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THE DEVELOPMENT OF TACHYPHYLAXIS TO ELECTRICAL STIMULATION IN GUINEA-PIG ILEAL LONGITUDINAL MUSCLES AND THE POSSIBLE PARTICIPATION OF ADENOSINE AND ADENINE NUCLEOTIDES

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1 Electrically (30 Hz) induced contractions of guinea-pig isolated ileal longitudinal muscles were reduced by tetrodotoxin (1 μM), atropine (1 μM), adenosine (30 μM) and morphine (10 μM).

2 When stimulated with 10 or 30 Hz for 10 s at 1 min intervals, a progressive decline of amplitude of the contraction was seen (development of tachyphylaxis). At this time, the contractile response to 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (10 μM) was also greatly reduced.

3 The smaller responses to electrical stimulation and DMPP during tachyphylaxis were restored to their initial amplitude by the addition of theophylline (10 μM). The appearance of tachyphylaxis was prevented by pretreatment with theophylline (1 to 10 μM) and was greatly accelerated by pretreatment with dipyridamole (0.1, 1 μM).

4 In [^{14}C]-choline or [^{3}H]-adenosine preloaded muscle strips, electrical stimulation (30 Hz) increased the ^{14}C- or ^{3}H-output, the effect being sensitive to tetrodotoxin blockade. The tachyphylaxis to electrical stimulation was accompanied by a considerable and sustained increase in ^{3}H-output, an effect that was accelerated by dipyridamole (1 μM). The ^{14}C-output initially increased but fell off gradually with the development of tachyphylaxis at which time theophylline (30 μM) reversed the fall.

5 There was a marked increase in the proportion of released [^{3}H]-adenosine to its derivatives during the development of tachyphylaxis. Approximately 60% of the released total radioactivity after tachyphylaxis was found to be [^{3}H]-adenosine.

6 These results suggest that the development of tachyphylaxis may be closely associated with the release of endogenous adenosine derivatives (mostly adenosine) which have presynaptic inhibitory actions on the cholinergic elements in guinea-pig ileum.

Introduction

Adenosine and adenine nucleotides have been known as effective smooth muscle relaxants for a long time. Physiological roles for these compounds have been proposed, including functions in the regulation of coronary blood flow (Berne, Rubio, Dobson & Cornish, 1971) and as transmitter substances in non-cholinergic or non-adrenergic nerves in various intestinal and other smooth muscles (Burnstock, Campbell, Satchell & Smithe, 1970; Burnstock, 1972) as well as in the central nervous system (McIlwain, 1974).

Recently adenosine and adenine nucleotides have been shown to exert a presynaptic inhibitory action in some tissues (Ginsborg & Hirst, 1972; McIlwain, 1974; Hedqvist & Fredholm, 1976). In the guinea-pig ileum, they have an antinicotinic action and significantly inhibit the evoked acetylcholine (ACh) release from cholinergic nerves (McDougal & Borowitz, 1972; Takagi & Takayanagi, 1972; Gintzler & Musacchio, 1975; Sawynok & Jhamandas, 1976; Hayashi, Yamada & Mori 1977). Previously we examined the effects of 21 purine compounds on the cholinergic nerves in guinea-pig isolated ileum and showed that adenosine derivatives among them reduced the electrically induced ACh release, the most potent being adenosine. The inhibitory effect of adenosine was competitively antagonized by methylxanthines such as theophylline and potentiated by dipyridamole and hexobendine (Hayashi, Mori, Yamada & Kunitomo, 1978).

In the present study, we found a progressive decline (tachyphylaxis) of twitch response when ileal longitudinal muscles of guinea-pigs were stimulated electri-

cally (10, 30 Hz) at 1 min intervals. The appearance of tachyphylaxis was significantly prevented by theophylline and accelerated by dipyridamole. Thus the participation of adenosine and adenine nucleotides in the appearance of tachyphylaxis was suggested. A preliminary report has been presented elsewhere (Hayashi, Yamada, Mori & Shinozuka, 1976).

