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THE EFFECTS OF 4-AMINOPYRIDINE ON THE ISOLATED VAS DEFERENS AND ITS EFFECTS ON THE INHIBITORY PROPERTIES OF ADENOSINE, MORPHINE, NORADRENALINE AND γ-AMINOBUTYRIC ACID

T.W. STONE

Department of Physiology, St. George's Hospital Medical School, University of London, London SW17 0RE

1 Adenosine, adenosine 5′-triphosphate (ATP), morphine, noradrenaline, γ-aminobutyric acid (GABA) phentolamine and amyl nitrite were used to inhibit electrically-evoked contractions of the isolated superfused vas deferens of the mouse.

2 The inhibitory effects of adenosine ATP, morphine, noradrenaline and GABA, which are thought to be due to presynaptic action, were reduced by perfusion with media containing 4-aminopyridine (4AP) or tetraethylaminonium (TEA) ions. The inhibitory effects of phentolamine and amyl nitrite were unaffected by 4AP or TEA.

3 Quinidine, which like 4AP and TEA produced some increase of twitch height, did not reduce responses to the various agonists, indicating that an increased muscle contraction was not itself responsible for the reduced responses.

4 It is concluded that antagonism between 4AP and adenosine is not a specific interaction, as had been suggested, but probably reflects an interaction with Ca^{2+} requiring processes in the presynaptic terminal.

Introduction

An examination of the effects of 4-aminopyridine (4AP) on neurones in the cerebral cortex has led to the suggestion that 4AP might act as an antagonist of the effects of adenosine, thus accounting for its suppression of potassium conductances, and increased transmitter release (Perkins & Stone, 1980; Stone, 1981). This possibility has now been investigated more directly in the isolated vas deferens of the mouse, in which adenosine depresses transmitter release. Adenosine has been compared with morphine, noradrenaline and γ-aminobutyric acid (GABA) which also depress transmitter release in the mouse vas (Henderson, Hughes & Kosterlitz, 1972; Jenkins, Marshall & Nasmyth, 1977; Bowery, Doble, Hill, Hudson, Shaw & Turnbull, 1979) and wth nitrites, thought to relax smooth muscle directly, and phentolamine which blocks transmitter action postsynaptically (Jones & Spriggs, 1975).

It has also been noted that quinidine, like 4AP, increases the size of electrically evoked contractions of the vas deferens and the effects of quinidine and 4AP on the agonist responses have therefore been compared.

Methods

Mice (TO strain) were killed by stunning and cervical dislocation and the vasa deferentia removed into cold medium of the following composition (mM): NaCl 118, KCl 4.70, $CaCl_2$ 2.48, $NaHCO_3$ 24, KH_2PO_4 0.85 and glucose 11.

Adhering connective tissue and blood vessels were removed from one vas and the tissue gently compressed to express luminal semen. The vas was transferred into a 5 ml organ bath through which solution of the above composition was perfused continuously at a rate of 3 ml per min. The solution was warmed before entering the bath so that the bath temperature was maintained between 35 and 37°C. The solution in the bath was gassed with a 95% O_2/5% CO_2 mixture.

The vas was attached to an isometric strain gauge transducer under a resting tension of approximately 0.5 g. Contractions of the vas were recorded on a Devices M4 pen recorder. Field stimulation of the vas was effected by a pair of parallel platinum wires placed on either side of the preparation, using pulses of 1–2 ms duration and supramaximal voltage (normally 80 V), delivered at 0.1 Hz.

Quinidine, 4AP and tetraethylammonium (TEA) were added to the perfusing solution at the required concentration. Other drugs were added directly into the bath in volumes of 0.05 or 0.1 ml. The concentrations quoted below are the estimated final concentrations attained in the bath.

