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Effects of 3,4-diaminopyridine and tetraethylammonium on the pre- and post-junctional α-adrenoceptor mediated inhibitory actions of noradrenaline in the guinea-pig ileum

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1 The effects of potassium channel blockers, 3,4-diaminopyridine (DAP) and tetraethylammonium (TEA) were studied on the pre- and post-junctional α_2-adrenoceptor mediated effects of noradrenaline in the guinea-pig proximal ileum.

2 Both DAP (4 to 500 μmol l^{-1}) and TEA (0.3 to 3 mmol l^{-1}) transiently increased the basal tension of the ileum. However, DAP also increased the amplitude of the smooth muscle twitches evoked by transmural nerve stimulation, whereas TEA marginally depressed them.

3 Atropine (2 μmol l^{-1}) antagonized the contractions induced by DAP but did not affect the similar effects of TEA. On the other hand, DAP restored the smooth muscle twitches depressed by atropine, while TEA did not.

4 DAP, in a concentration-dependent manner, reduced or abolished the prejunctional inhibitory α_2-adrenoceptor mediated effect of noradrenaline, whereas TEA (up to 3 mmol l^{-1}) was almost ineffective.

5 The postjunctional inhibitory α_2-adrenoceptor mediated effect of noradrenaline was attenuated even at the smallest TEA concentration used (0.3 mmol l^{-1}) and its postjunctional stimulatory α_1-adrenoceptor mediated effect was unmasked. However, DAP, was only marginally effective, even at the highest concentrations used (100 and 500 μmol l^{-1}).

6 From these results it would appear that in both the pre- and post-junctional inhibitory α_2-adrenoceptor mediated actions of noradrenaline in the guinea-pig ileum the primary step might be an increased potassium conductance. However, the potassium channels on the neuronal and the smooth muscle membrane have different sensitivities to DAP and TEA.

Introduction

The pre- and post-junctional inhibitory α-adrenoceptor mediated action of adrenoceptor agonists in the small intestine is well established (Ahlquist & Levy, 1959; Paton & Vizi, 1969; Kosterlitz *et al.*, 1970; Bauer, 1976; 1981; Anderson & Lees, 1976; Drew, 1977; Wikberg, 1978; Bauer & Kuriyama, 1982). It is also well known that an increase in the frequency of nerve stimulation may facilitate the secretory mechanisms (Vizi, 1979; Alberts, 1982) and oppose the receptor mediated depression of the transmitter secretion and smooth muscle twitches induced by nerve stimulation (Paton & Vizi, 1969; Vizi, 1979; Bauer *et al.*, 1982). The inhibition of transmitter secretion due to the activation of prejunctional α_2-adrenoceptors has been thought to involve restriction of nerve impulse-induced influx of calcium into the varicosities, where it acts as the trigger of their secretory mechanisms (Alberts, 1982).

The presence of inhibitory α-adrenoceptors was recently found not only on pre- but also on post-junctional structures of the longitudinal muscle layer of the guinea-pig ileum (Bauer, 1981; 1982; Bauer & Kuriyama, 1982).

It has become increasingly evident that an enhanced potassium conductance could play a pivotal role in both the pre- and post-junctional inhibitory α-adrenoceptor mediated effects of sympathomimetic amines. Rezvani *et al.* (1983) demonstrated that 4-amino-

pyridine, a potassium channel blocker, which increases the availability of calcium, antagonized the prejunctional inhibitory action of opiates in the guinea-pig ileum. Bauer & Rusko (1982) showed that changes in the tetraethylammonium sensitive potassium conductance are responsible for the postjunctional α-adrenoceptor mediated inhibition in the taenia coli.

The present study was carried out to analyse the interaction of 3,4-diaminopyridine and tetraethylammonium with the pre- and post-junctional inhibitory actions of noradrenaline in the guinea-pig ileum.

Methods

Guinea-pigs of either sex were stunned and bled. After the animals had been killed, pieces of the proximal ileum (more than 50 cm from the ileocaecal valve) of 3 cm length were quickly excised. The lumens of the preparations were flushed with Krebs-Eccles solution. The tissues were set-up in an organ bath filled with Krebs-Eccles solution of the following composition (mmol l^{-1}): Na^+ 137.9, K^+ 6.2, Ca^{2+} 2.7, Mg^{2+} 1.2, Cl^- 134.7, HCO_3^- 13.6, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2 and glucose 9.9. The solutions were gassed with 95% O_2 and 5% CO_2 and the pH was maintained at 7.2 to 7.4.

