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Reversible myocardial damage in gerbil brain ischaemia and its prevention by beta-adrenergic blockade*

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Summary. Acute cerebral infarction in gerbils, produced by unilateral carotid ligation, was used as a model to investigate secondary myocardial changes. The extent of the myocardial damage revealed by succinic dehydrogenase (SDH) histochemistry and by release of myocardial creatine phosphokinase (MB-CK) was measured in gerbils sacrificed from 3 to 48 h after either carotid ligation, carotid isolation only or skin incision only. For technical reasons dead animals were excluded from analysis. Of surviving ligated animals 74% developed neurological deficits related to brain ischaemia. A significant weight increase in the ipsilateral hemisphere was found at 6–10 h, and maximal histological damage at 16 h, both partially reversible thereafter. Non-ligated animals did not develop neurological changes, and showed neither brain swelling nor cerebral histopathology. Extensive cardiac damage was shown by the SDH method from 3 h postoperatively, and confirmed by the elevated serum levels of MB-CK in the carotid-ligated group. The SDH changes were identical with those described in the hearts of patients with acute intracranial lesions, and appeared to be reversible. The effect of beta-adrenergic blockade was assessed in this model. Metoprolol tartrate injected intraperitoneally 3 h before and 1 h after carotid ligation (10 mg/kg each dose) significantly decreased the extent of myocardial damage as estimated both with SDH histochemistry and MB-CK serum levels. It had no effect on the ischaemic brain changes. These results strongly support the concept of catecholamine mediation of myocardial injury resulting from acute brain lesions.

Keywords: reversible myocardial damage, succinic dehydrogenase, brain ischaemia, beta-1-adrenergic blockade

Myocardial damage has been documented in stroke patients in the form of ECG changes (Byer *et al.* 1947; Cropp & Manning 1960), increased serum levels of myocardial enzymes and increased frequency of cardiac arrhythmias and death (Norris 1983; Silver *et al.* 1984). It might be expected that widespread myocardial lesions would be necessary to cause these changes, yet classical morphological findings, such as dead myocardial fibres, were present in only 8–12% of patients dying of acute intracranial lesions (Connor 1968; 1970; Kolin & Norris 1984). Possibly these few necrotic muscle

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fibres represent only one extreme of a spectrum of functionally important molecular, ultrastructural and histological damage. Thus, the extensive changes in succinic dehydrogenase (SDH) staining pattern in the myocardium, observed in a majority of patients with acute intracranial lesions by Kolin & Norris (1984) may correlate better with both clinical data and biochemical observations.

Whilst individual factors linking brain and heart injury are not fully understood, increasing numbers of clinical and pathological observations suggest catecholamines as the agents reponsible for myocardial damage. Serum catecholamine levels are raised in acute stroke (Myers *et al.* 1981) as well as in experimental brain infarction where they seem to correlate with morphological evidence of myocardial necrosis (Hachinski *et al.* 1986).

To investigate the frequency and severity of myocardial changes following acute cerebral damage, and the putative pathogenetic role of catecholamines, we employed a model of unilateral carotid ligation in the gerbil.

Materials and methods

Adult (60–90 g) male gerbils were kept for at least seven days, prior to operation under standard animal house conditions, and given Ralston Purina LA 6-Chow and water *ad libitum*.

All animals were anaesthetized with intraperitoneal pentobarbital (50 mg/kg), and divided into three groups according to the operative procedure as follows:

Group 1 (Ligated). The left carotid bifurcation was exposed through a midline incision, and the common and external vessels were isolated and ligated with silk sutures. After recovery from anaesthesia the animals were continuously monitored on videotape, and neurological signs such as hypotonia, paresis or circling recorded. Food and water were available *ad libitum*. Animals which developed seizures and died spontaneously were eliminated from the study. Groups of 20–25 animals were sacrificed by decapitation at intervals of 3, 6, 10, 16, 24 and 48 h after operation.

Group 2 (Isolated). The left carotid artery was similarly exposed, and the common and external vessels isolated but not ligated. Animals were then observed as above, and groups of 10 were sacrificed at 3, 6, 10, 16, 24 and 48 h postoperatively.

Group 3 (Incised). A similar midline incision was made, but the carotid vessels were neither isolated nor ligated. Subsequent treatment was as above, and groups of 5–10 animals were sacrificed at 3, 6 and 24 h.

