Br. J. Pharmac. (1976), **57**, 51–57

EFFECTS OF SOME PURINE DERIVATIVES ON THE GUINEA-PIG TRACHEA AND THEIR INTERACTION WITH DRUGS THAT BLOCK ADENOSINE UPTAKE

R.A. COLEMAN

Department of Pharmacology, Allen & Hanburys Research Ltd, Ware, Hertfordshire SG12 0DJ

1 Adenosine, adenosine 5′-triphosphate (ATP), adenine, inosine and guanosine all caused concentration-dependent relaxations of guinea-pig tracheal smooth muscle *in vitro*. The relative potencies in descending order were: adenine ⩾ guanosine > inosine ⩾ adenosine ⩾ ATP

2 Responses to the purine compounds were unaffected by propranolol (1 μg/ml).

3 The spasmolytic potencies of adenosine and ATP were greatly enhanced in the presence of the adenosine uptake blocking drugs dipyridamole, hexobendine or Dilazep, whereas responses to adenine were unaffected and those to inosine and guanosine were reduced.

4 The spasmolytic potencies of noradrenaline, aminophylline, prostaglandin E_2 and glyceryl trinitrate were unaffected by dipyridamole, hexobendine and Dilazep.

5 It is suggested that an adenosine uptake process may exist in the trachea of the guinea-pig and that this process is inhibited by dipyridamole, hexobendine and Dilazep.

Introduction

Coleman & Levy (1974) demonstrated the spasmolytic activity of adenosine and adenosine 5′-triphosphate (ATP) in the guinea-pig isolated tracheal tube preparation. This spasmolytic activity was enhanced by three drugs reported to inhibit adenosine uptake, dipyridamole (Kolassa, Pfleger & Rummel, 1970), hexobendine (Kraupp, Wolner, Adler-Kastner, Chirikdjian, Ploszczanski & Tuisl, 1966) and Dilazep (Sano, Katsuki & Kawada, 1972; Buyniski, Losada, Bierwagen & Gardier, 1972). The present work is a more detailed study of spasmolytic activity in a range of purine derivatives on the guinea-pig isolated tracheal tube preparation and of the effects of drugs that block adenosine uptake on this activity.

Methods

Guinea-pigs weighing 300–400 g were killed by a blow on the head and the tracheas excised and set up in a manner similar to that described by Farmer & Coleman (1970). Two tracheal tube preparations were made from each animal by dividing the trachea halfway along its length. Each portion was mounted on a tracheal holder (Figure 1), modified from that described by Farmer & Coleman (1970). A high level of resting tone was induced in the tracheal tube as

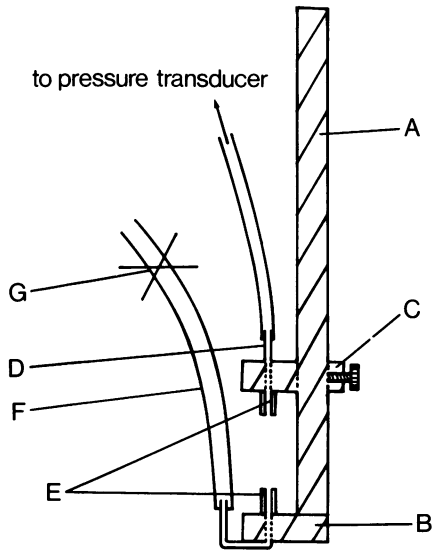

Figure 1 Diagram of tracheal holder. A: perspex rod. B: foot. C: adjustable collar. D: stainless steel tube (0.055 inch diam., 17 G). E: silicon rubber sleeve. F: silicon rubber tube. G: clip.