The ileum was stimulated transmurally (Paton, 1955) with supramaximal pulses of 0.1 ms duration at a frequency of 0.1 Hz. When the action of a drug on the responses to electrical stimulation was investigated, the stimuli were applied continuously throughout the experiment. The actual experiments were started after uniform twitches had been obtained. Increasing concentrations of noradrenaline were applied cumulatively to the bathing fluid. The interval between successive concentrations of noradrenaline was adjusted to allow the effect of each concentration to develop fully. A concentration-response curve to noradrenaline was first established, then exposure to noradrenaline was discontinued. When the twitch response and the basal tension had recovered, the potassium channel blockers were added to the bathing fluid for 5 min (the time required to reach steady state tension changes) and the concentration-response curve to noradrenaline was repeated.

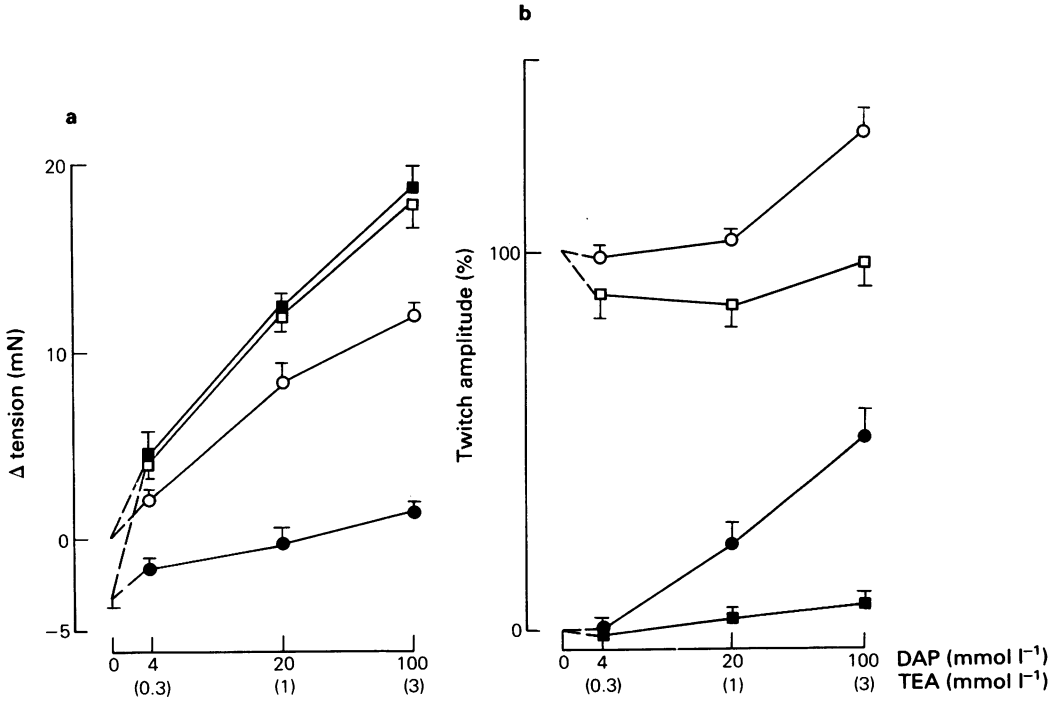

Figure 1 The effect of tetraethylammonium (TEA: □, ■) and 3,4-diaminopyridine (DAP: ○, ●) on the twitch amplitude (b) elicited by transmural stimulation and the basal tension (a) of the guinea-pig proximal ileum, before (open symbols) and after pretreatment with atropine (10 min; 2 μmol l^{-1}) (closed symbols). Each point represents the mean of 6 to 9 trials; vertical lines show s.e.mean.

In order to investigate the actions of 3,4-diaminopyridine and tetraethylammonium on the basal tension and evoked twitches, they were applied in single concentrations before and after pretreatment of the tissues with atropine for 10 min, the time required to reach the maximal effect.

Isometric smooth muscle contractions were recorded with a strain gauge transducer. The experiments were performed at 37°C under an initial tension of about 10 mN.

The following drugs were used: atropine sulphate (Spofa), 3,4-diaminopyridine (DAP; Fluka), (±)-noradrenaline hydrogentartrate (Spofa), tetraethylammonium chloride (TEA; Merck), tetrodotoxin (TTX; Sankyo).

