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ADENOSINETRIPHOSPHATE CONSERVATION BY INDORAMIN AND OTHER DRUGS

MICHAEL G. WYLLIE, PETER M. PACIOREK and JOHN F. WATERFALL
Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire, U.K.

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Abstract—The level of adenosinetriphosphate (ATP) in the cerebral cortex of rats was shown to be decreased on exposure to hypoxic conditions (4% O_2, 0.05% CO_2, 95.95% N_2). This effect of hypoxia was markedly inhibited by subhypotensive doses of indoramin (Wy 21901) and by dihydroergotoxine and naftidrofuryl. Slight inhibition was produced by prazosin, piracetam, phentolamine and clonidine. None of these drugs altered the ATP levels of rats exposed to normal oxygen levels (20%). A variety of other drugs including meclofenoxate, pemoline, diazepam and diphenhydramine were without effect on hypoxic ATP levels.

Results of *in vitro* experiments indicated that indoramin increased the rate of synthesis of ATP whereas dihydroergotoxine decreased the rate of utilisation under hypoxic conditions. Under the same conditions naftidrofuryl stimulated ATP synthesis and slightly inhibited the rate of utilisation. It is probable that these different effects on the turnover of ATP were responsible for the similar effects of the drug observed *in vivo* on cerebral ATP levels.

In a further series of *in vitro* experiments the rate of swelling of synaptosomes on exposure to hypoxic conditions was shown to be reduced by the addition of indoramin, dihydroergotoxine and naftidrofuryl. Cerebral microvessels behaved similarly. The degree of whole brain swelling in animals exposed to hypoxia was less in indoramin treated animals than in controls.

In anaesthetised rats indoramin and naftidrofuryl protected against cyanide induced reductions in EEG voltage, whereas dihydroergotoxine was inactive. These data are consistent with the actions described on ATP synthesis and utilisation.

Overall, indoramin resembled dihydroergotoxine and naftidrofuryl, agents used widely in cerebrovascular disease, in maintaining high energy phosphate levels under adverse conditions.

INTRODUCTION

A variety of models of cerebral ischaemia and their relevance to the ageing process and associated diseases have been described [1, 2] but no unitary hypothesis of drug action or agreement as to drug therapy involving "cerebral activators" has been developed. Two closely related areas which have received considerable attention are the "normalisation" of cerebral blood flow and the modification of intermediary metabolism and more especially high energy phosphate levels in the brain [3–6]. The models often involve complex surgical procedures or may reflect only post mortem changes.

We describe here a model of cerebral ischaemia which is reproducible biochemically and physiologically. The effects of drugs and hypoxia on rat brain adenosinetriphosphate (ATP) levels are described and a detailed biochemical and pharmacological profile of one of these, indoramin, is reported.

MATERIALS AND METHODS

Materials. Indoramin mesylate and clonidine were synthesized at Wyeth. Other materials were obtained as follows: ADP, ATP and strychnine sulphate from Sigma Chemical Co. (London U.K.); phenobarbitone sodium and ouabain octahydrate from BDH (Poole, U.K.); phentolamine mesylate (CIBA); meclofenoxate hydrochloride (Lloyds); dexamphetamine sulphate (SKF); chlorpromazine hydrochloride (May and Baker); dihydroergotoxine mesylate (Sandoz); naftidrofuryl oxalate (Lipha); pemoline hydrochloride (Medo); piracetam (Union Chemie Belge); prazosin hydrochloride (Pfizer); diphenhydramine hydrochloride (Parke-Davis); diazepam (from Roche); adenosine-5′-triphosphatase EC.3.6.1.3. from Sigma Chemical Co. (London, U.K.); adenosine-5′{α-^{32}P} triphosphate from Amersham/Searle (Arlington Heights, IL).

