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Metabolism of [^{14}C]Adenine and Derivatives by Cerebral Tissues, Superfused and Electrically Stimulated

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1. Uptake of [^{14}C]adenine and [^{14}C]adenosine from surrounding fluids to guinea-pig cerebral tissues was measured during incubation *in vitro*. Output of ^{14}C-labelled compounds from the loaded tissues to superfusion fluids occurred on continued incubation, at about 0.2% of the tissue's content/min, and this rate was increased about fourfold by electrical excitation of the tissue. 2. The compounds released from the tissue to superfusion fluids included adenine, adenosine, inosine and hypoxanthine with small amounts of nucleotides. Output of all these compounds, except adenine, increased on excitation. Media depleted of oxygen or glucose also increased the output of ^{14}C-labelled derivatives from [^{14}C]adenine-loaded tissues, and this augmented output was further increased by electrical stimulation. 3. [^{14}C]Adenosine was found as the main product from [^{14}C]ATP when this was added at low concentrations to fluids superfusing cerebral tissue. Metabolic and neurohumoural explanations of the liberation and action of adenosine derivatives in the tissue are discussed.

Special roles for relatively small proportions of the adenine derivatives of the brain are suggested by studies of isolated, electrically stimulated cerebral tissues. The adenosine 3′:5′-cyclic monophosphate (cyclic AMP) content of such tissues, normally about 2nmol/g, increases on stimulation and the increase is potentiated by agents including serotonin and catecholamines (Kakiuchi *et al.*, 1969). Moreover, this stimulation itself releases serotonin and catecholamines from the isolated tissue to superfusing fluids (McIlwain & Snyder, 1970; see Katz & Chase, 1970) and in certain other biological systems such release is accompanied by release of adenine derivatives (Douglas & Poisner, 1966; Iversen, 1967; Geffen & Livett, 1971). It has been suspected that release of adenine derivatives occurred on electrical excitation of cerebral tissues (Kakiuchi *et al.*, 1969; Sattin & Rall, 1970; Shimizu *et al.*, 1970; McIlwain, 1972; and see the Discussion section). In the present studies we establish that this release occurs and examine it in relation to characteristics of the electrical excitation and to metabolic conditions imposed on the tissue. Shimizu *et al.* (1970) and Shimizu & Daly (1970) adopted particular procedures for labelling the [^{14}C]adenine derivatives of cerebral tissues and for characterizing several of their metabolites; the examination and extension of these procedures is described first below.

Experimental

Methods

Tissue preparation and superfusion. The flow system of McIlwain & Snyder (1970) was employed. Tissue samples were cut as sheets, 0.35mm thick, from the outer surfaces of the neocortex or piriform cortex of the guinea-pig brain. When a defined part of the cortex was needed, it was dissected free with a scalpel before preparing the tissue sample. This was then floated from a supporting glass to a dish of incubation fluid at 38°C and manoeuvered to the transfer holder. The holder was then put in its incubating beaker also at 38°C and in a bath that held four such transfer holders. The beakers contained incubation fluid with chosen additions. Gas flow to each beaker was started when incubation commenced, and the tissues were normally incubated for 30–40min before other procedures were commenced.

After this preincubation, the flow of incubation fluid, also at 38°C and usually at 3.5–4ml/min, was commenced to each beaker and was maintained for up to 80min. Collections were usually made each 2min for the chosen periods of observation (see descriptions below of individual experiments), during which the incubation fluid or the gas mixture were changed or the tissue was electrically stimulated. After superfusion tissues were released from their holders, promptly transferred with a bent wire from the fluid to 6% (w/v) trichloroacetic acid in a test-tube homogenizer, and dispersed with a plunger.

The incubation fluid, unless otherwise specified, was a bicarbonate–glucose saline chosen for cerebral tissues and contained: NaCl, 120mM; KCl, 3.0mM; KH_2PO_4, 1.2mM; $MgCl_2$, 1.2mM; $CaCl_2$, 0.75mM; $NaHCO_3$, 25mM; and glucose, 10mM in equilibrium with O_2+CO_2 (95:5). Electrical stimulation was with the apparatus and procedures described by McIlwain & Snyder (1970).