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Results

Adenosine, morphine, adenosine 5'-triphosphate, noradrenaline, GABA, amyl nitrite and phentolamine all reduced the twitch height of the field stimulated vas deferens and representative records are shown in Figure 1. Dose-response curves were obtained for all compounds except ATP and nitrite (Figure 2). Dose-response curves could not be reliably obtained with nitrite because of the long duration of responses and difficulty in suspending the amyl nitrite in aqueous media. Responses were therefore compared for a single submaximal concentration of 250 μM (Figure 1).

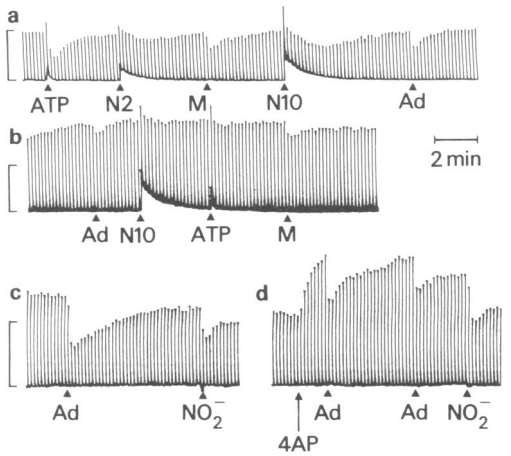

Figure 1 Sections of chart recordings showing the inhibitory effects of ATP 10 μM (ATP), noradrenaline 2 μM (N2) and 10 μM (N10), morphine 1 μM (M) and adenosine 5 μM (Ad). (a) Shows normal responses to these compounds; (b) shows the reduced inhibitory responses to adenosine, noradrenaline, ATP and morphine obtained in the presence of 4-aminopyridine (4AP) 10 μM. Note that the contractions produced by ATP and noradrenaline were not reduced by 4AP, and on occasions, such as this, showed a small increase. (c) Control responses to adenosine 10 μM and amyl nitrite 250 μM (NO_2^-). (d) Increase in twitch height produced as the perfusion medium containing 4AP 10 μM reached the vas and reduction of responses to adenosine, while the nitrite response is unchanged. The percentage inhibition by nitrite is 37% in (c) and 40% in (d). Calibrations: 200 mg tension; 2 min time bar.

4-Aminopyridine

During perfusion with medium containing 4AP, 10 μM, 100 μM or 1 mM, the twitch height was increased 62% (±11, s.e.; $n = 4$), 113% (±16 $n = 6$) and 192% (±24, $n = 6$) while responses to adenosine, morphine, noradrenaline, ATP and GABA were reduced in amplitude (Figures 1 and 2). The ID_{50} values for adenosine and morphine were correspondingly increased, while the maximum effect was greatly reduced from 100% inhibition of twitch height in the case of adenosine to a 42% reduction (Figures 1 and 2).

In contrast to these changes, the percentage depression of twitch height produced by nitrite or phentolamine was unaffected by the presence of 4AP (Figures 1 and 2).

Tetraethylammonium

Perfusion with TEA bromide 1 mM produced a large increase of twitch height (326% ± 51, $n = 6$) and also reduced the responses to adenosine, morphine and noradrenaline but not to phentolamine or nitrite (Figure 3).

Effect of quinidine

At concentrations of 10 μM or 50 μM quinidine initially increased twitch height by 85% (± 16, $n = 6$) and 125% (± 15, $n = 6$) respectively. However, as illustrated in Figures 4 and 5, the inhibitory effects of adenosine, morphine and noradrenaline were unchanged by quinidine or slightly enhanced compared to control responses.

4AP, 10 μM, had little effect on the contractile responses of the vas to noradrenaline or ATP applied exogenously (Figure 6) whereas quinidine 10 μM reduced responses to both agonists, noradrenaline being the more affected (Figure 4). Full dose-response curves were not constructed because desensitization to the excitant actions of these agonists causes problems of quantitation. However, contractions to standard concentrations of ATP (10 μM) were reduced to 76.9% (± 3.8, $n = 6$) while responses to 1 μM noradrenaline were abolished and responses to 10 μM noradrenaline were reduced to 20.9% (± 4.8, $n = 6$) (Figure 6).

