Acute Myocardial and Plasma Catecholamine Changes in Experimental Stroke

VLADIMIR C. HACHINSKI, M.D.,* KAREN E. SMITH, B.Sc.,* MALCOLM D. SILVER, M.D.,† CANDACE J. GIBSON, PH.D.,*† AND JOHN CIRIELLO, PH.D.*

SUMMARY  Focal cerebral ischemia in humans increases the incidence of cardiac arrhythmias, and serum cardiac enzyme and plasma norepinephrine levels. In addition, systemic administration of catecholamines causes myocardial damage. This suggests that cerebral ischemia may cause myocardial damage as a consequence of elevated plasma norepinephrine levels. Therefore, experiments were done in 23 chloralosed, paralyzed and artificially ventilated cats to investigate the effects of occluding (n = 17) or sham-occluding (n = 6) the left middle cerebral artery on the myocardium and on circulating levels of plasma catecholamines. After occlusion of the middle cerebral artery for 12–22 hr, 41% (7/17) of the hearts had either acute myocardial necrosis (3/7), focal hemorrhage (3/7), or both (1/7). In animals with acute myocardial damage the levels of plasma norepinephrine and epinephrine were significantly increased compared to pre-middle cerebral artery occlusion values (+46 ± 18% and +142 ± 45%, respectively). As well, in cats with acute myocardial damage, changes from initial levels of plasma norepinephrine and epinephrine were significantly increased over those of experimental cats without acute myocardial damage. In animals which did not have acute myocardial damage (10/17) the circulating plasma levels of catecholamines were not significantly different from pre-occlusion values. Similarly, sham occlusion did not alter plasma catecholamine levels. These data demonstrate that a percentage of animals subjected to middle cerebral artery occlusion have myocardial damage and an increase in plasma concentration of norepinephrine and epinephrine. This suggests that a rise in plasma catecholamine levels, due to increased sympathetic activity after middle cerebral artery occlusion, may cause myocardial damage.

PATIENTS who begin to recover neurologically after a stroke may perish unexpectedly from cardiac complications or sudden death.1 Although some of these deaths could be attributed to concomitant heart disease, stroke patients have an excess of cardiac arrhythmias, increased cardiac serum enzymes and raised plasma catecholamines when compared to similarly managed controls matched for age, sex and the presence of heart disease.2–5 Some patients dying from stroke show myocardial lesions,6 similar to those observed in animals infused with catecholamines7–8 and in animals with increased intracranial pressure,9 or intracranial hemorrhage.10–12 Taken together, these findings suggest that raised levels of catecholamines after cerebral ischemia, likely from increased sympathetic activity, contribute to myocardial damage. The present study was done to determine whether focal cerebral ischemia in the cat would produce cardiac lesions and whether changes in plasma levels of catecholamines were associated with the cardiac lesions.

Methods

Experiments were done in 23 adult cats of either sex weighing 2.0 to 3.5 kg. To isolate the middle cerebral artery (MCA) animals were anesthetized either with sodium pentobarbitol (Somnotol, M.T.C. Pharmaceuticals, Hamilton, Canada; 35 mg/kg i.p.) or ketamine (Rogarsetic, Rogar/STB, Montreal; 15–30 mg/kg i.m.). The carotid vein was cannulated and an infusion of lactated Ringer's solution (0.3 ml/min) was maintained throughout the surgical procedure. The animals were intubated, paralyzed with gallamine triethiodide (Flaxedil, Rhône Poulenc, Montreal; 2–4 mg/kg i.v. initially and additional doses when necessary) and artificially ventilated. End tidal CO₂ was monitored using a Beckman LB-2 gas analyzer and maintained between 3–4%. Rectal temperature was monitored and maintained at 37 ± 0.2 °C by a heating pad controlled by a Yellow Springs 73 temperature controller.

The head of the animal was placed in a Kopf stereotaxic frame and the left MCA was exposed by a transorbital approach modified from that of O'Brien and Waltz.13 An occluding device, which consisted of a 7–0 Prolene suture (Ethicon, Peterborough, Canada), was placed around the artery and the free ends of the suture pulled through a 4–5 cm piece of polyethylene-100 tubing. The occluding device was placed distal to the lenticulostriate arteries in preparation for occlusion at a later date without the need to re-expose the MCA. The incision in the dura and orbitosphenoid bone lateral to the optic foramen were covered with gelfoam and the orbit was partially filled with cotton balls and cyanoacrylate. The skin incision was closed and the eyelids sutured together. The animals were allowed to recover from the surgical procedure and given postoperative care.