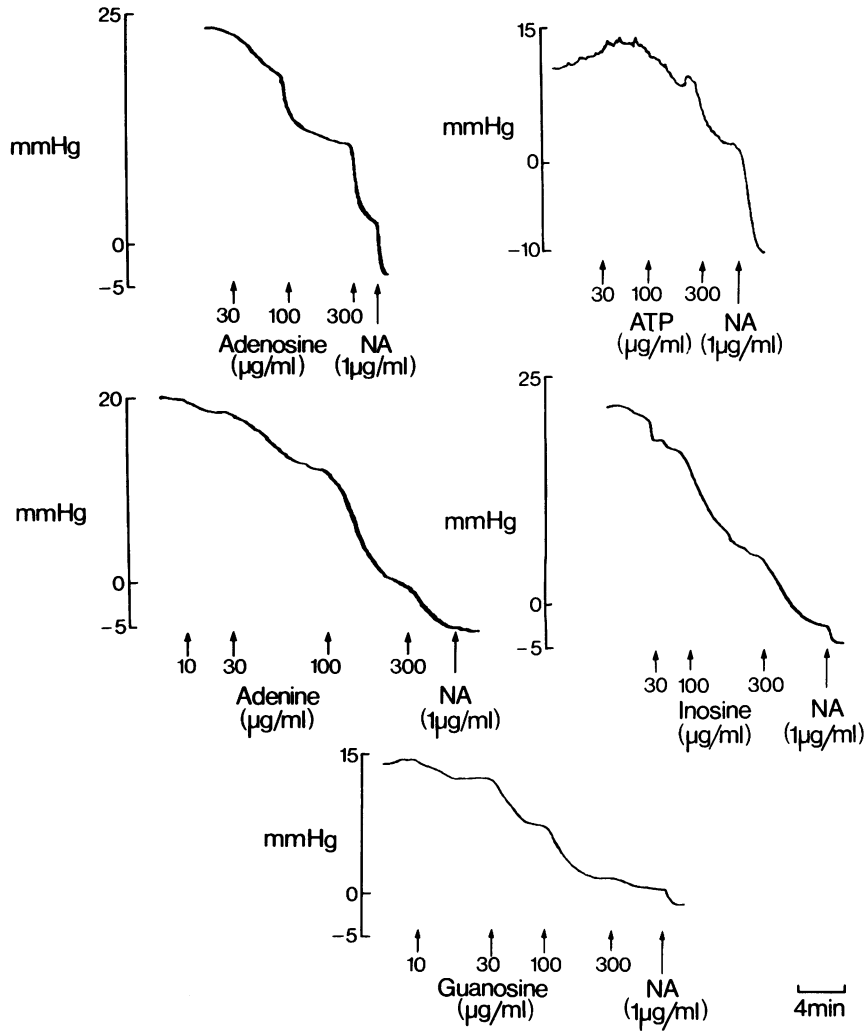

Figure 2 Guinea-pig isolated tracheal tube. Cumulative effects of adenosine, adenosine 5′-triphosphate (ATP), adenine, inosine and guanosine in relaxing spontaneous tone. NA = noradrenaline.

described by Coleman & Farmer (1971) and the intraluminal pressure (1 mmHg ≈ 133 Pa) was monitored continuously. Physiological salt solution maintained at 37°C was in contact with both inner and outer surfaces of the trachea. It had the following composition (g/l): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, glucose 2.0, $NaHCO_3$ 2.1, $CaCl_2.6H_2O$ 0.28.

Cumulative concentration-effect curves to spasmolytic drugs were determined. On completion of each curve a maximally effective concentration of noradrenaline (1 μg/ml) was added to the bathing solution to determine the 100% spasmolytic response.

In experiments in which the effects of drugs that block adenosine uptake were investigated, concentration-effect curves for the spasmolytic agents alone were repeated until constant results were obtained; the uptake blocking drug was then added to the bathing solution and the responses to the spasmolytic agents redetermined. Concentration-effect curves for the spasmolytic agents were repeated in the presence of the uptake blocking drug until any change in sensitivity became maximal. Spasmolytic responses to inosine, guanosine and prostaglandin E_2 were found to cause tachyphylaxis and so experiments were carried out on paired preparations, one half serving as

untreated control and the other being exposed to a drug blocking adenosine uptake.

Concentration-ratios were calculated from EC_{40} values before and after addition of the uptake blocking drug. EC_{40} values were used because 50% inhibition of spontaneous tone was not achieved in every experiment.

Drugs

The following drugs were used: adenine (Boehringer Mannheim); adenosine (Koch-Light); adenosine 5'-triphosphate (ATP, BDH); aminophylline (A & H); tetrahydro-1H-1, 4-diazepine-1,4 (5H)-dipropanol 3,4,5-trimethoxybenzoate (diester) (Dilazep, Asta Werke); dipyridamole (Boehringer Ingelheim); glyceryl trinitrate (BDH); guanosine (Koch-Light); hexobendine (Oestereichische Stickstoffwerke); inosine (Koch-Light); (–)-noradrenaline bitartrate (Winthrop); propranolol hydrochloride (ICI); prostaglandin E_2 (Cambrian).

