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TANIYAMA, Kohtaro

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ANTISPASMODIC MECHANISM OF NORADRENALINE IN THE ISOLATED RAT RECTUM, PARTICULARLY IN RELATION TO Ca

Kohtaro TANIYAMA, Nobuyoshi YOSHIDA, Nobuyuki TAKAHASHI, Juei-Tang CHENG and Hiromasa ARAKI

Department of Pharmacology
Kobe University School of Medicine

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rat rectum; noradrenaline; Na-free medium; Ca-free medium; Na-free, Ca-free medium; Ca release; concentration-action curve

Kohtaro TANIYAMA, Nobuyoshi YOSHIDA, Nobuyuki TAKAHASHI, Juei-Tang CHENG and Hiromasa ARAKI. *Antispasmodic Mechanism of Noradrenaline in the Isolated Rat Rectum, Particularly in Relation to Ca.* Kobe J. Med. Sci. **23**, 35-40, December 1977——Antispasmodic mechanism of noradrenaline (NA) was investigated in relation to the mobilization of Ca in the isolated rat rectum. The findings on the relaxing effects of NA on the contractions induced by increasing concentrations of K suggest that the antispasmodic mechanism of NA may be related to inhibition of the membrane activity concerning Ca mobilization, but not related to inhibition of the ability of contractile elements responding to Ca. The antispasmodic action of NA on the phasic contraction by acetylcholine in normal medium was similar to that in Ca-free medium. Thus, NA would inhibit the release of Ca from storage sites. Furthermore, analysis of the concentration-action curve of acetylcholine concerning phasic contraction affected by NA in normal and Ca-free media provides additional evidence for such an assumption. The antispasmodic action of NA was not significantly modified by treatment with the removal of external Na. However, the shift of concentration-action curve of acetylcholine concerning phasic contraction induced by NA was slightly attenuated by removal of external Na. From these findings, it appears that as a mechanism of its antispasmodic action, NA would inhibit the release of Ca from storage sites, and that the Ca release-inhibiting action of NA would be hard to be affected by removal of external Na.

INTRODUCTION

Several studies on the antispasmodic mechanisms of isoproterenol[1,6,7,8,11] and papaverine[2,3,5,7,11,12,13] in the smooth muscle have been recently reported. In the previous report,[11] the mechanisms of antispasmodic actions of β-receptor stimulant,

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isoproterenol, and non-receptor relaxant, papaverine, were investigated in relation to the mobilization of Ca in the isolated rat rectum. On the other hand, it has been proposed that adrenaline and noradrenaline act predominantly by their α-action, resulting in cessation of spike activity and hyperpolarization of membrane. However, little information is available concerning the mechanism of antispasmodic or relaxing action of α-receptor stimulant, noradrenaline, in relation to the mobilization of Ca. In the present study, the antispasmodic mechanism of noradrenaline against acetylcholine-induced contraction was investigated in the isolated rat rectum.

MATERIALS AND METHODS

Male rats, weighing approx. 250 g, were sacrificed by a blow on the neck and the rectum was dissected. Strips of the rectum, approx. 1 cm long, were fixed vertically in the organ bath containing 30 ml of nutrient solution. Isotonic contractions were recorded on a smoked paper with a magnification of approx. 10 times. The solution was maintained at $29\pm1^\circ$ C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the solution was as follows (g/ml): NaCl, 9×10^{-3}; KCl, 0.42×10^{-3}; $CaCl_2$, 0.24×10^{-3}; glucose, 10^{-3}; $NaHCO_3$, 0.4×10^{-3} (Locke's solution). Ca-free solution was prepared in the same fashion as Locke's solution except that $CaCl_2$ was omitted and 0.01 mM GEDTA was added. Na-free solution was made in the same way but that NaCl was substituted by LiCl for the isotonic adjustment, whereas the Na-free, Ca-free solution was prepared in the same way as the Na-free solution except that $CaCl_2$ was removed and 0.01 mM GEDTA was added. Preparations were allowed to equilibrate for 60 minutes in media before experiments were begun.

Noradrenaline hydrochloride (Sankyo), acetylcholine chloride (Tokyokasei) and glycoletherdiamine tetraacetic acid (GEDTA) (Wako Chemicals) were used. Drugs were added directly to the medium. Concentrations of drugs are expressed in term of g/ml of the salts, unless otherwise stated.

RESULTS

I. *Effect of the High K-Depolarizing Medium on the Relaxing Actions of Noradrenaline (NA).*

The addition of K to normal medium caused a contraction. The contraction by K augmented with increased concentrations, the maximal contraction by K being obtained in the concentration of 6×10^{-3}. The application of NA ($10^{-8}-10^{-5}$) during K-induced contraction caused a relaxation. The relaxing actions of 10^{-8}, 10^{-7} and 10^{-5} of NA progressively decreased with increased concentrations of K and they were abolished by 2×10^{-3} or over of K, by 6×10^{-3} or over of K and by 10^{-2} or over of K, respectively (Fig. 1). According to the method of earlier report,[11] it was investigated whether or not the effect of hyperosmolarity due to the addition of K to normal medium was related to the attenuation and abolishment of NA-induced relaxation. The comparison between the effects of high concentrations of K and sucrose on the NA-induced relaxations indicated that the action of NA was not influenced by the effects of hyperosmolarity.