ATP levels in the rat. Animals were exposed either to air or to a special gas mixture (4 per cent O_2, 0.05 per cent CO_2, 95.95 per cent N_2) for varying periods. The animals were then decapitated and the cortices removed within 15 sec of death and placed in 2 ml of 0.1 N PCA in methanol, on solid CO_2, (0°). The extracted ATP was assayed (i) by comparing the inorganic phosphate released by purified adenosinetriphosphatase (EC 3613) with that released from ATP standards (ii) fluorimetrically as described elsewhere [7] or (iii) by using luciferin luciferase [8]. Samples were normally assayed using the first method and routinely compared with the other methods. In a separate series of experiments animals were decapitated, the brains immediately placed in liquid nitrogen and ATP levels assessed. Drugs were administered orally in a 0.5% hydroxypropylmethylcellulose/0.9% saline (HPMC) vehicle (2 ml/kg) 2 hr before the animals were killed. The time course of drug action was also determined.

ATP synthesis and utilisation. ATP synthesis in rat cerebral cortex slices was measured manometrically

[9] in a Krebs-phosphate medium with glucose substrate. The Warburg flasks were gassed with either air or the special gas mixture prior to addition of drugs. The reaction was then monitored over the next 40 min.

ATP utilisation was measured in the same medium by following the removal of ^{32}P-ATP. A known amount of ^{32}P-ATP (1 μCi, 0.1 mM) was added. At the end of the experiment an aliquot was removed, spotted on a PEI-cellulose plate and separated by ascending chromatography in LiCl (1.2 M) [10]. The amount of ^{32}P-ATP relative to a control ATP spotting was assessed, and utilisation taken as the difference between this and the amount initially added.

Cell swelling experiments. Nerve terminals (synaptosomes) were isolated and their rate of swelling was monitored by following the change in extinction at 520 nm as described elsewhere [11, 12]. The synaptosomes were suspended in Krebs-bicarbonate medium and gassed with either 95 per cent O_2/5 per cent CO_2 or nitrogen and the extinction relative to a blank was measured at 520 nm in the presence or absence of drugs.

Cerebral microvessels were isolated [13] and intracellular water content was calculated from the differences in volume of distribution of raffinose and 3-orthomethylglucose using radiolabelled sugars.

Whole brain swelling was measured by comparing the wet:dry weight ratio on exposure to hypoxic conditions (4 per cent O_2, 0.05% CO_2, 95.95 per cent N_2).

Rat EEG. Female Charles River rats (200–250 g) were anaesthetised with pentobarbitone sodium (50 mg/kg i.p.) and artificially ventilated (60 strokes/min; 1 ml/100 g). Blood pressure and heart rate were monitored from the left femoral artery. Two EEG electrodes were implanted in the skull posterior to the bregma so that their tips just lay on the cortex and a third (earth) electrode was implanted anterior to the bregma. Shielded leads were used to join the electrodes to a wide band A.C. pre-amplifier and the calibrated EEG signal displayed on a polygraph (Grass Model 7).

Drugs or distilled water vehicle were administered as a bolus dose into the left femoral vein, 15 min before commencing an infusion of potassium cyanide in normal saline (0.4 mg/kg/min). The time taken for the EEG signal to decrease to an arbitrary voltage of 10 μV was measured.

The data were analysed statistically using an analysis of variance and then Student's *t*-test where appropriate.

RESULTS

Effects on brain ATP levels

Hypoxia. Exposure to hypoxic conditions resulted in a biphasic decline in brain ATP levels. There was a steep fall in levels between 3.5–5 min after the initial exposure. The levels then remained relatively stable for the following 10–12 min and finally there was a further decline in ATP levels (Fig. 1). The levels were found to be 80–85 per cent of those assessed by dropping the brains into liquid nitrogen and were not dependent on the technique used to analyse ATP levels (Table 1).

