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IONIC BASIS OF THE HYPERPOLARIZING ACTION OF ADENYL COMPOUNDS ON SINUS VENOSUS OF THE TORTOISE HEART

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SUMMARY

1. The ionic mechanism underlying the hyperpolarizing action of adenosine and adenine nucleotides was studied by measuring the efflux of ^{43}K or ^{86}Rb from sinus venosus and interauricular septum of tortoise heart. Preparations rendered quiescent by high-K (27 mM) Ringer solution were used.

2. Adenosine and ATP increased the efflux of ^{43}K and ^{86}Rb from sinus venosus. The magnitude of the responses varied from preparation to preparation, but in the same tissue adenosine and ATP were of equal efficacy. When dose–response relationships could be determined, the adenyl compounds were found to be of similar potency. K_m for adenosine was $6{\cdot}2 \times 10^{-6}$ M, for ATP $8{\cdot}3 \times 10^{-6}$ M.

3. Regional variations in the magnitude of the responses were observed. The largest responses were obtained from the muscular strip of sinus venosus near its junction with atrium, and from the right horn of the sinus venosus. In interauricular septum the adenyl compounds caused only a slight increase in isotope efflux. Acetylcholine, by contrast, produced large increases in ^{86}Rb efflux from all these preparations. Thus the distribution of the purinoceptors in the tortoise heart is more confined than that of the muscarinic receptors.

4. Antagonism of the response to adenyl compounds by theophylline and 8-phenyltheophylline was studied. The apparent K_i for theophylline was 10^{-5} M; that for 8-phenyltheophylline about 10^{-6} M. Atropine did not inhibit the responses to the adenyl compounds. These results indicate that the changes in K permeability produced by adenosine and ATP are mediated by P_1-purinoceptors.

5. The adenosine transport inhibitors, dipyridamole and nitrobenzylthioinosine (NBMPR), had no effect on the adenyl-induced responses, indicating that adenosine uptake is of little importance in tortoise sinus venosus.

6. The effects of phosphate-modified ATP analogues were studied. Adenylimidodiphosphate (APPNP) produced increases in ^{86}Rb efflux similar to those found with ATP, confirming that breakdown of ATP to adenosine is not obligatory for its action at P_1-purinoceptors. α–β methylene ATP (APCPP) and β–γ methylene ATP (APPCP) produced much smaller effects, which may be explained by their structural and chemical differences from ATP.

7. The use of ^{86}Rb as a tracer (Rb: K < 0·01 in load solution) gives qualitatively similar results to those obtained when ^{43}K is used to study the permeability increases

produced by the adenyl compounds or acetylcholine. Quantitative differences in the measures obtained with the two isotopes, however, become apparent when the efflux of both is studied simultaneously.

8. Replacement of extracellular K by Rb (2·7–27 mM), or the addition of Ba ions (5–10 mM) to the wash solution, blocks the responses to both the adenyl compounds and acetylcholine. Although adenosine and acetylcholine act at different receptors, the K channels opened evidently are similar in some of their characteristics.

INTRODUCTION

The negative chronotropic action of adenosine and adenine nucleotides on cardiac pace-maker tissue was first described by Drury & Szent-Györgyi (1929) and continues to be a topic of study (Szentmiklosi, Nemeth, Szegi, Papp & Szerkeres, 1980; Belardinelli, West, Crampton & Berne, 1983). In frog heart sinus venosus, Hartzell (1979) has shown it to be associated with a hyperpolarization similar to that produced by acetylcholine (Hutter & Trautwein, 1956). The magnitude of the hyperpolarization produced by adenyl compounds was found to depend on the extracellular K concentration, as with acetylcholine (Burgen & Terroux, 1953), which led Hartzell (1979) to suggest that they also increase the K permeability of pace-maker tissue. In the present experiments this hypothesis is borne out by the effect of the adenyl compounds on the efflux of ^{43}K and ^{86}Rb from tortoise heart sinus venosus. Occasion was taken to compare the distribution of purinoceptors and muscarinic receptors, to determine the affinity of agonists and antagonists, and to characterize the K channels opened.

METHODS

Preparation. Experiments were done on the sinus venosus and interauricular septum of Mediterranean tortoises (*Testudo graeca*) at room temperature (19–28 °C) in both summer and winter. The tortoises were decapitated, pithed and the heart removed. The sinus venosus and the interauricular septum were dissected free. The sinus venosus was opened into a U-shaped sheet of tissue which could be divided into a right and left horn. In some experiments a narrow muscular strip of the sinus venosus, near its junction with the atrium, was dissected as a separate preparation. All preparations continued to beat spontaneously throughout the dissection. A short length of fine wire was tied to one end of the tissue to ease transfer during the experiment. The tissue wet weight was 10–30 mg.

Solutions. The preparations were dissected in normal Ringer solution, containing 118 mM-NaCl, 2·5 mM-KCl, 2·0 mM-$CaCl_2$ and 2·0 mM-Tris buffer, pH 7·0. The rest of the experiment was done in high-K solution so as to render the preparation quiescent and to minimize changes in membrane potential. The K-efflux response to acetylcholine is best seen in high-K solution (Hutter, 1957). To obviate tissue swelling KCl was added to Ringer to give a solution containing 27 mM-KCl, 118 mM-NaCl, 2·0 mM-$CaCl_2$ and 1·8 mM-$NaHCO_3$, pH 7·9. The effect of external Rb was studied by replacement of KCl in this solution with RbCl, either partially (0·27 mM, 2·7 mM) or completely (27 mM). $BaCl_2$ (5 or 10 mM) was added to the high-K solution, with reduction in NaCl, to study the effect of external Ba ions.

