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INHIBITORY EFFECT OF ADENOSINE AND ADENINE NUCLEOTIDES ON POTASSIUM-EVOKED EFFLUX OF [^{3}H]-NORADRENALINE FROM THE RAT ISOLATED HEART: LACK OF RELATIONSHIP TO PROSTAGLANDINS

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1 In the isolated heart of the rat prelabelled with [^{3}H]-noradrenaline (NA) and perfused with Krebs solution, administration of potassium (K^+ 60 μmol) increased the efflux of total radioactivity and of [^{3}H]-NA in the perfusate.

2 Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine, but not inosine, dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP) or db cyclic GMP reduced the K^+-evoked overflow of total radioactivity and of intact [^{3}H]-NA, in concentrations too low to cause release of prostaglandins. ATP, ADP and adenosine did not affect tyramine-evoked overflow of tritium.

3 Blockade of prostaglandin synthesis with indomethacin did not alter the inhibitory effect of either ATP, ADP or adenosine on K^+-induced overflow of tritium, thereby indicating that these nucleotides inhibit adrenergic transmission by a mechanism unrelated to stimulation of prostaglandin synthesis.

4 Theophylline which increases entry of calcium (Ca^{2+}) across the cell membrane and reduces its binding in the cell, enhanced K^+-evoked overflow of tritium and diminished the inhibitory effect of ATP, ADP and adenosine on K^+-evoked overflow of tritium from the heart, presumably by interfering with transneuronal Ca^{2+} metabolism.

Introduction

Adenosine, adenosine triphosphate (ATP) and adenosine diphosphate (ADP) have been shown to inhibit the vascular responses to periarterial nerve stimulation and to injected noradrenaline (NA) in several tissues in concentrations that do not alter the basal vascular tone (Malik & McGiff, 1974; Verhaeghe, Vanhoutte & Shepherd, 1977; Enero & Saidman, 1977; Su, 1978). Since the vascular responses to nerve stimulation were reduced to a greater degree than those to injected NA, it was suggested that the nucleotides also inhibit release of the adrenergic transmitter. The ability of adenosine, ATP, and ADP to diminish the output of NA evoked by either sympathetic nerve or transmural stimulation has been demonstrated in several tissues of different species (Hedqvist & Fredholm, 1976; Verhaeghe *et al.*, 1977; Enero & Saidman, 1977; Su, 1978; Hedqvist, Fredholm & Ölundh, 1978; Wakade & Wakade, 1978). The demonstation that nucleotides (ATP, ADP) stimulate the biosynthesis of prostaglandins (Needleman, Minkes, & Douglas, 1974; Isakson, Raz, Denny, Pure & Needleman, 1977) and that prostaglandin E_2 (PGE_2) and PGI_2 inhibit the release of NA evoked by sympathetic nerve stimulation or by K^+ from the heart (Wennmalm, 1978; Khan & Malik, 1978a) raises the possibility that prostaglandins may participate in the modulatory effect of nucleotides on adrenergic transmission in the heart. To test this hypothesis we have examined the effect of several nucleotides on the basal and K^+- as well as tyramine-evoked release of [^{3}H]-NA and on the output of prostaglandins from the isolated heart of rat, perfused with Krebs solution. The studies were performed before and during inhibition of prostaglandin biosynthesis with indomethacin (Vane, 1971) to distinguish the direct actions of nucleotides on adrenergic transmission from those related to stimulation of prostaglandin synthesis. Thus, a modification of the effect of nucleotides on release of the neurotransmitter would indicate participation of a prostaglandin-mediated component. A

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preliminary account of the results has been published (Khan & Malik, 1978b).

Methods

Male rats (Wistar & Sprague–Dawley) weighing 250 to 275 g were anaesthetized with ether, and the thorax was opened. The heart was quickly removed and perfused, according to the method of Langendorff, with Krebs solution (composition, mM: NaCl 118.2; KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 5.5 and disodium edetate (EDTA) 0.02) maintained at 37°C, at a constant flow rate of 4 ml/min with a Harvard peristaltic pump (model 1203). Krebs solution was gassed with 95% O_2 and 5% CO_2.

[^{3}H]-NA 9.1 nmol was infused into the heart over a period of 20 min to label the endogenous store. Hearts were then perfused with [^{3}H]-NA-free Krebs solution for 1 h to wash the radioactivity from extraneuronal spaces. During this period, perfusates were collected for 5 min periods for 1 h. One ml from each of these samples was mixed with 10 ml of Insta-Gel emulsifier (Packard) and the radioactivity counted in a Liquid Scintillation Counter (Searle Mark III 6880). Potassium chloride (K^+) (60 μmol) or tyramine (7.2 μmol) contained in a volume of 0.2 ml saline (0.9% w/v NaCl solution) was then injected into the aortic cannula at 10 min intervals. The perfusate was collected for 2 min periods before and immediately after the administration of K^+ or tyramine: 1 ml of these samples was used to measure radioactivity as described above. The net efflux of tritium evoked by either K^+ or tyramine was calculated by subtracting the amount of tritium released spontaneously within the immediately preceding period from the efflux obtained by the administration of K^+ or tyramine, respectively. A total of five K^+ or tyramine injections was given into the heart. The first two samples, which were used to test the viability of the preparation in terms of release of tritium, were discarded and the subsequent samples were collected. The first period of basal (B_1) and K^+ (K_1^+)- or tyramine (T_1)-evoked overflow of tritium in this study represents the samples collected 20 min after washing. In each experiment the basal and K^+- or tyramine-evoked effluxes of tritium during second and third periods were expressed as a ratio of the corresponding effluxes obtained during the first period. Nucleotides were infused 7 min before the second period of K^+ or tyramine injection and terminated 2 min after the administration of K^+ or tyramine. In some experiments, nucleotides were infused during the third instead of second period of K^+ administration. Indomethacin and theophylline were infused 6 to 7 min before the second period of K^+ injection until the end of the experiment. In these experiments and in those where the nucleotides were infused alone, the mean ratios of the basal and K^+- or tyramine-evoked effluxes for the second and third periods to those in the first period, were calculated and compared to the corresponding mean ratios in control experiments. When the nucleotides were infused during the third period in the presence of either indomethacin, or theophylline, the mean ratio of tritium efflux during the third period to that in the second period was compared to the corresponding ratio obtained in the absence of nucleotides. In all experiments, the mean ratios were calculated from counts per minute obtained from 1 ml samples of the perfusate (8 ml) collected for 2 min.

