INTRODUCTION

Cerebral ischemia of thrombotic or embolic origin is still a major cause of morbidity and mortality, mainly because the cellular mechanisms that lead to primary and secondary brain damage are still incompletely understood. This has stimulated many authors to develop experimental models to study the modification of biological and neurophysiological parameters in cerebral ischemia.

The most common techniques are:
– microsurgical occlusion of the middle cerebral artery (MCA) by transorbital approach (1,2);
– intravascular embolization by microspheres or by homologous blood emboli (3-6);
– photothrombosis induced by Rose Bengal dye (7).

THE INTRAVASCULAR EMBOLIZATION METHOD

In recent years, at the C. Mondino Institute of Neurology in Pavia, we have studied experimental cerebral ischemia in rabbits using the intravascular embolization method. The embolization was obtained by means of the infusion of specially prepared microspheres (.30-.35 mm in diameter, Europharm, Italy) through a catheter inserted in the ostium of the right internal carotid artery (Fig. 1, see over). The injection of 1-5-10 microspheres made it possible to obtain various degrees of cerebral ischemia (focal, multifocal, massive embolization).

Looking at the time course of electroencephalogram (EEG), quantified EEG analysis (QEEG), somatosensory evoked potential (SEP), cerebral blood flow (CBF) and histological changes, we...
found that the parameters examined were characterized by a peculiar pattern in each group. In particular, the QEEG changes correlated well with the brain damage induced. In the group with focal ischemia, the increase in the slowest frequencies (delta activity) (Fig. 2) corresponded to a loss of cortical components of the SEPs (Fig. 3) and changes in CBF (Fig. 4). Similar changes, but more marked, were observed in the multifocal ischemia (Figs. 5 and 6) and massive embolization (Figs. 7 and 8) groups. The pathological pictures emerging in the 3 experimental groups ranged from a single ischemia, ipsilateral to the injected side, to widespread lesions and to massive brain edema (8) (Figs. 9 and 10, over).

The embolization method was found to be a good technique for developing different models of cerebral stroke (focal, multifocal ischemia and massive diffuse cerebral edema), as it resembles most closely the clinical situation in man. Using this experimental method we were able to evaluate changes in coagulability (9) and k opioid receptor binding (10) secondary to experimental cerebral ischemia in the rabbit.

EEG and QEEG proved useful for analyzing and monitoring in vivo the evolution of cerebral ischemia.

PHOTOTHROMBOSIS INDUCED BY ROSE BENGAL DYE

More recently, we have considered the model of photochemically induced focal cerebral ischemia, in rats. This well established method is based on photochemical induction of thrombotic stroke, using Rose Bengal dye administered intravenously (7). It has the advantage of being less invasive and traumatic than the other methods.

Owing to the complexity of the pathophysiological mechanisms involved in the evolution of cerebral ischemic events, several experimental studies, performed in different models, have been carried out in order to hypothesize new therapeutic strategies. As EEG monitoring has, in previous studies, shown a good correlation between the evolution of ischemic events and changes in cerebral electrical activity, both in the cerebral area submitted to stroke and in the contralateral
Fig. 4 - A: Typical tracing of 133Xe wash-out curves according to ischemic lesion degree (groups I, II, III, IV) one hour after embolization. B: Typical tracing of 133Xe wash-out curves recorded four hours after embolization (groups II, III).

Fig. 5 - QEEG in multifocal ischemia. Right: right cerebral hemisphere, left: Left cerebral hemisphere.

Fig. 6 - SEPs of a rabbit with multifocal ischemia. Right: right hemisphere, Left: left hemisphere.

Fig. 7 - QEEG in the group with massive embolization. Right: right hemisphere, Left: left hemisphere.

Fig. 8 - SEPs of a rabbit with massive embolization. Right: right hemisphere, Left: left hemisphere.

Fig. 9 - Spongy alteration and nuclear pyknosis 8 hours after embolization.
area, we performed an experimental protocol to evaluate this hypothetical correlation in photochemically-induced ischemia in rats. In this study, we used the well established Rose Bengal dye method to induce cerebral focal ischemia.

The above method is based on the photochemical induction of thrombotic stroke using Rose Bengal dye administered intravenously in the tail vein. Rose Bengal is the most efficient known photodynamic generator of singlet molecular oxygen which causes the formation of hydroperoxides in insaturated lipids (fatty acid autoxidation) (11). Photochemically induced cortical infarction has been demonstrated to be the consequence of primary endothelial membrane damage, which causes microvascular injury. The activation of platelet aggregation caused platelet thrombi within pial and parenchymal vessels, acutely depressing local cerebral blood flow (LCBF). The early blood-brain barrier breakdown increased brain water, which began at 15 min after irradiation, reached maximum values at 2 h and was still present at 5 days. The increase in [Na+] and decrease in [K+] was evident at 2 h, reached maximum values at 24 h, and was still present at 5 days (12). Another pathophysiological mechanism is glial swelling, which causes mechanical compression of adjacent microvessels. Similar results have been obtained by Lee et al. (13) using magnetic resonance imaging and examination of histological changes. Both techniques revealed reproducible primary and secondary damage characteristics.

The time course of the changes characterizing the evolution of the ischemic event in the Rose Bengal experimental model of brain ischemia has been studied by several authors. Yin et al. (14) investigated the role of GLAST mRNA (a subtype of the glutamate transporter system), which plays an important role in some pathological conditions (15). These authors postulated that elevation of GLAST mRNA expression in the cortex near the ischemic territory between 24 h and 72 h postischemia is correlated with the neuropathological process following the injury and may reflect a compensatory mechanism.

Inhibition of protein synthesis in the ischemic area has long been demonstrated (16).

In our study the EEG and the QEEG showed a significant increase in the slowest frequency bands (above all delta activity and to a lesser degree theta activity) in the cerebral area submitted to photochemical reaction and in the perilesional area. In the contralateral area, the increase in delta activity was evident 48-72 hours after the induction of cerebral ischemia (Fig.s 11-13). The values emerging from our statistical analysis are reported in Tables I-III.
Fig. 12 - QEEG in the area submitted to photochemical reaction (right Fr-PaM).

Fig. 13 - QEEG in the perilesional area.

Fig. 14 - QEEG in the contralateral area.

Table I - Statistical significance of the changes in power density values obtained over time in the four band frequencies in the ischemic area.

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<thead>
<tr>
<th></th>
<th>delta band</th>
<th>theta band</th>
<th>alpha band</th>
<th>beta band</th>
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<tr>
<td>basal vs 5 h</td>
<td>p&lt;0.000</td>
<td>p&lt;0.000</td>
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<td>basal vs 24 h</td>
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Table II - Statistical significance of the changes in power density values obtained over time in the four band frequencies in the perilesional ischemic area.

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<td>basal vs 5 h</td>
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<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.01</td>
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Abbreviations: n.s. = not significant.

Table III - Statistical significance of the changes in power density values obtained over time in the four band frequencies in the contralateral area.

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<td>n.s.</td>
<td>n.s.</td>
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<tr>
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Abbreviations: n.s. = not significant.
CONCLUDING REMARKS

We have considered two different methods in two different animal species. Although the mechanisms involved in the pathophysiology of the two models were different, the changes in cerebral electrical activity were similar. The time course of the evolution of ischemic events monitored by QEEG correlated well with biochemical and histological findings.

ACKNOWLEDGMENTS

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REFERENCES