In a second experiment, all animals were ligated as in group 1 above, but were divided on the basis of receiving or not receiving metoprolol tartrate injections, as follows:

Treated animals. These animals received metoprolol tartrate dissolved in physiological saline (1 mg/ml) by intraperitoneal injection, in two doses of 10 mg/kg each, given 3 h before and 1 h after operation. Groups of 12–15 animals were sacrificed by decapitation 6, 16 and 24 h postoperatively.

Control animals. These animals were treated similarly, but received saline injections instead of metoprolol.

Blood was collected from severed carotid arteries and centrifuged immediately to obtain serum for estimation of creatine kinase (CK) (Sampson *et al.* 1984) and its myocardial (CK-MB) and other isoenzymes using agarose electrophoresis and fluorimetric scanning with a Gelman Automatic Computing Densitometer 18 (Lum & Levy 1975). To establish the distribution of CK isoenzymes in normal gerbil tissues, brain, heart and skeletal muscle from two unoperated gerbils were homogenized in 10 volumes of Tris HCl buffer (50 mmol/l, pH 8.0) containing NaCl (100 mmol/l) and dithiothreitol (0.1 mmol/l) and centrifuged for 10 min at 12 800 *g*. The supernatant was used for determination of creatine kinase (Mercer 1974).

Brains were immediately and carefully removed after opening the skull and fixed in 10% buffered formol for at least two days. Cerebellum and brainstem were detached and the cerebral hemispheres carefully

separated in the midline. Excess fluid was blotted off and the hemispheres quickly weighed. Each of them was sliced coronally into six pieces by cuts 2, 4, 6, 8 and 10 mm anterior to the occipital pole. The most anterior part was discarded since it consisted mostly of olfactory nerve, the extent of which varied from brain to brain. The other five pieces were embedded anterior surface down for cutting, sectioned at 8 μm and stained with Luxol Fast Blue-haematoxylin and eosin. The extent of pathological changes was graded in a semi-quantitative fashion as follows:

Grade 0: No pathology.
Grade 1: Small areas of minimal pallor without sharp delineation.
Grade 2: Marked and well-defined pallor in one or two out of the four areas: thalamus, basal ganglia, neocortex and hippocampus.
Grade 3: Similar pallor in three or four of those areas.
Grade 4: As grade 3 with the addition of contralateral (usually medial frontal) lesions.

Freshly removed hearts were cut perpendicular to their main axis across the ventricles, removing two complete thin blocks. The first was sectioned at 8 μm in the cryostat and stained for SDH using Nitro Blue tetrazolium (Pearse 1968). Damaged myocardial fibres were recognized by changes of the normal 'myofibrillar' pattern of formazan deposits (dark blue) to a coarse 'granular' pattern (greenish-blue) (Kolin & Kvasnicka 1963; Kakari 1970), and damage was graded as follows:

0 Normal uniform myofibrillar pattern (Fig. 1).
1 Mixed myofibrillar and granular patterns, with the former predominating.
2 Mixed myofibrillar and granular patterns with the latter predominating (Fig. 2).
3 Uniformly granular pattern.

The second slice was fixed in 10% buffered formol, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Sections were evaluated by light and fluorescent microscopy (Siegel & Fishbein 1982).

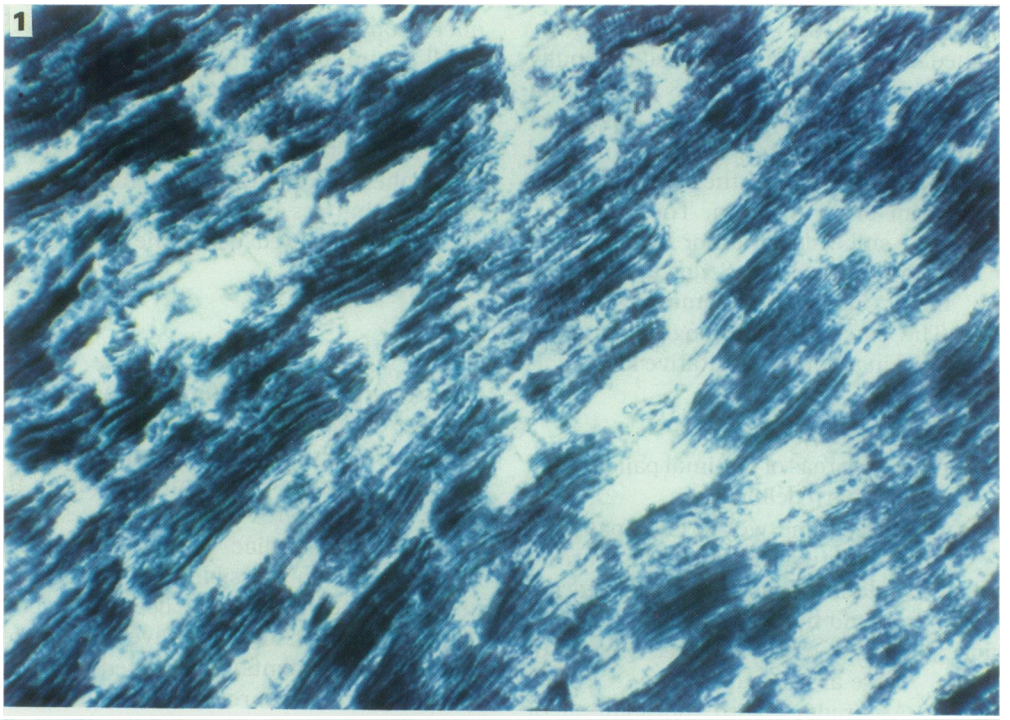
All serum, brain and heart samples were coded with random numbers and examined without knowledge of operative procedure, drug administration or postoperative course. Hemispheric weight ratios, extent of myocardial SDH changes and serum CK-MB percentage were compared using the Student's *t*-test.