After a survival period of 4 to 7 days, the cats were re-anesthetized with alpha-chloralose (60 mg/kg i.v. initially, supplemented by additional doses of 20 mg/kg at 10–12 h intervals) after ethyl chloride and ether induction. The trachea was cannulated and the

From the Departments of Physiology,* Clinical Neurological Sciences,* and Pathology,† Health Sciences Centre, University of Western Ontario, London, Ontario, Canada N6A 5C1

This work was supported by the Heart and Stroke Foundation of Ontario. Dr. V. Hachinski is a Research Associate of the Heart and Stroke Foundation of Ontario and Dr. J. Ciriello is a Canadian Heart Foundation Scholar.

Address correspondence to: Vladimir C. Hachinski, M.D., Room 7GE5 University Hospital, 339 Windermere Road, London, Ontario, Canada N6A 5A5.

Received July 30, 1985; accepted September 27, 1985.
animals were paralyzed with Flaxedil and artificially ventilated. End tidal CO₂ and temperature were monitored and maintained as previously described. Polyethylene-160 catheters were inserted into the femoral artery and vein to record arterial pressure and administer drugs, respectively. Arterial pressure was recorded through a Statham P23Db transducer and the heart rate was monitored with a 7P44B Grass tachograph triggered by the arterial pressure pulse. Both were continuously recorded on a Grass 7 polygraph. After control recordings of arterial pressure and heart rate, and the removal of a 3 ml sample of blood for catecholamine determination the MCA was permanently occluded in 17 animals by retracting the Prolene suture. The remaining six animals in which the MCA was not occluded were used as controls.

Plasma Catecholamine Determinations

Blood samples (3 ml) were taken from the cannulated femoral artery at timed intervals before (½ h) and after (2 and 5 h) the occlusion of the left MCA. The samples, containing 500 USP units of heparin, were centrifuged immediately at 2500 rpm for 20 min at 2°C and the plasma was collected and stored at −70°C until assayed for catecholamines. Plasma catecholamines (norepinephrine, dopamine and epinephrine) were measured by high performance liquid chromatography with electrochemical detection. Serum proteins were precipitated by the addition of 1/10 volume of 1 N perchloric acid, containing 1 mM EDTA and 0.005% ascorbic acid. Dihydroxybenzylamine (DHBA; Sigma Chemical Co., St. Louis, MO) was added as an internal standard (1000 pg/ml serum). The acidified supernatant was adjusted to pH 8.6 by adding 1 M TRIS-HCl, and the catecholamines were purified over mini-alumina columns. Half of the 0.5 N acetic acid eluate was placed on the liquid chromatography system. The system consisted of a Waters dual piston pump (Model 510; Waters Ass., Mississauga, Ont.), Rheodyne injector with 200 nl sample loop, precolumn, Altex Ultrasphere I.P. reverse-phase column (4.6 × 150 mm, 5 μ particles; Beckman-Altex, Toronto, Ont.), LC4-B amperometric controller and glassy carbon electrode (TL-5; Bioanalytical Systems, West Lafayette, IN). The mobile phase consisted of a 0.05 M sodium phosphate buffer, pH 3.3, with 75 mg/L sodium octyl sulfate (Eastman-Kodak, Fisher Scientific Co., Don Mills, Ont.) and 5% methanol and was pumped at a flow rate of 1.5 ml/minute. Detector potential was set at +0.8 V versus a Ag/AgCl reference electrode. The detection limits of pure catecholamine standards were 10 pg for norepinephrine (NE) and epinephrine (E) and 20 pg for dopamine (DA), and for the amines in serum following isolation and purification were 60 pg/ml for NE and E and 120 pg/ml for DA.

Histology

Twelve to twenty-two hours after the MCA occlusion, the animals were perfused via the femoral artery with 1 litre of 0.9% physiological saline followed by 1 litre of 10% buffered formalin — carbon dye solution. The brain and heart of each animal were removed and placed in a 30% sucrose-buffered formalin solution. The presence and extent of the cerebral infarcted area

**FIGURE 1 A-C.** Brightfield photomicrographs of hematoxylin and eosin stained transverse sections through the left ventricle showing acute myocardial damage in cats 12–22 hr after permanent occlusion of the MCA. A. Acute necrosis, with areas of dark staining and clumped eosinophilic fibers (arrow). There is a loose definition of the sarcomeres. Also visible are large areas of vacuolated tissue with associated pyknotic nuclei. On the lower right is a region of normal myocardium. B. Subendocardial hemorrhage suggestive of acute myocardial damage. Note the intact endothelial layer and extensive infiltration of blood cells. C. A variety of acute myocardial damage. The endothelium can be seen uplifted and separated from the underlying tissues. Subendocardial hemorrhage as well as eosinophilic and vacuolated myocardial tissue are present. Calibration in C, 100 μm, applies to all photomicrographs.
was determined by the gross observation of paler cortex in the area unstained by dye.

Each heart was cut into three transverse sections, one near the apex, one in mid-position, and one near the base. The sections included the right and left ventricular walls and the septum. They were embedded in paraffin, sectioned at 50 μm and stained with either hematoxylin and eosin or the Movat stain.