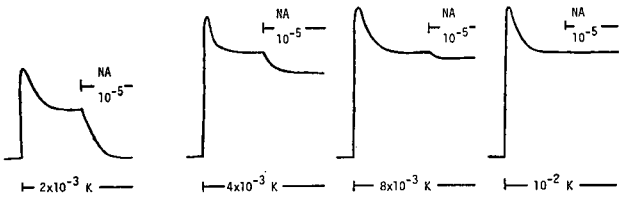

Fig. 1 Relaxing effect of noradrenaline (NA) on the contractions by increasing concentrations of K.

II. Effect of NA on the Acetylcholine (ACh)-Induced Phasic Contraction.

In this experiment, 5×10^{-8} ACh was used as the approx. ED60. In the normal medium, the addition of ACh to media caused a rapidly-developing contraction (phasic contraction, PC) followed by a slow contraction (tonic contraction). The effect of NA (pretreatment for 5 minutes) on the PC by ACh was investigated in normal medium. In Ca-free medium, the effect was examined as the following. The magnitude of ACh-induced PC in Ca-free medium 10 minutes later and that in Ca-free medium with preapplication of NA (for 5 minutes) 10 minutes later were measured in the same preparation.

10^{-9} NA modified ACh-induced PC neither in normal nor Ca-free medium, whereas 5×10^{-9} NA inhibited that by $15.4\pm2.7\%$ (N=7) in normal medium and by $20.2\pm3.0\%$ (N=7) in Ca-free medium, respectively. 10^{-8} NA inhibited more strongly the PC by ACh both in normal and in Ca-free media.

III. Effect of Noradrenaline on the Concentration-Action (C-A) Curve of Acetylcholine.

Effect of NA on cumulative C-A curve of ACh concerning phasic contraction was investigated by the pretreatment with NA for 5 minutes in normal medium. In Ca-free medium, effect of NA on cumulative C-A curve of ACh concerning PC at 10 minutes after Ca removal was examined by the pretreatment with NA for 5 minutes. NA ($10^{-8}-10^{-6}$) shifted the log C-A curve of ACh concerning PC to the right,

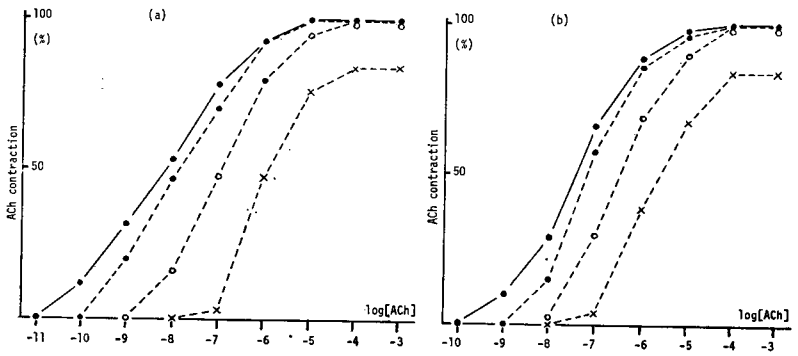

Fig. 2 Effect of noradrenaline (NA) on the concentration-action curve of acetylcholine (ACh) concerning phasic contraction in normal (a) and in Ca-free (b) media.
●—●: control, ●·····●: pretreatment with 10^{-8} NA, ○···○: 10^{-7} NA and ×···×: 5×10^{-6} NA.

whereas NA (5×10^{-6} and 10^{-5}) shifted it to the right and downward both in normal and in Ca-free media (Fig. 2).

IV. Effect of Removal of External Na on the Antispasmodic Action of Noradrenaline.

Effect of Na removal from normal medium on the antispasmodic action of NA was examined as the following. The magnitude of PC by 5×10^{-8} ACh in normal medium, that in normal medium with preapplication of 5×10^{-8} NA for 5 minutes, that in Na-free medium 10 minutes later and that in Na-free medium with preapplication of 5×10^{-8} NA (for 5 minutes) 10 minutes later were determined in the same preparation, respectively. In order to observe the effect of Na removal on the PC by ACh in Ca-free medium, the magnitude of PC in Ca-free medium 10 minutes later, that in Ca-free medium with preapplication of 5×10^{-8} NA (for 5 minutes) 10 minutes later, that in Na-free, Ca-free medium 10 minutes later and that in Na-free, Ca-free medium with preapplication of 5×10^{-8} NA (for 5 minutes) 10 minutes later were determined in the same preparation, respectively. The PC by 5×10^{-8} ACh was reduced by pretreatment with 5×10^{-8} NA by $46.1\pm4.1\%$ (N=7) in normal medium, by $42.7\pm4.0\%$ (N=7) in Na-free medium, by $59.9\pm4.8\%$ (N=6) in Ca-free medium and by $53.3\pm6.2\%$ (N=6) in Ca-free, Na-free medium, respectively. Thus, the NA-induced antispasmodic action was not significantly modified by removal of external Na.

