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RELEASE OF ADENOSINE, INOSINE AND HYPOXANTHINE FROM RABBIT NON-MYELINATED NERVE FIBRES AT REST AND DURING ACTIVITY

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SUMMARY

1. The composition of the efflux from desheathed rabbit vagus nerve, loaded with radioactivity by incubation in [^{3}H]adenosine, was studied at rest and during electrical activity and after application of inhibitors of ecto-enzymes and modifications of intermediary metabolism. In addition, the degradation of externally applied ATP and adenosine was examined.

2. [^{3}H]ATP applied to the incubation medium was degraded to ADP, AMP, adenosine and inosine. The hydrolysis to nucleosides was inhibited by α, β-methylene ADP; the appearance of AMP and nucleosides was slowed by β, γ-methylene ATP. Deamination of [^{3}H]adenosine was blocked by 2-deoxycoformycin.

3. The effluent from resting and stimulated preparations showed the presence of large amounts of inosine and hypoxanthine, smaller amounts of adenosine and adenine and traces of nucleotides. The composition of the effluent was not significantly altered by addition of α, β-methylene ADP; β, γ-methylene ATP or 2-deoxycoformycin.

4. Application of glucose-free solutions caused a large release of adenosine instead of inosine and hypoxanthine and a small increase in resting and stimulated efflux of ^{3}H. Addition of 2-deoxyglucose produced a large increase in resting efflux and increased liberation of adenosine. Cyanide, 2, 4-dinitrophenol, arsenate or salicylate increased the resting efflux of adenosine, inosine and hypoxanthine, and the effect of activity.

5. It is concluded that electrical activity leads to release of adenosine, inosine and hypoxanthine, in various proportions depending on metabolic state, and that there is practically no liberation of nucleotides from nerve axons.

INTRODUCTION

We have shown that mammalian non-myelinated nerve fibres, loaded with radioactivity by incubation in [^{3}H]adenosine release inosine, hypoxanthine and adenosine and that upon electrical stimulation the release of these compounds is increased (Maire, Medilanski & Straub, 1982). Of the collected compounds, inosine and hypoxanthine were by far the most important, adenosine accounting for only

a small proportion of the released radioactivity. On the other hand, Pull & McIlwain (1972) using guinea-pig neocortal tissue, found a large release of adenosine with comparatively small amounts of inosine and hypoxanthine in resting as well as in stimulated preparations. A large release of adenosine was also found in experiments on cat sensorimotor cortex *in vivo* (Sulakhe & Phillis, 1975), and in rat or guinea-pig cerebral cortical synaptosomes (Bender, Wu & Phillis, 1981; Daval & Barberis, 1981).

In view of a possible role of purines as neuromodulators or neurotransmitters (see Schubert, Reddington & Kreutzberg, 1979; Fredholm & Hedqvist, 1980; Stone, 1981, 1982; Burnstock, 1981; Phillis & Wu, 1981, Erulkar, 1983; Silinsky, 1984), it seemed interesting to study release in peripheral nerve in more detail, since compounds liberated from axons may well contribute to the effects of purines in the central nervous system. In this paper the effects of inhibitors of extracellular degradation of nucleotides and nucleosides are studied in order to see to what extent the composition of the efflux might be influenced by the activity of ecto-enzymes. In addition, the effects of withdrawal of glucose and of inhibitors of intermediary metabolism on the release of purines are examined since modifications in metabolism may well be reflected in the amount and nature of the liberated compounds.

METHODS

The experiments were performed *in vitro* on desheathed rabbit vagus nerves, a preparation rich in non-myelinated nerve fibres (Keynes & Ritchie, 1965). The methods have been described in detail (Maire *et al.* 1982). Briefly, after dissection, the preparations were loaded with radioactivity by incubation in Locke solution with [^{3}H]adenosine (0·1 μM) for 2 h at 37 °C and then mounted in an apparatus where they were continuously superfused with non-radioactive Locke solution (0·29 ml/min) and where they were stimulated and the action potentials recorded.

