Br. J. Pharmac. (1977), **60**, 221–231

DISSIMILARITY BETWEEN THE RESPONSES TO ADENOSINE TRIPHOSPHATE OR ITS RELATED COMPOUNDS AND NON-ADRENERGIC INHIBITORY NERVE STIMULATION IN THE LONGITUDINAL SMOOTH MUSCLE OF PIG STOMACH

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1 Transmural electrical stimulation (TMS) of longitudinal smooth muscle strips taken from the cardiac portion of the pig stomach produced biphasic responses consisting of initial contractions followed by relaxations. The excitatory component was enhanced by neostigmine and abolished by atropine. After atropine treatment, TMS and nicotine or 1,1-dimethyl-4-phenyl-piperazinium, caused a relaxation or a relaxation followed by an after-contraction. All of these responses were abolished or reduced reversibly with tetrodotoxin and cocaine, while hexamethonium only abolished the response to ganglion-stimulating agents.

2 The relaxation caused by TMS reached a maximum amplitude at 5–10 Hz, and was entirely resistant to the effects of α- and β-adrenoceptor blocking agents, or a combination of them, and also to guanethidine. These results strongly suggested that the relaxation was elicited by stimulation of intramural non-adrenergic inhibitory neurones.

3 In the presence of atropine and guanethidine, adenosine triphosphate (ATP, 5–20 μM) caused only a tonic contraction, and ATP (25–200 μM) or adenosine diphosphate (25–200 μM) produced a contractile response or a biphasic one (tonic contraction preceded by a slight relaxation). Adenosine monophosphate and adenosine caused only the tonic contraction over the range of concentrations (25–200 μM).

4 Stimulation of the intramural inhibitory neurones of the tissue consistently evoked an inhibitory junction potential, which showed a summation during repetitive stimulation. One the other hand, ATP elicited mainly a small depolarization of a few mV.

5 When the desensitization to ATP of the muscle was achieved in the presence of atropine and guanethidine, the relaxation induced by stimulation of the non-adrenergic inhibitory neurones could be evoked without any modification.

6 Dipyridamole neither potentiated the inhibitory responses due to stimulation of the intramural inhibitory neurones nor showed any consistent effect on the ATP-induced response.

7 From these results, it is unlikely that ATP, or any related compound, is the transmitter substance of the intramural inhibitory neurones in the longitudinal smooth muscle of the pig stomach.

Introduction

Adenosine triphosphate (ATP) or a related compound has been proposed by Burnstock, Campbell, Satchell & Smythe (1970) and Burnstock (1972) as a transmitter substance for the intramural inhibitory non-adrenergic, non-cholinergic neurones of the vertebrate gastrointestinal tract (purinergic nerves hypothesis). A parallelism between the effects of exogenously applied ATP or its analogues and the nerve stimulation was taken as evidence for this hypothesis (Burnstock *et al.*, 1970; Burnstock, Satchell & Smythe, 1972; Satchell, Lynch, Bourke & Burnstock, 1972; Satchell, Burnstock & Dann, 1973; Tomita & Watanabe, 1973). However, it has been reported that ATP cannot mimic the stimulatory effect of intramural inhibitory neurones in some gastrointestinal smooth muscle preparations (Burnstock *et al.*, 1970; MacKay & McKirdy, 1972;

Furness & Costa, 1973; Takewaki, Ohashi & Okada, unpublished results). Thus, further comparative experiments should be carried out to determine whether the stimulatory effects of the inhibitory neurones and ATP or its related compounds are the same in various other smooth muscle preparations.

Recently we found that isolated longitudinal smooth muscle from the pig stomach is useful for the investigation of the inhibitory mechanical responses because the tone of the preparation can be maintained at a high level for long periods without spontaneous fluctuations. The primary purpose of the present experiments was to clarify the intramural innervation of the smooth muscle, with special reference to the nature of the inhibitory neurones. It was found that the smooth muscle was supplied by excitatory cholinergic and inhibitory non-adrenergic, non-cholinergic postganglionic nerve fibres. Therefore, we tried to see whether there was any parallelism between the stimulatory effects of non-adrenergic inhibitory nerves and exogenously applied ATP or its analogues in this preparation. Some of these results were described at the 16th Congress of the Japanese Smooth Muscle Society, Fukuoka, 1974.

Methods

Experiments were carried out on isolated, longitudinal smooth muscles of the pig (Landrace) stomach. A part of the cardiac region of the stomach wall (5 x 5 cm) was removed from healthy adult pigs of either sex, which had been slaughtered at the local abattoir. The strips were then immersed in a cold saline solution and transported to the laboratory with a delay of approximately 60 minutes. After both the mucosal layer and the circular muscle coat were removed, the longitudinal smooth muscles were cut into segments of about 2.0 cm in length and 0.2 cm in width. A segment was placed vertically in an organ bath containing 5 ml of Krebs-Henseleit solution (mM: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.5; pH 7.3–7.4) equilibrated with a mixture of 95% O_2 and 5% CO_2. The solution was kept at 37–38°C and flowed continuously into the bath from a reservoir through polyethylene tubing connected to the bottom of the bath by a micropump (Tokyo Rika Kikai, MP-II). The overflowing fluid was removed by suction, and the volume of the bathing solution was kept constant.

The mechanical activities of the preparations were recorded isometrically with mechano-electronic transducers (Toshiba 5734A or Nihon Kohden, SB-IT) on the ink-writing oscillograph (Nihon Kohden, WI-260). The preparation was loaded with a tension of 2 g and allowed to equilibrate for 60–90 min before starting the experiment.

Transmural electrical stimulation (TMS) to the muscles was applied through two parallel, non-polarizing Ag-AgCl electrodes (5 x 25 mm, separated by 5 mm) placed at either side along the whole length of the preparations. An electronic stimulator (Nihon Kohden, MSE-3R) was used to deliver the rectangular pulses. Trains of pulses were applied for 10–20 s at intervals of not less than 3 min, unless otherwise stated.

In some experiments, changes in the membrane potential due to TMS or to added drugs were observed with the conventional sucrose-gap apparatus, as described by Stämpfli (1954) and Burnstock & Straub (1958). The method of stimulation of the intramural nerves was that described by Ohashi & Ohga (1967).

