0022-3565/81/2181-0260$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 218, No. 1

Printed in U.S.A.

Effects of 2-Nicotinamidoethyl Nitrate on Smooth Muscle Cells and on Adrenergic Transmission in the Guinea-Pig and Porcine Mesenteric Arteries[1]

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Accepted for publication March 20, 1981

ABSTRACT

Itoh, Takeo, Kenichiro Furukawa, Makoto Kajiwara, Kenji Kitamura, Hikaru Suzuki, Yushi Ito and Hirosi Kuriyama: *Effects of 2-nicotinamidoethyl nitrate on smooth muscle cells and on adrenergic transmission in the guinea-pig and porcine mesenteric arteries.* J. Pharmacol. Exp. Ther. **218:** 260–270, 1981.

Muscle membranes of the porcine but not guinea-pig mesenteric artery were hyperpolarized by applications of over 10^{-6} M 2-nicotinamidoethyl nitrate (SG 75; 2-NN) dose-dependently from −53 to −72 mV, due to an increase in the ionic conductance of the membrane. In the porcine mesenteric artery but not guinea-pig, 2-NN suppressed the K-induced contraction in concentrations below 20 mM $[K]_o$. The suppression of the K-induced contraction depended on the degree of hyperpolarization and the level of the resting membrane potential. The norepinephrine-induced contraction was suppressed by 2-NN with hyperpolarization of the membrane in the porcine mesenteric artery, but with no change in the membrane potential in the guinea-pig mesenteric artery, presumably due to suppression of the Ca-mobilization in the cell. The excitatory junction potential (ejp) could be recorded by perivascular nerve stimulation in both mesenteric arteries. Whether or not the membrane was hyperpolarized, 2-NN increased the amplitude of ejp in both tissues and increased the appearances of miniature ejp in the guinea-pig mesenteric artery. Nitroglycerin did not modify the membrane potential, the amplitude of ejp, electrical threshold and spike superimposed on the ejp, while sodium nitroprusside suppressed the amplitude of ejp, the facilitation process produced by repetitive stimulation and the spike superimposed on the ejp. Specific features of 2-NN actions on the muscle cell and adrenergic transmission of the mesenteric arteries in both species were discussed in relation to vasodilating actions, species differences and also in relation to other nitrite compounds.

2-NN (SG 75) showed a marked vasodilating action on the coronary artery of the pig or guinea pig and such was mainly due to the hyperpolarization of the membrane. A similar effect of 2-NN was obtained by using the dog atrial muscle (Yanagisawa and Taira, 1980). This action observed on the coronary artery was discussed in relation to other vasodilating agents, namely NG and NP, and elucidated the similarities between 2-NN and NP actions (Furukawa *et al.*, 1981).

Mackenzie and Parratt (1977) reported that in isolated vessels (dog femoral artery, saphenous vein and rat portal vein), both NG and sodium nitrate preferentially relaxed venous smooth muscle, particularly when the tissue was contracted with NE, and they suggested that the selective dilator effects of NG on venous smooth muscle might explain why the pain of the angina pectoris is alleviated.

Karashima (1980) observed the effects of NG on the peripheral vascular system (portal veins of the guinea pig and rat) and concluded that the response of muscle membrane differs with species while the mechanical response was consistently suppressed, presumably due to suppression of the Ca-mobilization in the cell. Häusler and Thoren (1976) stated that a concentration-dependent hyperpolarization of pulmonary artery smooth muscle may be of significance for the vasodilating action of NP. This conclusion was supported by Ito *et al.* (1978).

It is a general consensus of opinion that the effects of nitrites are derived primarily from their peripheral actions and not necessarily due to direct effects on the coronary circulation (Brunton, 1867; Voegtlin and Macht, 1913; Bogaert, 1972). On the other hand, other investigators have demonstrated that nitrites affect the regional distribution of coronary flow (Winbury *et al.*, 1969; Schnaar and Sparks, 1972; Vater and Heyndricks, 1975; Kreye and Gross, 1977). These results indicate that the beneficial effect of NG on the coronary artery has also appeared through its actions on the peripheral vascular bed,

Received for publication September 8, 1980.
[1] This work was supported by Grants 54408, 544020 and 577111 from the Ministry of Education and Welfare of Japan.

ABBREVIATIONS: 2-NN, 2-nicotinamidoethyl nitrate; NG, nitroglycerine; NP, sodium nitroprusside; NE, norepinephrine; ejp, excitatory junction potential; TEA, tetraethylammonium chloride; ACh, acetylcholine.

primarily on the venous side to reduce the preload of cardiac muscle activities.

The present experiments were an attempt to clarify the vasodilating mechanism of 2-NN on the peripheral arterial system, mesenteric arteries of the pig and guinea pig, in relation to the action of this agent on the coronary arteries. The mesenteric artery generates the ejp, thus enabling studies on the effects of 2-NN on the adrenergic transmission. The obtained results were also discussed in relation to vasodilating actions and adrenergic transmissions of NG and NP and also species differences.

Materials and Methods

A part of the mesenterium (10 × 10 cm) attached to the jejunum from adult pigs of either sex was excised in a local slaughter house and brought to our laboratory in oxygenated Krebs' solution at 12–15°C. The mesenteric artery was excised from membraneous material and connective tissue under a binocular microscope. Diameter of the tissue was 0.3 mm and a length of 10 mm was prepared by cutting the branches.

The guinea pigs, either sex, were stunned and bled. The mesenteric artery running in parallel with vein and lymph duct was dissected from the mesenterium of the jejunal region. The artery with a diameter between 0.1 to 0.15 mm was used. The vein and lymph duct were also attached to the mesenteric artery to prevent damages to the perivascular adrenergic nerves (Suzuki and Kuriyama, 1980).

The experimental procedures used in the present experiments were much the same as those described for measurements of the electrical and mechanical responses of the coronary artery (Furukawa *et al.*, 1981).

The microelectrode, double sucrose-gap methods and isometric tension recording method were used. The obtained value was expressed as the mean value with S.D.

To record the ejp, the perivascular nerves were stimulated by the partition-stimulating electrode (Abe and Tomita, 1968) with short pulses (0.1–0.3 msec). The experimental procedures were the same as those described by Kajiwara *et al.* (1981) and Suzuki and Kuriyama (1980).

The control solution was modified Krebs' solution (Bülbring, 1954) and the following drugs were used; 2-NN (SG 75; Chugai Company, Ltd., Tokyo, Japan), *dl*-NE (Sankyo Company, Ltd., Tokyo, Japan), NP (Wako Company, Ltd., Tokyo, Japan) and TEA (Daiichi Company, Ltd., Tokyo, Japan). The stock solution for drugs was freshly prepared just before the start of the experiments.

Results

Effects of 2-NN on the membrane potential and membrane resistance of smooth muscle cells of the porcine and guinea-pig mesenteric arteries. The membrane potentials of the porcine and guinea-pig mesenteric arteries were −53.2 ± 2.5 mV (S.D.) and −69.2 ± 2.3 mV (S.D.), respectively (n = 100). The length and time constants of the muscle membrane of the guinea-pig mesenteric artery were 0.81 mm and 129 msec, respectively (Kuriyama and Suzuki, 1981). The passive membrane property of the smooth muscle cell of the porcine coronary artery was not obtained.