The effluent of the part of the preparation that was in contact with the stimulating electrodes was discarded, so that effects of activity could be attributed entirely to propagated action potentials. Samples of the effluent were collected during periods of 5 min and counted after addition of Aquassure (New England Nuclear) in a liquid scintillation counter (Betamatic II, Kontron). At the end of the experiments the preparation was removed, homogenized, and the radioactivity measured. Calculation of the rate constant of efflux was based on the radioactivity found in the water-soluble fraction of the homogenate (see Ferrero, Jirounek, Rouiller & Straub, 1978). The effluent was analysed by thin-layer chromatography on silica gel plastic sheets, as described by Shimizu, Creveling & Daly (1970). For the analysis of nerve extracts, thin-layer chromatography on PEI cellulose sheets was used. The procedure described by Böhme & Schultz (1974) was followed with a slight modification: the sheets were developed by two successive runs with 0·05 M-acetic acid and with distilled water over the entire length and a third run with 4 M-sodium formate (pH 3·4) up to 10 cm above the origin. Separate tests, using internal standards, showed that counts were proportional to radioactivity.

All experiments were performed at 37 °C. Whenever possible, means and S.E. of means are given.

Solutions. The Locke solution had the following composition (mM): NaCl, 154; KCl, 5·6; $CaCl_2$, 0·9; $MgCl_2$, 0·5; Na orthophosphate, 0·2; D-glucose, 5; Tris (hydroxymethyl)aminomethane (Tris), 10. Glucose-free solution was prepared by omission of D-glucose. The pH was 7·4 in all cases. The following compounds were used: dipyridamole (Boehringer, Ingelheim), adenosine (Boehringer, Mannheim), Na cyanide (Fluka), 2-deoxycoformycin (Laboratoires Substancia, Courbevoie), 2,4-dinitrophenol, Na arsenate and Na salicylate (Merck), tubercidin (Serva), 2-deoxy-D-glucose, α,β-methylene ADP and β,γ-methylene ATP (Sigma). [2-^{3}H]adenosine and [2,8-^{3}H]ATP were obtained from New England Nuclear, their specific activities were 12–19 and 28·5 Ci/mmol respectively.

RESULTS

Extracellular hydrolysis of ATP and effects of inhibitors

It has been found that a number of enzymes are localized at the external surface of membranes (see Trams & Lauter, 1974). These ecto-enzymes may metabolize compounds released from nerve (Trams, 1974; Trams, Kaufmann & Burnstock, 1980), and as suggested by Pull & McIlwain (1972, 1973) liberated ATP may be the source of adenosine collected at the tissue surface. Previous experiments on vagus nerve demonstrated the hydrolysis of externally applied ATP (Maire *et al.* 1982), so that collected nucleosides could result from liberated ATP.

In order to study this further, ATP (1–200 μM) together with trace amounts of [^{3}H]ATP were added to solutions incubating a vagus nerve and aliquots examined for the presence of other nucleotides and nucleosides. Fig. 1*A* shows that with 1 μM-ATP labelled ADP and AMP appear, as well as different nucleosides. The same breakdown of ATP was found when the incubating solution contained, in addition, dipyridamole (2 μM), which at this concentration almost completely inhibits the uptake of nucleosides into vagus nerve fibres (Maire *et al.* 1982). Experiments with different ATP concentrations showed that the maximal rate of hydrolysis amounts to 429 nmol/g . min, the half-maximal rate was found with 444 μM-ATP (mean of two determinations). These values can be compared to the amount of released compounds. During a standard period of activity (10 Hz for 3 min), with a fractional release of $2{\cdot}3 \times 10^{-6}$/impulse (Maire *et al.* 1982) and a total nucleotide content of 2·2 μmol/g wet nerve (Chmouliovsky, Schorderet & Straub, 1969), the liberated compounds amount to 9·1 nmol/g wet nerve. If this amount were released in the form of ATP, the activity of the ecto-enzymes could have been sufficient for hydrolysis during the collection of the samples. It therefore appeared of interest to study the effect of inhibitors of extracellular ATP breakdown.

Fig. 1*B* shows experiments in which α,β-methylene ADP (200 μM), an inhibitor of 5′-nucleotidase (see Yount, 1975) was added to [^{3}H]ATP (1 μM). In these conditions a breakdown of ATP into ADP and AMP was found but only small amounts of nucleosides, showing that hydrolysis was blocked at the level of AMP. Essentially the same results were found with 10 and 100 μM-ATP. In contrast, when β,γ-methylene ATP, another inhibitor, was added to ATP, hydrolysis went beyond AMP and there was a substantial appearance of nucleosides. The incomplete effect of β,γ-methylene ATP could be due to its destruction by ATP pyrophosphohydrolase, an enzyme that has been found in liver plasma membrane (Flodgaard & Torp-Pedersen, 1978) and that appears to be present also at the outside of axon membrane (see Phillis & Wu, 1981).

