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A comparison of adenine and some derivatives on pig isolated tracheal muscle

Yvonne Bach-Dieterle, William E. Holden[1] & Alain F. Junod

Respiratory Division, Department of Medicine, Hôpital Cantonal, 1211, Geneva 4, Switzerland

1 We studied the muscle relaxation induced by adenine and several adenine derivatives in strips of tracheal smooth muscle from pigs; in addition their metabolism by the tissue was examined.

2 Adenine relaxed tissue which was contracted by carbachol, histamine, or KCl. Adenine's potency was similar to that of adenosine and ATP (threshold about 4×10^{-5} M).

3 In tissues with carbachol-induced tone, the adenine effect differed from adenosine and ATP by being slower in onset and in 'washout' time. Furthermore, neither dipyridamole nor theophylline modified the response to adenine.

4 The relationship was examined between pharmacological effects and the metabolism of [^{3}H]-adenosine and [^{3}H]-adenine. Both substrates were taken up by the tissue and converted to nucleotides, but relaxation correlated with nucleotide accumulation only in the case of [^{3}H]-adenine.

5 We conclude that the site and mechanism of adenine-induced relaxation is different from that of adenosine and ATP in porcine tracheal muscle.

Introduction

Adenine and its derivatives, adenosine and adenine nucleotides, are ubiquitous in mammalian tissue and have diverse cellular functions. Much attention has been focused on the effects of adenosine and adenosine-5′ triphosphate (ATP) on mammalian smooth muscle as putative neurotransmitters of the non-adrenergic inhibitory nervous system. By contrast, relatively little attention has been given to the relaxant effects on smooth muscle by the parent compound, adenine.

Farmer & Farrar (1976) and Coleman (1976) reported that adenine relaxed tracheal smooth muscle from guinea-pigs, and both suggested that the mechanism of adenine's effect may be different from that of adenosine and ATP. However, there are similarities in metabolism of these compounds by mammalian cells (Marz, Wohlhüter & Plagemann, 1979). ATP and adenosine are especially similar because exogenously applied ATP is converted by 5′-nucleotidase at the cell wall to adenosine which is then taken up by the cell and resynthesized into nucleotides (Lum, Marz, Plagemann & Wohlhüter, 1979). Exogenously applied adenine is also taken up by cells and converted to nucleotides intracellularly. However, the mechanism by which adenine and its derivatives relax smooth muscle remains unknown.

We have used pig trachealis muscle to compare the relaxation induced by adenine with that of several adenine derivatives. Although our findings show that adenine is of similar potency to adenosine and ATP, we have developed several lines of evidence suggesting that the mechanism and site of adenine's effect are different from those of adenosine and ATP. The adenine-induced relaxation correlates closely with its incorporation into the nucleotide pool within the tissue.

Methods

Tracheae from freshly killed pigs were transported to the laboratory in chilled Krebs-Ringer bicarbonate buffer (KRBB) the composition of which is described elsewhere (Dieterle, Ody, Ehrensberger, Stalder & Junod, 1978). Each trachea was cut longitudinally along the ventral wall, stripped of mucosa and placed in warm KRBB (37°C). For an experiment, we dissected approximately one-and-a-half tracheal rings (cartilage rings overlap in the pig) with interconnecting smooth muscle (about 80–100 mg) and fastened surgical suture clips or small stainless steel staples to

[1] Present address: Division of Pulmonary and Critical Care Medicine, Oregon Health Sciences University, Portland, Oregon, U.S.A. 97201.

the cartilage which had been trimmed on each side. The muscle was mounted in a 10 ml organ bath and tied with silk thread to a force displacement transducer (Grass, FT.03) at a tension of 0.5 g. The KRBB was kept at 37°C, bubbled with 95% O_2–5% CO_2 which maintained a pH of 7.38–7.44. An amplifier-recorder system (Hewlett-Packard 8802A and 7702B, or Grass Model 5 polygraph with driver amplifier and Model 5P1 preamplifier) was used to record tension of the muscle over time.

Each muscle contracted initially and then relaxed during an equilibration period of approximately 90 min and the tension on the muscle was continuously adjusted to 0.5 g. Several isometric contractions were then elicited with carbachol (CCh), 5×10^{-8} M, a concentration producing half-maximal contractions. All drugs were applied to the muscles by micropipetting a small volume (less than 1% total bath volume) of a concentrated stock solution into the bath. Similar volumes of KRBB, added to the bath, had no significant effect on the sustained tension caused by CCh.

Relaxation of tracheal smooth muscle by adenine and its derivatives

To cause relaxation, drugs were added in incremental concentrations (from 10^{-6} M to 5×10^{-4}M) at peak tension induced by CCh. The effect of each test dose was expressed as:

$$\% \text{ relaxation} = \frac{\dfrac{\text{amount of relaxation by test dose}}{\text{amount of tension before test dose added}}}{} \times 100$$

A mean cumulative concentration-response curve was plotted for each drug, calculated from the data from several muscles.