Results

Seizures and spontaneous death occurred in 22/154 carotid-ligated animals but in none of the carotid-isolated or skin-incised groups. These 22 animals were eliminated from the study, since seizures may themselves cause cerebral and cardiac pathology: furthermore, it was not possible to examine each animal immediately upon death and postmortem changes would have invalidated many measurements. Undoubtedly the elimination of the neurologically most severe cases has reduced the differences found between the ligated and other groups. Clinical neurological deficits occurred in 97/132 survivors of the carotid-ligated animals, but no such deficits occurred in either of the other groups.

Brain swelling was expressed as an increase in the ratio of left to right hemispheric weights (normal 0.995) and was maximal at 10–16 h (Table 1) in the carotid-ligated group, declining thereafter. Light microscopic changes developed rather more slowly, were maximal at 16 h (Table 2), and declined quickly up to 48 h. Neither brain swelling nor microscopic pathology were seen in the carotid-isolated or skin-incised groups.

The myocardial SDH pattern changed from myofibrillary (Fig. 1) to granular (Fig. 2) in the carotid-ligated animals, with maximal extent at 6 h and a slow decline thereafter. In the carotid-isolated group there was similar change, again maximal at 6 h, but with rapid decline almost to normal by 48 h. There was no significant difference between the ligated and isolated animals at 3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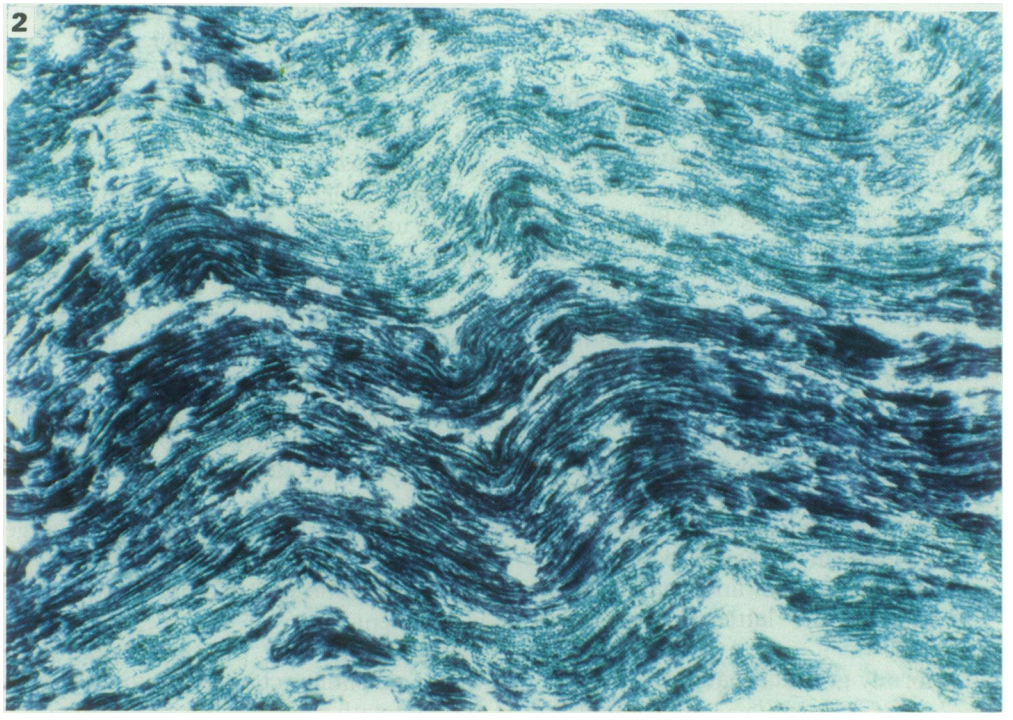
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Table I. Ratios of left/right brain hemisphere weights. Mean + SD