V. Effect of Na Removal on the Modification of Concentration-Action Curve of Acetylcholine by Noradrenaline.

Effect of NA on cumulative C-A curves of ACh concerning PC at 10 minutes after Ca removal and Ca and Na removal from normal medium was investigated. NA was applied by the pretreatment for 5 minutes method. In Na-free medium and in Na-free, Ca-free medium, NA ($10^{-8}-5\times10^{-6}$) shifted the log C-A curve of ACh to the right and 10^{-5} NA shifted that to the right and downward. Thus, although 5×10^{-6} NA shifted the log C-A curve of ACh to the right and downward in normal and in Ca-free media, yet it shifted that only to the right in Na-free and in Na-free, Ca-free media (Fig. 3).

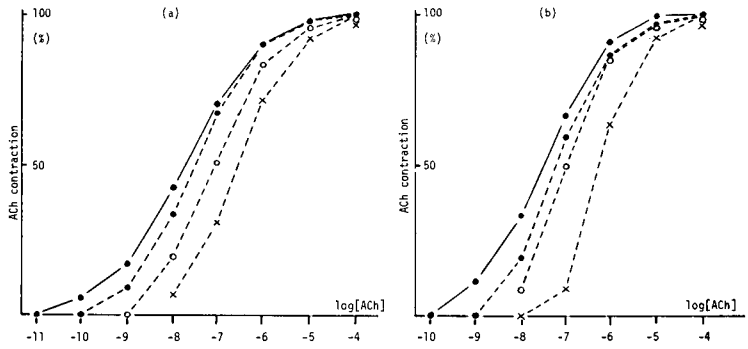

Fig. 3 Effect of noradrenaline (NA) on the concentration-action curve of acetylcholine (ACh) concerning phasic contraction in Na-free (a) and in Na-free, Ca-free (b) media.
●—●: control, ●···●: pretreatment with 10^{-8} NA, ○···○: 10^{-7} NA and ×···×: 5×10^{-6} NA.

DISCUSSION

The relaxing actions of NA ($10^{-8}-10^{-5}$) on contraction by K attenuated with increased concentrations of K and finally disappeared. Therefore, the relaxing action of NA would be due to inhibition of the membrane activity concerning Ca mobilization, not to inhibition of the contractile elements.

It was previously reported[10] that the PC by ACh was caused by the release of Ca from storage sites and by the passive influx of Ca in the isolated rat rectum. 10^{-9} NA inhibited the ACh-induced PC in neither normal nor Ca-free medium, whereas 5×10^{-9} NA inhibited that both in normal and Ca-free media. The inhibition of ACh-induced PC by 5×10^{-9} NA in Ca-free medium was similar in magnitude to that in normal medium. Thus, 5×10^{-9} NA would inhibit the release of Ca from storage sites induced by ACh. This assumption would be further supported by the fact that magnitudes of the right shifts induced by 10^{-8}, 10^{-7} and 5×10^{-6} of NA and of the downward shift induced by 5×10^{-6} NA in normal medium were similar to those in Ca-free medium. It is presumable that the shifts of log C-A curves by NA are produced by the functional antagonism between contracting agent, ACh, and NA concerning the mobilization of Ca.

Kuriyama[4] proposed that the relaxation of intestinal smooth muscle produced by adrenaline and noradrenaline was brought about by the cessation of spontaneous spike activity and the membrane hyperpolarization. In the present study, it is unclear whether or not NA inhibits the influx of Ca. However, it has been accepted that NA elicits the hyperpolarization of cell membrane in intestinal smooth muscle; therefore, it is possible that NA would reduce the ACh-induced influx of Ca.

As in previous report,[9] ACh-induced PC in Na-free medium was produced by the release of Ca from storage sites and the passive influx of Ca and that in Ca-free, Na-free medium was caused by the release of Ca in the isolated rat rectum. The inhibitory action of 5×10^{-8} NA on PC by ACh was not significantly modified by Na removal from normal and Ca-free media. On the other hand, although 5×10^{-6} NA shifted the log C-A curves of ACh in normal and in Ca-free media to the right and downward, in Na-free and in Na-free, Ca-free media it still shifted them only to the right. Thus, it appears that the inhibitory action of NA is slightly reduced by removal of external Na. It was demonstrated[11] that isoproterenol and papaverine accelerated the uptake of intracellular Ca by storage sites, resulting in generation of inhibitory action on the release of Ca and that Ca release-inhibiting actions of these relaxants were depressed by removal of external Na. Therefore, it would appear that unlike those of isoproterenol and papaverine the Ca release-inhibiting action of NA generates the inhibition against the release of Ca from storage sites presumably attached to the cell membrane by hyperpolarizing the cell membrane, not by accelerating Ca uptake into storage sites.

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