The drugs were applied as injections or as constant infusions. The final concentrations of drugs are expressed as g/ml or molar concentration (M).

Drugs used were: acetylcholine chloride (ACh), adenosine, adenosine-5′-diphosphate sodium salt (ADP), adenosine-5′-monophosphate sodium salt (AMP), adenosine-5′-triphosphate disodium salt (ATP), adrenaline bitartrate, atropine sulphate, carbamoylcholine hydrochloride (carbachol), cocaine hydrochloride, dibenamine hydrochloride, 1-1-dimethyl-4-phenyl-piperazinium (DMPP), dipyridamole, guanethidine monosulphate, hexamethonium chloride, isoproterenol hydrochloride, neostigmine methylsulphate, nicotine bitartrate, noradrenaline bitartrate, phenoxybenzamine hydrochloride, 5-(3-tert-butylamino-2-hydroxy)propoxy-3,4-dihydrocarbostyril hydrochloride (OPC-1085), tetrodotoxin citrate (TTX), and tyramine hydrochloride.

Results

The tone of the preparation increased during the equilibrium period of 60–90 min, and reached a steady level (5–10 g) of about 2.5–5 times the initial value and was maintained for several hours. Both the development and maintenance of the tonus were not affected either by atropine (1 μg/ml) or TTX (0.5 μg/ml). The tonus, however, fell to a low level without the calcium and at a low temperature (27–28°C).

Frequency-response relationships

Relationships between stimulation frequency (1–80 Hz) and responses were investigated by applying stimuli for 10 s with a fixed pulse of 1.0 ms duration and a supramaximal voltage. TMS caused only contractile responses over the range of frequencies (5–80 Hz) in the low tone preparations ($n=4$). The contractile response appeared at 5 Hz and reached a maximum at 40–80 Hz. In contrast, in the tonic preparations (Figure 1a), only a relaxation was obtained at a low range of frequencies (1–5 Hz,

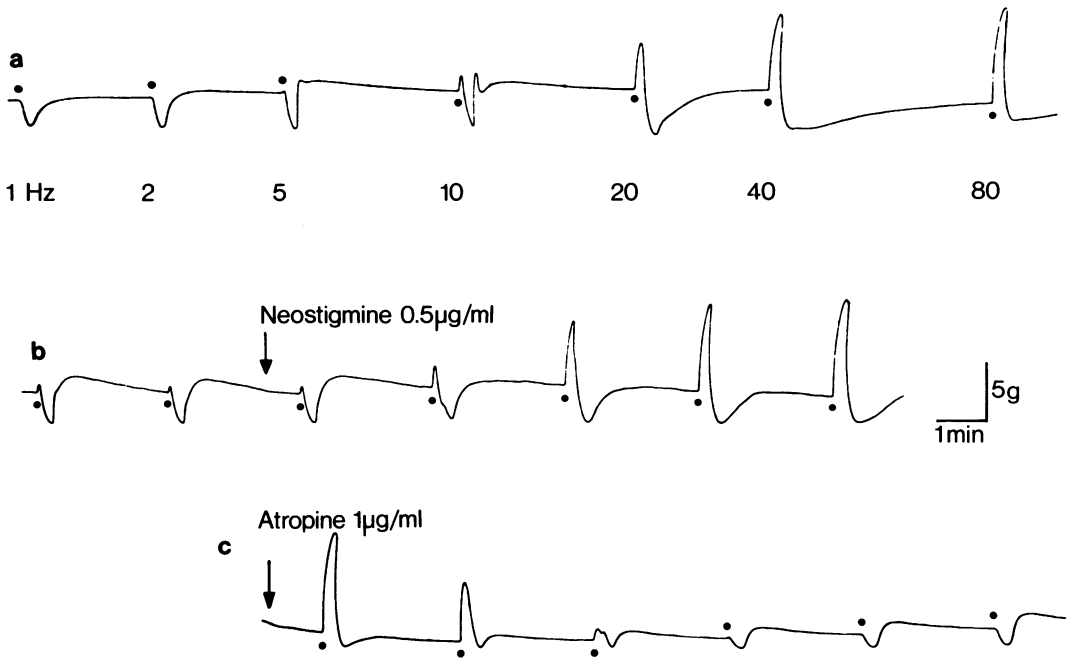

Figure 1 Relationships between frequency of transmural stimulation (TMS) and (a) types of responses and (b) effects of neostigmine and (c) atropine on contractile response to TMS in the longitudinal smooth muscle from the pig stomach. The records obtained from the same preparation are shown. TMS was applied at various frequencies (a) or 20 Hz (b,c) for 10 s every 3 minutes. Numbers under trace (a) refer to frequency of TMS and (●) indicate points of the stimulation. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

$n=18$). However, when the frequency was increased over 10 Hz, the response became somewhat variable. The response was classified into the following four types: initial rapid contraction followed by relaxation with after-contraction ($n=3$); the same without after-contraction ($n=5$); relaxation followed by after-contraction ($n=7$); pure relaxation ($n=3$). The initial contractile component observed during stimulation also reached a maximum amplitude at 40–80 Hz (Figure 1a).

The relationships between stimulation frequency (1–40 Hz) and relaxation response were also examined after treatment with atropine (0.5–1.0 μg/ml). In ten preparations, TMS elicited a relaxation over the range of frequency from 1–40 Hz. In general, the inhibitory responses were more marked, the greater the tone. Figure 2 shows the depth of relaxations increased with an increase in frequency until it reached a maximum at about 5–10 Hz. Sometimes, maximum relaxation occurred at 2 Hz. When the frequency was increased more than 5 Hz, the depth of the relaxation usually remained fairly constant, and the response was followed by a long-lasting after-contraction in eight preparations (Figure 2a). In two other cases, the relaxations were observed over the frequencies used (Figure 2b). The after-contraction began to develop at the end of each stimulus and increased in amplitude with increasing frequencies of up to 20–40 Hz.

All the responses described so far may be mediated by stimulation of the intramural nerve because TMS with a short pulse duration was effective. Thus, further experiments were carried out to study the nature of the neurones and the transmitter substances which might be involved in producing the present response.