Concentration and chromatography of adenine derivatives. To combined media samples (4–16ml), containing up to 25nCi of ^{14}C-labelled compounds derived from the tissue after incubation with radioactive precursors, were added 25μg each of non-radioactive adenine, adenosine, inosine, hypoxanthine, 3′:5′-cyclic AMP, 5′-AMP, ADP and ATP. The samples were shaken with activated charcoal (5mg) for 10min and the charcoal was then collected on a membrane filter and washed with 10ml of water. In several experiments with media samples derived from superfused tissues, with or without electrical stimulation or other modification, no more than 4% of the initial radioactivity present was found in the filtrate. Compounds adsorbed to the charcoal were eluted with 10ml of aq. 10% (v/v) pyridine drawn through the filter at a rate of 0.5–1 ml/min. The eluates were evaporated to dryness by rotary evaporation and 100μl of water was added to each to dissolve the residue. As the residue was not visible, trial experiments were done with different volumes of water; 100μl of water was sufficient to ensure complete solution and collection of the radioactive compounds. At this stage the recovery of radioactivity was 61–77% of that initially present in the media samples.

Purine bases and nucleosides were separated from nucleotides in the concentrated aqueous samples by t.l.c. on silica gel at room temperature, with a freshly prepared solvent mixture containing butan-1-ol–ethyl acetate–methanol–ammonia (sp.gr. 0.88) (7:4:3:4, by vol.) as described by Shimizu *et al.* (1970). The measured R_F values were: adenine, 0.79; adenosine, 0.68; hypoxanthine, 0.60; 3′:5′-cyclic AMP, 0.46; inosine, 0.38; whereas 5′-AMP, ADP and ATP remained at or near the origin. The u.v.-absorbing regions seen after development corresponded to the added marker compounds and were cut out. The gel was scraped off and the compounds were eluted by shaking with 1.3ml of 0.1M-HCl for 1h. After brief centrifugation 1ml samples of the supernatants were assayed for radioactivity. The radioactivity recovered from chromatography was normally 85–98% of that applied.

The recovery of radioactive nucleosides and bases added to incubation media not exposed to tissue was reproducible under the conditions used (Table 1). In preliminary experiments to appraise the charcoal separation, no systematic relationships were discerned between the different conditions applied to the tissue and the subsequent recovery of the radioactive compounds from the charcoal. Pyridine concentrations of 20% (v/v) did not give greater elution. Deactivation of charcoal with 8% (v/v) s-octanol in ethanol decreased the amount of ^{14}C retained by the charcoal after adsorption and elution of hypoxanthine, but the adsorption itself was less efficient and the overall recovery much less than under the conditions chosen.

Reported results have not been corrected for losses incurred during the above procedures. Spectrophotometric measurement of the added marker compounds in the final eluates was not satisfactory for assessing the final recovery of the individual compounds, because the apparent recovery of the nucleotides and of inosine determined in this way was greater than 100%.

Measurement of radioactivity. Samples of the superfusion media, tissue extracts, or acid extracts from chromatography were added to vials and the volume was adjusted to 1ml by adding water if necessary. A toluene–Triton X-100 (2:1, v/v) scintil-

Table 1. *Recovery of radioactive compounds added to tissue incubation media*

Samples of media (10ml) that contained 2nCi of each of the carrier-free radioactive compounds as specified in the text were treated as described in the Experimental section ('standard conditions') except when otherwise stated. The percentage recovery of each compound after charcoal adsorption and chromatographic separation is shown as the mean±S.D. of four replicate determinations. The overall recovery of radioactivity eluted from the charcoal was between 85 and 90% and the total radioactivity recovered from the chromatography plates was 95–97% of that applied. Mixt. *A* was ethanol–water–NH_3 (sp.gr. 0.88) (30:19:1, by vol.); Mixt. *B* was ethanol–water–pyridine (5:4:1, by vol.).

	Addition to medium			
Compound	As ^{14}C-labelled compound (pmol)	As carrier (nmol)	Elution conditions	^{14}C recovered (%, ±S.D.)
Inosine	38	93	Standard	68.3±3.5
Cyclic AMP	17	76	Standard	57.0±1.5
Hypoxanthine	33	184	Standard	58.9±4.8
Adenosine	3	93	Standard	76.3±0.7
Adenine	9	185	Standard	93.5±3.7
Adenine	9	185	Mixt. *A*	53
Adenine	9	185	Mixt. *B*	81

lation fluid (10ml) that contained 2,5-diphenyloxazole (0.55%, w/v) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.01%, w/v) was then added. The vials were cooled to the operating temperature of the counter and shaken vigorously to disperse the sample before counting for radioactivity in a Packard liquid-scintillation spectrometer. Quenching of the samples was monitored by the channels-ratio or by the external-standard method, and was found to be essentially constant; the efficiency of counting as determined by standards was 85–90%.