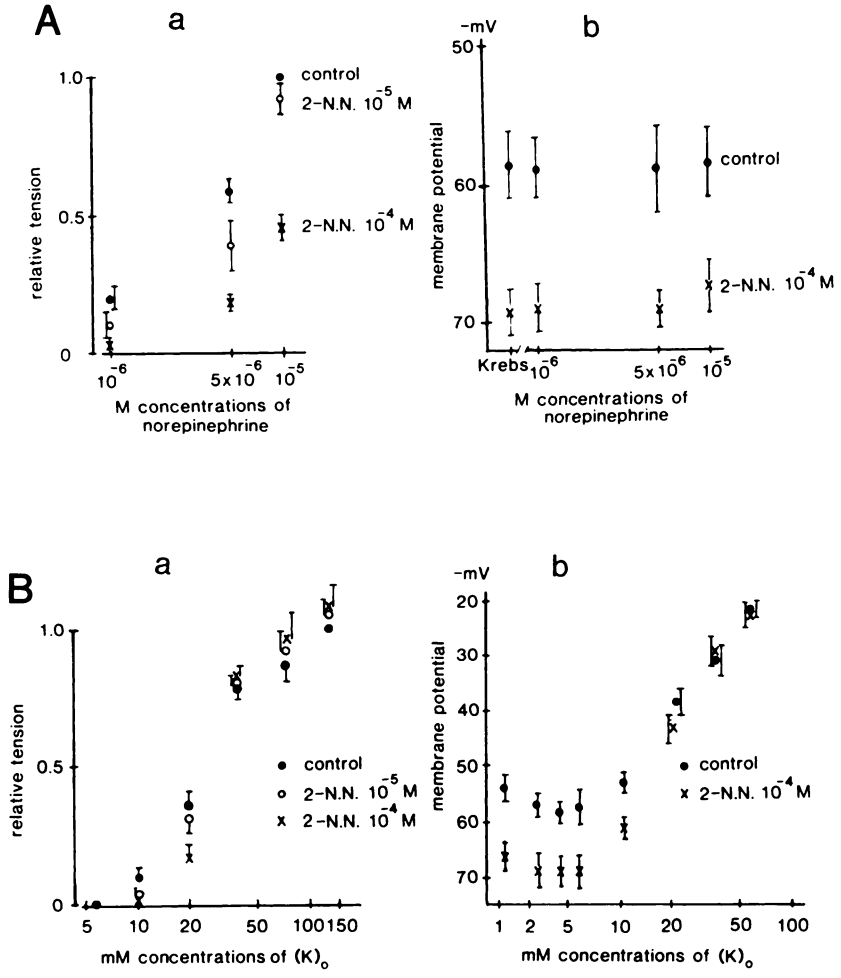

Fig. 1. A, effects of 2-NN on the membrane potential of smooth muscle cells of the porcine and guinea-pig mesenteric arteries. B, current-voltage relationship observed before and during application of 2-NN (2 × 10^{-5} M). The duration of current pulse was 3 sec. The microelectrode was inserted into the same cell throughout the experiment at 50 μm distance from the stimulating electrode (porcine mesenteric artery).

With application of 2-NN, the muscle membrane of the porcine mesenteric artery was hyperpolarized, but this compound did not modify the membrane potential in the guinea-pig mesenteric artery, in concentration ranges of 10^{-6} to 10^{-4} M.

Figure 1A shows the effects of 2-NN on the membrane potential in arteries from both species. In the guinea-pig mesenteric artery, 2-NN did not modify the membrane potential, however, in some preparations, the membrane potential was low (below −69 mV), in such cases 10^{-5} M 2-NN hyperpolarized the membrane to about −70 mV and increases in the concentration of 2-NN to 10^{-4} M did not further increase the membrane potential.

In the porcine mesenteric artery, the membrane was dose-dependently hyperpolarized to about −70 mV. To elucidate the nature of the hyperpolarization induced by 2-NN, the current-voltage relationship from the single cell of the porcine mesenteric artery before and during application of 2-NN was observed (fig. 1B). The microelectrode was inserted into the same cell throughout the experiment at a distance of 50 μm from the stimulating electrode. Applications of outward current pulse produced a marked rectifying property of the membrane. This property was the same as that observed in the case of the guinea-pig mesenteric artery (Kuriyama and Suzuki, 1981). In the presence of 2×10^{-5} M 2-NN, the membrane was hyperpolarized by about 10 mV in this particular cell and the current-voltage relationship observed with applications of various intensities of inward and outward current pulses became less steep compared with the control. Furthermore, the rectifying property appeared at low membrane potential levels with the control. The time constant of the membrane measured from the rising phase of the electrotonic potential was reduced in the presence of 2-NN (2×10^{-5} M) from 1168 ± 86 (n = 5) to 671 ± 49 msec (n = 7). These observations indicate that the hyperpolarization of the membrane in the porcine mesenteric artery is mainly due to increase in the membrane conductance, presumably due to increase in the K-conductance of the membrane.

Effects of 2-NN on the NE- and potassium-induced contractions in mesenteric arteries of both species. Figures 2A, a and b show the effects of 2-NN on the NE-induced contraction (a) and the membrane potential (b) in the porcine mesenteric artery in various concentrations of NE. In figure 2Aa, the contraction evoked by 10^{-5} M NE was registered as a relative tension of 1.0. Application of either 10^{-5} or 10^{-4} M 2-NN consistently suppressed the contraction evoked by 10^{-6} to 10^{-5} M NE. The membrane potential was little affected by NE but 2-NN hyperpolarized the membrane to about −70 mV, at any given concentration of NE. The maximum hyperpolarization induced by 2-NN in the presence of NE was much the same as that observed in Krebs' solution. These results indicate that NE produced the contraction with no change in the membrane potential and 2-NN suppressed the NE-induced contraction by hyperpolarizing the membrane. These findings were the same as those observed with ACh in the porcine coronary artery in the presence of 2-NN (Furukawa *et al.*, 1981). Figures 2B, a and b show the effects of 2-NN on the K-induced contraction and membrane potential in the porcine mesenteric artery. The amplitude of the K-induced contraction was suppressed by 2-NN (10^{-4} M) when the contraction was evoked with a dose less than 20 mM $[K]_o$. When the changes in the membrane potential were observed before and during application of 2-NN in various concentrations of $[K]_o$, the membrane was hyperpolarized only with concentrations of $[K]_o$ below 20 mM. The suppression of the contraction and hyperpolarization appeared with the same concentrations of $[K]_o$. These results suggest that the suppression of the K-induced contraction and the hyperpolarization of the membrane show a causal relation.

