2.5 Hypothalamus In Vitro

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INTRODUCTION

The hypothalamus is a region of major interest not only to neurobiologists and endocrinologists, as it serves the regulation of many functions of the whole organism. Its neurons can often be identified and their cellular function attributed to a circumscribed physiological task. This makes them attractive as experimental models and relatively easily accessible objects of rigorous electrophysiological investigation. The actions of many transmitters and hormones involved in the feedback of hypothalamic circuits and physiological conditions affecting the excitability and releasing activity of hypothalamic neurons can be studied; for instance, peptides, cytokines, effects of osmolarity, glucose level, temperature, and circadian rhythmus can be investigated in vitro.

EQUIPMENT

The brains are removed with standard equipment—special care must be taken if the hypophysis is to be retained on the preparation [Davis et al., 1985]. The cranial nerves should be cut with a spatula before levering out the brain from the skull. The brain is dropped into ice cold medium and the required tissue block is cut with razor blades. The whole hypothalamus and the explant of the median eminence are directly prepared in this way, and the blocks for slices are made larger than the final slice dimensions. Handcutting and chopping (McIlwain tissue chopper, Mickle Lab. Engineering Company, or homemade instrument) with fresh high quality razor blades are possible methods for making slices, especially when young animals are used (e.g., rats of less than 100 g), but vibrating knifes are preferable (Vibratome, Vibroslice; Brunswick Co.). Standard perfusion chambers (interface or submersion) may be used, but a chamber made specifically for the dimension of a particular slice is advantageous. Hypothalamic slices survive well for many hours in storage chambers at room temperature.

STRATEGIES

The in vitro investigation of the whole hypothalamus is an attractive and feasible possibility. Bourque and Renaud [1983] have used the isolated perfused hypothalamus. They introduce a fine pipette in the carotis interna, resting on an 8×8×2 mm block of rat hypothalamus, the median eminence directed towards the observing microscope. The medium enters the anterior and middle cerebral arteries and leaves the tissue through the veins. The optic chiasm, the arteries, and the hypophysis with its stalk allow a good orientation for placement of stimulating and recording electrodes. This preparation has mainly been used for investigation of neurohypophyseal neurons.

EXPLANT

Superficial regions of the ventral hypothalamus can also be studied in explants of the median eminence without vascular perfusion, especially when young and small rodents are used [Haas and Reiner, 1988]. We have recorded supraoptic (SON), suprachiasmatic (SCN), ventromedial, arcuate, mammillary and in particular the tuberomammillary (TM) neurons in such a preparation, which also allows paired recordings from synaptically connected nuclei, e.g., the histaminergic pathway from TM to SCN and SON. The explant is prepared by hand with a fresh high quality razor blade and placed in a superfusion chamber. A round cut parallel to the ventral surface delivers a piece of the basal hypothalamus of about equal thickness, up to 700 μm, which survives at 30 °C for several hours.

SLICES

Slices of the hypothalamus can be chopped from tissue blocks placed on the table with the ventral surface down. Thus the knife hits the cut surface first, which is not covered by pia. Better results are, however, obtained with vibrating knifes. A block of hypothalamic tissue is glued to the central part of a petri dish, which can be fixed to the micrometer-operated part of a vibratome. The vibroslice offers more flexibility with vibrating frequencies and is equally useful. It is important to avoid glue on the walls of the tissue block that is cut by the blade. A small amount of cyanoacrylic glue, preferably in gel-form, is sufficient to fix the block on the dish. After a few seconds, chilled medium is poured into the Petri dish. Size and direction of the tissue block, the angle of the blade, and the speed of its movement through the tissue are factors that should be optimized for a particular type of slice. Coronal slices are placed in the order of their manufacture in separate compartments or dishes where they can be subsequently identified

with the help of an atlas. Longitudinal slices are produced in the same way. The blocks are made slightly larger than needed, and the final cutting to size is done on the slice with two razor blades.

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