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The Anoxic Effect on 2-Deoxy-D-[^{3}H]Glucose Uptake in the Isolated Cerebral Capillaries[1] (40196)

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A decreased 2-deoxy-D-[^{3}H]glucose (^{3}H 2-DG) uptake was previously observed in cerebral capillaries isolated from the brain of gerbils subjected to bilateral cerebral ischemia (1). The purpose of this investigation has been to shed some light on the possible mechanism responsible for the described altered function of the cerebral capillaries during ischemia.

In this communication, we will demonstrate an anoxic reduction of capillary ^{3}H 2-DG uptake which was recovered in oxygen atmosphere or prevented by the presence of FFA-free bovine serum albumin (FFA-free BSA) in the anaerobic state.

Materials and methods. The cerebral capillaries were separated from the nonvascular brain tissue of gerbils according to the technique described by us (2). A duplicate aliquot of the isolated capillaries (0.5 ml containing 0.5 mg of protein) was incubated in 2 ml of 26 m*M* K phosphate–sucrose (147 m*M*) buffer or buffered Ringer containing ^{3}H 2-DG (specific activity 8.26 Ci/m*M*, New England Nuclear Corp., Boston, MA) in the atmosphere of breathing air or 100% nitrogen at pH 7.4 and 36° for 7.5, 15 and 30 min. The former one was used in all experiments but both solutions were used for the evaluation of the effect of different gas mixtures and metabolites on the ^{3}H 2-DG capillary uptake. The effect of various gas mixtures on the ^{3}H 2-DG uptake in the capillaries was evaluated by substituting breathing air for exhaled air (containing 5% CO_2 instead of 0.4% CO_2) or 100% oxygen or 95% N_2/5% O_2 and compared with the ^{3}H 2-DG uptake of capillaries exposed to pure 100% N_2 (all gases were supplied by NIH, which purchases the air and oxygen cylinders from Air Products, Allentown, PA and the pure 100% nitrogen from Burdett Oxygen, Norristown, PA). The influence of various substances on the specific ^{3}H 2-DG uptake in the capillaries exposed to either oxygen or nitrogen atmosphere was tested by adding the below listed compounds individually or in combination to the isotope solution at the beginning or midpoint of the incubation period. The nonspecific ^{3}H 2-DG capillary uptake was determined by addition of unlabeled 2-deoxy-D-glucose or 3-O-methyl–glucose to the incubating solution containing ^{3}H 2-DG (2).

The individual substances added to the isotope solution were as follows: (a) 2,4-dinitrophenol, KCN, NaF; (b) Na_2ATP, MgATP, ADP, cAMP, PEP [Sigma Chemical Co., St. Louis, MO, except cAMP, Boehringer-Mannheim Biochemicals, Indianapolis, IN]; (c) Disodium Ethylenediamine tetraacetate [(EDTA) Fisher Scientific Co., Fair Lawn, NJ]; (d) potassium chloride, sodium chloride, lithium chloride, calcium chloride and magnesium chloride; (e) free fatty acid salts (FFA) with addition of FFA-free BSA (see Table II); (f) FFA, each substance was dissolved in two drops of methanol and then diluted with K phosphate–sucrose buffer (the methanol itself had not any effect on the ^{3}H 2-DG capillary uptake); (g) FFA-free BSA (the final concentration of FFA-free BSA was 1%); (h) α and β globulin. (Both FFA and FFA-free BSA crystalized or Fraction V as well as α and β globulin were purchased from Sigma Chemical Co.) After incubation the samples were filtered and processed for liquid scintillation counting as described elsewhere (1, 2). The protein was determined by the method of Lowry with bovine albumin as standard (3).

Results. The exposure of cerebral capillaries to various oxygen containing mixtures showed no significant differences in their specific ^{3}H 2-DG uptake whether incubated for 7.5, 15 or 30 min. The actual rate was as

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follows: 55417.6 ± 1607, 87964.5 ± 4398, 105447.3 ± 7381 cpm/mgP at 7.5, 15 and 30 min of incubation in air respectively. The metabolic inhibitors such as DNP and KCN almost completely while NaF only slightly blocked the aerobic capillary ^{3}H 2-DG uptake (Fig. 1). Furthermore, a markedly reduced level of ^{3}H 2-DG capillary uptake was observed after 7.5 and 15 min of incubation in pure nitrogen atmosphere (reduction of 59.5 ± 1.5 and 71.1 ± 0.7% of control, respectively) as compared with either breathing or exhaled air or pure oxygen or 95% N_2/5% O_2 as can be surmised from the corresponding uptakes (Figs. 1 and 2). As may be seen from Table I a more pronounced reduction of ^{3}H 2-DG capillary uptake under anaerobic conditions was found when Na_2ATP or MgATP and PEP was added to the incubating isotope solution. Moreover ATP affected the ^{3}H 2-DG capillary uptake in the aerobic phase too. A similar effect was seen when 5 m*M* EDTA was used instead of ATP with labeled hexose solution [70.7 ± 3.2 percent of aerobic control (exp. 5)]. cAMP was the only metabolite that raised significantly the anaerobic ^{3}H 2-DG uptake in the cerebral capillaries (Fig. 1 and Table I). Neither mono or bivalent ions significantly influenced the aerobic or anaerobic

