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INTERACTION BETWEEN ADENOSINE TRIPHOSPHATE AND NORADRENALINE IN THE ISOLATED VAS DEFERENS OF THE GUINEA-PIG

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1 An interaction between adenosine triphosphate (ATP) and noradrenaline (NA) was investigated in the isolated vas deferens of the guinea-pig.

2 ATP (1.7 to 50 μM) enhanced contractions due to transmural electrical stimulation (ES; parameters: 30 Hz, 0.2 to 0.3 ms, 10 to 30 V; 1 s train duration). Responses to exogenous NA (12 μM) were also potentiated by ATP while contractions to acetylcholine (6 μM) were inhibited.

3 NA (1.2 μM) potentiated ATP-induced contractions and prevented the development of tachyphylaxis to ATP (510 μM).

4 Phentolamine (12.5 μM) prevented the potentiation by NA of ATP-induced contractions; these contractions were insensitive to phentolamine (up to 25 μM).

5 Removal of potassium chloride from the Tyrode solution for 10 min abolished the potentiating actions of both ATP and NA.

6 The present results suggest that the effect of ATP may be functionally closely related to that of NA at α-adrenoceptors in the vas deferens of the guinea-pig.

Introduction

The vas deferens contains an unusually high concentration of noradrenaline (NA) which is about 10 to 20 times that found in the brain, kidney, uterus and spleen, and five times that in the heart (Swedin, 1971; Holzbauer & Sharman, 1972). NA stored in the vesicular compartment of adrenergic neurones is closely bound in a macromolecular complex with adenosine triphosphate (ATP), calcium and a protein component (Hilarp, 1958; Winkler & Hörtnagl, 1973), although the molar ratios are still controversial (Winkler, 1976; Van Dyke, Robinson, Urquilla, Smith, Taylor, Trush & Wilson, 1977). There is increasing evidence that ATP may regulate the contractility of sympathetically innervated smooth muscle.

The picture of neurotransmission in the vas deferens differs considerably from that in most smooth muscle organs and is the subject of an unsolved controversy. Although the postganglionic innervation of this organ has been classified anatomically as sympathetic and physiologically as predominantly adrenergic (Huković, 1961; Burnstock & Holman, 1961; Birmingham & Wilson, 1963; Sjöstrand, 1965; Jones & Spriggs, 1975), some pharmacological results disagree with the prevalent view that the motor transmitter is noradrenaline (Ambache & Zar, 1971; Euler & Hedqvist, 1975). The unidentified motor transmitter may be released from the same neurone as noradrenaline and its release may be under the control of noradrenaline (Jenkins, Marshall & Nasmyth, 1976).

During nerve stimulation ATP and NA are simultaneously released by exocytosis from adrenergic nerves (Douglas, 1968; Geffen & Livett, 1971; Johnson, Thoa, Weinshilbaum, Axelrod & Kopin, 1971). Adrenergic nerves have been shown to take up tritiated adenosine and to release labelled nucleotides as well as NA; the release of both NA and ATP was prevented by guanethidine (Su, Bevan & Burnstock, 1971). Relatively large amounts of ATP are known to be present in adrenergic nerves and to be involved in both uptake and release of NA in isolated granules (Euler & Lishajko, 1963). ATP might also have several intraneuronal actions important for the release of NA (supply of energy for the transport of granules, role in formation of cyclic adenosine 3′,5′-monophosphate (cyclic AMP), role in fusion of granules with the plasma membranes; Poisner, 1973). Quite independent of adrenergic innervation, ATP may be a neurotransmitter released from purinergic nerves (Burnstock, 1972).

The aim of this study was to investigate possible interactions between ATP and NA in regulating the contractility of the smooth muscle of the vas deferens.

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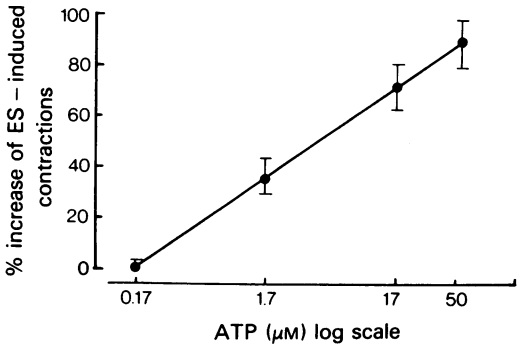

Figure 1 Potentiation by ATP of contractions elicited by electrical stimulation (ES) of the isolated vas deferens of the guinea-pig. Ordinate scale: percentage increase of ES-induced contractions. Abscissa scale: ATP concentration, log scale. Each point is the mean obtained from at least 6 experiments; vertical lines show s.e. mean.

