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Prejunctional inhibition mediated via α_2-adrenoceptors in excitatory transmission of canine small intestine

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Noradrenaline(NA) inhibited the contractile responses of longitudinal and circular muscles of canine small intestine to field stimulation (20 Hz, 1 ms, supramaximal voltage for 5 s). The purpose of this study was to see if NA-induced inhibitory action is mediated via α_1- or α_2-receptors and whether the inhibitory action involved prostaglandins. In both muscle layers the α_2-agonist, clonidine inhibited the contractile response to field stimulation more potently than did the α_1-agonists phenylephrine or methoxamine. It is suggested that NA-mediated prejunctional inhibition is mediated via α_2-receptors. Yohimbine, an α_2-adrenoceptor blocking drug antagonized NA-induced prejunctional inhibition, while E-643, an α_1-blocker, did not affect the inhibition. These results suggest that the inhibitory action of NA is mediated via α_2-receptors in both muscle layers. Indomethacin, an inhibitor of prostaglandin synthesis, did not affect NA-induced prejunctional inhibition in circular muscle, while augmenting it in longitudinal muscle. It is thought that NA-induced prejunctional inhibition is not mediated via prostaglandin release.

The release of acetylcholine(ACh) from guinea-pig ileum is inhibited by noradrenaline(NA) and adrenaline(Ad) (Paton & Vizi 1969; Kosterlitz et al 1970; Knoll & Vizi 1971) and ACh release from rabbit intestine is inhibited by sympathetic nerve stimulation (Vizi & Knoll 1971). The inhibitory effect of NA or Ad on ACh release is thought to be mediated via α_2-adrenoceptors (Drew 1978; Wikberg 1978; Andréjak et al 1980). These results mean that α_2-adrenoceptors exist in cholinergic nerve terminals and their excitation regulates ACh output from the terminals.

Recently, we examined the effects of prostaglandins on the cholinergic transmission of canine small intestine, and proposed that prostaglandin E exerts a negative feedback control of cholinergic transmission in circular but not longitudinal muscle (Nakahata et al 1980a, b). On the other hand, NA inhibited the contractile responses of longitudinal and circular muscles of canine small intestine to field stimulation, which releases ACh from postganglionic cholinergic fibres (Nakahata et al 1981). The site of action of NA is thought to be prejunctional, because NA potently inhibited the contractile response to field stimulation, but did not affect a contractile response to ACh with an amplitude similar to that of the response to field stimulation (Nakahata et al 1981). NA was more effective than isoprenaline in the inhibition of the response to field stimulation, suggesting that NA inhibited the response via α-adrenoceptors. We have now demonstrated the responses of the two kinds of α-receptors in the cholinergic nerve terminal of canine small intestine and we have also examined whether NA-induced prejunctional inhibition is mediated via the release of prostaglandins.

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MATERIALS AND METHODS

Mongrel dogs of either sex, 7–15 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.) and bled from carotid arteries. A jejunal segment of about 2 cm length was removed. The segment was cut longitudinally and the lumen was opened. The muscular layers were stripped from lamina muscularis mucosae, and were separated into longitudinal and circular muscles. Nicotinic response was observed in longitudinal and circular muscles. A strip (about 1·5 × 0·2 cm) of longitudinal or circular muscle was mounted between two platinum rings for field stimulation in organ baths containing 20 ml of Tyrode solution at 37 °C, gassed with 95% O_2 and 5% CO_2. The composition of the solution was (mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0, NaH_2PO_4 0·4, $NaHCO_3$ 11·9 and glucose 5·6. Tension development was recorded isometrically with an FD pick-up (Nihon Kohden, SB-1T) and a carrier amplifier (Nihon Kohden, RP-3). The resting tension was adjusted to 2·0 g at the beginning of the experiment and the tissue was allowed to equilibrate for more than 1 h. The resting tension decreased to

0·5 to 1·0 g during experiments. Field stimulation was applied with rectangular pulses of 1 ms duration at 20 Hz and supramaximal voltage for 5 s (using an electrical stimulator (Nihon Kohden, MSE-3R), except where mentioned otherwise.