Methods

Preparation of longitudinal muscle strips

Male guinea-pigs weighing 300 to 500 g were killed by a blow on the head and ileal longitudinal muscle strips with the attached myenteric plexus were prepared according to the method of Paton & Vizi (1969). A muscle strip (4 to 5 cm) prepared in this way weighed about 80 milligrams. The strip was set up in an organ bath (5 ml capacity) containing Tyrode solution at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode solution was described previously (Hayashi & Yamada, 1975).

Electrical stimulation

Electrical stimulation was carried out by a technique essentially similar to that described by Paton & Vizi (1969). The strip was stimulated by means of two electrodes, one at the top and one at the bottom of the organ bath. Rectangular pulses were used of 0.5 ms duration at the frequency of 0.1 to 30 Hz and strength sufficient to give a maximal response. The muscle strip was adjusted to an initial resting tension of approximately 0.5 g and allowed to stabilize for 60 minutes. The response was recorded isometrically by means of a force-displacement transducer on a recticorder (Nihon Kohden, RJG-3024).

Release of [^{14}C]-acetylcholine and [^{3}H]-adenosine derivatives

For radio-isotopic studies, an ileal longitudinal strip was equilibrated in Tyrode solution for 30 min and then incubated for 60 min at 37°C in Tyrode solution containing [^{14}C]-choline 30 μM (0.2 μCi/ml, sp. act. 56.85 mCi/mmol) or [^{3}H]-adenosine 10 μM (2 μCi/ml, sp. act. 23.2 Ci/mmol). After the incubation, the strip was transferred to a superfusion bath (2 ml capacity) equipped with platinum electrodes for electrical stimulation. It was superfused with prewarmed (37°C) Tyrode solution bubbled with a mixture of 95% O_2 and 5% CO_2, at a rate of 1 ml/min by means of a Watson Marlow MHRE 22 pump with 'Delta' attachment (Watson-Marlow, Ltd.). After 30 min superfusion, the output of ^{14}C or ^{3}H became relatively constant. The superfusates were collected (one 1 ml sample every minute) in liquid scintillation vials and after the addition of 5 ml scintillation solution, the radioactivity was measured in a Aloka scintillator and expressed as d min^{-1} 100 mg^{-1} tissue (wet wt.) per minute. The mechanical response was also recorded isometrically. The effects of several drugs were studied by their addition to the organ bath or the superfusion solution.

Analysis of [^{3}H]-adenine derivatives

The superfusates were collected and cooled immediately after an application of 3, 5 or 10 trains of stimuli (30 Hz, 0.5 ms, 15–20 V for 10 s at 1 min intervals) to [^{3}H]-adenosine preloaded muscle strips in the presence of dipyridamole 1 μM. They were analyzed for the [^{3}H]-adenine compounds in the following manner. Concentration and chromatography of adenine derivatives were carried out according to the methods of Pull & McIlwain (1972) and Stevens, Robinson, van Dyke & Stitzel (1972). Nonradioactive adenine derivatives (ATP, ADP, AMP, adenosine, inosine: 25 μg of each) were added to the perfusate samples. They were shaken with activated charcoal (5 mg) for 10 min and the charcoal was then collected on a membrane filter and washed with 10 ml of water. Compounds adsorbed to the charcoal were eluted with 10 ml of aq. 10% (v/v) pyridine at a rate of 0.5 to 1 ml/minute. The eluates were evaporated to dryness by rotary evaporation and 100 μl of water was added to each to dissolve the residue. They were spotted on polyethyleneimine cellulose ion-exchange thin-layer sheets (DC-Alufolien PEI-Cellulose F, Merck, Darmstadt). The sheet was developed by ascending chromatography with distilled water and again with lithium chloride (1 M); the separated compounds were eluted with NaOH (0.01 N) for scintillation counting. The recovery of radioactivity was 60 to 75% of that initially present in the samples.