Effect of drugs. Rats pretreated with indoramin exhibited a relatively steady decline in ATP levels from 3.5 to 20 min after exposure to hypoxic conditions. The absence of the rapid initial decline in ATP levels in indoramin treated animals was particularly noteworthy. Although compared to control animals ATP levels of indoramin treated rats were significantly greater at 5, 10 and 15 min, there was no significant difference at the end of a 20 min gassing period. Dihydroergotoxine, naftidrofuryl and piracetam like indoramin reduced the immediate effects of hypoxia on brain ATP levels in a dose dependent

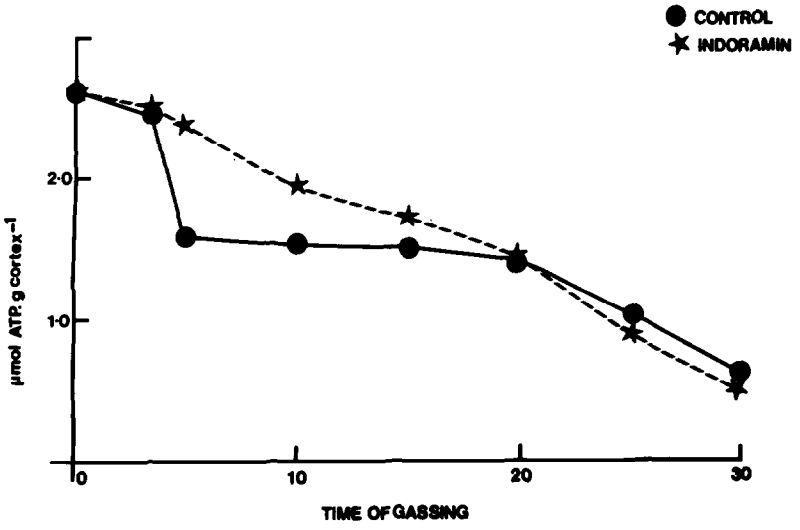

Fig. 1. Changes in brain ATP levels during hypoxia. The time of gassing with a mixture of 4 per cent, O_2, 0.05 per cent CO_2 and 95.95 per cent N_2 is shown in min. Each point represents the mean of 4–8 experiments. Standard errors are not shown as they were less than 5 per cent of the mean value. The effects of indoramin at 5, 10 and 15 min were significantly different from the control values.

Table 1. Comparison of different methods of assessment of ATP*

	ATP μmol Pi g^{-1} wet weight			
	Enzyme†	Enzyme and nitrogen	Luciferin†	Fluorimetry†
Control	2.6 ± 0.04	3.2 ± 0.03	2.8 ± 0.05	2.5 ± 0.04
Hypoxia	1.6 ± 0.02	1.95 ± 0.03	-	1.6 ± 0.03

* All results are the mean ± S.E.M. of analyses carried out on samples from 4 animals.
† Data from the same 4 animals.

manner, the maximum observed effect being after 5 min. By comparing the effects of a variety of doses of each drug after 5 min exposure of the rats to low pO_2 their ED_{50} doses (mg/kg) were shown to be dihydroergotoxine 0.34, indoramin 0.45, naftidrofuryl 17.5 and piracetam 32.8.

Effects of other drugs on normal and hypoxic animals. Table 2 shows that none of the other drugs tested had a similar profile of activity on ATP levels to that described for indoramin, dihydroergotoxine and naftidrofuryl. Although diphenhydramine and chlorpromazine (5 mg/kg) increased levels in normal animals, they had no effect on hypoxic animals. Phenobarbitone and strychnine decreased and ouabain elevated levels under both conditions. Piracetam and prazosin provided some degree of protection against the fall in ATP levels in hypoxia, but the dose or prazosin required was very high and the effect for both drugs was only approximately ⅓ of that achieved with indoramin, dihydroergotoxine or naftidrofuryl. Clonidine and phentolamine likewise resulted in slight protection against hypoxia.

Effects of drugs on ATP synthesis and utilisation. The effects of drugs on ATP synthesis are shown in Table 3.

Only ouabain, diphenhydramine, pemoline and ADP increased the rate of synthesis in normal animals whereas phenobarbitone decreased synthesis under the same conditions.