Potassium chloride

In the absence of electrical stimulation, the addition of KCl to the organ bath to a final concentration of 10 μM causes an initial contraction, usually followed by the development of a degree of rhythmic activity which remain until the K^+ concentration was restored to normal. This increased activity is thought to reflect increased excitability of the smooth muscle. When perfusing vasa with 4AP (10 μM) or TEA (1 mM), no difference was noted between the KCl-induced activity in such preparations compared with controls. However, in the presence of quinidine (50 μM) a substantial increase in the amplitude and duration of this activity was seen. It is extremely difficult to quantitate the KCl activity but visual inspection suggested that an approximately 3 fold increase occurred in the total time occupied by KCl-induced activity in the presence of quinidine.

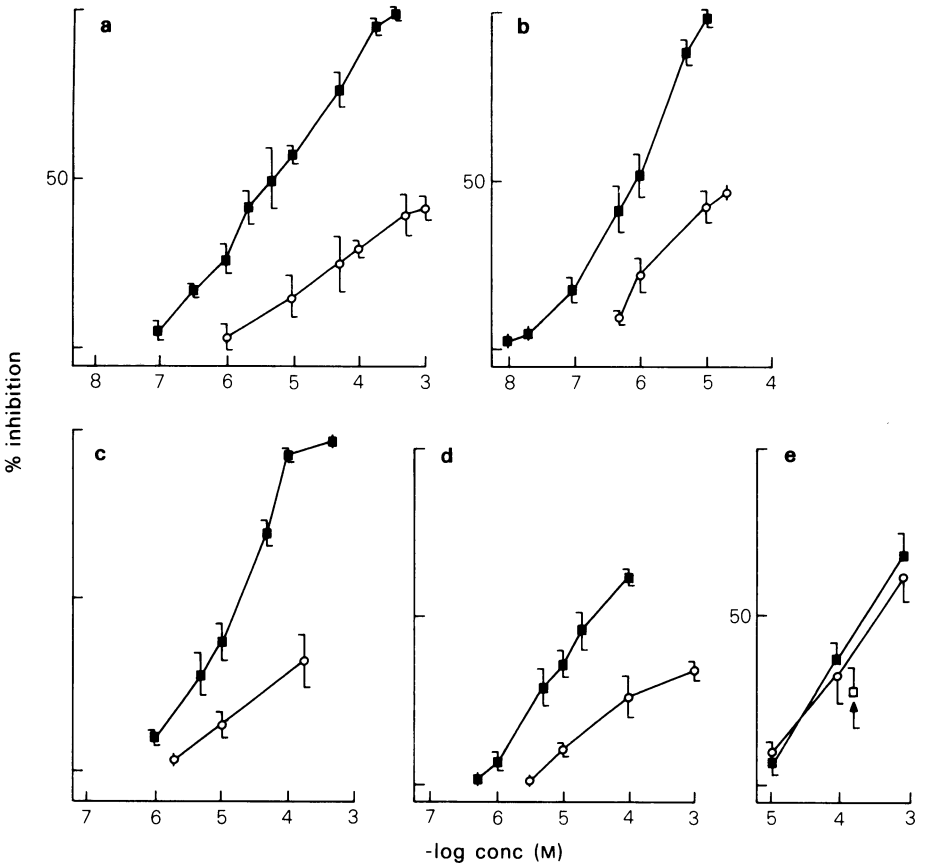

Figure 2 Dose-response curves for the inhibition of electrically-evoked contractions of the mouse vas deferens by (a) adenosine, (b) morphine, (c) noradrenaline, (d) GABA and (e) phentolamine (■, ○) and amyl nitrite (▲, □). In each case the control curves are shown by solid symbols. The open symbols indicate responses in the presence of 4-aminopyridine 100 μM. Each point is the mean of at least 4 responses; vertical lines show s.e. mean. All points on the control and 4-AP curves are significantly different in (a), (b), (c) and (d) ($P < 0.05$; Student's t test). None of the points in (e) are significantly different. The ordinate scale is percentage inhibition of the twitch; the abscissa scale is the negative log of the molar concentration.