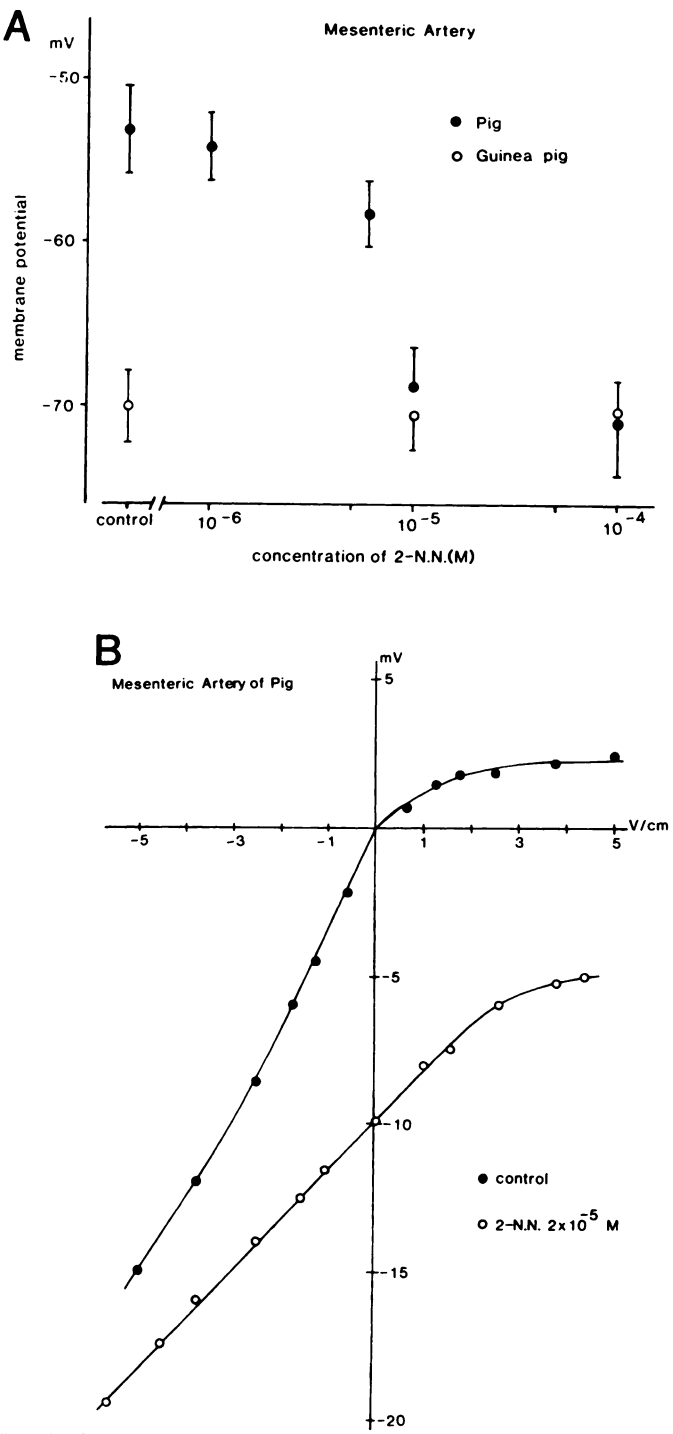

Fig. 2. A, effects of 2-NN on the NE-induced contraction and membrane potential of the porcine mesenteric artery in the presence of NE; a, effects of 2-NN (10^{-5} and 10^{-4} M) on the NE-induced contraction (concentrations of NE were 10^{-6}–10^{-5} M); b, effects of 2-NN (10^{-4} M) on the membrane potential in various concentrations of NE. B, effects of 2-NN on the K-induced contraction and membrane potential of the porcine mesenteric artery in various concentrations of $[K]_o$; a, effects of 2-NN on the K-induced contraction; b, effects of 2-NN (10^{-4} M) with various concentrations of $[K]_o$.

Figure 3 also shows the effects of 2-NN on the NE- and K-induced contractions in relation to the changes in the membrane potential in the guinea-pig mesenteric artery. As shown in figure 3Aa, the NE-induced contraction was markedly suppressed in the presence of 10^{-5} M 2-NN. In the guinea-pig mesenteric artery, the membrane potential was −70 mV and, therefore, neither NE nor 2-NN modified the membrane potential. For example, when the membrane potential was below −70 mV, 2-NN (10^{-5} M) hyperpolarized the membrane to about −70 mV and further increases in the concentrations of 2-NN no longer produced an increase in the membrane potential.

The appearance of NE-induced contraction had no relation to the change in the membrane potential but the amplitude was increased dose-dependently. Therefore, the suppression of the NE-induced contraction by 2-NN is presumably due to suppression of the Ca-mobilization from the storage sites in the cell.

Figures 3B, a and b show the effects of 2-NN on the K-induced contraction and on the membrane potential recorded from the guinea-pig mesenteric artery. In this tissue, 2-NN did not alter the K-induced contraction; however, 2-NN increased the membrane potential in concentrations of below 20 mM $[K]_o$. The differences seen between the two species may result from differences in the resting membrane potential level, namely, the resting membrane potential in the guinea-pig mesenteric artery was high and the depolarization required to produce the contraction was also high, thus requiring a much higher concentration of $[K]_o$ to produce the contraction other than that required in the case of the porcine mesenteric artery (fig. 2Ba *vs.* fig. 3Ba). Increases in $[K]_o$ to over 20 mM lowered the K-equilibrium potential and 2-NN no longer modified the membrane potential. As a consequence, the K-induced contraction was suppressed by 2-NN in the porcine mesenteric artery but not in the guinea-pig mesenteric artery. This means that the suppression of the K-induced contraction by 2-NN is dependent on the change in the membrane potential and the nature of the contraction differs from that observed on the NE-induced contraction in the guinea-pig and porcine mesenteric arteries.

When the effects of 2-NN on the mesenteric arteries were compared with those observed in the coronary arteries, the response of mesenteric arteries in both species to 2-NN was less than that observed in the coronary arteries. For example, the membrane potential of the porcine coronary and mesenteric arteries was much the same, while the hyperpolarization induced by 2-NN was much higher in the coronary artery than in the mesenteric artery when the concentrations were below 20 mM $[K]_o$.

Effects of 2-NN and other nitrite compounds on ejps recorded from the mesenteric artery. Figure 4 shows the effects of 2-NN on the ejp evoked by repetitive stimulation in the porcine mesenteric artery. To evoke the ejp, 0.2 msec in pulse duration, 100 Hz in stimulus frequency and various numbers of stimulations were applied. In this particular cell, the

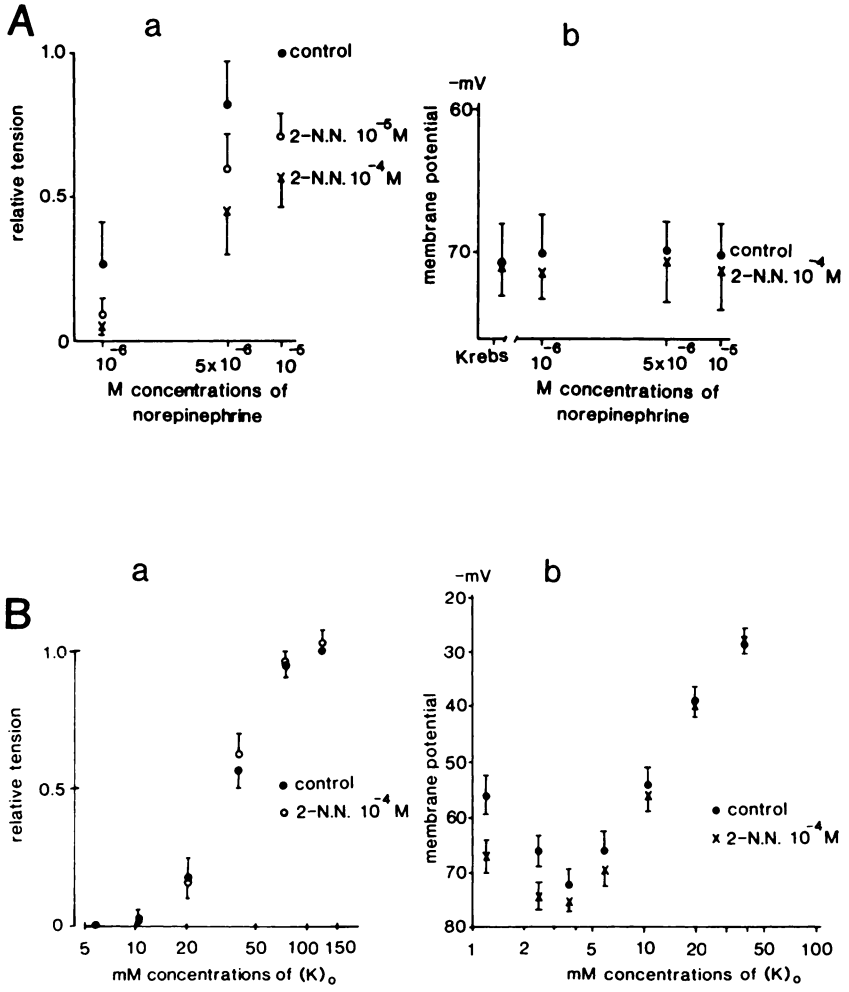

Fig. 3. A, effects of 2-NN on the NE-induced contraction and the muscle membrane potential of the guinea-pig mesenteric artery in the presence of various concentrations of NE; a, effects of 2-NN (10^{-5} and 10^{-4} M) on the NE-induced contraction (10^{-6}–10^{-5} M). The contraction evoked by 10^{-5} M NE was registered as a relative tension of 1.0; b, effects of 2-NN (10^{-4} M) on the membrane potential in various concentrations of NE (10^{-6}–10^{-5} M). B, effects of 2-NN on the K-induced contraction and the membrane potential in the guinea-pig mesenteric artery in various concentrations of $[K]_o$; a, effects of 2-NN (10^{-4} M) on the K-induced contraction. The contraction evoked by 118 mM $[K]_o$ was registered as a relative tension of 1.0; b, effects of 2-NN (10^{-4} M) on the membrane potential in various concentrations of $[K]_o$.