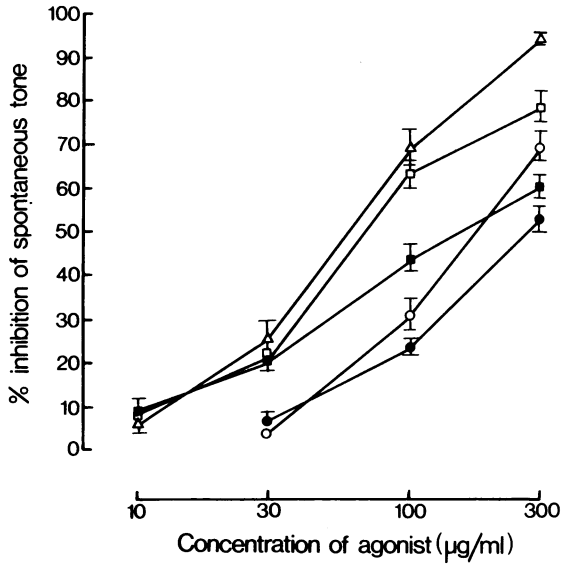

Figure 3 Guinea-pig isolated tracheal tube. Cumulative concentration-effect curves for the inhibition of spontaneous tone by adenosine (○), adenosine 5'-triphosphate (●), adenine (△), inosine (■) and guanosine (□). Each point represents the mean of at least 12 experiments and vertical bars indicate s.e. mean.

Results

Spasmolytic activity of purine derivatives

All the purines tested produced concentration-dependent spasmolytic responses in isolated tracheas (Figure 2) within the concentration range 10–300 μg/ml. Frequently ATP (30 μg/ml) and occasionally adenosine (30 μg/ml) elicited excitatory responses as previously reported (Coleman & Levy, 1974) at concentrations lower than those required for inhibition.

Adenine consistently caused 90–100% inhibition of spontaneous tone at the highest concentration (300 μg/ml) but none of the other purines achieved maximal inhibition of tone in concentrations up to 300 μg/ml, this being the practical limit of their solubilities. The responses to inosine and guanosine often caused tachyphylaxis but the concentration-effect curves for adenosine, ATP and adenine were reproducible. The order of spasmolytic potency over

Table 1 The effect of dipyridamole, hexobendine and Dilazep on concentration-effect curves to adenosine in the guinea-pig isolated tracheal tube

Compound	*Concentration μg/ml*	*No. of experiments*	*Potentiation ratio* $\frac{EC_{40}\ control}{EC_{40}\ treated}$	*95% Confidence limits*
Dipyridamole	0.1	4	9.9	2.8– 35.1
	1	8	44.5	23.8– 83.3
	10	8	123.2	59.4–255.6
Hexobendine	0.1	6	9.8	2.5– 38.8
	1	6	20.4	13.6– 30.6
	3	4	50.6	40.1– 63.8
	10	6	14.3	8.6– 23.7
Dilazep	0.1	5	26.8	12.9– 55.4
	1	5	29.8	16.4– 54.1
	10	5	33.8	22.5– 50.8

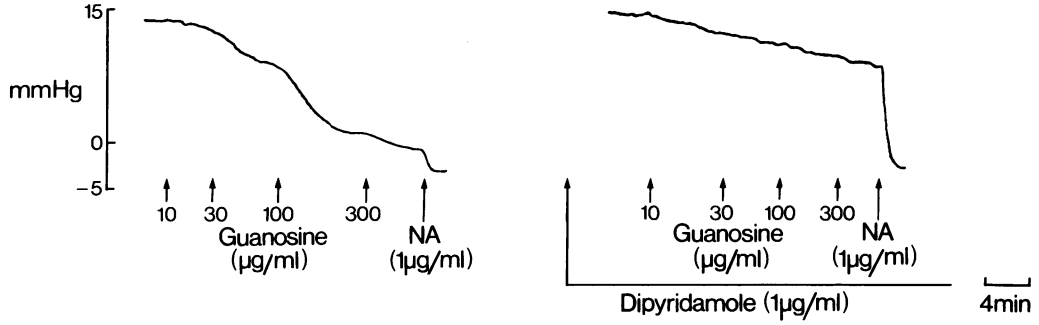

Figure 4 Guinea-pig isolated tracheal tube. Effect of dipyridamole (1 μg/ml) on cumulative effects of guanosine in relaxing spontaneous tone. NA = noradrenaline.

the whole series of experiments was adenine ⩾ guanosine > inosine ⩾ adenosine ⩾ ATP (Figure 3).

