No-reflow phenomenon in the cerebral circulation of the gerbil

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SYNOPSIS The no-reflow phenomenon has been produced in the cerebral hemispheres of the gerbil by 30 minutes of bilateral carotid artery occlusion. The no-reflow phenomenon was found to develop in relation to the fall in blood pressure which occurred on release of bilateral carotid clips. Metaraminol tartrate intravenously prevented the fall of blood pressure and significantly reduced the occurrence of the no-reflow phenomenon. Metaraminol tartrate, however, did not alter the morbidity or mortality of carotid artery occlusion for 30 minutes. There is thus no support from these experiments for the view that the no-reflow phenomenon plays an important functional role in the reversibility of the effects of severe cerebral ischaemia.

Ames suggested that a vascular factor might underly the inability of the brain to survive periods of total ischaemia (Cantu et al., 1969). He found that after five minutes of complete interruption of the cerebral circulation in the rabbit, reperfusion of the capillary bed was patchy. Regions failed to fill with a carbon black suspension (Cantu and Ames, 1969), and this incompleteness of microvascular filling after ischaemia has been called the 'no-reflow' phenomenon. Crowell and Olsson (1973) demonstrated that a similar defect of microvascular filling may follow temporary regional ischaemia in the monkey brain. In the cat, the no-reflow phenomenon was detected after eight minutes of total cerebral ischaemia but washing through the cerebral hemispheres with saline during the period of ischaemia prevented the development of this phenomenon (Olsson and Hossmann, 1971); in these animals the EEG and pyramidal response recovered more rapidly (Hossmann and Olsson, 1970).

The present experiments were designed to study further the functional significance of the no-reflow phenomenon in the cerebral circulation. Previous studies have employed intracardiac or aortic injections of a carbon suspension from a reservoir. A more physiological approach has been adopted in the present experiments by employing intravenous infusion of an isotonic carbon suspension.

METHODS

Severe ischaemia of the cerebral hemispheres was produced in adult Mongolian gerbils (Meriones unguiculatus) by bilateral clipping of the common carotid arteries in the mid-cervical region through a midline incision under pentobarbitone anaesthesia (5 mg/kg intraperitoneally). In some animals, arterial blood pressure was monitored by an intra-arterial cannula inserted in the femoral artery. At the end of a 30 minute period of occlusion of both carotid vessels, the clips were removed. Some animals were allowed to recover and were observed for signs of neurological damage and the morbidity and mortality of the procedure was assessed. In others, 10 minutes after release of the clips, 1.0 ml of a carbon suspension (isotonic 'biological' india ink, Pelikanwerke) was injected into a femoral vein. The animal was then killed by decapitation and the brain removed. One millimetre sections of the formalin-fixed brain were cleared in oil of wintergreen and viewed under a dissecting microscope. Any regions of the brain that did not show a normal capillary meshwork filled with carbon suspension were noted as regions of no-reflow.

In further groups of animals, a single injection of
2.0 µg metaraminol tartrate (Aramine, Merck, Sharp, and Dohme) was given either intramuscularly 7.5 minutes before the end of the period of carotid occlusion or intravenously immediately before release of the carotid clips in order to prevent post-ligation hypotension.

RESULTS

Mortality after temporary bilateral carotid occlusion In 10 animals, both carotid arteries in the neck were clipped for 30 minutes. Six died in the first eight hours after release of the clips. Of the four survivors, two showed abnormal circeling movements and died later. The overall mortality was thus eight out of 10.

The no-reflow phenomenon Areas of no reflow were found in 10 of the 18 brains (55%,) perfused with India ink 10 minutes after the end of 30 minutes' carotid clipping. Arterial blood pressure was monitored in nine of these animals. The pressure rose during the period of clipping in all animals. In seven, the removal of the clips occasioned an abrupt fall in the blood pressure and the no-reflow phenomenon was present in five of these seven animals. The two animals which had no such drop in pressure during the 10 minutes of restored circulation showed complete capillary filling with the carbon suspension.

In 11 animals, 2.0 µg metaraminol tartrate was given intramuscularly after 22.5 minutes of clipping. At 30 minutes the clips were removed, and 10 minutes later India ink perfusion showed the no-reflow phenomenon in only two cases. In 10 of these animals the blood pressure was monitored. The metaraminol tartrate prevented a fall in the mean arterial blood pressure during the period of restored circulation in seven. Of the three animals showing an abrupt drop, two showed regions of no reflow. None of the brains from the seven animals with a maintained level of blood pressure showed the no-reflow phenomenon.

Ten animals were given 2.0 µg metaraminol tartrate intravenously immediately before release of the carotid clips. Blood pressure was monitored in all. In none was an acute fall of blood pressure seen at the moment of restoration of the cerebral circulation. In one of these animals two small regions of capillary non-filling were detected.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Acute fall in blood pressure (no.)</th>
<th>No-reflow phenomenon (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>No fall in blood pressure (no.)</td>
<td>19</td>
</tr>
</tbody>
</table>

Thus, in 29 animals in which the blood pressure was monitored, the blood pressure fell abruptly after release of the clips in 10. Seven of these showed regions of no reflow. In 19 animals the pressure did not fall acutely and in only one of these was the no-reflow phenomenon seen ($\chi^2 = 10.7$, $P < 0.01$) (Table 1). The intravenous injection of metaraminol tartrate immediately before the release of clips reduced the number of animals showing a fall in blood pressure ($\chi^2 = 9.2$, $P < 0.01$) and reduced the proportion showing the no-reflow phenomenon ($\chi^2 = 3.8$, $P = 0.05$) (Table 2).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Mortality (no.)</th>
<th>No re-flow (no.)</th>
<th>Blood pressure fall (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>No met. tartrate</td>
<td>8/10 (80)</td>
<td>10/18 (55)</td>
<td>7/10 (70)</td>
</tr>
<tr>
<td>Met. tartrate*</td>
<td>9/10 (90)</td>
<td>1/10 (10)</td>
<td>0/10 (0)</td>
</tr>
<tr>
<td></td>
<td>$\chi^2 = 3.84$</td>
<td></td>
<td>$P = 0.05$</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td>$&lt; 0.01$</td>
</tr>
</tbody>
</table>

* Metaraminol tartrate intravenously at end of 30 minute period of 2.0 µg ischaemia.