In a few experiments, the effects of adenine, adenosine, and ATP were studied in tissues stimulated by histamine (5×10^{-5} M) or KCl (2×10^{-2} M).

To analyze differences between adenine and its derivatives, several methods were used. (a) The time was measured to onset of relaxation and to attainment of full relaxation after the compounds were placed in the bath at peak tension of CCh-induced contractions. (b) After an initial control CCh contraction, we placed adenine or one of its derivatives in the bath at the same time (T_0) or 10 min before (T_{-10}) CCh and measured the new peak tension. After the drugs were washed out, another control measurement was made and the two control values were averaged for comparison with the middle response. (c) We measured the time for the tissue to regain peak tension after the relaxant drug was washed out. The tissue was rinsed with KRBB containing CCh alone which thus regenerated the original tension.

(d) The effects of several drugs, propranolol, indomethacin, theophylline, or dipyridamole were tested on the cumulative concentration-response curves to adenine, adenosine, or ATP. After a control curve was determined, one of the above drugs was added to the bath just before CCh and another curve was measured. The concentration producing half-maximal relaxation (EC_{50}) was determined from each curve by a probit-log cumulative dose analysis (Colquhoun, 1971) and the EC_{50} value of each group of curves expressed as the geometric mean concentration (Fleming, Westfall, De La Lande & Jellett, 1972) of adenine, adenosine, or

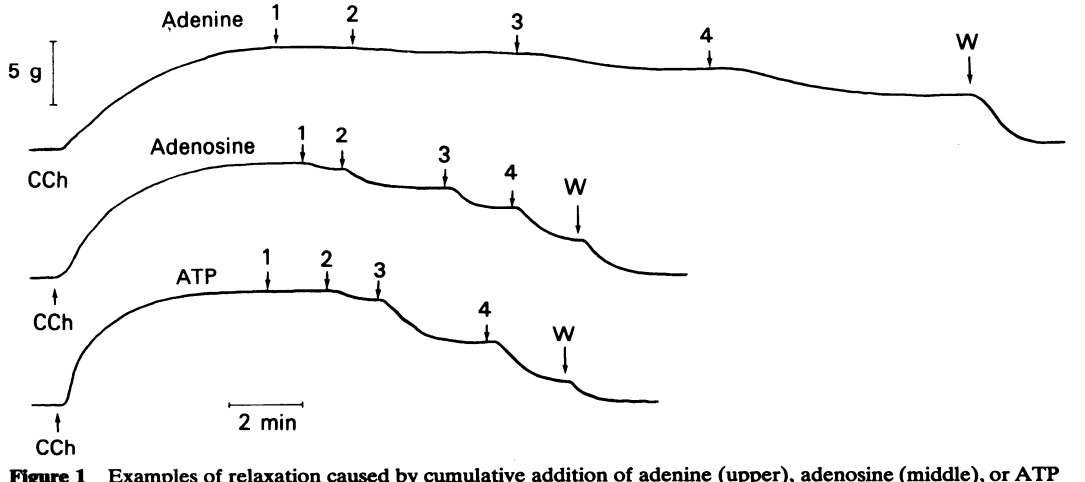

Figure 1 Examples of relaxation caused by cumulative addition of adenine (upper), adenosine (middle), or ATP (lower) on contractions of pig tracheal muscle strips produced by carbachol (CCh, 5×10^{-8} M). The vertical axis is tension (g) and the horizontal axis represents time (min). At arrows indicated by numbers 1 to 4 the muscle was exposed to the following concentrations of drugs: (1) 1.6×10^{-5} M; (2) 7.1×10^{-5} M; (3) 1.7×10^{-4} M; (4) 4.2×10^{-4} M. The bath was rinsed with fresh Krebs-Ringer bicarbonate buffer at W.

ATP producing half-maximal ralaxation. In the experiments with theophylline, and in a few indomethacin experiments, a slightly higher concentration of CCh (10^{-7} M) was used to obtain peak tensions equivalent to control contractions because of the muscle relaxant effects of these drugs.

Metabolic studies

In order to trace the metabolic events occurring concurrently with the relaxation effect of adenine and its derivatives, we performed experiments with radio-labelled compounds. In the first of these experiments, tissues contracted by CCh were exposed to 10^{-4} M adenine or derivative (containing respectively [^{3}H]-adenine, [^{3}H]-adenosine or [^{3}H]-ATP). The bath fluid was sampled 15 min later and analyzed for evidence of metabolism of the substrate.