Determination of the release of prostaglandins from the isolated heart of rat

Release of prostaglandin-like substance(s) from the isolated heart of rat during the infusion of adenosine and adenine nucleotides were detected by continuously superfusing the rat stomach strip with the perfusate from the heart according to the procedure of Vane (1964). The initial resting tension on the stomach strip was 2 to 3 g and its contraction was measured with a Harvard smooth muscle transducer and recorded on a Physiograph. The tissues were made insensitive to catecholamines, acetylcholine, histamine and 5-hydroxytryptamine (5-HT) by continuously infusing a mixture of the antagonists into the superfusing solution to give the final concentration of the active bases: hyoscine hydrobromide 0.1 μg/ml, pyrilamine maleate 0.1 μg/ml, methysergide 0.2 μg/ml, phenoxybenzamine hydrochloride 0.1 μg/ml and propranolol hydrochloride 2 μg/ml. Indomethacin (1.4×10^{-5} M) was also included in the superfusing fluid to block prostaglandin synthesis within the assay organ (Vane, 1971).

The perfusate from the heart before and during the administration of adenosine, ADP and ATP, in the absence and presence of indomethacin, was collected for 10 min periods. The effluent was adjusted to pH 3 with formic acid (1 N) and the lipids were extracted with ethylacetate. The ethylacetate was evaporated to dryness under vacuum; the extract was reconstituted in 1 ml of saline and assayed for prostaglandin-like activity on the rat stomach strip with authentic PGE_2 as the standard. The rat fundic strip was suspended in a glass chamber and superfused with Krebs solution (3 ml/min, 37°C) and its contraction was measured with a Harvard smooth muscle transducer and recorded on a Physiograph.

Separation of [^{3}H]-noradrenaline from its metabolites

Samples of the perfusate from the heart prelabelled with [^{3}H]-NA were collected for 2 min periods (8 ml) in ice-cold vials (containing 0.2 ml of 2 N HCl). ATP,

ADP and adenosine (9×10^{-6} M) were added to the fluid perfusing the heart. [^{3}H]-NA and its metabolites were separated by column chromatography. The catechols, noradrenaline (NA), 3,4-dihydroxymandelic acid (DOMA) and 3,4-dihydroxyphenylglycol (DOPEG) were separated from non-catechols, normetanephrine (NMN), 3-methoxy-4-hydroxymandelic acid (VMA) and 3-methoxy-4-hydroxyphenylglycol (MOPEG) by adsorption on alumina as described by Anton & Sayre (1966). [^{3}H]-NA was separated from DOMA and DOPEG and NMN was separated from VMA and MOPEG by applying these samples on two separate Dowex resin 50 Columns (Verhaeghe & Shepherd, 1976). The effluents containing DOMA plus DOPEG and VMA plus MOPEG from Dowex Columns were collected and the [^{3}H]-NA and NMN absorbed on separate Dowex Columns were eluted with 15 ml of 2 N HCl. No attempt was made to separate DOMA from DOPEG and VMA from MOPEG. To calculate the recovery, samples of [^{3}H]-NA (10 to 15×10^3 ct/min) were also dissolved in Krebs solution and were processed through the Columns. Radioactivity was measured in 1 ml samples mixed with 10 ml of Insta-Gel emulsifier (Packard Instruments Company, Illinois) and expressed as counts per minute per collection period.

Drugs

The following drugs were used: [^{3}H]-(−)-noradrenaline (specific activity 2.2 Ci/mmol, New England Nuclear); (−)-noradrenaline bitartrate (Levophed, Winthrop); tyramine hydrochloride, (±)-normetanephrine hydrochloride (NMN), (±)-3,4-dihydroxymandelic acid (DOMA), (±)-4-hydroxy-3-methoxymandelic acid (VMA), sodium metabisulphite, adenosine, adenosine 5-triphosphate disodium (ATP), adenosine-5-diphosphate (ADP), inosine, N^6,O_2-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid sodium salt (db cyclic AMP), N^2,O^2-dibutyryl guanosine-3′,5′-cyclic monophosphoric acid sodium salt (db cyclic GMP), theophylline and indomethacin (Sigma); guanethidine (CIBA); hyoscine hydrobromide (British Drug Houses); phenoxybenzamine hydrochloride (Smith, Kline & French); pyrilamine maleate (Pfaltz & Bauer); methysergide maleate (Sandoz); propranolol hydrochloride (Ayerst Labs); prostaglandins E_2 and prostacyclin sodium salt (PGI_2) (Upjohn Company). Concentrations of ATP, ADP, adenosine, inosine, db cyclic AMP, db cyclic GMP, and tyramine are expressed as bases.

Prostaglandin E_2 was dissolved in ethanol (1 mg/ml), and further dilutions were made in Krebs solution. PGI_2 was dissolved in Tris buffer pH 9.4 (0.05 mM). Control Krebs solution containing the same amount of ethanol (15 nl/ml) or Tris buffer (0.05 M) did not affect the assay organ. Indomethacin was dissolved in Krebs solution by vigorous shaking, whereas all other drugs were dissolved in saline. Tyramine was injected into the aortic cannula, whereas all other drugs were added to Krebs solution perfusing the heart to establish the final concentration.

