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RELEASE OF RADIOLABELLED ADENOSINE DERIVATIVES FROM SUPERFUSED SYNAPTOSOME BEDS

EVIDENCE FOR THE OUTPUT OF ADENOSINE

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Abstract—Synaptosomal 5′-mononucleotides were prelabelled by incubating synaptosome beds in the presence of [^{3}H] adenosine. The excess of precursor was removed by superfusion with glucose bicarbonate saline and superfusates were then collected and treated in order to analyse radiolabelled adenosine and related compounds by high pressure liquid chromatography. Electrical stimulation or depolarization with K^+ ions, ouabain or veratridine enhanced the output of adenosine derivatives and the proportion of effluent adenosine. The proportion of effluent adenosine remained preponderant when the synaptosomes were depolarized in the presence of 25 μM α,β-methylene ADP, an inhibitor of 5′-nucleotidase which indicated adenosine to be released as such from the synaptosomes. α,β-methylene ADP, as well as 2′-deoxycoformycin, an inhibitor of adenosine deaminase did not affect the proportion of the released inosine, which decreased in the presence of dipyridamole, an inhibitor of adenosine uptake. This indicated that extracellular nucleotides are not the precursor of inosine which is produced intracellularly. It is concluded that an appreciable proportion of the effluent [^{3}H]-adenosine, -inosine and -hypoxanthine derived from intracellular adenosine.

Evidence has been put forward that adenosine may play a role as a neurotransmitter in the nervous tissue [1, 2]. Thus, adenosine and various derivatives have been shown to be potent depressants of the spontaneous firing of rat cerebral cortical neurons [3] and of the synaptically evoked responses in slices of olfactory cortex [4] and hippocampal formation [5, 6]. For adenosine to have these actions, it must be released from the nerve cell. Such a 'release' of adenine derivatives has been shown.

When slices or nerve ending preparations (synaptosomes), isolated from guinea pig neocortex and preincubated in the presence of [^{14}C]adenine or [^{14}C]adenosine, were superfused, they 'released' an appreciable fraction of [^{14}C]adenine derivatives [1, 7, 8]. Electrical stimulation or depolarization induced by elevated extracellular K^+ concentration increased markedly the output of [^{14}C]adenine compounds. The source of the effluent adenine derivatives, which consisted mainly of adenosine, inosine and hypoxanthine has been shown to be the synaptosomal adenine 5′-mononucleotides [9]. Recently, it has been clearly established that isolated synaptosomes release small amounts of ATP upon KCl depolarization [10–12]; this extracellular ATP could be degraded into adenosine. cAMP has also been concluded to be the precursor, at least in part, of the effluent [^{14}C]adenosine, inosine and hypoxanthine after its 'release' from guinea pig cortex slices [13]. However, the efflux of adenosine as such by nerve terminals has never been demonstrated, thus making an apparent gap between physiological and biochemical studies.

In the present study, we consider the possibility of an efflux of adenosine from superfused brain nerve endings, as already concluded in studies using brain slices [14, 15]. Under depolarizing agents or electrical pulses intracellular ATP is dephosphorylated into adenosine, thus increasing the concentration of intracellular adenosine. Consequently, adenosine is partly 'released' into the extracellular space, and partly deaminated into inosine which is then 'released'. Preliminary results have previously been reported [16].

MATERIALS AND METHODS

Synaptosomal preparation, superfusion and stimulation. Synaptosome beds derived from the neocortex of guinea pigs (body wt 250–400 g) were prepared according to Gray and Whittaker, as described by Barberis and McIlwain [9]. The procedures used for deposition and superfusion were based on those of de Belleroche and Bradford, as previously described [8]. Four beds were separately incubated in 5 ml of medium containing isotopically labelled metabolites. These included [2-^{3}H]adenosine, [8-^{14}C]adenosine, or [adenine U-^{14}C] ATP, all from the Radiochemical Centre, Amersham, U.K. The standard glucose bicarbonate medium used for incubation and superfusion contained: 120 mM NaCl; 3.5 mM KCl; 1.25 mM KH_2PO_4; 1.2 mM $MgSO_4$; 0.75 mM $CaCl_2$; 25 mM $NaHCO_3$ and 10 mM glucose, equilibrated with a gas mixture of 95 per cent O_2–5 per cent CO_2. At chosen times during superfusion, depolarizing agents, electrical pulses or inhibitors of adenosine metabolism were applied to 2 beds. Alternating rectangular wave pulses [9] were employed, at 10 V

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(peak potential 0.4 ms) time constant and 50 Hz. Two control synaptosome beds superfused with only the standard medium, without any stimulation, were used in each experiment.

After superfusion, the beds were rapidly removed from their holder, promptly homogenized in 1 ml of chilled 10 per cent (w/v) trichloroacetic acid and centrifuged. Samples of the supernatant were then estimated for the radioactivity. The protein content of the pellet was determined after addition of 1.0 M NaOH according to Miller [17].

