4-Aminopyridine-Induced Changes in the Electrical and Contractile Activities of the Gastric Smooth Muscle

K. Boev, A. Bonev and M. Papasova

Institute of Physiology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., bl. 23, 1113 Sofia, Bulgaria

Abstract. Effects of 4-aminopyridine (4-AP) on the electrical and contractile activities of the fundus and antrum of the cat stomach were studied using the sucrose-gap technique. In the fundus, low concentrations of 4-AP (up to 1 mmol/l) induced membrane depolarization and appearance of spike potentials and phasic contractions. After preliminary administration of atropine, 4-AP produced an opposite effect: hyperpolarization and relaxation. On the background of tetrodotoxin (TTX) plus antagonists of cholinergic and adrenergic receptors, high concentrations of 4-AP (>5 mmol/l) caused membrane depolarization and appearance of spike potentials and phasic contractions. In the antrum, 4-AP in low concentrations (up to 1 mmol/l) decreased both the amplitude and the duration of the second component of the plateau-action potential, as well as those of the phasic contractions. This effect decreased in the presence of adrenergic receptor antagonists and was abolished by TTX. On this background, high concentrations of 4-AP (>5 mmol/l) led to the appearance of spike potentials superimposed on the second component of the plateau-action potentials, and to a further increase in the phasic contraction amplitudes. The present data suggest that 4-AP exerts its effects via an increase in neurotransmitter release (low concentrations) and/or directly on the smooth muscle cell membrane (high concentrations).

Key words: Smooth muscle — Stomach — 4-aminopyridine — Atropine — Tetrodotoxin

Introduction

It is known that the specific K-channels-blocking agent, 4-aminopyridine (4-AP), modulates the activity of the excitable cells of striated muscle (Gillespie and Hutter 1975; Fink and Wettewer 1978), heart muscle (Kenyon and Gibbons 1979) and the giant axon (Meves and Pichon 1975; Yeh et al. 1976). Low concentrations (up to 1 mmol/l) of 4-AP were also found to increase the contractile activity of the smooth muscle due to an increased release of neurotransmitters (Johns et al. 1976; Ito et al. 1980; Hara et al. 1980). Papasova et al. (1982) reported a 4-AP-induced activation or inhibition of electrical and contractile activities of muscle strips

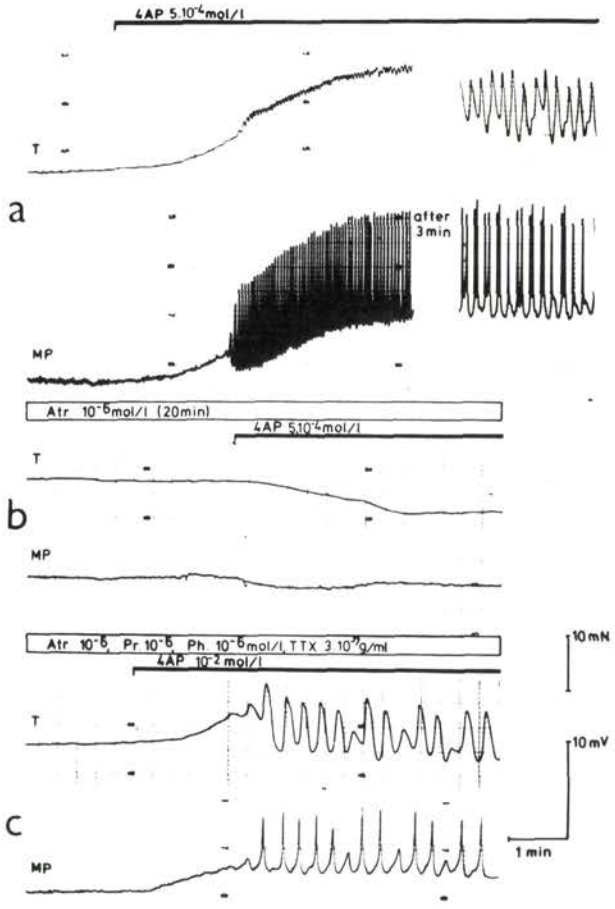

Fig. 1. Effect of 4-aminopyridine (4-AP) on spontaneously inactive preparations from cat stomach fundus. *a* — control condition; *b* — after atropine (Atr); *c* — effect of high concentrations of 4-AP following pretreatment with tetrodotoxin (TTX) plus atropine (Atr), propranolol (Pr) and phentolamine (Ph). Upper trace (T) — muscle tension; lower trace (MP) — membrane potential.

isolated from different stomach regions. These effects of 4-AP might be associated with the release of different neurotransmitters, depending on the stomach region studied. In the present work we attempted to evaluate the effects of low and high concentrations of 4-AP on smooth muscle strips isolated from the fundus and antrum of the cat stomach. We also studied changes in cell excitability following the administration of 4-AP under current clamp conditions.