Other analyses. Determinations of potassium and of phosphocreatine were as described by Swanson & McIlwain (1965). Lactate in samples of incubating fluid was determined by an automated fluorimetric method with lactate dehydrogenase and was based on the method of Antonis *et al.* (1966), which was adapted to measure lactate concentrations of 50μM.

Materials

[U-^{14}C]Adenosine (533mCi/mmol), [U-^{14}C]adenine (231mCi/mol), [8-^{14}C]hypoxanthine (60mCi/mmol), [*base*-U-^{14}C]ATP (196mCi/mmol), [*base*-U-^{14}C]adenosine 3′:5′-cyclic monophosphate (117mCi/mmol) and [8-^{14}C]inosine (53mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Non-radioactive nucleotides, nucleosides and bases were from Boehringer Corp. (London) Ltd., London W.5, U.K. The charcoal was a chromatographic grade from Hopkin and Williams Ltd., Chadwell Heath, Essex, U.K. Scintillation-grade Triton X-100 and other scintillation chemicals were from Koch–Light Laboratories Ltd., Colnbrook, Bucks., U.K. Additional reagents and solvents were of analytical grade and were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Pre-coated SilG/UV254 Polygram sheets (20cm × 20cm) were from Camlab Ltd., Cambridge, U.K. and the membrane filters were from Oxoid Ltd., London S.E.1, U.K.

Results

Tissues sampled after different periods of incubation and superfusion during the present experiments had a normal content of K^+ and phosphocreatine, and a normal glycolytic rate (see McIlwain & Snyder, 1970). After 30min of preincubation, guinea pig neocortex contained (mean±S.D., four samples) 68±2μequiv. of K^+/g and 2.6±0.2μmol of phosphocreatine/g. After 30min of preincubation followed by 40min superfusion, which included 4min of stimulation that was ended 6min before sampling, the K^+ content was 68±1μequiv./g and that of phosphocreatine 2.51±0.3μmol/g. The glycolytic rate during superfusion in the absence of stimulation was 25±3μmol of lactate/h per g of tissue; for values during stimulation, see Figs. 3 and 5.

Uptake and output of ^{14}C from adenine and adenosine

Uptake of the two ^{14}C-labelled compounds from 5μM solutions to cerebral-cortical tissues is shown in Fig. 1. Their uptake was approximately linear with time during the periods of incubation used in the present experiments, and was similar in the two cortical regions examined. At 30min, the tissue had acquired amounts of adenosine which, expressed as μCi/g, were 9 times those in the medium, expressed as μCi/ml; with adenine this value was 11.

Superfusion of the tissue with ^{14}C-labelled-precursor-free media was commenced after 30–40min of preincubation (Fig. 2). After a further 20min when 10–20 times the initial medium volume of fluid had flowed over the tissue, the tissue still retained most of the ^{14}C that it had taken up. Electrical stimulation

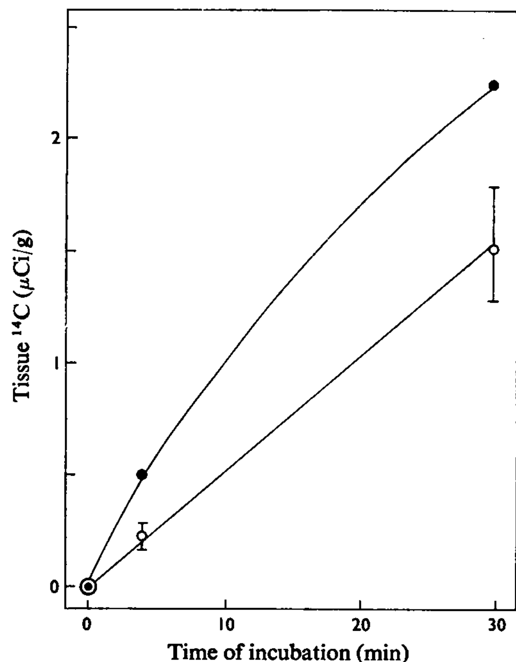

Fig. 1. *^{14}C content of guinea-pig neocortical tissues incubated with [U-^{14}C]adenine or [U-^{14}C]adenosine*

Tissues were from both anterior and posterior halves of the cortex and were incubated in 5ml of media containing 0.1μCi/ml of the ^{14}C-labelled adenosine (○) or 0.2μCi/ml of the ^{14}C-labelled adenine (●), with carrier adenine or adenosine to 5μM. Mean values from two or four tissues of 45 to 60mg are shown; in the latter case, vertical lines give the S.D.