EFFECT OF METARAMINOL TARTRATE ON MORTALITY

In another nine animals, 2.0 µg metaraminol tartrate was given at 22.5 minutes. Clips were removed at 30 minutes. Eight of the nine animals died. Of 10 animals given 2.0 µg metaraminol tartrate intravenously immediately before re-
moval of the clip, nine died. The overall morta-
tality after 30 minutes of bilateral carotid occlu-
sion (89%) was thus uninfluenced by metara-
minol tartrate despite its effect on blood pres-
sure and on the no-reflow phenomenon (Table 2).

DISCUSSION

The no-reflow phenomenon has been demon-
strated in a variety of organs since Ames's origi-
nal studies in the rabbit brain (Cantu and Ames,
1969). Several factors appear to be involved in
its development. Firstly, the duration of ischae-
mia is important. Ischaemia of less than about
five minutes produces no evidence of impaired
capillary reperfusion. Secondly, with prolonged
restoration of the circulation the regions of no
reflow diminish and the whole change proves
reversible (Cantu et al., 1969).

The presence of red blood cells is also critical.
Saline wash out of the capillary bed during
ischaemia prevents the development of the no-
reflow phenomenon (Olsson and Hossmann,
1971), and in the rabbit the no reflow is reduced
in extent if the haematocrit is reduced (Fischer
and Ames, 1972). The area of non-filling in the
post-ischaemic renal bed is greater if the perfu-
sate contains calcium treated red cells, which
are more resistant to deformation (Summers and
Jamison, 1971).

Post-ischaemic hypotension was thought to play
a role by Ames et al. (1968), and was shown
to be important by Klatzo et al. (1974) using
temporary clipping of one carotid artery in the
gerbil. The present study extends these observa-
tions and confirms the highly important role of a
period of low perfusion pressure in the immediate
post-ischaemic period, in provoking the no-
reflow phenomenon.

The picture that emerges from these studies,
and others in other tissues (Fischer, 1973), is of
trapping of red blood cells if perfusion be at low
pressure, in a vascular bed damaged by ischae-
mia. Ischaemic cells swell due to failure of met-
abolic ion pumps (Leaf, 1970) and endothelial
cell swelling and bleb formation (Chiang et al.,
1968) may compromise the lumen of small
capillaries. Red blood cells retained in the
ischaemic tissue during the period of arrested
flow may also suffer ischaemic damage and be-
come less mechanically deformable. This effect,
together with increased viscosity (Merrill, 1969),
may increase the risk of cell trapping in capillary
channels whose lumen has become irregular due
to bleb formation.

The no-reflow phenomenon can be avoided by
the prevention of ischaemic cell swelling by the
infusion of hypertonic solutions during ischae-
mia (Cantu and Ames, 1969), by reduction of
the haematocrit of the blood during the period
of restored circulation (Fischer and Ames,
1972), or by preventing post-ischaemic hypo-
tension (Klatzo et al., 1974; present study).

Hossmann and Olsson (1970) showed that
eight minutes of total ischaemia of the cat brain
caused the no-reflow phenomenon demonstrable by
a carbon suspension 20 minutes later. Saline
perfusion of the brain during ischaemia pre-
vented the development of the no-reflow pheno-
menon and the EEG and pyramidal responses
returned more rapidly and more completely.
These experiments might be taken to support
Ames's original view that a vascular factor in-
fluenced the vulnerability of the brain to ischae-
mia and was an important factor in the impaired
recovery from all but very brief periods of
ischaemia. Ginsberg and Myers (1972) similarly
felt that the no-reflow phenomenon contributed
to the neuropathology of cerebral circulatory
arrest.

The present experiments, on the other hand,
show that the prevention of the no-reflow pheno-
menon in the gerbil brain (by preventing post-
ischaemic hypotension) has no effect on the
animal's ability to survive. It seems possible,
therefore, that the beneficial effect of saline per-
fusion in Hossmann and Olsson's experiments
was due to the prevention of the accumulation of
metabolites rather than to the avoidance of the
no-reflow phenomenon.

The idea that the no-reflow phenomenon might
contribute to the neuropathology of ischaemia
has also been challenged on different grounds
by Brierley (1973) who has pointed out that it
fails to explain the selective vulnerability of
certain cells. Purkinje cells and neurones of the
third and fifth layers of the cortex for example
are particularly vulnerable during severe ischae-
mia of the brain. Eadie et al. (1971) have pre-
sented evidence that such differences in cell vul-
nerness may be related to the volume or
volume/area ratios of cells, as reflected in their
content of oxidative enzymes.
There is thus no evidence that the no-reflow phenomenon plays an important part in the recovery of neuronal function after cerebral ischaemia in the gerbil.

REFERENCES