Deamination of adenosine

Other experiments with adenosine (0·2–200 μM) and trace amounts of [^{3}H]adenosine showed the disappearance of adenosine, which was mainly transformed into inosine and hypoxanthine. Addition of dipyridamole (2 μM) did not alter the rate of breakdown. The maximum rate then was $44{\cdot}8 \pm 10{\cdot}9$ nmol/g . min ($n = 6$) and the half-maximal rate was found with $35{\cdot}6 \pm 6{\cdot}6$ μM ($n = 6$). 2-deoxycoformycin (20 μM),

an inhibitor of adenosine deaminase, almost completely blocked deamination of adenosine (0·2–2 μM) in nerve and homogenate of nerve.

Effects of inhibitors of 5′-nucleotidase and adenosine deaminase on efflux

In a number of experiments the ^{3}H efflux was analysed in the presence of inhibitors of 5′-nucleotidase or adenosine deaminase. The results of Table 1 show that addition of α,β-methylene ADP (200 μM) did not alter the composition of the resting and of

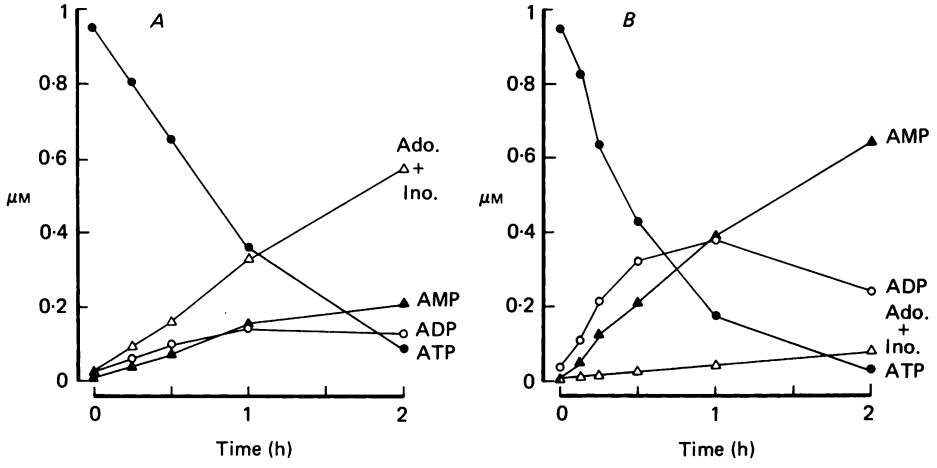

Fig. 1. Hydrolysis of [^{3}H]ATP in presence of vagus nerve. [^{3}H]ATP (1 μM) was added to 3 ml Locke solution bathing the desheathed vagus and aliquots were analysed at various times for ATP, ADP, AMP and nucleosides. *A*, [^{3}H]ATP alone; *B*, in addition α,β-methylene ADP (200 μM). Wet weights of preparations were 18·9 and 13·2 mg. Ado., adenosine; ino., inosine.

the increased efflux found after a period of activity. Further, α,β-methylene ADP did not increase the rate of efflux, whereas an increased efflux was found after addition of ADP or ATP. The effect of 20 μM-ATP was prevented by 200 μM-α,β-methylene ADP. In an experiment with β,γ-methylene ATP (200 μM) the rate and composition of the efflux were unchanged. In other experiments, the effect of 2-deoxycoformycin (20 μM) was tested and it was found that the composition of the effluent was similarly unchanged. The rate of efflux also was not affected. The finding that the composition of the effluent is not altered by inhibitors of ATP and adenosine breakdown shows that extracellular transformation of released compounds is of minor importance in the conditions of our experiments.

Effects of glucose-free solution and metabolic inhibitors

Resting efflux and liberation during activity. In order to test whether changes in energy metabolism could affect the release of purines, the effects of low-glucose or glucose-free solutions were studied. In addition, a number of metabolic inhibitors were applied during the wash-out period.