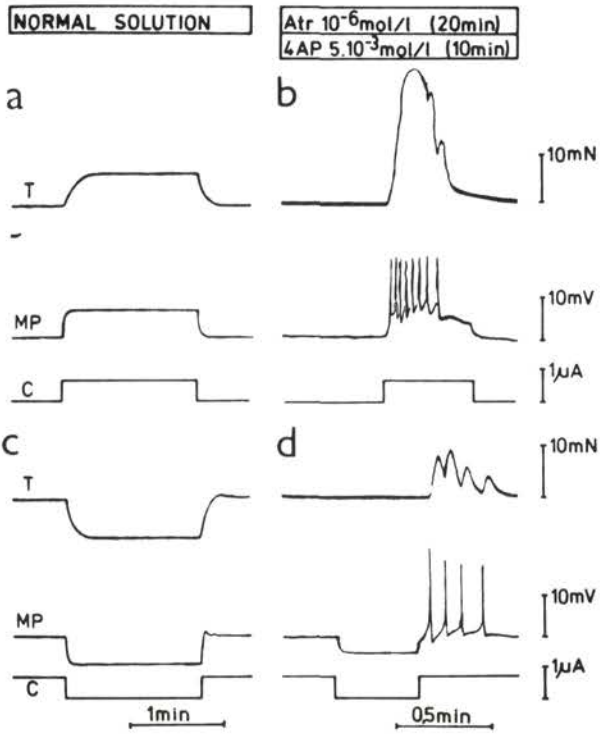

Fig. 2. Electrical and contractile responses of fundic smooth muscles to direct electrical stimulation under current-clamp conditions. *a*, *c* — control condition; *b*, *d* — in the presence of 4-aminopyridine (4-AP) following pretreatment with atropine (Atr). T — muscle tension; *MP* — membrane potential; C — *polarizing currents*.

Materials and Methods

Cats of both sexes were used. The animals were let to starve for 24 hours and the stomachs were subsequent removed under chloralose (60—70 mg/kg) anaesthesia. Circular muscle strips, 20 × 2 mm, were cut from the fundus and antrum. Electrical and contractile activities were simultaneously recorded by the sucrose-gap technique (Boev and Golenhofen 1974). The preparations were allowed to equilibrate for 80—120 min before any measurements were started. The modified Krebs solution contained (in mmol/l): Na^+ 137.5; K^+ 5.94; Mg^{2+} 1.2; Ca^{2+} 2.5; Cl^- 134.15; $H_2PO_4^-$ 1.19; HCO_3^- 15.5; glucose 11.5.

Drugs used: 4-aminopyridine (Merck-Schuchardt), atropine sulphate (Merck), propranolol (Sigma), phentolamine (Hoffman—La Roche), tetrodotoxin (Sankyo), methoxyverapamil (D600) (Knoll).

Results

The fundus preparations did not show spontaneous electrical or contractile activities. Within the initial 2—3 min low concentrations (up to 1 mmol/l) of 4-AP

produced depolarization with superimposed high (10—15 mV) spike potentials. This effect was manifested by an increase in the tone and by the appearance of phasic contractions preceded by spike potentials (Fig. 1*a*). Following pretreatment by atropine, 4-AP in low concentrations induced slight hyperpolarization and muscle relaxation (Fig. 1*b*). Tetrodotoxin (TTX) in the presence of antagonists of cholinergic and adrenergic receptors abolished all effects of low 4-AP concentrations. On this background, high concentrations of 4-AP (>5 mmol/l) induced depolarization and appearance of spike potentias and phasic contractions (Fig. 1*c*).

Under control conditions depolarizing currents produced membrane depolarization and gradual tonic contractions (Fig. 2*a*). Hyperpolarizing currents produced an opposite effects (Fig. 2*c*). However, following the administration of 4-AP in the presence of atropine, both the switching on (Fig. 2*b*) and off (Fig. 2*d*) of polarizing currents resulted in the appearance of spike potentials and phasic contractions of spontaneously inactive fundic strips.

Under control conditions the antrum smooth muscle generated spontaneous plateau-action potentials accompanied by low-amplitude phasic contractions. Low concentrations (up to 1 mmol/l) of 4-AP produced hyperpolarization and decreased both the amplitude and the duration of the second component of the plateau-action potential as early as within the initial few minutes following the drug application (Fig. 3*a*). This was accompanied by a decrease in the amplitude of the phasic contractions. The effect of low 4-AP concentrations decreased in the presence of adrenergic receptor antagonists and was not observed in preparations pretreated with TTX plus antagonists of cholinergic and adrenergic receptors (Fig. 3*b*). However, higher concentrations (5—10 mmol/l) of 4-AP produced membrane depolarization and appearance of bursts of spike potentials superimposed on the second component of the plateau-action potential. The appearance of spike potentials led to a further increase in amplitude of phasic contractions (Fig. 4*a*). Methoxyverapamil (D600) antagonized the effect of 4-AP in the antrum smooth muscle: it inhibited the spike potentials, and decreased the amplitude of phasic contractions (Fig. 4*b*).