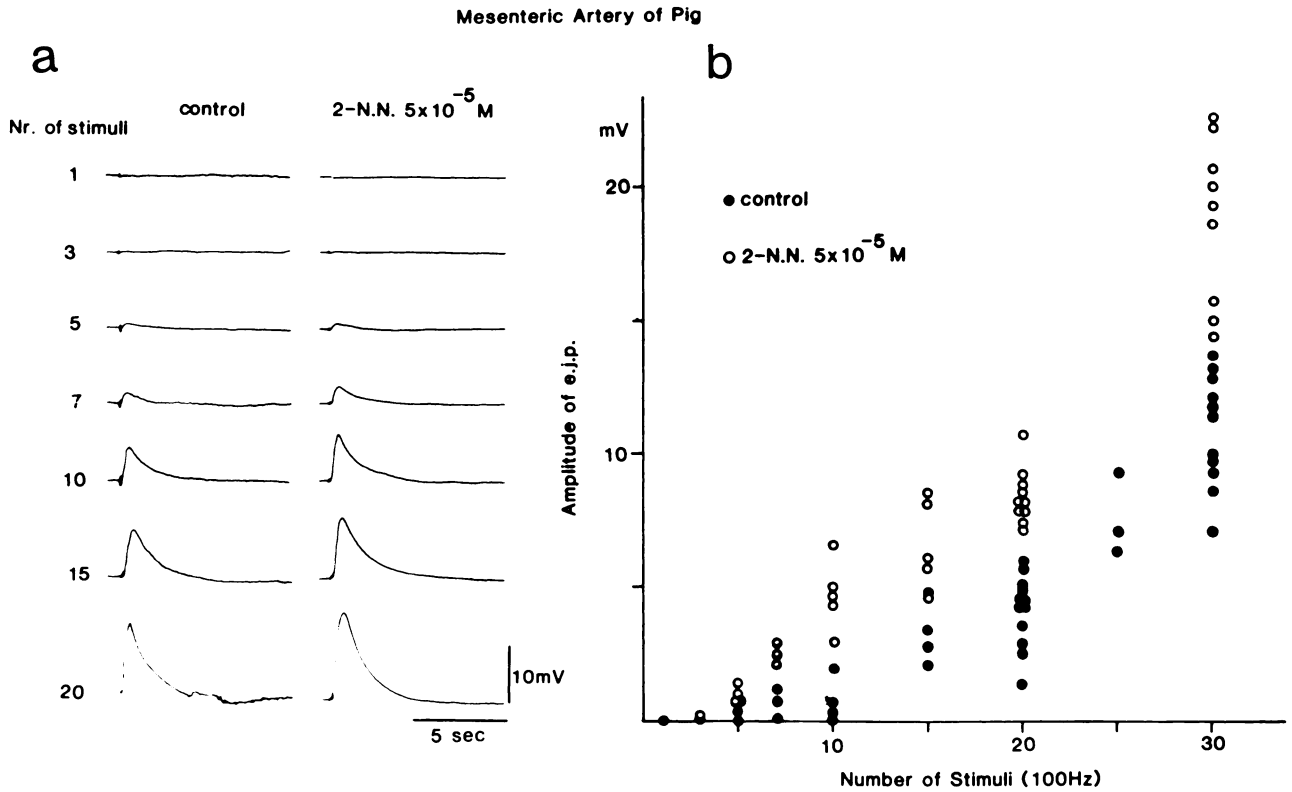

Fig. 4. Effects of 2-NN on the ejp evoked by repetitive stimulation in the porcine mesenteric artery. a, ejps recorded before and during application of 5×10^{-5} M 2-NN. b, Relationship between the amplitude of ejp and the stimulus number before and during application of 2-NN (5×10^{-5} M). The stimulus condition was 50 Hz in frequency and 0.2 msec in pulse duration.

visible amplitude of ejp was only recorded in the case of five stimulations. When the number of stimulations was increased, the amplitude of the ejp was also increased. In the presence of 5×10^{-5} M 2-NN, amplitudes of ejp were further increased by application of any number of stimulation, compared with the amplitudes evoked in Krebs' solution. When the relationship between the amplitude of ejp generated by various numbers of stimulations was assessed before and during application of 2-NN, this amplitude of ejp was found to be consistently increased in the presence of 2-NN (5×10^{-5} M). In the presence of 2-NN, the membrane resistance was reduced. Therefore, to determine the relationship between the amplitude of ejp and the membrane resistance before and during application of 2-NN, the time constant of the membrane and the time constant of the falling phase of ejp were measured from the same cell at a distance of 50 μm from the stimulating electrode. The electrotonic potential (5 sec in pulse duration) and the ejp evoked by repetitive stimulation (0.2 msec in pulse duration, 50 Hz in the stimulus frequency and 30 times in the stimulus number) were applied (fig. 5). The amplitude and the time constant of the muscle membrane were reduced in the presence of 2-NN (2×10^{-5} M) compared with the amplitude observed in Krebs' solution.

The mean value of the time constant of the membrane was 1168 ± 86 msec ($n = 5$) in Krebs' solution and 671 ± 49 msec ($n = 7$) in the presence of 2×10^{-5} M 2-NN. However, when the time constant of the falling phase of ejp (e^{-1}) was measured before and during application of 2-NN, the values were 652 and 625 msec, respectively (the mean values were 650 ± 57 msec, $n = 5$ and 659 ± 77 msec, $n = 6$, respectively). No difference between before and during application of 2-NN was observed on the time constant of the falling phase of ejp (fig. 5). This result indicates that the increase in the amplitude of ejp produced by 2-NN is mainly due to an increase in the NE release from nerve terminals in the porcine mesenteric artery.

General features of the ejp recorded from the guinea-pig mesenteric artery were studied in detail by Kuriyama and Suzuki (1981). Figure 6 shows the effects of 2-NN (10^{-4} M) on the ejp recorded from the guinea-pig mesenteric artery. When the different stimulus frequencies (0.25, 0.5 and 1.0 Hz) of electrical stimulation (0.2 msec in pulse duration) were applied to the tissues, the evoked response increased the amplitude smoothly up to a certain level of frequency. The steady maximum amplitude and the time required to reach the steady amplitude of ejp after repetitive stimulation were dependent on the frequency and intensity of the stimulation. With application of 10^{-4} M 2-NN, the amplitude of ejp was consistently increased. The facilitation process which appeared with repetitive stimulation in the presence of 2-NN remained unaffected.

