PROSTACYCLIN REDUCES EARLY ISCHEMIC CHANGES IN CENTRAL NERVOUS SYSTEM

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Abstract. The authors made a study of the effect of prostacyclin (PGI₂) on ischemic morphological changes in the brain, extracellular calcium concentration (Ca²⁺e) and the blood-brain barrier (BBB) permeability. This was combined with physiological and neurophysiological measurements. Complete cerebral ischemia (CCI) lasting 15 or 20 min was produced by the ligation of the brachiocephalic trunk, the left subclavian and both internal thoracic arteries. The experiments with PGI₂ were carried out in two groups. In group I, the rabbits received PGI₂ 3 min before CCI, during it and for 15 min after it. In group II PGI₂ was infused in the last 3 min of CCI and for 40 min after it. Control animals with CCI of the same duration were not given PGI₂ medication. The rabbits treated with PGI₂ were found to have recovery of the bioelectric activity of the brain in half the time that its return took in the untreated cases. PGI₂ was noted to have a positive effect on some parameters of the peripheral blood system after CCI. Application of PGI₂ reduced the depth of ischemia-evoked drop of Ca²⁺e by 50% without accelerating recovery during recirculation; the post-ischemic increase of BBB permeability to fluorescein was also diminished. PGI₂ reduced edema and the spectrum of neuronal changes and decreased the number of pathologically changed neurons in the brain. In the group that re-
received PGI₂ ischemic ultrastructural changes in the cytoplasm of neurons were abolished, but PGI₂ did not prevent pathological changes in the neuronal nuclei after CCI. Those changes were manifested in numerous vesicular structures and nuclear inclusions. Hence, the described data indicate that the beneficial action of PGI₂ is directed only to early changes in the neuronal cytoplasm and reflects a transient facilitation of functional recovery rather than permanent brain protection after complete cerebral ischemia.

INTRODUCTION

Cerebral infarct is one of the commonest causes of death and personal disability in industrialized societies. Medical treatment has not been particularly successful so far, and so great interest was aroused by the report of Gryglewski et al. (3) that patients with a stroke benefited significantly from an intravenous infusion of prostacyclin (PGI₂), a potent vasodilator and antiplatelet agent (2). Their findings have been supported by some controlled studies (12) but not by all such studies (7, 11).

In various animal models it has been demonstrated that an infusion of PGI₂ limits experimentally induced changes in the ischemic brain (13, 14, 16, 18). Its pharmacological properties suggest that PGI₂ may be helpful in bringing improvement in the ischemic disease which particularly involves platelet aggregation with deposition in ischemic zones (1) and vascular spasms (5) during and after ischemia (6, 20).

The aim of this study was to investigate the curative effects of PGI₂ on the changes in the bioelectric activity of the brain, the calcium unbalance and the impairment of the blood-brain barrier (BBB) function which develop during and after complete cerebral ischemia, and to confront these effects with the pathomorphological consequences of transient complete cerebral ischemia in the rabbit during the acute phase (6 h) following total arrest of the circulation in the central nervous system.

MATERIAL AND METHODS

Rabbits (n = 24; 2.5-3.5 kg) of both sexes were implanted with trans-hippocampal microdialysis fibre (19) one day before the principal experiments. On the next day they were anesthetized with 30 mg/kg of pentobarbital with 2.5 ml noraminophenazonum methanosulfonicum natrium and Xylocain as painkillers, and tracheostomized, relaxed with gallaminum triaethididum (3 mg/kg i.v.) and artificially ventilated (15, 17, 18). Global 15- or 20-min cerebral ischemia was induced by the occlusion
of the brachiocephalic trunk, the left subclavian and both internal thoracic arteries (15, 17). In order to avoid collateral blood supply to the brain, the systemic arterial blood pressure was lowered to 60 ± 10 mm Hg by controlled bleeding from the femoral artery into a pressure compensator (15, 17). Dialysis perfusion of hippocampus was performed before complete cerebral ischemia, during 15 min of its duration and for following 2 h. The changes of extracellular calcium concentration (Ca$^{2+}$e) were measured with $^{45}$Ca (10), the fluorescence in the dialysate was measured after an i.v. injection of Na-fluorescein (10 mg/kg) (19). During the experiments general physiological parameters and EEG were recorded. This was combined with morphological changes in the light and the electron microscope (14, 18).

Throughout the experiment prostacyclin (Sigma) was used. Immediately before application PGI$_2$ was dissolved in 0.1 M glycine buffer, pH 10.5 to a final concentration of 1 mg/ml. Next, 100 µl of the standard solution was diluted in 10 ml of 50 mM Tris buffer, pH 7.5. After being cooled to 4°C, the PGI$_2$ solution was administered continuously by an infusion pump into the femoral vein in a dose 2 µg/kg/min.

