The Effect of "Ouabain" on the Ultrastructure of Cerebral Arterioles and Surrounding Tissue, Studied by a Cannulation of a Cerebral Artery

D. LOWE, CHR. SCHIEWS, W. MEIER-RÜGE, D. BANGER TER and J.R. WOLFF
Department of Basic Medical Research, SANDOZ LTD., Basel, Switzerland and MAX-PLANCK-INSTITUTE of Biophysical Chemistry, Department of Neurobiology and Neuroanatomy, Göttingen, Germany

Received April 30, 1975

Summary: A technique for cannulation of a parietal branch of the middle cerebral artery is described by which high but local concentrations of substances can be achieved in cortical vessels. Using this technique it was shown that ouabain, a specific inhibitor of the Na⁺-K⁺-ATPase enzyme system, can produce alterations in the blood brain barrier (BBB) permeability as seen by the passage of Evans Blue into cortical tissue. Electron microscopy revealed changes in the endothelium of cerebral arterioles ranging from an increase in the number of vesicles and vacuoles to complete breakdown of cytoplasm and membranes. Swelling of the peri-arteriolar end feet of protoplasmic astroglia and of dendrites was characteristic of tissue surrounding affected arterioles. Swollen fibrous astrocytes, oligodendrocytes and microglia were not seen even in areas of vasogenic edema. These results are discussed in terms of current ideas of the BBB and astroglial function.

Key words: Ouabain - CNS - Electron microscopy - Vessels - Cannulation technique

The effect of ouabain on neural tissue is of long standing interest due to its specific inhibitory effect on the Na⁺-K⁺-ATPase system. Morphological studies of cultured neuroglia have established an ouabain sensitivity of the astrocytes (RENKA WECK et al., 1970; SOLHEID and PALLADINI 1974), findings which are in agreement with the high Na⁺-K⁺-ATPase activities found in astroglia enriched fractions and cultures (MEDZIH RADSKY et al. 1971; HENN et al. 1972; KIMELBERG, 1974; NAGATA et al., 1974). In vivo morphological studies with ouabain generally support these results but precise interpretation is somewhat complicated by the intracerebral injection method employed to administer the substance (CORN OG et al., 1967; TONFIGHI and GONATAS, 1973). BALDY-MOU LINIER and HUMEAU (1974) report swelling of the astroglia following intraventricular ouabain perfusion, whilst TANAKA (1969) using a superfusion method found only denritic swelling. In view of the increasing importance placed on the Na⁺-K⁺-ATPase system in brain ionic regulation we felt further experiments with ouabain were justified in an attempt to define the in vivo response of the various brain cell types.
To this end a technique was developed for cannulating an artery on the brain surface so that ouabain could be introduced in high concentrations into a limited region of the brain without introducing any of the artefacts of direct intracerebral injection. Such a technique of local application is necessary because ouabain exerts lethal effects on the organs controlling circulation and respiration when administered via the general blood stream. In this paper we discuss the features of this method and present our findings on the ouabain induced changes seen around arterioles. This study is a continuation of previous work in which the effect of ouabain on pericapillary tissue was reported (MEIER-RUGE et al., 1974b; WOLFF et al., 1975).

MATERIAL AND METHODS

A Cannulation of the middle cerebral artery

Cats weighing 2.5 - 3.5 kg were premedicated with 0.2 mg/kg Combelen i.m. and anaesthetized 30 minutes later with 30 mg/kg Nembutal. The depth of the anaesthesia was increased, when necessary, by further doses of 10 mg/kg administered through the femoral vein. Rectal temperature was monitored continuously by an Alfos animal thermostat (Sales Agency A. Bollinger, CH-8610 Uster, Switzerland) to which heating pads and infra red lamps were connected.

A tracheotomy was performed and the femoral artery cannulated for measuring blood pressure and pulse rate via an A-Statham element. The right side of the head lay in a holder adjusted to its shape. After exposure of the skull trephination was performed using a dentist's drill (crown cutter), a section of bone about 20 mm x 20 mm, straddling the line of junction of the temporal squama and the sphenoid bone being cut out and removed. After diagonal incision the dura was retracted to expose the Sylvian gyrus. Bleeding from the dural vessels was stopped by microcoagulation. In the Sylvian sulcus one or two parietal branches of the middle cerebral artery are located and quite often show individual variations in respect of course and size.

For cannulation of this vessel the front end of a no. 22/V2A cannula, ground free of burr edges with a wet stone, was bent into a fish hook shape and connected to a 10 ml perfusion syringe (Perfusor-Braun, Melsungen) with a flexible silicone tube of 0.6 mm outer diameter. A circumscribed area of the vessel was carefully freed of its arachnoidal fibers and the fish hook cannula inserted into the vessel, using a fine ophthalmological needle holder. The cannula was held in place only by the position of the flexible tube, such that no compression was exerted on the nearby vessels.

Ouabain was infused into the middle cerebral artery by means of the perfusor. The injection rate was so adjusted that not more than 10 to 20 % of the blood column in the artery was displaced by the infusion solution. The ideal injection rate was 0.5 ml in approximately 3 min.

In order to check the distribution of the infusion fluid in the brain area supplied by the cannulated artery, the infusion fluid was coloured with a 2% Evans blue solution.

This was necessary, because the infusion fluid is transported partly in the laminar boundary flow and therefore becomes distributed in the supply areas of the arterial branches served by this flow. Areas of increased BBB permeability remained marked after the end of the infusion and perfusion fixation, thus making it possible to assess which subcortical areas had been affected. Any