Kuriyama and Suzuki (1981) have compared the time constant of the membrane and the time constant of the falling phase of ejp of the guinea-pig mesenteric artery and found that the latter showed consistently larger values. When the time constant of the falling phase of ejp was measured before and during application of 2-NN, the value was reduced in the presence of 10^{-4} M 2-NN. Figure 7 shows an example of effects of 2-NN on the shape of ejp and the time constant of the falling phase of ejp before and during application of 10^{-4} M 2-NN. The time constant was reduced despite an increase in the amplitude of ejp. The reduction in the time constant of the falling phase of ejp was attributed to a reduction in the membrane resistance. This result also parallels observations made on the porcine

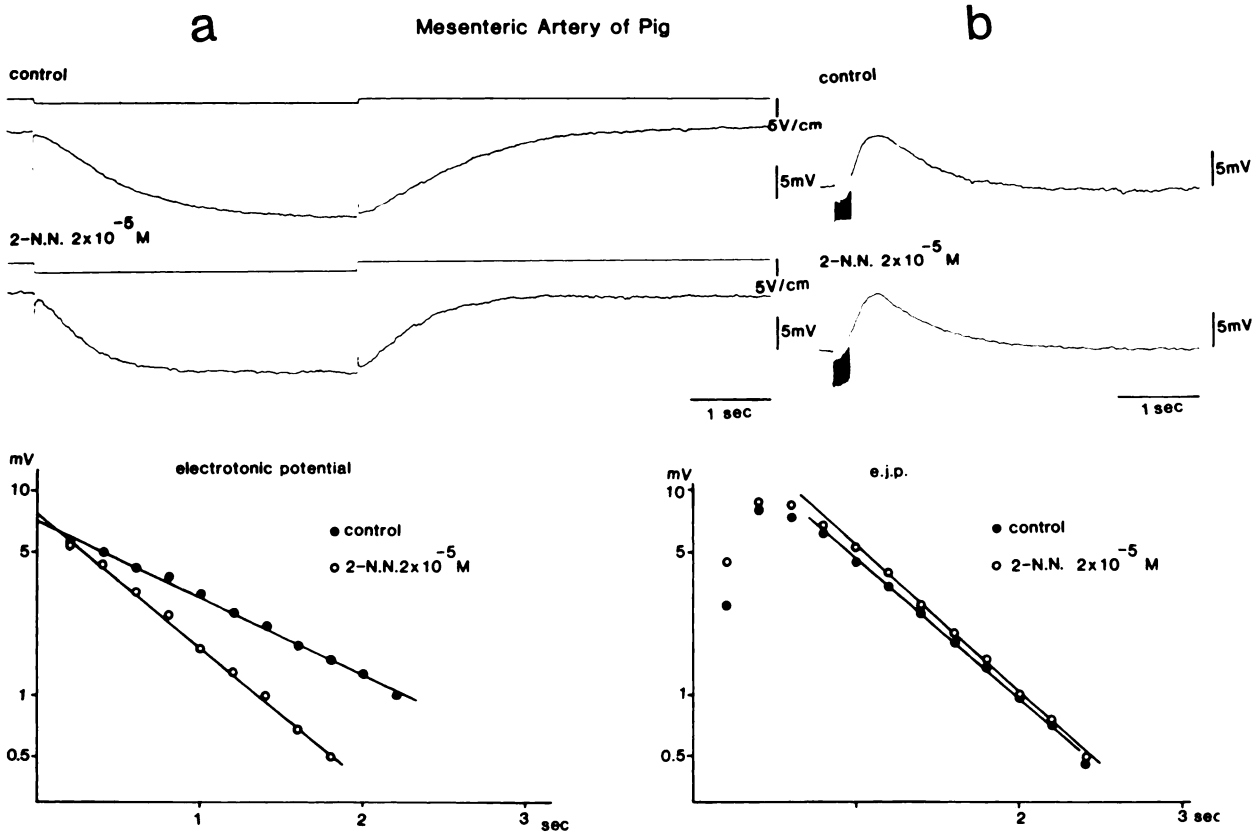

Fig. 5. Effects of 2-NN on the ejp evoked from the muscle cells of the porcine mesenteric artery. a, Electrotonic potential evoked by inward current pulse (5 sec) before and during application of 2-NN (2×10^{-5} M). The relationship between the rising time of electrotonic potential plotted as a log scale against the time after the stimulations. The calculations were made from a. In a, applied stimulus intensities differed before and during application of 2-NN. b, ejps recorded by repetitive stimulation (50 Hz, 0.2 msec and 30 stimulations) before and during application of 2-NN (2×10^{-5} M). Falling phase of ejps was plotted on a log scale against the time before and during application of 2-NN. Calculation was made from b.

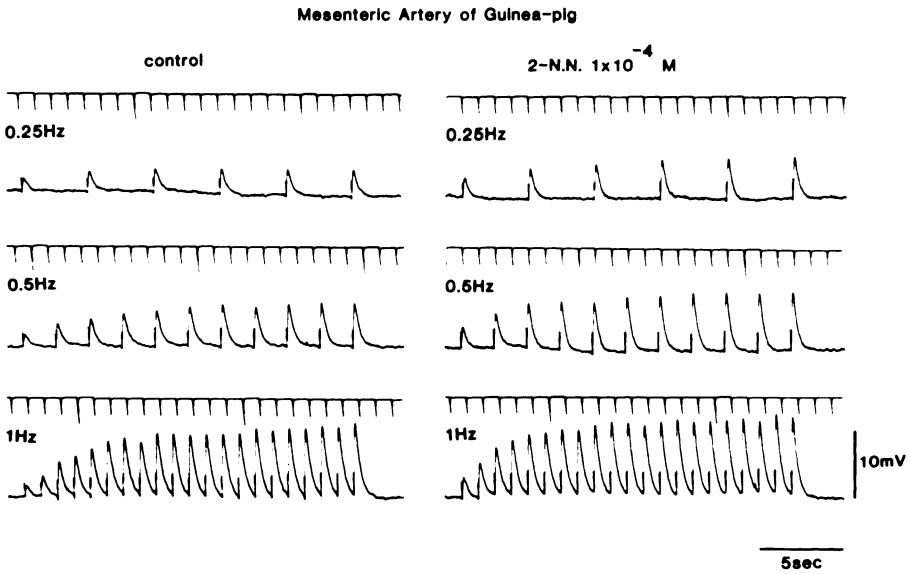

Fig. 6. Effects of 2-NN (10^{-4} M) on the ejp evoked by 0.25, 0.5, 1.0 Hz stimulation in the guinea-pig mesenteric artery. The stimulus conditions were 0.2 msec in pulse duration and above stimulus frequencies. Stimulus duration was about 20 sec.

coronary artery in which there was an increase in the amplitude of ejp with application of 2-NN and such was apparently due to an increase in release of NE from the nerve terminals.

The spontaneously released miniature ejp was only recorded rarely from the guinea-pig mesenteric artery (Suzuki and Kuriyama, 1980). Generations of the miniature ejp were increased in the presence of 2-NN. Figure 8 shows the effects of 2-NN (10^{-4} M) on the spontaneously generated miniature ejp in the guinea-pig mesenteric artery. The frequency of generations was markedly increased. These potential changes were markedly suppressed by treatment with phentolamine (Suzuki and Kuriyama, 1980), therefore, such were due to a release of NE from