Fresh stock solutions were prepared in distilled water. All concentrations are indicated as mol of base l^{-1}.

Results are expressed as arithmetic means ± s.e.mean. Differences between means were assessed using Student's t test for paired observations (Delaunois, 1973).

Results

The effect of noradrenaline on twitch response and basal tension

Cumulatively applied noradrenaline (1 nmol l^{-1} to 10 μmol l^{-1}) led to a concentration-dependent inhibition of smooth muscle twitches elicited by supramaximal electrical stimulation of the intramural 'postganglionic' nerve fibres of the guinea-pig ileum. The basal tension of the proximal ileum was decreased by 1 nmol l^{-1} to 1 μmol l^{-1} of noradrenaline, while the highest noradrenaline concentration used (10 μmol l^{-1}) induced a contraction of the smooth muscle, as described earlier (Bauer, 1981; 1982).

The effect of tetratethylammonium and 3,4-diaminopyridine on twitch response and basal tension

TEA (0.3 to 3 mmol l^{-1}) evoked a concentration-dependent, transient contraction and slightly decreased the amplitude (by 10 to 20%) of smooth muscle twitches elicited by transmural stimulation (Figure 1). After the initial contraction the smooth muscle tension gradually declined and the sustained tension reached the initial level (0.01 ± 0.48 mN; $n = 6$) in the presence of 0.3 mmol l^{-1} TEA, or it dropped below the initial level (− 1.83 ± 0.42 and − 3.62 ± 0.48 mN; $n = 6$) in the presence of 1 and 3 mmol l^{-1} TEA in the bathing fluid for 5 min, respectively.

DAP (4 to 500 μmol l^{-1}) also induced a contraction of the smooth muscle. Moreover, it increased the amplitude of evoked smooth muscle twitches. The initial fast contraction (phasic response) was also concentration-dependent (Figure 1). The sustained increase in tension (tonic response) was only marginally dependent on the concentration of DAP (4, 20, 100 and 500 μmol l^{-1}) and reached the values of 4.80 ± 0.59 ($n = 7$), 4.51 ± 0.34 ($n = 9$), 5.39 ± 0.73 ($n = 8$) and 6.57 ± 0.51 ($n = 7$) mN, respectively.

The effects of atropine on tetraethylammonium and 3,4-diaminopyridine-induced contractions and changes in twitch amplitude

Atropine (2 μmol l^{-1}) lowered the basal tension of the guinea-pig ileum by 3.2 ± 0.4 mN and fully abolished the evoked smooth muscle twitches ($n = 12$).

Pretreatment of the ileum with atropine had no significant effect on the TEA-induced smooth muscle contractions. In the presence of atropine TEA failed to restore the responses of the ileum to transmural stimulation. High concentrations of TEA (higher than 3 mmol l^{-1}) evoked rhythmic smooth muscle contractions; therefore, their effect on the evoked twitches was not tested. These rhythmic contractions were of

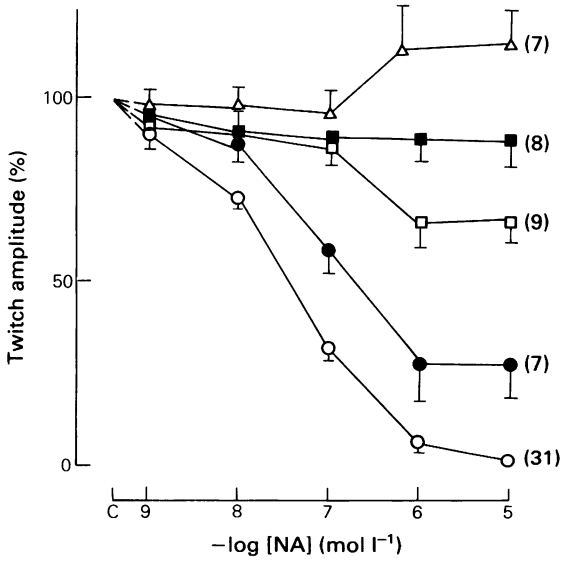

Figure 2 The effect of 3,4-diaminopyridine (DAP) on the inhibitory effect of noradrenaline on the twitch response of the guinea-pig proximal ileum. The control responses (○) are the inhibitory effects induced by noradrenaline (NA) alone. The concentrations of DAP used are: (●) 4 and (□) 20 μmol l^{-1}; (■) 0.1 and (△) 0.5 mmol l^{-1}. Each point represents the mean; vertical lines show s.e.mean; number of trials is given in parentheses.

myogenic origin, as they were not affected by TTX ($1\,\mu mol\,l^{-1}$) or atropine ($2\,\mu mol\,l^{-1}$) ($n = 7$).