Some receptor and ionic mechanisms which might underly the interactions have also been considered.

Methods

Vasa deferentia were removed from guinea-pigs weighing 300 to 600 g. The organs were carefully desheathed rendering them ganglion-free, and were suspended in a 10 ml organ bath containing Tyrode solution of the following composition (mM): NaCl 137, KCl 2.67, $NaHCO_3$ 12, $CaCl_2$ 1.80, $MgCl_2$ 0.11, NaH_2PO_4 0.42 and glucose 5.56. The solution was bubbled with pure O_2 and kept at a constant temperature of 36°C. For the electrical stimulation (ES) experiments, platinum electrodes were placed coaxially: one in the lumen of the organ while the other was positioned parallel to the first electrode in the bath. The repetitive transmural ES was carried out at 30 Hz with square wave pulses of 0.2 to 0.3 ms duration and voltage of 10 to 30 V; the differences are due to variations in sensitivity of preparations. Trains of pulses (1 s duration) were delivered from a Grass S8 electronic stimulator; they were spaced at 100 s intervals. Contractor responses were recorded on a smoked drum by means of an auxotonic frontal writing lever with a 20 fold magnification or an isometric transducer and amplifier (Microdynamometer 7001, Ugo Basile). The same result was obtained by either method of recording. Agonists were added to the bath at 3 to 5 min intervals. ATP was allowed 15 s in contact with the preparation while the other agonists were left in contact for 30 s.

The following drugs were used: acetylcholine chloride, adenosine triphosphate disodium salt (Koch-Light), adrenaline hydrochloride, noradrenaline bitartrate, phentolamine methanesulphonate, potassium chloride and guanethidine chloride.

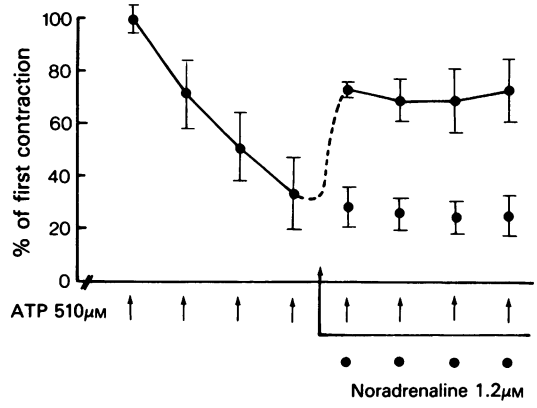

Figure 2 Reversal of tachyphylaxis to ATP by incubation with noradrenaline (NA). Ordinate scale: magnitude of ATP-induced contractions expressed as % of the first control contraction produced by ATP. Arrows indicate application of ATP 510 μM. After the broken line the application of ATP was preceded for 30 s by NA 1.2 μM of (upper line). Unconnected points are the corresponding control responses to ATP alone. Each point is the mean from 6 experiments; vertical lines show s.e. mean.

Results

Facilitation of neurotransmission by ATP

ATP (1.7 to 50 μM) singificantly potentiated the ES-induced contractions (Figure 1). In this series of experiments ATP was added to the bath during the 100 s intervals between the trains of pulses and left in contact for 6 to 10 min (4 to 6 trains of pulses). Responses to ES were readily blocked by guanethidine (10 μM) and therefore could be regarded as nerve-mediated contractions. There was some tachyphylaxis to the ATP-induced potentiation in a few experiments.