Drugs used were noradrenaline hydrogen tartrate (Seikagaku Kogyo), methoxamine hydrochloride (Nihon Shinyaku), phenylephrine hydrochloride (Kohwa), 2-(4-(n-butyl)-homopiperazine-1-yl)-4-amino-6,7-dimethoxyquinazoline(E-643, Eizai), yohimbine hydrochloride (Wako Pure Chemical) and indomethacin (Sumitomo Chemical). Indomethacin was dissolved in a weak basic solution in a concentration of 1 mg ml^{-1} by adding in NaOH to pH 8·5, and was diluted with 0·9% NaCl. E-643 was dissolved in 20% dimethyl sulphoxide to make a concentration of 5 mg ml^{-1}, and diluted with 0·9% NaCl.

The statistical difference of the values obtained was determined using Student's *t*-test.

RESULTS

Effects of α-adrenoceptor stimulants on contractile responses to field stimulation

Electrical field stimulation (20 Hz, 1 ms, supramaximal voltage for 5 s) produced contractions of the longitudinal and circular muscles of canine jejunum that were inhibited dose-dependently by NA 10^{-9} to 10^{-5} M (Figs 1, 2). The response of longitudinal muscle was unaffected by the α_1-stimulants phenylephrine or methoxamine, but inhibited by clonidine, an α_2-stimulant, indicating that NA may have its effect on α_2-receptors. In the circular muscle, the response to field stimulation was slightly inhibited by methoxamine or phenylephrine, and strongly inhibited by clonidine and NA. These results indicate that α-adrenoceptors regulating prejunctional transmitter release are mainly of α_2-type in both muscle layers.

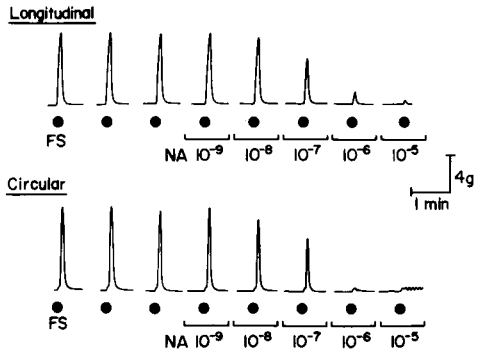

FIG. 1. Typical responses of longitudinal (upper) and circular (lower) muscles to field stimulation (FS, ●) of 20 Hz, 1 ms, supramaximal voltage for 5 s, and the effect of NA on the responses. NA(10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M) was applied 5 min before each field stimulation.

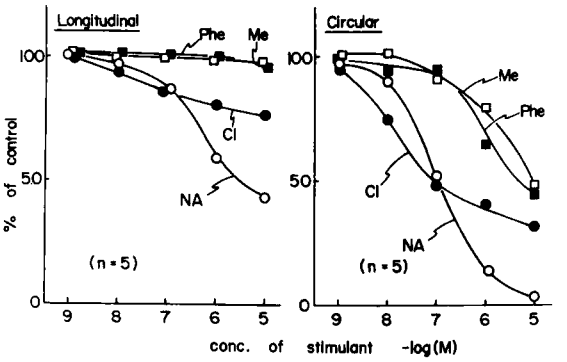

FIG. 2. Effects of α-stimulants on the contractile responses of longitudinal (left) and circular (right) muscles to field stimulation. Phe: phenylephrine. Me: methoxamine. Cl: clonidine. NA: noradrenaline. Ordinate: % of control (the response to field stimulation before α-stimulants application was taken as 100). Abscissa: concentration of α-stimulant -log(M). Each point represents the mean value from 5 observations.

Effects of E-643 and yohimbine on NA-induced inhibition of contractile responses to field stimulation

E-643, a new drug, has potential use as a blocker of α_1-, adrenoceptors (Shoji et al 1980; Shoji 1981). Before experiments on canine intestine, the drug's potency was checked by monitoring its inhibitory effect on NA-induced contractions in rabbit aorta (α_1-response) and its antagonistic effect on NA-induced inhibition of twitch responses of guinea-pig ileum to electrical field stimulation (α_2-response) (Fig. 3). In these experiments, E-643 was confirmed as a blocker of α_1-adrenoceptors.