Statistical analysis

Statistical analysis was performed according to the procedure described by Kirk (1968). The data are expressed as means ± s.e. mean throughout this study. Analysis of variance was performed to compare the difference between more than two means and the source of variance was determined by the Newman Keuls test. An unpaired *t*-test was used to determine the statistical significance of a difference between two means. $P < 0.05$ was considered to be significant.

Results

Effect of K^+ on the output of tritium from the isolated heart of rat

Analysis of the perfusate from the heart showed that the spontaneous overflow of tritium reached almost steady levels in about 50 to 60 min after the termination of the infusion of [^{3}H]-NA into the heart. Injections of K^+ into the heart were associated with an increased overflow of tritium which was related to the dose of K^+. The maximum increase in the overflow of tritium was produced by 70 μmol of K^+, and further increments in doses of K^+ caused increases of smaller magnitude in the overflow of tritium (Figure 1a). Repeated administration of K^+ in 60 μmol doses at 10 min intervals increased the overflow of tritium; this increment being progressively reduced (Figure 1b). Although the basal as well as the K^+-evoked output of tritium varied among different hearts, the ratio for the second and third as well as subsequent periods (10 min intervals) to the first period remained constant among different preparations. In 6 hearts the mean ratios calculated for the basal efflux of tritium for the second and third periods to the first period were 0.915 ± 0.003 and 0.839 ± 0.004, respectively; whereas the mean ratios for the K^+-evoked efflux of tritium for the second and third periods to the first period were 0.936 ± 0.008 and 0.877 ± 0.006, respectively. The first period for the basal and K^+-evoked overflow and those where the drugs were infused represents the period 20 min after washing with [^{3}H]-NA-free Krebs solution.

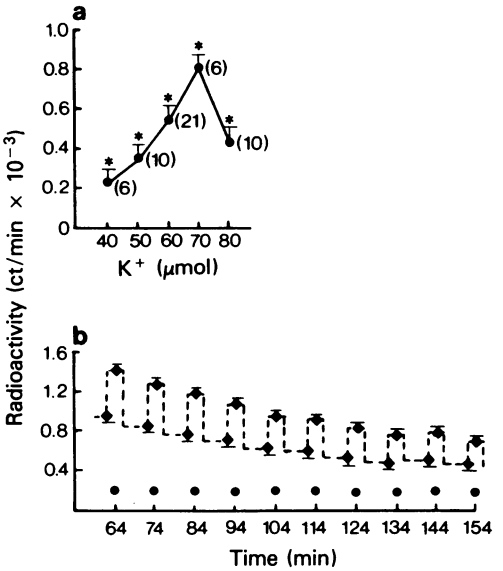

Figure 1 Effect of K^+ on the overflow of tritium from the rat isolated heart perfused with Krebs solution. (a) The increase in the overflow of tritium produced by different doses of K^+ (40 to 80 μmol). (b) The increase in overflow of tritium produced by repeated administration of K^+ (●, 60 μmol) at 10 min intervals. Vertical bars indicate s.e. mean. * Denotes increase from the basal efflux ($P < 0.001$). Number of experiments are shown in parentheses in (a); in (b) $n = 6$.

Effect of calcium removal from the perfusion medium and guanethidine of K^+-evoked overflow of tritium from the rat isolated heart

In 6 hearts, perfused with calcium (Ca^{2+})-free Krebs solution, injections of K^+ (60 μmol) failed to alter the basal output of tritium (873 ± 114 ct/min). The mean ratio of the basal efflux during the second period in the absence of Ca^{2+} to that in the first period (0.935 ± 0.03) was not significantly different ($P > 0.05$) from the corresponding ratios obtained in the presence of Ca^{2+} (0.915 ± 0.003). In 6 additional hearts perfused with normal Krebs solution, injection of K^+ (60 μmol) increased the output of tritium from 603 ± 68 ct/min to 994 ± 118 ct/min during the first period, whereas during the second period in the presence of guanethidine (2.5×10^{-5} M), the K^+-evoked efflux of tritium but not the basal efflux was diminished. The mean ratio of the basal efflux during the second period in the presence of guanethidine to that in the first period (0.941 ± 0.025) was not different ($P > 0.05$) from the corresponding ratios obtained in the absence of this drug (0.915 ± 0.003). In contrast, the mean ratio of the K^+-evoked efflux during the second period in the presence of guanethidine to that in the first period (0.280 ± 0.058) was significantly lower ($P < 0.001$) than the corresponding ratios obtained in the absence of guanethidine (0.936 ± 0.008).

Effect of ATP, ADP, adenosine, inosine, db cyclic AMP, db cyclic GMP on K^+-evoked efflux of tritium

Infusion of either ATP, ADP or adenosine for 7 min did not alter the basal efflux of tritium ($P > 0.05$) but reduced the K^+-evoked release of tritium. Inosine, db cyclic AMP and db cyclic GMP, which also did not alter the basal efflux of tritium, produced variable effects on K^+-evoked overflow of tritium (Figure 2 and Tables 1 and 2).

Effects of ATP, ADP and adenosine on the overflow of [^{3}H]-noradrenaline and its metabolites evoked by K^+ from the heart of rat

Column chromatographic analysis of the perfusate from 6 rat hearts prelabelled with [^{3}H]-NA was performed to separate the amine from its metabolites. The recovery of [^{3}H]-NA after column chromatography was 69 ± 4% (±s.e. mean, $n = 6$), whereas the rest of the radioactivity appeared in fractions corresponding to various metabolites. Of the total radioactivity present in the perfusate during control periods, 15% was accounted for by the metabolites (NMN, VMA, DOMA, DOPEG and MOPEG). Administration of K^+ to the heart increased the output of intact [^{3}H]-NA and this was significantly reduced during the infusion of either ATP, ADP or adenosine (Table 3).