Drugs. Fresh solutions of the drugs used were prepared before each experiment. Nitrobenzylthioinosine (NBMPR) was a gift from Professor A. R. P. Paterson, University of Alberta. 8-Phenyltheophylline was supplied by Calbiochem; all other drugs from Sigma Chemical Company.

Isotopes. ^{43}K (half-life 22·3 h) was obtained from the MRC Cyclotron Unit, Hammersmith Hospital, as 'carrier-free' isotope in normal saline solution. ^{86}Rb was supplied by Amersham International Ltd. in aqueous solution of RbCl, with specific activity of 1·68–8·77 mCi/mg

(62·2–324·5 MBq/mg). The concentration of Rb in the load solutions ranged from 0·045 to 0·217 mM. In view of its conveniently long half-life (18·7 days) ^{86}Rb was used in the majority of the experiments. In a few experiments the tissues were loaded with both ^{43}K and ^{86}Rb to compare the efflux of these isotopes.

Procedure. After dissection the preparations were immersed for 1–3 h in the load solution containing the isotope. This loading period was followed by a 5 min wash period to remove extracellular isotope. The tissues were then transferred to vials containing 2 ml high-K solution at consecutive 5 or 10 min intervals. Drugs, dissolved in 20 μl, were added to the appropriate vial immediately before the tissue was transferred into it, with intervals of 20–30 min between drug additions. At the end of the efflux period, the tissue was weighed and then immersed in 2 ml water in a final vial. Scintillation fluid (8 ml Scintol-2, Triton X-100 and toluene mixture) was added to each vial, and the radioactivity counted with a liquid scintillation spectrometer. By summation of the counts the total uptake of isotope by the tissue was estimated, and the rate coefficient, i.e. the fraction of the isotope content of the tissue which escapes per minute, was calculated for each sample during the efflux period.

In the experiments loaded with both ^{43}K and ^{86}Rb the samples were recounted 10 days later, when the activity of ^{43}K had decayed to background levels. Subtraction of the ^{86}Rb counts, corrected for decay, from the original combined activity allowed calculation of the individual efflux rates of the two isotopes.

Errors related to the magnitude of the sample counts were mainly less than 2%, and never more than 4%.

Analysis. Responses were expressed quantitatively by the increment in rate coefficient as a multiple of the rate coefficient during the preceding 10 min efflux period, i.e. taking the rate coefficient in the presence of the drug as D, and the preceding base-line value as B, then

$$\text{Response} = \frac{D-B}{B}.$$

Statistical analysis of drug responses was done using Wilcoxon's rank sum test; dose–response curves were compared by applying a t test to regression analysis. Curve fitting was by the method of non-linear least squares.

RESULTS

Adenosine and adenine nucleotides

The extent to which adenosine and adenine nucleotides increased ^{43}K and ^{86}Rb efflux was characterized by a high degree of variability from preparation to preparation. Since the magnitude of the responses could not be anticipated, nor readily monitored during an experiment, it was not always possible to resolve all quantitative questions posed. In the first instance we present results from preparations that showed relatively large effects, so that dose–response relationships and variations in regional sensitivity within a given preparation could be investigated.

Dose–response relationships for adenosine and ATP. An experiment in which adenosine was added to the wash solution for interspaced 5 min periods is illustrated in Fig. 1*A*. For concentrations of adenosine from 2×10^{-6} to 2×10^{-4} M a graded increase in ^{86}Rb efflux from the sinus venosus was observed. The increase in the rate coefficient in response to adenosine was expressed as a multiple of the rate coefficient during the preceding 10 min efflux period. The maximal response in this experiment was an increment in ^{86}Rb efflux of 3·7 times the resting rate. ATP also produced an increase in ^{86}Rb efflux from sinus venosus when added to the wash solution in varying concentration, at 30 min intervals (Fig. 1*B*). In this example the maximum response was an increment in ^{86}Rb efflux of only 2·0 times the resting value. This reflects the variation in the magnitude of the responses in different preparations, rather than a