The inhibitory effect of NA on contractile responses to field stimulation was then tested in the presence of E-643, (10^{-7}–10^{-6} M) and in longitudinal and circular muscles. The drug did not affect the inhibitory action of NA (Fig. 4). However, with longitudinal and circular muscles, inhibition of the response to field stimulation induced by NA at high concentrations (10^{-5} M) was significantly antagonized by pretreatment with yohimbine, an α_2-adrenoceptor blocking drug (Doxey et al 1977), 10^{-7} or 10^{-6} M (Fig. 5) suggesting that NA-induced inhibition of the field stimulation was mediated via

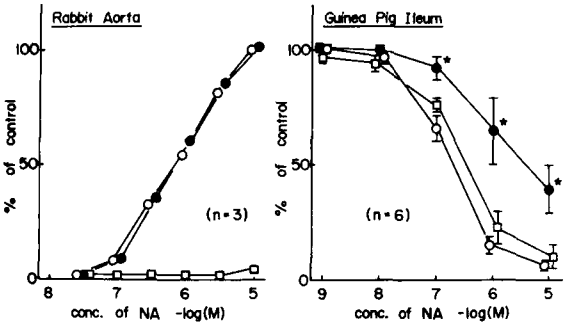

FIG. 3. Modification by E-643 and yohimbine of NA effect in rabbit aorta and guinea-pig ileum. Left: NA-induced contraction and effects of E-643 and yohimbine in rabbit aorta. NA-induced contraction (○——○) was strongly antagonized by treatment with 10^{-6} M E-643 (□——□) but not 10^{-6} M yohimbine (●——●). Ordinate: % of control (response to 10^{-4} M NA was taken as 100). Abscissa: concentration of NA. Each point represents the mean value from 3 observations. Right: NA-induced inhibition on the contractile response of guinea-pig ileum to field stimulation (1 Hz, 1 ms, supramaximal voltage). NA-induced inhibition (○——○) was strongly inhibited by treatment with 10^{-6} M yohimbine (●——●) but not 10^{-6} M E-643 (□——□). Ordinate: % of control (the response to field stimulation before NA application was taken as 100). Significant difference from NA alone (*$P < 0.05$).

α_2-receptors. The dose-response curve for NA in the presence of yohimbine did not show a parallel shift to the right.

Effect of indomethacin on NA-induced inhibition of the contractile response to field stimulation

Indomethacin has an inhibitory action on prostaglandin synthesis (Vane 1971; Hanberg & Samuelsson

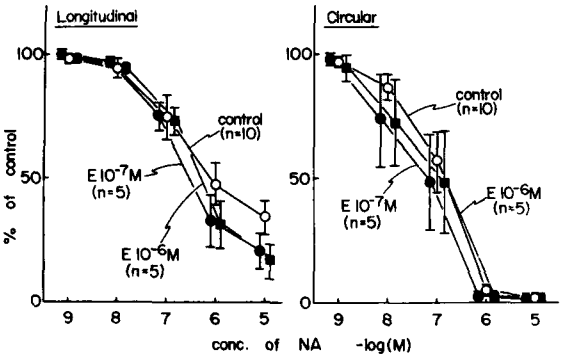

FIG. 4. Effect of E-643 on NA-induced inhibition of field stimulation. Left: longitudinal muscle. Right: circular muscle. Ordinate: % of control (the response to field stimulation before NA application was taken as 100). ○——○: NA alone. E-643 (10^{-7} M) (●——●) or (10^{-6} M) (■——■) was treated 5 min before NA application. Abscissa: concentration of NA-log(M). Each point represents the mean value from 5 or 10 observations with ± s.e. as a vertical line.

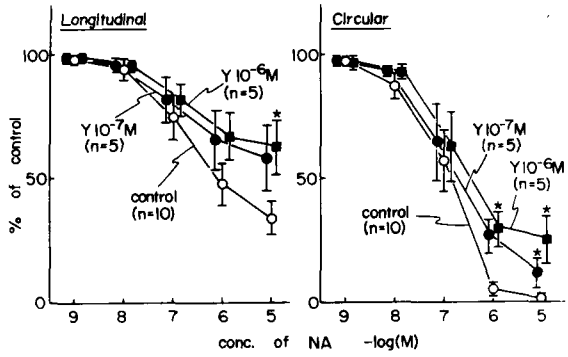

FIG. 5. Effect of yohimbine on NA-induced inhibition of field stimulation. Left: longitudinal muscle. Right: circular muscle. ○——○: NA alone. Yohimbine (10^{-7} M) (●——●) or (10^{-6} M) (■——■) was added 5 min before NA application. Yohimbine significantly restored the NA-induced inhibition (*$P < 0.05$). Ordinate: % of control (the response to field stimulation before NA application was taken as 100). Abscissa: concentration of NA-log(M). Each point represents the mean value from 5 or 10 observations with ± s.e. as a vertical line.