### Data Analysis

Plasma catecholamine levels at 2 and 5 h after occlusion or sham occlusion of the MCA were averaged and expressed as means ± standard error of the means. Statistical comparisons between pre- and post-occlusion levels of plasma catecholamines were done using a two-way ANOVA and Student’s t-test. A p value of less than 0.05 was considered statistically significant.

During the histological examination of the myocardium, the pathologist was not informed of the procedure done on each cat. Histological results and catecholamine data were related subsequently.

### Results

**Myocardial Histology**

Various morphological changes were observed in the myocardium of animals with MCA occlusion. "Acute" changes were considered to be those that followed a time course compatible with the length of the MCA occlusion and that had occurred during the 12–22 hours before sacrifice. These morphological changes included acute myocardial necrosis, (both fiber acidophilia and coagulation necrosis with polymorphonuclear leukocyte exudate) and focal hemorrhage. Fiber acidophilia indicated that the necrosis had been present for a very short time, while leukocyte exudation was related to necrosis present for a few hours to a day. In addition, more chronic myocardial changes were observed. These included both focal cellular necrosis of recent origin showing organization and acellular necrosis, both of which may have been due to previous myocardial damage. "Myocarditis" was also noted. However, this was difficult to distinguish from early healing myocardial necrosis of several days duration.

The animals with MCA occlusion were divided into two groups based on histological findings in the myocardium; those with acute changes (fig. 1A–C), and those with chronic or no changes. Forty-one percent (7/17) of the MCA-occluded animals had acute myocardial damage; three acute necrosis (fig. 1A), three focal hemorrhage (fig. 1B) and one animal had both acute necrosis and focal hemorrhage (fig. 1C).

In the sham-stroked control animals, five of the six animals had no acute myocardial damage. However, one cat showed myocardial necrosis.

### Plasma Catecholamines

The plasma catecholamine concentrations in the MCA-occluded animals were divided into two groups based on the presence or absence of acute myocardial damage within the donor animal. As shown in table 1, plasma levels of NE and E were significantly increased (46 ± 18% and 142 ± 45%, respectively) in animals with acute myocardial damage compared to pre-occlusion values. In addition, although there was a trend towards an increase in the plasma concentration of DA in MCA-occluded animals with acute myocardial damage, this increase was not significant. In animals without myocardial damage, the plasma levels of the three catecholamines were not significantly altered from pre-occlusion values. Sham occlusion of the MCA did not significantly alter the plasma levels of NE, E and DA. In the one sham-occluded animal in which myocardial damage was observed, NE and E decreased by approximately 37% and 33%, respectively, and DA increased by approximately 195% as compared to initial levels in that animal.

### Discussion

These data have demonstrated that a percentage of animals subjected to occlusion of the MCA have myocardial lesions and increased circulating levels of plasma NE and E. Moreover, this study indicates a direct relationship between the level of plasma NE and E and the likelihood of cardiac effects. The myocardial damage observed in these animals was similar to that seen in patients dying from ischemic stroke and to that induced by raised intracranial pressure. In addition,
these changes in the myocardium were similar to those observed in animals after chronic systemic infusion of catecholamines. This evidence, combined with the present data suggest that focal brain ischemia produces cardiac damage as a result of increased circulating levels of NE and E.

The origin of these increased plasma catecholamines is not known. Although it may be argued that they are of central origin, this possibility is unlikely as it has been shown that catecholamines do not breach the blood brain barrier and the blood brain barrier does not break down acutely. On the other hand, it is likely that the increased NE and E are the result of increased sympathetic nerve discharge. This suggestion is supported by the recent demonstration of increased responsiveness of the sympathetic nervous system after experimental stroke. Smith et al demonstrated that somatosympathetic reflexes are increased in animals after occlusion of the MCA.

Although the central pathways which are involved in mediating the increased release of NE and E after focal brain ischemia are not known, it has been shown that electrical stimulation of the posterior orbital cortex, posterior hypothalamus and brain stem reticular formation results in myocardial damage and increased levels of plasma catecholamines. Greenhoot and Reichenbach also showed that adrenalectomy did not prevent the myocardial damage resulting from stimulation of the brain stem reticular formation. This suggests that the increased release of NE from nerve terminals is responsible for the cardiac effects. This suggestion of a direct effect of NE on the heart is supported by the finding that stroke patients with the highest levels of NE also had the highest levels of cardiac creatine kinase isoenzyme.

In summary, the results of the present study suggest that activation of the sympathetic nervous system and the increased release of catecholamines after ischemic stroke represents an important mechanism in the myocardial damage and cardiac complications associated with stroke.

References

Acute myocardial and plasma catecholamine changes in experimental stroke.
V C Hachinski, K E Smith, M D Silver, C J Gibson and J Ciriello

Stroke. 1986;17:387-390
doi: 10.1161/01.STR.17.3.387

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/17/3/387

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at: http://stroke.ahajournals.org/subscriptions/