Omission of glucose slightly increased the efflux of ^{3}H-labelled compounds from

resting preparations and produced a large effect on the composition of the released material by increasing the adenosine content to more than 50 % (Table 2). The effect of glucose-free solution on the rate and composition of the resting efflux developed slowly and was complete after 30 min. Glucose-free solution also increased the release on stimulation, and adenosine was then also prevailing. An effect on the efflux was found only in the complete absence of glucose: when the glucose concentration was lowered from 5 mM to 2·5 or 1·25 mM the composition and rate of efflux were not modified.

TABLE 1. Composition of efflux during exposure to inhibitors of ecto-enzymes

	Locke solution		α,β-methylene ADP	
	R	S	R	S
Adenosine	5·5±2·0	5·5±2·4	4·7±0·8	6·3±3·5
Inosine	67·3±4·2	75·7±5·9	64·0±5·6	68·1±7·2
Hypoxanthine	23·1±1·1	16·7±0·7	24·4±3·6	22·7±0·6
Adenine	1·9±1·1	1·2±0·7	4·1±1·5	1·0±0·4
Nucleotides	2·1±1·8	0·9±0·8	2·8±1·6	1·9±0·4
n =	4	4	4	4
	Locke solution		Deoxycoformycin	
	R	S	R	S
Adenosine	4·8±2·4	4·1±3·1	5·5±3·2	4·9±2·7
Inosine	64·8±6·5	68·3±2·8	69·3±6·5	71·1±2·1
Hypoxanthine	29·7±4·0	26·4±1·9	23·8±1·3	22·4±1·1
Adenine	0·4±0·3	0·8±0·5	0·6±0·5	0·9±0·6
Nucleotides	0·3±0·2	0·4±0·3	0·8±0·3	0·7±0·3
n =	4	4	4	4

The composition of resting (R) and stimulated (S) efflux was determined (as percentage of radioactivity) before and after the application of α,β-methylene ADP (200 μM) or deoxycoformycin (20 μM) and during the exposure to these compounds, when two periods of stimulation were applied. Values are means of two determinations in four samples; for each drug, two different preparations were used. Stimulation was 10 Hz for 3 min. Values with test solutions are not significantly different from corresponding values with Locke solution.

When 2-deoxyglucose was added to the glucose-free solution there was a further increase in the rate of resting release and the composition of the effluent was also modified, adenosine making up a smaller percentage than in glucose-free solution (Table 2). In absolute amounts, however, the release of adenosine increased above the value in glucose-free solution. Stimulation hardly increased the release.

As seen in Figs. 2 and 3, the first period of activity in a ^{3}H-loaded nerve leads to a large liberation of radioactivity which is followed on the second stimulation by a much smaller release. As shown previously, the same stimulated release is then maintained for subsequent periods of activity (Maire *et al.* 1982): the comparison of the effect of different compounds on the release during activity was therefore based on the second and the following periods of activity.

The effect of cyanide (2 mM) is seen in Table 2. With arsenate (1 mM) the normalized resting efflux increased 1·7 times; adenosine accounted for 13·6 %, inosine for 49 %, hypoxanthine for 36·2 %, and the nucleotides for 1·2 %; for the stimulated efflux the

corresponding numbers were 2·9; 14, 61·2, 23·9 and 1 %. Addition of 2,4-dinitrophenol (0·2 mM) caused a large increase in resting efflux (by a factor of 36) (see Fig. 2), adenosine accounted for 26·1 %, inosine for 37·1 %, hypoxanthine for 34·9 % and adenine for 2 %. The normalized stimulated efflux was also greatly increased (Fig. 2) and mounted to 6·8 (means of two experiments). When salicylate (5 mM) was added,

TABLE 2. Effect of glucose-free solution, 2-deoxyglucose, and cyanide on rate and composition of efflux