In a second series of experiments, we added the same solutions at peak tension of CCh-induced contractions, waited for a predetermined period (2.5, 7, or 15 min), and quickly removed the muscle from its cartilaginous attachments, gently blotted the tissue dry, weighed it, and then homogenized the tissue in 1 ml of perchloric acid solution (0.4 N). The homogenate was centrifuged at 30,000 *g* for 60 min at 4°C and then analyzed for total radioactivity and the fractions representing the metabolites of adenine, adenosine, or ATP. Analysis of the metabolites was done by thin layer chromatography on PEI-cellulose F and cellulose F plates as described previously (Randerath, 1961; Randerath & Randerath, 1964). In two other series of eight experiments with [^{3}H]-adenosine and [^{3}H]-adenine, the tissue was immediately frozen in liquid nitrogen, homogenized in 1 ml perchloric acid and the suspension centrifuged. The supernatant was used for ATP determination by the luciferin-luciferase method (Stanley & Williams, 1969) and for the measurement of its radioactive content and the analysis of the metabolites, and the precipitate for protein determination (Lowry, Rosebrough, Farr & Randall, 1951). A linear regression analysis was used to relate percentage of muscle relaxation to the amount of ^{3}H-nucleotides formed in the tissue.

Compounds used in the study were purchased from Sigma (CCh, (±)-propranolol, histamine, and hypoxanthine) or Boehringer (adenosine 5'-triphosphate, adenine, adenosine, inosine). Dipyridamole was kindly provided by Boehringer and indomethacin by Merck, Sharp and Dohme. [2-^{3}H]-adenosine 5'-triphosphate (ammonium salt, 16 Ci mmol^{-1}), [2-^{3}H]-adenosine (24 Ci mmol^{-1}), and [2-^{3}H]-adenine (22 Ci mmol^{-1}) were purchased from Amersham.

Comparison between groups was made by paired Student's *t* test. Differences were considered significant at $P < 0.05$.

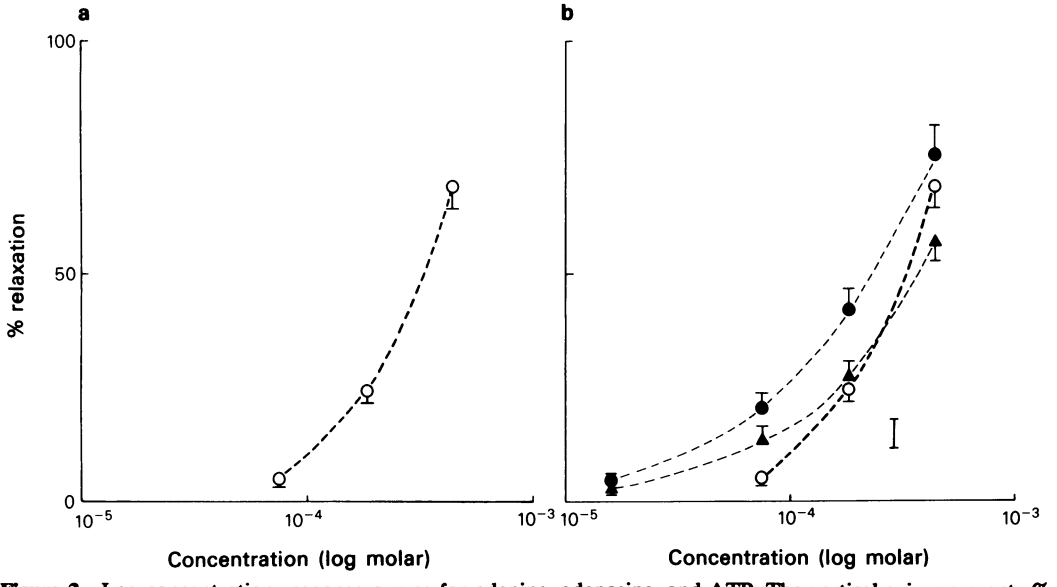

Figure 2 Log concentration-response curves for adenine, adenosine, and ATP. The vertical axis represents % relaxation and the horizontal axis is the molar concentration plotted on a logarithmic scale. (a) Curve for adenine ($n = 18$). (b) Curve for adenine (○) and the curves for adenosine (●, $n = 17$) and ATP (▲, $n = 17$). Each point represents the mean; vertical lines show s.e.mean.