Discussion

The presence of 4AP clearly brings about a reduction of the inhibitory effects of adenosine, ATP, morphine, noradrenaline and GABA on the mouse isolated vas deferens. These agonists have in common that their site of action in depressing the size of the electrically-evoked twitch is presynaptic (Henderson *et al.*, 1972; Henderson & Hughes, 1976; Clanachan, Johns & Paton, 1977; Jenkins *et al.*, 1977; Marshall, Nasmyth, Nicholl & Shepperson, 1978, Bowery *et al.*, 1979; Stone, 1981). Amyl nitrite however, is known to have direct relaxant effects on smooth muscle, and phentolamine reduces twitch height by a postsynaptic blockade of neurally released noradrenaline (Henderson *et al.*, 1972; Jones & Spriggs, 1975). These two compounds which were unaffected by 4AP, have a different site of action, implying that the interaction between 4AP and the other agonists occurs at a presynaptic site.

4AP has been shown to block K^+ channels in neural membranes, thus causing a prolongation of the action potential with, in nerve terminals, consequent increase in the Ca^{2+} influx and thus transmitter release (Llinas, Walton & Bohr, 1976; Illes & Thesleff, 1978; Horn, Lambert & Marshall, 1979; Thesleff, 1980). The presynaptic inhibitory effects of adenosine, morphine and noradrenaline have been shown to be reduced by procedures which enhance Ca^{2+} influx, such as increasing the extracellular Ca^{2+} concentration or increasing the frequency of electrical stimulation (Drew, 1978; Bennett & Lavidis, 1980; Stone, 1981). It is therefore highly probable that the blockade of these agonists' effects by 4AP is the consequence of

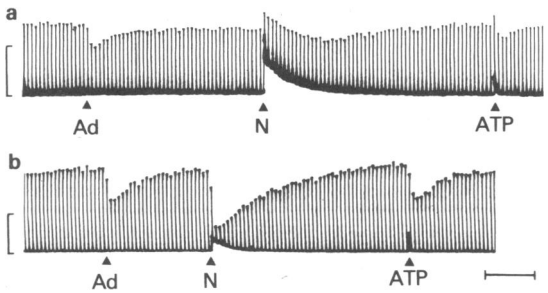

Figure 4 Sections of chart recordings showing the effects of adenosine 10 μM (Ad), noradrenaline 10 μM (N) and ATP 10 μM on the mouse vas deferens. (a) Control responses; (b) responses of the same preparation during perfusion with quinidine 50 μM. Note the increase of twitch height, with the inhibitory responses to adenosine and ATP being typically unchanged or enhanced. The adenosine depression is 27% in (a) and 35% in (b). Note also that the large increase in the inhibitory effect of noradrenaline is probably partly due to the reduction of the contractile response. Calibration 200 mg and 2 min.

