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A Qualitative Study on Changes in Local Brain pH Due to Discrete Cerebral Microembolism

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ABSTRACT

In this work autoradiography of ^{14}C-5,5-dimethyl-2,4-oxazolidinedione (^{14}C-DMO) was used to trace changes in local cerebral pH in embolized awake rabbits. One hour after i.v. injection of ^{14}C-DMO small cerebral ischemic foci were produced in rabbits by injecting plastic beads into the left heart ventricle under short-acting anaesthesia, and after another hour the animals were put to death and their brains processed for autoradiography of ^{14}C-DMO.

Evidence of acidosis was in general not found in the microischemic regions, though there were a few possible exceptions. However in the hippocampus a diffuse acidosis involving a large part of the structure, could be found in 2 of the 4 experiments. This hippocampal phenomenon probably reflected the same process as has been observed using autoradiography of 2-deoxyglucose (reflecting cellular glucose uptake) on the same ischemic model – increased 2-deoxyglucose phosphorylation. Because the hipocampus is invloved in the memory function and the fact that small infarcts are coupled to dementia, this phenomenon should be drawn into focus for further studies.

INTRODUCTION

Small subcortical (diameter less than 1.5 cm) cerebral infarctions are common in humans (1, 13). Their presence is closely coupled to dementia (2, 24), which in its advanced stages presents with symptoms like gait difficulties and pseudobulbar palsy (3, 10, 24). For decades, most human subcortical infarctions have been considered to be caused mainly by mural changes of the small penetrating cerebral arteries (5, 6, 14, 18, 20). Nowadays it seems that small emboli account for a significant number of these infarctions (6, 18). However, since this kind of stroke is not life-threatening, it is difficult to correlate embolism to the individual infarcts using autopsy material. However, there seems to be a correlation between dementia and atrial fibrillation (11) – a well-known risk factor for cerebral embolism.

Many aspects of cerebral microembolism have been studied (4, 7, 8, 9, 19, 21, 23, 30, 32, 33, 35 - 38, 40, 42, 43). However, the rate of follow-up on these studies is pore. Moreover the number of emboli in those studies were too large for studying discrete areas of microischemia. However, a model of irreversible cerebral microischemia, employing plastic beads as emboli, has recently been introduced (25). In that study it was found that emboli trapped in the brain stem and diencephalon affected the local tissue metabolism more often than emboli reaching the cortex. Further, from 24-h experiments it was concluded that infarcts tend to appear mainly in the subcortical structures. It was suggested that this might be due to the deeper regions having a less efficient collateral vascular network. Moreover, it was reported from acute experiments that in some ischemic foci, mainly located in the basal ganglia, there was a central "dip" in the 2-deoxy-D-[^{14}C]glucose (a tracer of cellular glucose uptake (34)) activity profile, and it was suggested that this dip may be due to central glucose depletion (27) or a depressed cellular function caused by acidosis.

The aim of this work was to come closer to an understanding of the pathophysiological events taking place in the embolized brain of rabbits by using

autoradiography on the tissue activity of ^{14}C-5,5-dimethyl-2,4-oxazolidinedione (^{14}C-DMO) to trace changes in tissue pH. The ischemic model used has been characterized in a series of papers (25 - 29). On an average, about 25 discrete ischemic regions were obtained per brain.

MATERIALS AND METHODS

Animals

4 New Zealand rabbits, 3-4 kg b.w., of either sex were used. Before the experiments the animals were housed in individual cages at a specialized animal department where they were allowed free access to food and water. The experimental design has been approved by the local ethics committee.

The ^{14}C-DMO procedure

^{14}C-5,5-dimethyl-2,4-oxazolidinedione (^{14}C-DMO) has been used for decades to investigate pH in normal as well as ischemic brain tissue (12, 15, 31, 41). Somewhat simplistic, the higher tissue concentration of ^{14}C-DMO the higher the tissue pH. In this study, ^{14}C-DMO (Amersham Int.) was used to trace possible emboli-induced tissue acidosis. The rabbits were fitted with an ear venous catheter to allow injections, and were then injected i.v. with about 250 μCi ^{14}C-DMO. After about 60 min the beads were administered as described below. Arterial blood was collected by using a needle and a piece of tubing. About 60 min after the injection of ^{14}C-DMO, the rabbits were killed with a large dose of pentobarbital sodium i.v. The heads were then submerged as quickly as possible in hexane mixed with blocks of carbon dioxide and proceesed for autoradiography as described below. No attempt was made to calculate tissue pH.