Collection and separation of 'released' [^{3}H]adenine derivatives. Each synaptosome bed was incubated in the presence of [2-^{3}H]adenosine (3.4 μM–20 μCi) for 30–40 min and then superfused for 20 min to remove the excess of [2-^{3}H]adenosine. Superfusate medium was then collected for three successive periods of 6, 10 and 10 min. At the beginning of the second collection period, the beds were electrically stimulated or depolarized for 10 min. The [^{3}H]adenine derivatives were collected from the pooled superfusates using Norite A Charcoal [1]. The charcoal was collected on membrane filters, well washed with 10 ml of water and then slowly eluted with 10 ml of aq. 10 per cent (v/v) pyridine. The eluates were evaporated to dryness by rotary evaporation and the residue was then dissolved in 100 μl of water.

Purine bases and nucleotides were separated from nucleotides in the concentrated aqueous samples by high pressure liquid chromatography (Waters, model 46 K Universal injector, 6000 A solvent delivery system, 440 u.v. absorbance detector) according to Nordström *et al.* [18]. A nonpolar column (μ Bondapak C-18) was used as the stationary phase and a mixture of 5 mM diammonium hydrogen phosphate and 10 per cent methanol, pH 7.1, as the mobile phase (reverse phase operating mode). The absorbance was measured at 254 nm. 20 μl of the sample were injected directly into the column. The retention times, with an eluant flow of 2.0 ml.min^{-1} were: adenosine, 9.5 min; adenine, 5.3 min; inosine, 4.0 min; hypoxanthine, 2.7 min; nucleotides, 1.0–2.5 min.

Measurement of radioactivity. Samples of the superfusion media, beds extracts, or effluent from chromatography were added to vials and the volume was adjusted to 1 ml by adding water if necessary. The radioactivity was estimated using a LS 150 Beckman liquid scintillation spectrometer [8].

Chemicals. α,β-Methylene ADP was from P.L. Biochemicals Inc. (Milwaukee, Wisconsin, U.S.A.); adenosine deaminase, free of ammonium sulphate was obtained from Boehringer (Mannheim, West Germany); 2'-deoxycoformycin (pentostatin) were kindly provided by Dr Douros, NCI, NIH (Bethesda, MD, U.S.A.) and by Dr Dion, Warner-Lambert/Parke-Davis (Detroit, MI, U.S.A.). Dipyridamole (Persantin) was a gift from Boehringer-Ingelheim (Reims, France).

RESULTS

Identity of [^{3}H]adenosine derivatives 'released' on stimulation or depolarization

In those experiments, we have analysed the different ^{3}H-labelled adenosine derivatives 'released' by the synaptosome beds, preincubated with [^{3}H]adenosine. The net recovery of radioactivity following charcoal adsorption, elution and high pres-

Table 1. Efflux of [^{3}H]adenosine derivatives from synaptosome beds: effect of depolarizing agents and metabolic inhibitors

Experimental conditions	^{3}H Efflux (nCi/100 mg protein)	
Control	4070 ± 610	(6)
25 μM α,β-methylene ADP	4220	(2)
50 mM potassium	7880 ± 1830	(6)
50 mM potassium +25 μM α,β-methylene ADP	9070	(2)
80 μM veratridine	9770	(2)
80 μM veratridine +25 μM α,β-methylene ADP	11,240	(2)
0.1 mM ouabain	7540 ± 1490	(4)
5 μM dipyridamole	4580 ± 1130	(4)
0.2 μM deoxycoformycin	4520 ± 490	(4)
50 mM potassium +0.2 μM deoxycoformycin	7810 ± 1710	(4)
0.1 mM adenosine	7070	(2)
0.1 mM adenosine +5 μM dipyridamole	5260 ± 490	(4)
0.1 mM adenosine +50 mM potassium	13,970	(2)
0.1 mM adenosine +50 mM potassium +5 μM dipyridamole	8570 ± 910	(4)
50 mM potassium +5 μM dipyridamole	8470 ± 1000	(4)
0.1 mM adenosine +50 mM potassium +0.1 mM theophylline	11,120 ± 1100	(4)

The synaptosome beds (1.5–2.1 mg protein) were separately incubated with [^{3}H]adenosine and superfused as stated in Fig. 1. After 26 min superfusion, superfusate specimens were collected for 10 min and the radioactivity analysed. When used, metabolic inhibitors were added to the superfusion medium at the beginning of superfusion. Depolarizing agents and adenosine were added after 26 min superfusion. Results are averages ±S.D. with number of separate experiments in parentheses.