	Locke solution		Glucose-free solution	
	R	S	R	S
Normalized rate	1	1	7±3	4±2
Adenosine	4·3±1·3	5·1±1·1	52·3±2·0*	57·4±0·1*
Inosine	66·0±6·8	74·7±2·8	28·7±1·0*	27·8±1·7*
Hypoxanthine	27·0±6·2	19·1±3·0	16·3±2·5*	12·8±1·6
Adenine	0·6±0·4	0·4±0·3	1·9±0·6	1·3±0·5
Nucleotides	1·9±0·9	0·6±0·4	1·2±0·8	0·8±0·5
n =	4	5	3	3
	2-deoxyglucose		Cyanide	
	R	S	R	S
Normalized rate	77±9	2±1	76±12	6±3
Adenosine	6·7±1·6	7·8±1·2	4·4±1·3	18·1±3·0*
Inosine	68·1±3·1	62·4±1·3	72·7±6·0	45·4±5·5*
Hypoxanthine	23·1±2·3	27·6±2·5	22·3±3·5	35·2±4·7*
Adenine	0·4±0·3	0·7±0·4	0·2±0·1	0·3±0·1
Nucleotides	1·7±0·9	1·4±0·9	0·5±0·1	1·0±0·3
n =	3	3	3	3

Effect of applied solution on rate of resting efflux is normalized with respect to rate in Locke solution (taken as unity), using peak of observed effect; for normalized efflux during activity, the second period of stimulation in Locke solution is used as the basis for comparison (see Fig. 3). Composition is given as the percentage of collected radioactivity. Glucose-free solution was applied for 30 min, 2-deoxyglucose was then added (5 mM) and determinations repeated after another 30 min. Cyanide (5 mM) was applied in Locke solution for 30 min. Values marked * are significantly different from corresponding values with Locke solution ($P < 0·05$).

the increase in resting efflux was relatively small but the effect of stimulation was large (Fig. 3). The effect of salicylate on ^{3}H efflux was thus different from its effect on ^{32}P liberation, where a large but transient increase in the resting efflux is seen (Ritchie & Straub, 1980).

Intracellular distribution. In some experiments, preparations loaded by incubation with [^{3}H]adenosine were treated as in efflux experiments but removed from the apparatus for analysis of their intracellular content. The results are summarized in Table 3.

Influx and labelling of intracellular compounds. In other experiments, preparations were exposed to glucose-free solution or to metabolic inhibitors for 30 min, [^{3}H]adenosine was then added and the *uptake* of radioactivity measured after 15 min. Compared to the influx in Locke solution (0·12 nmol/g . min), there was a lowering to

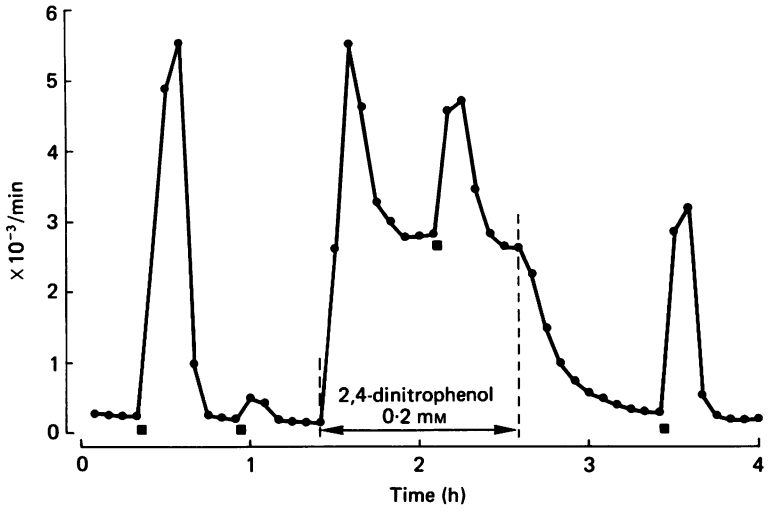

Fig. 2. Effect of electrical activity (■) and 2,4-dinitrophenol on the efflux of radioactivity in ^{3}H-pre-loaded rabbit vagus nerve. The preparation was loaded for 2 h in 0·1 μM-[^{3}H]adenosine Locke solution and then washed for 1 h in non-radioactive Locke solution before collection began. Ordinate indicates rate constant of efflux of radioactivity, abscissa is time. A large increase in efflux is seen after first stimulation (10 Hz, 180 s) and a much smaller effect after second period of activity. Application of 2,4-dinitrophenol increased both resting and stimulated efflux.

