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EVIDENCE FOR PURINERGIC INNERVATION OF THE ANOCOCCYGEUS MUSCLE

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1 Fluorescence histochemical localization of quinacrine (which binds to adenosine 5'-triphosphate (ATP)) revealed nerve fibres running singly and in bundles in both rat and rabbit anococcygeus muscle. Single neurone cell bodies and ganglia containing between 2 and 50 cells were also observed.

2 Catecholamine fluorescence studies revealed a dense adrenergic ground plexus, but no adrenergic ganglion cells were detected. No acetylcholinesterase-positive nerve fibres or ganglion cells were seen in the rat.

3 When the tone was raised with guanethidine, a relaxation in response to field stimulation was revealed, which was unaffected by atropine but blocked by tetrodotoxin.

4 Release of ATP increased 3 to 6 times above background during stimulation of these non-adrenergic, non-cholinergic, inhibitory nerves.

5 Neither quinacrine staining nor the release of ATP during inhibitory nerve stimulation was affected by 6-hydroxydopamine treatment, which abolished catecholamine fluorescence.

6 Exogenous ATP produced relaxation in high tone preparations of the rabbit anococcygeus muscle. ATP produced either contraction or a small relaxation followed by a contraction of the rat anococcygeus muscle, but treatment with low concentrations of the prostaglandin synthesis inhibitor indomethacin, converted the contraction to a relaxation.

7 These data are consistent with the view that the anococcygeus muscle is innervated by purinergic inhibitory nerves.

Introduction

The anococcygeus is a thin, paired smooth muscle band anchoring the distal rectum to the upper coccygeal vertebrae (Gillespie, 1972). In the cat, rabbit and rat, this muscle receives a dense adrenergic excitatory innervation as well as an inhibitory innervation whose transmitter has not yet been identified (Gillespie, 1972; Gillespie & McGrath, 1974; Creed, Gillespie & McCaffery, 1977; Creed & Gillespie, 1977).

Non-adrenergic, non-cholinergic, inhibitory nerves are present throughout the mammalian gastrointestinal tract (see Burnstock, 1969; Campbell, 1970). Evidence has been presented that a purine nucleotide, probably adenosine triphosphate (ATP), is the transmitter released by some of these nerves (Burnstock, Campbell, Satchell & Smythe, 1970; Burnstock, 1975; Burnstock, Cocks, Kasakov & Wong, 1978b) and they have therefore been called 'purinergic' (Burnstock, 1971). Although ATP is a powerful inhibitory agent in the rabbit anococcygeus (Creed *et al.*, 1977), in the rat it causes contraction (Gillespie, 1972), while in the cat, high concentrations of ATP are required to produce relaxation (Gillespie & McGrath, 1974). Since it was argued that the inhibitory transmitter is likely to be the same in all three species, ATP was therefore regarded as an unlikely candidate (Gillespie & McGrath, 1974; Creed *et al.*, 1977).

In this study, three criteria for the investigation of ATP as the inhibitory transmitter in the rat and rabbit anococcygeus muscle have been examined: storage, release and postjunctional action of ATP. Fluorescence histochemical localization of quinacrine has been carried out since it has been shown to bind to ATP (Irvin & Irvin, 1954) and has been employed to localize non-adrenergic, non-cholinergic nerves in both the gastrointestinal tract (Olson, Ålund & Norberg, 1976) and bladder (Burnstock, Cocks, Crowe & Kasakov, 1978a), where experimental evidence for purinergic innervation has been presented (Burnstock, 1975). Release of ATP during stimulation of intramural inhibitory nerves was examined by the sensitive firefly-ATP assay method (Strehler, 1968). The effect of indomethacin on the postjunctional action of ATP

in the rat was examined, since ATP is a potent stimulant of prostaglandin synthesis in a wide variety of tissues (Needleman, Minkes & Douglas, 1974; Burnstock, Cocks, Paddle & Staszewska-Barczak, 1975; Kamikawa, Serizawa, Mizutani & Shimo, 1976) and prostaglandins cause contraction of the rat anococcygeus (Gillespie & McGrath, 1974).