Hours post operation	Carotid artery: Isolated ($n=10$)	Carotid artery: Ligated ($n=20$–25)	P values	Skin incised ($n=5$–10)
3	0.9982±0.0348	1.0137±0.0336	ns	0.9966±0.032
6	0.9860±0.0390	1.0317±0.0838	ns <0.06	0.9608±0.396
10	0.9791±0.0260	1.0189±0.0460	<0.025	—
16	0.9860±0.0294	1.0303±0.0424	<0.005	—
24	0.9811±0.0436	1.0033±0.0374	ns	0.9805±0.0141
48	1.0043±0.0316	1.0000±0.0283	ns	—

Normal value ($n=10$) 0.9954±0.406.
ns Not significant.

or 6 h, but later changes were significantly more extensive in the ligated group. Minimal change in the SDH pattern was observed in the skin-incised group (Table 3). No necrotic or contracted myocardial fibres could be found in any of the groups by either light or fluorescent microscopy.

As any type of stress causes a nonspecific

Table 2. Summary of brain pathology in 132 carotid-ligated gerbils

Grade	Hours: 3	6	10	16	24	48
1	1	3	0	1	0	0
2	4	2	3	3	2	0
3	1	2	4	6	3	1
4	0	1	0	2	0	0
Number in group	20	20	24	25	23	20
1–4 as percentage of total	30	40	29	48	22	5
Average L/R ratio*	1.049	1.054	1.049	1.046	0.996	0.942

* In brains with grade 1–4 changes. For definition of grades see text.

Figure 1. Normal myocardium (Grade 0). Fine dark blue formazan granules are deposited between myofibrils outlining cellular details (myofibrillar pattern). SDH reaction. Magnification, × 72.

Figure 2. Damaged myocardium (Grade 2). Formazan deposits in damaged fibres (upper and lower part of illustration) are greenish blue and coarse, not allowing recognition of cell details. SDH reaction. Magnification, × 40.

rise in serum total CK, leakage from the myocardium was assessed by estimating the percentage of the total which was CK-MB. Studies of homogenized normal gerbil tissues showed that the MB isoenzyme made up 28% of the total CK in the myocardium, but only 4% in the brain and 5% in skeletal muscle. In the ligated animals the CK-MB% was markedly raised from 3 to 16 h, with a slow fall up to 48 h. In the carotid-isolated animals, there was a similar rise at 3 h, but with a rather precipitous subsequent decline. The values for ligated animals were significantly higher than those for isolated animals from 6 to 24 h. No increases were found in the incised group (Table 4).

In the experiment concerning the effects of beta-adrenergic blockade (using metoprolol tartrate) in carotid-ligated animals, seizures and spontaneous death occurred in 7/83 animals (3/41 in metoprolol-treated and 4/42 control animals). Of the survivors 65% developed clinical neurological deficits (73% of the metoprolol group and 58% of controls). Ischaemic brain lesions were seen by light microscopy in 43% of animals (37% of metoprolol-treated and 50% of controls). None of these results are statistically significant. As shown in Table 5, left hemispheric swelling induced by ipsilateral carotid ligation did not differ significantly in the two groups.

Table 3. Extent of SDH changes in myocardium. Mean ± SD

Hours post operation	Carotid artery: Isolated ($n=10$)	Carotid artery: Ligated ($n=20$–25)	P values	Skin incised ($n=5$–10)
3	1.70 ± 1.06	2.00 ± 1.12	ns	0.00 ± 0.00
6	2.09 ± 0.94	2.30 ± 0.86	ns	0.40 ± 0.54
10	1.00 ± 0.82	1.88 ± 1.15	<0.025	—
16	0.60 ± 0.97	1.48 ± 1.15	<0.025	—
24	0.53 ± 0.74	1.91 ± 1.31	<0.005	0.20 ± 0.42
48	0.20 ± 0.42	1.50 ± 1.05	<0.025	—

Normal value ($n=10$) 0.00.
ns Not significant.

