Pathophysiological Responses to Acute Cerebral Ischemia in the Gerbil

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Abstract: Pathophysiological Responses to Acute Cerebral Ischemia in the Gerbil

The left carotid artery of healthy, male gerbils was surgically ligated to induce a state of cerebral ischemia. The animals were sacrificed two, four, six, 12, 24 and 48 hours postligation to determine whether the induced cerebral ischemia would be reflected by changes in blood chemistry. The gerbil, unlike the rat, has a well-defined circle of Willis and cerebral arterial branches which resemble the arrangement of cerebral arteries found in man.

The gerbils withstood the induced cerebral ischemia relatively well with few overt signs of cerebral damage, e.g., muscular paralysis, and a low mortality rate. However, intense lipid mobilization accompanied by depletion of peripheral adipose tissue sites and fatty infiltration of the liver developed very promptly. Marked cerebral edema and definitive foci of cerebral infarction occurred in both the frontal and medial lobes of the brain. The adrenal cortices of the gerbil were markedly depleted of lipid concomitant with severe thymus gland involution indicative of intense corticosteroid release.

The serum enzymes, creatine phosphokinase and glutamic oxaloacetic transaminase, both rose promptly, indicating cerebral and hepatic damage respectively. Lipids, i.e., triglycerides, free fatty acids and total cholesterol, all rose promptly and to significantly above-normal levels and remained super-normal throughout the 48-hour course of the experiment. Concomitant with the hyperlipidemia and other metabolic changes there were definite hyperglycemia and elevation of BUN levels in a brisk response to the induced, acute cerebral ischemia. The gerbil adrenal gland produces hydrocortisone predominantly. Blood levels of hydrocortisone rose quickly to super-normal levels in response to the stress of cerebral ischemia but fell to significantly below-normal levels 24 and 48 hours after carotid artery ligation. These findings demonstrate that acute cerebral ischemia constitutes a severe stress in the gerbil which is reflected, almost quantitatively, by temporal changes in such blood constituents as enzymes, lipids, glucose, and adrenal stress hormones.

ADDITIONAL KEY WORDS circle of Willis carotid artery ligation cerebral infarcts cerebral edema fatty liver CPK SGOT triglycerides free fatty acids total cholesterol glucose BUN

In earlier publications in this journal Wexler et al.1,2 reported that the experimental induction of acute cerebral ischemia by carotid artery ligation in arteriosclerotic breeder rats versus nonarteriosclerotic virgin rats was accompanied by metabolic changes which were commensurate with the severity of the experi-
mentally induced cerebral ischemia. That is, unilateral carotid artery ligation caused acute alterations in the serum levels of enzymes, i.e., CPK, SGOT, lipids, i.e., triglycerides, free fatty acids and cholesterol, hyperglycemia and elevated adrenal corticosterone (compound B) production.1 Bilateral carotid artery ligation or more severe cerebral ischemia constitutes a severe stress and that, in the rat, the severity of cerebral ischemia may be “biologically titrated” by changes in blood enzymes, lipids, glucose and adrenal steroids in much the same way that the course of myocardial ischemia may be followed clinically through the use of these same serum constituents. In addition, it was also found that the arteriosclerotic subjects reflected much more deleterious cerebral ischemia when their sclerotic carotid arteries were ligated by virtue of the greater excursion of these blood constituents compared to nonarteriosclerotic virgin rats.1,2 The implications drawn from these findings are that the assumed impeded cerebral blood flow through the arteriosclerotic arteries added to the stress of the induced cerebral ischemia.

One of the drawbacks in using the rat as a subject for the investigations of cerebrovascular disease is that the anatomical arrangement of the circle of Willis and its branches is not as well defined as it is in man. Despite the more rudimentary nature of the circle of Willis (and its branches) in the rat, it is difficult to produce bona fide cerebral infarcts in the rat. Therefore, we elected to use the gerbil whose cerebral arterial system is anatomically distributed in a pattern which closely resembles the cerebrovascular pattern found in man, e.g., a well-defined circle of Willis with anterior, middle and posterior cerebral arterial branches. Acute cerebral ischemia was induced in gerbils by ligating one carotid artery and the pathophysiological changes which followed were measured, on a temporal basis, to determine whether a species with a more highly evolved cerebral vascular system would react more or less dynamically to acutely induced cerebral ischemia than a species (rat)1,2 with a more rudimentary cerebral arterial supply.