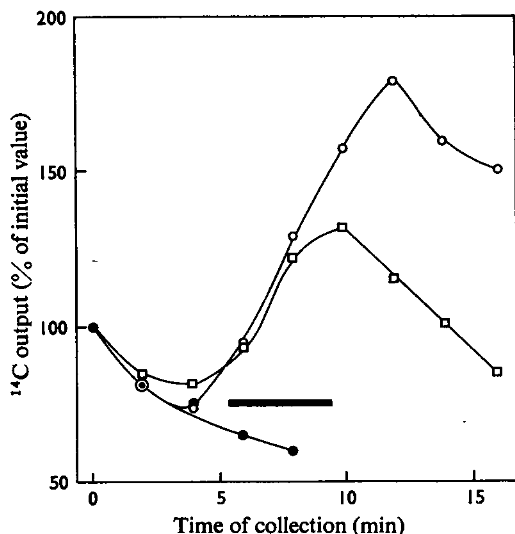

Fig. 2. *Output of ^{14}C from guinea-pig neocortical tissues*

Tissues from the anterior (○) and posterior (□) halves of the neocortex were pre-incubated with [U-^{14}C]-adenosine under the conditions described in Fig. 1 and subsequently subjected to a flow of glucose–bicarbonate saline at 3.5ml/min. After 20min of superfusion (zero time on the diagram), samples of fluid were collected in successive periods of 2min and electrical stimulation was applied for the 4min period shown by the bar. Anterior tissues not subjected to electrical stimulation (●) were similarly treated. The initial liberation, 100% on the diagram, was 50–60pCi/2min per tissue sample and mean values are shown from two or three tissues; values agreed within 15% of the mean.

then greatly accelerated the gradual loss of ^{14}C, which was still in progress. When stimulation was stopped, the output fell towards its previous value. These changes could be repeated with the same tissue sample, and were correlated in time with the other response to stimulation that was measured, namely the tissue's output of lactate (Fig. 3). The response of ^{14}C output to stimulation took place in tissues pre-incubated with [^{14}C]adenosine (Fig. 2) and [^{14}C]-adenine (Fig. 3), and the response of tissues from anterior neocortical areas was greater than that from posterior areas.

Characterization of ^{14}C-labelled derivatives in the effluent

Effluent media from experiments done as shown in Fig. 3, with [^{14}C]adenine as precursor, were pooled in two groups: samples collected immediately before stimulation and those just after, and subjected to the adsorption and chromatography described in the Experimental section. The distribution of ^{14}C found in this way is shown in Fig. 4. Purines and nucleosides were preponderant in the effluents. Stimulation increased the output mainly of adenosine and its two immediate metabolites, inosine and hypoxanthine. In parallel experiments, similar products, in approximately the same ratios, were obtained by electrical stimulation of tissues from the piriform lobe of guinea pigs. Preliminary experiments to determine the net change in adenine derivatives on stimulation and superfusion under these conditions indicated an output of $<0.05\mu$mol of adenosine/375mg of tissue during 20min.

Little of the effluent ^{14}C was as nucleotides, though these predominate in the tissue (see the Discussion section). Accordingly, the products from [^{14}C]ATP added to the fluid superfusing normal, unstimulated tissues were examined (Table 2). The major non-nucleotide product was adenosine with little or no formation of adenine. Although radioactive inosine and hypoxanthine were formed, the amount of ^{14}C in these two compounds was a smaller proportion of the total ^{14}C in the media than was found in effluents from electrically excited tissues (Fig. 4).

Output of ^{14}C-labelled compounds and lactate with stimuli of different parameters

Stimulating pulses of chosen frequencies were applied to neocortical tissues for times from a few seconds to a few minutes and the output of ^{14}C-labelled metabolites was measured (Fig. 5). As an indication of satisfactory experimental conditions, output of lactate was determined in the same experiments and showed (Fig. 5*a*) regular relationships implying the additional formation of 1.5–2nmol of lactate/stimulating pulse per g of tissue.