Effect of indomethacin on the inhibitory effect of nucleotides on K^+-evoked efflux of tritium from the heart of rat

Infusion of indomethacin (1.4×10^{-6} M) into the heart for 10 min did not alter the basal efflux of tritium (804 ± 64 ct/min, $n = 10$). The mean ratios of basal efflux during the second and third period in the presence of indomethacin to that in the first period (0.912 ± 0.004 and 0.847 ± 0.015, respectively) were not significantly different ($P > 0.05$) from the corresponding ratios obtained in the absence of indomethacin (0.915 ± 0.003 and 0.839 ± 0.004, $n = 6$, respectively). In contrast, this concentration of indomethacin significantly increased the K^+-evoked efflux of tritium (Figure 3a). During indomethacin (1.4×10^{-6} M) infusion, ATP, ADP or adenosine inhibited the K^+-evoked efflux of tritium to a similar degree as in the absence of indomethacin ($P > 0.9$) (Figure 3b and c).

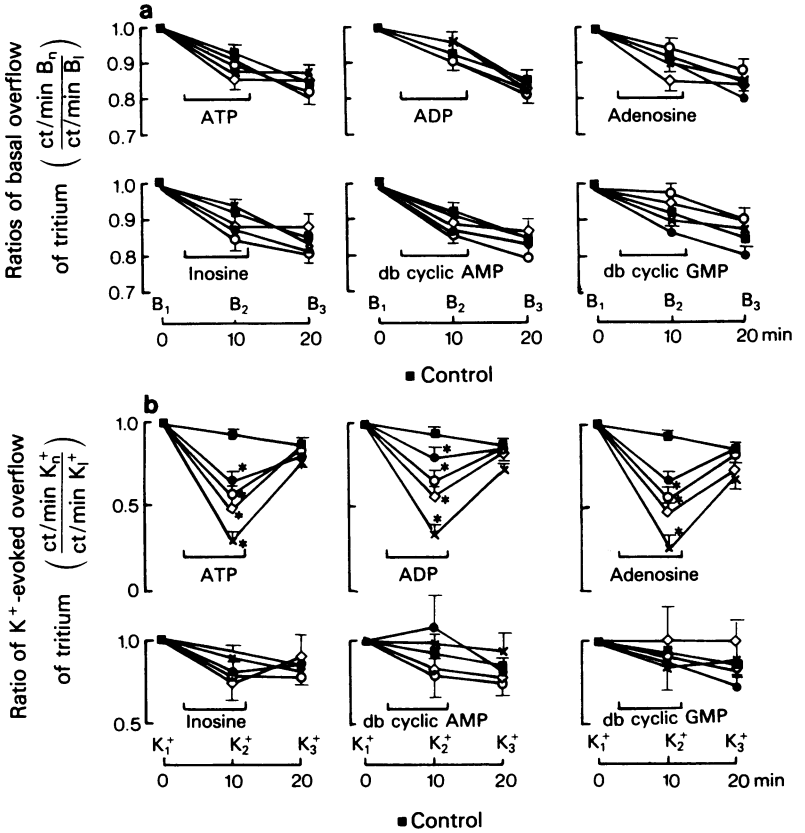

Figure 2 Effect of ATP, ADP, adenosine, inosine, db cyclic AMP and db cyclic GMP on the output of basal (a) and K^+ (60 μmol) evoked overflow (b) of tritium from the isolated heart of rat, prelabelled with [^{3}H]-noradrenaline and perfused with Krebs solution. Basal efflux (B_1, B_2 and B_3) represents the amount of tritium released spontaneously in the perfusate collected immediately before the injections of K^+ (K_1^+, K_2^+ and K_3^+, respectively). In control experiments the absolute values for the basal efflux of tritium (B_1) and the increase in the overflow of tritium evoked by K^+ (K_1^+) were 774 ± 63 and 405 ± 16, respectively. In experiments where nucleotides were infused the absolute values for the basal efflux and the increase in the overflow of tritium evoked by the injection of K^+ during the first period (B_1 and K_1^+) before the infusion of nucleotides is given in Tables 1 and 2, respectively. The mean ratio of the basal efflux during second period in the presence of nucleotides to that in the first period (B_2/B_1) was not significantly different ($P > 0.05$) from the corresponding mean ratio of the basal efflux obtained in the absence of nucleotides (■, control, B_2/B_1). The mean ratio of the increases in the K^+-evoked efflux during second period in the presence of ATP, ADP and adenosine but not inosine, db cyclic AMP and db cyclic GMP to that in the first period was significantly less (*$P < 0.05$) than the corresponding mean ratios obtained in the absence of these nucleotides (■, control K_2^+/K_1^+).

Table 1 Basal efflux of tritium during first period (B_1) before infusion of nucleotides (see also Figure 2a)

Nucleotide	(●) 9×10^{-7} M	(○) 1.8×10^{-6} M *ct/min in 1 ml of perfusate*	(◇) 4.5×10^{-9} M	(×) 9×10^{-6} M
ATP	784 ± 65 (7)	651 ± 84 (10)	722 ± 60 (5)	716 ± 76 (6)
ADP	694 ± 33 (6)	765 ± 91 (6)	680 ± 68 (7)	716 ± 21 (7)
Adenosine	782 ± 57 (7)	660 ± 26 (8)	670 ± 99 (6)	757 ± 53 (15)
Inosine	789 ± 38 (9)	796 ± 139 (7)	733 ± 61 (5)	752 ± 33 (13)
db cyclic AMP	699 ± 62 (7)	658 ± 85 (7)	704 ± 96 (9)	659 ± 80 (7)
db cyclic GMP	644 ± 71 (6)	737 ± 39 (10)	690 ± 51 (7)	752 ± 66 (7)

Number in parentheses denotes the number of hearts.