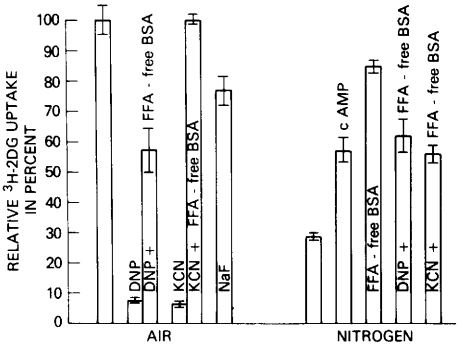

FIG. 1. 2-Deoxy-D[^{3}H]glucose uptake in the cerebral capillaries under aerobic and anaerobic conditions. The cerebral capillaries were incubated in ^{3}H 2-DG containing buffer for 15 min. The final concentration of ^{3}H 2-DG was 1.2 μ*M*. The following substances were added to the incubation mixture: FFA-free BSA (1%), DNP (1 m*M*), KCN (1 m*M*), NaF (2 m*M*) and cAMP (1 m*M*). The results represent the mean percentage of ^{3}H 2-DG capillary uptake of 6–55 experiments. The actual control aerobic ^{3}H 2-DG uptake was as follows: 87964.5 ± 4398, 87822.7 ± 7580, 83680.2 ± 2068 cpm/mg protein after exposure to air, pure oxygen and 95% N_2/5% O_2, respectively.

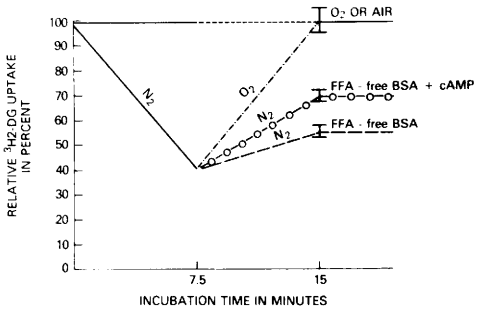

FIG. 2. Similar conditions as in Fig. 1 during the first half of the incubation period. Thereafter the changed procedures were as follows: (a) either oxygen was substituted for nitrogen atmosphere or (b) FFA-free BSA alone or with 1 m*M* cAMP was added to the incubation medium for the second half of the experimental time.

TABLE I. EFFECTS OF METABOLITES ON CAPILLARY ^{3}H 2-DG UPTAKE UNDER AEROBIC AND ANAEROBIC CONDITIONS.[a]

Addition	Concentration (m*M*)	Percent of aerobic control	Percent of anaerobic control
none	—	100.0 ± 5.0	100.0 ± 7.0
Na_2ATP^+	2	69.9 ± 5.5**	55.6 ± 6.5**
	5	34.1 ± 1.8**	18.9 ± 0.8**
Na_2ATP^{++}	5	63.5 ± 3.4**	64.2 ± 3.1**
$MgATP^{++}$	5	6 8 ± 0.7**	4.1 ± 0.4**
ADP	2	92.0 ± 4.2	97.3 ± 10.6
	5	99.9 ± 5.7	90.9 ± 4.4
cAMP	1	92.9 ± 7.4	170.7 ± 12.7**
	2	84.4 ± 4.8	198.9 ± 22.0**
PEP	2	98.4 ± 8.1	104.0 ± 11.0
	5	92.3 ± 8.0	76.4 ± 3.1*

[a] Each number represents the mean ± SE of 3–55 experiments incubated for 15 min. Control uptake: aerobic 87964.5 ± 4398 cpm/mgP (55). Anaerobic 25421.7 ± 1780 cpm/mgP (55).

* <0.01; $^+$ incubated in buffered Ringer solution only.

** <0.001; $^{++}$ incubated in K phosphate–sucrose buffer only.

uptake of ^{3}H 2-DG except for $MgCl_2$ (2 m*M*) which increased the ^{3}H 2-DG capillary uptake in the anaerobic phase [187 ± 8.9% of anaerobic control, taken as 100% (exp. 6)].