Methods

Wistar rats of either sex (200 to 250 g) and female Dutch rabbits (1 to 2 kg) were stunned by a blow to the back of the head and exsanguinated. The anococcygeus muscle was prepared as described by Gillespie (1972). Tissues were bathed with a modified Krebs solution (Bülbring, 1953) either by superfusion, or by incubation in a 20 ml organ bath maintained at 37°C. Isometric tension was recorded with a Grass model 7D polygraph and a Grass force-displacement transducer. An initial load of 0.2 to 0.5 g was placed on each preparation and 60 min allowed for equilibration. Electrical field stimulation was applied by means of a pair of platinum ring electrodes (approx. 3 mm apart) placed around the tissue. Rectangular pulses were delivered by a Grass S44 electronic stimulator.

Histochemistry

The anococcygeus was dissected out and immediately placed in ice-cold Krebs solution.

Quinacrine fluorescence Tissues were incubated in Krebs solution containing 0.5 μM quinacrine dihydrochloride for 1 h at 37°C and bubbled continuously with 95% O_2 and 5% CO_2. They were rinsed in quinacrine-free, ice-cold Krebs (Olson *et al.*, 1976). Whole mounts of tissue were stretched and mounted in liquid paraffin and viewed through a Zeiss photomicroscope fitted with an epifluorescence condensor 111RS and selected areas were photographed on Ilford HP5 film.

Monoamine fluorescence Stretch preparations of the anococcygeus were dried over phosphorus pentoxide for 1 h and then treated with paraformaldehyde vapour (relative humidity 80% at 80°C) for 1 h as described by Falk, Hillarp, Thieme & Torp (1962). They were then mounted in paraffin for fluorescence photomicroscopy as described for quinacrine fluorescence.

In some experiments, 6-hydroxydopamine (250 mg/kg) was injected 24 h before the animals were killed (see Malmfors & Thoenen, 1971). Catecholamine fluorescence was abolished by this treatment.

Acetylcholinesterase staining Whole mounts of the anococcygeus were fixed in formol-calcium for 20 min and stained for acetylcholinesterase as described by Karnovsky & Roots (1964). Selected areas were viewed by Zeiss photomicroscopy.

ATP assay

Fractions of superfusate were assayed for ATP by the firefly luciferin-luciferase reaction, which is specific for ATP (McElroy & Seliger, 1963; Strehler, 1968) with a Du Pont luminescence biometer. All reagents for the assay were supplied by Du Pont.

Drugs used were: adenosine 5'-triphosphate disodium salt (Sigma), atropine sulphate (Sigma), guanethidine sulphate (Ciba), 6-hydroxydopamine (Sigma), 5-hydroxytryptamine creatinine sulphate (May & Baker), indomethacin (Sigma), noradrenaline bitartrate (BDH), prostaglandins, E_1, E_2, & $F_{2\alpha}$ (Upjohn), quinacrine dihydrochloride (Sigma), tetrodotoxin (Sigma) and thiocholine iodide (Sigma). Indomethacin and prostaglandins were made up in 70% ethanol as concentrated stock solutions and diluted to the required concentration in the organ bath by the addition of 1 to 10 μl amounts. All other drugs were prepared as fresh aqueous solutions at the beginning of each experiment.

Results

Histochemistry

Treatment of whole mount preparations of the rat and rabbit anococcygeus with quinacrine (0.5 μM) revealed numerous green fluorescent nerve cell bodies found either singly or in ganglia containing 2 to approximately 50 cells. Larger ganglia were found predominantly on the medial surface of the paired muscle bands (Figure 1a) and on the ventral surface of the band of muscle passing over the distal rectum. Smaller groups of nerve cell bodies were found within the muscle. The nerve cell bodies within these ganglia were spherical. Their nuclei did not exhibit any fluorescence. Fluorescent nerve bundles were also present on the surface and throughout the muscle layer (Figure 1b); occasionally they could be seen to be associated with ganglia (Figure 1c). Fine varicose fibres were commonly seen in the rabbit anococcygeus (Figure 2a), but were only occasionally observed in the anococcygeus of the rat.

Catecholamine fluorescence studies revealed a dense network of fine, varicose nerve fibres throughout the muscle layer and around blood vessels in both species (Figure 2b). No ganglion cells were observed either within the muscle or on its surface. Treatment of the animals with 6-hydroxydopamine (250 mg/kg