Excitatory pathway

ACh (0.1–10 μg/ml) or carbachol (10 ng–1 μg/ml) contracted the preparations, depending on the concentrations used. TTX (0.25 μg/ml) abolished the contractile response caused by TMS in low tone preparations, but did not affect that induced by ACh. Hexamethonium (100 μg/ml) had no significant effect on the contraction induced by TMS. The contractions in low tone preparations and the initial brief excitatory component of biphasic or triphasic response in tonic preparations caused by TMS, and the contractions produced by ACh were greatly enhanced by neostigmine (0.1–0.5 μg/ml, $n=7$, Figure 1b) and

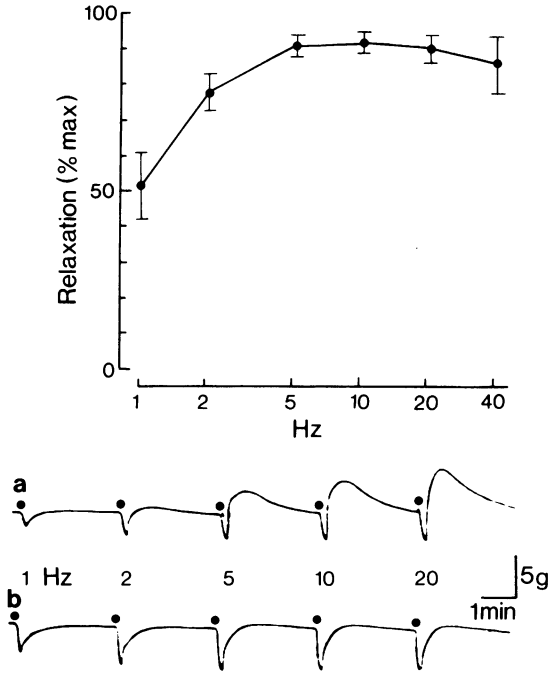

Figure 2 Relationships between frequency of transmural stimulation (TMS) and amplitude of relaxation of the longitudinal smooth muscle from the pig stomach (in the presence of atropine 0.5 μg/ml). Ordinate scale: amplitude of relaxations expressed as a percentage of the maximum relaxation. Each point is the mean of 10 preparations. Vertical bars show s.e. means. Abscissa scale: frequency of TMS on a logarithmic scale. Traces (a,b): two types of responses elicited by TMS. TMS was applied at various frequencies for 10 s every 3 min; (●) indicate points of the stimulation. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

abolished by atropine (0.5–1.0 μg/ml, $n=8$, Figure 1c). After application of atropine, pure relaxations or relaxations followed by after-contractions were observed in response to TMS (Figures 1c and 2a,b).

Inhibitory pathway

In order to analyze the inhibitory responses, the following experiments were performed in the presence of atropine (0.5–1.0 μg/ml). Both TTX (0.25–0.5 μg/ml, $n=8$) and cocaine (50–100 μg/ml, $n=10$) reversibly abolished the inhibitory responses induced by TMS but not those induced by adrenaline (0.25–1.0 μg/ml, Figure 3) and isoproterenol (1 μg/ml).

Nicotine (5–50 μg/ml, $n=23$) and DMPP (5–20 μg/ml, $n=21$) relaxed the preparations in a dose-dependent manner, and the relaxations were also sensitive to TTX and cocaine. Generally, the relaxations developed after a delay of about 10 s and reached their maximum response within a minute.

As shown in Figure 3, the inhibitory responses produced by TMS were little affected by hexamethonium (100–250 μg/ml, $n=11$), which completely abolished the relaxations induced by the ganglionic stimulants ($n=5$). Furthermore, the relaxation in response to TMS and nicotine or DMPP was entirely resistant to α-adrenoceptor blocking agents (phenoxybenzamine 0.5–2.0 μg/ml, $n=3$, dibenamine 5.9 μg/ml, $n=2$) and β-blockers (propranolol 1–5 μg/ml, $n=19$, OPC-1085 1–2 μg/ml, $n=3$) or the combined use ($n=18$) of α- and β-blockers and an adrenergic neurone blocking agent (guanethidine 1–5 μg/ml, $n=10$) (Figures 3 and 4). However, the combined use of α- and β-blocking agents reduced or abolished the relaxations caused by adrenaline (Figure 4) or other sympathomimetic drugs.

The relaxations in response to TMS (0.5–5 Hz) and to ganglionic stimulants were not potentied by cocaine (2–25 μg/ml, $n=7$). Tyramine (2–400 μg/ml, $n=10$) was ineffective in producing the relaxations.

Figure 5a shows that a single pulse of 0.3 ms width or less with a supramaximal intensity gave a transient hyperpolarization of up to 5 mV in the quiescent preparations in the presence or absence of atropine (1 μg/ml, $n=8$). The latency varied between 150–300 ms, and the time required to reach a maximum hyperpolarization was approximately 500 ms or more. When repetitive stimulation (1–10 Hz) was applied to the preparations, individual hyperpolarizations summed with each other to give a larger hyperpolarization (Figure 5a). The amplitude and the rate of hyperpolarization of up to about 15 mV was produced at 10 Hz, the highest frequency used. The hyperpolarization was associated with a relaxation, the amplitude of which was proportional to the size of the hyperpolarization. When repetitive stimulation with a frequency of over 5 Hz was terminated, the membrane potential was reversed transiently to a depolarization of a few mV. At this period, the recovery of the relaxation was facilitated, and after-contractions were often observed.

As shown in Figure 5b, the hyperpolarization was reversibly abolished by TTX (0.25–0.5 μg/ml, $n=4$) and cocaine (50–100 μg/ml, $n=2$), but not significantly affected by hexamethonium (100 μg/ml, $n=2$), guanethidine (1 μg/ml, $n=4$) and the combined use ($n=4$) of phenoxybenzamine (0.5 μg/ml) and OPC-1085 (1 μg/ml).

These pharmacological and electrophysiological analyses suggested that the smooth muscle was supplied with non-adrenergic inhibitory nerves, and that hyperpolarization evoked by TMS is an inhibitory junction potential (i.j.p.) resulting from the excitation of the inhibitory nerves.

