Comparative Effects of Unilateral and Bilateral Carotid Artery Ligation in the Spontaneously Hypertensive Rat

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SUMMARY Male and female, spontaneously hypertensive rats (SHR) with blood pressures ranging from 190–210 mmHg were subjected to unilateral or bilateral carotid artery ligation. Representative numbers of animals were killed 2, 4, 6, 8, 10, 12, 24 and 48 hours later. Severe cerebral ischemia caused a significant and protracted increase in the pre-existent high blood pressure, the enzymes CPK, SGOT and LDH triglycerides, free fatty acids, glucose, and corticosterone. Despite these marked pathophysiologic changes, the brains of these animals were free of real damage except for cerebral edema and scattered petechiae. Some of the animals developed massive atrial thrombi and myocardial infarcts. It is suggested that severe cerebral ischemia precipitated the myocardial infarcts through the aegis of the hypothalamic-pituitary-adrenal stress response.

methods

Male and female, 100 day old, spontaneously hypertensive rats born and raised in our Animal Research Colony were used. These animals were derived from the original Okamoto-Aoki (Kyoto) strain provided through the generosity of Dr. Carl T. Hansen, Animal Genetics Division, N.I.H. The animals were fed a commercial rat chow diet ad libitum which is relatively low in fat (4%). The animals were housed in air-conditioned quarters where light, heat, and humidity were controlled and monitored.

Male (280 ± 10 gms) and female (203 ± 5 gms) SHR, selected randomly, served as controls and experimental animals. Just prior to surgery, the systolic blood pressure of each animal was determined under light Seconal (secobarbital) anesthesia (1 to 2 mg/100 gm bw, ip) using the Friedman:Freed microphonic manometer and tail cuff which measures systolic blood pressure. The experimental animals were given a supplemental injection of secobarbital (4 mg/100 gm bw, sc) and were then subjected to unilateral or bilateral carotid artery ligation as previously described.1-3 The carotid artery was carefully separated from the jugular vein and vagus nerve and a single ligation placed about the common carotid artery 2 cm below the bifurcation of the carotid artery into the external and internal carotid arteries. The ligature was tied snugly to occlude but not damage the vessel. (Previous experience demonstrated that sham carotid artery manipulation did not cause any significant changes in the blood constituents measured. For this reason, all of the animals prepared surgically were used as experimental subjects.) A large number of animals were used to ensure a sufficient number of survivors, i.e., 12 animals for each control group and a minimum of 8 for each experimental group. Following carotid artery ligation, the animals were killed 2, 4, 6, 8, 10, 12, 24 and 48 hours later to establish a dynamic

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record of the pathophysiologic sequelae which attend acute cerebral ischemia. The blood pressure was recorded again at each of the time intervals. The animals were killed by instant decapitation and blood was collected from the severed neck vessels. The blood of each animal was spun in a refrigerated centrifuge and the plasma frozen and stored until time for analysis. The following blood chemistries were measured by means of automated techniques (Auto-analyzer, Technicon): creatine phosphokinase (CPK), glutamic oxaloacetic and pyruvic transaminases (SGOT and SGPT), lactic dehydrogenase (LDH), triglycerides, free fatty acids, total cholesterol, glucose, and blood urea nitrogen (BUN). Circulating corticosterone, the main adrenocortical steroid in the rat, was also measured by an automated fluorometric method as an index of adrenocortical secretion. At autopsy each animal was examined carefully for any evidence of cerebral or cardiovascular damage. Pertinent tissues such as brain, heart, thymus, adrenal, liver, and kidney were weighed and fixed in 10% neutral formalin for histopathological examination. Tissues were embedded in paraffin and sectioned at 3μ. Frozen sections for demonstration of lipids were cut at 10μ. Adjacent sections were stained with hematoxylin and eosin for routine analysis, alcin blue and toluidine blue for metachromasia, the Hale stain for mucopolysaccharides, the von Kossa method to demonstrate calcium, oil red O and Sudan black B for lipids, and the Klüver-Barrera stain for brain tissue.

