Studies of the Development and Subsequent Reduction of Swelling of Mammalian Cerebral Cortex under Isosmotic Conditions in vitro

ROBERT S. BOURKE

Laboratory of Neurochemistry, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014 (USA), and Georgetown University Division of Neurosurgery, District of Columbia General Hospital, Washington, D.C. (USA)

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Summary. The ionic parameters of incubation media which foster both the development and subsequent reduction of swelling of slices of cerebral cortex under isosmotic conditions of incubation 
in vitro

are described. A linear relationship between increasing chloride concentrations in incubation media and progressive swelling of tissue slices (under conditions of constant temperature and K+ concentrations and isotonicity of incubation media) is demonstrated. Subsequent reduction of chloride concentration in incubation media together with reciprocal replacement by isethionate is associated with significant and characteristic reduction in the volume of tissue swelling when all other conditions of incubation, including isotonicity of the media, are kept constant. The ionic composition of the fluid of swelling under different conditions of incubation is derived together with the ionic composition and expected transmembrane potentials of the neuronal compartment of cerebral cortex in vitro. Mechanisms involved in the development and subsequent reduction of swelling of cerebral cortex in vitro are discussed, and proposals for possible clinical applications are suggested.

Key Words: Cerebral cortex in vitro — Edema — Fluid compartmentation — Chloride transport — Cat

Introduction

The studies reported here were carried out in conjunction with investigations of the kinetics of chloride transport in slices of cerebral cortex of the adult cat incubated in vitro (BOURKE 1969). Particular attention has been given to effects of various anions in incubation media on the development, compartmentation and subsequent reduction of swelling of slices of cerebral cortex under isosmotic conditions of incubation in vitro. In so far as possible, complete data for each experiment have been obtained for swelling of slices, for content of electrolytes, and for extent of fluid spaces of tissue slices accessible to inulin, isethionate and chloride.

Material and Methods

The materials and methods employed have been fully described (BOURKE 1969; BOURKE and TOWER 1966a) with the exception of certain additional information provided here.
Isotopically-labeled compounds were all obtained from New England Nuclear Corp. (Boston, Mass.) with the following specifications: [Carboxyl-\(^{14}\text{C}\)] inulin (S.A. 1.14 \(\mu\text{c} / \mu\text{mole}\)); sodium \([^{38}\text{S}]\) isethionate (S.A. corrected for decay, 11.9 \(\mu\text{c} / \mu\text{mole}\)); and \([^{14}\text{C}\] toluene standard source (4.45 \(\times\) 10\(^5\) disintegrations/min/ml). The isotopically-labeled inulin and isethionate were made up in stock aqueous solutions containing 20 \(\mu\text{c} / \mu\text{l}\) and were stored frozen. For use, 0.2 ml was added to 5 ml of incubation medium, and 0.5 ml of the mixture added to 2.0 ml of medium in each Warburg flask. The final amount of solute present during incubation corresponded to 0.4 \(\mu\text{c}\) in 2.5 ml of incubation medium. In order to determine the content of isethionate in slices of cerebral cortex, the activity of \([^{38}\text{S}]\) isethionate (counts/min/g) in tissue was corrected for specific activity (counts/min/\(\mu\text{mole} / \mu\text{g}\)) as described in the accompanying paper (BOURKE 1969).

The osmolarity of the various incubation media was determined by the method of freezing point depression.

**Results**

**Swelling of Incubated Slices of Cerebral Cortex**

*Dependence on the Concentrations of \(K^+\) and \(Cl^-\) in Incubation Media.* Under isotonic conditions of incubation *in vitro*, the swelling of slices of cerebral cortex of the adult cat is a linear function of the concentration of \(K^+\) in the incubation media over the range of 27—125 mM (BOURKE and TOWER 1966a). However, when the principal anion of the incubation media, chloride, was replaced by isethionate (all other conditions being kept constant) no swelling of tissue occurred.

The correlation of the swelling of tissue slices with the concentration of \(K^+\) in the incubation medium is quantitatively dependent upon the concentration of chloride therein (Fig. 1). For example, under conditions of constant \(K^+\) (\(\sim\) 54 mM) and varied chloride concentrations, the swelling is a linear function of the concentration of chloride over the range 6.8—132 mM. In these studies, the isosmolarity and total anion concentration of the incubation media were maintained constant by reciprocal replacement of chloride with isethionate. No threshold concentration of chloride was apparent at which swelling of the tissue was initiated. The intercept of the plotted curve at 88 \(\mu\text{l} / \mu\text{g}\) (Fig. 1) presumably reflects adherence of media to tissue slices and/or hydration of protein-rich membranes (BOURKE and TOWER 1966a; VARON and MCILWAIN 1961; TOBIAS 1964).

After preincubation for 1 h in isotonic, chloride-rich media and subsequent transfer to identical media containing less chloride and reciprocally more isethionate, the tissue slices lost fluid during incubation for a second hour. The resultant swelling was within the range of values observed in slices incubated only in media similar to those used for the final hour of incubation during which the fluid loss occurred (Fig. 1). The concentrations of neither \(K^+\) nor \(Cl^-\) alone but both in concert determine the magnitude of swelling of incubated slices of cerebral cortex in *vitro*. Moreover, once the characteristic volume of swelling of slices of cerebral cortex is attained *in vitro*, it is maintained (under isotonic conditions) only if the initial relative concentrations of \(K^+\) (BOURKE and TOWER 1966a) and/or \(Cl^-\) in incubation media are maintained. A decrease in the concentration of either ion in the incubation medium (isosmolarity and temperature being kept constant) is attended by an absolute loss of tissue fluid compared to controls. Tissue solids remain constant under these conditions (BOURKE and TOWER 1966a). The loss of tissue fluid is complete within a relatively short period of incubation; the exact period being related to the degree of previous swelling (Table 1, Figs. 2 and 3).

16  Exp. Brain Res. Vol. 8