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Uptake of Peroxidase from the Third Ventricle by Ependymal Cells of the Median Eminence*

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Summary. Peroxidase injected into the subarachnoid space in mice is absorbed by ependymal cells of the median eminence. The ependymal cells of the median eminence of the rat and Japanese quail absorb peroxidase injected into the third ventricle. The processes of these ependymal cells terminate at the capillaries of the primary plexus or those surrounding the ventromedial nucleus of the hypothalamus. In all three species, peroxidase is absorbed by the ependymal cells of the paraventricular organ and by those in close proximity to it. Some ependymal cells send processes to the capillaries in the lateral nucleus of the hypothalamus. These phenomena are discussed in relation to adenohypophysial function.

Key words: Ependymal cell — Median eminence — Peroxidase uptake — Primary capillary plexus.

Introduction

Secretion by the ependymal cells of the median eminence into the third ventricle has been suggested by many investigators (Vigh *et al.*, 1962; Knowles, 1967; Leonhardt, 1969; Knowles and Anand Kumar, 1969; Kobayashi *et al.*, 1970). In females, this ependymal function seems to change with reproductive state (Leveque *et al.*, 1966; Anand Kumar, 1968; Kobayashi and Matsui, 1969; Kobayashi *et al.*, 1970). However, its physiological meaning is as yet unknown.

On the other hand, absorption of ventricular fluid by the ependymal cells of the median eminence has been suggested by a few investigators (Löfgren, 1960; Knowles and Anand Kumar, 1969; Kobayashi *et al.*, 1970). The ventricular fluid contains neurohypophysial hormones (Heller, 1969), noradrenaline (Dencken and Häggendal, 1969) and seems to contain gonadotropin-releasing factors. The latter is suggested by the hypertrophy of basophils in adenohypophysial tissue implanted in the third ventricle (Szentágothai *et al.*, 1968). From these findings it is postulated that the ependymal cells of the median eminence absorb these substances from the ventricle and transport them to the capillaries of the primary plexus through their processes terminating on the capillaries of the primary plexus. We present in this paper direct evidence of a capacity of the ependymal cells to absorb substances from the ventricular fluid.

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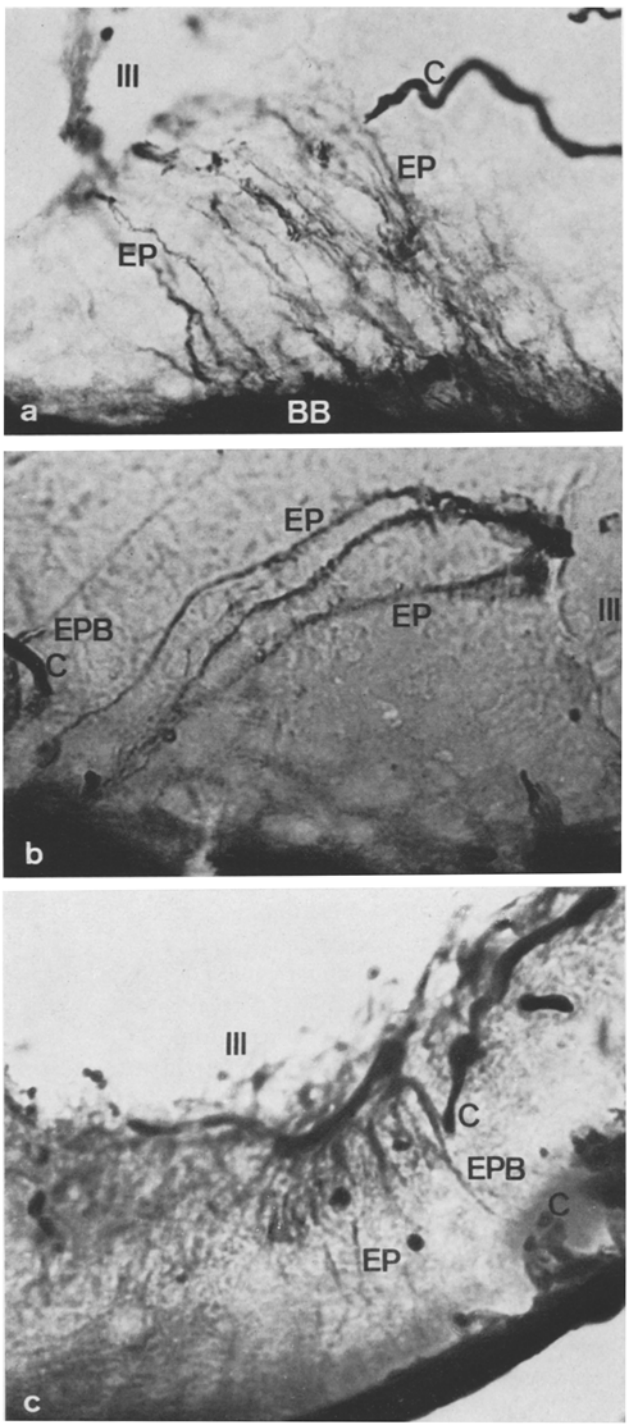