All of the data were subjected to analysis of variance using Student's t-test to determine statistical significance; p values less than 0.05 were considered non-significant.10

Results

A. General Observations

None of the SHR subjected to unilateral carotid artery occlusion manifested any outward signs of cerebral ischemia whereas 82% of the bilaterally ligated SHR (male and female) either convulsed, displayed Horner's syndrome, paralysis, or extensor rigidity.

Only 2 males and 3 females died of the 64 male and 64 female SHR subjected to unilateral carotid artery ligation; 13 male and 15 female SHR died of the 64 male and 64 female SHR subjected to bilateral carotid artery ligation. Of special note was the fact that female SHR subjected to bilateral carotid artery ligation did not recover from the anesthesia until 8 h post-ligation. Whereas male SHR (bilateral ligation) began to succumb immediately after ligation, females (bilateral ligation) did not die until they began to recover from the anesthesia. Nine of the 15 deaths among the female SHR (bilateral ligation) occurred between 24 and 48 h post-ligation.

B. Changes in Systolic Blood Pressure

Although blood pressures were elevated greatly in these spontaneously hypertensive rats, the acute induction of cerebral ischemia caused the blood pressure to rise even further (fig. 1). Mild cerebral ischemia (unilateral) caused an acute rise in the blood pressure of male and female SHR but this increase was evanescent in the male and prolonged in the female (fig. 1). Severe cerebral ischemia (bilateral) caused a very significant and sustained increase in blood pressure in both males and females, attaining levels of 240 mm Hg (fig. 1).

C. Changes in Blood Chemistry

Enzymes

Using the rise and fall of circulating enzymes (CPK, SGOT, SGPT and LDH) as an index of cerebral, myocardial, and hepatic damage, we found definite increases in circulating CPK levels with maximally elevated levels in male SHR subjected to bilateral carotid artery ligation (fig. 2). The zenith of CPK elevation occurred between 4 and 12 h post-ligation. The SGOT levels of all animals rose progressively following the induction of cerebral ischemia, reaching a zenith 10 to 12 h post-ligation, promptly receding to normal thereafter (fig. 3). There was no apparent pattern or significant change in circulating SGPT levels. Male and female SHR displayed peak levels of lactic dehydrogenase at 6 and 12 h, respectively (fig. 4). There was little differentiation between enzyme increase and severity of cerebral ischemia.
Lipids

All of the animals manifested a brisk and statistically significant ($p < 0.001$) increase in circulating triglycerides within 2 to 4 h after acute cerebral ischemia (fig. 5). The maximum increase in triglyceride levels occurred in those animals subjected to severe cerebral ischemia. All of the animals developed an acute elevation of free fatty acids in their blood with a supernormal increase in male SHR sub-

![Figure 2. Changes in plasma creatine phosphokinase levels.](image)

![Figure 4. Changes in plasma lactic dehydrogenase levels.](image)

![Figure 3. Changes in plasma glutamic oxaloacetic transaminase levels.](image)

![Figure 5. Changes in plasma triglyceride levels.](image)
jected to mild cerebral ischemia. SHR subjected to severe cerebral ischemia displayed unusually high and prolonged elevation of free fatty acid levels (fig. 6). Circulating total cholesterol levels ranging between 72 and 113 mg % (normal = 93 mg % for males, 104 mg % for females) did not exhibit any significant changes during the 48 h course of the experiment.

**Glucose**

SHR of our strain are spontaneously hyperglycemic (glucose = 150 ± 5 mg %); these SHR were normoglycemic (fig. 7). Those animals exposed to mild cerebral ischemia manifested acute and significant hyperglycemia; those subjected to severe cerebral ischemia exhibited a more delayed rise but became much more hyperglycemic and on a more sustained basis (fig. 7).

**Blood Urea Nitrogen**

BUN levels ranged between 15 and 31 mg % (normal = 26 mg %) during the 48 h period. There was no significant pattern of change in the BUN levels.