Hypoxia resulted in a reduction in synthesis (75

Table 2. Effects of test drugs on brain ATP levels in the rat

Drug	Dose (mg/kg p.o.)	ATP concn. (μmol/g cortex ± S.E.M.) Normal	Hypoxia
Vehicle		2.6 ± 0.04	1.6 ± 0.02
Indoramin	3	2.6 ± 0.1	2.4 ± 0.03***
	1		2.3 ± 0.02***
	0.3		1.8 ± 0.03***
(1) Dihydroergotoxine	3	2.6 ± 0.07	2.4 ± 0.04***
	1		2.4 ± 0.03***
	0.3		1.9 ± 0.02***
(1) Naftidrofuryl	100	2.6 ± 0.05	2.4 ± 0.07***
	10		1.9 ± 0.02***
	1		1.5 ± 0.04
(1) Meclofenoxate	10	2.6 ± 0.03	1.5 ± 0.03
(1) Pemoline	10	2.5 ± 0.03	1.5 ± 0.03
(1) Piracetam	50	2.6 ± 0.03	1.9 ± 0.04**
(2) Clonidine	3	2.7 ± 0.1	1.7 ± 0.04*
(2) Prazosin	10	2.6 ± 0.04	1.9 ± 0.05***
(2) Phentolamine	3	2.7 ± 0.03	1.7 ± 0.06*
(3) Chlorpromazine	5	2.9 ± 0.05**	1.5 ± 0.07
	0.1	2.4 ± 0.03**	1.4 ± 0.03**
(3) Diphenhydramine	10	2.8 ± 0.03*	1.6 ± 0.05
(3) Diazepam	0.5	2.5 ± 0.05	1.6 ± 0.06
(3) Phenobarbitone	100	2.0 ± 0.07**	1.4 ± 0.06**
(3) Ouabain	1	2.9 ± 0.03***	2.4 ± 0.07***
(3) Strychnine	2	2.4 ± 0.02**	1.4 ± 0.04**

Rats were exposed to the hypoxic gas mixture for 5 min prior to killing.
Where single doses are shown these represent the maximum observed effect of that drug.
(1) Cerebral "activators".
(2) Antihypertensives.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Table 3. Effects of drugs on ATP synthesis in rat cerebral cortical slices

Drug	Concentration (mM)	μmol ATP produced/g cortex/hr	
		Normal	Hypoxia
Control		239 ± 3 (6)	58 ± 3 (6)
Indoramin†	0.1	229 ± 7 (4)	92 ± 5 (4)***
Dihydroergotoxine†	0.1	230 ± 11 (4)	56 ± 11 (4)
Naftidrofuryl†	1.0	229 ± 9 (3)	71 ± 3 (3)*
Pemoline	0.57	269 ± 5 (3)**	49 ± 4 (3)
Meclofenoxate	1.0	232 ± 19 (3)	60 ± 6 (3)
Chlorpromazine	0.1	229 ± 9 (3)	62 ± 6 (3)
Prazosin	1.0	–	58 ± 3 (3)
Phentolamine	1.0	241 ± 3 (3)	62 ± 3 (3)
Phenobarbitone	1.0	185 ± 3 (3)***	53 ± 2 (3)
Diphenhydramine	1.0	327 ± 6 (3)***	61 ± 3 (3)
Ouabain	1.0	294 ± 5 (3)***	80 ± 4 (3)**
ADP	1.0	261 ± 4 (3)*	86 ± 4 (3)**

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
† Lowest concentration resulting in the maximum observed effect.

per cent). Under hypoxic conditions several drugs increased synthesis. These included indoramin, naftidrofuryl, ouabain and ADP.

Examination of the actions of drugs on utilisation of ATP under normal and hypoxic conditions (Table 4) showed that no drug increased the rate of utilisation. On the other hand a variety of compounds reduced utilisation in both normal and hypoxic conditions namely dihydroergotoxine, chlorpromazine, ouabain and ADP. Pemoline reduced normal utilisation and hypoxic utilisation was decreased by naftidrofuryl.

Turnover

The action of test drugs on ATP turnover was assessed by calculating the ratio of the rates of ATP synthesis (Table 3) and utilisation (Table 4) and the results are presented in Table 5. In normally oxygenated tissue, diphenhydramine and ouabain markedly increased the synthesis to utilisation showing reduced ATP turnover. Chlorpromazine, ADP and pemoline slightly reduced turnover and phenobarbitone increased turnover.