Effect of indomethacin on the release of prostaglandins produced by nucleotides from the isolated heart of rat

To determine if ATP, ADP and adenosine stimulate the synthesis of prostaglandins in the rat heart and that the concentration of indomethacin, 1.4×10^{-6} M, used did inhibit the output of prostaglandins, we superfused rat stomach strip with the perfusate. Application of PGE_2 or PGI_2 directly into the fluid superfusing the rat stomach strip produced contraction. PGE_2 was about 10 times more active than PGI_2 in causing contraction of rat stomach strip. ADP (4.5 to 9×10^{-6} M) infused into the heart released a prostaglandin-like substance(s) which caused contraction of the stomach strip. Application of ADP directly to the stomach strip also caused contraction. However, this was much smaller than that produced by the infusion of ADP through the heart. Infusion of ATP and adenosine (4.5 to 9×10^{-6} M) through the heart also caused the release of a prostaglandin-like substance(s) (5 hearts). Low concentrations of either ATP, ADP or adenosine failed to release a prostaglandin-like substance(s) (5 hearts). Infusion of either inosine, db cyclic AMP or db cyclic GMP (9×10^{-6} M) did not release a prostaglandin-like material (6 hearts). During the infusion of indomethacin (1.4×10^{-6} M) through the heart, ATP, ADP and adenosine failed to release a prostaglandin-like substance(s) from the rat heart, as indicated by a decreased contraction of the rat stomach strip (Figure 4). The blockade induced by indomethacin of output of prostaglandin-like substance(s) evoked by either ATP, ADP or adenosine (9×10^{-6} M) from the heart was also confirmed by acidic lipid extraction of the perfusate and bioassay on rat stomach strip. In two experiments, the basal output of prostaglandin-like substance(s) from the rat heart was 0.02 ng/min average which increased to 0.15 ng/min. Extracts obtained

Table 2 Increases in the overflow of tritium evoked by the injection of K^+ during first period (K_1^+) before the infusion of nucleotides (see also Figure 2b)

Nucleotide	(●) 9×10^{-7} M	(○) 1.8×10^{-6} M	(◇) 4.5×10^{-9} M	(×) 9×10^{-6} M
	ct/min in 1 ml of perfusate			
ATP	432 ± 65 (7)	390 ± 31 (10)	385 ± 66 (6)	430 ± 70 (6)
ADP	425 ± 65 (6)	434 ± 92 (6)	428 ± 28 (7)	385 ± 95 (7)
Adenosine	398 ± 48 (7)	388 ± 70 (6)	390 ± 60 (6)	478 ± 65 (15)
Inosine	388 ± 50 (11)	591 ± 140 (9)	400 ± 30 (10)	410 ± 66 (13)
db cyclic AMP	435 ± 27 (7)	404 ± 26 (9)	383 ± 40 (9)	410 ± 53 (7)
db cyclic GMP	395 ± 59 (10)	368 ± 49 (8)	435 ± 28 (6)	398 ± 10 (10)

Number in parentheses denotes the number of hearts.

Table 3 Column chromatographic analysis of noradrenaline and its metabolites in the perfusate from 6 isolated hearts of rat prelabelled with [^{3}H]-noradrenaline†

Product	*Standard* [^3H]-*NA*‡	*Basal*	K^+ (60 μmol)	K^+ (60 μmol) plus *ATP* 9×10^{-6} M	K^+ (60 μmol) plus *ADP* 9×10^{-6} M	K^+ (60 μmol) plus *Adenosine* 9×10^{-6} M
	ct/min $\times 10^{-3}$ *recovered after column chromatography*					
Noradrenaline (NA)	9.39 ± 0.70	0.95 ± 0.10	4.78 ± 0.52	1.75 ± 0.25*	0.87 ± 0.13**	0.98 ± 0.09**
DOMA + DOPEG	0.57 ± 0.05	2.39 ± 0.32	2.83 ± 0.37	2.24 ± 0.11	1.96 ± 0.35	1.77 ± 0.38
NMN	0.25 ± 0.03	0.22 ± 0.04	0.28 ± 0.04	0.21 ± 0.01	0.22 ± 0.01	0.19 ± 0.01
VMA + MOPEG	0.59 ± 0.04	1.43 ± 0.15	1.81 ± 0.23	1.37 ± 0.10	1.32 ± 0.22	1.18 ± 0.08

† Perfusate was collected for 2 min (8 ml) from each heart: 1 ml of the sample was taken for counting the radioactivity and rest of the sample applied on the column.
Data are expressed as mean ± s.e. mean; ‡ = samples were not perfused through the heart.
* $P < 0.01$ and ** $P < 0.001$ denote significance of difference between K^+-evoked release of intact [^{3}H]-NA in the absence and presence of nucleotides.
DOMA = 3,4-dihydroxymandelic acid; DOPEG = 3,4-dihydroxyphenylglycol; NMN = normetanephrine; VMA = 3-methoxy-4-hydroxymandelic acid; MOPEG = 3-methoxy-4-hydroxyphenylglycol.