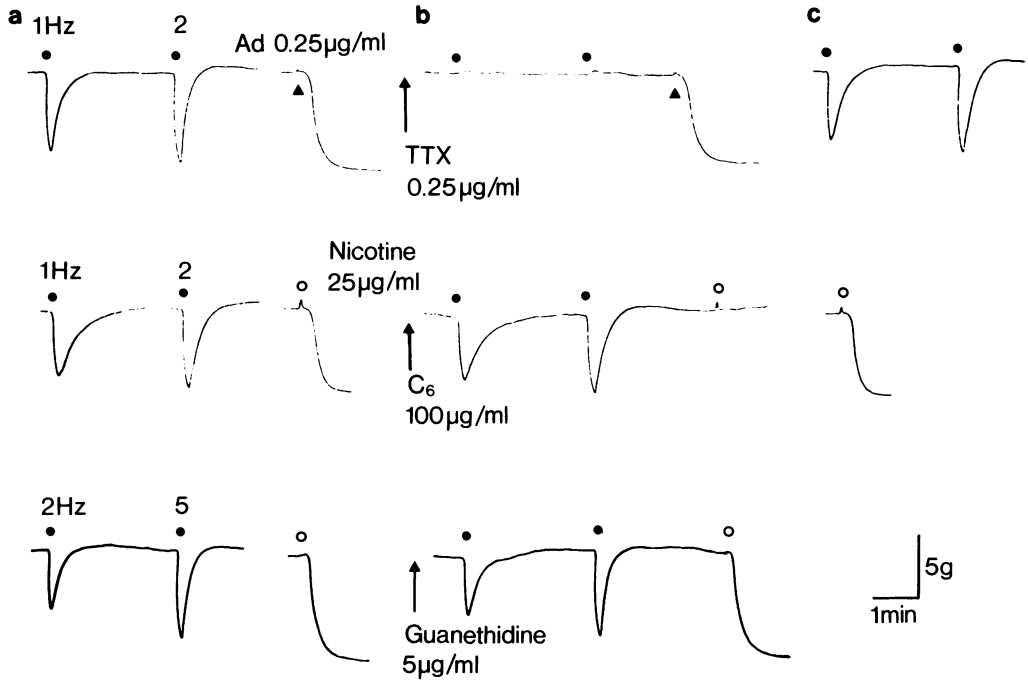

Figure 3 Effects of tetrodotoxin (TTX), hexamethonium (C_6) and guanethidine on inhibitory responses to transmural stimulation (TMS), nicotine and adrenaline (Ad) of the longitudinal smooth muscle from the pig stomach (in the presence of atropine 0.5 μg/ml). (a) Control responses to TMS (●), nicotine (25 μg/ml, ○) and Ad (0.25 μg/ml, ▲). (b) Responses after treatment with TTX (0.25 μg/ml), C_6 (100 μg/ml) and guanethidine (5 μg/ml). (c) Responses after washing out TTX and C_6. TMS was applied for 10 s every 3 min, and the preparations were exposed to each agonist for 60–90 seconds. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

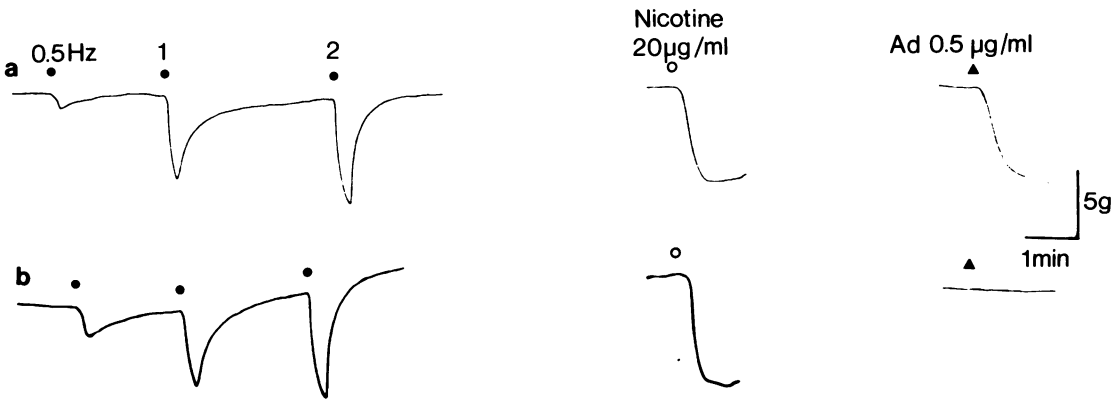

Figure 4 Effects of phenoxybenzamine plus OPC-1085 on inhibitory responses to transmural stimulation (TMS), nicotine and adrenaline of the longitudinal smooth muscle from the pig stomach (in the presence of atropine 0.5 μg/ml). (a) Control responses to TMS (●), nicotine (20 μg/ml, ○) and adrenaline (Ad, 0.5 μg/ml, ▲). (b) Responses after treatment with phenoxybenzamine (0.5 μg/ml) and OPC-1085 (2 μg/ml). TMS was applied for 10 s every 3 min and the tissues were exposed to the agonist for 90 seconds. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams.