The drugs used were obtained from the following sources: acetylcholine chloride (Daiichi), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Aldrich), atropine sulphate (Merck), morphine hydrochloride (Takeda), tetrodotoxin (Sankyo), dipyridamole (Tanabe), theophylline (Kohjin), adenosine (Kohjin). [^{14}C]-choline and [^{3}H]-adenosine were obtained from the New England Nuclear, Boston, U.S.A. Drugs were dissolved in distilled water and the concentrations refer to the weights of the salts.

The number(n) of experiments represents that of the preparation isolated from different animals. Some data were statistically analyzed by Student's t test.

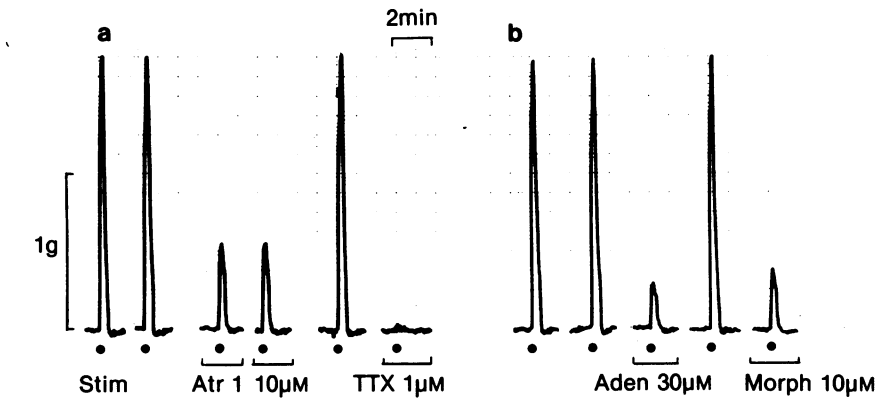

Figure 1 Effects of atropine (Atr), tetrodotoxin (TTX), adenosine (Aden) and morphine (Morph) on the contractile responses of guinea-pig isolated ileal longitudinal muscles (a, b) to electrical stimulation. Dots indicate the electrical stimulation (Stim, frequency: 30 Hz, pulse width: 0.5 ms, voltage: 15 V, stimulation periods: 10 s) at 10 min intervals and the bars indicate the presence of blocking agents added 5 or 10 min before Stim. Time marker: 2 min, vertical calibration: 1 gram.

Results

Appearance of tachyphylaxis

The effects of some drugs were investigated on the electrically (30 Hz) induced contraction of guinea-pig ileal longitudinal muscles. The contractile response was blocked by tetrodotoxin (1 μM) and partially (70%) reduced by atropine (1 μM) (Figure 1a). Little or no further depression was seen even at higher concentrations of atropine (3 or 10 μM). Such an atropine-resistant contraction of the intestine in the guinea-pig was described by Ambache & Freeman (1968). Both adenosine (30 μM) and morphine (10 μM) reduced the contractile response by 70 to 90% (Figure 1b).

When electrical stimulation (30 Hz for 10 s) was applied to the muscle at 1 min intervals tachyphylaxis developed, the contractile response progressively declining, as shown in Figure 2, until 20 to 30 min later when it was, at most, only 30 to 40% of the initial response amplitude. At this time, the contractile response to DMPP (10 μM) was reduced more than that to ACh (1 μM), the responses being 34.4 ± 4.6 ($n = 3$) and 82.5 ± 3.8 ($n = 3$) % of the initial amplitude respectively (Figure 3). The occurrence of tachyphylaxis also depended upon the stimulus frequency, developing only when a strip was stimulated at 10 or 30 Hz, but not at 0.1 or 1 Hz.

Effects of theophylline and dipyridamole on tachyphylaxis

Theophylline and dipyridamole have been known to antagonize and potentiate the inhibitory actions of adenosine and adenine nucleotides in the guinea-pig ileum and taenia coli (Satchell, Lynch, Bourke & Burnstock, 1972; Sawynok & Jhamandas, 1976; Okwuasaba, Hamilton & Cook, 1977; Hayashi *et al.*, 1978). In an attempt to test the possibility that endogenous adenosine derivatives may participate in the appearance of tachyphylaxis to nerve mediated responses, we have examined how the development of tachyphylaxis is influenced by theophylline and dipyridamole.