Embolization procedure

Black-dyed beads (Styrene/2%/Divinylbenzene/170-200 mesh, from Bangs Laboratories, Inc.), size range 75-90 μm, were employed as emboli. About 120 mg of the delivered sphere suspension (10 mg/ml) was mixed with 12 g Macrodex® (60 mg/ml). To allow insertion of a needle into the heart the rabbits were anaesthetized with Brietal® (metohexital, 10 mg/ml), 1 ml/kg, administered i.v. during about 20 s. Then, by using a syringe and a needle (ø=0.8 mm) connected to a piece of tubing about 0.67 ml/kg of the final suspension of beads was administered transthoracically into the left heart ventricle. Within 5 min after the Brietal® injection, the animals were conscious – sitting upright and moving their heads and extremities.

Autoradiography

The autoradiographic technique was in accordance with Ullberg (39). Briefly, whole brains were sectioned *in situ* in 40 μm thick sections, very few sections being lost in the process. The consecutive series of sections was dehydrated through storage at -20°C and then, some days later, put on film at room temperature for exposure at -20°C. The exposed films were then developed, fixed and rinsed.

RESULTS

4 foci with decreased ^{14}C-DMO activity (suggesting a decreased tissue pH) located in subcortical structures could be detected – two of them were found in one of the rabbits and the other two were found in two other animals (Fig. 1). Thus, these experiments did not indicate that an acidosis was generally present in these small ischemic regions. However, an interesting finding was that in two out of the four animals one of the hippocampi showed a lower ^{14}C-DMO activity than

the other (Fig. 1). This finding seemed to correspond to the diffusely increased 2-deoxyglucose (a tracer for cellular glucose uptake (34)) previously found in the hippocampi of embolized rabbits (Fig. 2).

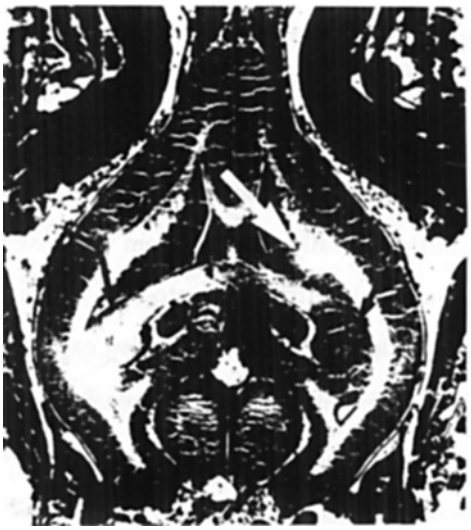

Figure 1. A ^{14}C-DMO autoradiogram. A focus of somewhat decreased ^{14}C-DMO activity indicating a lower tissue pH can bee seen (white arrow). Further it can be seen that one of the hippocampi has a lower ^{14}C-DMO activity than the other (black arrows) – compare with Fig. 2.

Figure 2. 2-deoxy-D-[^{14}C] glucose autoradiograms from a previous study on conscious rabbits [29]. a) A region with enhanced 2-deoxy-D-[^{14}C] glucose (2-DG), located in the right caudate nucleus, can be seen. The focus has a central dip in the 2-DG accumulation. b) This figure shows diffusely increased hippocampal 2-DG accumulation (arrow). This region probably does not reflect ischemia, but rather an increased cellular activity. Published with permission.

DISCUSSION

Changes in the pH are considered to reflect alterations in cerebral circulation and metabolism and are believed to be of importance to the final ischemia related damages (67). This work did not supply support for that acidosis is of any general importance in the process of microinfarction.

The sparseness (4 foci in a model generating about 25 ischemic regions per brain (28, 29)) of the foci with decreased ^{14}C-DMO activity may, for instance, be explained by the protons from an increased glycolysis being effectively buffered by buffers locally at hand from the onset of the ischemic process and by buffers continuously diffusing into the ischemic regions from the surroundings. A glucose deficiency (27) may also explain why ischemia does not cause acidosis. Another reason may be a low sensitivity of the ^{14}C-DMO method used.

Reconsidering Figs. 1 and 2, we see that in the hippocampus the increased 2-deoxyglucose accumulation and the decreased ^{14}C-DMO activity may reflect the same process – increased glycolysis. Whether these observations reflect the presence of processes affecting important general brain functions remains to be studied (it is well known that the hippocampus is of importance for memory). Such "activations" may well reflect processes of importance for the development of brain dysfunction, e.g. dementia. In fact we have preliminary results showing increased selective neuronal death in the hippocampi of embolized rabbits. This is an interesting finding worthy of further attention.

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