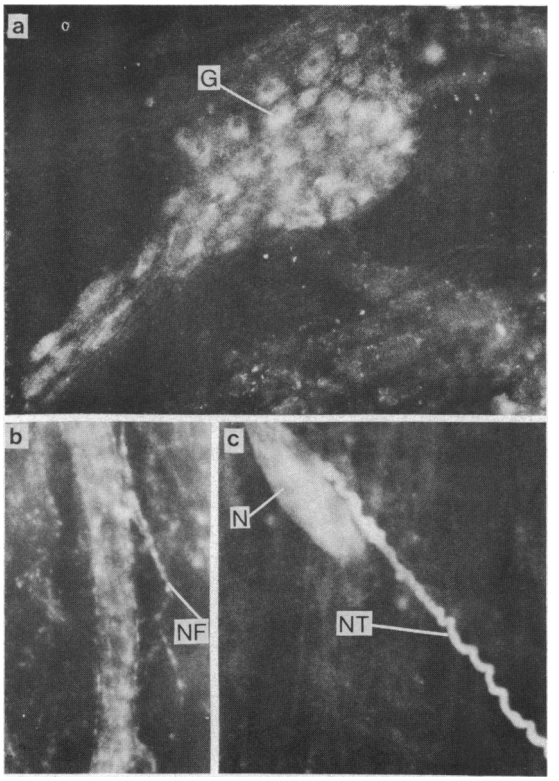

Figure 1 Fluorescence histochemical localization of quinacrine on whole mount stretch preparation of the rat anococcygeus. Tissues were taken from animals treated with 6-hydroxydopamine. (a) A large group of approximately 30 ganglion cells (G) on the serosal surface of the muscle layer (×200). (b) A small nerve trunk located within the muscle layer. Note the varicose nerve fibre (NF) attached to the trunk. Other varicose nerve fibres can be seen within the nerve trunk (×200). (c) A small ganglion located within the muscle layer. Weakly fluorescent areas (N) are probably nuclei of the ganglion cells. The ganglion is associated with a fluorescent nerve trunk (NT) (×200).

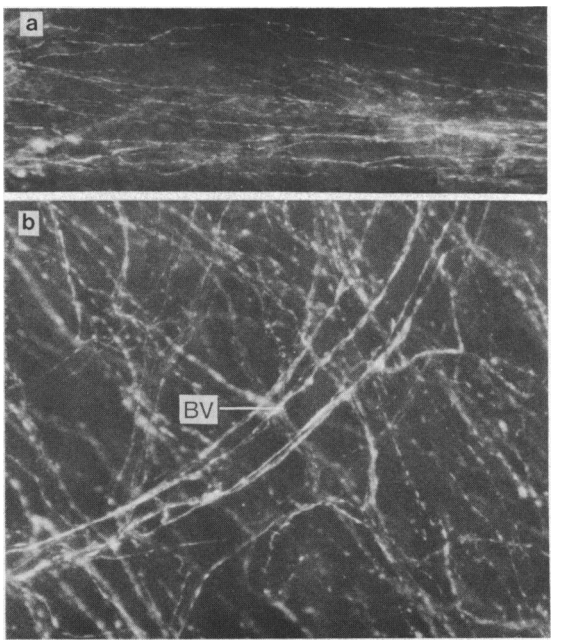

Figure 2 (a) Fluorescence histochemical localization of quinacrine on a whole mount stretch preparation of the rabbit anococcygeus. The animal was treated with 6-hydroxydopamine. Fine varicose nerve fibres can be seen within the muscle layer (×200). (b) Fluorescence histochemical localization of monoamines on a whole mount stretch preparation of the rat anococcygeus. A dense network of brightly fluorescent varicose nerve fibres can be seen both throughout the muscle layer and around blood vessels (BV) (×230).

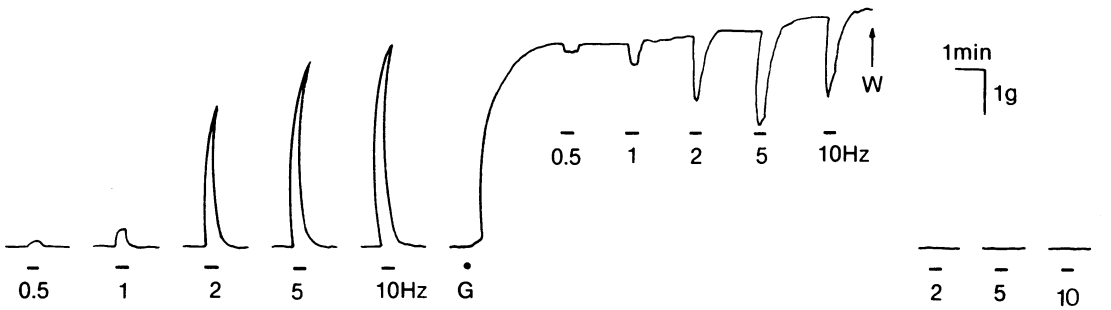

Figure 3 Responses of rat anococcygeus to stimulation of intramural excitatory and inhibitory nerves (0.2 ms duration pulses delivered at the frequencies indicated and supramaximal voltage of 50 V for 20 s). Guanethidine (G, 34 μM) was used both to block the contractile response to adrenergic nerve stimulation and to raise the tone of the preparation. Atropine (1.4 μM) was present throughout. At W, guanethidine was washed out of the bath; after 10 min the tone was lowered but block of adrenergic responses persisted.