Table 4. CK-MB isoenzyme levels as percentage of total serum CK. Mean ± SD

Hours post operation	Carotid artery: Isolated ($n=10$)	Carotid artery: Ligated ($n=20$–25)	P values	Skin incised ($n=5$–10)
3	8.95 ± 5.97	12.78 ± 5.84	ns	0.44 ± 0.23
6	3.42 ± 1.98	10.27 ± 6.76	<0.005	2.72 ± 1.52
10	1.86 ± 1.13	9.32 ± 7.46	<0.001	—
16	5.65 ± 3.35	11.49 ± 7.92	<0.01	—
24	0.38 ± 0.51	4.83 ± 4.10	<0.001	1.26 ± 1.48
48	4.43 ± 2.55	4.61 ± 3.76	ns	—

Normal value ($n=10$) 2.08 ± 1.65.
ns Not significant.

Table 5. Effect of metoprolol treatment on hemispheric weight ratios, extent of SDH changes and percentage CK-MB (Mean ± SD)

Hours post operation	Brains L/R ratio			SDH changes			Percentage MB-CK		
	Metoprolol and ligation	Saline and ligation	*P* values	Metoprolol and ligation	Saline and ligation	*P* values	Metoprolol and ligation	Saline and ligation	*P* values
6	1.01 ± 0.04 (n=12)	1.02 ± 0.04 (n=12)	ns	1.75 ± 0.96 (n=12)	1.33 ± 1.15 (n=12)	ns	5.12 ± 2.17 (n=12)	9.94 ± 7.05 (n=12)	<0.025
16	1.02 ± 0.02 (n=13)	1.04 ± 0.04 (n=14)	ns	0.38 ± 0.50 (n=13)	1.07 ± 0.99 (n=14)	<0.025	3.26 ± 3.63 (n=13)	8.20 ± 8.67 (n=14)	<0.05
24	0.94 ± 0.27 (n=13)	1.00 ± 0.05 (n=12)	ns	0.38 ± 0.76 (n=13)	1.50 ± 0.79 (n=12)	<0.005	3.60 ± 1.40 (n=13)	6.60 ± 6.01 (n=12)	<0.05

ns Not significant.

On the other hand, both indices of myocardial damage showed significantly less injury in the metoprolol-treated group (Table 5). Again, no evidence of myocardial necrosis could be detected.

Discussion

In previous studies (Levine & Sohn 1969; Kahn 1972; Levy & Brierley 1978) acute unilateral carotid ligation has caused brain ischaemia, as judged by clinical or morphological criteria, in 32–53% of animals. In the survivors of our carotid-ligated group brain damage occurred in 73.5% judged by clinical observation, but only 47% showed light microscopic lesions. These were most frequent at 16 h, and had declined sharply to only 5% at 48 h. Together with the fact that 26.5% of animals showed clinical signs but no pathological changes, this decline suggests that ischaemic oedema resulting from temporary hypoperfusion during a period of circulatory re-adjustment, rather than irreversible damage such as selective necrosis or infarction, was often the cause of both cerebral and secondary myocardial changes. Such hypoperfusion might be expected to be associated with maximal vasodilatation causing blood–brain barrier damage and oedema, which would account for the pallor and spongiosis seen microscopically. Since we did not examine the brains of the 22 animals that died spontaneously, we cannot exclude more severe and potentially permanent damage in these animals. Such widely varying results from a standardized procedure are probably accounted for by the known variation in gerbil vascular anatomy (Donadio *et al.* 1982), especially in the morphology of the anterior cerebral arteries.

Extensive myocardial damage, as judged by altered pattern of SDH staining, occurred during the first six hours in both the carotid-ligated and the carotid-isolated groups, but was not seen in the skin-incised group. These observations suggest that the early myocardial changes were at least in part due to manipulation of the peri-arterial sympathetic plexus of the carotid vessels. However, staining changes persisted in the ligated group longer and at higher levels than in the isolated group indicating more profound damage requiring longer recovery. The extent of myocardial damage shown by semi-quantitative assessment of SDH staining at 16 h (when brain changes were maximal) is significantly greater in those cases with observed brain pathology than in those without (Table 6). It appears that even when no cerebral pathology was seen by light microscopy, there must have been a degree of cerebral ischaemia sufficient to be reflected in myocardial damage. The change in SDH staining pattern seen in carotid-ligated gerbils is analogous to that reported in patients dying with acute intracranial lesions (Kolin & Norris 1984), and similar changes were observed in dogs given toxic doses of norepinephrine (Kolin & Kvasnicka 1963) and in rats given isoproterenol (Ferrans *et al.* 1964; Niles *et al.* 1968) or reserpine (Kakari 1970). Kakari has shown that fat accumulation is responsible for the changed SDH pattern, which she claimed to be a highly sensitive indicator of myocardial damage.

