on the e.j.p. was not investigated in detail. However, diltiazem reduced the amplitude of e.j.ps without changing the membrane potential ($<10^{-5}$ M). Therefore, these seem to be interactions between diltiazem and the Ca influx at the nerve terminal underlying the release of the chemical transmitter. In the guinea-pig mesenteric artery, diltiazem reduced the amplitude of the e.j.ps without change in the membrane potential, membrane resistance and conduction of the excitation in peripheral nerves contributing to the generation of e.j.p. (Suzuki *et al.*, 1981).

The present results show that contraction could be initiated by the generation of the e.j.p., and that the amplitude of the contraction was in proportion to the amplitude of e.j.ps evoked by repetitive perivascular nerve stimulation. This was also true in the presence of various concentrations of diltiazem. Presumably activations of α-adrenoceptors distributed on the muscle membrane may directly or indirectly increase the Ca-influx and this increase in the Ca-influx is suppressed by diltiazem.

The contraction evoked by excess $[K]_o$, by NaCl-free solution or by ATP was abolished in Ca-free solution and the amplitude of contraction evoked by caffeine was slightly suppressed. Therefore, the Ca-influx is mainly responsible for the contraction produced by these agents, except for caffeine. In the guinea-pig mesenteric artery, diltiazem reduced the contraction evoked by noradrenaline or excess $[K]_o$ but did not suppress the contraction evoked by NaCl-free solution (Suzuki *et al.*, 1981). In this tissue, the amplitude of the contraction produced by NaCl-free

solution is 0.8 times the maximum contraction evoked by excess $[K]_o$ and a high concentration of diltiazem reduces the contraction evoked by NaCl-free solution. In the canine basilar artery, the maximum amplitude of the contraction in NaCl-free solution was about 0.1 times the amplitude of that produced by 128 mM $[K]_o$. The reduction in $[Na]_o$ may not play an important role in regulation of the $[Ca]_i$ in this muscle tissue, i.e. the Na-Ca exchange diffusion process suggested by Reuter, Blaustein & Haeusler (1973) or the Na-Ca competition on the ion channel postulated by Casteels (1980) may not play an important role for the Ca-regulation in the myoplasm. These effects also differed from results obtained with the canine coronary artery (Hirata *et al.*, 1981), where NaCl-free solution does not evoke a contraction. The effects of diltiazem on the NaCl-free contraction also indicate that diltiazem selectively inhibits the Ca-channel. The contraction evoked by excess $[K]_o$ was reduced by diltiazem in concentrations above $3 \times 10^{-7}$ M and this concentration is much lower than that which was effective in the guinea-pig mesenteric artery (Suzuki *et al.*, 1981).

The ATP-induced contraction was reduced by diltiazem but a much higher concentration was required to reduce this contraction than the K-induced contraction. In concentrations above $10^{-5}$ M, ATP depolarized the membrane and produced the contraction. In the guinea-pig basilar artery, ATP also depolarizes the membrane (Karashima & Kuriyama, 1981), while in the guinea-pig coronary artery, ATP hyperpolarizes the membrane because of an increase in the K-conductance of the membrane (Takata & Kuriyama, 1980). We did not demonstrate whether this ATP-induced contraction was due to activation of $P_1$-receptors (Burnstock, 1980; 1981). In the canine basilar artery, adenosine, an agonist of $P_2$-receptors, relaxes the tissue (reduces the resting tone) and reduces the contraction evoked by excess $[K]_o$ (Itoh, personal communication).

The caffeine-induced contraction was slightly reduced by diltiazem, and this reduction may be caused by a reduction in the Ca-influx but not by a reduction in the release of Ca stored in the cell. As shown in Figures 10 and 11, preincubation with 128 mM $[K]_o$ produced a larger caffeine-induced contraction than that produced after preincubation with 5.9 mM $[K]_o$ in the presence of 2.5 mM $[Ca]_o$. The reduction of the caffeine-induced contraction by diltiazem was more pronounced after preincubation with 128 mM $[K]_o$. However, when the effects of diltiazem on the amplitudes of caffeine-induced contractions after pretreatment with 5.9 mM $[K]_o$ and 128 mM $[K]_o$ were compared, the absolute amplitude of the caffeine-induced contractions were the same, i.e. the increased Ca-influx induced by excess $[K]_o$ and the Ca influx in the resting condition were reduced by diltiazem but release of the Ca stored in the cell was not affected.

In conclusion, in the canine basilar artery, diltiazem inhibits the Ca-influx during the activation of the membrane (i.e. it acts as a Ca-spike blocker) and there was a voltage-dependent Ca-influx induced by application of excess $[K]_o$ or ATP. Diltiazem slightly reduced the amplitude of the caffeine-induced contraction in Krebs solution, and since in the presence of diltiazem the caffeine-induced contractions evoked in Krebs solution and in excess $[K]_o$ solution were of similar amplitude, diltiazem may also suppress the Ca-influx in the resting membrane. Diltiazem reduced the e.j.p. evoked by perivascular nerve stimulation and this inhibitory action may be related to Ca-influx at the nerve terminal rather than to Ca mobilization in the nerve terminal, as there was little or no effect on the facilitation or depression process observed by repetitive stimulation of perivascular nerves.

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