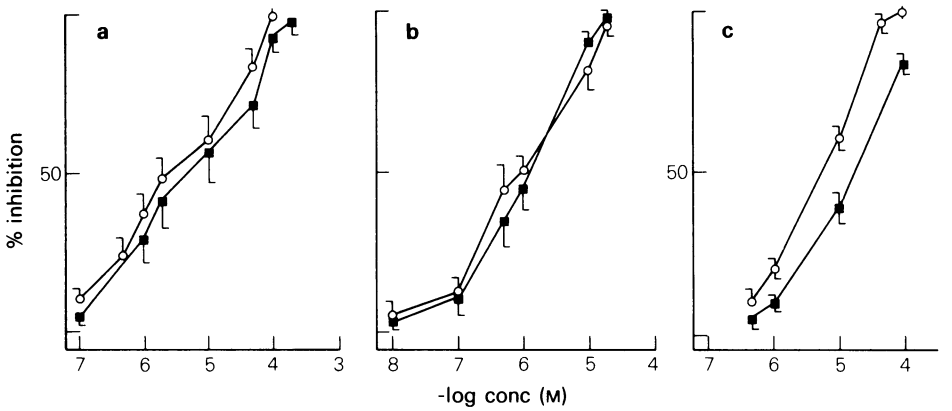

Figure 5 Dose-response curves for the inhibitory effects of (a) adenosine, (b) morphine and (c) noradrenaline on the mouse vas deferens. Control curves are shown by solid symbols, responses in the presence of quinidine 50 μM by open symbols. Details as for Figure 2. None of the points in (a) and (b) are significantly different on the two curves. All points except the lowest are significantly different in (c) ($P < 0.05$; Student's t test).

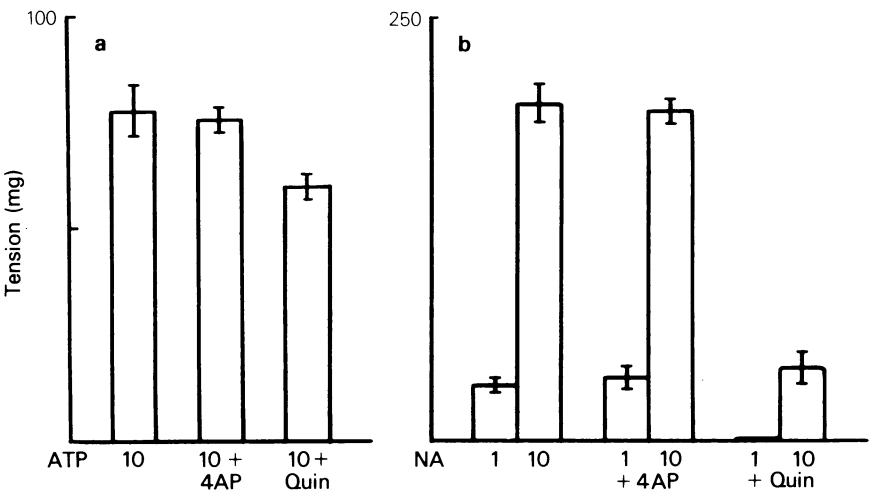

Figure 6 Histograms showing the size of contractile responses to (a) ATP 10 μM and (b) noradrenaline 1 and 10 μM, in control conditions, and in the presence of 4-aminopyridine (4AP) 100 μM, or quinidine 50 μM. Quinidine but not 4AP, produced a significant reduction of these responses ($P < 0.05$; $n = 6$; Student's t test).