Discussion

The effect of 4-AP on excitable cells is explained by its ability to inhibit the K-outward current which in turn results in an increased Ca^{2+} influx through the cell membrane (Lundh and Thesleff 1977). The mechanism of action of 4-AP is very similar to that of tetraethylammonium (Haeusler et al. 1980). It has been suggested that nerve terminals are more sensitive to 4-AP compared to smooth muscle cells (Ito et al. 1980). Changes observed in the electrical and contractile activities of the gastric smooth muscle under the effect of low 4-AP concentrations in the present

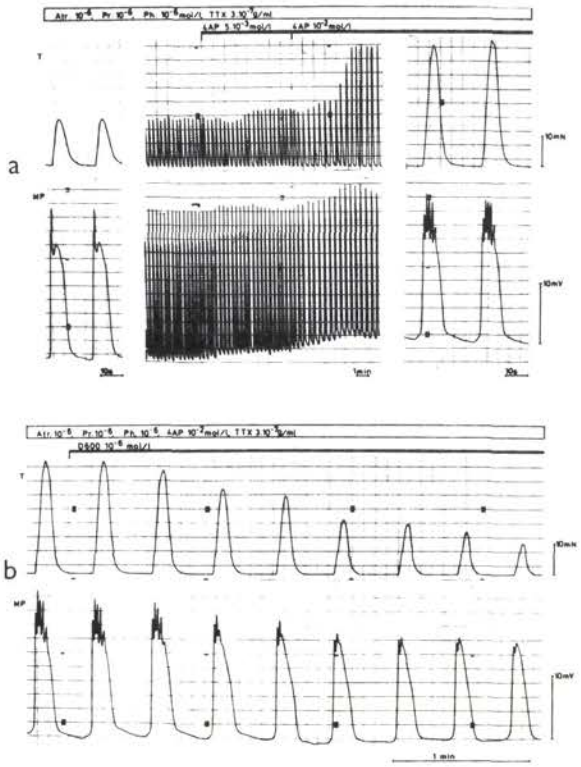

Fig. 3. Effect of 4-aminopyridine (4-AP) on the spontaneous electrical and contractile activities of cat stomach antrum. *a* — control condition; *b* — after atropine (Atr), propranolol (Pr), phentolamine (Ph) and tetrodotoxin (TTX). Upper trace (T) — muscle tension; lower trace (MP) — membrane potential.

study were likely due to an increased neurotransmitter release. In the fundic smooth muscle 4-AP produced depolarization, appearance of spike potentials and phasic contractions, while in the antrum it induced hyperpolarization and decrease in both the amplitude and the second component of the spontaneous plateau-action potential. A pharmacological analysis showed that 4-AP induced neurotransmitter release from cholinergic, adrenergic and non-adrenergic, non-cholinergic nerve terminals. Hara et al. (1980) and Ito et al. (1980) observed a potentiating effect of 4-AP on noradrenaline release. The inhibitory effect of 4-AP in the antrum is probably due to an increase in noradrenaline release. This view is supported by the fact that antagonists of adrenergic receptors reduced the 4-AP effect. The latter becomes completely abolished after TTX, suggesting the participation of non-adrenergic, non-cholinergic nerve terminals.

In the fundic smooth muscle, 4-AP affects mainly cholinergic nerve terminals,

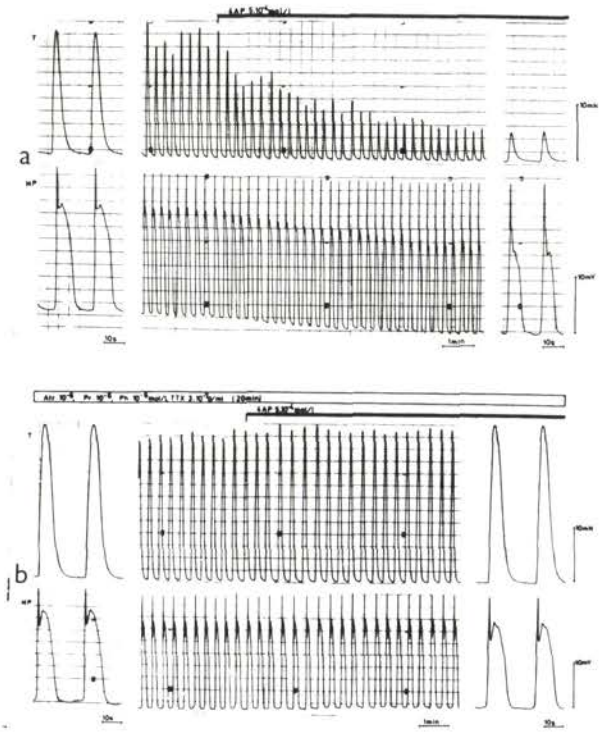

Fig. 4. Effect of high concentrations of 4-aminopyridine (4-AP) on the spontaneous electrical and contractile activities of the antrum following pretreatment with atropine (Atr), propranolol (Pr), phentolamine (Ph) and tetrodotoxin (TTX) (*a*); and methoxyverapamil (D600) (*b*).

