Acute Myocardial and Plasma Catecholamine Changes in Experimental Stroke

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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, and Pathology.3 This work was supported by the Heart and Stroke Foundation of Ontario and Dr. J. Ciriello is a Canadian Heart Foundation Scholar.

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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.15 An occluding device, which consisted of a 7-0 Prolene suture (Ethicon, St. Louis, Missouri; 15-30 mg/kg i.v.i.). The cephalic 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.i. initially and additional doses when necessary) and artificially ventilated. End tidal CO2, was monitored using a Beckman LB-2 gas analyzer and maintained between 34-40%. Rectal temperature was monitored and maintained at 37 ± 0.2 °C by a heating pad controlled by a Yellow Springs 73 temperature controller.

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 cianoacrylate. 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.i. 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...
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 µl sample loop, precolumn, Altex Ultrasphere I.P. reverse-phase column (4.6 x 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 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...
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.

**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.

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**TABLE 1** | Changes in Plasma Catecholamine Concentrations after Experimental Stroke in Animals with and without Myocardial Damage

<table>
<thead>
<tr>
<th>Experimental stroke</th>
<th>Pre-occlusion concentration (ng/ml plasma)</th>
<th>Cats with acute myocardial damage (ng/ml plasma)</th>
<th>Cats without acute myocardial damage (ng/ml plasma)</th>
<th>Sham stroke (ng/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>1.47 ± 0.25 (n = 23)</td>
<td>+1.16 ± 0.61* (n = 7)</td>
<td>−0.18 ± 0.21 (n = 10)</td>
<td>+0.18 ± 0.24 (n = 6)</td>
</tr>
<tr>
<td>E</td>
<td>0.57 ± 0.12 (n = 22)</td>
<td>+0.45 ± 0.15* (n = 7)</td>
<td>−0.15 ± 0.17 (n = 9)</td>
<td>−0.08 ± 0.17 (n = 6)</td>
</tr>
<tr>
<td>DA</td>
<td>0.37 ± 0.08 (n = 19)</td>
<td>+0.56 ± 0.40 (n = 6)</td>
<td>−0.21 ± 0.14 (n = 8)</td>
<td>+0.09 ± 0.03 (n = 5)</td>
</tr>
</tbody>
</table>

Values are means ± se. n, number of animals.

*p < 0.05; significantly different from experimental cats without acute myocardial damage.
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 also 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

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