Effect of propranolol on responses to purine derivatives

The β-adrenoceptor antagonist propranolol (1 μg/ml), had no effect on the spasmolytic potency of the purine derivatives ($n = 4-8$).

Effects of dipyridamole, hexobendine and Dilazep on responses to adenosine

The spasmolytic potency of adenosine was clearly potentiated by all three drugs blocking adenosine uptake (Table 1). The potentiating effect of dipyridamole (0.1–10 μg/ml) was concentration-dependent as was that of hexobendine up to 3 μg/ml, but hexobendine was less active at 10 μg/ml than at 3 μg/ml. The potentiating effect of Dilazep was virtually the same over the range tested (0.1–10 μg/ml).

Effects of dipyridamole, hexobendine and Dilazep on responses to ATP, adenine, inosine and guanosine

The concentrations of drugs blocking adenosine uptake used in these experiments had been shown (Table 1) to potentiate the action of adenosine. The effects of these agents on the responses to the other purines are summarized in Table 2.

The effects of ATP were potentiated in much the

Table 2 The effect of dipyridamole (1 μg/ml), hexobendine (3 μg/ml) and Dilazep (1 μg/ml) on concentration-effect curves to adenosine 5′-triphosphate (ATP), adenosine, inosine and guanosine in the guinea-pig isolated tracheal tube

Purine derivative	*Treatment*	*No. of experiments*	*Potentiation ratio* $\frac{EC_{40}\ control}{EC_{40}\ treated}$	*95% Confidence limits*
ATP	Dipyridamole	4	63.3	28.8–139.1
	Hexobendine	4	55.7	40.4– 76.8
	Dilazep	6	22.7	12.9– 39.9
Adenine	Dipyridamole	4	1.5	1.1– 2.1
	Hexobendine	4	1.3	0.8– 2.3
	Dilazep	4	1.4	0.7– 2.7
Inosine	Dipyridamole	4	<1	–
	Hexobendine	3	<1	–
	Dilazep	4	<1	–
Guanosine	Dipyridamole	6	<1	–
	Hexobendine	4	<1	–
	Dilazep	4	<1	–

same way as those of adenosine but the spasmolytic potency of adenine was not much affected by the adenosine uptake blocking drugs. Surprisingly, the responses to both inosine and guanosine were reduced but the effect was difficult to quantify because the maximal response to each drug was reduced in the presence of the uptake blocking agents. A typical experiment showing the interaction between guanosine and dipyridamole is illustrated in Figure 4.

Effects of dipyridamole, hexobendine and Dilazep on responses to other spasmolytic drugs

The effects of the three drugs blocking adenosine uptake on the spasmolytic potencies of noradrenaline, aminophylline, prostaglandin E_2 and glyceryl trinitrate are summarized in Table 3. The only significant potentiation was a modest 2.4-fold increase in the activity of glyceryl trinitrate in the presence of dipyridamole.

Discussion

Adenosine and certain related compounds cause relaxation of the smooth muscle in a number of structures including that in the gastrointestinal tract (Burnstock, 1972) and the coronary vasculature (Berne, 1964), and tracheal bronchial smooth muscle (Bennett & Drury, 1931; Florey & Wells, 1931; Lendle, 1937; Meves, 1953; Bianchi, de Natale & Giaquinto, 1963; Bertelli, Bianchi & Beani, 1973). Some of these effects of adenosine are potentiated by drugs such as dipyridamole, hexobendine and Dilazep which were originally developed as coronary vasodilators. All of these compounds have been reported to potentiate the effect of adenosine on the coronary vasculature (Kraupp, Heistracher, Wolner & Tuisl, 1964; Hilger, 1969; Kolassa *et al.*, 1970; Buyniski *et al.*, 1972; Sano *et al.*, 1972) and dipyridamole and hexobendine to potentiate the negative chronotropic effect of adenosine on cardiac muscle (Hockerts & Bögelmann, 1959; Hopkins & Goldie, 1971; Kolassa, Pfleger & Träm, 1971). The mechanism of the potentiation of adenosine by these agents may involve inhibition of adenosine uptake since Pfleger, Volkmer & Kolassa (1969) and Hopkins (1973) have shown that dipyridamole and hexobendine inhibit the uptake of [^{14}C]-adenosine into guinea-pig hearts. However, not all of the effects of adenosine are potentiated by drugs of this kind. For example, they do not potentiate adenosine-induced relaxation of rabbit gastro-intestinal smooth muscle (Stafford, 1966; Hulme & Weston, 1974) or portal vein (Hughes & Vane, 1970). Presumably, the adenosine uptake process is present in some but not all tissues.