In contrast, pretreatment of the preparations with atropine significantly ($P < 0.05$) attenuated the effect of DAP on the basal tension, and DAP in a concentration-dependent manner restored the evoked smooth muscle twitches suppressed by atropine (Figure 1).

The effects of 3,4-diaminopyridine on the inhibitory responses elicited by noradrenaline

Incubation of the ileum with DAP reduced the potency of noradrenaline at inhibiting electrically induced smooth muscle twitches. As shown in Figure 2, DAP (4 and $20\,\mu mol\,l^{-1}$) displaced the noradrenaline concentration-response curve to the right and significantly ($P < 0.05$) reduced or abolished its maximal inhibitory effect. In the presence of high concentrations of DAP (0.1 and $0.5\,mmol\,l^{-1}$) noradrenaline up to $10\,\mu mol\,l^{-1}$ either did not affect the amplitude of evoked twitches or in concentrations of $1\,\mu mol\,l^{-1}$ and higher slightly increased their amplitude.

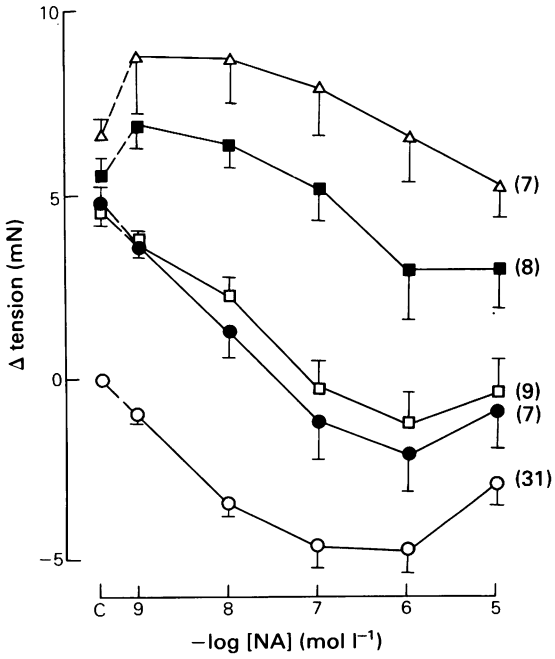

Figure 3 The effect of 3,4-diaminopyridine (DAP) on smooth muscle relaxation induced by noradrenaline (NA) in the guinea-pig proximal ileum. (○) Control concentration-response curve to NA. The concentrations of DAP used are: (●) 4 and (□) $20\,\mu mol\,l^{-1}$; (■) 0.1 and (△) $0.5\,mmol\,l^{-1}$. Each point represents the mean; vertical lines show s.e.mean; number of trials is given in parentheses.

The shape and intensity of the inhibitory action of noradrenaline on the smooth muscle basal tension was unchanged by small concentrations of DAP (4 and $20\,\mu mol\,l^{-1}$). High concentrations of DAP (0.1 and $0.5\,mmol\,l^{-1}$), however, prevented the reduction of the basal tension by noradrenaline (up to $1\,\mu mol\,l^{-1}$). Only the highest concentration of noradrenaline used ($10\,\mu mol\,l^{-1}$) significantly ($P < 0.05$) reduced the basal tension of the ileum (Figure 3).

The effects of tetraethylammonium on the inhibitory responses elicited by noradrenaline

Incubation of the ileum with TEA either slightly increased ($0.3\,mmol\,l^{-1}$) or reduced ($1\,mmol\,l^{-1}$) the inhibitory effect of noradrenaline on the evoked smooth muscle twitches. The highest TEA concentration ($3\,mmol\,l^{-1}$) used, however, significantly ($P < 0.05$) attenuated the inhibitory effect of noradrenaline and shifted the noradrenaline concentration-response curve to the right (Figure 4). In contrast, the effect of TEA was much more pronounced against the noradrenaline induced smooth muscle relaxation. Even $0.3\,mmol\,l^{-1}$ of TEA reduced the inhibitory effect of noradrenaline on the smooth muscle tension. Higher concentrations of TEA (1 and $3\,mmol\,l^{-1}$) not only fully abolished the noradrenaline evoked smooth muscle relaxation, but also reversed its action to a concentration-dependent contraction (Figure 5).