injected i.p. 24 h before they were killed) completely abolished catecholamine fluorescence, whereas the fluorescence associated with quinacrine-positive nerves and ganglion cells was unaffected (4 animals).

Treatment of the anococcygeus for detection of acetylcholinesterase activity failed to reveal any structures which resembled either nerves or ganglion cells.

Non-adrenergic, non-cholinergic inhibitory nerves and release of ATP

To demonstrate relaxation of the rat anococcygeus to intramural nerve stimulation, the tone of the preparation was raised with guanethidine (34 μM) in the manner described by Gillespie (1972). At this concentration, guanethidine blocked the contractile response to adrenergic nerve stimulation and produced a sustained, tonic contraction (Figure 3). Under these conditions, stimulation of intramural nerves caused rapid, maintained relaxation of the muscle (Figure 3) which was unaffected by atropine (1.4 μM) but blocked by tetrodotoxin (3 μM). This nerve-mediated inhibitory response was accompanied by a 3 to 6 times increase in the release of ATP above the prestimulation level (Figure 4). Atropine (1.4 μM) was present during all release experiments. The spontaneous background release of ATP was not affected by increasing the tone with guanethidine. Following injection of the animals with 6-hydroxydopamine, which resulted in complete loss of catecholamine fluorescence, the increase in ATP release during intramural nerve stimulation was not significantly affected. Similar results were obtained with the rabbit anococcygeus.

Action of exogenous ATP

When the tone was raised with guanethidine, noradrenaline or 5-hydroxytryptamine, ATP (1 to 2000 μM) caused a powerful relaxation of the *rabbit* anococcygeus, which was similar to that produced by intramural nerve stimulation (Figure 5a), in confirmation of Creed *et al.* (1977). In some experiments with the *rat* anococcygeus, ATP (1 to 2000 μM) caused weak,

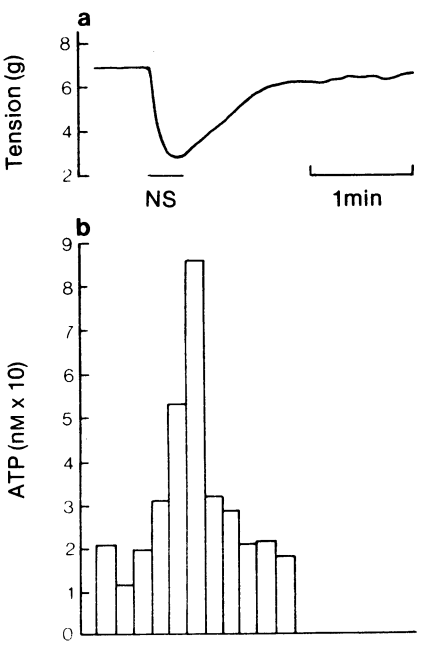

Figure 4 Release of ATP from the superfused rat anococcygeus during stimulation of intramural inhibitory nerves (NS: 0.2 ms duration pulses delivered at 10 Hz and 40 V for 20 s). (a) Isometric mechanical activity of the muscle; (b) concentration of ATP in consecutive 10 s fractions of superfusate. The rate of superfusion was 3 ml/min. Guanethidine (34 μM) was used to raise the tone and block adrenergic nerves. Atropine (1.4 μM) was present throughout.