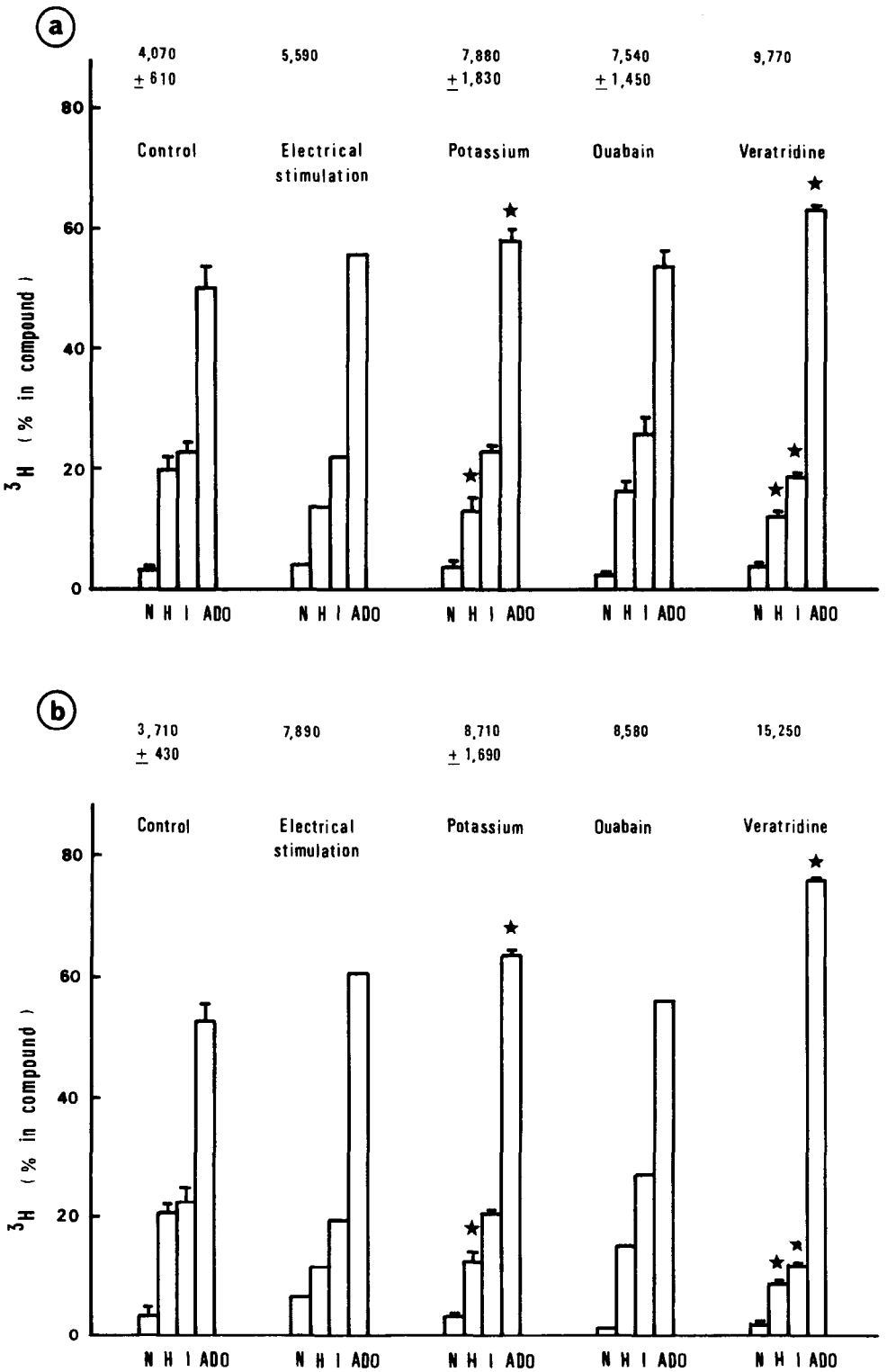

Fig. 1. Output of adenosine derivatives from guinea pig neocortex synaptosomes. Four synaptosome beds were separately preincubated for 30 min with [^{3}H]adenosine and superfused. 26 min after the beginning of superfusion, electrical pulses or depolarizing agents were applied for 10 min and the superfusion was continued for a further 10 min. Collections of superfusate were made after 20 min superfusion and the effluent [^{3}H]adenosine derivatives were analysed (see Experimental Section). Mean values for control, electrical stimulation, 50 mM potassium, 0.1 mM ouabain and 0.08 mM veratridine are quoted from 6, 2, 6, 4 and 4 separate experiments respectively. The S.D. is given by the vertical bars. Values quoted above each group of bars represent the total radioactivity output ±S.D. (a) During stimulation; (b) after stimulation. N: nucleotides; H: hypoxanthine; I: inosine; ADO: adenosine. ★ Significant ($P < 0.01$) differences from control values by Student's *t*-test for paired observations.

sure liquid chromatography of each released derivatives; i.e. [^{3}H]adenosine, [^{3}H]inosine, [^{3}H] hypoxanthine, [^{3}H]nucleotides was 50–60 per cent. No attempt was made to correct for losses incurred during these procedures. Since we have also analysed the products from ATP and adenosine added to synaptosome beds in the presence or absence of adenosine metabolism inhibitor, it was judged important that the synaptosomes employed should be in satisfactory metabolic condition and comparable to those subjected to stimulation in previous experiments [9].