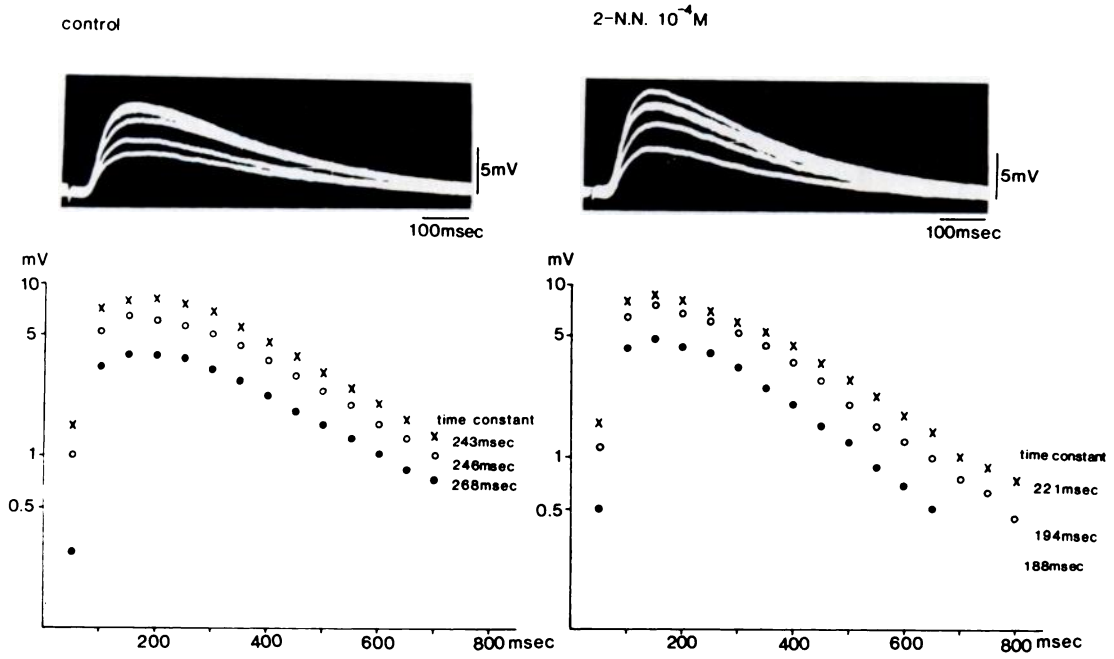

Fig. 7. Effects of 2-NN on the shape of ejp and the time constant of falling phase of ejp of the guinea-pig mesenteric artery before and during application of 2-NN (10^{-4} M). Similar intensity with five successive stimulations (pulse duration was 0.2 msec) was applied to the guinea-pig mesenteric artery before and during application of 2-NN (10^{-4} M). The time constant of the falling phase of ejps was measured from three ejps from the above actual records. Measured values of the time constant are inserted in the figure.

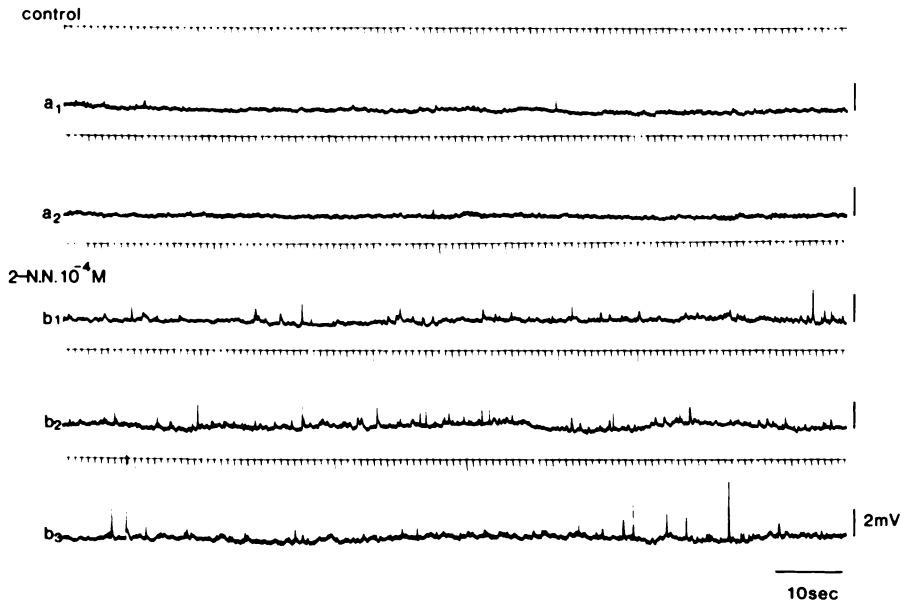

Fig. 8. Effects of 2-NN on the spontaneously generated miniature ejps recorded from the guinea-pig mesenteric artery. a_1 and a_2, control. b_1–b_3, in the presence of 10^{-4} M 2-NN (after about 5 min).

the nerve terminals. These findings further support the postulation that 2-NN increases the amplitude of ejp after a release of NE from the nerve terminals.

The graded response or spike was recorded from the muscle cell of the guinea-pig mesenteric artery by application of direct stimulation or by generation of the ejp. In the presence of 2-NN (10^{-4} M), the ejp-generated spike was still recordable, thus 2-NN did not suppress the Ca-carrier system which elicits the spike.

Figure 9A shows the effects of 10^{-6} M NG on the ejp and the spike evoked on the ejp. To produce the ejp and the spike, three repetitive stimulations with various intervals (0.3 msec in pulse duration) were used. Application of 10^{-6} M NG did not change the membrane potential (-68.3 ± 2.8 mV in the control and -67.8 ± 3.4 mV in the presence of 10^{-6} M NG, $n = 30$) and no effect was observed on the amplitude of ejp and the spike superimposed on the ejp.

Figure 9B shows the effects of NG on the ejp and spike superimposed on ejp evoked from the guinea-pig mesenteric artery in the presence of 10^{-3} M TEA. Here the minimum concentration of TEA required to produce the spike was much lower than that induced in the guinea-pig and porcine coronary arteries (10^{-2} M or more). Application of 10^{-3} M TEA did not modify the membrane potential or enhance the amplitude of ejp and the spike was produced within 1 min. TEA not only suppressed the K-conductance of postsynaptic muscle mem-