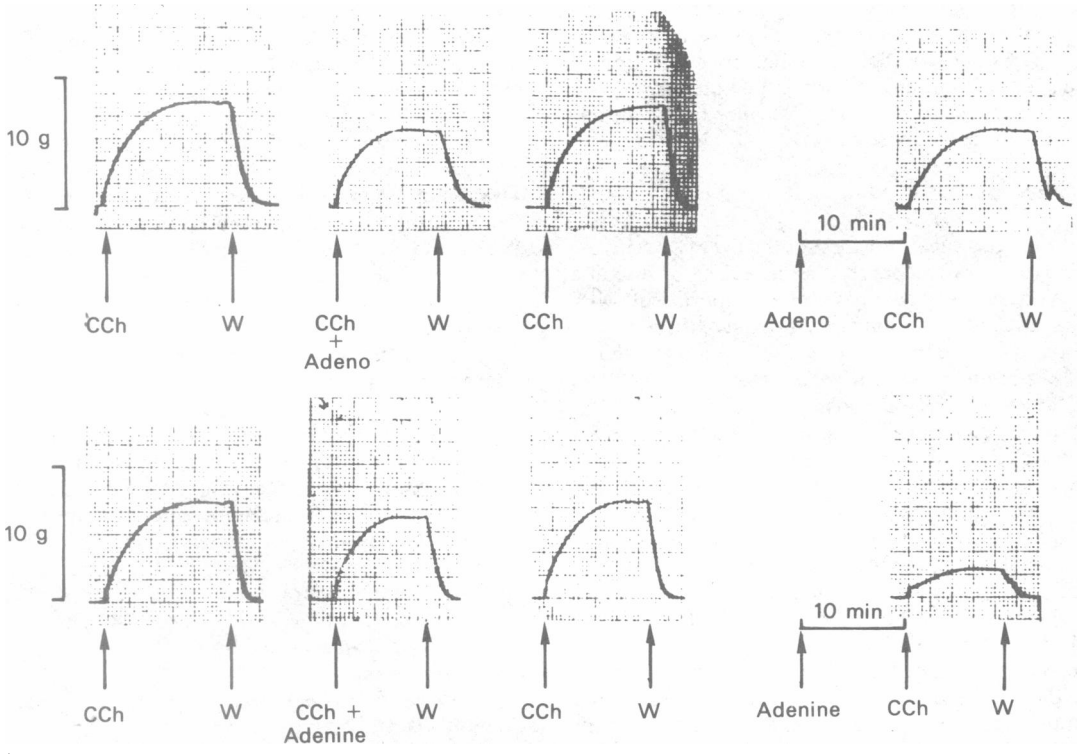

Figure 3 Example of an experiment comparing the effects of adenine and adenosine added simultaneously with (T_0) or 10 min before (T_{-10}) carbachol (5×10^{-8} M). In the top panel (from left to right) a control contraction was followed by a test contraction in which adenosine (Adeno, 10^{-4} M) was added at the same time (T_0) as CCh. The bath was washed with fresh KRBB solution at W. Another control contraction followed and then adenosine was added 10 min before CCh (T_{-10}). The effect of adenosine was the same at both T_0 and T_{-10} in this experiment (in several other experiments, the amount of relaxation at T_{-10} was less than the T_0 value). In the lower panel the same sequence was repeated for adenine (10^{-4} M); the record is continuous. For adenine, the effect at T_{-10} was much greater than at T_0.

Results

Relaxation by adenine of pig tracheal smooth muscle

When applied at peak tension of CCh-induced contractions, adenine relaxed pig tracheal smooth muscle (Figure 1, upper panel). Peak tension of control contractions decreased very slightly (less than 2%) in some of the muscles but was nearly constant over a 15 min contraction in most. The log concentration-mean response curve for adenine (Figure 2a) was quite steep and suggested a threshold concentration near 4×10^{-5} M. Adenine (10^{-4} M) also relaxed tissues contracted by histamine ($1-5 \times 10^{-5}$ M) or KCl (2×10^{-2} M) although full dose-response curves were not performed because these contractions were less sustained than those induced by CCh.

Differences between adenine and its derivatives

Inosine and hypoxanthine were ineffective or produced barely detectable relaxation at relatively large concentrations (i.e., 10^{-3} M). Adenosine and ATP relaxed pig tracheal muscle in a concentration-related manner (Figure 1, lower panels; Figure 2b) over a similar concentration range to adenine but the slopes of their concentration-mean response curves were not as steep. The time course of drug effects also differed: for example, the time to onset of the adenine effect (78 ± 9 s, mean $\pm$ s.e.mean, $n = 4$) was significantly longer than either adenosine (9 ± 1 s, $n = 5$, $P < 0.01$) or ATP (10 ± 1 s, $n = 3$, $P < 0.01$). Also, the adenine effect took several minutes to reach a plateau, whereas adenosine and ATP reached plateaus more quickly.