as shown by depolarization and the appearance of spike potentials and phasic contractions. The finding that after atropine pretreatment 4-AP produces hyperpolarization and relaxation of the smooth muscle suggest an additional effect of 4-AP on adrenergic nerve terminals which are sensitive to adrenoreceptor blockers. The differences in the effects of 4-AP between the antrum and fundus could be related to different ratios of nerve terminals between various stomach regions (Lolova and Papasova 1981).

It is known that 4-AP inhibits the K-outward currents in both the nerve terminals and the smooth muscle cell membrane (Mironneau and Savineau 1980; Imaizumi and Watanabe 1983). Thus, the effects of high 4-AP concentrations might be explained by the direct action of 4-AP on the cell membrane of the gastric smooth muscle. In vitro the fundus smooth muscle do not exhibit spontaneous electrical and contractile activities and have a lower electrical excitability. In voltage-clamp experiments, Bury and Boev (1980) could show that this lower

electrical excitability of the fundic cells was due to the early activation of the channels determining the K-outward currents. The inhibition of the K-outward currents by high concentrations of 4-AP may create conditions for an increase in the cell excitability as well as for the appearance of spike potentials in spontaneously inactive fundic muscle.

References

Boev K., Golenhofen K. (1974): Sucrose-gap technique with pressed rubber membranes. Pflügers Arch. **349**, 227—283

Bury V., Boev K. (1980): Studies on the transmembrane ion currents in the smooth muscle cells of the gastric fundus. Experientia **36**, 216—217

Fink R., Wettewer E. (1978): Modified K-channel gating by exhaustion and the block by internally applied TEA and 4-aminopyridine in muscle. Pflügers Arch. **374**, 289—292

Gillespie J. I., Hutter O. F. (1975): The action of 4-AP on the delayed potassium current in skeletal muscle fibres. J. Physiol. (London) **252**, 70—71

Haeusler G., Kuhn H., Thorens S. (1980): The effect of tetraethylammonium chloride on calcium fluxes in smooth muscle from rabbit main pulmonary artery. J. Physiol. (London) **303**, 225—241

Hara I., Kitamura K., Kuriyama H. (1980): Actions of 4-aminopyridine on vascular smooth muscle tissues of the guinea pig. Brit. J. Pharmacol. **68**, 99—106

Imaizumi Y., Watanabe M. (1983): Effect of 4-aminopyridine on potassium permeability of canine tracheal smooth muscle cell membrane. Jpn. J. Pharmacol. **33**, 201—208

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. Brit. J. Pharmacol. **69**, 453—460

Johns J., Golks D. S., Lanson P. A., Paton D. M. (1976): The potentiating effects of 4-aminopyridine on adrenergic transmission in the rabbit vas deferens. J. Pharmacol. **38**, 71—78

Kenyon J., Gibbons W. R. (1979): 4-aminopyridine and the early outward current of sheep cardiac Purkinje fibres. J. Gen. Physiol. **73**, 139—157

Lolova I., Papasova M. (1981): Comparative quantitative estimation of the granular vesicles in the intramural ganglia of Auerbach's plexus in the nonsphincter and sphincter parts of cat alimentary tract. Acta Physiol. Pharmacol. Bulg. **7**, 50—59

Lundh H., Thesleff S. (1977): The mode of action of 4-aminopyridine and guanidine on transmitter release from motor nerve terminals. Eur. J. Pharmacol. **42**, 411—412

Meves H., Pichon Y. (1975): Effects of 4-aminopyridine on the potassium current in internally perfused giant axons of squid. J. Physiol. (London) **251**, 60P—62P

Mironneau J., Savineau J. P. (1980): Effects of calcium on outward membrane currents in rat uterine smooth muscle. J. Physiol. (London) **302**, 411—425

Papasova M., Bonev A., Boev K., Zafirov D., Gachilova S. (1982): Effect of 4-aminopyridine on neurotransmission in smooth muscle. Agressologie **23**, 99—103

Yeh J. Z., Oxford G. S., Wu C. H., Narahashi T. (1976): Dynamics of aminipyridine block of potassium channels in squid axon membrane. J. Gen. Physiol. **68**, 519—535

Received July 23, 1984/Accepted November 15, 1984