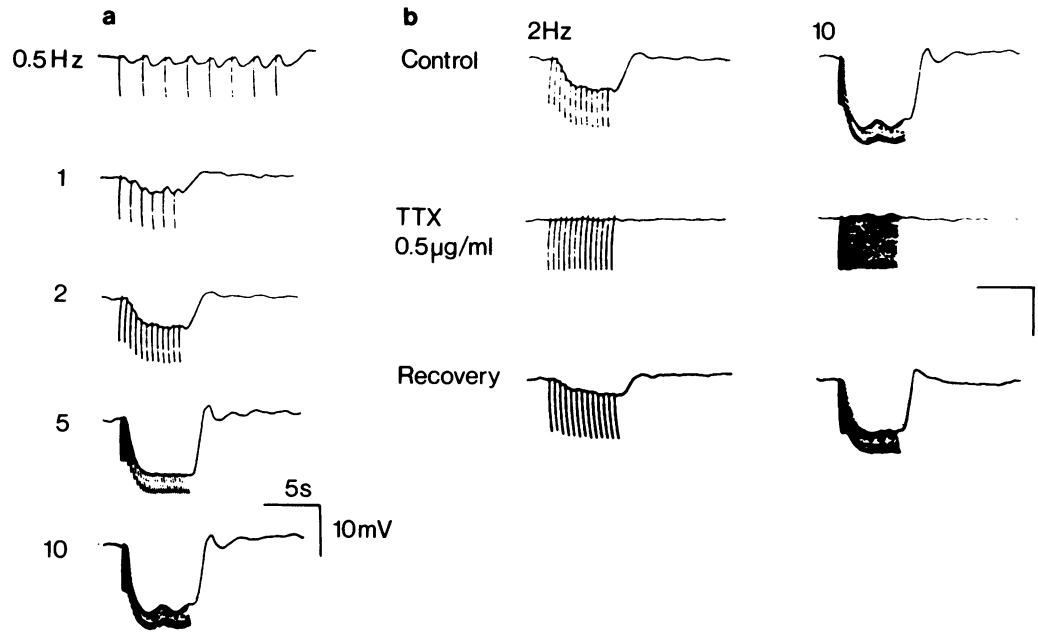

Figure 5 Sucrose-gap records from the longitudinal smooth muscle of the pig stomach. The records from the same preparation are shown. (a) Hyperpolarization induced by transmural stimulation (TMS) at different frequencies (0.5–10 Hz). (b) Effect of tetrodotoxin (TTX, 0.5 μg/ml) on the hyperpolarization. TMS with a brief pulse duration (<0.3 ms) was applied at the various frequencies (0.5–10 Hz) for about 5 seconds. The horizontal bar shows the scale for 5 s and the vertical bar the scale for 10 mV.

Comparison between effects of transmural stimulation and exogenously applied ATP or its related compounds

Burnstock and his colleagues have proposed that the neurohumoral transmitter of the intramural non-adrenergic inhibitory neurones in the gut is an adenine nucleotide, probably ATP. If this is the case, exogenously applied ATP should mimic the response caused by the stimulation of the inhibitory neurones in this prearation. To make certain of this, the responses to TMS and to ATP or its related compounds were compared in the presence of atropine (0.5–1.0 μg/ml) plus guanethidine (1 μg/ml).

There was a distinct difference between the responses to adenine nucleotides or to adenosine and those to the inhibitory nerve stimulation. As illustrated in Figure 6a, ATP (50 μM) caused only a contractile response, despite the fact that TMS at a lower frequency (<5 Hz) produced a marked relaxation.

ATP at a concentration lower than 2 μM elicited no response at all in any of the 15 preparations. ATP (5–20 μM) evoked only a contractile response in 46 out of 70 observations. A threshold dose to induce a contraction was usually between 5 to 10 μM. In general, the contractile response consisted of a tonic contraction with or without spontaneous activity superimposed on it. The contractions began slowly, developed gradually, and attained a peak amplitude within 1–2 minutes. In 24 other cases, ATP in these concentrations induced no response. With a higher dose of ATP (25–200 μM), in addition to a pure tonic contraction, a biphasic response (a tonic contraction preceded by a slight relaxation) was observed. The tonic contractile response to ATP in this range of concentrations was observed in over 57% of the responses, and the biphasic response was noted in 35% of the 153 observations. Such biphasic responses were observed predominantly with a much higher dose of ATP (500 μM or more, in 17 out of 18 observations). The dose-dependency of the slight relaxation in the biphasic response, however, was not clear. TTX (0.5 μg/ml, $n=4$) did not affect the contraction or the biphasic response to ATP. Thus, the responses appeared to be mediated by the direct action of ATP on the smooth muscle.

In contrast to the effects of ATP, TMS at lower frequencies (0.5, 1 and 2 Hz) caused a pure relaxation in about 70% and biphasic responses (marked relaxations followed by after-contractions) in about 30% of the 103 observations. In response to TMS at higher frequencies (5, 10, 20 and 40 Hz) relaxation was observed in 39% of the cases, and a biphasic response in 61% of the 117 observations.

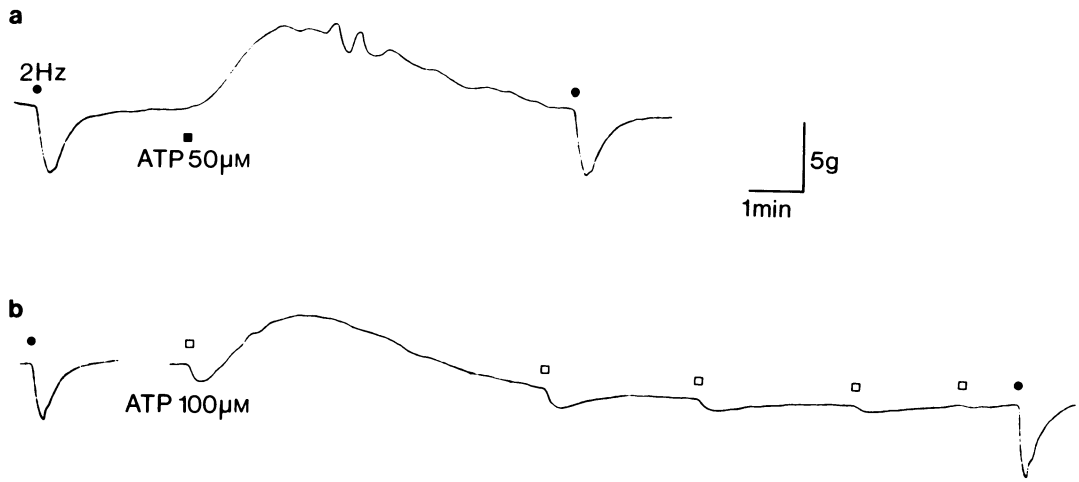

Figure 6 Dissimilarity between effects of transmural stimulation (TMS) and ATP in the presence of atropine (1 μg/ml) and guanethidine (2 μg/ml). (a) ATP (50 μM, ■) caused a tonic contraction while TMS (●) with a frequency of 2 Hz evoked only a relaxation. (b) Effects of desensitization to ATP (100 μM, □) on relaxation induced by TMS. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 5 grams. Note that the inhibitory response induced by TMS was observed without any modification, even after the biphasic responses caused by ATP were completely abolished.

