Goldthioglucose and the Blood-Brain Barrier

It has been postulated that the ventromedial nucleus (VMN) of the hypothalamus is a satiety area containing glucoreceptors. The evidence in favor of this view has been recently reviewed[1]. One important finding supporting this evidence was the demonstration that goldthioglucose produces lesions in the ventromedial area of mice[2] followed by hyperphagia and obesity. The effect is similar to that which can be induced by electrolytic lesions[3]. These chemically-induced lesions will be obtained only if the glucose moiety is attached to gold by the sulfur bridge. Goldthiogalactose, goldthiosorbitol and other goldthio compounds do not produce lesions[4]. Studies using the technique of neutron radioactivation of gold, have shown that gold does accumulate in the VMN when goldthioglucose is injected, but does not when goldthiomalate is administered under the same conditions[5].

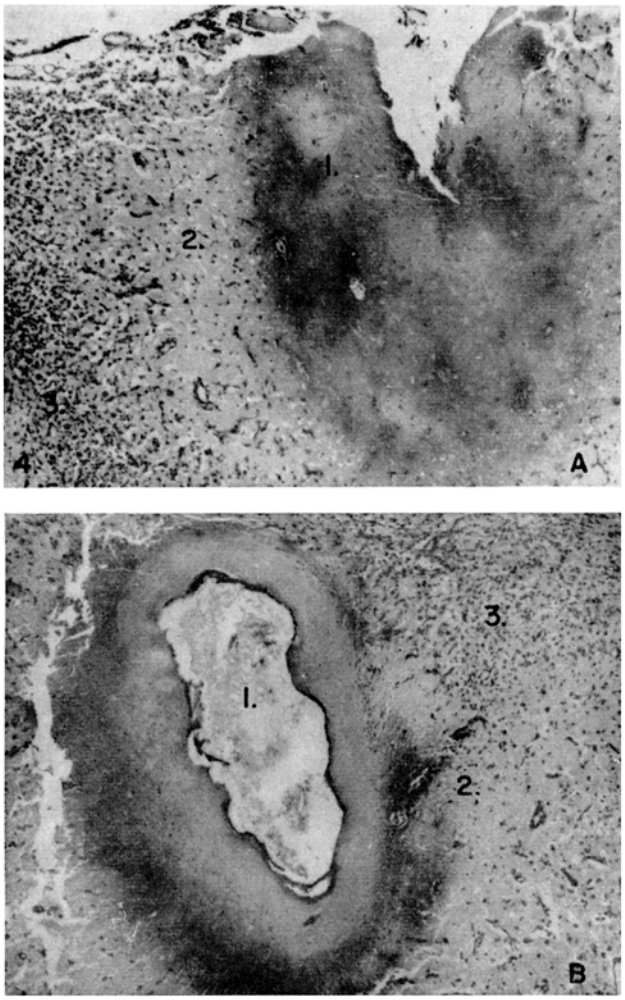
In spite of the evidence for the chemical specificity of the action of goldthioglucose, PERRY and LIEBELT[6] have stated that goldthioglucose-induced lesions occur not because the VMN has a peculiar affinity for glucose, but because of a weakness in the blood-brain barrier at this site. Their hypothesis would lead to the expectation that similar lesions should occur in other areas of the brain in which the properties of the blood-brain barrier have been purposely altered. The aim of the study reported here was to test PERRY and LIEBELT's alternate hypothesis.

It has been well established that many substances, such as electrolytes, dyes, or more complex molecules which do not enter the normal brain, will do so readily at the site of any focal brain lesion[7]. In particular, those lesions which cause large areas of cerebral necrosis have been shown to increase the permeability of the blood-brain barrier at the lesioned site lasting at least several days[8].

In this study, 2 methods were used to induce lesions in the cerebrum. First, 13 female rats (Charles River CD) weighing between 175–225 g were lesioned electrolytically by passing a direct current of 2 mA for 15 sec through an insulated stainless steel electrode that was bared at the tip for 0.5 mm and which had been inserted to a depth of 1 mm in the cerebrum through a small hole drilled in the skull. These lesions were placed at the level of bregma, 1 mm lateral to the midline.

Approximately 18 h after lesioning, 8 of the 13 rats were injected i.p. with goldthioglucose in concentrations equal to 1 mg/g of body weight. The other 5 rats were used as controls, with 2 of the 5 injected with 0.5 mg of glucose/g body weight. The other 3 rats received no injections. About 48 h later, all rats were sacrificed and sectioned in a manner previously described[9]. Microscopic examination of sections stained with the Nissl method showed no evidence that goldthioglucose reacted with cortical tissue adjacent to the electrolytic lesion. No difference was apparent between animals injected with goldthioglucose and those that served as controls. All cortical lesions were approximately the same size and showed a relatively large necrotic region surrounded by a lightly staining area consisting mostly of gitter cells. A more densely packed band of glia cells separated this region from normal cortical tissue. The concentration of goldthioglucose used was adequate, since 7 of 8 injected rats developed lesions in VMN of hypothalamus. Typical sections from a goldthioglucose-injected rat and a control are shown in the Figure.