The experiments with PGI$_2$ were carried out in two groups. In group I the rabbits received PGI$_2$ 3 min before 20-min ischemia, during it and for 15 min after it. In group II PGI$_2$ was infused for the last 3 min of ischemia and for 40 min after 15-min period of ischemia. In both cases the vehicle control was run.

RESULTS

Pathophysiological observations

An analysis of the changes in the physiological parameters characterizing the cardiovascular and respiratory status of the animals before ischemia showed that they were within the limits of normality. During ischemia in the treated and the untreated rabbits the arterial blood pressure was maintained at 70-50 mm Hg with noradrenaline infusions. In the treated cases, the heart rate balanced between 156 and 290/min and in the untreated ones between 180 and 340/min. The physiological parameters were stable during the whole period course of the study, although there was consistent moderate hypotension and bradycardia at all times in the untreated animals following ischemia.

In the treated and the untreated rabbits a sudden arrest of blood flow to the brain led within 7-20 s to a halt in the bioelectric activity of the cerebral cortex. The activity of vasomotor centres disappeared 3-6 min after the onset of ischemia. During ischemia, in all cases the
isoelectric EEG record continued and no vasomotor or respiratory activity was apparent.

The time and character of the recovery of the bioelectric activity of the cerebral cortex after ischemia differed between the groups. In the treated groups, the earliest signs of recovery were noted 2-18 min after ischemia, and the continuous EEG record after 7-45 min. In the untreated groups, the recovery occurred 30-55 and 55-93 min after ischemia, respectively. Normalization of the EEG record in the treated rabbits, regard to frequency, occurred between 2 and 3 h of recirculation. In the untreated animals it occurred within 6 h after ischemia. In all cases the EEG record reached 50-80% of the initial amplitude within 6 h of observation after ischemia. The activity of vasomotor centres returned after 5 and 20 min of recirculation in the brain. In all cases the basic reflex reactions were observed; in the treated animals they recovered faster. After ischemia the infusion of PGI₂ was stopped, but this did not interfere with the accelerated recovery of the function.

The infusion of PGI₂ in the rabbits of the second group did not modify the initial (5-10%) rise of Ca²⁺ in the hippocampus during ischemia. The 30% drop of Ca²⁺ in the untreated group was reduced to about 15% in the animals treated with PGI₂. The restoration of the basal Ca²⁺ level in the rabbits receiving PGI₂ was attained at the same time after ischemia as in the untreated cases.

The concentration of fluorescein in the dialysate from the hippocampus was estimated as a measure of BBB permeability for this micromolecular compound, which was earlier injected i.v. In the untreated animals the increased permeability of BBB to fluorescein, varying from 140 to 180% of the initial value was noted during the postischemic period up to the end of 2 h observation. An infusion of PGI₂ significantly delayed and reduced the increase of fluorescein concentration in the dialysate during recirculation. Two hours after ischemia fluorescein concentration in the dialysate was at the 115% level of the baseline.

Pathomorphological observations

In the untreated animals symptoms of edema were accompanied by those of cerebral hyperemia such as perivascular extravasations around vessels distended with blood. In the treated rabbits macroscopic abnormalities in the brain were not noted.

The perivascular swelling was much less conspicuous in the PGI₂-treated cases. PGI₂ reduced the spectrum of neuronal changes and decreased the number of pathologically changed neurons in the cerebral hemispheres. Sporadically, the Purkinje cells underwent homogenization
Fig. 1. Neuron. Cytoplasm with reduced electron density, contains single swollen mitochondria and fragments of endoplasmic reticulum. Vesicular structure (arrow) in the nucleus (N). Temporal cortex. 20-min ischemia; time of observation 3 h. ×10800.
Fig. 2. Neuron. In the cytoplasm, abundant ribosomes accumulated in the direct vicinity of the nucleus, well-preserved canals of rough endoplasmic reticulum, numerous normally looking mitochondria and well-developed Golgi apparatus. Vesicular structures (arrows) in the nucleus (N). Occipital cortex. 20-min ischemia + PGI$_2$; time of observation 3 h. × 14400.
Fig. 3. Neuronal nucleus. Intranuclear filamentous inclusion. Motor cortex. 20-min ischemia; time of observation 3h. ×180000.
Fig. 4. Neuronal nucleus. Intranuclear filamentous inclusion. Motor cortex. 20-min ischemia + PGI$_2$; time of observation 6h. $\times 90,000$. 
without shrinkage of the cytoplasm. The bulbar motoneurons were morphologically normal in the animals treated with PGI₂.