Immediate results for ^{14}C in the effluents from stimulated tissues, corresponding to the area below output curves including that of Fig. 3, did not show as clear a relationship. This was judged to be due to the several compounds contributing to such efflux and in particular to the varying contribution made by residual amounts of the [^{14}C]adenine used as precursor. Chromatographic separation was therefore done with a number of the fluids from experiments in which stimuli had been applied at two different frequencies and for two different periods, with results shown in Fig. 5(*b*). Though necessarily more limited than the results for lactate output, the values suggest liberation of 0.16–0.19pCi of ^{14}C/stimulating pulse. The greater part of the recovered ^{14}C was as adenosine; the contributions made by hypoxanthine and inosine decreased with increasing frequency of stimulation. Output of adenosine from tissues of the piriform lobe also increased on excitation, and

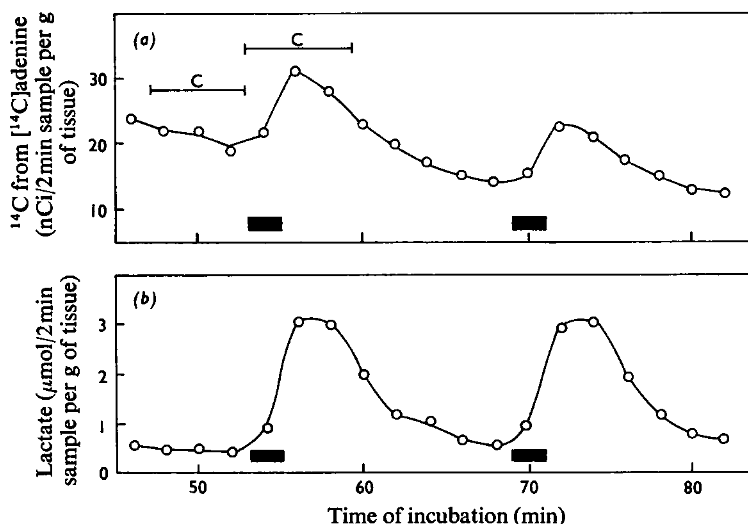

Fig. 3. *Output of [^{14}C]adenine derivatives and of lactate on excitation of guinea-pig neocortical tissue*

Preincubation of two samples of guinea-pig anterior neocortex, totalling 110mg, was in glucose–bicarbonate saline with 4.8μM-adenine (2μCi/10ml). Superfusion was at 3.1ml/min starting after 30min of incubation. Samples were collected as indicated and 0.1ml was taken for determination of lactate. Stimulation was for 2min during the two periods shown, and was at 40Hz (pulses/s). The samples marked C were pooled for chromatography (see Fig. 4).

did so to a greater extent at 40pulses/s than at 10pulses/s.

Other circumstances causing tissue output of [^{14}C]-adenine derivatives

Many adverse conditions deplete cerebral tissues of adenine nucleotides (see McIlwain & Bachelard, 1971) and the output of adenine derivatives to effluent fluids has now been examined under two such conditions (Fig. 6). After uptake of [^{14}C]adenine by neocortical tissue and subsequent superfusion under normal conditions until the spontaneous release of ^{14}C was small and relatively constant, the O_2+CO_2 supplied to the tissue was replaced by N_2+CO_2 (95:5). This was followed by an increased ^{14}C output, but marked further increase was caused by electrical excitation (Fig. 6*a*). Similar phenomena were shown when tissues that had assimilated [^{14}C]adenine under normal conditions were stimulated after superfusion with fluids that lacked glucose (Fig. 6*b*).

Discussion

We consider first how far the observed metabolite changes are within known enzyme capacities of the brain, and subsequently the possible role of adenosine, the major compound released.

Observed metabolites

In rat cerebral-cortical tissues exposed to [^{14}C]-adenine, most of the ^{14}C not in its original form was found as adenine nucleotides (Santos *et al.*, 1968). On superfusion of similarly treated tissues, the present experiments showed that release of [^{14}C]adenine was considerable, but after electrical excitation it formed a much smaller proportion of the effluent ^{14}C. The excitation increased the output of [^{14}C]-adenine nucleotides and of compounds capable of production from them by known routes: that is, it increased the effluent adenosine, inosine and hypoxanthine. Reported rates and K_m values for enzymic steps interconnecting the adenine nucleotides were discussed by McIlwain (1972); the operation in cerebral systems of subsequent stages is noted now. Adenosine deaminase (EC 3.5.4.4) catalyses the direct formation of inosine from adenosine and can operate in rodent brain at a rate of 50–60μmol/h per g of whole brain (Jordan *et al.*, 1959). Deamination is, however, more rapid at the nucleotide level, adenylate deaminase (EC 3.5.4.6) yielding inosine 5′-phosphate with calf brain preparations at a rate of 420μmol/h per g of tissue (Setlow & Lowenstein, 1967). The subsequent dephosphorylation to inosine is catalysed by a cerebral 5′-nucleotidase (Ipata, 1968). Hydrolysis of inosine to hypoxanthine occurred at a rate of about 8μmol/h per g of tissue