After a 20 min preincubation, the synaptosome beds have been found to contain 61.0 ± 2.3 μeq.K^+/100 mg protein. Their adenylate energy charge was 0.53 ± 0.01 [9]. After 30 min incubation followed by 34 min superfusion, they contained 58.0 ± 9.1 μeq K^+/100 mg protein and their adenylate energy charge was 0.54 ± 0.04. Lactic acid has been found to be formed at some 7 μmol/100 mg protein per hour and the lactate formation has been shown to be selectively stable during 30 min superfusion [8]. These observations indicated that tissue respiration and oxidative phosphorylation, although not measured in the present experiments, was adequate for tissue maintenance [9].

When the synaptosome beds were incubated for 20 min at 37° with [^{3}H]adenosine, this precursor was very rapidly incorporated into adenine nucleotides [7, 19]. About 50 and 90 per cent of the ^{3}H incorporated by the beds was found as [^{3}H]ATP and [^{3}H]nucleotides respectively, while 9 per cent was found as [^{3}H]adenosine. During superfusion, these preparations released labelled adenosine derivatives at a constant rate after 15–20 min. The effluent adenosine derivatives consisted mainly of adenosine (51–53 per cent), inosine (23 per cent) and hypoxanthine (20 per cent) with only 3 per cent of nucleotides ([7] and Fig. 1(a) and (b)). The efflux of adenine was very low and did not change in any condition.

Electrical stimulation and 50 mM K^+ depolarization increased the efflux of [^{3}H]adenosine derivatives by up to 113 and 135 per cent respectively (Table 1). In both cases, the proportion of adenosine in the superfusate slightly increased while that of hypoxanthine decreased [Fig. 1(a)]. These changes were even more pronounced after stimulation had ceased [Fig. 1(b)].

Ouabain (100 μM) also enhanced the output of purine compounds by its inhibitory effect on sodium, potassium-activated ATPase (Table 1). The increase in the efflux of [^{3}H]adenosine derivatives produced by ouabain almost entirely resulted from increases in all adenosine derivatives detected in the superfusate since their proportion did not change very much except for hypoxanthine which slightly decreased (Fig. 1). By contrast, the very large enhancement of the efflux of purine compounds observed in the presence of 80 μM veratridine (Table 1), was largely due to an increase in adenosine, the proportion of which raised to 76 per cent, while that of inosine and hypoxanthine decreased to about 10 per cent (Fig. 1).

Products from [^{14}C]ATP added to synaptosome beds: effects of α,β-methylene ADP and adenosine deaminase

When [^{14}C]ATP was added in a small volume of saline to make the fluid surrounding the bed approximately 10 nM or 1 μM in added ATP, this yielded adenosine in the fluid as its main product, with no further conversion to inosine and hypoxanthine

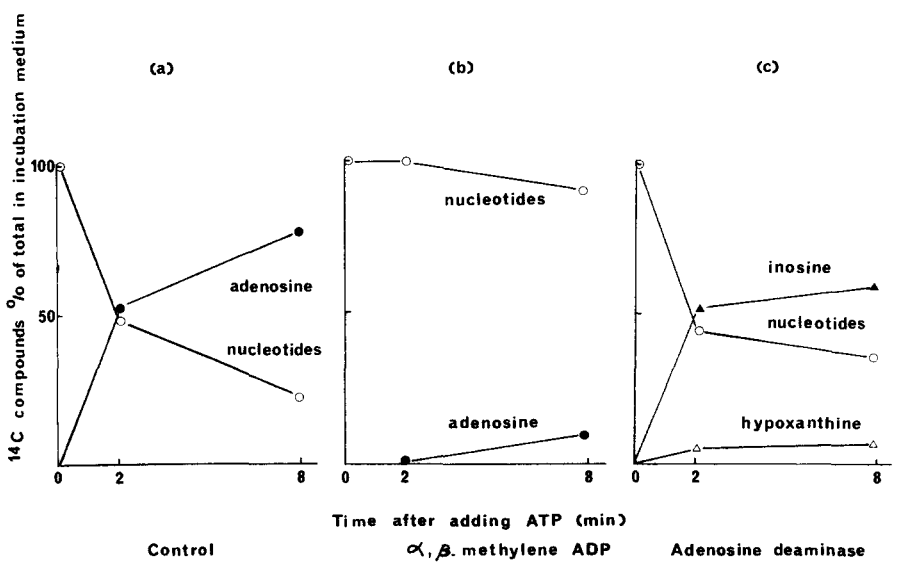

Fig. 2. Products from [^{14}C]ATP at neocortical synaptosome beds from the guinea pig, incubated in glucose bicarbonate saline. Synaptosome beds were preincubated in glucose bicarbonate for 30 min and superfused for 18 min. Then 10 nCi of [^{14}C]ATP (10 nM) were added and incubation continued for 2 or 8 min. Media samples were then analysed by high pressure liquid chromatography and the mean values from two separate experiments in each case are reported. When present, α,β-methylene ADP (25 μM) (b) and adenosine deaminase (10 μg/ml) (c) were added at the beginning of superfusion.