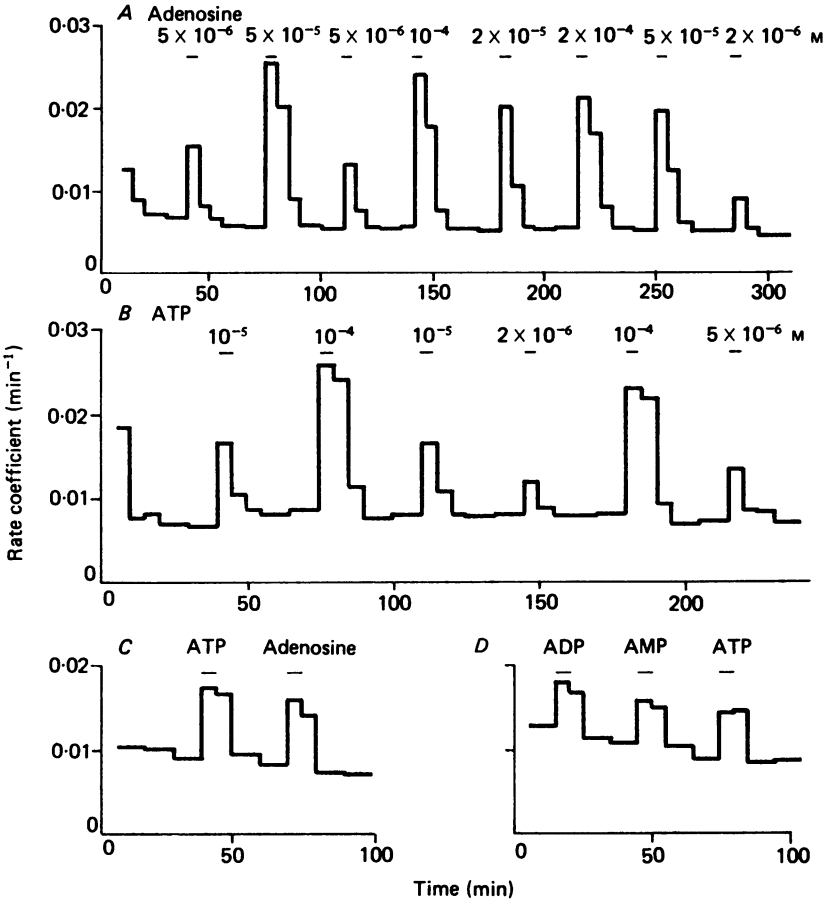

Fig. 1. Effects of adenosine and adenine nucleotides on ^{86}Rb and ^{43}K efflux from sinus venosus of tortoise heart. Ordinate, efflux rate coefficient (min^{-1}). Abscissa, time (min) from start of wash period. *A*, increases in rate coefficient of ^{86}Rb efflux produced by addition of adenosine (2×10^{-6}–2×10^{-4} M) to the wash solution for periods indicated. *B*, increases in ^{86}Rb efflux by ATP (2×10^{-6}–10^{-4} M). *C*, comparison of effects of adenosine (10^{-4} M) and ATP (10^{-4} M) on ^{86}Rb efflux. *D*, increases in ^{43}K efflux produced by AMP, ADP and ATP (10^{-4} M).

difference in efficacy between ATP and adenosine. When both adenosine and ATP, at 10^{-4} M, were applied to the same tissue as part of the experimental design (Fig. 1*C*) there was no significant difference between the paired responses ($P > 0{\cdot}05$; $n = 38$). Similarly the increases in efflux produced by AMP, ADP and ATP (10^{-4} M) were of comparable magnitude (Fig. 1*D*).

Dose–response relationships for the adenyl compounds could be estimated from individual experiments, such as those in Figs. 1*A* and *B*; but the small number of drug additions possible in any one experiment limited their accuracy. Responses to adenosine and ATP, from eight different experiments with each, were therefore expressed as a percentage of the maximal response produced by them in that tissue, and plotted against the concentration of adenosine or ATP (Fig. 2). On the

assumption that we are dealing with a first-order reaction between agonist and receptor, the results were fitted to the relation

$$q = (1 + \exp 2{\cdot}3\,(\log K_m - \log [\mathrm{P}]))^{-1},$$

where q is the fraction of receptors occupied, i.e. response/maximal response, [P] is the molar adenyl concentration, and K_m is the concentration for half-maximal response. The resultant best-fit value of K_m for adenosine was $6{\cdot}2 \times 10^{-6}$ M (S.E. $+0{\cdot}8 \times 10^{-6}$ M, $-0{\cdot}7 \times 10^{-6}$ M); for ATP it was $8{\cdot}3 \times 10^{-6}$ M (S.E. $+1{\cdot}1 \times 10^{-6}$ M, $-1{\cdot}0 \times 10^{-6}$ M). Regression analysis applied to the data for the linear portion of the curves indicated that this difference is not significant ($0{\cdot}1 > P > 0{\cdot}05$).

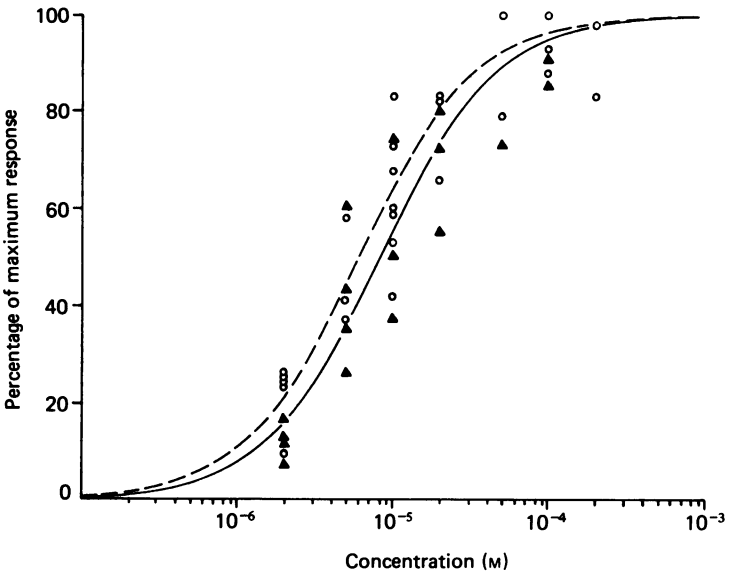

Fig. 2. Dose–response curves for increase by adenosine and ATP of ^{86}Rb or ^{43}K permeability. Normalized results from eight sinus venosus preparations with each adenine compound are plotted. ○, adenosine; ▲, ATP. First-order reaction curves were fitted by method of non-linear least squares. Interrupted curve, adenosine; continuous curve, ATP.

Regional variation in responses. The magnitude of the responses produced by the adenyl compounds varied not only from animal to animal, but with the different regions of the same heart. Such regional variations were best seen in hearts that were highly responsive to adenosine and ATP. In the subject of Fig. 3 for instance, adenosine 10^{-4} M increased the efflux of ^{86}Rb from the muscular strip preparation by 6·2 times, and by 4·4 times from the right horn of the sinus venosus; on the other hand, with the left horn the response was only 1·3 times, and with the interauricular septum the ^{86}Rb efflux was scarcely increased. The results of all the experiments done are summarized in Fig. 4 to bring out their variability. It will be recognized that the responses in Fig. 3 were exceptionally large. In experiments in which the addition of adenyl compounds gave only small increases in isotope efflux, the differences in regional sensitivity were less striking, but they remain discernible when the population

of results is surveyed. Thus the majority of the strip and right horn of sinus venosus preparations gave efflux increments of greater than 0·25, whilst most left horn and interauricular septum preparations gave smaller responses (Fig. 4). In some of these experiments the effect of a near-maximal dose of acetylcholine (10^{-6} M) was also tested. By contrast to the adenyl compounds, a clear-cut increment in isotope efflux was observed in all the preparations studied. The experiments illustrated in Fig. 3 bring out how differently the purinoceptors and muscarinic receptors may be distributed. In that heart the strip preparation gave a larger response to adenosine, whilst all other parts (and especially the interauricular septum) gave larger responses to acetylcholine.