Methods
Mature, male gerbils (Meriones unguiculatus) weighing 60 ± 5 gm were purchased from Tumblebrook Farms, Brant Lake, New York. They were housed in our air-conditioned, humidity-temperature- and light-controlled Animal Research Colony. After several weeks’ adjustment to our animal quarters and diet of commercial rat chow, which is relatively low in fat (4% fat content), we culled all animals which appeared sick in any way to insure the use of healthy animals only. Mortality due to carotid artery ligation is relatively low in the gerbil (15%) compared to the Sprague-Dawley rat (50%). However, because of the comparatively small volume of blood in the gerbil it was necessary to pool the blood from three gerbils to have sufficient serum to adequately measure all of the various serum constituents described in this report. Therefore, a total of 216 gerbils had to be used in order to insure an adequate number of survivors after carotid artery ligation and to provide sufficient blood for the chemical analyses. Accordingly, 72 gerbils served as baseline controls whose carotid arteries were manipulated but not ligated, i.e., sham operation. Since the blood of three animals was required this provided us with 24 pools to establish baseline control values.

The animals were anesthetized with secobarbital (Seconal). A longitudinal incision (1 cm) was made lateral to the midline over the hyoid muscles. Fascia and muscles were displaced to expose the triad of carotid artery, jugular vein, and vagus nerve. The carotid artery was carefully separated from this complex, and a single ligature placed about the common carotid artery 0.5 cm below the bifurcation of the carotid artery into the external and internal carotid arteries. The ligature was tied snuggly to occlude but not damage the vessel. Muscle and skin were closed as separate layers. The animals recovered promptly from their light secobarbital anesthesia, were returned to their cages, and were allowed to have food and water on an ad libitum basis.

The animals were sacrificed by decapitation to avoid the stress of anesthesia, and blood was collected from the severed neck vessels. The animals were sacrificed, sequentially, two, four, six, 12, 24 and 48 hours after unilateral carotid artery occlusion. The blood of each animal was spun in a refrigerated centrifuge and the serum frozen and stored until time of analysis. The following serum parameters were measured by means of automated techniques (Auto-Analyzer, Technicon): creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), total cholesterol, free fatty acids, and glucose. In addition, serum hydrocortisosterone (compound F) levels, the main adrenocortical steroid in the gerbil were also measured.8
At autopsy, each animal was carefully examined for any evidence of cerebral damage or other complications. In addition, the site of ligation was carefully checked; animals showing questionable ligation were discarded. Pertinent tissues, such as brain, heart, aorta, carotid arteries, thymus, adrenal, liver, and kidney, were weighed and fixed in 10% neutral formalin (Lillie) for histopathological examination. Tissues were embedded in paraffin and sectioned at 3 μ. Frozen sections for demonstration of lipids were cut at 10 μ.

Results

A. GENERAL OBSERVATIONS

We have had extensive experience with carotid artery ligation in the rat. In the rat, there is a very prompt onset of signs similar to Horner's syndrome, blanching of the eye, convulsions, extensor rigidity, paraplegia and high mortality following carotid artery occlusion. In the gerbil, these adverse effects were not particularly manifest.

At autopsy, all of the animals exhibited severe fatty metamorphosis of the liver within four to six hours after carotid artery ligation. This condition of fatty liver was transient and became less pronounced so that from 12 to 48 hours postligation all animals displayed some extra hepatic fat, but this was found histopathologically and was no longer grossly visible. All of the animals manifested progressively worsening cerebral edema after carotid artery ligation and gross inspection of their brain tissue suggested the strong likelihood of the presence of cerebral infarction, of diffuse distribution, but confined to the frontal and

Liver of a gerbil four hours after carotid artery ligation. Concomitant with involution of peripheral adipose tissue and hyperlipidemia the hepatic parenchyma shows intense fatty infiltration of diffuse distribution. Hematoxylin and eosin, X150.
midcerebral areas. Evidence of grossly visible cerebral damage was found as early as two hours after carotid artery ligation.