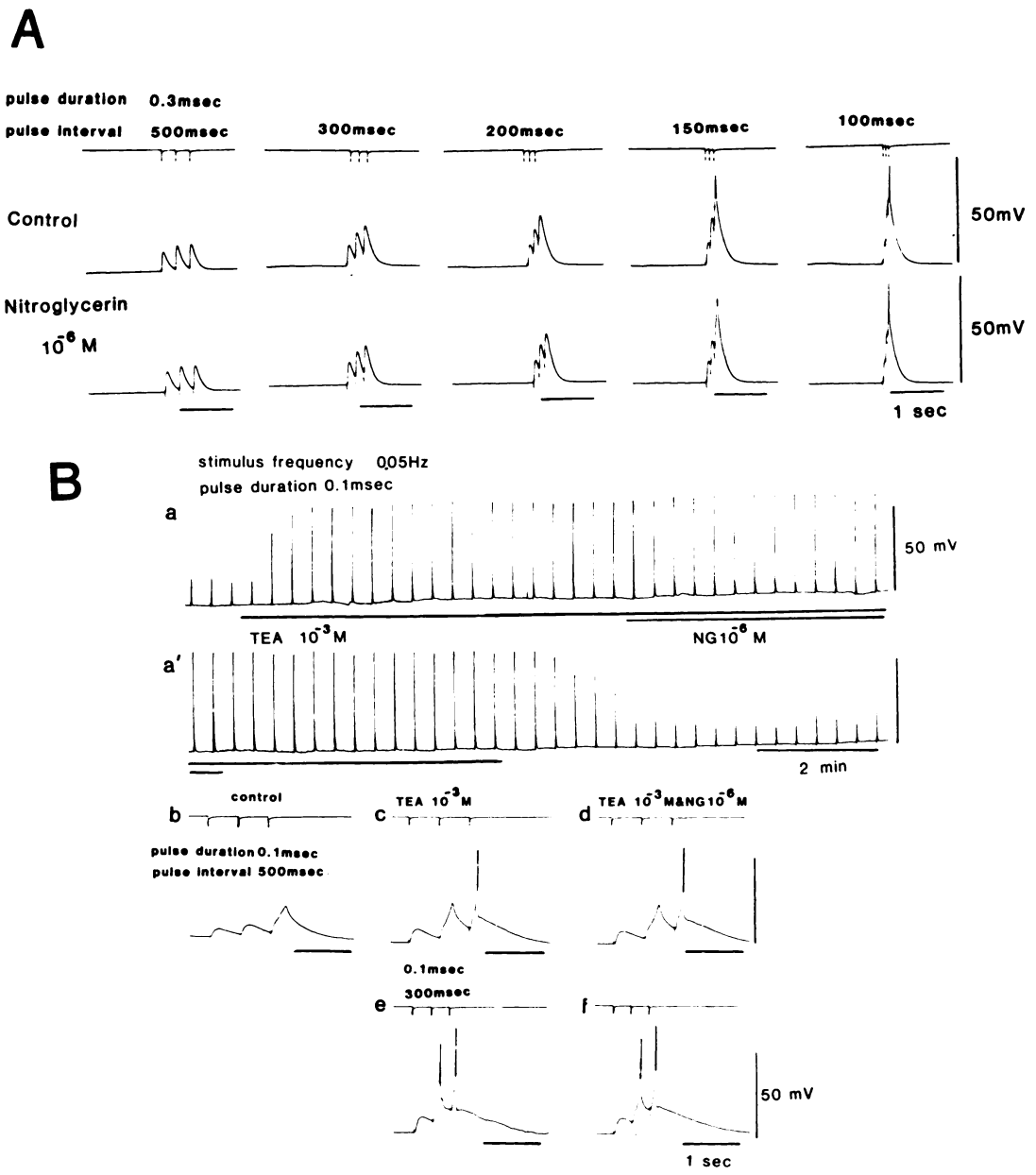

Fig. 9. A, effects of NG on the ejp and the spike evoked from the muscle cell of the guinea-pig mesenteric artery. The stimulus condition: 0.3 msec in pulse duration, three stimulations with various stimulus intervals. B, effects of NG (10^{-6} M) on the ejp and spike with overshoot of the guinea-pig mesenteric artery evoked by 0.05 Hz stimulation under pretreatment with 10^{-3} M TEA; a and a' are successive recordings (0.1 msec in pulse duration and 0.05 Hz in stimulus frequency); b–f, fast speed recordings of the effects of NG under pretreatment with TEA; b–d, control, in the presence of TEA and TEA with NG, respectively. The stimulus conditions were 0.1 msec in pulse duration, 500 msec in stimulus interval and three stimulations; e–f, the condition was the same as that of c and d, respectively, except for the stimulus interval of 300 msec.

brane but also increased the NE release from the nerve terminals. The spike with overshoot generated in the presence of TEA was not affected by treatment with 10^{-6} M NG. These affects of NG indicate that this compound has no effect on the release of NE or on the electrical threshold required to evoke the spike and the Ca-carrier system during the active state of the membrane.

Figure 10A shows the effects of NP on ejps generated in the guinea-pig mesenteric artery. Application of NP (5×10^{-8} M) did not modify the membrane potential, however, increased concentrations of NP (10^{-6} M) slightly hyperpolarized the membrane (from -67.5 ± 3.2 to -71.8 ± 2.1 mV, $n = 20$). With application of NP in concentrations over 5×10^{-8} M, the amplitude of ejp was suppressed and the facilitation process produced by 0.5 and 1.0 Hz of stimulation (pulse duration was 0.2 msec), *i.e.*, the time required to reach the maximum amplitude of ejp required an increased number of stimulations, as compared to the control. Figure 10B shows the effects of NP on the ejp and spike generation of the guinea-pig mesenteric artery. To evoke the ejp and the spike, 0.2 msec in pulse duration, 20 Hz in stimulus frequency and various numbers of stimulations were given, both before and during application of 10^{-6} M NP. The amplitude of ejp and spike were consistently suppressed in the presence of NP. The spike could be evoked on the ejp when 5 times the amount of stimulation was given, but in the presence of 10^{-6} M NP, the spike was not evoked even with application

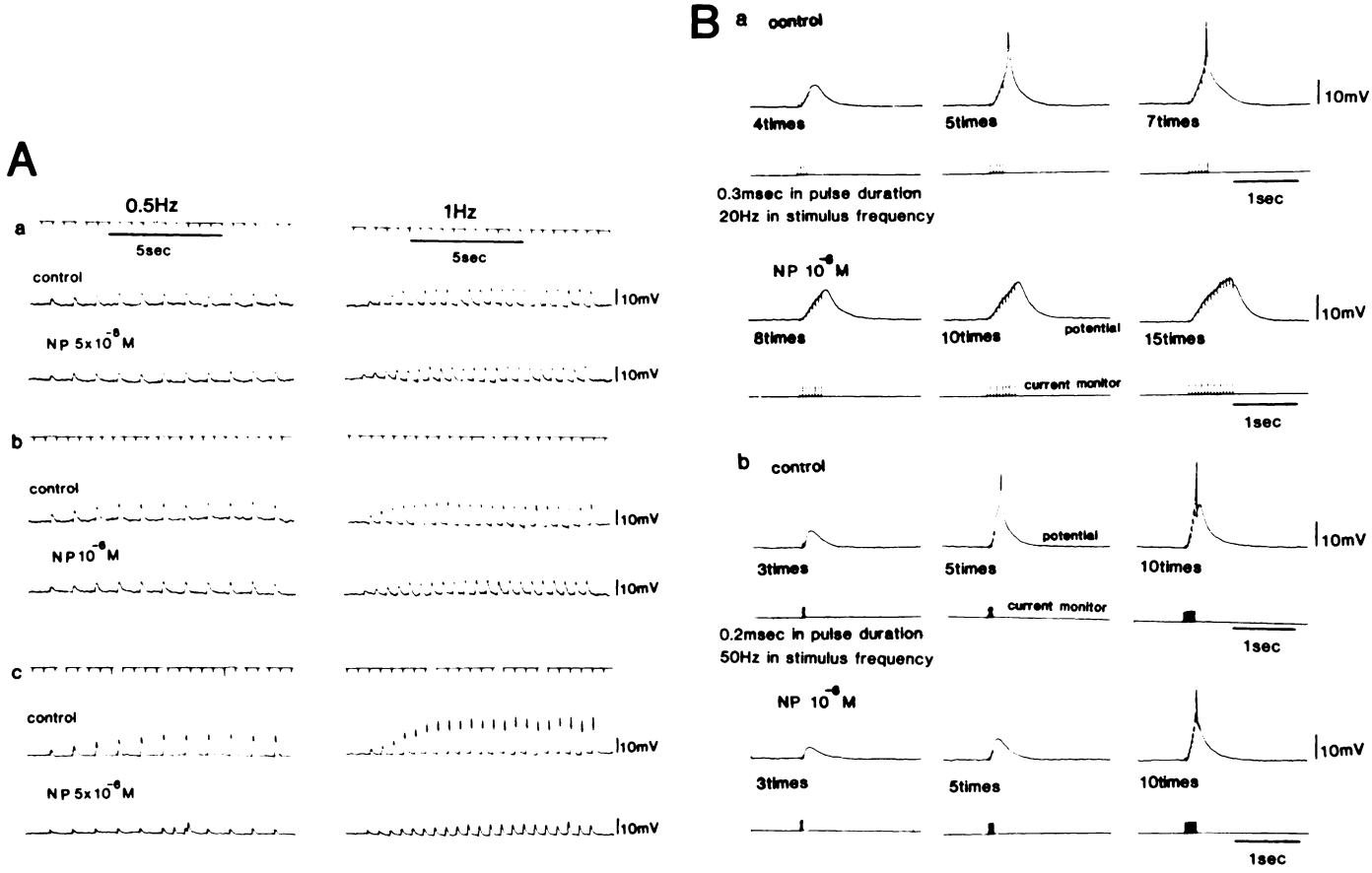

Fig. 10. A, effects of NP on the ejp evoked by repetitive stimulation in the guinea-pig mesenteric artery; a–c, ejps were recorded from three different cells; a, before and during application of 5×10^{-8} M NP; b, before and during application of 10^{-6} M NP; c, before and during application of 5×10^{-5} M NP. Two different stimulus frequencies were applied (pulse duration was 0.2 msec). B, effects of NP on the ejp and spike evoked by repetitive stimulation from the guinea-pig mesenteric artery; a, 0.3 msec in pulse duration, 20 Hz in stimulus frequency and various stimulus numbers were applied before and during application of 10^{-6} M NP; b, effects of NP (10^{-6} M) on the ejp and spike evoked by repetitive stimulation in the guinea-pig mesenteric artery (0.2 msec in pulse duration, 50 Hz in stimulus frequency and various stimulus numbers were applied) before and during application of 10^{-6} M NP.

of 15 times the amount of stimulation. On the other hand, as shown in figure 10Bb, when the stimulus frequency was increased to 50 Hz, the spike was evoked on the ejp in the presence of 10^{-6} M NP when a greater number of stimulations were given. Therefore, the suppression of the generation of the spike in the presence of NP was due to suppression of the amplitude of ejp or to the hyperpolarization of the membrane. Here the possibility of suppression of the Ca-carrier system in the membrane can be ruled out.