Theophylline (10 μM) had little effect on the initial responses to electrical stimulation or DMPP (10 μM) but fully restored them after the development of tachyphylaxis while leaving responses to ACh (1 μM) unaffected (Figures 2 & 3). The reversal by theophylline was concentration-dependent and was abolished in the presence of atropine (3 μM). There was no significant tachyphylaxis when muscles were pretreated with theophylline (10 μM) (Figure 4). By contrast, in the presence of dipyridamole (0.1, 1 μM) tachyphylaxis developed more rapidly, the response to electrical stimulation being reduced to 30–40% of the initial amplitude within 5–15 min (Figure 4). Furthermore, within this time the response to DMPP (10 μM) was reduced by 60 to 70% and that to ACh (1 μM) by 10 to 20%.

Release of [^{14}C]-acetylcholine and [^{3}H]-adenine derivatives

In [^{14}C]-choline or [^{3}H]-adenosine preloaded muscles, the effects of electrical stimulation or DMPP on ^{14}C- and ^{3}H-output were investigated. Electrical stimulation increased ^{14}C-output, the effect being more pronounced as the frequency was increased (0.1, 1, 10 and 30 Hz). In contrast, ^{3}H-output increased only with frequencies of 10 and 30 Hz. Tetrodotoxin

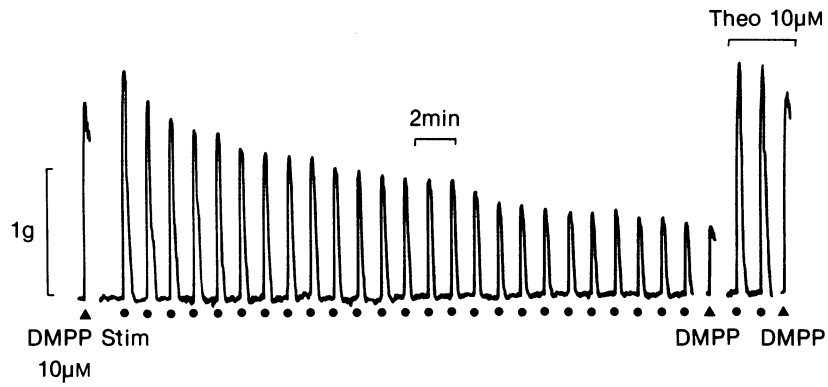

Figure 2 Development of tachyphylaxis in ileal longitudinal muscle to electrical stimulation (Stim) and addition of dimethylphenylpiperazinium (DMPP, 10 μM); reversal by theophylline (Theo, 10 μM). Dots indicate electrical stimulation (Stim, 30 Hz, 0.5 ms, 15 V, for 10 s) at 1 min intervals and the bar indicates the presence of theophylline. Time marker: 2 min, vertical calibration: 1 gram.

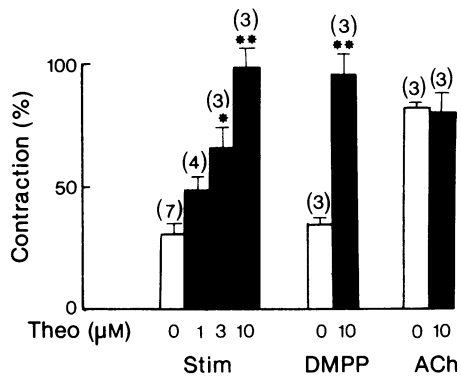

Figure 3 Contractile responses produced by the stimulation (Stim), dimethylphenylpiperazinium (DMPP, 10 μM) and acetylcholine (ACh, 1 μM) in the absence (open columns) and presence (closed columns) of theophylline (Theo, 1 to 10 μM) measured when tachyphylaxis of ileal longitudinal muscles had developed to electrical stimulation (30 Hz, 0.5 ms, 15-20 V, for 10 s) at 1 min intervals; responses are expressed as percentage of their initial response amplitudes. Each column represents the mean of 3 to 7 experiments as indicated in parentheses and vertical bars indicate s.e. mean. An asterisk indicates a significant difference of values between contractions in the absence and presence of theophylline (*$P < 0.05$; **$P < 0.01$).