Fig. 1a–c

Material and Methods

As experimental animals, ten mice, five rats and ten Japanese quail (*Coturnix coturnix japonica*) were used. In the mouse, peroxidase was injected into the subarachnoidal space while the animals were under Nembutal anaesthesia; in the rat and quail, peroxidase was injected into the third ventricle with the aid of a stereotaxic instrument, the recipients being under Nembutal anaesthesia. Although in some quail, peroxidase was injected without anaesthesia, the results were almost the same as in the anaesthetized quail. Each animal received 0.2–3 mg horseradish peroxidase (60 U/mg, Carl Roth) dissolved in 0.02 ml of physiological saline. Control animals received the same amount of saline. The animals were killed from 5 to 10 minutes after injection. The hypothalamic tissue was rapidly removed and fixed in ice-cold 5% glutaraldehyde-1/15 M phosphate buffer, pH 7.4, for 2 to 4 hours. The tissue was then washed in cold 0.1 M Tris buffer, pH 7.6, for 2 to 4 hours and cut at 20–40 μm with a freezing microtome for light microscopic observations. Then the sections were incubated in the medium consisting of 3,3′-diaminobenzidine-tetrahydrochloride (Sigma Chemical Co.), 0.05% and hydrogen peroxide, 0.01% in 0.05 M Tris buffer at pH 7.6. After incubation, the sections were rinsed in distilled water and fixed in 10% formaline for 15 minutes and then dehydrated in graded ethanol solutions and mounted in balsam for light microscopic observations.

Results and Discussion

The brown peroxidase reaction occurred in the ependymal cell bodies and their processes in the median eminence of the mouse (Fig. 1a, b). The capillaries in the brain also showed strong reaction. Since this capillary reaction occured in all parts of the brain, the reaction seems to be due to endogenous peroxidase. In the hypothalamus of control mice which were not injected with peroxidase, the ependymal cells did not show any reaction; however the capillaries did. From these findings, it may be concluded that the ependymal reaction in the experimental mice was due to the peroxidase that was absorbed by the ependymal cells from the third ventricle. In the mice, in which peroxidase was injected into the subarachnoidal space, the reaction was also found at the base of the brain (Fig. 1a). There is the possibility, therefore, that the ependymal cells absorbed peroxidase from the base of the brain through their processes.

In the rat, peroxidase was injected into the third ventricle. The peroxidase reaction occurred in the capillaries, in the ependymal cells of the median eminence and a weak reaction was also found at the base of the brain (Figs. 1c, 2a). However, in the quail the reaction was very weak at the base of the brain. Even so, the ependymal cells showed a strong reaction in this species (Figs. 2b, c). These findings show that the ependymal reaction was due to peroxidase that was absorbed by the ependymal cells from the third ventricle. Phenninger (1969), in a brief report, found that the ependymal cells of the subfornical organ absorbed peroxidase injected intraventricularly.

Fig. 1. a Ependymal cells and processes (*EP*) of the median eminence of the mouse showing peroxidase reaction. *BB*, Base of brain; *C*, capillary; *III*, third ventricle. ×570. b High magnification of ependymal cell processes (*EP*) of the mouse showing peroxidase reaction. *EPB*, an ependymal process terminating on a capillary (*C*). *III*, third ventricle. ×900. c Ependymal cells and processes (*EP*) of the median eminence of the rat showing peroxidase reaction. *EPB*, an ependymal process terminating on a capillary (*C*). *III*, third ventricle. ×480