The serum levels of CK-MB correlate with the SDH staining changes. At 3 h there is a marked rise in both the carotid-ligated and the carotid-isolated groups, likely due to stimulation of the peri-arterial sympathetic plexus. From 6 to 48 h levels fall in both groups, but remain significantly higher in the ligated group from 6 to 24 h postoperatively, apparently related to brain ischaemia. Similarly, serum CK-MB is higher in cases with observed brain pathology than in those without (Table 6). While increased levels of CK-MB are generally considered as a consequence of myocardial necroses, our findings of CK-MB release without irreversible myocardial damage is in keeping with other conclusions (Gebhard *et al.* 1977; Hearse 1980; Piper *et al.* 1984) about enzyme leakage from nonlethally damaged heart muscle fibres.

No myocardial necroses were observed in the ligated gerbils using the standard histolo-

Table 6. Correlation of cerebral ischaemic changes with myocardial damage and hemispheric weight ratios after 16 h ligation

	Brain pathology positive ($n=12$) Mean ± SD	Brain pathology negative ($n=13$) Mean ± SD	*P* values
SDH	2.16 ± 0.93	0.84 ± 0.98	<0.005
Percentage CK-MB	15.13 ± 9.69	8.13 ± 3.73	<0.010
Brain L/R ratio	1.047 ± 0.046	1.015 ± 0.033	ns

ns Not significant.

gical criteria of nuclear pyknosis, cytoplasmic eosinophilia and leucocyte migration. In contrast, necrotic fibres or myocytolytic foci have been found in human autopsy studies (Connor 1968; 1970; Kolin & Norris 1984) and in the hearts of cats following ligation of the middle cerebral artery (Hachinski *et al.* 1986). However, the data from cat experiments were not quantitative, and necrotic fibres in human hearts were infrequent and affected only a miniscule fraction of the myocardial mass. Clinical heart abnormalities observed in acute stroke patients are probably better explained by reversible metabolic damage of muscle fibres than by infrequent scattered necrotic fibres. ECG changes and the release of myocardial enzymes into the circulation, both reported in acute stroke patients (Norris 1983) presumably require involvement of a large portion of the myocardium. There is no evidence of significant myocardial necrosis in patients with acute stroke, or of detectable diffuse myocardial fibrosis in patients who have recovered from acute stroke. It is therefore probable that metabolic damage of moderate degree and almost complete reversibility is the basis of the clinical heart abnormalities. Histoenzymatic reaction for SDH using Nitro-BT or electron microscopy, are most suitable to detect this.

There is clearly a cause and effect relationship between acute brain lesions and myocardial damage. Doshi & Neil-Dwyer (1980), in patients with acute subarachnoid haemorrhages, correlated heart injury with the presence of hypothalamic lesions. Both clinical (Myers *et al.* 1981) and experimental (Hachinski *et al.* 1986) evidence indicates that catecholamines may be the link between brain and heart damage, the latter group having specifically correlated a rise in serum catecholamines with focal myocardial necroses. In our second experiment, beta-adrenergic blockade has been shown to ameliorate the myocardial effects of unilateral carotid ligation in spite of having no effect on the frequency or severity of the brain lesions. Since the main effect of metoprolol tartrate is the blockade of myocardial beta-1-adrenergic receptors (Ablad *et al.* 1973; Weiner 1980) our results reaffirm the importance of catecholamines as the link between acute brain lesions and myocardial damage. The role of sympathetic hyperactivity is strongly suggested by our findings in the carotid-isolated animals, in whom the myocardial changes differed from those in ligated animals only in being less severe and of shorter duration. Whether there is release of catecholamines solely at myocardial nerve endings, or the phenomenon is more generalized, remains to be elucidated.

Hopefully, these results may in time lead to therapeutic or prophylactic measures for the cardiac complications (often fatal, as shown by Silver *et al.* 1984) of acute intracranial disasters.

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