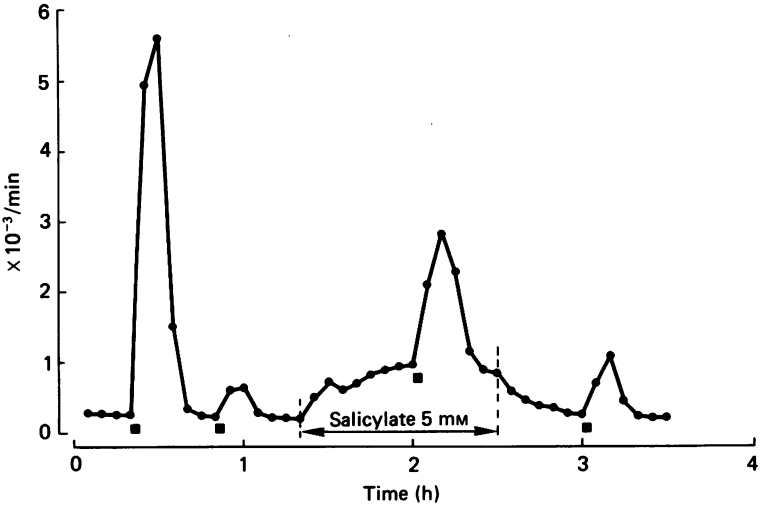

Fig. 3. Effect of electrical activity (■) and salicylate on the efflux of radioactivity in ^{3}H-pre-loaded rabbit vagus nerve. The preparation was loaded for 2 h in 0·1 μM-[^{3}H]adenosine Locke solution and then washed for 1 h in non-radioactive Locke solution before collection began. Ordinate indicates rate constant of efflux of radioactivity, abscissa is time. A large increase in efflux is seen after first stimulation (10 Hz, 180 s). Application of salicylate increased resting and stimulated efflux.

0·05 nmol/g . min in Locke solution with either 2,4-dinitrophenol (0·2 mM) or cyanide (5 mM) and to 0·07 nmol/g . min with arsenate (1 mM); glucose-free Locke solution or 2-deoxyglucose in glucose-free Locke solution had no effect.

A large effect was found when the intracellular distribution of radioactivity was studied. In the presence of metabolic inhibitors a decrease in the amount of labelled

TABLE 3. Distribution of intracellular radioactivity after exposure to glucose-free solution, 2-deoxyglucose or cyanide (as percentage of total radioactivity)

	Locke solution	Glucose-free solution	2-deoxyglucose	Cyanide
Adenosine–hypoxanthine	0·2±0·01	0·4±0·2	1·2±0·2*	1·8±0·4*
Inosine	1·4±0·03	2·0±0·1*	3·9±0·3*	3·2±0·2*
Adenine	0·6±0·1	0·5±0·04	0·8±0·03	0·8±0·1
AMP	2·0±0·9	4·1±0·8	10·8±2·9*	20·0±0·4*
ADP	9·0±0·4	13·3±0·6*	18·3±3·5*	18·6±0·4*
ATP	86·8±0·3	76·6±0·5*	64·9±5·8*	55·6±1·2*

Nerves incubated in [^{3}H]adenosine Locke solution for 2 h were washed with label-free Locke solution (1 h), exposed for 30 min to the solutions indicated and then analysed by thin-layer chromatography on cellulose. Separate determinations on silica gel confirmed the results and gave the following percentage of adenosine in the adenosine–hypoxanthine fraction: Locke solution, 17; glucose-free, 13; 2-deoxyglucose, 30; cyanide, 24. Means are based on two determinations in two separate experiments. 2-deoxyglucose (5 mM) was applied in glucose-free solution, cyanide (5 mM) in Locke solution. Values marked * are significantly different from corresponding values with Locke solution ($P < 0·05$).

ATP was observed, and a corresponding increase in the amounts of AMP and ADP. With 2,4-dinitrophenol or cyanide there was also an increase in the content of adenosine, hypoxanthine, and inosine. In glucose-free solution or glucose-free 2-deoxyglucose solution the amount of labelled nucleosides was not affected.