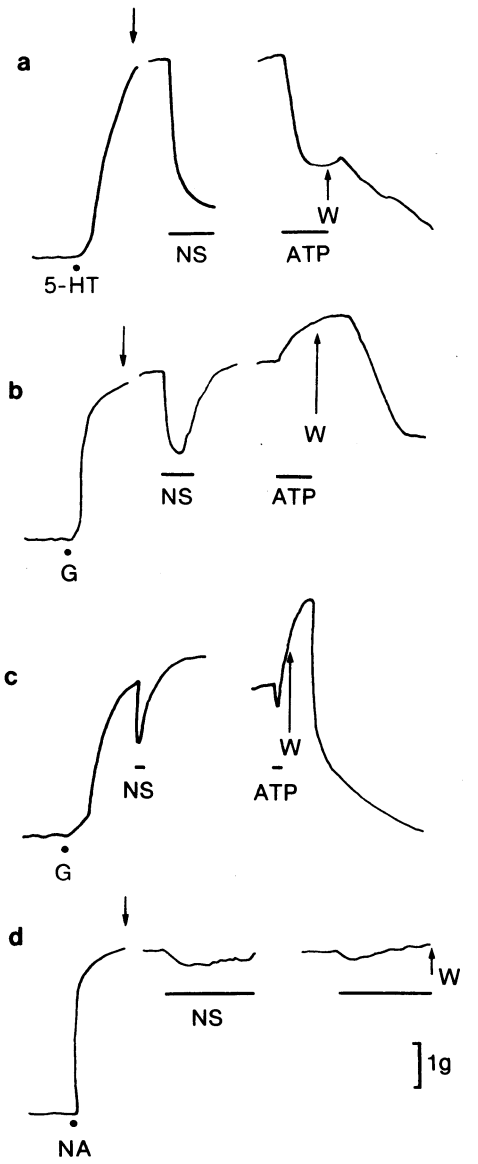

Figure 5 Inhibitory responses of the isolated anococcygeus muscle to intramural nerve stimulation (NS: 0.2 ms duration pulses delivered at frequencies indicated and supramaximal voltage (40 V)) and exogenous ATP. The tone of the preparation was raised with 5-hydroxytryptamine (5-HT: 2.5 μM), guanethidine (G: 34 μM), or noradrenaline (NA: 1 μM). These agonists were washed out (W) after ATP application. Atropine (1.4 μM) and low concentrations of guanethidine (3.4 μM) were present throughout. (a) Rabbit: NS (2 Hz), ATP (100 μM); (b) rat: NS (2 Hz), ATP (100 μM); (c) & (d) rat: NS (10 Hz), ATP (50 μM). Periods of stimulation (horizontal bars) 20 s; vertical bar, 1 g.

slow contractions (Figure 5b), whilst in other experiments it caused an initial rapid relaxation followed by a tonic contraction (Figure 5c, d). Both types of responses were unaffected by atropine (1.4 μM).

Effects of indomethacin

Prostaglandins E_1, E_2, and $F_{2\alpha}$ elicited weak, sluggish contractions of the rat anococcygeus (confirming the results of Gillespie & McGrath, 1974) similar to those produced by exogenously applied ATP in some preparations. However, after incubation of the anococcygeus in low concentrations of indomethacin (2.8 to 14 μM) for periods of between 60 and 90 min and after raising the tone with either noradrenaline or 5-hydroxytryptamine, ATP (1 to 1000 μM) caused relaxations similar to those produced by intramural nerve stimulation (Figure 6a, b). The inhibitory response to ATP was not affected by either atropine (1.4 μM) or tetrodotoxin (3 μM).

Discussion

Quinacrine fluorescent histochemistry has revealed a population of nerve fibres and ganglion cells in both the rabbit and rat anococcygeus. These nerves are not adrenergic because this plexus is much denser and no adrenergic ganglion cells were seen. Furthermore, treatment of the animal with 6-hydroxydopamine abolished catecholamine fluorescence without affecting quinacrine fluorescence. The quinacrine-positive nerves are not likely to be cholinergic, since no acetylcholinesterase activity in nerves was demonstrated (see also Gillespie, 1972); and neither atropine nor neostigmine affected the motor or the inhibitory response to nerve stimulation (Gillespie, 1972; Gillespie & McGrath, 1974). Quinacrine fluorescence was not seen in the iris or the vas deferens, where both catecholamine fluorescence and acetylcholinesterase activity were abundant (see Burnstock *et al.*, 1978a).