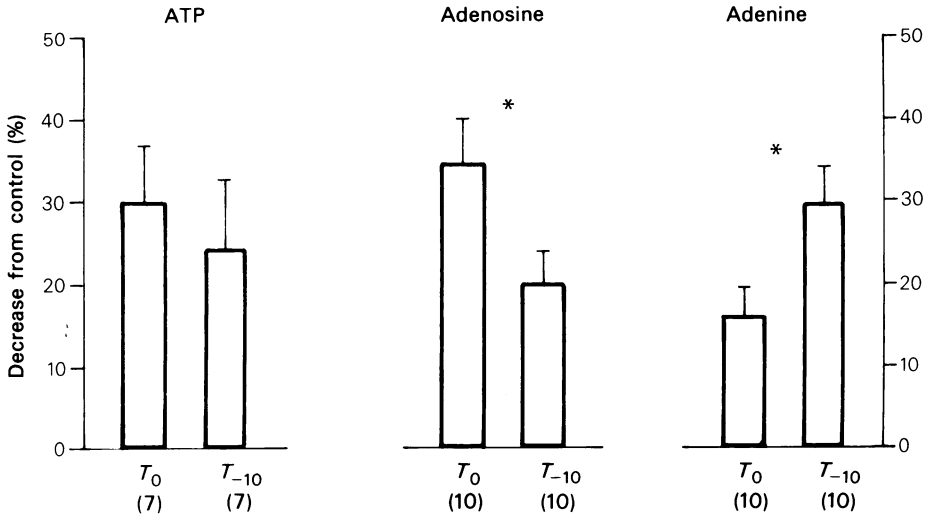

Figure 4 Comparison of adenine, adenosine, and ATP (1×10^{-4} M) on pig tracheal smooth muscle when each was added at the same time (T_0) or 10 min before carbachol (T_{-10}). The vertical axis represents the % decrease in tension as compared to control contractions. Each column represents the mean and vertical lines s.e.mean of n experiments (in parentheses). Significant differences between T_0 and T_{-10} effects for adenosine and adenine are indicated by an asterisk (*).

Another difference between the drug-induced relaxations was seen when each drug was applied to the muscles simultaneously with (T_0) or 10 min before CCh (T_{-10}); see Figure 3. All three compounds relaxed the muscles when each was applied at either T_0 or T_{-10}, but the adenine effect increased at T_{-10}, whereas the adenosine effect decreased and the ATP effect was unchanged (Figure 4).

Next, we measured the tissue recovery time after washing out the relaxant drugs; a typical experiment is seen in Figure 5. The time needed for the muscle to redevelop peak tension was significantly longer for

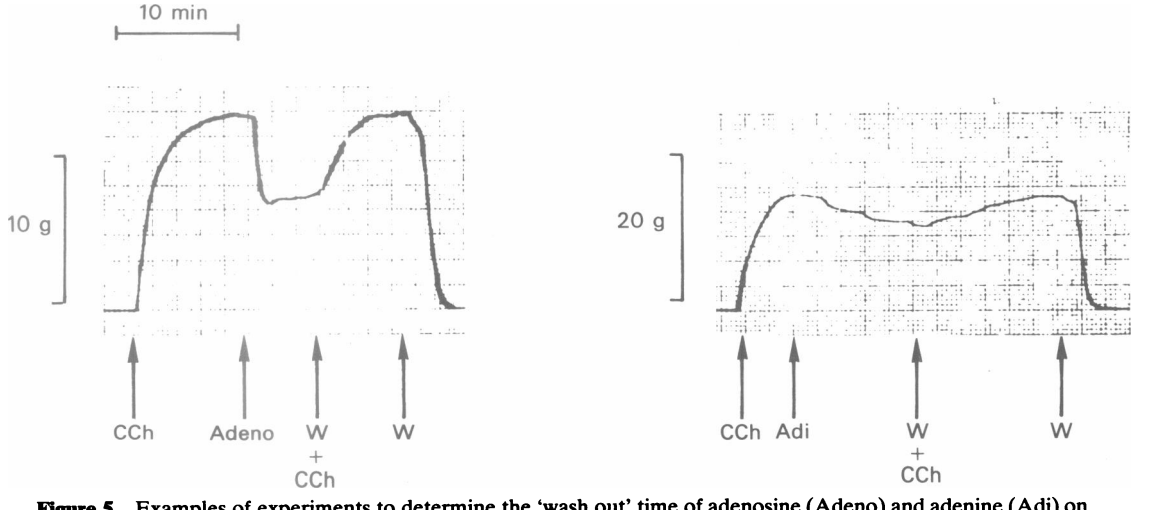

Figure 5 Examples of experiments to determine the 'wash out' time of adenosine (Adeno) and adenine (Adi) on pig tracheal smooth muscle in which contractions were induced by carbachol (CCh, 5×10^{-8} M). The vertical axis represents tension (g) and the horizontal axis represents time (min). Adenosine (left panel) or adenine (right panel) (1×10^{-4} M) was added at peak tension induced by CCh. When maximum relaxation had occurred, a fresh solution of KRBB containing CCh was rinsed into the bath (W + CCh), thus 'washing out' adenosine or adenine. The 'wash out' time was measured from W + CCh until the muscle redeveloped peak tension.

adenine (14 ± 3 min, mean $\pm$ s.e.mean, $n = 5$) than for either adenosine (4 ± 1 min, $n = 6$, $P < 0.01$) or ATP (6 ± 1 min, $n = 5$. $P < 0.05$).