The effects of ADP, AMP and adenosine were also observed under similar experimental conditions. ADP (25–200 μM) caused a tonic contractile response or a biphasic one. The percentage appearance of the contractile response and the biphasic response to ADP ($n=53$) was about the same as that caused by ATP. The same AMP and adenosine doses elicited only a contractile response in all 40 and 49 observations, respectively. The contractile responses to ADP, AMP and adenosine were also dose-dependent, and there was no significant qualitative difference in their time course. The order of potency of adenine nucleotides and adenosine in their ability to elicit contractile responses was ADP > ATP > AMP > adenosine (Figure 7).

Figure 8 shows the change in membrane potential due to the application of ATP. In the spontaneously active preparations, ATP (50–100 μM) increased the frequency of spike discharges accompanied by tension development (Figure 8a). In the quiescent muscles, ATP caused depolarization of the cell membrane,

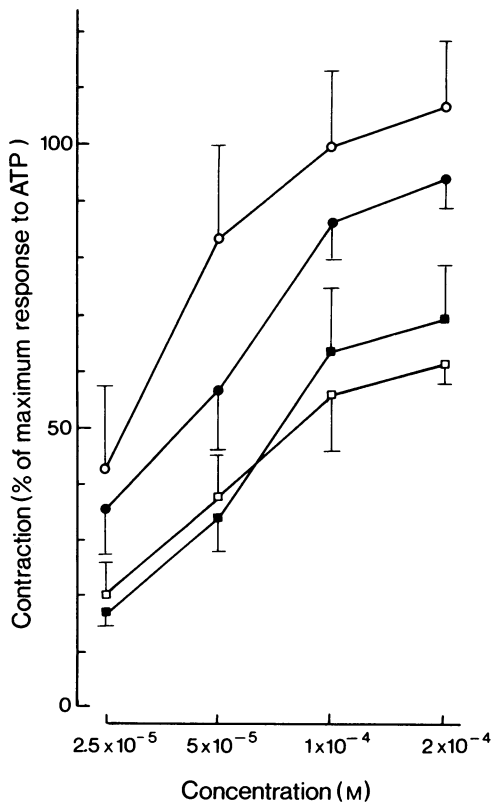

Figure 7 Dose-response curves for adenine nucleotides and adenosine in causing contractions of longitudinal smooth muscle from the pig stomach. Ordinate scale: amplitude of contractions expressed as a percentage of the maximum contraction to ATP. Each point is the mean of at least 6 preparations. Vertical bars show s.e. mean. Abscissa scale: molar dose of the drugs. Dose-response curves are shown for ATP (●), ADP (○), AMP (■) and adenosine (□).

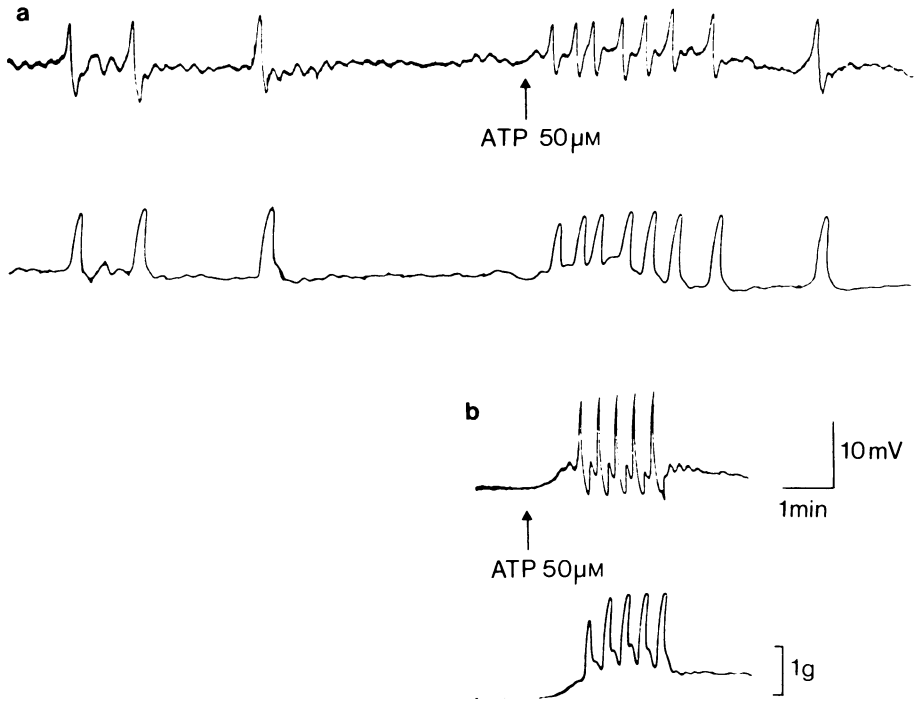

Figure 8 Sucrose-gap records from the longitudinal smooth muscle of the pig stomach showing excitatory responses to ATP (50 μM) in (a) spontaneously active preparations and in (b) quiescent preparations. Upper trace: membrane potential. Lower trace: tension. Arrows refer to points of the drug application. The vertical bars are the scale for 10 mV and 1 g, and the horizontal bar the scale for 1 min, respectively.

which was usually superimposed with action potentials (Figure 8b). The depolarization was followed by a tonic contraction. When action potentials were generated, phasic contractions were superimposed upon the tonic contraction.

Tachyphylaxis to ATP

It has been reported that stimulation of non-adrenergic inhibitory nerves fails to induce the inhibitory responses of the rabbit ileum which has been desensitized to ATP by exposure to a high dose of the drug (Burnstock *et al.*, 1970). This result is considered as evidence supporting the purinergic nerves hypothesis. In the rabbit ileum, however, exposure to high concentrations of either ATP or adenosine desensitized the preparation to ATP, ADP, AMP and adenosine, but the inhibitory responses to the intramural or sympathetic nerve stimulation and to sympathomimetic agents were not affected (Weston, 1973). We, therefore, investigated whether the pig stomach preparations could be desensitized to ATP and TMS at the same time.