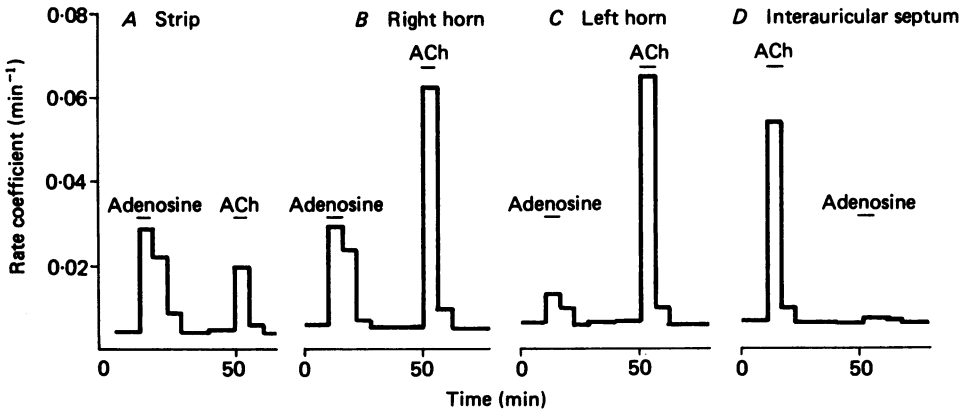

Fig. 3. Regional variation in ^{86}Rb efflux responses produced by adenosine (10^{-4} M) and ACh (10^{-6} M) when applied to four regions of the same heart. *A*, muscular strip of sinus venosus, near junction with atrium. *B*, right horn of sinus venosus. *C*, left horn of sinus venosus. *D*, interauricular septum.

Antagonism of adenyl-induced response

Addition of theophylline (5×10^{-4} M) to the wash solution reduced the ^{86}Rb efflux response to adenosine, with no effect on the resting efflux (Fig. 5*A*). Results from experiments with theophylline at 10^{-4} M and 5×10^{-4} M, plotted on a double reciprocal (Lineweaver–Burke) plot, are shown in Fig. 5*B*. The lines are drawn to cross the *Y*-axis at 0·01, corresponding to the normalized maximum response of 100 %, consistent with competitive inhibition. The apparent inhibition constant, K_i, for theophylline can be calculated from the *X*-intercepts of the lines, and also for individual experiments, from:

$$K_i = \frac{K_m[T]}{\frac{[P]}{q} - [P] - K_m},$$

where K_m is the apparent dissociation constant for adenosine and [T] the molar concentration of theophylline. The estimated value of K_i for theophylline from these experiments was $1{\cdot}0 \times 10^{-5}$ M (S.D. $\pm 4{\cdot}7 \times 10^{-6}$ M, $n = 8$). A few experiments were also done with 8-phenyltheophylline, which reduced the responses to adenosine