The decrease in anaerobic specific ^{3}H 2-DG capillary uptake was fully or partially recoverable, when either the pure nitrogen was substituted for one of the gas mixtures containing oxygen or FFA-free BSA alone or with cAMP was added during the anaerobic incubation, respectively (Fig. 2).

The anoxic effect on the specific ^{3}H 2-DG uptake in the cerebral capillaries could also

be prevented by the addition of FFA-free BSA to the labeled hexose mixture during the exposure of the capillaries to pure nitrogen (Fig. 1 and Table II). However, α and/or β globulins did not show such effect. The FFA-free BSA effect could also be disturbed by DNP, KCN and various individually present FFA in the incubating mixture. As may also be seen from this table, oleate, stearate and palmitate affected the ^{3}H 2-DG capillary uptake under aerobic conditions too. The aerobic interference with the capillary ^{3}H 2-DG uptake was complete with the addition of oleate while it was partial with DNP, KCN, stearate and palmitate, but with the last one in high concentrations only (Fig. 1 and Table II). The specific uptake of ^{3}H 2-DG in the capillaries was also fully abolished by the individually tested unsaturated FFA and inhibited (70%) by 1.5 m*M* palmitate when incubated without the FFA-free BSA under aerobic and anaerobic conditions.

Discussion. These studies indicate that the uptake of ^{3}H 2-DG, the partially metabolizable glucose analogue into the cerebral capillaries is oxygen dependent, since the observed anoxic reduction of ^{3}H 2-DG could be restored to the control aerobic levels by exchanging the capillary exposure from pure nitrogen to oxygen atmosphere (Fig. 2). Moreover, energy dependence of the capillary hexose uptake is also suggested by marked DNP and KCN in contrast to slight NaF inhibition of ^{3}H 2-DG uptake in the cerebral capillaries. It is also possible that the necessary energy is derived from oxidative phosphorylation rather than electron flow since the uptake of ^{3}H 2-DG was greater in the capillaries exposed to KCN with FFA-free BSA than to DNP with FFA-free BSA under aerobic but not anaerobic conditions (Fig. 1). The improved anaerobic capillary ^{3}H 2-DG uptake in presence of cAMP would strengthen the phosphorylation concept if cAMP would be proved to be involved in the phosphorylation of the capillary plasma membrane as it has been known to stimulate the phosphorylation of the isolated rat liver plasma membrane by protein kinase (4). This process is probably unrelated to the 2-DG metabolism since the free form was found in the cerebral capillaries [97% as compared to about 95% described by Goldstein *et al.* (7)].

TABLE II. 2-DEOXY-D-[^{3}H] GLUCOSE UPTAKE IN CEREBRAL CAPILLARIES.[a]

Addition	Uptake in %	
	Aerobic	Anaerobic
none	100.0 ± 5.0	28.9 ± 0.6
FFA-free BSA	100.0 ± 5.0	84.6 ± 1.6
FFA-free BSA + α Butyrate	99.1 ± 4.0	64.0 ± 5.4
FFA-free BSA + β Butyrate	103.0 ± 10.5	25.7 ± 1.7
FFA-free BSA + Palmitate 1.5 m*M*	36.3 ± 3.6	34.2 ± 3.6
FFA-free BSA + Palmitate	99.7 ± 4.5	38.8 ± 3.7
FFA-free BSA + Stearate	48.8 ± 5.9	52.6 ± 2.8
FFA-free BSA + Oleate 1.5 m*M*	0	0
FFA-free BSA + Linoleate	96.5 ± 9.8	30.7 ± 4.9
FFA-free BSA + Arachidonate	95.8 ± 3.9	32.5 ± 3.8

[a] The concentration of the added substrates was mole per mole of FFA-free BSA. Each number represents a mean ± SE of 3–55 experiments incubated for 15 min. Control uptake: aerobic = 87964.5 ± 4398 cpm/mgP. Anaerobic = 25421.7 ± 1780 cpm/mgP. Anaerobic uptake in presence of FFA-free BSA = 74156 ± 1187 cpm/mgP (14).