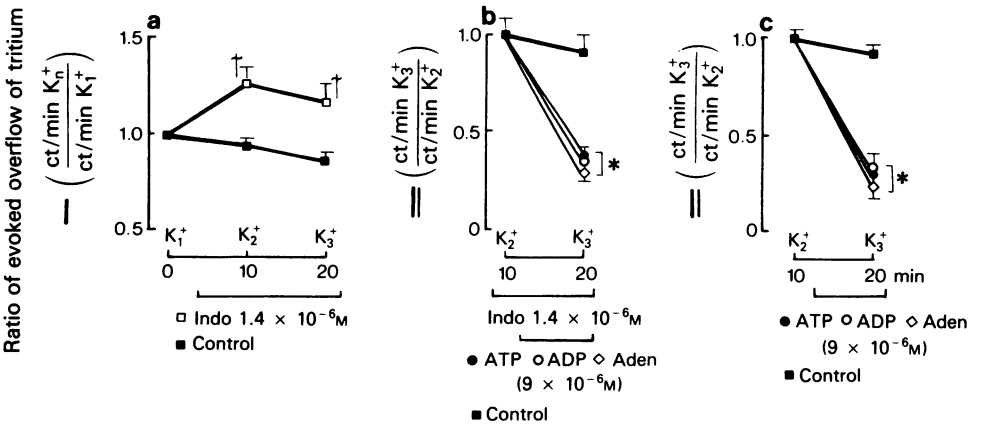

Figure 3 Effect of indomethacin (Indo) on K^+-evoked overflow of tritium (a) and the effect of ATP, ADP and adenosine (Aden) on K^+-evoked overflow of tritium in the presence (b) and absence of indomethacin (c). (a) The absolute overflow of tritium in response to K_1^+ was 422 ± 91 ct/min (±s.e. mean, $n = 10$). Infusion of indomethacin increased the overflow of tritium evoked by K^+. The mean ratios of K^+-evoked overflow of tritium during the second and third period in the presence of indomethacin to that in the first period (K_2^+/K_1^+ and K_3^+/K_1^+) were significantly greater ($\dagger P < 0.05$) than the corresponding mean ratios obtained in the absence of indomethacin (■, control). (b) The overflow of tritium in response to K_2^+ before the infusion of (●) ATP, (○) ADP and (◇) adenosine was 600 ± 66 ct/min ($n = 6$), 550 ± 55 ct/min ($n = 6$) and 530 ± 40 ct/min ($n = 6$) (±s.e. mean), respectively. The mean ratios of K^+-evoked overflow of tritium during third period in the presence of nucleotides to that in the second period (K_3^+/K_2^+) was significantly less ($^*P < 0.001$) than the corresponding ratio obtained in the absence of nucleotides (■ control, K_3^+/K_2^+). (c) The absolute overflow of tritium in response to K_2^+ before the infusion of ATP, ADP and adenosine was 380 ± 40 ct/min ($n = 6$), 375 ± 37 ct/min ($n = 8$) and 395 ± 60 ct/min ($n = 7$), respectively. The mean ratio of K^+-evoked overflow of tritium during third period in the presence of these nucleotides to that in the second period (K_3^+/K_2^+) was significantly less ($^*P < 0.001$) than the corresponding ratio obtained in the absence of nucleotides (■, control, K_3^+/K_2^+).

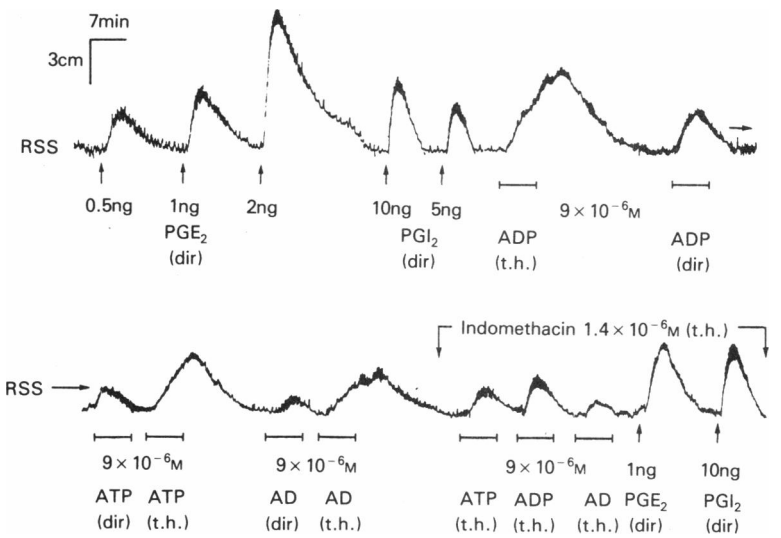

Figure 4 Release of prostaglandin-like substance(s) from the rat isolated heart produced by ADP, ATP and adenosine (Aden) (9×10^{-6} M). Prostaglandin E_2 (PGE_2) and PGI_2 were applied directly (dir) into the fluid superfusing the rat stomach strip (RSS). ADP, ATP and adenosine were added to fluid perfusing through the heart (t.h.) or infused directly into Krebs solution superfusing the RSS for 7 min.