Discussion

Effects of 2-NN on the membrane and mechanical properties of mesenteric arteries. Furukawa *et al.* (1981) stated that 2-NN suppresses the mechanical response induced by excess $[K]_o$ in the porcine and guinea-pig coronary arteries mainly by hyperpolarization of the membrane. In the present experiment, the K-induced contraction in the porcine mesenteric artery was suppressed by 2-NN but not in the guinea-pig artery. However, the lack of effect of 2-NN on the guinea-pig mesenteric artery does not rule out the possible action of 2-NN on the membrane property. Since the membrane potential was already close to the K-equilibrium potential in the resting state, the increase in the K-conductance of the membrane by 2-NN no longer hyperpolarizes the membrane, thus there was no effect on the K-induced contraction. Namely, the membrane potential of the porcine mesenteric artery was −53 mV, and in the guinea-pig mesenteric artery it was −69 mV. The K-equilibrium potential in the guinea-pig coronary artery was thought to be located at about −70 mV (Kitamura and Kuriyama, 1979; Takata and Kuriyama, 1980). In the present experiment and also in studies on the coronary artery, the maximum hyperpolarization induced by 2-NN in 5.9 mM $[K]_o$ (control solution) reached about −70 mV. The depolarization required for the generation of the K-induced contraction was high and therefore higher concentrations of excess $[K]_o$ were required in the guinea-pig mesenteric artery. These observations support the idea that the principal action of 2-NN on the K-induced contraction is dependent on hyperpolarization of the membrane.

On the other hand, the NE-induced contractions of mesenteric arteries in both species were dose-dependently suppressed by 2-NN. Although NE did not modify the membrane potential, 2-NN hyperpolarized the membrane in the porcine but not in the guinea-pig mesenteric artery. The suppression of the Ca-mobilization in the cell is probably the main action of 2-NN on the NE-induced contraction in the mesenteric arteries, as was postulated in the case of the contraction evoked by ACh in the coronary arteries (Furukawa *et al.*, 1981).

In the saphenous artery, Holman and Suprenant (1979) reported that exogenously applied NE produced the contraction

with no depolarization of the membrane, but NE endogenously released from the nerve terminal depolarized the membrane and produced the contraction. Therefore, the possibility of two different adrenoceptors at the membrane was discussed. Ito *et al.* (1977) reported that the K-induced (depolarization) contraction and chemically induced (NE or ACh) contractions differ in nature as was suggested from the voltage and current clamp experiments. The action of 2-NN is also probably related to different mechanisms, an activation of the K-channel in the membrane and an activation of chemically sensitive Ca-mobilization processes in the cell.

Effects of 2-NN, NG and NP on the neuromuscular transmission. In the mesenteric artery, the ejp generated by perivascular nerve stimulation was due to release of NE from the nerve terminal because tetrodotoxin completely suppresses the generation of ejps and *alpha* adrenoblocking agents also suppressed the amplitude of ejp (Suzuki and Kuriyama, 1980). In the guinea-pig mesenteric artery, 2-NN did not alter the membrane potential, yet it increased the amplitude of ejp. In contrast, 2-NN hyperpolarized the membrane and increased the ionic conductance of the membrane of the porcine mesenteric artery, yet it increased the amplitude of ejp. The time constant of the membrane in the porcine mesenteric artery, measured in the presence of 2-NN, was consistently reduced as expected from change in the membrane conductance, but the time constant of the falling phase of ejp remained much the same. The ejp in the porcine mesenteric artery was evoked by brief repetitive stimulation with high frequency, thus the falling phase of ejp does not strictly represent the absolute value of falling phase of ejp evoked by single stimulus and may not be directly comparable with the time constant of the muscle membrane. On the other hand, the time constant of the falling phase of ejp evoked by single stimulus in the guinea-pig mesenteric artery was also reduced by 2-NN compared to the value obtained in the control under the enhanced amplitude of ejp and the reduced membrane time constant. This finding was given additional support by the fact that in the presence of 2-NN (10^{-4} M) there was an increase in the number of miniature ejps. In both species, 2-NN increased the amplitude of ejp, probably due to an increase in the release of NE from adrenergic nerve terminals.

Effects of NG and NP on the adrenergic transmission were compared with that observed by treatment with 2-NN. NG did not modify either the amplitude of ejp or the electrical threshold to generate the spike and there was no change in the membrane potential. In the concentration of 10^{-10} M, NG relaxed the porcine coronary artery in the presence of 11.7 mM $[K]_o$ without affecting the K-induced depolarization (Ito *et al.*, 1980). In the mesenteric artery, NG suppressed the K-induced contraction in both guinea-pig and porcine arteries (the present authors, unpublished observations). This agent did not affect the adrenergic transmission and the spike evoked on the ejp, thus the Ca-carrier system at the muscle membrane is probably not affected by NG. Similar effects were also observed in the rat portal vein, *i.e.*, NG did not affect the spontaneously generated spike, but did suppress the contraction evoked by the spike. This means that NG acts as an uncoupler of the excitation-contraction coupling in the rat portal vein. In contrast, in the guinea-pig portal vein, NG hyperpolarized the membrane, suppressed the spike generation and reduced or blocked the contraction. The species differences are apparent (Karashima, 1980).

NP only slightly hyperpolarized the membrane, but did suppress the amplitude of ejp in the guinea-pig mesenteric artery. When the time constant of the falling phase was measured in the presence of NP, the reduction in the falling phase of ejp was less than the reduction in the amplitude. NP also suppressed the facilitation process. If the NP-induced suppression of ejp is mainly due to an increase in the conductance of muscle membrane, the facilitation seen in the presence of NP should show a parallel shift of the facilitation observed in the control. Presumably, suppression of ejp by NP is mainly due to a suppression of the NE release.

From the present experiments, we conclude that 2-NN suppresses the K- and NE-induced contractions of the porcine and guinea-pig mesenteric arteries. The K-induced contraction was suppressed by change in the membrane potential before application of 2-NN and was dependent on the resulting hyperpolarization. The NE-induced contraction was suppressed by inhibitions of the Ca-mobilization in the cell with an additional change in the membrane potential. These results equate findings in the case of 2-NN actions on the coronary artery. Nevertheless, the effects of 2-NN appeared to be dominant in the porcine and guinea-pig coronary arteries rather than in the mesenteric arteries. Furthermore, there were clear differences in actions of 2-NN, NG and NP on the neuromuscular transmission, *i.e.*, 2-NN enhances, NG does not affect and NP suppresses the amplitude of ejp generated by nerve stimulation; yet all these agents produce vasodilating actions through direct actions on the muscle cell.

Acknowledgments

We thank M. Ohara, Kyushu University, for reading of the manuscript. 2-Nicotinamidoethyl nitrate was kindly provided by Chugai Pharmacy, Ltd.

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