B. MICROSCOPICAL OBSERVATIONS
There were no histopathological changes in any of the organs examined except the liver, brain, adrenal and thymus glands. Hepatic lipid droplets, without any definitive distribution pattern, i.e., centrolobular or peripheral, was found in all lobules, becoming particularly dense four hours postligation (fig. 1). The suspected brain damage, as viewed by gross inspection, was found to be bona fide cerebral infarcts (figs. 2-4). The infarcts were splotty and scattered in their distribution; occasionally these infarcts appeared to be confluent, and none of the necrotic foci showed evidence of margination. Although the brain of each animal was examined histopathologically it was not possible to completely section each brain for extensive microscopical scrutiny. Rather, random sections were taken. However, on this basis of random microscopical examination 76% of the brains were found to have some evidence of cerebral necrosis, and virtually all of the brains exhibited some degree of cerebral edema. The adrenal cortices showed progressively increasing lipid depletion, first from the inner cortical zones, i.e., zona reticularis, and then, when the biochemical analyses showed subnormal circulating compound F levels, the cortical lipid depletion extended into the upper portions of the zona fasciculata. The thymi were virtually totally involuted.

C. PATHOPHYSIOLOGICAL CHANGES IN THE SERUM

Enzymes
There was a very prompt and striking increase in serum CPK levels which reached a peak at six hours postligation, and although the CPK levels began to decline they had not returned to normal within the 48-hour time course of this experiment (fig. 5). Because of insufficient serum it was not possible to run an adequate number of SGOT analyses to make any definitive conclusions. However, it was apparent that SGOT levels were also elevated four to 12 hours postligation.

Lipids
There was a very marked increase in serum triglycerides four hours postligation, peaking at
GERBIL: CEREBRAL ISCHEMIA

Changes in serum creatine phosphokinase activity (in adult male gerbils sacrificed two, four, six, 12, 24 and 48 hours after unilateral carotid artery ligation to induce cerebral ischemia). The control animals had been subjected to sham carotid artery ligation only. Each point on the graph depicts the mean ± standard error; the numbers in parentheses indicate the number of pools used for that determination; each pool consists of the combined blood of three individual animals. This same format applies to figures 6 to 11.

12 hours but remaining substantially elevated throughout the course of the experiment (fig. 6). There was an unusually rapid and high elevation of free fatty acid levels which persisted throughout the course of the experiment (fig. 7). Cholesterol levels were similarly elevated (fig. 8).

Carbohydrate and Blood Urea Nitrogen (BUN)
In keeping with the marked hyperlipidemia there was definite hyperglycemia which reached a zenith at six hours postligation and returned to normal 48 hours postligation (fig. 9). The BUN level was significantly elevated within two hours postligation. Because of the paucity of serum, analyses for BUN levels could not be completed for the 24 and 48 postligation groups. However, the pattern of excursion of BUN levels indicated a downward trend in BUN levels after an initial steep rise (fig. 10).

Adrenal Steroids
The gerbil adrenal produces compound F predominantly in contrast to the rat whose adrenal produces compound B. In keeping with the stress of acute cerebral ischemia the circulating compound F levels showed an acute and significant increase two to 12 hours postligation followed by significantly below-normal levels 24 and 48 hours postligation (fig. 11).

Discussion
These experimental findings demonstrate that the acute cerebral ischemia induced by unilateral carotid artery ligation in the gerbil will induce severe cerebral edema and infarction. The pathophysiological changes which accompany the cerebral ischemia bear a close resemblance to the spectrum of pathophysiological changes which attend myocardial ischemia, e.g., elevated enzyme levels (CPK, SGOT), hyperlipidemia, hyperglycemia and increased adrenocorticoid production. Further, the pathophysiological changes which attend the in-
Reduced cerebral ischemia in the gerbil are highly reminiscent of those changes which we have observed in the rat under similar conditions. However, it is of interest that the time of onset of these pathophysiological changes is much more acute in the gerbil than in the rat. Whether these differences between the two species could be a reflection of the more highly evolved circle of Willis and cerebral arterial supply in the case of the gerbil versus the rat is a moot question. In this same connection, there is an apparent paradox in that the rat displays more overt signs of cerebral damage as demonstrated by the appearance of Horner's syndrome, extensor rigidity, paraplegia, convulsions and a significantly greater mortality rate after carotid artery ligation, whereas the gerbil manifests little or none of these overt changes. The gerbil, in contradistinction to the rat, exhibits much more acute changes in blood chemistry and a significantly greater incidence of cerebral infarction. As a matter of conjecture, these observations could be interpreted to mean that the more highly evolved cerebrovascular system of the gerbil is much more susceptible to acute carotid artery constriction than the more primitive cerebral vascular system of the rat.