**Corticosterone**

Because these animals were lightly anesthetized during the initial recording of blood pressure, the control blood corticosterone levels were above normal, i.e., anesthesia constitutes a mild stress and in hyperreactive SHR will cause substantial increases in corticosterone (fig. 8). There was a distinct dichotomous pattern in adrenal secretory patterns between those animals subjected to mild vs severe cerebral ischemia. Mild cerebral ischemia caused only a slight and evanescent increase in adrenal secretory activity, whereas severe cerebral ischemia was mirrored by supernormal increases in corticosterone 2 to 12 h post-ligation (fig. 8).

**D. Gross and Microscopic Pathology**

Concomitant with their hyperlipidemia, all of the animals showed progressively worsening fatty infiltr-
tion of the liver accompanied by grossly visible disappearance of peripheral adipose tissue sites, e.g., peri-adrenal and mesenteric fat. The fatty liver condition was much more intense in animals exposed to severe cerebral ischemia.

There were no additional untoward changes found in the SHR subjected to mild ischemia. At autopsy, several SHR with severe cerebral ischemia had extravasated thoracic fluid, i.e., hydrothorax, and 12% of the males and 7% of the females had massive myocardial infarcts (fig. 9) which occurred late, post-ligation. Many of the hearts also displayed large, occlusive, atrial thrombi (fig. 10).

Despite the ubiquitous presence of advanced cerebral edema and scattered petechiae in SHR with severe cerebral ischemia, there was no evidence of necrosis or damage by gross and microscopic examination.

Discussion

In our earlier work, normotensive Sprague-Dawley rats manifested many untoward changes in response to unilateral carotid artery occlusion. Paradoxically, despite the pre-existent, severe hypertension in SHR, the induction of acute cerebral ischemia did not cause any cerebral necrosis. We have found that SH rats are usually resistant to alloxan-induced diabetes, myocardial infarction, increased dietary saline, and the induction of arteriosclerosis. This would suggest that SHR have different hormonal, metabolic, and cardiovascular attributes than normotensive strains of rats. Ogata et al., and I examined the cerebral cortex, corpus striatum, callosal radiation, hippocampus and midbrain but Ogata et al., found bilateral, diffuse and extensive cerebral infarcts in their SHR involving the frontal, medial, and occipital regions. Our animals were 100 days old and had equally severe hypertension, whereas Ogata's animals ranged from 5 to 9 months of age. This would suggest that aging or the chronicity rather than the severity of hypertension is the prime vector in conditioning necrosis of the cerebrum. The fact that the mild cerebral ischemia of unilateral carotid artery ligation, combined with hypertension, caused only brain edema, petechiae, paralysis and a few deaths could be due to the fact that collateral cerebral arteries can sustain brain tissue under conditions of impeded supply. SHR have adequate connecting arteries between the carotid and vertebrobasilar systems. It has been shown that experimental occlusion of one carotid artery will cause a 100% increase in basilar artery blood flow; occlusion of both common carotid arteries causes a 300% increase in basilar artery flow. Although male rats are much more prone to succumb to acute myocardial infarction, there was no apparent sex difference in response to the acutely-induced cerebral ischemia. It is noteworthy that female rats in general have less efficient hepatic capacity than males to metabolize barbiturates and the more prolonged anesthesia in the female SHR apparently protected them from the untoward effects of cerebral ischemia until they began to recover from the anesthesia in the later hours post-ligation.

The slight increase in the pre-existent high blood pressure following mild cerebral ischemia and the protracted and greatly exacerbated high blood pressure...
CAROTID ARTERY LIGATION IN SHR/Wexler

Figure 10. Left atrium of same animal shown in Fig. 9. The entire atrial chamber is occluded by a massive thrombus and the atrial tissue itself shows extensive hemorrhage and wbc infiltration. H & E, X 25

Pressure following severe cerebral ischemia is in keeping with experimental and clinical observations. For example, during the temporary occlusion of the carotid artery during the procedure of carotid endarterectomy, systemic blood pressure often rises to dangerously high levels. This pressor response is attributed to activation of the sympathetic nervous system due to ischemia or ligation-induced blockade of the baroreceptor endings.