(1 μM) greatly reduced both the mechanical responses and the evoked output of ^{14}C or ^{3}H (Figure 5). Atropine (3 μM) reduced the contractile response by approximately 70% but had no significant effect on the output of ^{14}C or ^{3}H. DMPP (10 μM) increased considerably both the ^{14}C- and ^{3}H-output. These results suggest that the increased output of ^{14}C and ^{3}H following electrical stimulation is primarily neurogenic and not secondary to muscle contraction.

A progressive decline of contractile response was seen when electrical stimulation (30 Hz for 10 s at 1 min intervals) was applied to [^{3}H]-adenosine preloaded muscle strips ($n = 3$) in the presence of low concentration (0.1 μM) of dipyridamole. The development of tachyphylaxis was accompanied by a large and sustained increase in ^{3}H-output, mostly up to approximately twice the pre-stimulation level (Figure 6). In the presence of dipyridamole (1 μM) there was a more rapid increase in both ^{3}H-output and the appearance of tachyphylaxis (Figure 7). Thus the development of tachyphylaxis appears to be closely associated with the increased release of [^{3}H]-adenine derivatives.

In [^{14}C]-choline preloaded muscle strips ($n = 3$), ^{14}C-output was initially increased but fell off gradually with the development of tachyphylaxis (Figure 6). At this time, theophylline (30 μM) greatly increased the ^{14}C-output with the prevention of tachyphylaxis. In contrast, ^{14}C-output was greatly diminished in the presence of dipyridamole (Figure 7).

Identification of the released [^{3}H]-adenine derivatives

Samples of the superfusate fraction from [^{3}H]-adenosine preloaded muscle strips were analyzed for their contents of labelled adenine derivatives. As summarized in Table 1, [^{3}H]-adenosine and [^{3}H]-adenine nucleotides [^{3}H]-ATP, ADP, AMP accounted for 31.1 ± 5.0% and 51.8 ± 4.1% ($n = 4$) respectively of the released total radioactivity after 3 trains of stimuli, by which time tachyphylaxis had not yet developed.

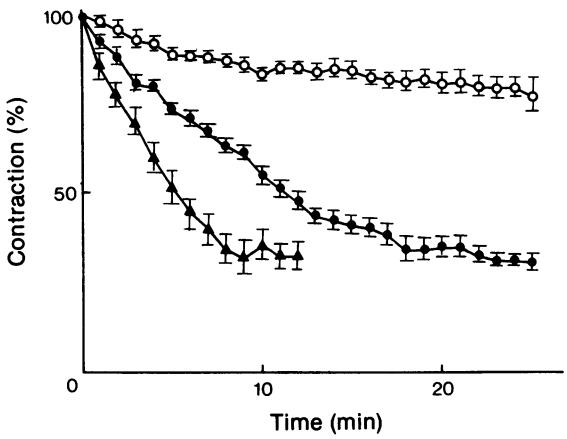

Figure 4 Effects of pretreatment with theophylline or dipyridamole on the development of tachyphylaxis of ileal longitudinal muscles to electrical stimulation. Control (n = 4) (●); in the presence of theophylline (10 μM, n = 5) (○); in presence of dipyridamole (1 μM, n = 3) (▲). Ordinate scale: percentage of initial response amplitude. Abscissa scale: time (min) after initiating electrical stimulation (30 Hz, 0.5 ms, 15-20 V, for 10 s) at 1 min intervals. Each point represents the mean of 3 to 5 experiments and vertical bars indicate s.e. mean.