Finally, we examined the interactions of several different compounds on the tracheal muscle. Propranolol (10^{-6} M) and indomethacin (10^{-4} M) had no discernible effect on the relaxation produced by each of the three compounds. The effects of theophylline and dipyridamole are shown in Table 1. At 10^{-5} M, theophylline had no effect, but at 10^{-4} M reduced the relaxation caused by adenosine and by ATP but the latter reduction was not statistically significant. Dipyridamole (10^{-6} M) potentiated the effects of adenosine; and at 10^{-5} M potentiated both adenosine and ATP. Neither theophylline nor dipyridamole had a discernible effect on the relaxation of tracheal muscle by adenine.

Metabolic studies

Because previous studies in our laboratory suggested that pig tracheal muscle hydrolyzes ATP to adenosine in the medium (unpublished results), we analyzed the metabolism of [^{3}H]-adenine, [^{3}H]-adenosine and [^{3}H]-ATP in the medium as well as in the tissue over a 15 min period (Table 2). The concentration of each drug used was 10^{-4} M with a concentration of label of $1-2 \times 10^{-7}$ M. Uptake into the tissue was 109 ± 11, 88 ± 18, and 79 ± 14 nmol g^{-1} tissue (mean $\pm$ s.e.mean, $n = 5$) for [^{3}H]-adenine, [^{3}H]-adenosine and [^{3}H]-ATP respectively. This uptake represented approximately 1% of the label for each drug.

Adenine was essentially unchanged in the medium, whereas about 8% of the ATP was metabolized to adenosine and a similar amount of adenosine was converted to inosine. In the case of ATP, it was impossible to distinguish the proportion of nucleotides in the tissue as opposed to nucleotides in the medium, so we limited our studies of tissue metabolism to adenosine and adenine. Over the 15 min period, both [^{3}H]-adenine and [^{3}H]-adenosine were converted to nucleotides in the tissue, although the proportion of nucleotides formed from [^{3}H]-adenosine was greater. The amount of tissue ATP determined by the luciferin-luciferase method (Stanley & Williams, 1969) was similar (2.8 ± 0.9 and 2.9 ± 1.2 nmol g^{-1} protein, mean $\pm$ s.d., $n = 8$) for [^{3}H]-adenosine and [^{3}H]-adenine respectively.

Finally, we studied the metabolism of [^{3}H]-adenine and [^{3}H]-adenosine in the tissues at several intervals (2.5, 7, and 15 min) and correlated the

Table 1 Effects of theophylline or dipyridamole on relaxation induced by adenine, adenosine, or ATP in pig tracheal smooth muscle

	Adenine EC_{50}		*Adenosine* EC_{50}		*ATP* EC_{50}	
Theophylline						
10^{-5} M						
C	$3.3 (\pm 1.4) \times 10^{-4}$	(9)	$2.9 (\pm 2.6) \times 10^{-4}$	(8)	$3.7 (\pm 1.5) \times 10^{-4}$	(8)
E	$3.5 (\pm 1.5) \times 10^{-4}$		$3.5 (\pm 3.3) \times 10^{-4}$		$4.9 (\pm 3.4) \times 10^{-4}$	
10^{-4} M						
C	$2.1 (\pm 1.0) \times 10^{-4}$	(4)	$4.6 (\pm 3.0) \times 10^{-4}$ *	(7)	$4.2 (\pm 3.1) \times 10^{-4}$	(8)
E	$2.8 (\pm 2.2) \times 10^{-4}$		$8.7 (\pm 5.7) \times 10^{-4}$		$8.9 (\pm 7.9) \times 10^{-4}$	
Dipyridamole						
10^{-6} M						
C	$3.0 (\pm 1.4) \times 10^{-4}$	(7)	$4.4 (\pm 3.6) \times 10^{-4}$ *	(6)	$4.2 (\pm 1.9) \times 10^{-4}$	(5)
E	$3.7 (\pm 1.6) \times 10^{-4}$		$7.9 (\pm 4.3) \times 10^{-5}$		$7.2 (\pm 7.0) \times 10^{-4}$	
10^{-5} M						
C	$2.5 (\pm 7.3) \times 10^{-4}$	(3)	$3.1 (\pm 2.4) \times 10^{-4}$ *	(5)	$2.9 (\pm 7.0) \times 10^{-4}$ *	(4)
E	$4.1 (\pm 3.0) \times 10^{-4}$		$1.3 (\pm 1.2) \times 10^{-4}$		$7.3 (\pm 6.2) \times 10^{-5}$	

Lines C represent control values (antilog of geometric mean values) for adenine, adenosine or ATP alone. Lines E give corresponding effects in presence of theophylline or dipyridamole. Number of experiments are shown in parentheses; asterisk (*) shows significant difference at $P < 0.05$ for paired Student's t test.