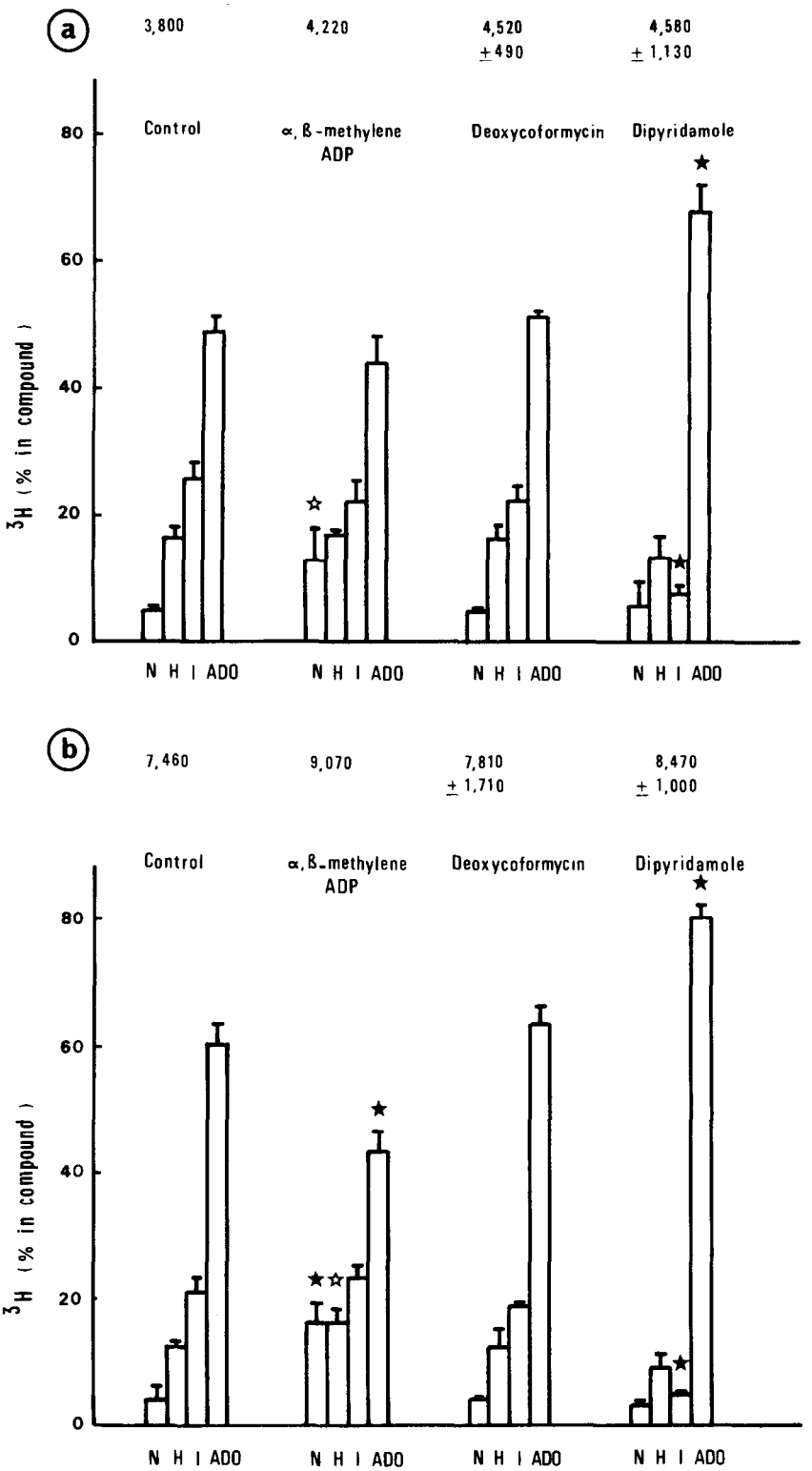

Fig. 3. Characterization of [^{3}H]adenosine and derivatives 'released' from superfused neocortical synaptosomes. Effect of inhibitors of adenosine metabolism. Data are recorded and derived as stated in Fig. 1. When used, 25 μM α,β-methylene ADP, 0.2 μM 2'-deoxycoformycin or 5 μM dipyridamole were added at the beginning of superfusion and the synaptosomes were depolarized for 10 min with 50 mM K^+ after 26 min superfusion. Mean values are quoted from 6 separate experiments. The standard deviation is given by vertical bars. Values quoted above each group of bars represent the total radioactivity output ±S.D. (a) Before depolarization; (b) during depolarization. ★ Significant ($P < 0.01$) differences from control values by Student's t-test for paired observations. ☆ Significant ($P < 0.05$) differences from control values by Student's t-test for paired observations.