All the purine derivatives employed in this investigation relaxed guinea-pig tracheal smooth muscle by a mechanism not involving stimulation of β-adrenoceptors. These agents were about 1,000 times less active than noradrenaline. Furthermore, the potentiation of adenosine responses in the trachea by the uptake blocking drugs suggests that an uptake process for this nucleoside, similar to that present in the heart and coronary vasculature may also exist in the trachea.

The spasmolytic potency of ATP was similar to

Table 3 The effect of dipyridamole (1 μg/ml), hexobendine (3 μg/ml) and Dilazep (1 μg/ml) on concentration-effect curves to noradrenaline, aminophylline, prostaglandin E_2 and glyceryl trinitrate in the guinea-pig isolated tracheal tube

	Treatment	*No. of experiments*	*Potentiation ratio* $\frac{EC_{40}\ control}{EC_{40}\ treated}$	*95% Confidence limits*
Noradrenaline	Dipyridamole	4	1.2	(0.9–1.6)
	Hexobendine	4	0.8	(0.5–1.2)
	Dilazep	4	1.2	(0.8–1.8)
Aminophylline	Dipyridamole	5	0.8	(0.5–1.4)
	Hexobendine	4	0.7	(0.5–0.9)
	Dilazep	4	1.3	(0.7–2.6)
Prostaglandin E_2	Dipyridamole	12	1.3	(0.9–2.0)
	Hexobendine	8	1.3	(0.8–2.3)
	Dilazep	5	1.0	(0.7–1.6)
Glyceryl trinitrate	Dipyridamole	7	2.4	(1.3–4.5)
	Hexobendine	7	0.7	(0.5–1.0)
	Dilazep	5	1.0	(0.7–1.5)

that of adenosine and responses to these two purines were potentiated to a similar extent by drugs which block adenosine uptake. The latter effect is somewhat surprising since ATP, unlike adenosine, does not readily cross cell membranes (Lowy, Davoll & Brown, 1952; Hoffman & Okita, 1965; Hattori, Miyazaki & Nakamura, 1969). A possible explanation is that the spasmolytic effect of ATP depends on its first being hydrolysed to adenosine at the cell surface, a reaction which is known to occur rapidly in the heart (Baer & Drummond, 1968) and in the gut (Burnstock, 1972) and may, therefore, also occur in the trachea.

In contrast, the spasmolytic potency of adenine was virtually unaltered by drugs that block adenosine uptake. This suggests that adenine is not actively transported into tracheal smooth muscle cells or that its uptake process is not sensitive to dipyridamole-like drugs. It may be significant that adenine uptake into guinea-pig heart muscle is not inhibited by dipyridamole (Kolassa *et al.*, 1970) and so in this tissue at least, adenine and adenosine enter the cell by different processes.

Since inosine uptake into guinea-pig heart muscle has been reported to be inhibited by dipyridamole (Kolassa *et al.*, 1970) it was expected that dipyridamole would enhance the spasmolytic potency of inosine in the guinea-pig trachea. In fact, its activity was greatly reduced by dipyridamole and by the other two adenosine uptake blocking drugs. The mechanism of the spasmolytic action of inosine may, therefore, be intracellular and quite different from that of adenosine. Dipyridamole and similar drugs would then antagonize the effects of inosine by preventing its uptake into cells. The same explanation could also apply to guanosine, which behaves like inosine. Tachyphylaxis to the relaxant effects of inosine and guanosine on the trachea develops rapidly and may, therefore, depend on their releasing some endogenous spasmolytic substance from a depletable intracellular store, but the nature and origin of such a substance are not known.