Table 2 Metabolism of [^{3}H]-adenine, [^{3}H]-adenosine and [^{3}H]-ATP by pig tracheal smooth muscle

	In medium (%) (3 Experiments)				*In tissues* (%) (5 Experiments)					
	Nuc	Ado	Adi	I	Nuc			Ado	Adi	I
Substrate										
Adenine (10^{-4} M)	1	1	97	1	13			2	83	2
					* ATP	ADP	AMP			
					50	31	19			
Adenosine (10^{-4} M)	0	91	1	8	*	53		21	0	26
					39	34	27			
ATP (10^{-4} M)	90	8	0	2	†					

Values are % of radiolabel in medium or tissue.
Substrate concentration includes $1-2 \times 10^{-7}$ M radiolabel.
Metabolites are abbreviated as follows: Nuc = nucleotides, Ado = adenosine, Adi = adenine and I = inosine.
* % of each nucleotide in nucleotide pool.
† Not determined because intra- and extracellular nucleotide were indistinguishable.

functional event, i.e., relaxation of smooth muscle, with the metabolic products found in the tissue. A correlation with relaxation was found only for the nmol g^{-1} tissue of nucleotides formed from [^{3}H]-adenine (Figure 6). No correlation between relaxation and metabolic products was demonstrated in the case of adenosine.

Discussion

Our study confirms the findings of others (Farmer & Farrar, 1976; Coleman, 1976; Christie & Satchell, 1980; Jones, Lefcoe, & Hamilton, 1980) of adenine's relaxant effect on tracheal smooth muscle; its potency is similar to that of adenosine and ATP in pig

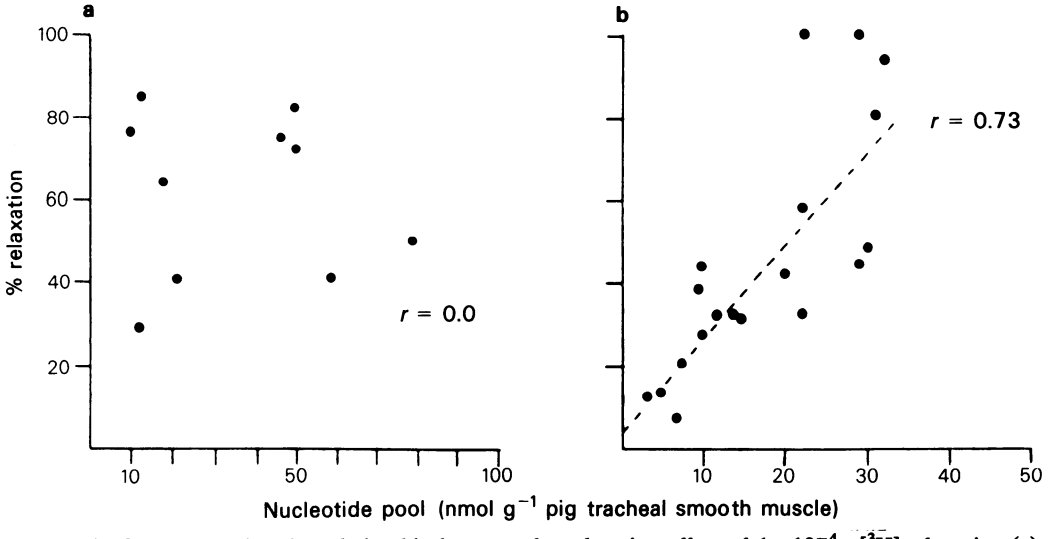

Figure 6 Graphs showing the relationship between the relaxation effect of 1×10^{-4} M [^{3}H]-adenosine (a) or $0.5-5 \times 10^{-4}$ M [^{3}H]-adenine (b) and the incorporation of each into the intracellular nucleotide pool. [^{3}H]-adenosine or [^{3}H]-adenine was added to the tissue bath at peak tension of carbachol-induced contractions. After different time intervals (see text), the % relaxation was recorded, the muscle removed, and the incorporation of label into the nucleotide pool measured. Each point represents one muscle strip. Linear least square analysis was performed and the correlation determined for the line of best fit.

trachea. We found a direct correlation between adenine's pharmacological action and its conversion to nucleotides within the tissue. Since no such relationship could be demonstrated for adenosine, we postulate that the sites of action and mechanisms of relaxation effects are different for these two compounds.