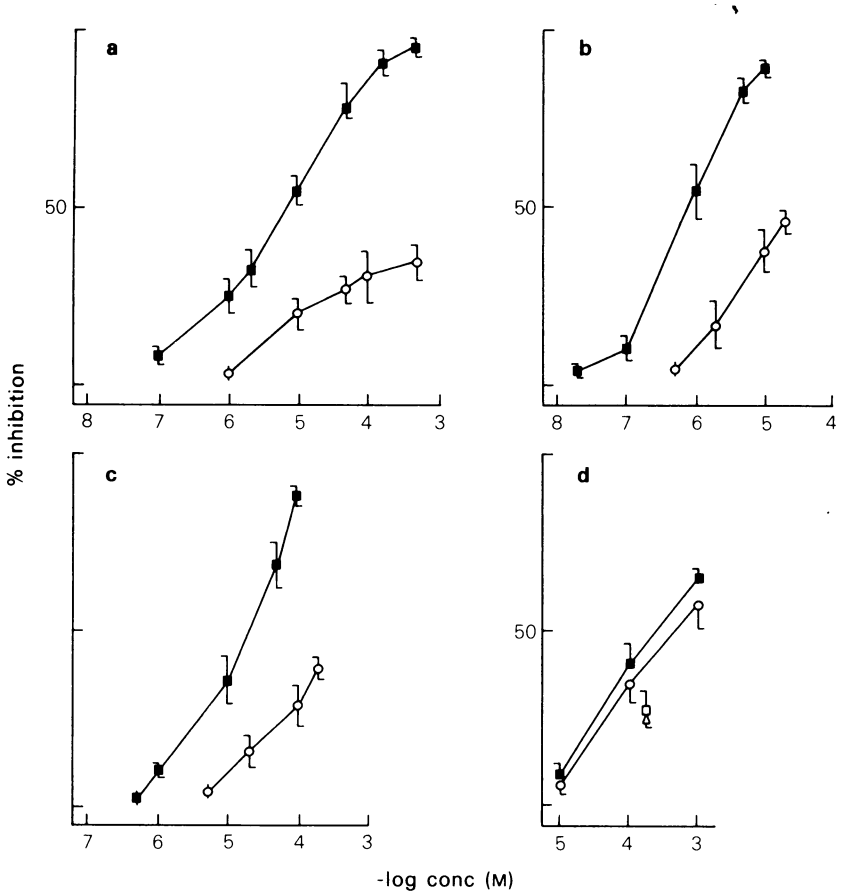

Figure 3 Dose-response curves for (a) adenosine, (b) morphine, (c) noradrenaline and (d) phentolamine (■, ○) and amyl nitrite (▲, □). Control curves are shown by solid symbols, the open symbols indicate responses in the presence of tetraethylammonium (TEA) 1mM. Details as for Figure 2. All points in (a), (b) and (c) are significantly different on control and TEA curves ($P < 0.05$; Student's t test). None of the points in (d) are significantly different.

the changes of Ca^{2+} concentrations, rather than a receptor interaction.

This conclusion is supported by the similarity of action of TEA and 4AP, as the former compound also blocks K^+ channels and increases Ca^{2+} influx (Hille, 1970; Thompson, 1977; Meves & Pichon, 1977; Hermann & Gorman, 1979). Quinidine on the other hand does not have this action and, while we have found it to increase twitch size, it does not reduce the responses to any of the agonists examined. Indeed the responses to adenosine and noradrenaline may even be slightly enhanced, though a definite conclusion is made difficult for noradrenaline, as the blockade by quinidine of the amine's excitatory effect tends to increase the apparent inhibition.

The increase of twitch size *per se* therefore is unlikely to be responsible for the reduced agonist responses produced by 4AP and TEA. Although it is not yet entirely clear why quinidine should increse twitch size in the first place, as it appears to reduce Na^+ permeability in some tissues (Brown, Giles, Hume & Lee, 1980), the apparent potentiation of the KCl-evoked activity of the vas strongly suggests that quinidine is increasing directly the excitability of the muscle. This may also be taken to support indirectly the concept of a presynaptic site of interaction of 4AP with adenosine, morphine and noradrenaline.

Thus the antagonism of 4AP towards adenosine responses noted by Perkins & Stone (1980) in the central nervous system, is probably not a unique interaction but reflects the interaction of compounds having opposite effects on Ca^{2+} movements. As a corollary to this, the depressant effects of adenosine on central neurones, (Phillis, Kostopoulos & Limacher, 1974; Stone & Taylor, 1977) may well result from a presynaptic site of action (Edstrom & Phillis, 1976; Stone, 1981).

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References

BENNETT, M.R. & LAVIDIS, N.A. (1980). An electrophysiological analysis of the effects of morphine on the calcium dependence of neuromuscular transmission in the mouse vas deferens. *Br. J. Pharmac.*, **69**, 185–191.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S. & TURNBULL, M.J. (1979). Baclofen; a selective agonist for a novel type of GABA receptor. *Br. J. Pharmac.*, **67**, 444–445P.