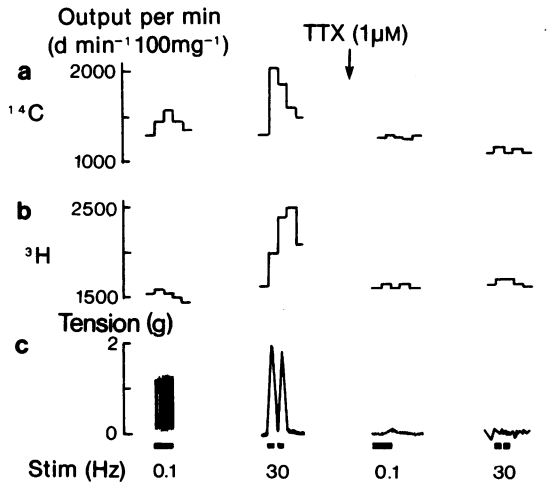

Figure 5 ^{14}C- and ^{3}H-output from [^{14}C]-choline or [^{3}H]-adenosine preloaded ileal longitudinal muscle strips in response to electrical stimulation (Stim) at frequency of 0.1 (2 min) and 30 (2 stimuli, 30 s) Hz in the absence and presence of tetrodotoxin (TTX). (a) ^{14}C- and (b) ^{3}H-output per minute (d min^{-1} 100 mg^{-1} tissue (wet wt.)) in superfusion fluids; (c) mechanical response (g) of the [^{3}H]-adenosine preloaded strip. At arrow, tetrodotoxin 1 μM was added to the superfusion solution.

In contrast, after the development of tachyphylaxis (10 trains of stimuli) 60.9 ± 7.0% (n = 6) of the released total radioactivity was found to be [^{3}H]-adenosine and 29.5 ± 4.5% to be [^{3}H]-nucleotides, the remainder being mainly [^{3}H]-inosine.

Discussion

Adenosine triphosphate (ATP), or a related purine compound, has been proposed as the transmitter substance for the inhibitory autonomic nerves, which have consequently been termed 'purinergic' (Burnstock, 1972). In the present study, we found that the contractile response of guinea-pig isolated ileal longitudinal muscles to electrical stimulation at 30 Hz was not abolished even by high concentrations (< 10 μM) of atropine. The atropine-resistant contraction may be similar to the longitudinal muscle spasms due to excitation of noncholinergic neurones as previously reported by Ambache & Freeman (1968).

When the same electrical stimulation (30 Hz) was applied to longitudinal muscles at 1 min intervals, a progressive decline of amplitude of the contraction was seen (tachyphylaxis). It developed only through high frequency stimulation of 10 and 30 Hz at 1 min intervals. Three possible explanations for the tachyphylaxis to electrical stimulation should be considered: (1) a fatigue of muscle itself; (2) an exhaustion of transmitter substance (ACh) in the nerve terminals; or (3) a depression of ACh release by any endogenous substance. It is unlikely that a fatigue of muscle itself is responsible since no significant depression of the direct muscle response to ACh developed. During tachyphylaxis, theophylline restored the responses to both electrical stimulation and DMPP possibly through increasing the ACh release, suggesting that an exhaustion of ACh in the nerve terminals is unlikely to be responsible for the tachyphylaxis. Therefore the tachyphylaxis to nerve mediated responses may be explained by assuming that one or more liberated endogenous substances reduce ACh release from cholinergic nerve elements.

The antagonism by methylxanthines, such as theophylline, of adenosine and adenine nucleotides has been demonstrated in some organs (De Gubareff & Sleator, 1965; Afonso, 1970; Sattin & Rall, 1970; Osswald, 1975; Ally & Nakatsu, 1976; Sawynok & Jhamandas, 1976; Okwuasaba *et al.*, 1977). In contrast, dipyridamole and hexobendine have been shown to potentiate the inhibitory effect of adenosine and adenine nucleotide in the heart, intestine and trachea (Stafford, 1966; Kolassa, Pfleger & Rummel, 1970;