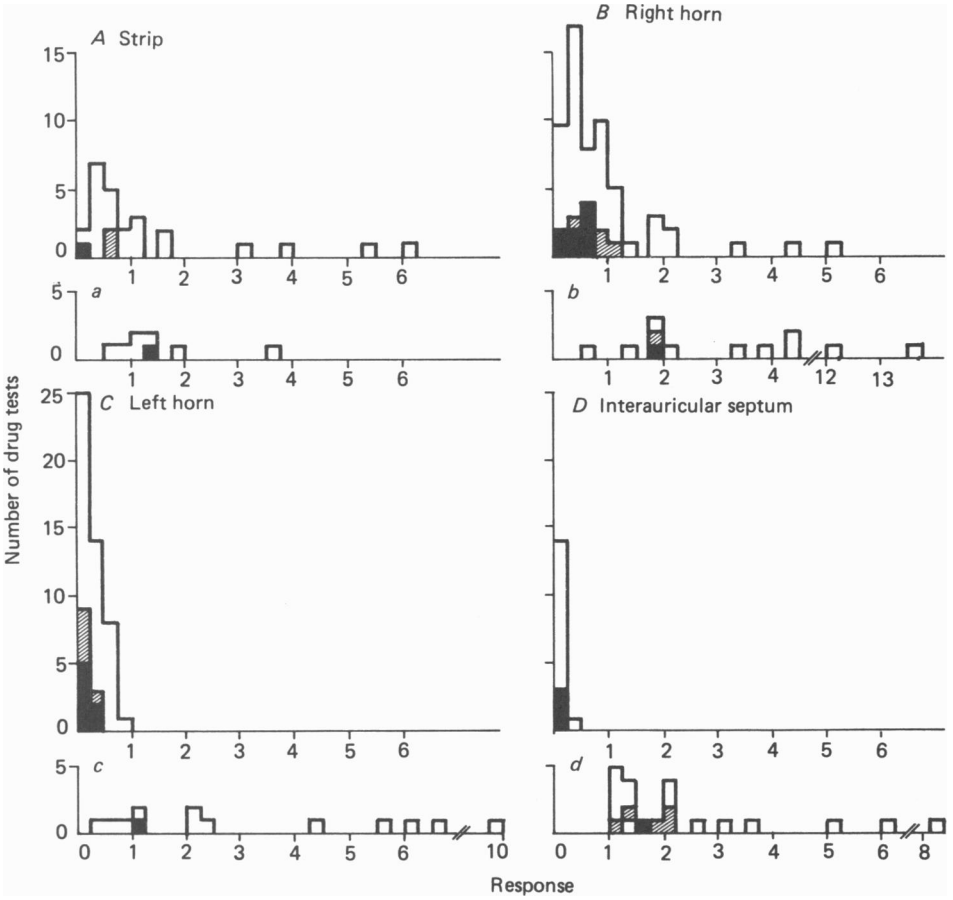

Fig. 4. Distribution of magnitude of responses produced by 10^{-4} M-adenosine or ATP (*A*, *B*, *C* and *D*) or 10^{-6} M-acetylcholine (*a*, *b*, *c* and *d*) on sinus venosus strip preparation (*A* and *a*), right horn of sinus venosus (*B* and *b*), left horn of sinus venosus (*C* and *c*) and interauricular septum (*D* and *d*). Ordinate, number of tests with adenyl compounds or acetylcholine producing similar responses. Abscissa, magnitude of responses grouped in intervals of 0·25 times resting efflux. Filled blocks, ^{42}K efflux experiments; hatched blocks, ^{42}K efflux from experiments loaded with both ^{42}K and ^{86}Rb; all others, ^{86}Rb efflux experiments.

and ATP, with an estimated K_i of about 10^{-6} M. These results compare with similar K_i values for theophylline ($1{\cdot}2 \times 10^{-5}$ M) and 8-phenyltheophylline (6×10^{-7} M) obtained from tension experiments with guinea-pig atria (Griffith, Meghji, Moody & Burnstock, 1981). Atropine, which antagonized the isotope efflux increase produced by acetylcholine, had no effect on the adenyl-induced responses.

Adenosine transport inhibitors

Dipyridamole, an inhibitor of adenosine uptake, when added to the wash solution in concentrations of 10^{-5}–10^{-4} M, had no significant effect on isotope efflux responses to adenosine or ATP ($P > 0{\cdot}05$; $n = 23$). NBMPR in a concentration of 5×10^{-5} M

also left adenyl-induced responses unaffected. These results are in contrast to the potentiation by dipyridamole of the action of adenosine on frog heart sinus venosus (Hartzell, 1979). This probably signifies adenosine uptake as of lesser importance in tortoise heart sinus venosus. At all events, the small responses to adenosine sometimes obtained cannot be ascribed to its avid uptake. Rather, a paucity of purinoceptors in some preparations is indicated.

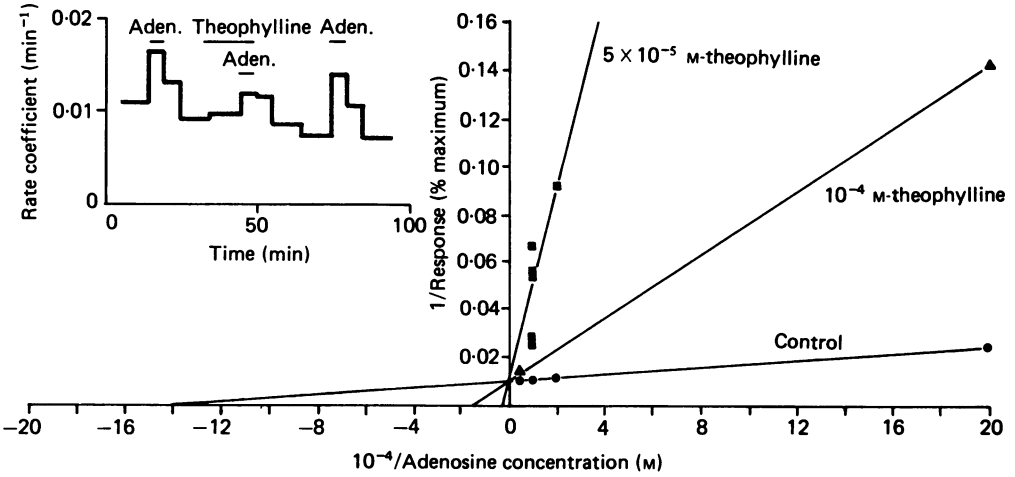

Fig. 5. Antagonism by theophylline of the effect of adenosine on ^{86}Rb and ^{43}K efflux from sinus venosus. *A*, inset, ^{86}Rb efflux responses to adenosine (10^{-4} M) in presence and absence of theophylline. Theophylline (5×10^{-4} M) present in wash solution for 10 min prior to, and during the period of adenosine addition. *B*, results from two experiments with 10^{-4} M-theophylline, and six experiments with 5×10^{-4} M-theophylline plotted on double reciprocal (Lineweaver–Burke) plot. ●, control; ▲, 10^{-4} M-theophylline; ■, 5×10^{-4} M-theophylline. Lines fitted by method of least squares.

Phosphate-modified ATP analogues

Adenyl imidodiphosphate (APPNP) is an ATP analogue in which a nitrogen replaces the oxygen linking the β and γ phosphorus atoms in the triphosphate chain, making it resistant to degradation by ATPases (Yount, Babcock, Ballantyne & Ojala, 1971). When added to the wash solution, APPNP (10^{-4} M) was found to produce an increase in ^{86}Rb efflux similar to that produced by ATP (10^{-4} M) (Fig. 6*A*). Substitution of a methylene group for the oxygen linking either the α and β, or β and γ phosphorus atoms in the triphosphate chain gives α–β methylene ATP (APCPP) and β–γ methylene ATP (APPCP), which are also resistant to breakdown (Yount, 1975). These analogues produced much smaller increases in ^{86}Rb efflux than did adenosine or ATP (Fig. 6*B*). The mean response to nine applications of 10^{-4} M-APCPP was 30% of the responses produced by 10^{-4} M-ATP in the same tissues. In some experiments the response to APCPP was reduced by atropine (10^{-5} M), to a mean of 15% of the ATP response (Fig. 6*C*). This is consistent with evidence that APCPP can lead to the release of acetylcholine from cholinergic nerve endings (Moody & Burnstock, 1982). APPCP also produced small increases in ^{86}Rb

efflux, the mean response to five applications at 10^{-4} M being 30 % of the ATP (10^{-4} M) responses. Atropine did not affect these responses, but they were subject to inhibition by 8-phenyltheophylline (Fig. 6*D*).