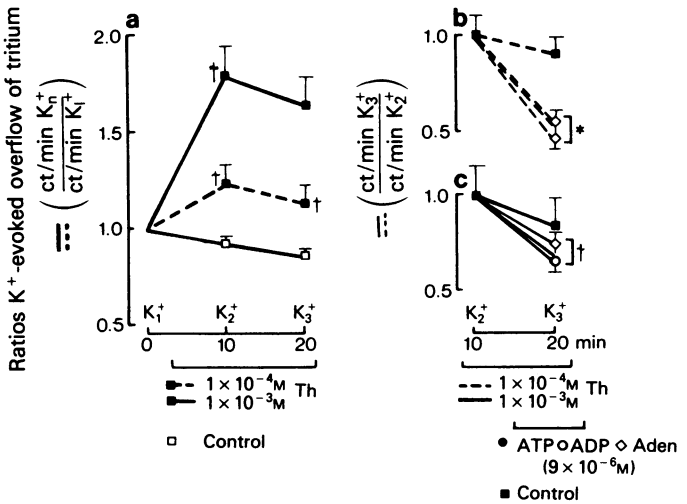

Figure 5 (see also Table 4) Effect of theophylline on K^+-evoked overflow of tritium (a) and the effect of ATP, ADP and adenosine (Aden) on K^+-evoked overflow of tritium in the presence (b and c) of theophylline (Th). (a) The absolute increase in overflow of tritium in response to (■——■) K_1^+ was 430 ± 27 ct/min (n = 7) and (■ - - ■) K_1^+ was 421 ± 31 ct/min (n = 9) (±s.e. mean). Infusion of theophylline (1×10^{-4} M and 1×10^{-3} M) into the heart increased the K^+-evoked overflow of tritium. The mean ratios of K^+-evoked overflow during the second and third period in the presence of theophylline 1×10^{-4} M and 1×10^{-3} M to that in the first period (K_2^+/K_1^+ and K_3^+/K_1^+) were significantly greater (†$P < 0.05$, ‡$P < 0.01$, *$P < 0.001$) compared to the corresponding mean ratios obtained in the absence of theophylline (□, control). (b and c) Increase in overflow of tritium in response to K_2^+ before infusion of nucleotides. In these experiments with theophylline, the mean ratio of K^+-evoked overflow of tritium during the third period in the presence of nucleotides to that in the second period (K_3^+/K_2^+) was significantly less (†$P < 0.05$ and *$P < 0.001$) than the corresponding mean ratios obtained in the absence of nucleotides (■, control, K_3^+/K_2^+).

from the hearts pretreated with indomethacin did not cause contraction of the rat stomach strip.

Effect of theophylline on the inhibitory effect of ATP, ADP and adenosine on K^+-evoked overflow of tritium

Theophylline, in concentrations of 1×10^{-4} and 1×10^{-3} M, did not alter the basal efflux of tritium. The basal efflux during the first period in the absence of theophylline was 654 ± 44 ct/min (n = 7) and 606 ± 63 ct/min (n = 10), respectively. The mean ratios of the basal efflux during the second and third period in the presence of theophylline 1×10^{-4} and 1×10^{-3} M (0.842 ± 0.051 and 0.782 ± 0.062, and 0.895 ± 0.045 and 0.832 ± 0.045, respectively) were not significantly different from the corresponding mean ratios obtained in the absence of theophylline (0.915 ± 0.003 and 0.834 ± 0.004, n = 6, respectively). Theophylline in similar concentrations increased K^+-evoked overflow of tritium (Figure 5a). Infusion of either ATP, ADP or adenosine in the presence of theophylline (1×10^{-4} or 1×10^{-3} M) reduced K^+-evoked overflow of tritium; however, the degree of reduction was significantly less ($P < 0.05$ and $P < 0.01$, respectively) than that produced by these

Table 4 Increase in the overflow of tritium in response to K_2^+ in the presence of theophylline before the infusion of nucleotides (see also Figure 5)

Theophylline	(●) *ATP*	(○) *ADP*	(◇) *Adenosine*
	ct/min in 1 ml of perfusate ±*s.e. mean*		
1×10^{-4} M	525 ± 65 (7)	575 ± 70 (5)	505 ± 17 (6)
1×10^{-3} M	815 ± 17 (10)	780 ± 71 (6)	809 ± 40 (11)

Number in parentheses denotes the number of hearts.

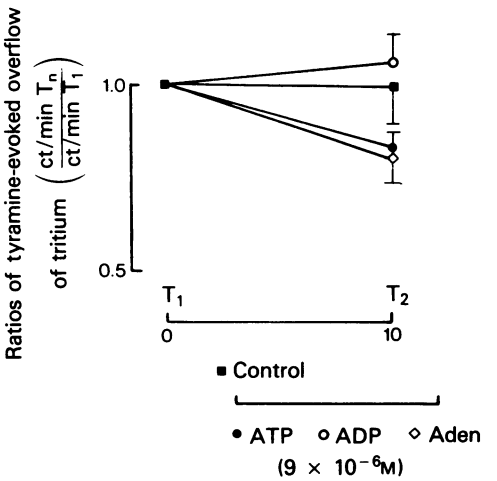

Figure 6 Effect of ATP, ADP and adenosine (Aden) on the overflow of tritium evoked by tyramine (T). In experiments where nucleotides were infused, the absolute overflow of tritium in response to T_1 before the infusion of (●) ATP, (○) ADP and (◇) adenosine was 1029 ± 75 ct/min (±s.e. mean, $n = 6$), 834 ± 78 ct/min (±s.e. mean, $n = 6$) and 926 ± 54 ct/min (±s.e. mean, $n = 6$), respectively. The mean ratio of the tritium released by T during the second period to that in the first period in the absence of nucleotides (■, control) was not significantly different ($P > 0.05$) from the corresponding ratio obtained in the presence of either ATP, ADP or adenosine.

nucleotides in the absence of theophylline (Figure 5b and c and Figure 3c).

Effect of ATP, ADP and adenosine on the overflow of tritium evoked by tyramine

Injection of tyramine in a dose of 7.2 μmol into the heart increased the overflow of tritium from the basal 647 ± 114 ct/min to 1724 ± 263 ct/min (6 experiments) ($P < 0.001$). Infusion of either ATP, ADP or adenosine (9×10^{-6} M) which inhibited K^+-evoked release of tritium (Figure 2), failed to alter the tyramine induced release of tritium from the rat heart (Figure 6).