Discussion

The ability of α-adrenoceptor agonists to inhibit electrically induced twitches and relax the guinea-pig ileum has been demonstrated to be due to the reduction of the amplitude of the cholinergic excitatory junction potential (e.j.p.) (Bauer & Kuriyama, 1982), acetylcholine release (Paton & Vizi, 1969) and hyperpolarization of the smooth muscle membrane (Bauer & Kuriyama, 1982), respectively. Both pre- and postjunctional inhibitory α-adrenoceptor mediated effects of adrenoceptor agonists in the guinea-pig ileum are the result of activation of α_2-adrenoceptors (Bauer, 1981; 1982; Bauer & Kuriyama, 1982).

There is considerable evidence suggesting that α_2-adrenoceptor activation of neurones in the myenteric plexus affects calcium-sensitive potassium conductance (Morita & North, 1981). Activation of inhibitory α-adrenoceptors enhances potassium efflux in sympathetic and enteric neurones (Brown & Caulfield, 1979; North, 1982). Since this would lead to hyperpolarization of the membrane, the possibility that prejunctional α-adrenoceptor mediated inhibition depressed influx of calcium into nerve terminals by promoting efflux of potassium was considered. DAP

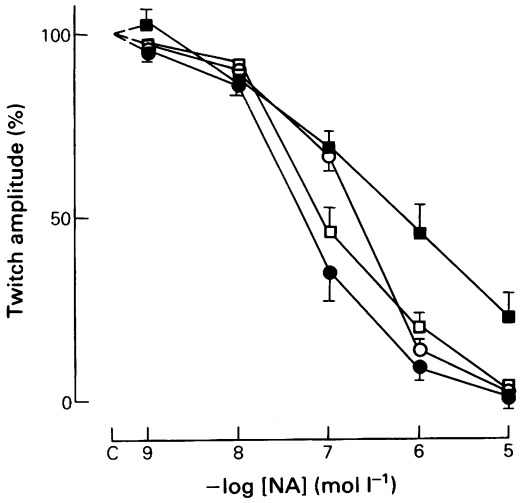

Figure 4 The effect of tetraethylammonium (TEA) on the inhibitory effect of noradrenaline on the twitch response of the guinea-pig proximal ileum. (□) Twitch inhibition induced by noradrenaline (NA) alone. The concentrations of TEA used are: (●) 0.3, (○) 1 and (■) 3 mmol l^{-1}. Each point represents the mean of 6 trials (control, 12 trials); vertical lines show s.e.mean.

and TEA have previously been found to block potassium conductances (Armstrong & Birnstock, 1965; Hille, 1967; Lundh & Thesleff, 1977; Ito *et al.*, 1980). The effects of DAP and TEA observed in this study suggest that the inhibition of acetylcholine release induced by the activation of prejunctional α_2-adrenoceptors results from an increased potassium conductance and is similar to that observed following activation of prejunctional opiate receptors (Rezvani *et al.*, 1983). Whether shortening of action potentials in enteric nerve terminals and the ensuing reduced influx of calcium, or hyperpolarization of the membrane of terminal varicosities by noradrenaline, is responsible for its interaction with stimulus-evoked release of acetylcholine should be further analysed. The facilitation of calcium entry and augmentation of acetylcholine release seems to be important (Löffelholz & Weide, 1982) for the more pronounced prejunctional antagonistic effect of DAP, compared to that of TEA, on the noradrenaline induced twitch inhibition. This suggestion is consistent with the presumption that catecholamines elicit their prejunctional inhibitory effect by blocking calcium uptake (Alberts, 1982) as the result of an increased potassium conductance.

In a separate series of experiments (Bauer & Matušák, unpublished results) we have demonstrated that quinine, recently shown to block selectively calcium activated potassium conductance in mammalian enteric neurones (Cherubini *et al.*, 1984), relaxed the guinea-pig ileum and attenuated or fully abolished the smooth muscle twitches elicited by transmural nerve stimulation at concentrations 2 to 20 times lower than those used by Cherubini *et al.* (1984). These concentrations of quinine did not significantly affect the contractions induced by acetylcholine and histamine whereas they decreased those induced by noradrenaline in the terminal ileum by 10 to 35%. The same concentrations of quinine also slightly attenuated (by 5 to 25%) the relaxation evoked by noradrenaline on the proximal ileum. Because of the above mentioned marked inhibitory effect of quinine itself, its effects at higher concentrations (more than 5 μmol l^{-1}) on the pre- and post-junctional inhibition induced by noradrenaline could not be studied.