Table 2. Efflux of [^{3}H]adenosine from synaptosome beds

Experimental conditions	Amount of [^{3}H]adenosine 'released' (nCi/100 mg protein/10 min)
(a)	
Control	1900
50 mM potassium	4620
25 μM α,β-methylene ADP	1690
25 μM α,β-methylene ADP +50 mM potassium	4020
(b)	
Control	2050
80 μM veratridine	6350
25 μM α,β-methylene ADP	1370
25 μM α,β-methylene ADP +80 μM veratridine	5510

The synaptosome beds were incubated and superfused as stated in Table 1. The collected superfusates were analysed by high pressure liquid chromatography and scintillation spectrometry as stated in the Experimental Section.

(a) Data are the mean of 2 values obtained from individual experiments using 4 beds. Another separate experiment, using [^{14}C]adenosine gave the same results.

(b) Data are the mean of 2 values obtained from individual experiments using 4 beds.

[Fig. 2(a)]. This was also the case when 12.7 nM- or 127 nM-adenosine was the added compound. The conversion of [^{14}C]ATP to [^{14}C]inosine and [^{14}C]hypoxanthine by the synaptosomes was obtained when adenosine deaminase (10 μg/ml) was present in the superfusion medium [Fig. 2(c)]. This shows that synaptosomes are unable to convert extracellular adenosine into inosine and hypoxanthine.

A specific inhibitor of 5′-nucleotidase (25 μM α,β-methylene ADP) [20] was included in the superfusion fluids of several experiments in order to explore the possibility of a release of 5′-nucleotides from the synaptosomes. It almost completely abolished the extracellular breakdown of added [^{14}C] ATP [Fig. 2(b)].

Output of [^{3}H]*adenosine derivatives yielded on depolarization*

Effect of inhibitors of adenosine metabolism. When added to the superfusion medium of synaptosome beds prelabelled with [^{3}H]adenosine, α,β-methylene ADP did not change the total labelled adenosine derivatives released (Table 1) but it enhanced the proportion of 5′-nucleotides yielded in the superfusate by the synaptosomes in the presence or absence of 50 mM K^+ [Figs. 3(a) and (b)]. However, the proportion of adenosine remained preponderant, although α,β-methylene ADP blocks the extracellular degradation of ATP as seen in Fig. 2(b). In the experiments performed in the absence of α,β-methylene ADP, the amount of adenosine found in the superfusate of K^+-stimulated synaptosomes was 4620 nCi/100 mg protein/10 min [Table 2, Fig. 3(b)]. In the presence of α,β-methylene ADP, a condition where extrasynaptosomal production of adenosine from nucleotides is completely inhibited, the amount of adenosine in the superfusate of the unstimulated synaptosomes was 1690 nCi/100 mg protein/10 min and it was 4020 nCi/100 mg protein/10 min in the superfusate of K^+-stimulated synaptosomes. Thus, the increase by the K^+ depolarization in the amount of adenosine of possible intrasynaptosomal origin was 4020 − 1690 = 2330 nCi/100 mg protein (Table 2) and the amount of adenosine of extrasynaptosomal origin under K^+-stimulated condition was 4620 − 4020 = 600 nCi/100 mg protein. Thus the amount of adenosine produced outside synaptosomes by potassium depolarization is sizably smaller than that of adenosine which is produced inside synaptosomes and is subsequently 'released' into the superfusate.

The difference was even greater when the synaptosomes were depolarized with 80 μM veratridine (Table 2), since the amount of adenosine of intrasynaptosomal origin produced by veratridine was 5510 − 1370 = 4140 nCi/100 mg protein and the amount of adenosine of extrasynaptosomal origin under veratridine condition was 6350 − 5510 = 830 nCi/100 mg protein.

2′-Deoxycoformycin (pentostatin) at a concentration of 2×10^{-7} M, which inhibits the activity of adenosine deaminase by more than 80 per cent*, did not affect very much neither the total amount nor the proportion of the [^{3}H]adenosine derivatives found in the superfusate [Figs. 3(a) and (b)].

Five micromolars of dipyridamole, which inhibits the uptake of adenosine by 85 per cent*, did not change the total efflux of [^{3}H]adenosine derivatives but it greatly increased the proportion of [^{3}H]adenosine which became 80 per cent in the superfusate [Figs. 3(a) and (b)]. By contrast, that of

* J. L. Daval and C. Barberis, unpublished results.