The specificity of action of the adenosine uptake blocking drugs on the trachea is clear, since they greatly enhance the spasmolytic effect of adenosine and ATP but have little or no effect upon that of adenine, noradrenaline, aminophylline, prostaglandin E_2 or glyceryl trinitrate. However, the small enhancement of the response to glyceryl trinitrate seen with dipyridamole (1 μg/ml) is of interest as dipyridamole has also been reported to enhance responses to glyceryl trinitrate on guinea-pig parametrial artery by an unexplained mechanism (Bell, 1974).

A considerable amount of evidence exists for the presence, in gastro-intestinal smooth muscle, of intramural inhibitory nerves which are neither adrenergic nor cholinergic (Burnstock, 1972) and Burnstock and his co-workers have proposed that adenosine or an adenine nucleotide is the neurotransmitter involved. The presence of non-adrenergic, non-cholinergic inhibitory nerves in the guinea-pig trachea has also been reported (Coburn & Tomita, 1973; Coleman & Levy, 1974) and there is some evidence that the neurotransmitter may be an adenyl derivative (Coleman & Levy, 1974). A possible criticism of the idea of purinergic nerves in the trachea is that exogenously applied adenosine derivatives appear to be less active than known nerve transmitters. However, the present findings reveal that the 'true' potency of adenosine and related nucleotides may be considerably greater than originally thought. The order of potency of the purine derivatives in the untreated trachea—adenine $\geqslant$ guanosine $>$ inosine $\geqslant$ adenosine $\geqslant$ ATP—becomes adenosine $\geqslant$ ATP $\gg$ adenine $\gg$ guanosine = inosine after blockade of the adenosine uptake process. In the presence of dipyridamole, the potency of adenosine approaches that of the sympathetic neurotransmitter noradrenaline. Adenosine and adenine nucleotides need no longer be precluded as possible neurotransmitters on the grounds of low potency.

I wish to thank Dr G.P. Levy for valuable discussion in the preparation of the manuscript.

References

BAER, H.P. & DRUMMOND, G.I. (1968). Catabolism of adenine nucleotides by the isolated perfused rat heart. *Proc. Soc. exp. Biol. Med.*, **127**, 33–36.

BELL, C. (1974). Effects of dipyridamole on neurogenic and non-neurogenic dilator responses of arterial smooth muscle. *Br. J. Pharmac.*, **52**, 13–17.

BENNETT, D.W. & DRURY, A.N. (1931). Further observations relating to the physiological activity of adenine compounds. *J. Physiol., Lond.*, **72**, 288–320.

BERNE, R.M. (1964). Regulation of coronary blood flow. *Physiol. Rev.*, **44**, 1–29.

BERTELLI, A., BIANCHI, C. & BEANI, L. (1973). Effect of inosine on guinea-pig bronchial muscle. *J. Pharm. Pharmac.*, **25**, 60–64.

BIANCHI, A., de NATALE, G. & GIAQUINTO, S. (1963). The effects of adenosine and its phosphorylated derivatives upon the respiratory apparatus. *Arch. int. Pharmacodyn.*, **145**, 498–517.

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

BUYNISKI, J.P., LOSADA, M., BIERWAGEN, M.E. & GARDIER, R.W. (1972). Cerebral and coronary vascular effects of a symmetrical N,N'-disubstituted hexahydrodiazepine. *J. Pharmac. exp. Ther.*, **181**, 522–528.

COBURN, R.J. & TOMITA, T. (1973). Evidence for nonadrenergic inhibitory nerves in the guinea-pig trachealis muscle. *Am. J. Physiol.*, **224**, 1072–1080.

COLEMAN, R.A. & FARMER, J.B. (1971). The inducement

of tone and its inhibition in isolated tracheal muscle. *J. Pharm. Pharmac.*, **23**, 220–222.

COLEMAN, R.A. & LEVY, G.P. (1974). A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. *Br. J. Pharmac.*, **52**, 167–174.

FARMER, J.B. & COLEMAN, R.A. (1970). A new preparation of the isolated intact trachea of the guinea-pig. *J. Pharm. Pharmac.*, **22**, 46–50.