The activation of postjunctional α_2-adrenoceptors in the proximal ileum (more than 50 cm from the ileocaecal valve) results in an increase of membrane conductance, pronounced hyperpolarization of the smooth muscle membrane, cessation of action potential discharge and smooth muscle relaxation (Bauer, 1981; Bauer & Kuriyama, 1982). These postjunctional inhibitory effects of α-adrenoceptor agonists have also been well established in other smooth muscles of the gut, e.g. taenia coli (Bülbring & Tomita, 1969a,b;

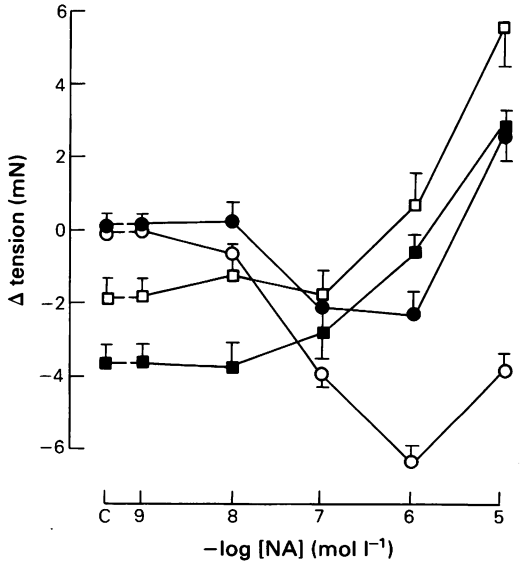

Figure 5 The effect of tetraethylammonium (TEA) on the smooth muscle relaxation induced by noradrenaline (NA) in the guinea-pig proximal ileum. (○) Control concentration-response curve to NA. The concentrations of TEA used are: (●) 0.3, (□) 1 and (■) 3 mmol l^{-1}. Each point represents the mean of 6 trials (control, 12 trials); vertical lines show s.e.mean.

Bauer & Zakhari, 1977). The increase in membrane conductance in taenia coli was suggested to be due to an increase in potassium conductance (Bülbring *et al.*, 1966; Shuba & Klevetz, 1967, Tomita *et al.*, 1974) across the TEA sensitive potassium channels (Bauer & Rusko, 1982). It is well recognized that an increase in internal calcium concentration results in the activation of a potassium conductance in a wide variety of cells from unicellular organisms to erythrocytes and vertebrate neurones (cf. review by Meech, 1978). There have been a number of suggestions that such an effect also occurs in vertebrate smooth muscles (Bülbring & Tomita, 1969b; Inomata & Kao, 1979; Walsh & Singer, 1980). TEA blocks calcium-activated potassium conductance in isolated smooth muscle cells and tissue preparations (Mironneau *et al.*, 1977; Walsh & Singer, 1980). Therefore, the effects of DAP and TEA on the smooth muscle relaxation induced by noradrenaline in segments of the proximal ileum provide evidence supporting the view that the postjunctional inhibitory α-adrenoceptor mediated action of adrenoceptor agonists in smooth muscles of the gut are due to the increased calcium-activated TEA-sensitive potassium conductance. This is also supported by our recent findings in taenia coli of the guinea-pig (Bauer & Rusko, 1982; Rusko *et al.*, 1984) and accords with the proposed mechanism of the inhibitory α-adrenoceptor mediated action of adrenoceptor agonists in other tissues, e.g. parotid gland (Putney, 1979), liver cells (Egashira, 1980) and myenteric neurones (Grafe *et al.*, 1980; Morita & North, 1981).

From the interactions of atropine with DAP and TEA it would appear that in the guinea-pig ileum the effect of DAP is predominantly due to an increased release of acetylcholine, whereas that of TEA is due to a direct effect on the smooth muscle membrane. We have recently found that excitatory postjunctional α_1-adrenoceptors are more densely distributed in the terminal (close to the ileocaecal valve) than in proximal ileum. However, these receptors are also distributed in the proximal regions and could be revealed either after the blockade of postjunctional α_2-adrenoceptors by selective antagonists (Bauer, 1982; Bauer & Kuriyama, 1982) or after the inhibition of processes linking the activation of α_2-adrenoceptors and smooth muscle relaxation, as shown with the use of TEA in the present study.