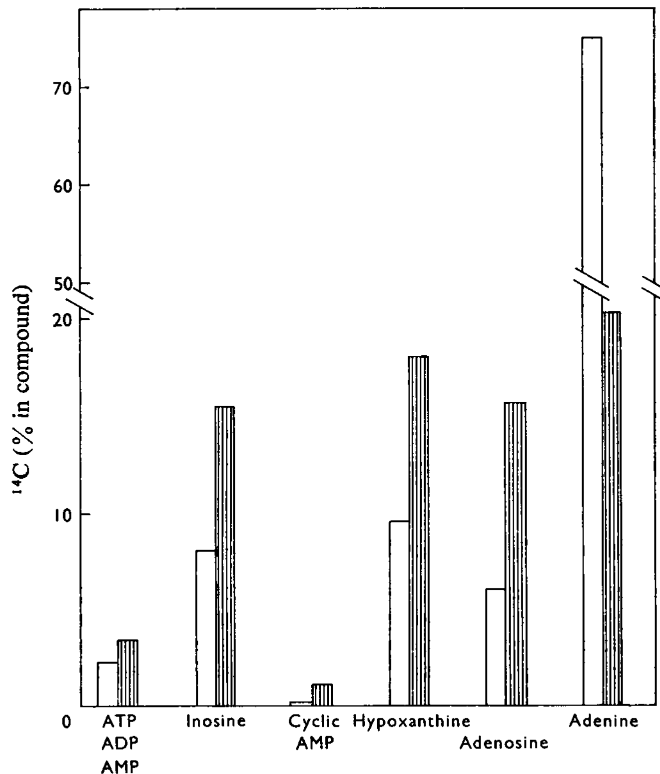

Fig. 4. *Characterization of* [^{14}C]*adenine and derivatives released from superfused neocortical tissues*

Effluent media from duplicate samples of guinea-pig tissue (anterior region) were collected as shown in Fig. 3; grouped samples before and during the period of stimulation and immediately after it were taken for chromatography as described in the Experimental section. Total nCi of ^{14}C in 37ml samples: 1.79 before and 2.15 after stimulation. Charcoal adsorption was done with 68% yield and thin-layer silica gel separation with 92% yield. □ Before stimulation; ▥ during and after stimulation; mean values are quoted from duplicates that agreed within 12%.

with a supernatant from dispersions of rat brain (Santos *et al.*, 1968).

Present observations suggest that there are additional functions for enzymes concerned with degradation of adenosine derivatives in tissues from the brain. One can consider the deamination as contributing to the removal of adenosine when it appears extracellularly, such removal being important through the property of adenosine in augmenting cyclic AMP by one of the routes considered below. The degradative enzymes could thus to some extent be significant in the same way as cholinesterases.

Role of adenosine

The observed output of adenine derivatives on electrical excitation can be considered in terms of three possible processes; one or more of these may be operating in a given situation, but they are stated separately here. Thus (1) electrical excitation increases the tissue content of less phosphorylated adenine derivatives (Minard & Davis, 1962; McIlwain & Bachelard, 1971). Supposing that there is an unchanged distribution of such substances between intracellular and extracellular phases, electrical excitation would lead to their increased output. (2) Output of adenine derivatives may be in association with noradrenaline or another neurohumour, as noted at the beginning of this paper. (3) In view of the marked effects of adenosine on cyclic AMP contents in cerebral tissues (Sattin & Rall, 1970; McIlwain, 1972) it is feasible that adenosine itself may be a neurotransmitter in the brain. Adenine nucleotides are suspected to be neurotransmitters in at least two

Table 2. *Products from [^{14}C]ATP added to neocortical tissues*

Tissues were pre-incubated in glucose–bicarbonate saline for 40min, and superfused at 3.1 ml/min for 18min and then 10nCi of [^{14}C]ATP was added by one of the two procedures. In Expts. (1), which lacked tissue, and Expts. (2) superfusion was stopped after the 18min and the [^{14}C]ATP added and mixed with the whole incubation fluid, giving an ATP concentration of 10nM; incubation was continued for 2 or 5min as indicated. In Expts. (3), superfusion was continued and the [^{14}C]ATP was rapidly injected into the inflowing stream of media bringing it to the tissue at an initial concentration of 1 μM which declined to about 0.3nM during the subsequent 6min of superfusion. Media samples were analysed after the time shown and the mean values from two experiments in each case are reported.