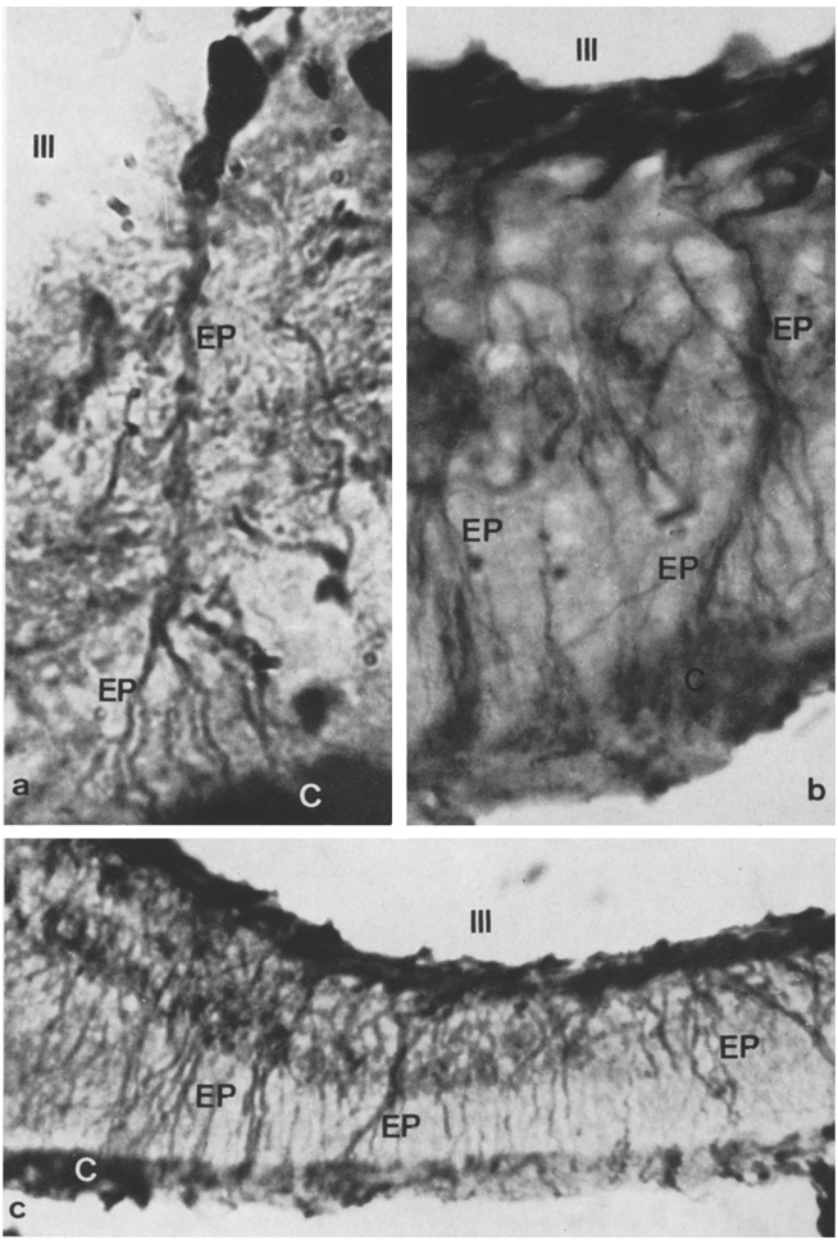

Fig. 2. a High magnification of an ependymal process (*EP*) in the median eminence of the rat showing strong peroxidase reaction. *C*, capillary; *III*, third ventricle. ×1630. b Ependymal cells and processes (*EP*) showing peroxidase reaction in the median eminence of the Japanese quail. *C*, capillary; *III*, third ventricle. ×1300. c Ependymal cells and processes (*EP*) of the median eminence of the quail showing peroxidase reaction. *C*, capillary; *III*, third ventricle. ×530