Tachyphylaxis to ATP and influence of noradrenaline

ATP contracted the isolated vas deferens if sufficiently high concentrations of the nucleotide were used. Although very sensitive preparations responded well to 10 to 20 μM, most preparations required 200 to 800 μM ATP to elicit a contraction. The response to ATP was rapid and the contraction was followed by relaxation; both responses took place within 10 to 20 s. The magnitude of these contractions progressively diminished to about 20 to 30% of the initial control

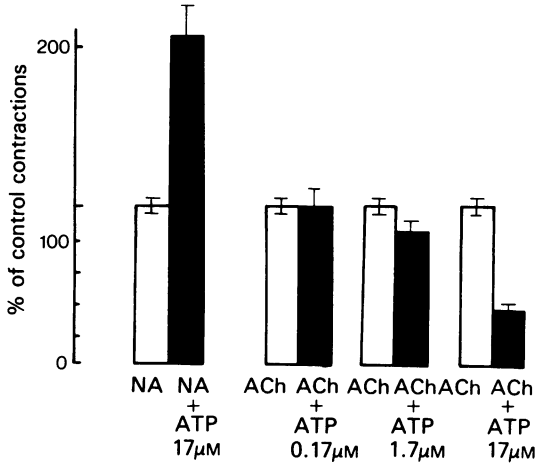

Figure 3 Differential effects of ATP on contractor responses to noradrenaline (NA) and acetylcholine (ACh). Open columns are control responses to either NA (12 μM) or ACh (6 μM); filled columns are responses in the presence of ATP shown as percentages of control responses. Heights of the columns are the means from 6–8 experiments; vertical lines show s.e. mean.

contraction (Figure 2) or sometimes disappeared completely.

Since the twitch-like responses to ATP closely resembled responses to ES, experiments were performed to see whether tachyphylaxis developed to both phenomena. Contractions produced by ES were not significantly reduced ($P > 0.05$; t-test) when tachyphylaxis to ATP was fully developed.

To see whether NA was involved in the development of tachyphylaxis, the preparation was incubated with NA 20 μM for 5 min intervals between applications of ATP (NA was washed out 60 s before application of ATP) and this procedure prevented the development of tachyphylaxis or restored the ATP contractions to the initial level. In addition, when tachyphylaxis to ATP had developed, successive administrations of NA (1.2 μM) 30 s before ATP restored the contractions almost to control levels (Figure 2). This concentration of NA was below that producing a contraction.

When the order of drug administration was reversed, ATP significantly potentiated the contractions produced by exogenously applied NA. ATP (17 μM) potentiated the responses to NA (12 μM) by 108 ± 20% (Figure 3); ATP was added 60 s before NA. A satisfactory dose-response curve could not be obtained because the ability of ATP to produce this potentiation diminished during repeated application of increasing concentrations of the nucleotide; this resembled tachyphylaxis observed with the use of ATP as agonist.

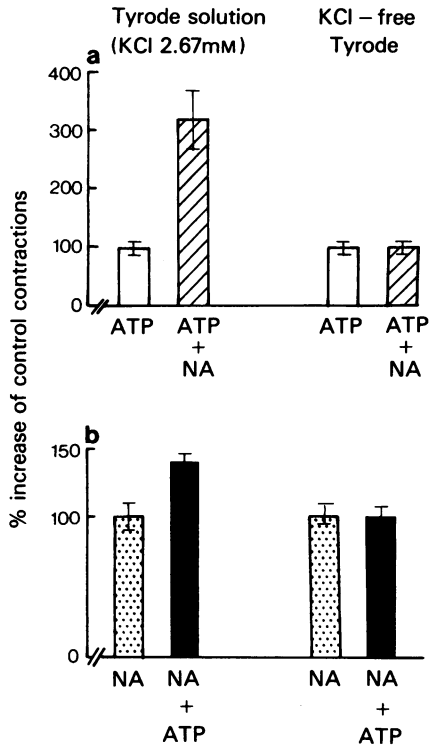

Figure 4 Potassium dependence of the potentiation of ATP-induced contractions by noradrenaline (NA) and *vice versa*. Ordinate scale: percentage increase in magnitude of (a) ATP-induced contractions and (b) NA-induced contractions. In (a) open columns represent contraction to ATP (510 μM) alone; hatched columns show effect of NA (12 μM) given 30 s before ATP (510 μM). In (b) stippled columns show effect of NA (12 μM) alone; solid columns show effect of NA (12 μM) plus ATP (17 μM). Each column is the mean of 6 experiments; vertical lines show s e. mean.

To see whether this ATP-NA interaction was specific, experiments with acetylcholine were performed. Acetylcholine-induced contractions were significantly inhibited in a dose-dependent manner: 1.7 μM ATP produced an inhibition of 16 ± 6% and 17 μM ATP of 63 ± 4% (Figure 3).