FLOREY, H. & WELLS, A.Q. (1931). A method of photographic registration of small volume changes with some results when applied to the trachea. *J. Pharmac. exp. Ther.*, **42**, 133–141.

HATTORI, E., MIYAZAKI, T. & NAKAMURA, M. (1969). Incorporation of adenosine and adenosine triphosphate into rat myocardium. *Jap. Heart J.*, **10**, 47–52.

HILGER, H.H. (1969). Experimentelle Prüfung der Wirkung von Coronardilatatoren am Menschen. *Arch. exp. Path. Pharmak.*, **263**, 168–184.

HOCKERTS, T. & BÖGELMANN, G. (1959). Untersuchungen uber die Wirkung von 2,6-Bis (diaethanolamino)-4,8-dipiperidino-pyrimido (5,4-d)-pyrimidin auf Herz und Kreislauf. *Arz. Forsch.*, **9**, 47–49.

HOFFMANN, P.C. & OKITA, G.T. (1965). Penetration of ATP into the myocardium. *Proc. Soc. exp. Biol. Med.*, **119**, 573–576.

HOPKINS, S.V. (1973). The potentiation of the action of adenosine on the guinea-pig heart. *Biochem. Pharmac.*, **22**, 341–348.

HOPKINS, S.V. & GOLDIE, R.G. (1971). A species difference in the uptake of adenosine by heart. *Biochem. Pharmac.*, **20**, 3359–3365.

HUGHES, J. & VANE, J.R. (1970). Relaxations of the isolated portal vein of the rabbit induced by nicotine and electrical stimulation. *Br. J. Pharmac.*, **39**, 476–489.

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.

KOLASSA, N., PFLEGER, K. & RUMMEL, W. (1970). Specificity of adenosine uptake into the heart and inhibition by dipyridamole. *Eur. J. Pharmac.*, **9**, 265–268.

KOLASSA, N., PFLEGER, K. & TRÄM, M. (1971). Species differences in action and elimination of adenosine after dipyridamole and hexobendine. *Eur. J. Pharmac.*, **13**, 320–325.

KRAUPP, O., HEISTRACHER, P., WOLNER, E. & TUISL, E. (1964). Die Wirkung von N,N′-Dimethyl-N,N′-bis-[3-(3′,4′,5′-trimethoxybenzoxy)-propyl]-äthylen-diamin auf Herz- und Kreislaufdynamik sowie O_2-Versorgung des Herzmuskels und des Gehirnes. *Arz. Forsch.*, **14**, 1086–1098.

KRAUPP, O., WOLNER, E., ADLER-KASTNER, L., CHIRIKDJIAN, J.J., PLOSZCZANSKI, B. & TUISL, E. (1966). Die Wirkung von Hexobendin auf Sauerstoffverbrauch, Energetik und Substratstoffweschsel des Herzens. *Arz. Forsch.*, **16**, 692–696.

LENDLE, L. (1937). Über spasmolytische Wirkungen von kreislaufwirksamen Gewebsextrakten am künstlichen Bronchospasmus. *Ark. exp. Path., Pharmak.*, **187**, 371–377.

LOWY, B.A., DAVOLL, J. & BROWN, G.B. (1952). The utilisation of purine nucleosides for nucleic acid synthesis in the rat. *J. biol. Chem.*, **197**, 591–600.

MEVES, H. (1953). Die wirkung von adrenalin und adrenalinverwandten auf gefässe und muskulatur der froschlunge. *Pflüg. Arch.*, **258**, 200–210.

PFLEGER, K., VOLKMER, I. & KOLASSA, N. (1969). Hemmung der Aufnahme von Adenosin und Verstärkung seiner Wirkung am isolierten Warmblüterherzen durch coronarwirksame substanzen. *Arz. Forsch.*, **19**, 1972–1974.

SANO, N., KATSUKI, S. & KAWADA, M. (1972). Enhancement of coronary vasodilator action of adenosine by 1,4-bis-[3-(3,4,5-trimethoxybenzoyloxy)-propyl]-perhydro-1,4-diazepine (Dilazep I.N.N.). *Arz. Forsch.*, **22**, 1655–1658.

STAFFORD, A. (1966). Potentiation of adenosine and the adenine nucleotides by dipyridamole. *Br. J. Pharmac. Chemother.*, **28**, 218–227.

(Received September 30, 1975.)