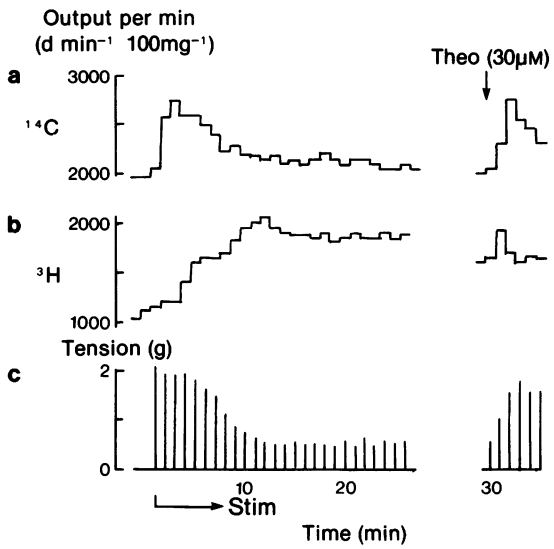

Figure 6 ^{14}C- and ^{3}H-output per minute [^{14}C]-choline or [^{3}H]-adenosine preloaded longitudinal muscle strips (a, b) with a development of tachyphylaxis to electrical stimulation (Stim) in the presence of dipyridamole 0.1 μM, and the effect of theophylline (Theo). Ordinate scales: (a) ^{14}C- and (b) ^{3}H-output per minute (d min^{-1} 100 mg^{-1} tissue (wet wt.)) in superfusion fluids; (c) mechanical response (g) of the [^{3}H]-adenosine preloaded muscle strip (b). Abscissa scale: time (min) after initiating electrical stimulation (Stim, 30 Hz, 0.5 ms, 15 V, for 10 s) at 1 min intervals. At arrow, theophylline 30 μM was added to the superfusion solution.

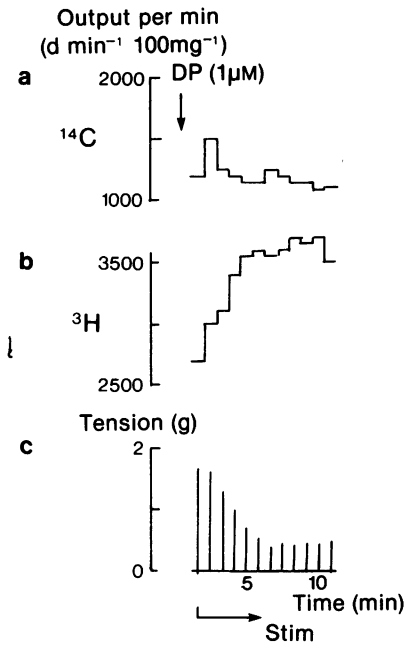

Figure 7 Effect of pretreatment with dipyridamole (DP) on ^{14}C- and ^{3}H-output from [^{14}C]-choline or [^{3}H]-adenosine preloaded longitudinal muscle strips (a, b) in response to electrical stimulation (Stim). Ordinate scale: (a) ^{14}C- and (b) ^{3}H-output per minute (d min^{-1} 100 mg^{-1} tissue (wet wt.)) in superfusion fluids; (c) mechanical response (g) of the [^{3}H]-adenosine preloaded muscle strip (b). Abscissa scale: time (min) after initiating electrical stimulation (Stim, 30 Hz, 0.5 ms, 15 V, for 10 s) at 1 min intervals. At arrow, dipyridamole 1 μM was added to the superfusion solution.

Satchell *et al.*, 1972; Coleman, 1976). Our previous experiments with guinea-pig ileum demonstrated that the inhibitory effect of adenosine on cholinergic nerves was specifically antagonized by theophylline and potentiated by dipyridamole and hexobendine. Thus it was suggested that adenosine reduced ACh release from intramural cholinergic nerves possibly in a specific manner (or through a specific receptor site) different from that of tetrodotoxin, adrenaline, strychnine and morphine (Hayashi *et al.*, 1978).