Another remarkable finding in these experiments, which we have also encountered under similar stressful circumstances of both cerebral and myocardial ischemia, is the transient appearance of severe, fatty metamorphosis of the liver. The fatty infiltration of the liver concomitant with the hyperglycemia and acutely increased adrenal steroid levels could be indicative of a "metabolic reflex" in response to acute cerebral ischemia, i.e., steroid-induced glucose and lipid mobilization, as a body defense mechanism.

Insofar as the enzyme changes are concerned the pattern of change in the CPK levels are certainly commensurate with the onset of acute cerebral ischemia and impending infarction. The CPK changes suggest that cerebral ischemia reached a zenith of intensity six hours postligation but that the consequences of the cerebral ischemia persisted as indicated by the persistently elevated CPK.

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FIGURE 7
Changes in free fatty acids.

FIGURE 8
Changes in total cholesterol.
GERBIL: CEREBRAL ISCHEMIA

Changes in glucose.

Changes in blood urea nitrogen.

levels. Although our SGOT analyses are incomplete, the delayed rise of this enzyme was probably not only due to the consequences of cerebral ischemia but also could be indicative of the severe fatty infiltration of the liver which was so pronounced in these animals. That is, although CPK is a reasonably good index of cerebral, myocardial and skeletal muscle damage, SGOT levels can be elevated due to a greater variety of causes and especially in response to liver impairment.

The striking increase in serum triglycerides, free fatty acids and total cholesterol also attests to the intense metabolic changes which accompany the induced cerebral ischemia. The intense fatty infiltration of the liver is probably due to the intense lipid mobilization. It is of interest that the peripheral adipose tissue sites, e.g., peri-adrenal and carcass fat, etc., were all greatly depleted and watery in appearance during this period of intense hyperlipidemia.

The acute hyperglycemia which also accompanied the cerebral ischemia could not be accounted for by any obvious changes in islet beta cell granulation, i.e., insulin secretion. Therefore, it would be more reasonable to ascribe the hyperglycemia, like the hyperlipidemia, to the gluconeogenic effects of the observed increased compound F production.

The greatly elevated BUN levels also attest to the stressful nature of the cerebral ischemia. The acute elevation of the other metabolic parameters concomitant with increased BUN levels is indicative not only of the on-going necrosis and catabolism but also of possible concurrent renal pathophysiology. Azotemia is the hallmark of shock and renal failure.

Although the gerbil is well suited for the experimental investigation of cerebrovascular disease, few investigators have availed themselves of its use. One report, however, by Levine and Payan,\(^7\) corroborates our finding of the gerbil's high susceptibility to cerebral ischemia by carotid artery ligation alone. They also made the noteworthy finding of vacuolation of the white matter of the optic tract in the hemisphere contralateral to the ligation site, Wallerian degeneration of retinal ganglion cell axons, and ischemic retinopathy.

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Finally, the greatly elevated compound F levels reinforce our contention that cerebral ischemia constitutes a severe stress and that this is reflected in the production of unusual quantities of adrenal hormones and that the unusual lipid mobilization observed here and gluconeogenesis may be a reflection of this intense stimulation of increased steroidogenesis. It is particularly interesting that this initial increased adrenocorticoid production was eventually followed by adrenal steroidogenic failure, i.e., so-called "adrenal exhaustion," as indicated by greatly depressed or subnormal compound F levels. In our previous experiments with induced cerebral ischemia in rats we had observed the same hypersecretion of compound B in the early stages of cerebral ischemia followed by greatly depressed compound B levels in the latter stages of cerebral ischemia.1,2

References
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