	Addition of [^{14}C]ATP to:			
	(1) No tissue	(2) Tissues after superfusion		(3) Tissues during superfusion
Measured ^{14}C-compounds	(5min)	(2min)	(5min)	(6min)
^{14}C in tissue (nCi/g)	0	3.60	8.85	1.28
^{14}C in fluid (nCi)	10.08	9.48	9.02	9.65
^{14}C in fluid, distribution, %:				
ATP, ADP, AMP	99.1	72.1	67.2	94.6
Inosine	0.15	0.92	1.16	0.22
Cyclic AMP	0.08	0.17	0.14	0
Hypoxanthine	0	0.71	0.62	0
Adenosine	0.13	25.7	30.1	4.76
Adenine	0.52	0.49	0.69	0.51

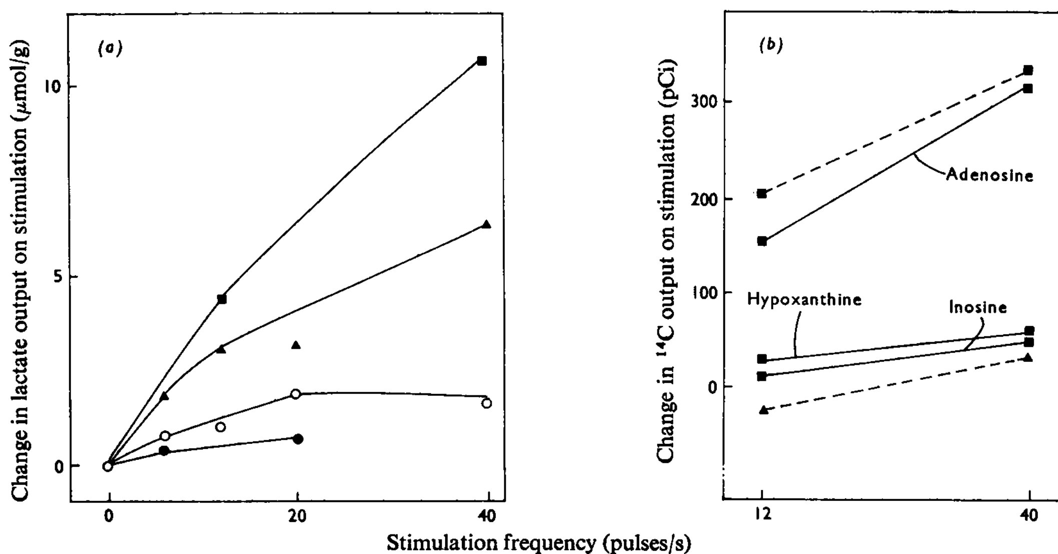

Fig. 5. *Stimulation parameters and the output of lactate and of ^{14}C from adenine*

Twelve samples of anterior neocortex from six guinea pigs were employed in three experiments, each arranged similarly to that of Fig. 3 except that the successive periods of stimulation differed in duration and pulse frequency. Stimuli were of 10V peak potential, time constant 0.4ms, and were applied for: ●, 0.25min; ○, 0.5min; ▲, 2min and ■, 4min. Effluent media were sampled (*a*) for lactate and (*b*) for determination of ^{14}C, and pooled samples were chromatographed as described in Fig. 4. Values for lactate and ^{14}C output of samples collected before stimulation were subtracted from those of samples collected after stimulation; points in the diagrams give mean results from two or three values so obtained. ----, Total output of ^{14}C.