To insure that the electrolytic method used to lesion was not masking any effects that the goldthioglucose may have made on the cortex, a second lesioning method was used. Here the electrode was simply inserted and removed from the cortex in 5 additional female rats, producing a smaller but definite lesion. Approximately 6 h after lesioning, 3 of the rats were injected i.p. with a 1 mg/g body weight concentration of goldthioglucose, while the other 2 received no injection. Likewise in these animals,



Portion of rat cortex lesioned electrolytically. (A) Goldthioglucose-injected. (B) Control. (1) Necrotic area; (2) lightly staining region consisting mostly of gitter cells; (3) dense band of glia cells; (4) normal neuronal tissue.

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no difference was observed around the lesioned site between any of the 5 animals. In all 5 animals, only a slight increase in the number of gitter cells was observed in the area immediately surrounding the lesion. Normal tissue, without any further obvious increase in glia cells, was found adjacent to this area.

The results show that merely increasing the permeability of the blood-brain barrier is not sufficient to permit the induction of a localized goldthioglucose lesion. These findings give no support to the hypothesis of PERRY and LIEBELT[6] (which incidentally does not explain why only goldthioglucose would go through a 'weakened' blood-brain barrier site). Their hypothesis also is inconsistent with the prevention of lesions in rats made diabetic by alloxan and then administered goldthioglucose[10]. The view that the VMN contains glucoreceptors remains the more probable. That goldthioglucose may be found to act in other localized areas may mean that such areas also contain glucoreceptors. The multiplicity of physiological functions dependent on neural glucose sensors (e.g. control of epinephrine secretion, supererogatory control of insulin secretion) make the existence of a number of such glucoreceptive areas possible[11,12].

Résumé. Après avoir augmenté la perméabilité corticale sang-cerveau chez des rats, on a donné à ces animaux une injection d'aurothioglucose. Aucune lésion due à l'aurothioglucose n'a été decouverte dans aucun des sites de perméabilité accrue. Les résultats montrent que la production de lésions par l'aurothioglucose n'est pas due à une permeabilité non-specifique dans certains sites hypothalamiques; ils confirment la théorie glucostatique qui suggère qu'il existe des glucorécepteurs à affinité définie situés à ces sites.

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The Fiber Spectrum of the Cat VI Nerve to the Lateral Rectus and Retractor Bulbi Muscles

The VI nerve of the cat supplies the lateral rectus muscle and the 4 slips of the retractor bulbi. Histologically, 3 muscle fiber types have been found in the lateral rectus on the basis of sarcoplasmic reticulum, myofibril size, and size and distribution of mitochondria[1]. In the retractor bulbi, only a single fiber type has been observed[2].

Although VI nerve fiber spectrum studies have been reported[3,4], they have not included the supply to the retractor bulbi. In view of the differences between lateral rectus and retractor bulbi muscle fiber types, the spectrum of the nerve fiber supply and the innervation ratio were investigated, in order to correlate extraocular nerve and muscle fiber types.

Methods. In 4 cats (2.3–2.6 kg in weight), following a lethal dose of Diabutal, the VI nerve was fixed in situ by perfusion via the carotid arteries with 5% glutaraldehyde buffered with 0.15 m sodium cacodylate and post fixed in osmium tetroxide buffered with phosphate. Nerve cross-sections were taken (1) at the exit of the VI nerve from the brainstem, (2) at the entrance of the VI nerve to the lateral rectus, and (3) of the branch to the retractor bulbi at its emergence from the VI nerve. All sections were made before the occurrence of intramuscular splitting. Specimens were embedded in araldite. Cross-sections of 0.5–0.7 μ were cut and stained by Richardson methylene blue method. Fiber diameter, including myelin sheath, was measured by superimposing clear plastic circles of known diameter over photographs enlarged 2000–2500 times. 4 nerves were analyzed at each of the above 3 levels. Total fiber number was counted and a sample of 500 fibers/nerve was measured, except in the retractor bulbi supply, where the total supply, always under 500, was measured.

ALVARADO (unpublished results) has determined the muscle fiber count of 1 superior lateral slip of the retractor bulbi. To derive an innervation ratio utilizing his count, it was necessary to determine the proportion of the total retractor bulbi contributed by the superior lateral slip. Thus, in 3 cats, the 4 slips were dissected out and weighed.

Results. The Figure presents the distribution of fiber diameters at the 3 levels of the VI nerve. Data are based on averages from 4 cats of measurements of 500 fibers per nerve (except for the retractor bulbi) and are plotted as % of total fibers at each level in 1 μ increments. Fiber diameters ranged from 1–21 μ for each level, with the distribution in the branch to the lateral rectus being similar to that of the cranial section of the VI nerve. Spectra at these 2 levels indicate an initial peak at 3–4 μ, a secondary peak at 6 μ and a smooth extension to 21 μ. In contrast, the branch to the retractor bulbi reveals a concentration of fibers between 6 and 14 μ.

The Table presents the total fiber count of each 4 nerves at the cranial level and in the branches to the lateral rectus and retractor bulbi. It will be noted that approximately 7 times as many fibers innervate the lateral rectus as the retractor bulbi.

Many fibers from 1–5 μ in size in the cranial and the lateral rectus sections had thin myelin sheaths in contrast to the thick myelin around the larger axons. The ratio of

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