1974). Therefore, the effect of indomethacin on the inhibitory action of NA was examined to see whether this was mediated via a release of prostaglandins. In longitudinal muscle, NA-induced inhibition was significantly *potentiated* by pretreatment with indomethacin (3×10^{-7} M) for 20 min. In the circular muscle, NA-induced inhibition was unaffected by pretreatment with indomethacin for 20 min (Fig. 6).

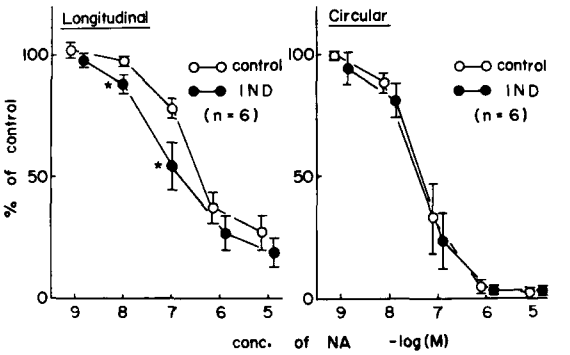

FIG. 6. Effect of indomethacin (3×10^{-7} M) on NA-induced inhibition of field stimulation. Left: longitudinal muscle. Right: circular muscle. Indomethacin (IND) was treated 20 min before application. ○——○: NA alone. ●——●: effect of NA in indomethacin-treated preparations. Ordinate: % of control (the response to field stimulation before NA application was taken as 100). Abscissa: concentration of NA -log(M). Each point represents the mean value from 6 observations with ± s.e. as a vertical line. When the longitudinal muscle was treated with indomethacin, NA more strongly inhibited the contractile response to field stimulation (*$P < 0.05$).

DISCUSSION

α-Adrenoceptors are classified into α_1- and α_2-types (Langer 1974; Starke et al 1975). It has been reported that those inhibiting ACh-release in guinea-pig ileum are of the α_2-type (Drew 1978; Wikberg 1978). In the preparations of longitudinal and circular muscles of canine small intestine, NA reduced the contractile response to field stimulation mediated via α-adrenoceptors (Nakahata et al 1981). In the present experiment, clonidine, an α_2-stimulant, was more effective in inhibiting the contractile responses of longitudinal and circular muscles to field stimulation than the α_1-stimulants methoxamine and phenylephrine. On the other hand, the NA-induced inhibitory effect on the contractile responses of both muscle layers to field stimulation was significantly antagonized by treatment with yohimbine, an α_2-adrenoceptor blocking drug, but not by treatment with E-643, an α_1-blocker. Therefore, it is thought that the inhibitory effect of NA is mediated via α_2-receptors. Yohimbine did not induce a parallel shift of the dose-response curve for NA-induced inhibition of the contractile responses of either longitudinal or circular muscles to field stimulation. However, the possibility that the NA-induced inhibition is mediated via β-adrenoceptors is small, because isoprenaline only slightly inhibited the contractile response to field stimulation (Nakahata et al 1981).

We have already reported that prostaglandins E_1 and E_2 inhibit the contractile response of circular but not of longitudinal muscle (Nakahata et al 1980a, 1980b). The prostaglandin-mediated presynaptic inhibition differed from the α_2-receptor-mediated inhibition observed in longitudinal and circular muscles since NA-induced inhibition of the contractile response of circular muscle to field stimulation was unaffected by pretreatment with indomethacin. Therefore, NA inhibited the contractile response by a mechanism other than via prostaglandins. Hedqvist (1974) suggested that in adrenergically innervated tissue, presynaptic α-adrenoceptor (α_2) agonists and prostaglandin act on different levels of the process of excitation-secretion coupling. On the other hand, indomethacin *potentiated* the inhibitory effect of NA on the contractile response of longitudinal muscle to field stimulation. Indomethacin inhibits cholinergic transmission in longitudinal muscle of guinea-pig ileum (Ehrenpreis et al 1973; Kadlec et al 1974; Bennett et al 1975) so its potentiating effect on NA-induced inhibition may be due partly to this inhibition of cholinergic transmission. In conclusion, the prejunctional inhibitory effect of NA on the contractile response to field stimulation may be mediated via α_2-adrenoceptors rather than by prostaglandin release.

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