The present experiments provide evidence supporting the view that pre- and post-junctional α_2-receptor mediated effects of adrenoceptor agonists are the result of an increased potassium conductance. The neuronal potassium channels are more sensitive to DAP, whereas the potassium channels in the smooth muscle membrane are more affected by TEA.

References

AHLQUIST, R.P. & LEVY, B. (1959). Adrenergic receptive mechanism of canine ileum. *J. Pharmac. exp. Ther.*, **127**, 146–149.

ALBERTS, P. (1982). Mechanisms of facilitation and muscarinic or α-adrenergic inhibition of acetylcholine and noradrenaline secretion from peripheral nerves. *Acta physiol. scand.*, (Suppl.) **506**, 3–39.

ANDERSON, A.A. & LEES, G.M. (1976). Investigation of occurence of tolerance to bronchodilator drugs in chronically pretreated guinea-pigs. *Br. J. Pharmac.*, **56**, 331–338.

ARMSTRONG, C.M. & BIRNSTOCK, L. (1965). Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. gen. Physiol.*, **48**, 859–872.

BAUER, V. (1976). Action of adrenotropic drugs on intestinal and ureteral smooth muscle. *Abstr. of Int. Symp. on Physiol. Pharmac. Smooth Muscle (Varna)* p. 4.

BAUER, V. (1981). Distribution and types of adrenoceptors in the guinea-pig ileum: the action of α- and β-adrenoceptor agonists. *Br. J. Pharmac.*, **72**, 201–210.

BAUER, V. (1982). Distribution and types of adrenoceptors in the guinea-pig ileum: the action of α- and β-adrenoceptor blocking agents. *Br. J. Pharmac.*, **76**, 569–578.

BAUER, V. & KURIYAMA, H. (1982). Homogenous and non-homogenous distributions of inhibitory and excitatory adrenoceptors in the guinea-pig ileum. *Br. J. Pharmac.*, **76**, 603–611.

BAUER, V., MATUŠÁK, O. & KURIYAMA, H. (1982). Non-cholinergic, non-adrenergic responses to nerve stimulation of the guinea-pig small intestine. *Naunyn Schmiedebergs Arch. Pharmac.*, **319**, 115–120.

BAUER, V. & RUSKO, J. (1982). TEA-sensitive potassium conductance changes induced by α_1-adrenoceptor and ATP-receptor activation in guinea-pig taenia coli. *Gen. Physiol. Biophys.*, **1**, 89–102.

BAUER, V. & ZAKHARI, S. (1977). Pharmacological studies with beta-adrenoceptor blocking agents. I. Effect on the smooth muscle of the taenia coli of the guinea-pig. *Life Sci.*, **21**, 683–694.

BROWN, D.A. & CAULFIELD, M.P. (1979). Hyperpolarizing α_2-adrenoceptors in rat sympathetic ganglia. *Br. J. Pharmac.*, **65**, 435–445.

BÜLBRING, E., GOODFORD, P.J. & SETEKLIEV, J. (1966). The action of adrenaline on the ionic content and on sodium and potassium movements in the smooth muscle of the guinea-pig taenia coli. *Br. J. Pharmac. Chemother.*, **28**, 296–307.

BÜLBRING, E. & TOMITA, T. (1969a). Increase of membrane conductance by adrenaline in the smooth muscle of guinea-pig taenia coli. *Proc. R. Soc. B.*, **172**, 89–102.

BÜLBRING, E. & TOMITA, T. (1969b). Suppression of spontaneous spike generation by catecholamines in the smooth muscle of the guinea-pig taenia coli. *Proc. R. Soc. B.*, **172**, 103–119.

CHERUBINI, E., NORTH, R.A. & SURPRENANT, A. (1984).

Quinine blocks a calcium-activated potassium conductance in mammalian enteric neurones. *Br. J. Pharmac.*, **83,** 3–5.

DELAUNOIS, A.L. (1973). *Biostatistics in Pharmacology*. pp. 675–738. Oxford: Pergamon Press.

DREW, G.M. (1977). Pharmacological characterization of presynaptic α-adrenoceptors which regulate cholinergic activity in guinea-pig ileum. *Br. J. Pharmac.*, **59,** P513.

EGASHIRA, K. (1980). Hyperpolarization by noradrenaline in guinea-pig liver cells: Effects of ouabain and external Ca^{2+}. *Jap. J. Physiol.*, **30,** 473–485.