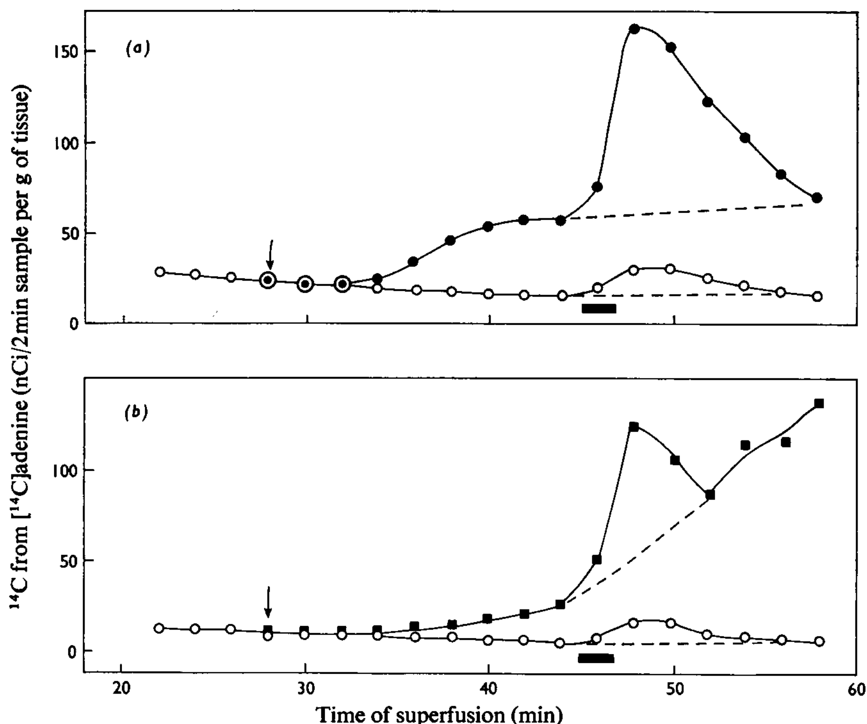

Fig. 6. *Effect of stimulation on output of ^{14}C-labelled derivatives of adenine into media lacking oxygen or glucose*

Tissues from the anterior portion of the guinea-pig neocortex were pre-incubated in normal oxygenated glucose–salines with [^{14}C]adenine, and were superfused. At the time indicated by the arrows some of the superfusion solutions were exchanged for solutions in which N_2 replaced O_2 (*a*) or which lacked glucose (*b*). Tissues deprived of O_2 (●) or of glucose (■) together with tissues exposed to unmodified media (○) were electrically stimulated at 32 or 40pulses/s for 2min as indicated by the bars. Mean values, from four tissues before alteration in the media and of two tissues subsequently, are shown.

other neural systems: in sensory nerves of the rabbit ear (Holton & Holton, 1954; Holton, 1959) and in non-adrenergic inhibitory nerves of the gut (Burnstock *et al.*, 1970).

Present findings contribute to characterizing some of these roles in tissues from the mammalian brain. Thus (1) an output of adenosine derivatives, secondary to intracellular production of the less phosphorylated compounds, is supported by the results of experiments done in hypoxia and in glucose-free media (Fig. 6). The hypoxic or hypoglycaemic tissues, however, are still capable of responding to electrical excitation by further output of ^{14}C from [^{14}C]-adenine. (2) In sympathetic neural systems the adenine derivative occurring and liberated with catecholamines is probably ATP (Douglas & Poisner, 1966; see Geffen & Livett, 1971) though, again, the adenine derivatives observed extracellularly are less phosphorylated. A comparable role for part of the ATP in the brain is thus compatible with the products now found on addition of [^{14}C]ATP to cerebral tissues. These products were similar to those detected after electrical stimulation and thus ATP itself may be the compound released extracellularly on stimulation. (3) Present observations of both uptake and output of adenosine are consistent with a role in which it arises by liberation from one cellular compartment, possibly with catecholamines or cognate compounds, and increases the cyclic AMP in another compartment. In considering the route of increase in cyclic AMP formation, an interaction of adenosine with adenylate cyclase has been considered (Sattin & Rall, 1970) and also an interaction dependent on the increased ATP concentration likely to occur at points of adenosine uptake (McIlwain, 1972). It is not known whether one or both of these two mechanisms operate (Shimizu & Daly, 1970).

By whatever mechanism cerebral cyclic AMP arises

from adenosine it is noteworthy that in specific systems of the brain, cyclic AMP has both immediate effects on cell-firing and also can induce long-term changes in enzyme make-up. Thus cyclic AMP depresses the firing of cerebellar Purkinje cells (Siggins *et al.*, 1969). Coma accompanies hypoglycaemia and glucose lack is now observed to liberate adenosine; resultant cyclic AMP may mediate in producing the coma. On the other hand, convulsive episodes result from hypoxia and in isolated tissues hypoxic conditions are now known to release adenosine; resulting cyclic AMP may limit the convulsive tendency. Cyclic AMP can induce increased formation of an *N*-acetyltransferase in pineal cultures, the increased enzyme activity lasting for some days (Klein & Berg, 1970). Electrical excitation of the brain *in situ* by stimuli similar to those now shown to liberate adenosine has long-lasting effects on the neural threshold for excitation (Essig *et al.*, 1961). Intermediation by adenosine in these various processes merits investigation.

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