Electron-microscopic observations 3 and 6 h after ischemia showed the cytoplasm of the neurons in the untreated rabbits to be devastated and almost void of cytoplasmic organelles (Fig. 1). It contained single organelles, such as swollen mitochondria and fragments of smooth and granular endoplasmic reticulum. There was a fall in the electron density of the cytoplasm of the neurons. In the animals treated with PGI₂ the neurons exhibited a normal distribution and configuration of the cellular structures (Fig. 2). In the cytoplasm, the granular endoplasmic reticulum was always rich. It consisted of long and short canals covered with numerous ribosomes. The number of both ribosomes and polyribosomes significantly increased as compared with norm. The Golgi apparatus was very well developed. The neurons contained more vesicular smooth endoplasmic reticulum than the normal ones. The small and the large mitochondria were almost normal.

Changes in the neuronal nuclei were observed in both the treated and the untreated animals. The nuclei were large and irregularly shaped with randomly dispersed chromatin aggregated in various amounts. They contained large aggregations of interchromatin. The nucleous was segregated with visible fibrillar and granular parts, and it was not always visible. The nuclei contained vesicular structures of various shapes, single or in groups of some half a dozen (Figs. 1 and 2). They were enveloped by a single smooth membrane. The vesicles were mostly electron-lucent or contained floccular masses or granular material with a visible light central area. The vesicular structures were often accompanied by inclusions of rod, lattice and cluster types (Figs. 3 and 4). The inclusions were formed of filaments. Passages between the vesicular structures and inclusions were not observed. Intraneuronal inclusions had no contact with the nucleolus and the nuclear envelope. The number of inclusions and vesicular structures increased with time after ischemia.

DISCUSSION

There are contradictory data in the literature concerning the improvement of the neurological state of humans and animals with ischemic brain diseases treated with PGI₂ (3, 4, 7, 8, 11-16, 18). In our study we limited the observation period after ischemia to 6 hours of recirculation, which was sufficient to develop early pathological changes most probably connected with postischemic hypoperfusion (20). Within that period of recirculation there was marked improvement, due to PGI₂ treatment, in the morphological appearance of neurons in the brain. The EEG
activity after ischemia also returned significantly sooner and the final recordings after 2 hours of recirculation were much better than in the untreated animals. Since the changes of the EEG frequency and amplitude reflect the efficiency of the cerebral blood flow (CBF) and the oxygen delivery to the brain tissue (21), our results are in conformity with the findings showing that PGI₂ prevents the impairment of postischemic CBF (4). This does not exclude, however, a direct influence of PGI₂ on neuronal cells (14, 22).

To evaluate the metabolic effects of the PGI₂ treatment, we measured the ischemia-evoked changes in Ca²⁺ and the penetration of fluorescein through the BBB. The PGI₂ infusion reduced the maximal ischemic drop of Ca²⁺ by 50%. On the other hand, PGI₂ diminished the increased BBB permeability to fluorescein. This may serve as additional evidence of the protective effects of PGI₂ early after the ischemic episode. It seems that there is a direct causal relation between the early effects of PGI₂ on calcium homeostasis and BBB permeability and the final effect of this treatment: the protection or, more probably, facilitation of postischemic recovery. Huczyński et al. (7) report that in patients to whom PGI₂ was administered a significant alleviation of the ischemia-evoked neurological deficit was recorded 6 and 54 h after treatment. Our results support this observation and suggest a complex character of the mechanisms of the PGI₂ action in the early period following the ischemic episode, resulting in the facilitation of postischemic recovery.

The beneficial effects of the PGI₂ treatment on the early postischemic functional recovery of the brain neurons and on the ultrastructural changes in their cytoplasm contrast with the ineffectiveness of the treatment for the development of ultrastructural changes in the nuclei. Those pathological changes of the karyoplasm which developed with time consisted in "vesicular degeneration" — the occurrence of vesicular structures and fibrillar inclusions in the neuronal nuclei. It is rational to relate these changes to the still obscure mechanism of the "maturation phenomenon" (9), i.e., the delayed development of postischemic neuronal death. It may be hypothetized that postischemic structural changes in the nuclei, if they reflect disturbance in the normal function of the cell nucleus above all of the protein biosynthesis, could cause a severe secondary metabolic disorder in the whole cell, which further leads to far-reaching functional changes, resulting in the death of the neurons. Our data show that the pathological ultrastructural processes in the neuron nuclei initiated during or after ischemia are not affected by PGI₂ under the above experimental conditions. In agreement with this investigation, Huczyński et al. (7) showed that, at the end of a 2-week observation period, in patients treated with PGI₂
the neurological improvement lost its statistical significance. These results strongly suggest that the early beneficial effect of PGI₂ may be transient and limited to the neuronal cytoplasm, whereas the delayed death of the neurons is preceded by irreversible changes in the nuclei.

REFERENCES