Desensitization to ATP could be quite easily achieved in this preparation by repeated administration of ATP (50–100 μM, $n=6$) at short intervals without washing out the bath. As shown in Figure 6b, a long-lasting tonic contraction preceded by a slight relaxation disappeared more quickly than the inhibitory response. Even after a second application of ATP at intervals of 2–3 min, a further addition of ATP failed to cause the contractile response. The residual inhibitory component also decreased in amplitude and disappeared after a few subsequent administrations of the drug. When the ATP desensitization of the preparations was completed, however, the TMS (1–5 Hz) still evoked a relaxation without any significant modification in the presence of atropine plus guanethidine (Figure 6b). The effect of desensitization to ATP was abolished by washing out the bath solution for about 30 minutes.

Effect of dipyridamole

Satchell *et al.* (1972) have reported that dipyridamole and hexobendine selectively potentiated the inhibitory response of the guinea-pig taenia coli to non-adrenergic nerve stimulation simultaneously with ATP. We also attempted to observe the effect of dipyridamole on the responses to TMS and ATP and

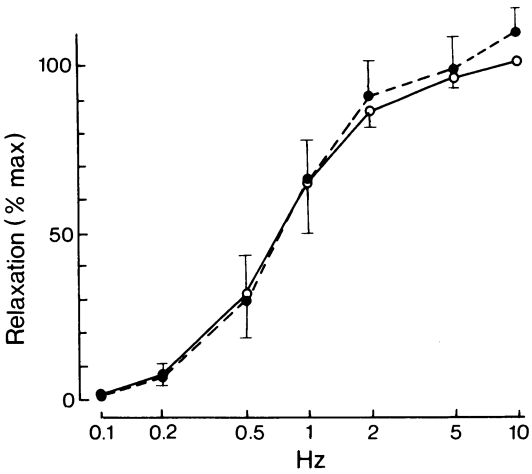

Figure 9 Effects of dipyridamole on inhibitory responses caused by transmural stimulation (TMS) at various frequencies in the presence of atropine (0.5 μg/ml) and guanethidine (1 μg/ml). Ordinate scale: amplitude of relaxations expressed as a percentage to the maximum relaxation. Each point is the mean of 5 preparations. Vertical bars show s.e. mean. Abscissa scale: frequency of TMS on a logarithmic scale. Frequency-response curves are presented for the control (○), and for that after treatment with dipyridamole (0.2–1 μg/ml, ●).

noradrenaline. As illustrated in Figure 9, the inhibitory response to TMS (0.1–10 Hz) in the presence of atropine and guanethidine was not potentiated by dipyridamole (0.2–1 μg/ml) in any of the 15 cases. Furthermore, dipyridamole did not cause any consistent effect on the responses to ATP (20–200 μM, $n=18$). More specifically, the tonic contractions ($n=13$) or the contractile components of the biphasic responses ($n=5$) caused by ATP were unaffected ($n=9$), potentiated ($n=4$) or inhibited ($n=5$), and the inhibitory components remained unaffected in all of the observations. The effect of dipyridamole on the inhibitory response to noradrenaline (0.5 μg/ml, $n=6$) was also variable, i.e., it was reduced or reversed to a contractile response.

Discussion

The present experiments show that the longitudinal smooth muscle of the pig stomach is innervated at least with intrinsic cholinergic excitatory and non-adrenergic inhibitory nerves, and ATP (or its related compounds) is probably not the transmitter substance in the inhibitory neurones.

Biphasic responses induced by TMS were reversibly abolished by TTX and cocaine, but they were not significantly affected by hexamethonium. The contractile component of the response was enhanced by neostigmine and abolished by atropine. After treatment with atropine, TMS and ganglionic stimulants consistently produced a relaxation. The physiological and pharmacological properties of the relaxation were similar to those caused by excitation of the non-adrenergic inhibitory neurones in the atropinized gut smooth muscle preparations isolated from various animals (see list of references in Burnstock, 1972). The similarity was that stimulation at a low range of frequencies and ganglionic stimulants were effective in causing a relaxation; either a single or repetitive stimulation (<10 Hz) caused hyperpolarization (i.j.p.). All the mechanical and electrical responses were insensitive to the adrenergic neurone blocking agent, and to α- and β-adrenoceptor blocking agents. From these similarities, it may be suggested that the nerves responsible for inducing the relaxations of the present preparation are similar to the intramural non-adrenergic inhibitory neurones, as reported for various other preparations.

There was no parallelism between the effects of stimulation of non-adrenergic inhibitory neurones and of exogenously applied ATP or its related compounds in the present preparation. In the presence of atropine and guanethidine, ATP (5–20 μM), AMP and adenosine (25–200 μM) caused only a tonic contraction, despite the fact that TMS with low frequencies (>5 Hz) usually produced a marked relaxation. The contractile response induced by ATP was always preceded by membrane depolarization. However, both ATP and ADP in a higher concentration of 25–200 μM, caused a tonic contraction preceded by a slight relaxation in 35% out of the 153 and 53 observations, respectively. The incidence of a marked relaxation with an after-contraction was increased by higher frequency stimulation (<5 Hz) of the intramural inhibitory nerves. Burnstock *et al.* (1970) have pointed out that there may exist two different ATP receptors for excitation and for inhibition in the gastrointestinal smooth muscle. The contractions elicited by adenine nucleotides and adenosine in this preparation increased in amplitude in a dose-dependent manner, but the relaxation did not. On the other hand, the amplitude of relaxation induced by TMS was increased with an increase in frequency until it reached a maximum at about 5–10 Hz. In addition, even with a high concentration such as 1 mM, ATP never produced a relaxation having a comparable amplitude with those caused by TMS at the optimum frequency (1–5 Hz). The dissimilarity between the response induced by stimulation of the intramural inhibitory nerve and that induced by ATP or its related compounds has been reported in the longitudinal smooth muscle of the rabbit rectum (MacKay & McKirdy, 1972) and in the rat stomach (Heazell, 1969, cited by Furness & Costa, 1973; Burnstock *et al.*, 1970) and in the chicken colon and rectum (Takewaki, Ohashi & Okada, unpublished

results). Moreover Rikimaru, Fukushi & Suzuki (1971), Suzuki, Fukushi & Rikimaru (1971) and Saito (1972) have reported that phentolamine and imidazole greatly reduced the relaxations of the guinea-pig taenia coli induced by ATP without affecting those due to the excitation of the non-adrenergic inhibitory nerves.