^{86}Rb as a tracer for K fluxes

Acetylcholine will produce a sizeable increase in the rate of efflux of ^{86}Rb from sinus venosus (Hutter, 1961), and the present experiments show that adenyl compounds will do likewise. ^{86}Rb had the advantages of economy and convenience, but since Rb

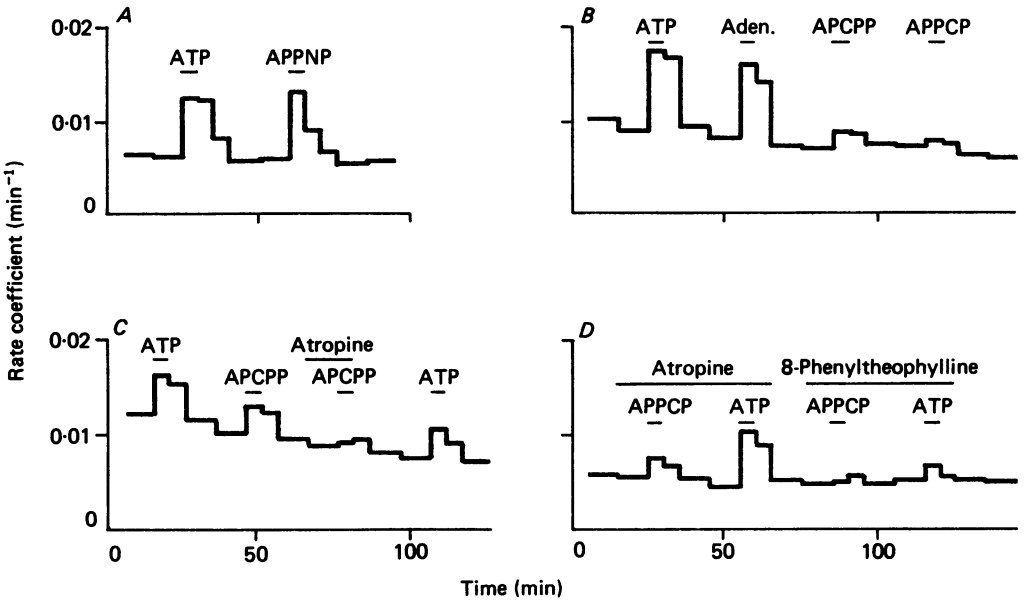

Fig. 6. Effects of phosphate-modified ATP analogues on ^{86}Rb efflux from sinus venosus. *A*, similarity of responses to adenyl imidodiphosphate (APPNP), and ATP (10^{-4} M). *B*, reduced responses produced by α–β methylene ATP (APCPP) and β–γ methylene ATP (APPCP) compared to adenosine (Aden.) and ATP (10^{-4} M). *C*, reduction in effect of APCPP (10^{-4} M) by atropine (10^{-5} M). *D*, antagonism of responses to ATP and APPCP (10^{-4} M) by 8-phenyltheophylline (10^{-5} M) but not by atropine (10^{-5} M). 10^{-4} M-APPCP produced the same effect in absence of atropine 1 h before illustrated response in presence of atropine.

may be handled differently from K in cardiac tissue (Müller, 1965) the use of ^{86}Rb as a tracer for K required evaluation. In so far as the responses produced by adenosine and ATP in experiments with ^{43}K were within the range of those found with ^{86}Rb (Fig. 4) no great differences between these isotopes seemed indicated. In view of the variability of these responses from animal to animal and tissue to tissue, however, a more quantitative assessment of ^{86}Rb was advisable. Experiments were therefore done with a load solution containing both ^{43}K and ^{86}Rb (Fig. 7). Such experiments on sinus venosus showed that (i) the efflux of ^{86}Rb in the resting preparation in fact proceeds at a lower rate than does the resting efflux of ^{43}K, and (ii) the relative increments in efflux produced by both acetylcholine and adenosine were somewhat