Table 1 Proportions of [^{3}H]-adenine derivatives in superfusates from [^{3}H]-adenosine preloaded ileal longitudinal muscle strips before and after development of tachyphylaxis to electrical stimulation

	Released radioactivity (% of released total tritium)		
^{3}H-material	3 trains (n = 4)	5 trains (n = 4)	10 trains (n = 6)
Adenosine	31.1 ± 5.0	53.5 ± 8.9	60.9 ± 7.0*
AMP, ADP, ATP	51.8 ± 4.1	34.4 ± 6.5	29.5 ± 4.5*
Inosine	17.2 ± 5.4	12.1 ± 3.6	9.5 ± 2.9

The muscle strips were incubated with [^{3}H]-adenosine for 60 min, superfused for 30 min and stimulated electrically (30 Hz for 10 s at 1 min intervals) in the presence of dipyridamole (1 μM). The superfusates were analyzed for the [^{3}H]-adenine compounds. Each value is shown as a percentage (mean ± s.e. mean, n = 4 to 6) of the released total tritium. An asterisk indicates a significant difference of values between 3 and 10 trains of stimuli (*$P < 0.05$).

In the present experiments, the appearance of tachyphylaxis to nerve mediated responses was significantly prevented by theophylline and accelerated by dipyridamole. In addition, in [^{3}H]-adenosine preloaded muscle strips, tachyphylaxis was accompanied by a marked and sustained increase in the release of [^{3}H]-adenine derivatives. By contrast, the evoked [^{14}C]-ACh release from [^{14}C]-choline preloaded muscle strips decreased gradually with the development of tachyphylaxis, at which time theophylline increased the [^{14}C]-ACh release and restored the contractions. In dipyridamole pretreated muscle strips, a more rapid release occurred of the [^{3}H]-adenine derivatives to electrical stimulation together with an acceleration in the appearance of tachyphylaxis; and the [^{14}C]-ACh release decreased. These data suggest that the appearance of tachyphylaxis may be closely associated with the release of endogenous adenosine derivatives which have pre-synaptic inhibitory actions on the cholinergic elements in guinea-pig ileum.

The [^{3}H]-adenine derivatives released during tachyphylaxis to electrical stimulation were determined. There was an increase in the proportion of adenosine to its derivatives as tachyphylaxis developed, [^{3}H]-adenosine accounting for approximately 60% of the released total radioactivity in the later stages. Therefore, it seems likely that adenosine itself plays a most important role for the development of tachyphylaxis in ileal longitudinal muscles to electrical stimulation and DMPP. This concept is also supported by the fact that adenosine was the most potent of a number of purine compounds in depressing cholinergic nervous activity (Hayashi *et al.*, 1978). It has been proposed that theophylline prevents the appearance of tachyphylaxis by competing with adenosine, presumably at the receptor site and that dipyridamole accelerates it by blocking adenosine uptake into the tissues (Satchell *et al.*, 1972; Hayashi *et al.*, 1978).

Berne *et al.* (1971) suggested that adenosine or adenine nucleotides play a role in regulation of cardiac and skeletal muscle blood flow. Other workers have suggested that ATP or related compounds may function as inhibitory modulators in the portal vein and brain (Pull & McIlwain, 1972; McIlwain, 1974; Su, 1975). ATP is located with ACh in synaptic vesicles of cholinergic nerves supplying the electric organ of torpedinoid rays (Bohan, Boyne, Guth, Narayanan & Williams, 1973; Zimmerman & Whittaker, 1974), and it has been also shown that ATP is released together with ACh from the phrenic nerves in the rat diaphragm (Silinsky, 1975). Further, Pull & McIlwain (1972) calculated that the adenosine concentration in the extracellular space during stimulation might be upwards of 100 μM, being a concentration high enough to produce a large degree of inhibition of neural activity. Therefore the present findings led us to the hypothesis that adenosine derivatives (mostly adenosine) may act as inhibitors or physiological modulators in a feed-back system which regulates the release of ACh caused by nerve stimulation in guinea-pig ileum. These results are also consistent with the concept that the purine compounds play two physiological roles in neural control of the ileal smooth muscle tone: one role as an inhibitory modulator in association with the cholinergic innervation and the other as a transmitter substance for the purinergic innervation in the gut (Burnstock, 1972).

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