Several separate lines of evidence indicate that quinacrine binds to ATP. Irvin & Irvin (1954) have demonstrated that quinacrine binds more readily to ATP than to other adenylates. Intense quinacrine staining occurs where there are high levels of ATP, e.g. adrenal medulla and in ATP granules of blood platelets (Lorez, Da Prada & Launay, 1977). Preliminary observations in this laboratory have shown that quinacrine binds to mast cells, which are known to contain high levels of ATP (Johansen, 1977). Also, microsomal fractions obtained by differential and sucrose-density gradient centrifugation of homogenates of purinergically-innervated preparations, preloaded with [^{3}H]-adenosine and [^{14}C]-quinacrine, show peaks of ^{14}C which correspond to peaks of [^{3}H]-ATP (Cocks, Crowe, Stitzel, Edgar & Burnstock, unpub-

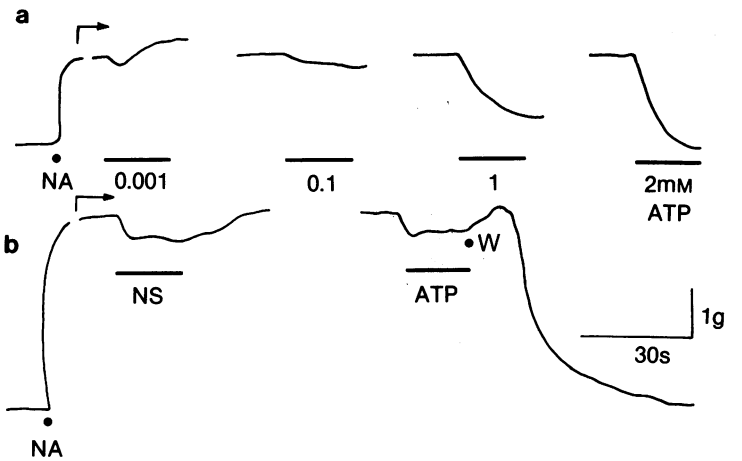

Figure 6 Responses of the rat anococcygeus to field stimulation of intramural inhibitory nerves and exogenously applied ATP following incubation for 90 min with indomethacin (2.8 μM) and after the tone was raised with noradrenaline (NA: 1 μM). Atropine (1.4 μM) was present throughout. Time calibration (horizontal bar) = 30 s, except for the short initial segments before the bent arrows. (a) Responses to increasing concentrations of ATP; (b) comparison of the responses to intramural nerve stimulation (NS: 0.2 ms duration pulses delivered at 5 Hz and supramaximal voltage (50 V) for 20 s) and exogenous ATP (0.05 mM). Note that tone falls as noradrenaline is washed out (W) of bath.

lished observations). Olson *et al.* (1976) have shown quinacrine-positive nerve fibres in the gastro-intestinal tract which has been shown to be purinergically innervated (Burnstock, 1972; 1975). This correlation has also been demonstrated in the guinea-pig urinary bladder (Burnstock *et al.*, 1978a), gall bladder (Davison, Al-Hassani, Crowe & Burnstock, unpublished observations), and portal vein (Burnstock, Crowe & Wong, 1978c).

Release of ATP increased 3 to 6 times above background during the relaxations produced by stimulation of the non-adrenergic, non-cholinergic nerves. The source of this ATP is unlikely to be cholinergic nerves, since no histochemical evidence for acetylcholinesterase activity was found in the present study or by Gillespie (1972). It is also unlikely to be adrenergic nerves since release was unaffected by treatment with 6-hydroxydopamine, known to produce degeneration of adrenergic nerve terminals (see Malmfors & Thoenen, 1971). However, in the present experiments, while catecholamine fluorescence was shown to be abolished, no electronmicroscopic studies of fibre degeneration were carried out. Thus it is conceivable that some ATP, which is known to accompany noradrenaline in adrenergic nerves (see Smith, 1972), remains and can be released from undamaged fibres after catecholamine depletion (see Langer & Pinto, 1976; Westfall, Stitzel & Rowe, 1978). ATP is unlikely to come from smooth muscle as a result of changes in tension, since in studies in the guinea-pig taenia coli and bladder it has been shown that changes in tension in response to direct muscle stimulation do not result in release of ATP (Burnstock *et al.*, 1978b). The possibility that the released ATP is from sensory nerves in response to antidromic stimulation (see Holton, 1959) cannot be excluded but seems unlikely as there is no evidence for ATP release from sensory nerves in the gut (see Burnstock, 1972) and there is no ultra-structural evidence for sensory innervation of the rat anococcygeus (Gillespie & Lüllman-Rauch, 1974). Thus the possibility remains that ATP is being released from non-adrenergic, non-cholinergic inhibitory nerves.