DISCUSSION

The present results show that ATP is degraded by extracellular enzymes of the vagus nerve and that the hydrolysis can be blocked by α,β-methylene ADP at the AMP level. Further, there is evidence of extracellular deamination of adenosine, which in turn can be prevented by deoxycoformycin. However, the application of these inhibitors does not alter the composition of the collected effluent: it therefore appears that in our experiments extracellular enzymes did not to a measurable extent interfere with the compounds released from resting or stimulated nerve. The analysis of the effluent thus closely reflects the composition of the efflux; or, in other words, resting or stimulated nerve fibres release inosine, hypoxanthine and to a small degree, adenosine and adenine; there is practically no release of nucleotides. This agrees with conclusions based on comparison between efflux of ^{3}H and ^{32}P (Maire & Straub, 1981). In addition, the finding that application of 2-deoxycoformycin, which also blocks intracellular deamination of adenosine, does not alter the composion of resting and stimulated efflux suggests that the collected inosine is derived from inosine-5′-phosphate (IMP) and not formed by deamination of adenosine. Thus, the difference

between the compounds released from brain slices and nerve axons may be due to the release of ATP from nerve endings and its extracellular degradation to adenosine.

The experiments also show that the rate and composition of the efflux depend on the metabolic state of the preparation. Electrical activity increases the efflux and causes a small change in its composition (Table 2); adenosine becoming in most experiments slightly more important at the expense of inosine and hypoxanthine. These changes are amplified when the preparation is treated with substrate-free solution or with metabolic inhibitors: in these cases a large increase in total efflux is accompanied by an increase in the relative amount of adenosine.

The change in efflux appears to be caused by the change in internal nucleotides: a lowering in labelled ATP and a corresponding increase in ADP and AMP (Table 3). These changes correspond to changes in the absolute amounts of these compounds, as judged from the total radioactivity of the preparation at the time of analysis.

The liberated compounds adenosine, inosine and hypoxanthine appear to be derived mainly from AMP, adenosine resulting from hydrolysis by 5′-nucleotidase. This reaction is normally reversed by re-phosphorylation of adenosine by adenosine kinase, which in the presence of a sufficiently high ATP concentration maintains a low intracellular concentration of adenosine (see Arch & Newsholme, 1978). The main reason for the increased intracellular concentration and liberation of adenosine after activity or inhibition of metabolism may well lie in the increase of AMP which together with the lowered ATP (see Chmouliovsky *et al.* 1969 and Table 3) leads to increased production of adenosine. The increase in AMP may also lead by way of IMP to increased production of inosine and hypoxanthine which accords with the increase in these compounds found in rat brain after a period of intense electrical stimulation (Schultz & Lowenstein, 1976). The rate of transformation of AMP to IMP by AMP deaminase depends on numerous activators and inhibitors (e.g. Sammons, Henry & Chilson, 1970), among which inorganic phosphate, nucleotides and 2,3-phosphoglyceric acid appear to be particularly important. It is difficult to predict the effect of the interplay between these factors. Some features however merit further consideration. For instance, the relatively large liberation of adenosine in glucose-free solution may be caused by the increase in AMP (Table 3) and in internal P_i (see Wu & Racker, 1959, Yushok, 1971), an inhibitor of AMP deaminase. The increase in internal P_i in vagus nerve gives rise to a large efflux of inorganic phosphate (P. Jirounek & R. W. Straub, unpublished experiments). With 2-deoxyglucose there is a lowering in internal P_i (Coe & Lee, 1969) and a decrease in phosphate efflux (Ritchie & Straub, 1980). The large liberation of inosine and hypoxanthine is explained by a high AMP content (Table 3) and a high activity of AMP deaminase, with rapid production of IMP (see Lomax, Bagnara & Henderson, 1975) and subsequently of inosine and hypoxanthine. When oxydative phosphorylation is inhibited (2,4-dinitrophenol, arsenate, salicylate) and in cyanide-produced anoxia, the large liberation of adenosine (Table 2) may be due to the increased AMP and the inhibition of adenylate deaminase by high P_i. A large efflux of P_i is found after application of these inhibitors (Ritchie & Straub, 1980) and the inhibition of AMP deamination has been observed by Newby (1980) in rat leucocytes.

The dependence of the amount of released compounds on the metabolic state which we observed in the present experiments may have physiological implications, as

adenosine appears to be physiologically more active than inosine and hypoxanthine, particularly as a neuromodulator in the central nervous system (e.g. Phillis & Wu, 1981; Stone, 1981, 1982).

The present findings show that release of purines is not restricted to nerve endings or other specialized regions of axons: a large proportion of the nucleosides collected in experiments on the central nervous system may well originate from nerve axons and the compounds liberated from them contribute to the central effects of purines.

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