Furthermore, even after both the inhibitory and excitatory components of the biphasic response to ATP were abolished, TMS was still effective in producing the relaxation without any modification. Similar results have been reported in the rabbit duodenum (Weston, 1973) and chicken colon and rectum (Takewaki, Ohashi & Okada, unpublished results). Quite recently, Hooper, Spedding, Sweetman & Weetman (1974) have reported that 2-2'-pyridylisatogen tosylate (PIT) is a specific antagonist to ATP. This compound was found to inhibit the relaxation of guinea-pig taenia coli elicited by ATP without affecting the inhibitory responses to stimulation of non-adrenergic inhibitory nerves (Spedding, Sweetman & Weetman, 1975).

Satchell *et al.* (1972) and Satchell & Burnstock (1975) have suggested that dipyridamole and hexobendine potentiated the inhibitory responses of the isolated guinea-pig taenia coli induced by both the stimulation of non-adrenergic inhibitory nerves and ATP, by inhibiting the uptake of adenosine. In the present preparation, however, dipyridamole was not found to be effective in either potentiating the inhibitory responses induced by TMS or in causing any consistent effect on the ATP-induced responses. The ineffectiveness of dipyridamole in enhancing the relaxations resulting from the excitation of non-adrenergic inhibitory nerves in the rabbit duodenum (Hulme & Weston, 1974) and in the rat stomach (Heazell, 1975) has also been reported.

It appears, therefore, that the purinergic nerves hypothesis proposed by Burnstock *et al.* (1970) and Burnstock (1972) is not applicable to intramural inhibitory transmission from non-adrenergic inhibitory neurones to the smooth muscle.

The authors wish to thank Dr Y. Nakazato and Mr K. Saito for useful suggestions and comments on this manuscript. We also are grateful to Tanabe Seiyaku for supplying the dipyridamole, to Sumitomo kaguka Kogyo for the guanethidine, and to Otsuka Seiyaku for the OPC-1085.

References

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

BURNSTOCK, G., CAMPBELL, G., SATCHELL, D. & 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., 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.

BURNSTOCK, G. & STRAUB, R.W. (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. *J. Physiol., Lond.*, **140**, 156–167.

FURNESS, J.B. & COSTA, M. (1973). The nervous release and the action of substances which affect intestinal muscle through neither adrenoceptors nor cholinoreceptors. *Phil. Trans. R. Soc. Lond. B.*, **265**, 123–133.

HEAZELL, M.A. (1975). Is ATP an inhibitory neurotransmitter in the rat stomach? *Br. J. Pharmac.*, **55**, 285–286P.

HOOPER, M., SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1974). 2-2'-Pyridylisatogen tosylate: an antagonist of the inhibitory effects of ATP on smooth muscle. *Br. J. Pharmac.*, **50**, 458–459P.

HULME, M.E. & WESTON, A.H. (1974). Some effects of dipyridamole hexobendine and lidoflazine on inhibitory processes in rabbit duodenum. *Br. J. Pharmac.*, **50**, 609–611.

MACKAY, D. & McKIRDY, H.C. (1972). Effect of vasopressin and of adenosine triphosphate on the flat preparation of rabbit rectum. *Br. J. Pharmac.*, **44**, 366–367P.

OHASHI, H. & OHGA, A. (1967). Transmission of excitation from the parasympathetic nerve to the smooth muscle. *Nature, Lond.*, **216**, 291–292.

RIKIMARU, A., FUKUSHI, Y. & SUZUKI, T. (1971). Effects of imidazole and phentolamine on the relaxant responses of guinea-pig taenia coli to transmural stimulation and to adenosine triphosphate. *Tohoku J. exp. Med.*, **105**, 199–200.

SAITO, K. (1972). Effects of extracellularly applied ATP and its related nucleotides on the membrane potential of the guinea-pig taenia coli. *Jap. J. Smooth Muscle Res.*, **8**, 32–39.

SATCHELL, D.G. & BURNSTOCK, G. (1975). Comparison of the inhibitory effects on the guinea-pig taenia coli of adenine nucleotides and adenosine in the presence and absence of dipyridamole. *Eur. J. Pharmac.*, **32**, 324–328.

SATCHELL, D.G., BURNSTOCK, G. & DANN, P. (1973). Antagonism of the effects of purinergic nerve stimulation and exogenously applied ATP on the guinea-pig taenia coli by 2-substituted imidazolines and related compounds. *Eur. J. Pharmac.*, **23**, 264–269.

SATCHELL, D.G., LYNCH, A., BOURKE, P.M. & BURNSTOCK, G. (1972). Potentiation of the effects of exogenously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridamole and hexobendine. *Eur. J. Pharmac.*, **19**, 343–350.

SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1975). Antagonism of adenosine 5′-triphosphate-induced relaxation by 2-2′-pyridylisatogen in the taenia of guinea-pig caecum. *Br. J. Pharmac.*, **53**, 575–583.

STÄMPFLI, R. (1954). A new method for measuring membrane potentials with external electrodes. *Experientia*, **10**, 508–509.

SUZUKI, T., FUKUSHI, Y. & RIKIMARU, A. (1971). Relaxant effect of adenosine triphosphate and its related nucleotides on the guinea-pig taenia coli. *Jap. J. Smooth Muscle Res.*, **7**, 207–212.

TOMITA, T. & WATANABE, H. (1973). A comparison of the effects of adenosine triphosphate with noradrenaline and with the inhibitory potential of the guinea-pig taenia coli. *J. Physiol., Lond.*, **231**, 167–177.

WESTON, A.H. (1973). Nerve-mediated inhibition of mechanical activity in rabbit duodenum and the effects of desensitization to adenosine and several of its derivatives. *Br. J. Pharmac.*, **48**, 302–308.

(Received November 29, 1976)