GRAFE, P., MAYER, C.J. & WOOD, J.D. (1980). Synaptic modulation of calcium-dependent potassium conductance in myenteric neurones in the guinea-pig. *J. Physiol.*, **305,** 235–248.

HILLE, B. (1967). The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. gen. Physiol.*, **50,** 1287–1302.

INOMATA, H. & KAO, C.Y. (1979). Ionic mechanisms of repolarization of guinea-pig taenia coli as revealed by the actions of strontium. *J. Physiol.*, **297,** 443–462.

ITO, Y., KORENAGA, S. & TAJIMA, K. (1980). Comparative study of the effects of 4-aminopyridine and tetraethylammonium on neuroeffector transmission in the guinea-pig vas deferens. *Br. J. Pharmac.*, **69,** 453–460.

KOSTERLITZ, H.W., LYDON, R.J. & WATT, A.J. (1970). The effects of adrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39,** 398–413.

LÖFFELHOLZ, K. & WEIDE, W. (1982). Aminopyridines and the release of acetylcholine. *Trends in Pharmac. Sci.*, **3,** 147–148.

LUNDH, H. & THESLEFF, S. (1977). The mode of action of 4-aminopyridine and guanidine on transmitter release from motor nerve terminals. *Eur. J. Pharmac.*, **42,** 411–412.

MEECH, R.W. (1978). Calcium-dependent potassium activation in nervous tissues. *Ann. Rev. Biophys. Bioeng.*, **7,** 1–18.

MIRONNEAU, J., SAVINEAU, J.P. & RAHETY, A. (1977). Evidence for a component of outward current mediated by calcium influx in uterine smooth muscle. In *Excitation-contraction Coupling in Smooth Muscle*, ed. Casteels, R., Godfraind, T. & Rüegg, J.C. pp. 117–122, New York: Elsevier.

MORITA, K. & NORTH, R.A. (1981). Clonidine activates membrane potassium conductance in myenteric neurons. *Br. J. Pharmac.*, **74,** 419–428.

NORTH, R.D. (1982). Electrophysiology of the enteric nervous system. *Neuroscience*, **7,** 315–325.

PATON, W.D.M. (1955). The response of the guinea-pig ileum to electrical stimulation by coaxial electrodes. *J. Physiol.*, **127,** 40–41P.

PATON, W.D.M. & VIZI, E.S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35,** 10–28.

PUTNEY, J.W. (1979). Stimulus-permeability coupling: Role of calcium in the receptor regulation of membrane permeability. *Pharmac. Rev.*, **30,** 209–245.

REZVANI, A., HUIDOBRO-TORO, J.P. & WAY, E.L. (1983). Effect of 4-aminopyridine and verapamil on the inhibitory action of normorphine on the guinea-pig ileum. *Eur. J. Pharmac.*, **86,** 111–115.

RUSKO, J., BAUER, V. & BOŠKA, D. (1984). The role of Ca^{2+} on the inhibitory effect of catecholamines in taenia coli. *Čs. Fysiol.* (In Slovak), **33,** 179–180.

SHUBA, M.F. & KLEVETZ, M.J. (1967). Ionic mechanism of inhibitory action of adrenaline and noradrenaline on smooth muscle cells. *Fiziol. Zh. Kiev.*, **13,** 3–11.

TOMITA, T., SAKAMOTO, Y. & OHBA, M. (1974). Conductance increase by adrenaline in guinea-pig taenia coli studied with voltage clamp method. *Nature*, **250,** 432–433.

VIZI, E.S. (1979). Presynaptic modulation of neurochemical transmission. *Prog. Neurobiol.*, **12,** 181–190.

WALSH, J.V. & SINGER, J.J. (1980). Penetration-induced hyperpolarization as evidence for Ca^{2+} activation of K^+ conductance in isolated smooth muscle cells. *Am. J. Physiol.*, **239** (Cell Physiol. 8), C182–C189.

WIEGEL, R.J., CONNOR, J.A. & PROSSER, C.L. (1979). Two roles of calcium during the spike in circular muscle of small intestine in cat. *Am. J. Physiol.*, **237** (Cell Physiol. 6), C247–C256.

WIKBERG, J. (1978). Differentiation between prejunctional and post-junctional alpha receptors in guinea-pig ileum and rabbit aorta. *Acta physiol. scand.*, **103,** 225–239.

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