Purine receptors and adrenoceptors

The residual ATP contraction remaining after the full development of tachyphylaxis was resistant to the α-adrenoceptor antagonist, phentolamine (up to 25 μM) which completely abolished contractor responses to exogenous NA (20 μM). Phentolamine was added to the bath 60 s before ATP or NA.

NA (0.6, 1.2 and 12 μM) potentiated contractions to ATP (62 ± 6, 97 ± 12 and 230 ± 50% respectively).

Phentolamine (12.5 μM) prevented the potentiation of ATP-induced contractions by NA (1.2 μM).

Effect of changes in the ionic composition of the Tyrode solution

In the search for a mechanism of the ATP-NA interactions observed in the present experiments, we investigated the influence of changes in the Tyrode solution of magnesium and potassium, ions which play a role in the action of ATP on smooth muscle (Bennett, Burnstock & Holman, 1963; Daniel & Irwin, 1965).

The omission of magnesium resulted in an enhanced contractor response to agonists, but the potentiating ability of either ATP or NA was not impaired. In contrast, a brief removal (10 min) of potassium chloride produced no change in control contractions but the potentiation of both ATP-induced contractions by NA and NA-induced contractions by ATP was completely abolished (Figure 4).

Discussion

The idea that adenine nucleotides could be locally active has recently been suggested (Van Dyke *et al.*, 1977; Holck & Marks, 1978). In the present study, interactions between ATP and NA were observed in the isolated vas deferens of the guinea-pig. Both compounds were shown to potentiate contractions induced by ATP, NA or by repetitive ES. The interaction appeared to be selective, because the concentration of ATP that doubled the responses to exogenous NA significantly inhibited acetylcholine-induced contractions.

In many tissues purine compounds have been shown to produce a presynaptic inhibition of the mechanical response and of neurotransmitter release in the sympathetic nervous system (Kažić, 1974; Enero & Saidman, 1977), and in the cholinergic system (Sawynok & Jhamandas, 1976; Vizi & Knoll, 1976). In the vas deferens, Hedqvist & Fredholm (1976) have shown that adenosine inhibits adrenergic neurotransmission presynaptically and potentiates it by a postsynaptic action. Since in the present study low concentrations of ATP enhanced neurotransmission (potentiated ES-induced contractions) and potentiated the contractions produced by exogenous NA, we assume that the observed interaction in both cases took place at the postsynaptic membrane, i.e. via postsynaptic purine receptors and α-adrenoceptors. In support of this view, the ATP-induced contractions were insensitive to the α-adrenoceptor antagonist, phentolamine, in concentrations that completely abolished the responses to NA. The potentiation of ATP-induced contractions by NA was selectively prevented by phentolamine. Having observed the same, Holck & Marks (1978) proposed that the site of action of phentolamine was on the postsynaptic membrane because the action of phentolamine was unaffected by reserpine pretreatment. Therefore, an unchanged integrity of postsynaptic α-adrenoceptors appeared to be an essential prerequisite for the ATP-NA interaction.

One of the most impressive features in the action of ATP on the vas deferens was the development of autodesensitization or tachyphylaxis. However, rather high concentrations of ATP (0.1 to 1 mM) were required to produce a contraction which was fast in onset, of short duration and thus resembled the twitch response to ES, which is supposed to be due to an activation of junctional receptors by the released transmitter (Hotta, 1969). Thus, ATP-induced contractions could have been at least partly due to presynaptic stimulation and NA release followed by depletion. However, these contractions were insensitive to phentolamine and could not be regarded as responses to released NA. It has been shown that ATP and adenosine inhibit the efflux of labelled NA from the rat portal vein and the guinea-pig vas deferens, respectively (Enero & Saidman, 1977; Hedqvist & Fredholm, 1976). However, our finding that neurotransmission was still well maintained when the tachyphylaxis to ATP was fully developed shows that the tachyphylaxis was not due to a depletion of NA stores. If any depletion was present at all, it was not sufficient to impair neurotransmission, or alternatively might imply that the motor transmitter is not noradrenaline.

The facts that the observed tachyphylaxis could be overcome by incubating the preparation with NA or by exposing it to low and subthreshold concentrations of NA, strongly suggest that the effect of ATP might be coupled with NA action. In addition to this, Holck & Marks (1978) found that adenosine and AMP (which are otherwise devoid of agonist activity) produced contractions in the presence of NA; the contractions produced by ATP and ADP were profoundly enhanced.