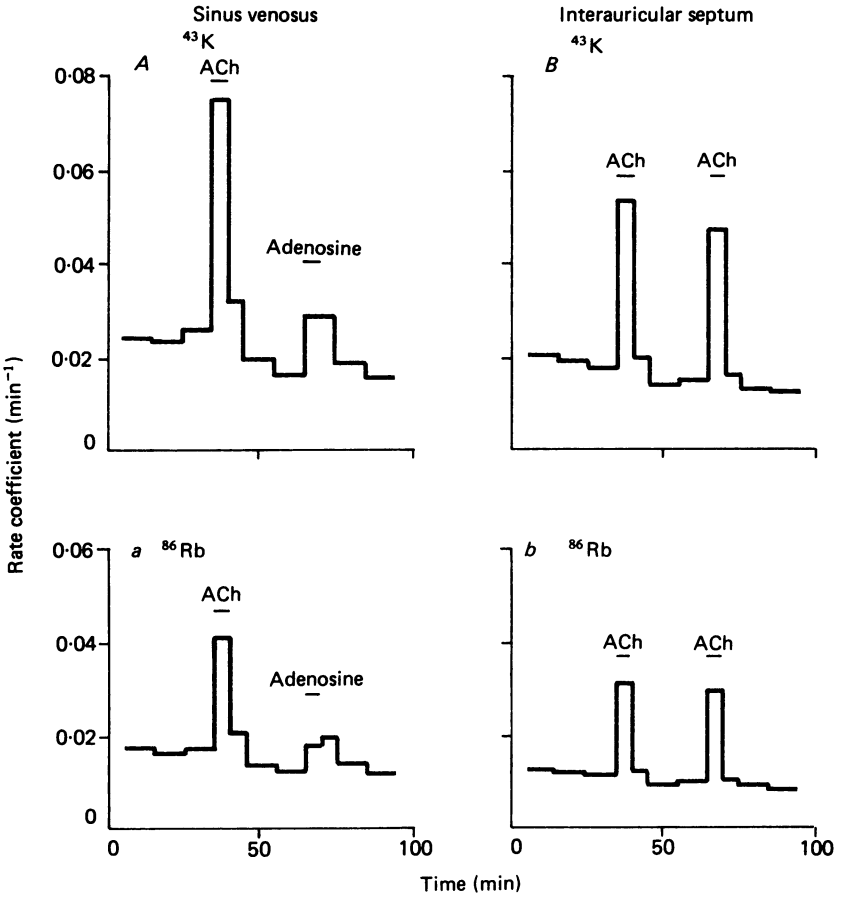

Fig. 7. Efflux of isotopes from tissues loaded with both ^{86}Rb and ^{43}K. Comparison of ^{43}K efflux (*A* and *B*) and ^{86}Rb efflux (*a* and *b*) from right horn of sinus venosus (*A* and *a*), and interauricular septum (*B* and *b*). 10^{-6} M-ACh and 10^{-4} M-adenosine added to wash solution for periods indicated. The load solutions contained 27 mM-K and 0·06 mM-Rb (*A* and *a*) or 0·17 mM-Rb (*B* and *b*).

greater for ^{43}K than ^{86}Rb. With the interauricular septum preparations, by contrast, the relative increment in efflux produced by acetylcholine was the same for ^{43}K and ^{86}Rb. This pattern of differences was obtained with double-loaded preparations from each of three hearts. The implication is that the chemosensitive channels in the sinus venosus discriminate against Rb more severely than do the background channels, whilst in atrial fibres both background and chemosensitive channels discriminate similarly between the two cations. On the present evidence we cannot say whether the difference lies between the background or between the chemosensitive channels in the two tissues.

Effects of rubidium and barium

We have also examined the effect of extracellular Rb on the background efflux of ^{43}K or ^{86}Rb and on the increase in isotope efflux produced by acetylcholine and

adenosine. At a low concentration (0·27 mM) Rb had little effect, but when the external K is partially or totally replaced by Rb, to give Rb concentrations of 2·7 and 27 mM, there was a clear inhibitory effect on the isotope efflux, especially the drug-induced increase (Fig. 8). A plausible interpretation of these findings is that Rb ions, when present in more than trace concentration, interfere with the efflux of K ions, as happens in skeletal muscle (Sjodin, 1959). In the absence of information on

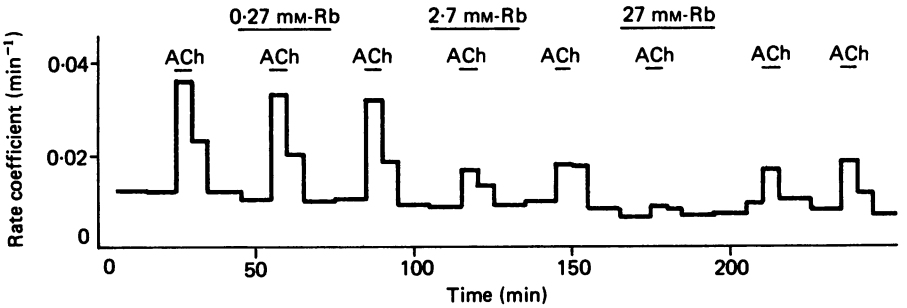

Fig. 8. Effect of extracellular Rb on ^{43}K efflux from interauricular septum. External K was substituted by Rb in progressively greater concentrations (0·27, 2·7 and 27 mM) for periods indicated. 10^{-5} M-ACh added to wash solution at intervals.

the behaviour in the membrane potential, however, it remains uncertain whether the greater effect of Rb on the efflux of ^{43}K in the presence of ACh signifies a greater sensitivity of the chemosensitive channels to Rb as compared to the channels mediating the background K flux. In this connexion it may be noted that in frog atrium under voltage-clamp conditions Cs is known to block background and carbachol-induced K currents to an equal extent (Argibay, Ildefonse, Ojeda, Rougier & Tourneur, 1981). Similar experiments with Rb are necessary to resolve the questions raised by the present results.

By way of further characterization of the chemosensitive channels opened by the adenyl compounds, the effect of the addition of Ba ions to the wash solution was also studied. At 5–10 mM, Ba totally blocked the increase in ^{86}Rb efflux produced by adenosine; the same was found with the response to acetylcholine.