Discussion

In the isolated heart of the rat labelled with [^{3}H]-NA and perfused with Krebs solution, administration of K^+ (60 μmol) consistently increased the overflow of tritium and [^{3}H]-NA. Since the release of tritium evoked by K^+ from the rat heart was abolished by the removal of Ca^{2+} from the perfusion medium and was markedly inhibited by the administration of the adrenergic neurone blocking agent, guanethidine, it appears that the release of NA produced by K^+ involves a similar Ca^{2+}-dependent process to the release of NA evoked by stimulation of sympathetic nerves (Kirpekar & Wakade, 1968; Haeusler, Thoenen, Haefely & Huerlimann, 1968). ATP, ADP and adenosine but not inosine, db cyclic AMP or db cyclic GMP, reduced the K^+-evoked release of tritium. These observations are consistent with the inhibitory effect of ATP, ADP and adenosine on adrenergic transmission reported in various tissues (for references see introduction).

Our present study indicates that the inhibitory effect of ATP, ADP and adenosine on the release of tritium evoked by K^+ from the rat heart is unrelated to stimulation of prostaglandin synthesis. Thus, ATP, ADP and adenosine inhibited the overflow of tritium in concentrations which failed to enhance the output of prostaglandin-like material from the rat heart. Moreover, blockade of prostaglandin synthesis with indomethacin did not alter the inhibitory effect of these nucleotides on the overflow of tritium evoked by K^+. Although higher concentrations of ATP, ADP and adenosine (9×10^{-6} M) enhanced the efflux of a prostaglandin-like substance(s) from the rat heart, the concentration of released prostaglandins reaching the nerve terminal was probably insufficient to affect the output of adrenergic transmitter evoked by K^+.

The release of tritium from the rat heart evoked by tyramine, which liberates NA from adrenergic nerves by a process independent of Ca^{2+} (Lindmar, Löffelholz & Muscholl, 1967), was not altered by ATP, ADP or adenosine. The inhibitory effect of these nucleotides on release of the adrenergic transmitter presumably results from decreased entry of Ca^{2+} into the nerve fibre. In support of this view are the observations that increased concentrations of Ca^{2+} antagonized the inhibitory effect of ATP and adenosine on adrenergic transmission in the isolated mesenteric arteries of the rat (Malik & McGiff, 1974) and the guinea-pig ileum (Kažić & Milosavljević, 1976), respectively. Alternatively, ATP, ADP and adenosine may inhibit release of NA by affecting the intraneuronal Ca^{2+} metabolism as a consequence of increased cellular levels of cyclic AMP (McIlwain, 1972). However, this seems to be unlikely because the overflow of tritium evoked by K^+ was altered neither by db cyclic AMP nor db cyclic GMP in the rat heart. In the isolated vas deferens of guinea-pig, db cyclic AMP enhanced release of the adrenergic transmitter (Wooten, Thoa, Kopin & Axelrod, 1973). Furthermore, theophylline, which inhibits the activity of phosphodiesterase and increases cellular levels of cyclic AMP (Butcher & Sutherland, 1962), did not inhibit but rather, enhanced the overflow of tritium evoked by K^+ from the rat heart. In addition, theophylline

antagonized the inhibitory effect of ATP, ADP and adenosine on the overflow of tritium evoked by K^+ from the heart. A similar antagonsim by theophylline of the inhibitory effect of nucleotides on adrenergic transmission has been reported in the canine blood vessels and in the isolated kidney of rabbit (Verhaeghe *et al.*, 1977; Hedqvist *et al.*, 1978). The latter investigators showed that the ability of theophylline to antagonize the inhibitory effect of adenosine on adrenergic transmission was also unrelated to phosphodiesterase inhibition, for the two potent inhibitors of phosphodiesterase, RO 20-1724 and ZK 62-711 failed to antagonize the action of adenosine on release of the adrenergic transmitter in the kidney of rabbit. Since (1) theophylline in the coronary vessels and cardiac tissue has been shown to produce its effect by acting on the surface of the cell membrane (Belleman & Scholz, 1974; Olsson, Davis, Khouri & Patterson, 1976) and (2) adenine nucleotides do not easily cross the normal cell membrane, it is possible that theophylline antagonizes the inhibitory effects of ATP, ADP and adenosine on release of the neurotransmitter by acting on the 'purine receptors' located on the adrenergic nerve terminal. The mechanism by which theophylline enhances release of the adrenergic transmitter and diminishes the inhibitory effect of ATP, ADP and adenosine on release of NA is presumably due to alterations in the transneuronal Ca^{2+} metabolism, because theophylline increases the entry of Ca^{2+} across the cell membrane and reduces its binding within the cell (Blinks, Olson, Jewell & Braveny, 1972).

Our findings that adenosine, ADP and ATP in concentrations that are likely to be reached at the adrenergic neuroeffector junction during increased sympathetic nerve activity (Su, 1975) inhibited the K^+-evoked release of [^{3}H]-NA together with the observations that: (1) adenosine and adenine are taken up by adrenergic nerves (Su, 1975; Fredholm & Hedqvist, 1978); (2) ATP and its breakdown products including adenosine is released with catecholamines during sympathetic nerve stimulation (Douglas, 1968; Su, 1975; Fredholm & Hedqvist, 1978) and that (3) the release of labelled nucleotides as well as NA from the rabbit aorta evoked by transmural stimulation was blocked by guanethidine (Su, 1975), supports the hypothesis that adenosine and adenine nucleotides may function as modulators of the adrenergic transmission (Malik & McGiff, 1974; Enero & Saidman, 1977; Su, 1978; Hedqvist *et al.*, 1978). Further, the interaction of these nucleotides with the adrenergic nervous system in the heart may have implications for the regulation of the coronary blood flow and cardiac function. Enhanced formation of nucleotides in conditions such as ischaemia and hypoxia, could influence the coronary circulation by producing coronary vasodilatation (Berne, 1963) and more importantly, modulating the activity of the adrenergic nervous system in the myocardium.

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