Adenine is ubiquitous in higher organisms and necessary for synthesis of adenine nucleotides and nucleic acids. Purine salvage pathways, particularly the reaction involving adenine phosphoribosyl transferase, prevent degradation of adenine and provide a mechanism for continuous reutilization by cells. Adenine occurs in free or uncombined form in trace amounts in cells and plasma (approximately 70 nmol l^{-1}; De Verdier, Ericson, Niklasson & Westman, 1977). It seems unlikely that free adenine influences vascular smooth muscle tone directly at these plasma levels, but an indirect effect through its conversion to nucleotides via purine salvage pathways is possible. Such reactions are stimulated when adenine is added to stored human blood to prolong shelf life, presumably by increasing ATP levels within red blood cells. Also, adenine has been given orally to treat patients with the Lesch-Nyhan syndrome (Van der Zee, Lommen, Trijbels & Schrethlen, 1970). More recently, others (Akintonwa, Auditore & Green, 1979) have cautioned against such treatment because of adenine's toxicity in rats.

Adenosine, like adenine, occurs in trace amounts in cells, but may be released under certain circumstances, e.g., hypoxia (Berne, 1963; Dobson, Rubio & Berne, 1971; Mentzer, Rubio & Berne, 1975). Nucleotides, however, occur as free forms intracellularly in significant amounts. When applied exogenously to smooth muscle, including pig tracheal smooth muscle explants in our laboratory (unpublished results), ATP is hydrolyzed to adenosine which is then taken up by the cells and converted to nucleotides. Thus, the metabolism of adenosine and ATP by smooth muscle is quite similar.

There were many similarities in magnitude and timing of the response to adenosine and ATP and in the influence of theophylline and dipyridamole on them. However, subtle differences support the hypothesis (Burnstock, 1981) for two populations of receptors, P_1 for adenosine and P_2 for ATP, of which the adenosine receptors are more numerous in tracheal smooth muscle (Christie & Satchell, 1980).

Several differences between adenine and its derivatives, adenosine and ATP, may provide insight into the site and mechanism of action of these compounds. For example, the rapid onset and early plateau of responses and rapid reversal during 'wash out' of ATP and adenosine contrasted with the slow and constant effect of adenine which was only reversed slowly by 'wash out'. Longer substrate exposure time had no effect (for ATP) or decreased the effect (of adenosine) while the adenine effect was increased. Dipyridamole potentiated both adenosine and ATP but not adenine. Similar findings were obtained by others in guinea-pig intestinal (Spedding & Weetman, 1976) and tracheal smooth muscle (Farmer & Farrar, 1976; Coleman, 1976), although the magnitude of the dipyridamole effect for adenosine differs depending on the type of preparation used (Jones *et al.*, 1980). Also, theophylline (10^{-4} M) inhibited the adenosine effect but not that of adenine. These findings are consistent with different sites of action of the drugs, possibly the plasma membrane for ATP and adenosine as opposed to an intracellular site for adenine.

In the experiments where we analyzed the metabolism of adenine and adenosine in relation to their pharmacological effects, the extent of muscle relaxation was linearly related to the amount of intracellular nucleotides formed from adenine. No such relationship was found for adenosine even though more intracellular nucleotides were formed. The proportion of nucleotides ATP, ADP and AMP was quite similar whether adenine or adenosine was the substrate and in each case ATP was the predominant metabolite. That the amount of nucleotides formed from adenine correlated with relaxation events suggests, but does not prove, a cause and effect relationship. Intracellular nucleotides, especially ATP, have been implicated in smooth muscle relaxation by findings that microsomes and mitochondria accumulate Ca^{2+} in the presence of nucleotides (Clyman, Manganiello, Lovell-Smith & Vaughan, 1976; Webb & Bhalla, 1976). Since the state of muscle contraction is thought to be related to free Ca^{2+} in smooth muscle cytosol, this process or the known chelating properties of ATP might result in muscle relaxation by decreasing free Ca^{2+} concentration.

Our study also suggests that intracellular nucleotides formed from adenosine are not involved in the relaxation process, although nucleotides could have been formed from existing substrate pools inside the cells due to adenosine stimulation of adenyl cyclase at the plasma membrane. Since the relaxation induced by adenine, but not adenosine, correlated directly with amounts of intracellular nucleotides formed, we can postulate the existence of more than one intracellular nucleotide pool. Evidence in favour of intracellular compartmentation of nucleotides has been presented for other cellular systems (Rapaport & Zamecnik, 1976; Schrader & Gerlach, 1976). Further analysis of this possibility awaits the development of methods to study ATP accumulation in different regions of the cell. Use of adenine and adenosine to increase intracellular ATP may be useful in such experiments.

In summary, our study confirms that adenine causes relaxation of tracheal smooth muscle from pigs and establishes that its potency is similar to that of adenosine and ATP. Several lines of evidence suggest that the mechanism of adenine's effect is different from and occurs at a different site from that of adenosine and ATP. Although the molar concentrations of adenine causing relaxation are much greater than the concentration known to exist *in vivo*, we think that adenine may have a role in control of smooth muscle tone through purine salvage reactions leading to ATP synthesis in smooth muscle cells. The nucleotides synthesized from adenine may be pooled in an area of the smooth muscle cell influencing the state of contraction.

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