Under hypoxic conditions, the turnover ratio dropped to 0.44 indicating that utilisation outstripped synthesis by at least a factor of two. A variety of substances notably indoramin, dihydroergotoxine, naftidrofuryl, prazosin, ouabain and ADP decreased the turnover. Of the drugs effective only under hypoxic conditions, indoramin had the most pronounced effect. In the presence of 0.1 mM indoramin, the ratio of synthesis/utilisation under hypoxic conditions more closely resembled that found under conditions of normal oxygenation, than was found with any other drug.

Table 4. Effects of drugs on ATP utilisation in rat cerebral cortex

Drug	Concentration (mM)	μmol ATP metabolised/g cortex/hr	
		Normal	Hypoxia
Control		233 ± 6 (6)	132 ± 5 (6)
Indoramin†	0.1	235 ± 6 (4)	129 ± 8 (4)
Dihydroergotoxine†	0.1	206 ± 2 (3)**	103 ± 3 (3)**
Naftidrofuryl†	1.0	202 ± 7 (3)	119 ± 3 (3)*
Pemoline	0.57	205 ± 6 (3)*	125 ± 4 (3)
Meclofenoxate	1.0	223 ± 8 (3)	137 ± 4 (3)
Chlorpromazine	0.1	186 ± 3 (3)**	123 ± 3 (3)*
Prazosin	1.0	–	107 ± 4 (3)**
Phentolamine	1.0	225 ± 6 (3)	125 ± 4 (3)
Phenobarbitone	1.0	235 ± 5 (3)	–
Diphenhydramine	1.0	175 ± 7 (3)**	–
Ouabain	1.0	180 ± 3 (3)**	93 ± 5 (3)**
ADP	1.0	213 ± 4 (3)*	94 ± 5 (3)**

* $P < 0.05$.
** $P < 0.01$.
† Lowest concentration resulting in the maximum observed effect.

Table 5. Turnover of ATP

Drug	Concentration (mM)	Ratio synthesis: utilisation	
		Normal	Hypoxia
Diphenhydramine	1.0	1.87*	–
Ouabain	1.0	1.63*	0.87*
Pemoline	0.57	1.31	0.39
Chlorpromazine	0.1	1.29	0.48
ADP	1.0	1.22	0.92*
Naftidrofuryl	1.0	1.13	0.59*
Dihydroergotoxine	0.1	1.12	0.55*
Phentolamine	1.0	1.06	0.49
Meclofenoxate	1.0	1.04	0.44
Control		1.03	0.44
Indoramin	0.1	0.97	0.71*
Phenobarbitone	1.0	0.78*	–
Prazosin	1.0	–	0.54*

* Significantly different from control $P < 0.05$.

Tissue swelling

Gassing nerve terminals with nitrogen resulted in their swelling. The extent of the swelling was similar to that achieved with the metabolic inhibitors KCN or DNP or the Na^+,K^+-ATPase inhibitor ouabain. Of the drugs tested, only indoramin, dihydroergotoxine and naftidrofuryl protected against this swelling (Table 6). The effect of the drugs was dependent on their concentration in the incubation medium.

Hypoxic conditions also resulted in a significant increase in whole brain wet weight (13.4 ± 0.3 per cent) which was prevented by pretreatment with indoramin (3.0 mg/kg) ($P < 0.05$).

Exposure of microvessels to hypoxic conditions doubled the intracellular water volume of microvessels (Table 7). This was partially prevented by inclusion of indoramin or dihydroergotoxine in the incubation medium. These drugs had no effect on intracellular water volume of microvessels under conditions of normal tissue oxygenation.

Table 6. Effect of indoramin, dihydroergotoxine and naftidrofuryl on synaptosomal swelling induced by hypoxia

Drug		Swelling index ± S.E.M.
None		153 ± 3.0
Indoramin	0.1 mM	122 ± 7.5*
Dihydroergotoxine	0.1 mM	130 ± 3.8**
Naftidrofuryl	0.1 mM	134 ± 6.7*
Meclofenoxate	0.1 mM	159 ± 9.1
Pemoline	0.1 mM	167 ± 4.1*
DNP	0.1 mM	178 ± 5.4**
Ouabain	0.1 mM	193 ± 9.0**

The swelling index is related to the optical density change per mg of synaptosomal protein. The swelling index of synaptosomes under normal oxygenation was found to be 78 ± 4.1.