DISCUSSION

Adenyl-induced increase in K permeability and membrane potential

Hartzell (1979) has studied the mode of action of adenosine and adenine nucleotides on cardiac pace-maker tissue by electrophysiological means. In the present work the problem is approached with a complementary method. However, only a guarded comparison of results is possible. A tissue such as the sinus venosus cannot be regarded as perfectly uniform. With a micro-electrode a highly responsive chemosensitive area can be studied. Isotope flux measurements, by comparison, have a lesser spatial definition, though they give a more direct measure of permeability. These inherent differences between the two methods complicate quantitative comparison; and the variable magnitude of the responses to the adenyl compounds adds to the

difficulty. In an attempt, nevertheless, to relate the available electrophysiological results to the present flux measurements the Goldman equation (Hodgkin & Katz, 1949) was used in the rudimentary form

$$E_m = 58 \text{ mV} \log \frac{[K]_o + A}{[K]_i},$$

where A is the term which causes the membrane potential, E_m, to depart from E_K owing to the finite concentration of permeable cations, other than K, in the extracellular solution. Taking E_m as -58 mV at a $[K]_o$ of 2 mM, and E_K as -93 mV (Hartzell, 1979) yields A equal to 6. For the membrane potential to undergo hyperpolarization to -84 mV (Fig. 3: Hartzell, 1979) requires A to fall to 0·85. This would happen if the membrane permeability to K ions increased by 6/0·85, i.e. about 7-fold. An increase in efflux of such magnitude was in fact observed in a few of the present experiments, but in the majority the increase in efflux was much smaller. To some extent this may have been due to the use of ^{86}Rb in place of K (see Fig. 7), but the most probable reason is the admixture of tissue poorly endowed with purinoceptors. It could also be that the hyperpolarizing effect of an increase in K permeability less than that calculated on the above basis is magnified by inward rectification of the resting and chemosensitive components of the K conductance (Hutter & Noble, 1960; Noble, 1965; Garnier, Nargeot, Ojeda & Rougier, 1978; Noma & Trautwein, 1978).

As the present experiments were done in a K-rich solution, little hyperpolarization will have attended the increase in K permeability produced by the adenyl compounds (see Burgen & Terroux, 1953). The flux increment may therefore be considered as closely similar to the permeability increment. In trying to relate the observed hyperpolarization to the observed flux increment along the above lines, it was assumed that the adenyl compounds act only by increasing the permeability to K ions. In principle, the hyperpolarization could be due in part also to a decrease in inward current. If so, the above estimate of the required K-permeability increase would be misleading. It should be recalled, however, that Hartzell (1979) used Mn^{2+}-treated preparations, so that in his experiments the slow inward current was suppressed throughout. If the non-selective inward current activated by hyperpolarization, i_f, were suppressed by adenyl compounds, hyperpolarization due to an increase in K permeability would proceed against less opposition and the above estimate of the relation between membrane potential and K permeability change would remain valid.

Considered within their own confines, the present isotope-flux results allowed the relation between dose of adenyl compounds and membrane response to be determined more directly than hitherto. The variations in peak response from preparation to preparation were regarded as due to differences in the density of chemosensitive channels and the results pooled on that basis. For ATP the present results give a K_m value of $8{\cdot}3 \times 10^{-6}$ M which compares with a K_m value of $4{\cdot}6 \times 10^{-6}$ M obtained by Hartzell (1979) after correction for the non-linear summation of the hyperpolarizing effect of ATP. The corroboration is useful, because the two methods are prone to error at opposite ends of the dose–response curve: with the isotope-flux method the error is greatest for small increments of permeability, whereas hyperpolarization is a least-linear measure when the permeability nears saturation.

Purinoceptor characterization

A feature of the present results was the similar potency of adenosine and ATP in their action on K permeability. In the context of the purinoceptor theory (Burnstock,

1978) such equipotency, taken together with the competitive antagonistic action of theophylline and 8-phenyltheophylline, points to the involvement of P_1-purinoceptors. But it is necessary to suppose either that ATP is rapidly degraded to adenosine, or that ATP can also act on P_1-receptors. Regarding the first possibility, Collis & Pettinger (1982) have provided evidence that breakdown of ATP is not obligatory for its negative inotropic action on guinea-pig atrium. The ineffectiveness of the stable APCPP, which has been interpreted as supporting the opposite view (Burnstock & Meghji, 1981), may be attributed to the chemical and structural differences between the methylene analogues and ATP (Yount, 1975). More compelling is the positive finding that the stable imido analogue, APPNP, is as potent as ATP: it argues in favour of a direct action of ATP on P_1-purinoceptors.

Possible physiological and pathological role of adenyl compounds

Sensitivity of pace-maker and nodal tissue to adenyl compounds is found in many species, although in some, in particular the guinea-pig, the atrioventricular node is more sensitive than is the sinus node (Green & Stoner, 1950). In man, both transient sinus bradycardia and heart block may result from intravenous administration of adenosine or ATP (Honey, Ritchie & Thomson, 1930; Leclerq & Coumel, 1978). It remains uncertain, however, whether adenyl compounds play a physiological role in the control of cardiac tissue. It is known that ATP is released together with noradrenaline from sympathetic nerves (see Burnstock, 1983). Inasmuch as noradrenaline also increases K permeability (Boyden, Cranefield & Gadsby, 1983) ATP would reinforce this effect which may be designed to preserve the availability of inward current systems (Gadsby, 1983). In this connexion, it is intriguing to speculate whether the distribution of purinoceptors, which clearly does not follow the pattern for muscarinic receptors (see Fig. 3), might be more in keeping with the distribution of adrenoceptors.

A plausible case can be made for a pathophysiological role for the negative chronotropic action of the adenyl compounds. Sinus bradycardia is a common feature of the early stage of acute myocardial infarction, and while it is often responsive to atropine, this is not always so (Adgey, Geddes, Mulholland, Keegan & Pantridge, 1968; Dauchot & Gravenstein, 1976). Experimental ischaemia of the sinus node in dogs similarly produces a bradycardia which is unresponsive to atropine (Billette, Elharrar, Porlier & Nadeau, (1973). Since myocardial hypoxia causes an increase in the release of adenosine (Berne, 1963) such bradycardia could be caused by local accumulation of adenyl compounds. The use of ATP in the treatment of paroxysmal supraventricular tachycardia (Greco, Musto, Arienzo, Alborino, Garofalo & Masico, 1982) presumably also owes to an increase in K permeability.

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