The FFA-free BSA protection of ^{3}H 2-DG capillary uptake in the anaerobic phase is of great interest especially since this effect was abolished by the addition of individually tested saturated and unsaturated free fatty acids (Table II). Some of these substrates like palmitate, butyrate and acetate were described as inhibitors of accelerated glucose transport in aerobic heart muscle. However, oxidation of these compounds appeared to be essential for this inhibition because such an effect was not seen in the anoxic heart (5, 6). Palmitate was also reported to accelerate the K^+ uptake into the cerebral capillaries, which was inhibited by 4-pentenoic acid (8), a known inhibitor of fatty acid metabolism (9). As far as the ^{3}H 2-DG capillary uptake is concerned palmitate (1.5 m*M*), stearate and oleate (mole per mole of FFA-free BSA) not only disturbed the protective effect of FFA-free BSA in anaerobic phase but interfered with the normal hexose uptake under aerobic conditions. Therefore, some of these substances mentioned may be effective inhibitors in the metabolized and nonmetabolized form. The drastically different interference of ^{3}H 2-DG capillary uptake in the presence of oleate and FFA-free BSA than those of other FFA (Table II) is unclear but it might be related

to the high concentration of this substrate used for the amount of FFA-free BSA since the same effect was seen when the unsaturated FFA were incubated without FFA-free BSA. Based on our studies, there is no doubt that in the cerebral capillaries the anaerobic ^{3}H 2-DG uptake can almost reach the aerobic ^{3}H 2-DG levels providing the incubating medium contains FFA-free BSA. The question then remains what kind of specific role FFA plays in altering the capillary glucose uptake. Before we could stipulate its possible role we would like to discuss also the effect of ATP in these experiments. The ATP reduced the 2-DG capillary uptake both in aerobic and anaerobic cells contrary to the described anoxic effect of ATP in avian erythrocytes which showed an increased uptake (5, 10). Since the chelating agent EDTA produced the same reaction as ATP and although the inhibition of the ^{3}H 2-DG capillary uptake was greater with MgATP than Na_2ATP, it appears that chelation of bivalent cations from the membrane could have taken place. This possibility is substantiated to a certain degree by the increased 2-DG uptake found in the capillaries when incubated in the presence of $MgCl_2$. Some nonspecific reaction under anaerobic conditions can not be excluded since high concentration of PEP lowered significantly the already reduced ^{3}H 2-DG uptake in the capillaries (Table I). Therefore the factors which have to be taken into consideration are (a) free fatty acid albumin, (b) free fatty acid substrates, (c) ATP and (d) cAMP. Most likely there are several possibilities by which these substances could interact in relationship to the hexose uptake by the cerebral capillaries but few will be considered here. It appears that under anoxic conditions a release of free fatty acid may occur irrespective of its origin whether from the endothelial cell proper or from the plasma membrane since the protective effect of the FFA-free BSA in the ^{3}H 2-DG was decreased or abolished by the presence of free fatty acid in the incubating medium. In support of this concept are the reports of free fatty acid release in so-called cerebral ischemia produced by decapitation and convulsions in contrast to microwave irradiation (11–14). Even though the free fatty acids are part of normal cycle in CNS lipid metabolism, the membrane function may be impaired following their accumulation in ischemia (12, 13). The protective effect of FFA-free BSA could be either the result of simple surface coating which would prevent a physical and/or chemical structural change in the plasma membrane or it could have a more specific action binding and/or serving as a transporting vehicle for the released fatty acids and/or its metabolites. Although the lipoproteins are associated with α and β globulin, many various molecules preferentially and reversibly combine with serum albumin which has a definite saturation capacity for different substances including anions of fatty acids (15). In support of the preferred binding of FFA to FFA-free BSA has been the protective and partial recovery function of ^{3}H 2-DG capillary uptake in the presence of FFA-free BSA but not of α and β globulin in the anaerobic medium. The improved cAMP anaerobic hexose uptake could be related to the metabolism of free fatty acids and/or to the carrier or the protein regulating sugar transport because this substance was similarly effective in the presence or absence of FFA-free BSA in anoxia. But all these possibilities have to be reconciled with the ATP and EDTA effect on the ^{3}H 2-DG uptake by the cerebral capillaries. Based on our findings reduced sugar uptake may be the result of chelation with EDTA and most likely with ATP. Therefore, the inhibition of hexose entry into the capillaries may be due to structural chemical alteration of the membrane in which the appearance of free fatty acid may be the result or the cause of such a change affecting the carrier mediated uptake.

In conclusion, the anoxic reduction of ^{3}H 2-DG uptake into the cerebral capillaries is suggested to result from structural chemical alteration of the plasma membrane. Therefore, the accepted concept of oxygen and energy independent transport of glucose across the blood brain barrier will have to be reexamined and modified appropriately.

Summary. The uptake of ^{3}H 2-DG into the isolated cerebral capillaries was investigated under aerobic and anaerobic conditions. The capillary exposure to pure nitrogen atmosphere decreased the ^{3}H 2-DG uptake. This reduction in ^{3}H 2-DG uptake was either prevented by addition of FFA-free BSA into the incubating media under nitrogen or it could be recovered by substituting the nitrogen by

oxygen containing gas mixture.

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