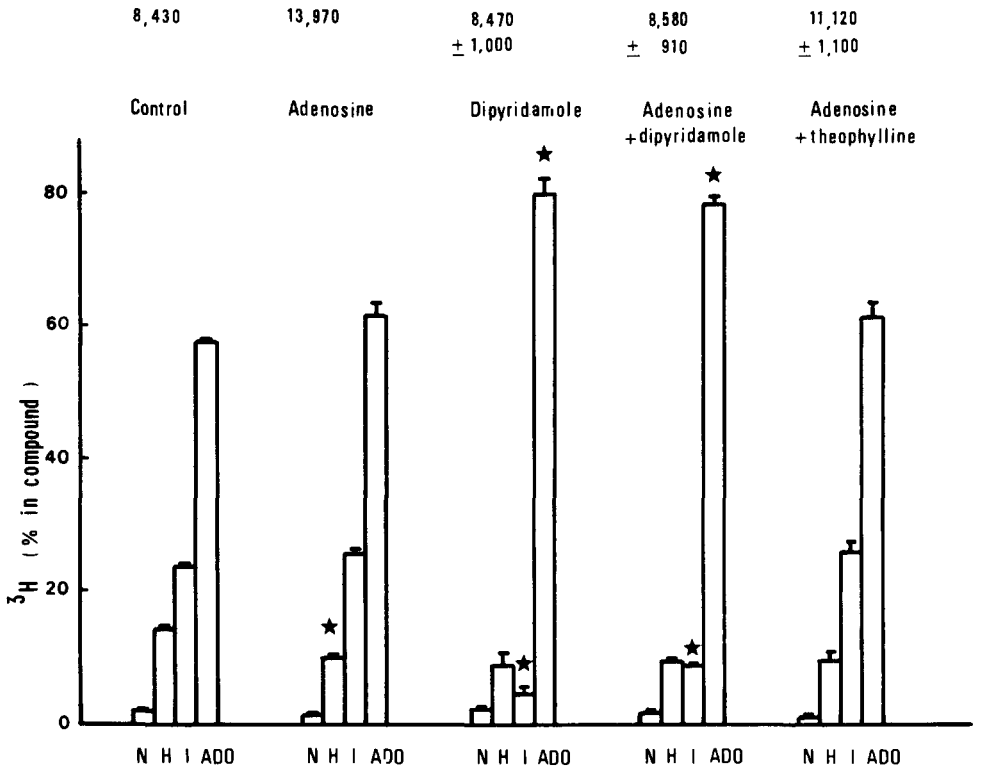

Fig. 4. Characterization of [^{3}H]adenosine and derivatives released from superfused neocortical synaptosomes. Data are recorded and derived as stated in Fig. 1. Five μM dipyridamole or 0.1 mM theophylline were added at the beginning or superfusion. 0.1 mM adenosine was added after 26 min superfusion and 50 mM K^+ applied after 36 min superfusion. The effluent media were then collected for 10 min and analyzed. Mean values are quoted from 5 separate experiments. The standard deviation is given by the vertical bars. Values quoted above each group of bars represent the total radioactivity output ± ★ S.D. Significant ($P < 0.01$) differences from control values by Student's t-test for paired observations.

inosine dramatically fell down, while that of hypoxanthine did not change significantly.

Effect of extracellular adenosine. Synaptosomes preincubated with [^{3}H]adenosine and then superfused with standard medium to remove unassimilated ^{3}H, on continued superfusion 'released' some 0.5 per cent of their ^{3}H/min [7, 8]. This could be increased by adding 0.1 mM adenosine to the standard medium (Table 1). K^+-depolarization in the presence of extracellular adenosine gave greater output of [^{3}H]adenosine derivatives. The total radioactivity 'released' by 50 mM K^+ in the presence of 0.1 mM adenosine increased its resting value by 240 per cent (Table 1). This resulted from increases in all adenosine derivatives since their proportion did not change very much (Fig. 4).

The enhanced efflux evoked by adenosine was completely blocked by 5 μM-dipyridamole (Table 1), which also markedly reduced the proportion of inosine, while that of adenosine increased. By contrast, 10^{-4} M theophylline appeared to influence neither the enhanced efflux evoked by adenosine (Table 1), nor the proportion of the different purine compounds (Fig. 4).

DISCUSSION

Superfused synaptosome beds, prelabelled with [^{14}C]adenosine, 'released' adenosine derivatives which consisted mainly of adenosine, inosine and hypoxanthine [7]. Since the source of the effluent adenosine derivatives has been shown to be the synaptosomal 5'-adenine mononucleotides [9], there is a choice between output of ATP with its subsequent extracellular breakdown or intracellular breakdown of ATP followed by diffusion of its products.

In judging which is the main precursor of the preponderant products, effects of depolarizing agents are adequate. Preceding papers [1, 4, 21–23] have demonstrated that during incubations of cortical slices or synaptosomes previously labelled with [^{14}C]adenine or [^{14}C]adenosine, spontaneous release of [^{14}C]adenosine is increased by electrical stimulation, potassium, ouabain and veratridine; and that the increase elicited by electrical stimulation or potassium is Ca^{2+}-dependent [4, 24].