Although the waxing and waning of the various serum enzyme levels, CPK, SGOT, and LDH, did not correlate well with the severity of induced cerebral ischemia following carotid artery ligation, the dynamic rise and fall of these enzymes indicated that cerebral damage became manifest immediately after ligation and reached a zenith between 4 to 12 h with restoration to normal 12 to 48 h post-ligation. In our previous work with normotensive Sprague-Dawley rats and the gerbil, we found these serum enzymes to be an excellent index of the severity of cerebral ischemia as well as the time of greatest damage.

In all previous investigations, our strain of SHR was consistently hyperglycemic and hyperlipidemic. It is difficult to explain why these SHR were normoglycemic and normolipidemic. Nonetheless, it is of interest that like the gerbil and normotensive rats, these SHR responded to the acute duress of cerebral ischemia by a most dynamic dissolution of peripheral adipose tissue sites, hypertriglyceridemia, supernormal free fatty acid levels, no change in circulating total cholesterol, and the rapid appearance of a fatty liver. Most likely, the great increase in adrenocortical secretion (see below) which attends the duress of cerebral ischemia exercised intense lipid-mobilizing effects causing dissolution of peripheral adipose tissue sites and active transport of liberated triglycerides and free fatty acids to the liver.

It is well known that many patients who suffer an acute myocardial infarction will manifest abnormal glucose tolerance during the immediate myocardial infarct period becoming normoglycemic during the myocardial repair phase. Similar findings have been made in patients who have survived acute cerebral infarction. Since cerebrovascular damage is often associated with disruption of normal hypothalamic-pituitary trophic hormone release, it is tempting to suggest that in patients and in these cerebral ischemic SHR, the protracted hyperglycemia was due to derangement of hypothalamic-pituitary trophic hormone release, e.g., ACTH, growth hormone, etc.

It should be emphasized that the circulating corticosterone levels of these SHR prior to carotid artery ligation were above normal due to the unavoidable handling of the animals and the use of anesthesia prior to surgery. SHR are hyperreactive and show extra responsiveness to relatively mild stress compared to normotensive rats. Nonetheless, the supernormal increase in circulating corticosterone levels coincident with the rise in blood pressure, the increase in CPK levels, hypertriglyceridemia and hyperglycemia, served as the best index of the stressful nature of the cerebral ischemia induced by bilateral vs unilateral carotid artery ligation.

Another intriguing finding in this investigation is the appearance of copious (5 to 6 ml) thoracic fluid exudate suggestive of congestive heart failure, occlusive atrial thrombi, and massive myocardial infarction. In ancillary investigations, it has been found that the
combined insult of myocardial infarction (isoproterenol) superimposed upon cerebral ischemia (carotid artery ligation) are synergistic and greatly exacerbate the usual pathophysiologic changes which would attend each maneuver by itself. All of the above pathophysiologic changes are even further exacerbated if pre-existent chronic hypertension had been present. We and others have suggested that cerebrovascular damage may give rise to extra adrenocortical steroid and catecholamine production which would pave the way for myocardial damage, i.e., a brain-heart interaction. Further, it has been amply demonstrated experimentally that carotid artery ligation will cause ACTH release, increased blood pressure, tachycardia, increased myocardial contractility, and increased cardiac output all of which would favor myocardial anoxia, ischemia, and infarction.

Fujishima et al. assert that when strokes occur in chronically hypertensive patients, the cortical lesions are usually found deeply situated whereas strokes in normotensive patients show large cortical and subcortical damage. Diligent inspection by gross and microscopic examination failed to discern any real damage in the brains of these severely hypertensive animals with acute ischemia other than advanced edema and scattered petechiae. The consensus is that carotid artery ligation in the rat, as in higher forms of life, need not be detrimental to cerebrovascular flow. It is also well known that sensitivity to cerebral ischemia can range greatly according to animal strain, age, level of blood pressure, and hormonal influences. Pregnant women with stroke suffer a much higher mortality than men or nonpregnant women. Adrenal and gonadal hormones greatly influence cerebral blood flow. The current epidemiologic emphasis on hypertension, diabetes, age, sex, and the world-wide use of the contraceptive medication, calls for much more intensive investigation of the interrelationship of the above vectors and cerebrovascular disease. The availability of such a unique animal model as the SHR provides an unprecedented opportunity for investigators.

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References


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