Contraction of the rat anococcygeus by exogenously applied ATP is probably mediated by prostaglandins since the prostaglandin synthesis inhibitor, indomethacin (Vane, 1971), unmasked an inhibitory response to ATP and ATP is known to stimulate prostaglandin synthesis (Needleman *et al.*, 1974; Burnstock *et al.*, 1975; Kamikawa *et al.*, 1976). Furthermore, prostaglandins cause sluggish contractions of the rat anococcygeus, similar to the response to exogenously applied ATP. The high concentration of ATP required to give an inhibitory response of similar magnitude to that produced by intramural nerve stimulation could be due to physiological antagonism by noradrenaline and 5-hydroxytryptamine, both of which were used to raise the tone after treatment with indomethacin. This is supported by the finding that when the tone was raised with either

agonist, an inhibitory response to intramural nerve stimulation was difficult to obtain (see also Gillespie, 1972). It may also explain the findings of San San Wai & Coupar (1976) who reported that relatively high concentrations of ATP were required to relax the rabbit anococcygeus when they used phenylephrine to raise the tone. The reason why relaxations to nerve stimulation of the rat anococcygeus were easier to elicit than those to ATP in the absence of indomethacin, may relate to the geometry of the nerve-muscle junctions. The high concentrations of transmitter reached at smooth muscle receptor sites in neuromuscular junctions would produce a dominating inhibitory action relative to the excitatory action of the prostaglandin synthesized following transmitter release. In contrast, exogenous application of transmitter may provide non-physiological conditions where the competition at receptor sites between the transmitter and the prostaglandin produced may be equal or indeed favour the excitatory response.

The previous objections to ATP as the inhibitory transmitter in the anococcygeus were based largely on the findings that ATP causes contraction in the rat and relaxation in the cat only in relatively high concentrations (Gillespie, 1972; Gillespie & McGrath, 1974; Creed *et al.*, 1977). However, in the rabbit, ATP was found to be a powerful inhibitory agent, mimicking closely the responses to non-adrenergic, non-cholinergic inhibitory nerve stimulation (Creed *et al.*, 1977; this paper). The present finding that ATP can cause a relaxation in the rat anococcygeus after treatment with indomethacin not only suggests the involvement of prostaglandins in the contractile response to ATP, but appears to answer the objection to consideration of ATP as the inhibitory transmitter in the anococcygeus. Furthermore, the inhibitory junction potentials demonstrated by Creed & Gillespie (1977) in smooth muscle cells of the anococcygeus in response to single pulses in the non-adrenergic, non-cholinergic nerves, show a close resemblance to the inhibitory junction potentials recorded in smooth muscle of intestine to non-adrenergic, non-cholinergic ('purinergic') nerve stimulation (Bennett, Burnstock & Holman, 1966).

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References

BENNETT, M.R., BURNSTOCK, G. & HOLMAN, M.E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol.*, **182**, 541–558.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.*, **122**, 111–134.

BURNSTOCK, G. (1969). Evolution of the autonomic innervation of visceral and cardio-vascular systems in vertebrates. *Pharmac. Rev.*, **21**, 247–324.

BURNSTOCK, G. (1971). Neural nomenclature. *Nature*, **229**, 282–283.

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

BURNSTOCK, G. (1975). Purinergic transmission. In *Handbook of Psychopharmacology*, Vol 5, ed. Iversen, L.L., Iversen, S.D. & Snyder, S.H., pp. 131–194. New York: Plenum Press.

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D.G. & SMYTHE, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *Br. J. Pharmac.*, **40**, 668–688.

BURNSTOCK, G., COCKS, T., PADDLE, B. & STASZEWSKA-BARCZAK, J. (1975). Evidence that prostaglandin is responsible for the 'rebound contraction' following stimulation of non-adrenergic, non-cholinergic ('purinergic') inhibitory nerves. *Eur. J. Pharmac.*, **31**, 360–362.

BURNSTOCK, G., COCKS, T., CROWE, R. & KASAKOV, L. (1978a). Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmac.*, **63**, 125–138.

BURNSTOCK, G., COCKS, T., KASAKOV, L. & WONG, H.K. (1978b). Direct evidence for ATP release from non-adrenergic, non-cholinergic ('purinergic') nerves in the guinea-pig taenia coli and bladder. *Eur. J. Pharmac.*, **45**, 144–149.

BURNSTOCK, G., CROWE, R & WONG, H. (1978c). Comparative pharmacological and histochemical evidence for purinergic inhibitory innervation of the portal vein of the rabbit but not guinea-pig. *Br. J. Pharmac*, (in press).