The potassium and ouabain-evoked increases in efflux of adenine derivatives were much smaller than that evoked by veratridine, as already observed [16]. It was due mainly to increments in adenosine and to a lesser extent in inosine. By contrast more than 75 per cent of the radioactivity 'released' in the presence of veratridine was contained in adenosine, while inosine proportion dramatically decreased. This effect resembles that of dipyridamole, although this compound did not change the total efflux of [^{3}H]adenosine. The difference in the effects of oua-

bain and veratridine on the efflux of adenosine derivatives may reflect an effect of veratridine on adenosine uptake by the synaptosomes or on the activity of adenosine deaminase (see below). [^{14}C]-adenosine was shown to be produced by cortical synaptosomes on addition of [^{14}C]ATP [Fig. 2(a)]. This extracellular breakdown of 5'-nucleotides was almost completely blocked in the presence of α,β-methylene ADP which did not affect the efflux of total [^{3}H]adenosine derivatives elicited by K^+. Moreover, the additional presence, under potassium- or veratridine-stimulated condition, of 25 μM α,β-methylene ADP limited the amount of adenosine of extrasynaptosomal origin to about 17–20 per cent of the total adenosine 'released' in the superfusate by the depolarization. This indicates that an appreciable proportion of [^{3}H]adenosine derivatives found extracellularly on depolarization derive from adenosine and could do so after adenosine itself had appeared extracellularly, as already suggested [14]. A similar conclusion has recently been obtained by Pons *et al.* [15] who found that hydrolysis of 'released' adenine nucleotides has no significant role as a source of the extracellular adenosine that stimulates cyclic AMP accumulation in depolarized guinea pig cerebral cortical slices.

As to the spontaneous efflux of inosine in the extracellular medium, we can notice that the synaptosomes are not able to deaminate extracellular adenosine as shown in Fig. 2. Moreover, 2'-deoxycoformycin, a potent inhibitor of brain adenosine deaminase [25] does not affect the proportion of inosine found in the superfusate. These two lines of evidence indicate that inosine is formed intracellularly, as suggested by the subcellular localization of adenosine deaminase [26] and that the deamination of adenosine is predominantly catalysed by a soluble cytoplasmic enzyme [27]. In superfused synaptosomes, the 'released' adenosine is probably taken up by a high affinity uptake system [19, 28–29], thus increasing the intracellular concentration of adenosine. Inhibition of transport of adenosine by dipyridamole reduced deamination of intrasynaptosomal adenosine as seen by the decreased proportion of inosine appearing in the superfusate (Fig. 3). This is due to a reduction in the intracellular concentration of adenosine [30]. By contrast, inhibition of extracellular dephosphorylation of AMP by α,β-methylene ADP did not affect the effluent inosine. We can conclude that extracellular nucleotides which are supposedly not taken up by the cells, are not the source of adenosine that is deaminated into inosine. Then the only possibility is that free intracellular adenosine produced both by the dephosphorylation of intracellular nucleotides and the reuptake of released adenosine is the precursor of the effluent inosine.

The concentration of free adenosine in nerve terminals is not known, owing to the small amounts present in the nervous tissue. In isolated cerebral tissue, free adenosine occurs in small but fairly stable concentrations, about 2 nmoles/g of tissue [31]. Electrical excitation markedly increased the adenosine content of the isolated cerebral tissues [31]. It also induced a loss of AMP and ATP [9] and an increased efflux of purine derivatives [7] from superfused synaptosomes. It is thus possible that the changes caused by electrical stimulation or depolarizing agents (i.e. the dephosphorylation of ATP to ADP and AMP) also cause a further increase in the activity of the 5'-nucleotidase which can hydrolyse intracellular AMP. This would lead to the increase of intracellular adenosine. In conclusion, we propose that on depolarization of superfused synaptosome beds, intrasynaptosomal 5'-nucleotides are degraded into adenosine which is either 'released' directly into the extracellular space or deaminated into inosine which is 'released'. Indeed, the addition of adenosine to the superfusion medium significantly enhanced the efflux of radioactivity, largely accounted for by an increase in adenosine, inosine and hypoxanthine (Fig. 4). It seemed likely that this effect of adenosine resulted from its uptake and displacement of intracellular adenosine, since the augmentation of efflux of radioactivity elicited by adenosine was blocked by dipyridamole, an inhibitor of adenosine uptake, but was not altered by theophylline, which is thought to antagonize the action of adenosine on a membrane receptor.These findings suggest that the enhanced efflux of [^{14}C]adenosine derivatives produced by adenosine was dependent on its uptake rather than on an extracellular action. It also supports the suggestion that the efflux of adenosine from synaptosomes on depolarization is a manifestation of an intracellular conversion of adenine nucleotides to adenosine.

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