BROWN, A.M., GILES, W., HUME, J.R. & LEE, K.S. (1980). Voltage clamp analysis of lidocaine and quinidine induced depression of the sodium current in isolated rat ventricular cells. *J. Physiol.*, **307**, 62–63P.

CLANACHAN, A.S., JOHNS, A. & PATON, D.M. (1977). Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in rat vas deferens. *Neuroscience*, **2**, 597–602.

DREW, G.M. (1978). The effect of different calcium concentrations on the inhibitory effect of presynaptic α-adrenoceptors int he rat vas deferens. *Br. J. Pharmac.*, **63**, 417–419.

EDSTROM, J.P. & PHILLIS, J.W. (1976). The effects of AMP on the potential of rat cerebral cortical neurones. *Can. J. Physiol. Pharmac.*, **54**, 787–790.

HENDERSON, G. & HUGHES, J. (1976). The effects of morphine on th release of noradrenaline from the mouse vas deferens. *Br. J. Pharmac.*, **57**, 551–557.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuroeffector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmac.*, **46**, 764–766.

HERMANN, A. & GORMAN, A.L.F. (1979). External and internal effects of TEA on voltage-dependent and calcium-dependent K^+ current components in molluscan pacemaker neurones. *Neurosci. Lett.*, **12**, 87–92.

HILLE, B. (1970). Ionic channels in nerve membranes. *Progr. Biophys. mol. Biol.*, **21**, 1–32.

HORN, A.S., LAMBERT, J.J. & MARSHALL, I.G. (1979). A comparison of the facilitatory actions of 4-aminopyridine methiodide and 4-aminopyridine on neuromuscular transmission. *Br. J. Pharmac.* **65**, 53–62.

ILLES, P. & THESLEFF, S. (1978). 4-aminopyridine and evoked transmitter release from motor nerve endings. *Br. J. Pharmac.*, **44**, 623–629.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1977). An inhibitory role for noradrenaline in the mouse vas deferens. *Br. J. Pharmac.*, **61**, 649–655.

JONES, M.E.L. & SPRIGGS, T.L.B. (1975). Noradrenaline and motor transmission in the vas deferens of the mouse. *Br. J. Pharmac.*, **53**, 323–331.

LLINAS, R., WALTON, K. & BOHR, V. (1976). Synaptic transmission in squid giant synapses after K^+-conductance blockade with external 3- and 4-aminopyridine. *Biophys. J.*, **16**, 83–86.

MARSHALL, I., NASMYTH, P.A., NICHOLL, C.G. & SHEPPERSON, N.B. (1978). α-adrenoceptors in the mouse vas deferens and their effects on its responses to electrical stimulation. *Br. J. Pharmac.*, **62**, 147–151.

MEVES, H. & PICHON, Y. (1977). The effect of internal and external 4-aminopyridine on the potassium currents in intracellularly perfused squid giant axons. *J. Physiol.*, **268**, 511–532.

PERKINS, M.N. & STONE, T.W. (1980). 4-aminopyridine blockade of neuronal depressant responses to ATP. *Br. J. Pharmac.*, **70**, 425–428.

PHILLIS, J.W., KOSTOPOULOS, G.K. & LIMACHER, J.J. (1974). Depression of corticospinal cells by various purines and pyrimidines. *Can. J. Physiol. Pharmac.*, **52**, 1226–1229.

STONE, T.W. (1981). Physiological roles for adenosine and ATP in the nervous system. *Neuroscience*, **6**, 523–555.

STONE, T.W. & TAYLOR, D.A. (1977). Microiontophoretic studies on the effects on cyclic nucleotides on excitability of neurones in the rat cerebral cortex. *J. Physiol.*, **226**, 523–543.

THESLEFF, S. (1980). Aminopyridines and synaptic transmission. *Neuroscience*, **5**, 1413–1420.

THOMPSON, S.H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol.*, **265**, 465–488.

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