* $P < 0.05$.

** $P < 0.01$.

Effect of drugs on cyanide induced depression of rat EEG

The effect of an infusion of cyanide on the EEG signal of an anaesthetised rat is shown in Fig. 2. In control experiments EEG voltage was decreased to ≤ 10 μV in 6.19 ± 0.27 min. Indoramin (1.5, 3 and 6 mg/kg) significantly prolonged the time taken for the signal to decrease to this arbitrary voltage (Figs 2 and 3). Significant protection against cyanide was also afforded by naftidrofuryl (6 mg/kg), ATP (1 and 3 mg/kg), ouabain (0.3 mg/kg) and chlorpromazine (0.75 and 1.5 mg/kg), the effect with the latter agent being especially marked. Dexamphetamine (2 and 4 mg/kg), dihydroergotoxine (0.3 and 3 mg/kg), phentolamine (6 mg/kg) and meclofenoxate (10 mg/kg) had no significant effects on the EEG signal. A higher dose of meclofenoxate (100 mg/kg) significantly potentiated the effects of cyanide ($P < 0.05$).

DISCUSSION

The normal animal was resistant to alterations in cerebral ATP levels and there was no evidence that any of the compounds described as "cerebral activators" altered ATP levels in normal animals. This is at variance with some other published results [5]

Table 7. Alterations in the degree of cerebral microvessel swelling by indoramin and dihydroergotoxine

Treatment	Intracellular water volume μlH_2O/mg protein
None	2.6 ± 0.2 (3)
Exposure to hypoxia	5.1 ± 0.2 (3)
Exposure to hypoxia + indoramin (0.1 mM)	4.0 ± 0.2 (3)
Exposure to hypoxia + dihydroergotoxine (0.1 mM)	4.1 ± 0.3 (3)

Fig. 2. Protective action of indoramin against cyanide induced reduction of electroencephalogram (EEG) voltage in anaesthetised rats. The upper trace shows the effect of an infusion of KCN (0.4 mg/kg/min) on the EEG signal of rats pretreated (15 min) with distilled water. Voltage was reduced to ≤10 μV in 7 min. The lower trace shows the protective effect of indoramin (1.5 mg/kg) administered 15 min before the start of the KCN infusion. The time taken to reduce the EEG signal to ≤10 μV was increased to 10 min.

but not entirely unexpected, in that alterations in ATP levels are normally localised in discrete areas and compensated for by rapid changes in energy producing or consuming systems [14].

In the normal animal any imbalance in cerebral ATP levels is quickly restored [4] and the balance between utilisation and synthesis has to be dramatically altered before changes are observed. This is immediately apparent if one compares the data in Tables 2 and 5 where only drugs which altered the ratio of utilisation to synthesis by a large proportion eg. diphenhydramine (1.87) and ouabain (1.63) significantly altered normal cerebral ATP levels whereas pemoline (1.31) did not. Chlorpromazine (1.29) is anomalous as it decreased ATP levels at low doses but evoked an increase at higher levels. On the other hand the hypoxic animal was more sensitive to changes in turnover. The decreased ratio (0.44) in the hypoxic control reflects a much higher turnover than in the normal animal. This is because although both synthesis and utilisation were reduced compared to the normal animal, synthesis was more drastically reduced than utilisation; turnover was therefore increased and ATP levels fell. Under these