The structure of neuromuscular junctions in the vas deferens distinguishes this organ from intestinal and vascular smooth muscle. In the guinea-pig vas deferens, about one half of the smooth muscle cells have close neuromuscular contacts with a gap width of about 200 Å. The junctional gap in the intestinal and vascular muscle is rarely less than 800 Å (Burnstock, 1970). Thus, the minimum separation of nerve and muscle membranes in the vas deferens might provide the morphological basis for the development of tachyphylaxis to ATP. Although the present results rule out the possibility of the presynaptic action of ATP, it is still intriguing that tachyphylaxis to ATP has not been seen in the intestinal muscle where the junctio-

nal gap is several times wider (Burnstock, Satchell & Smythe, 1972; Kažić, 1973; 1974).

Catecholamines are generally believed to produce depolarization and contraction due to an increased membrane conductance to several ions. In the vas deferens, NA has been shown to increase ion conductance of the membrane to K^+, Na^+ and Ca^{2+} (Shuba, Gurkovskaya, Klevetz, Kochemasova & Taranenko, 1976). We have found that both potentiating abilities of ATP on NA contractions and *vice versa* are potassium-dependent, which might imply that these two substances share a common mechanism. Finally, the removal of potassium from the bathing solution did not change the magnitude of control contractions to ATP or NA, but selectively prevented the potentiations. These data suggest that potassium ions play a more important role in the purinergic-adrenergic coupling than in the contractor responses to either ATP or NA.

The tachyphylaxis observed to ATP in the Tyrode solution of normal composition, was also seen in the modified Tyrode (double potassium or potassium-free) solution. In addition to this, the same type of tachyphylaxis was observed in experiments with vasa taken from the rabbit or the rat (unpublished results).

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References

AMBACHE, N. & ZAR, M. ABOO (1971). Evidence against adrenergic motor transmission in the guinea-pig vas deferens. *J. Physiol.*, **216**, 259–389.

BENNETT, M.R., BURNSTOCK, G. & HOLMAN, M.E. (1963). The effect of potassium and chloride ions in the inhibitory potential recorded in the guinea-pig taenia coli. *J. Physiol.*, **169**, 33–34P.

BIRMINGHAM, A.T. & WILSON, A.B. (1963). Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferencs preparation. *Br. J. Pharmac.*, **21**, 569–580.

BURNSTOCK, G. (1970). Structure of smooth muscle and its innervation. In *Smooth Muscle*, ed. Bülbring E., Jones, A. & Tomita, T. pp. 1–69. London: Edward Arnold.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 509–581.

BURNSTOCK, G. & HOLMAN, M, E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.*, **155**, 115–133.

BURNSTOCK, G., SATCHELL, D. G. & SMYTHE, A. (1972). A comparison of the excitatory and inhibitory effects of non-adrenergic, non-cholinergic nerve stimulation and exogenously applied ATP on a variety of smooth muscle preparations from different vertebrate species. *Br. J. Pharmac.* **46**, 234–242.

DANIEL, E.E. & IRWIN, J. (1965). On the mechanism whereby certain nucleotides produce contractions of smooth muscle. *Can. J. Physiol. Pharmac.*, **43**, 89–136.

DOUGLAS, W.W. (1968). Stimulus secretion coupling: the concept and clues from chromaffin and other cells. *Br. J. Pharmac.*, **34**, 451–474.

ENERO, M.A. & SAIDMAN, B.Q. (1977). Possible feedback inhibition of noradrenaline release by purine compounds. *Naunyn-Schiedebergs Arch. Pharmac.*, **297**, 39–46.

EULER, U.S. VON & HEDQVIST, P. (1975). Evidence for an alpha- and beta-receptor mediated inhibition of the twitch response in the guinea-pig vas deferens by noradrenaline. *Acta physiol. scand.*, **93**, 572–573.

EULER, U.S. VON & LISHAJKO, F. (1963). Effect of adenine nucleotides on catecholamine release and uptake in isolated adrenergic nerve granules. *Acta physiol. scand.*, **59**, 454–461.

GEFFEN, L.B. & LIVETT, B.G. (1971). Synaptic vesicles in sympathetic neurones. *Physiol. Rev.*, **51**, 98–157.