CAMPBELL, G. (1970). Autonomic nervous supply of effector organs. In *Smooth Muscle*, ed. Bülbring, E., Brading, A., Jones, A. & Tomita, T. pp. 451–495. London: Edward Arnold.

CREED, K.E. & GILLESPIE, J.S. (1977). Some electrical properties of the rabbit anococcygeus muscle and a comparison of the effects of inhibitory nerve stimulation in the rat and rabbit. *J. Physiol.*, **273**, 137–153.

CREED, K.E., GILLESPIE, J.S. & McCAFFERY, H. (1977). The rabbit anococcygeus muscle and its response to field stimulation and to some drugs. *J. Physiol.*, **273**, 121–135.

FALK, B., HILLARP, N.A., THIEME, G. & TORP, A. (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.*, **10**, 348–354.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

GILLESPIE, J.S. & LULLMAN-RAUCH, R. (1974). On the

ultrastructure of the rat anococcygeus muscle. *Cell Tiss. Res.*, **149,** 91–104.

GILLESPIE, J.S. & McGRATH, J.C. (1974). The response of the cat anococcygeus muscle to nerve or drug stimulation and a comparison with the rat anococcygeus. *Br. J. Pharmac.*, **50,** 109–118.

HOLTON, P. (1959). The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. *J. Physiol.*, **145,** 494–504.

IRVIN, J.L. & IRVIN, E.M. (1954). The interaction of quinacrine with adenine nucleotides. *J. biol. Chem.*, **210,** 45–56.

JOHANSEN, T. (1977). Dependence of histamine release from rat mast cells induced by the ionophore A23187 on endogenous adenosine triphosphate. *Br. J. Pharmac.*, **59,** 474–475P.

KAMIKAWA, Y., SERIZAWA, K., MIZUTANI, M. & SHIMO, Y. (1976). Responses to adenosine 5′-triphosphate (ATP) of the isolated esophageal and gastrointestinal smooth muscles of guinea-pig. *Jap. J. Pharmac.*, **76** suppl., 61P.

KARNOVSKY, M.J. & ROOTS, L. (1964). A 'direct'-colouring thiocholine method for cholinesterases. *J. Histochem. Cytochem.*, **12,** 219–221.

LANGER, S.Z. & PINTO, J.E.B. (1976). Possible involvement of a transmitter different from norepinephrine in residual responses to nerve stimulation of cat nictitating membrane after pretreatment with reserpine. *J. Pharmac. exp. Ther.*, **196,** 697–713.

LOREZ, H.P., DAPRADA, M. & LAUNAY, J.M. (1977). Fluorescence microscopy of 5HT organelles in normal and storage pool deficient blood platelets (P). Abstract *9th Annual Meeting of the Unions of Swiss Societies of Experimental Biology*, Zurich, April.

McELROY, W.D. & SELIGER, H.H. (1963). The chemistry of light emission. *Adv. Enzymol.*, **25,** 119–166.

MALMFORS, T. & THOENEN, H. (1971). *6-Hydroxydopamine and Catecholamine Neurons.* ed. Malmfors, T. & Thoenen, H. Amsterdam and London: North-Holland Publ. Co.

NEEDLEMAN, P., MINKES, M.S. & DOUGLAS, J.R. (1974). Stimulation of prostaglandin biosynthesis by adenine nucleotides. Profile of prostaglandin release by perfused organs. *Circulation Res.*, **34,** 455–460.

OLSON, L., ÅLUND, M. & NORBERG, K.A. (1976). Fluorescence microscopical demonstration of a population of gastrointestinal nerves fibres with a selective affinity for quinacrine. *Cell Tiss. Res.*, **171,** 407–423.

SAN SAN WAI & COUPAR, I.M. (1976). The inhibitory response of the rabbit anococcygeus muscle. *Clin. exp. Pharmac. Physiol.*, **3,** 141–145.

SMITH, A.D. (1972). Subcellular localization of noradrenaline in sympathetic neurons. *Pharmac. Rev.*, **24,** 435–457.

STREHLER, B.L. (1968). In *Methods of Biochemical Analysis*, Vol. 16. ed. Glick, D. pp. 99–181. New York: Interscience.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature, New. Biol.*, **231,** 232–235.

WESTFALL, D.P., STITZEL, R.E. & ROWE, J.N. (1978). The post-junctional effects and neural release of purine compounds in the guinea-pig vas deferens. *J. Pharmac. exp. Ther.* (in press).

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