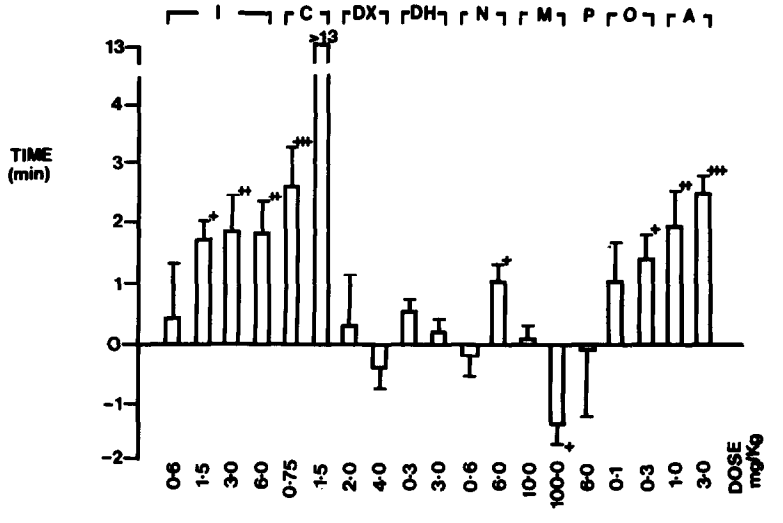

Fig. 3. Effect of drugs on changes in electroencephalogram (EEG) voltage evoked by cyanide. Histogram bars show differences (+S.E.M.) from distilled water vehicle control in time (min) taken for the EEG signal to decrease to ≤10 μV in response to an infusion of KCN (0.4 mg/kg/min). Drugs were administered 15 min before commencing the cyanide infusion. I = indoramin; C = chlorpromazine; DX = dexamphetamine; DH = dihydroergotoxine; N = naftidrofuryl; M = meclofenoxate; P = phentolamine; O = ouabain; A = ATP. +$P < 0.05$; ++$P < 0.01$; +++$P < 0.001$.

conditions indoramin, dihydroergotoxine, naftidrofuryl, ouabain, prazosin and ADP all increased the synthesis to utilisation ratio indicating that there was a decreased turnover of ATP (relative to untreated hypoxic animals) and consequent increase in ATP levels. The result with ADP is interesting as although under hypoxic conditions all compensatory mechanisms would be expected to be operational, i.e. maximal possible synthesis and minimal utilisation, a metabolite of ATP normally associated with feedback control can still further stimulate ATP production and inhibit metabolism.

It is probably fortuitous that the turnover index in the normal animal is 1.03. The method chosen for measurement of ATP utilisation in these experiments does not include any breakdown of endogenous ATP synthesized during the experimental procedure and probably underestimates utilisation due to the relative impermeability of cells to ATP. The turnover index should therefore be considered best as an index of drug action and not necessarily as a quantitative reflection of the balance between synthesis and utilisation. Also to be considered are changes due to alterations in neuronal activity as observed with strychnine and phenobarbitone.

As described elsewhere [15–17] exposure to hypoxia reduces ATP levels. The absolute levels of ATP found in this study agree well with the data from those groups. This reduction is presumably due to a decreased oxygen availability for oxidative phosphorylation. The 4 per cent O_2 in the special gas mixture used in this study would result in only partial saturation of haemoglobin [18] and subsequently in a reduction in tissue oxygenation. The initial fall in ATP levels probably reflects this decreased tissue oxygenation, the subsequent fall representing cellular death [15].

Of the drugs tested which had effects only under hypoxic conditions indoramin was most effective in restoring the energy balance of the hypoxic animal. The turnover ratio (0.71) indicates that although synthesis did not match utilisation (ratio 1.0) there is undoubtedly an improvement when compared to the untreated hypoxic condition (ratio 0.44).

The decreased turnover observed with indoramin, prazosin, dihydroergotoxine and naftidrofuryl could have been produced by either alterations in utilisation or synthesis or both. Dihydroergotoxine decreased utilisation in agreement with published data [19] as did prazosin, indoramin increased synthesis and naftidrofuryl had a strong effect on synthesis and a weak one on utilisation. The net effect with each drug was however an increased turnover ratio (decreased turnover) and subsequent increase in ATP levels. The qualitative differences of the four drugs may account for differences in their profile of activity in other tests. Similar actions on turnover have been ascribed to piracetam [3].