HEDQVIST, P. & FREDHOLM, B.B. (1976). Effects of adenosine on adrenergic neurotransmission; prejunctional inhibition and postjunctional enhancement. *Naunyn-Schmiedebergs Arrch. Pharmac.*, **293**, 217–223.

HILLARP, N.A. (1958). Adenosine phosphates and inorganic phosphates in adrenaline and noradrenaline containing granules of the adrenal medulla. *Acta physiol. scand.*, **42**, 321–332.

HOLCK, M.I. & MARKS, B.H. (1978). Purine nucleotide and nucleoside interactions on normal and subsensitive alpha adrenoceptors responsiveness in guinea-pig vas deferens. *J. Pharmac. exp. Ther.*, **205**, 104–117.

HOLZAUBER, M. and SHARMAN, D.F. (1972). The distribution of catecholamines in vertebrates. In *Catecholamines.* ed. Blaschko, H. & Muscholl, E. pp. 110–185. Berlin-Heidelberg-New York: Springer.

HOTTA, Y. (1969). Some properties of the junctional and extrajunctional receptors in the vas deferens of the guinea-pig. *Agents Actions*, **1**, 13–21.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1976). Is noradrenaline the motor transmitter in the mouse vas deferens? *J. Physiol.*, **254**, 49P.

HUKOVIĆ, S. (1961). Responses of the isolated sympathetic nerve-ductus deferens preparation of the guinea-pig. *Br. J. Pharmac. Chemother.*, **16**, 188–194.

JOHNSON, D.G., THOA, N.B., WEINSHILBAUM, R.M., AXELROD, J. & KOPIN, J.I. (1971). Enhanced release of dopamine beta-hydroxylase from sympathetic nerves by calcium and phenoxybenzamine and its reversal by prostaglandines. *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2227–2230.

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

KAŽIĆ, T. (1973). Some pharmacological actions of ATP on the isolated guinea-pig ileum. *Iugoslav. Physiol. Pharmac. Acta*, **9**, 391–398.

KAŽIČ, T. (1974). Interaction of ATP with the effects of acatecholamines and coaxial electrical stimulation of the isolated terminal ileum of the guinea-pig. *Iugoslav. Physiol. Pharmac. Acta*, **10**, 315–322.

POISNER, A.M. (1973). Mechanisms of exocytosis. In *Frontiers in Catecholamine Research.* ed. Usdin, E. & Snyder, S. pp. 477–482. New York: Pergamon Press.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine: antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197**, 379–390.

SHUBA, M.F., GURKOVSKAYA, A.V., KLEVETZ, M.J., KOCHEMASOVA, N.G. & TARANENKO, V.M. (1976). Mechanism of the excitatory and inhibitory actions of catecholamines on the membrane of smooth muscle cells. In *Physiology of Smooth Muscle.* ed. Bülbring, E. & Shuba, M.F. pp. 347–355. New York: Raven Press.

SJÖSTRAND, N.O. (1965). The adrenergic innervation of the vas deferens and the accessory male genital glands. *Acta physiol. scand.*, Suppl. 257, **65**, 2–82.

SU, C., BEVAN, J.A. & BURNSTOCK, G. (1971). ^{3}H-adenosine triphosphate release during stimulation of enteric nerves. *Science*, **173**, 336–339.

SWEDIN, G. (1971). Studies on neurotransmission mechanisms in the rat and guinea-pig vas deferens. *Acta physiol. scand.*, Suppl. **369.**

VAN DYKE, K., ROBINSON, R., URQUILLA, P., SMITH, D., TAYLOR, M., TRUSH, M. & WILSON, M. (1977). An analysis of nucleotides and catecholamines in bovine medullary granules by anion exchange high pressure liquid chromatography and fluorescence. Evidence that most of catecholamines in chromaffin granules are stored without associated ATP. *Pharmacology*, **15**, 377–391.

VIZI, E.S. & KNOLL, J. (1976). The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. *Neurosci.*, **1**, 391–398.

WINKLER, H. (1976). The composition of adrenal chromaffin granules. An assessment of controversial results. *Neurosci.*, **1**, 65–80.

WINKLER, H. & HÖRTNAGL, H. (1973). Composition and molecular organisation of chromaffin granules. In *Frontiers in Catecholamine Research.* ed. Usdin, E. & Snyder, S. pp. 415–421. New York: Pergamon Press.

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