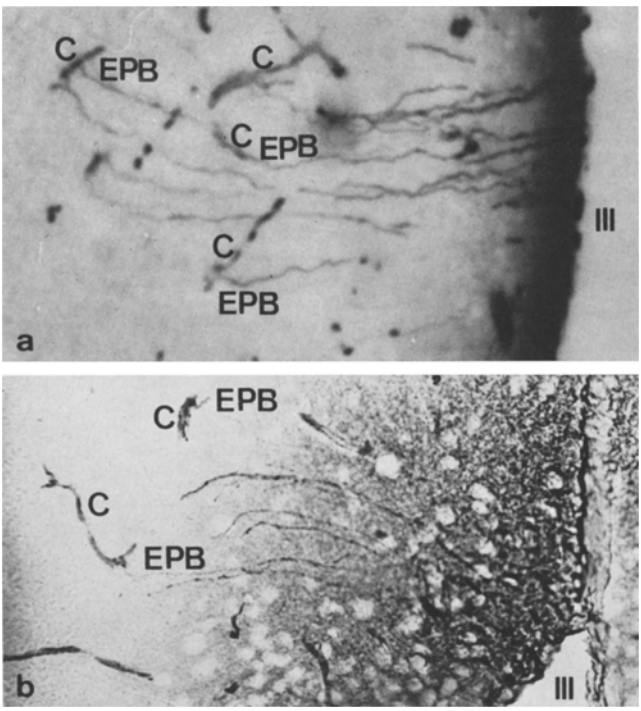

Fig. 3. a Ependymal processes (*EPB*) near the paraventricular organ showing peroxidase reaction and terminating on capillaries in the lateral nucleus of the hypothalamus of the Japanese quail. *C*, capillary; *III*, third ventricle. ×330. b Ependymal processes (*EPB*) of the median eminence of the mouse (see Kobayashi *et al.*, 1970, for a definition of the median eminence) terminating on the capillaries in the ventromedial nucleus of the hypothalamus. *C*, capillary; *III*, third ventricle. ×300

The number of ependymal cells showing the peroxidase reaction was greater in the quail than in the mouse and rat. The reason for this is not known at the moment. All of the ependymal cells with peroxidase reaction in the median eminence send processes to the capillaries of the primary plexus in the mouse, rat and quail. Therefore it is possible that these cells absorb some substances from the ventricular fluid and secrete them into the capillaries of the primary plexus of the hypophysial portal vessels. As mentioned above, the possibility is not excluded that the ependymal processes absorb some substances from the capillaries of the primary plexus at the base of the hypothalamus and secrete it into the ventricle. Further studies are necessary to obtain evidence for this phenomenon.

Some ependymal cells in and near the paraventricular organ show a strong reaction and their processes showing the peroxidase reaction terminate on the capillaries in the lateral nucleus of the hypothalamus (Fig. 3a). Some ependymal cells of the upper portion of the median eminence (see Kobayashi *et al.*, 1970, for a definition of the median eminence) also show a strong reaction; their processes with peroxidase reaction terminate on the capillaries in the ventromedial

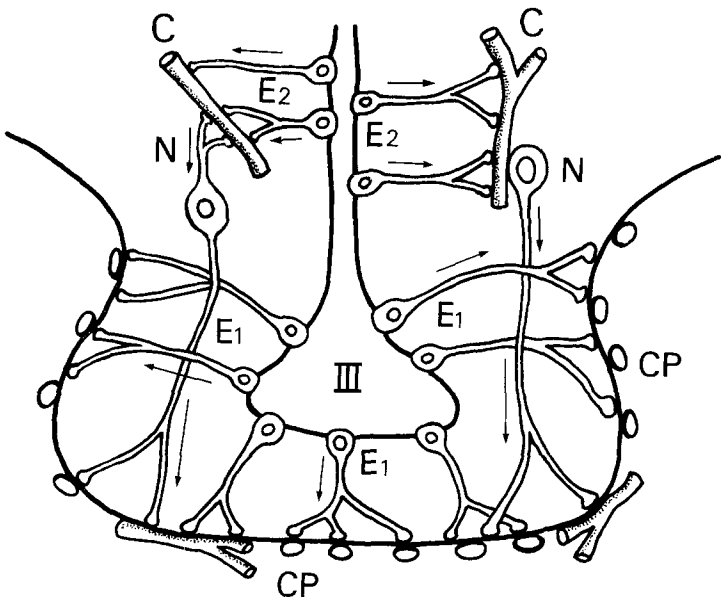

Fig. 4. Diagram showing a probable functional connection (*E1*) between cerebrospinal fluid in the third ventricle (*III*) and capillaries (*CP*) of the primary portal plexus, and connection (*E2*) between the ventricular fluid and neurons (*N*), whose axons may terminate on *CP*. In the latter connection (*E2*), capillaries (*C*) intervene between the ventricle and the neurons

nucleus of the hypothalamus (Fig. 3b) or in the arcuate (mouse and rat) and tuberal (quail) nuclei. These findings indicate that those ependymal cells absorb some substances from the ventricle and transfer them to the capillaries surrounding the neurons in the mentioned hypothalamic nuclei. Thus, it is probable that the ependymal cells transfer humoral information from the third ventricle to the neurons via the capillaries. Some of these neurons, whose processes terminate in the median eminence, may transfer this information to the median eminence, perhaps being involved in the regulation of adenohypophysial function. These probable processes are depicted diagrammatically in Fig. 4.

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