The effects of indoramin, dihydroergotoxine and naftidrofuryl were also seen *in vitro*. The swelling of synaptosomes also observed by others [20] in the presence of ouabain and metabolic inhibitors suggests that swelling due to hypoxia was due to a decrease in nerve terminal ATP. The monovalent cation pumping linked to Na^+, K^+-ATPase would be reduced and sodium and water loading results. As isolated nerve terminals are capable of synthesizing and metabolising ATP [21] the three drugs probably act on this situation in a manner identical to their effect on brain slices, by decreasing ATP turnover and therefore preventing the effects of hypoxia. Interestingly indoramin also protected against hypoxic cell swelling in whole brain indicating that *in vitro* nerve terminal swelling may be a useful model for the *in vivo* situation.

Although drugs which have effects on blood flow e.g. prazosin, clonidine and phentolamine partially protected against the effects of hypoxia, this was a lesser effect than that observed with indoramin. Indoramin does have effects on the vasculature [22] but it is unlikely that its effect could be completely accounted for by regional blood flow changes. It is also interesting to note that prazosin reduced turnover of cerebral ATP. It is possible that the effects on blood flow might therefore be secondary to alterations in cellular metabolism.

All too often there is no immediately apparent physiological correlate of biochemical events. The effects of ATP on the excitability of the CNS are however reflected in changes in the EEG. Cyanide evokes a cytotoxic hypoxia which is manifest as a reduction in the EEG voltage. Cyanide-induced changes in the EEG were prevented by pretreating animals with ATP or ouabain. Although it was not possible in these experiments to determine if pretreatment with ATP or ouabain resulted directly or indirectly in increased cerebral ATP levels, it is likely that the KCN induced EEG events are related to ATP levels. ATP could either increase brain ATP levels directly or if broken down to ADP or AMP would stimulate synthesis. Ouabain a known inhibitor of Na^+, K^+-ATPase would increase ATP levels by reducing turnover. In any event the effects on the EEG are consistent with the biochemical indications of an energy sparing effect of indoramin and naftidrofuryl [23].

It is an apparent anomaly that dihydroergotoxine which increased brain ATP levels did not protect against KCN whereas chlorpromazine had largely the opposite actions. Cyanide uncouples oxidative phosphorylation and this may be a partial explanation of why a drug such as dihydroergotoxine which acts only on ATP utilisation is ineffective. If no ATP was being synthesised a reduced utilisation would not be expected to prevent the hypoxic effect. On the other hand drugs such as indoramin and naftidrofuryl which act on synthesis might be expected to compete more effectively. The actions of chlorpromazine in these experiments were not clearly understood, but may reflect a diverse pharmacology.

The cell swelling studies of microvessels raise an interesting possibility. There is no doubt that these microvessels originate from inside the brain [13] and may be a model for the blood brain barrier and local blood supply and tissue oxygenation. Under hypoxic conditions these vessels are observed to swell as monitored by an increase in intracellular water volume. A similar model of the biochemical and morphological changes associated with hypoxia has recently been suggested although little experimental evidence was offered [6]. Presuming this swelling is equal in all directions partial closure of the vessel

lumen would result. This would lead to a situation in which there were competing forces namely an optimisation of overall blood flow to the brain, but a restriction on local blood flow because of cellular swelling. A drug such as indoramin might therefore be useful in regulating local blood flow. Experiments are planned to determine if this effect on microvessels is also apparent in the periphery. These changes may be difficult to measure in the normal animal because of compensatory mechanisms and even under hypoxic conditions changes may be discretely localised and therefore difficult to detect.

Apart from this effect on capillary endothelial swelling, the findings for indoramin, dihydroergotoxine, piracetam and naftidrofuryl should also be considered in the light of major central and peripheral roles of ATP. These include roles as a general energy supply, as a complex with noradrenaline [24], as an inhibitory neurotransmitter/neuromodulator [25] or as having an involvement in the synthesis of replacement CNS and other protein [26].

In conclusion, these experiments show that indoramin decreased ATP turnover in hypoxic animals by increasing ATP synthesis. This was apparent whether direct measurements of ATP synthesis were undertaken or more indirect indices including *in vitro* synaptosomal swelling or cyanide-induced EEG changes were monitored. In most respects indoramin resembled naftidrofuryl and dihydroergotoxine, agents used widely in cerebrovascular diseases. The similarities suggest that indoramin may be worth clinical appraisal in cerebrovascular diseases characterised by poor oxygenation.

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