NOTE

Hypertonic Solutions Induce Hemorrhage in the Anterior Pituitary in Mice

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Abstract

Acute and intense hemorrhage in the anterior lobe of the pituitary was observed in mice treated with hypertonic solutions. The onset of hemorrhage and the spontaneous recovery phase were studied with light and electron microscopes.

Ten minutes after ip injection of 9% NaCl, 8% NaHCO₃, 3 M glucose or 3 M sucrose at a dose of 0.03 ml/g B. W. there was selective bleeding in the anterior lobe of the pituitary. The maximum extent of hemorrhage was reached 12 h after the injection of hypertonic solution and lasted for approximately 3 days. Ontogenetic study indicated that no pituitary hemorrhage occurred in mice younger than 2 weeks of age.

Hypophyseal cleft was found to be not merely a residual lumen but an active site providing for accumulation and digestion of blood cells which flowed out of the bleeding anterior pituitary.

By the end of a week, the necrotic tissue in the center of the anterior pituitary was replaced by granulation tissue consisting of fibroblasts, macrophages, capillaries and a stack of excess basal lamina.

Pituitary hemorrhage (pituitary apoplexy) has been reported in patients with pituitary adenomas and with some other pituitary disorders (Fujimoto et al., 1981, Wakai et al., 1981. See also references, Yamaji et al., 1981). Little is known, however, about experimental pituitary apoplexy except for prolactin-producing adenoma in rats given an excess amount of estrogen (Fujimoto, personal communication).

During the course of experiments in which the effects of non-specific noxious stimuli on the adenohypophysis were studied, acute and intense hemorrhage was observed selectively in the anterior pituitary of mice when treated with hypertonic solutions of either electrolytes or non-electrolytes. This preliminary study describes light and electron microscopic observation of the onset and the repair phase of hemorrhage in the anterior pituitary of the mouse.

Materials and Methods

Male and female mice of the strains of ICR, dd and C57BL/Tw were kept under controlled lighting (L: 700-2100) and air conditioning (22±1°C) with free access to mouse chow and water. In most of the experiments, ICR mice were used unless otherwise indicated.

Hypertonic solutions tested were 6, 7, 8 and 9% NaCl, 8% NaHCO₃, 3 M glucose and 3 M sucrose. The animals were injected intraperitoneally with one of these solutions at a dose of 0.03 ml/g body weight, and sacrificed by decapitation 10 min after the injection. In addition, mice given 9% NaCl were
sacrificed 12 h, 1, 3, 5 and 7 days after the injection. Pituitaries were removed and fixed with 3% glutaraldehyde (pH 7.2) followed by 1% OsO₄, and, after dehydration, specimens were embedded in Quetol 812. Thick sections were stained with toluidine blue for the light microscopy and thin sections were contrasted with uranyl acetate and lead citrate, and examined with an 11-E Hitachi electron microscope.

Results

1. Hypertonic solutions which cause hemorrhage in the anterior pituitary.

Ten minutes after intraperitoneal injection of 9% NaCl, 8% NaHCO₃, 3 M glucose or 3 M sucrose at a dose of 0.03 ml/g body weight there was marked anterior pituitary hemorrhage which was readily recognized by the dark-red color of the anterior lobe with an edematous appearance at autopsy. No bleeding was observed histologically in the neuro-intermediate lobe of the pituitary, thyroid gland, pancreas, adrenal gland, liver and kidney so far examined.

None of the animals died following the injection when young adult mice (6–8 weeks of age) were used, but animals older than 3 months of age had a mortality rate approximately of 30%.

2. Concentration of NaCl in producing hemorrhage in the anterior pituitary.

NaCl was chosen as representative of hypertonic solutions. 6% NaCl and 7%
NaCl at a dose of 0.03 ml/g BW had no effect. 8% NaCl was the minimum effective concentration with somewhat individual variation. 9% NaCl (10 times of normal saline) was the concentration which gave positive results constantly. The degree and extent of pituitary hemorrhage with 9% NaCl were comparable in two albino strains of ICR and dd mice, whereas 9% NaCl at the same dose was relatively less effective in C57BL/Tw black mice.

3. Age dependent incidence of hemorrhage in the anterior pituitary.

Immature mice at 1, 2 and 3 weeks of age and adult mice were used. Animals at 1 week of age (10 mice) and 2 weeks of age (16 mice) showed no pituitary bleeding for 24 h after the injection of 9% NaCl solution. However, anterior pituitary hemorrhage was observed in 9 out of 20 mice (45%) at 3 weeks of age, and 80 out of 81 adult mice (ca 100%). An additional finding was that immature mice younger than 2 weeks of age frequently showed small cerebral hemorrhage after 9% NaCl treatment.

4. Light and electron microscopy.

Parasagittal section of the pituitary gland appeared to be nearly normal in outline at low magnification after 10 min of 9% NaCl injection (Fig. 1). The hypophyseal cleft pierced the adenohypophysis, separat-
ing the anterior lobe from the intermediate lobe. After 12 h of the injection, the hypophyseal cleft was extremely expanded by the accumulation of blood cells which flowed out of the necrotic anterior pituitary (Fig. 2). These histological changes lasted for 3 days. After 5 days of the treatment, the hypophyseal cleft was filled with blood cells and blood plasma (Fig. 3). A small number of cellular elements were observed in the still distended hypophyseal cleft 7 days after the injection (Fig. 4).

Higher magnification of the anterior pituitary of saline control mice showed normal structure in which blood cells were exclusive to the lumen of blood vessels (Fig. 5) while marked bleeding in the whole anterior pituitary with apparent extravascular erythrocytes among denatured glandular cells was observed after 10 min of 9% NaCl injection (Fig. 6). Structural signs of spontaneous recovery from hemorrhage were recognized after 7 days of the injection. The necrotic tissue in the center of the anterior lobe was replaced by granulation tissue which consisted mainly of mesenchymal cells and capillaries (Fig. 7).

Electron microscopic examination of the anterior pituitary 10 min after 9% NaCl injection revealed that intact capillaries were filled with blood cells and that extravascular erythrocytes were located adjacent to the glandular cells (Fig. 9). In the repair phase, the granulation tissue consisted of fibroblasts, macrophages, capillaries and a stack of basal lamina (Fig. 10). At the same time, spherical macrophages and small lymphocytes appeared in the cavity of Rathke's pouch (Fig. 8).

Discussion

The present study demonstrated that ip injection of hypertonic solution, either electrolytes or non-electrolytes, produced acute and intense hemorrhage selectively in the anterior lobe of the mouse pituitary. The tissue specificity of hemorrhage in the anterior lobe may be due to the specialized structure and/or system of hypothalamic-pituitary vasculature. Seven efferent routes from the capillary bed of the neurohypophysis to the brain, adenohypophysis and the systemic circulation are suggested in the monkey (Bergland and Page, 1978). Retrograde blood flow toward the hypothalamus through the pituitary stalk is demonstrated in the rat (Oliver et al., 1977). Such anastomosing vasculature may account for this hemorrhage specific for the anterior pituitary. Constriction or infarction of some efferent veins in the pituitary might occur due to the injection of hypertonic solution. The theoretical consequences of regional vasoconstriction of the pituitary have been reported (Bergland and Page, 1979). In the present study, however, it is uncertain which regional vasoconstriction of efferent veins would occur in the pituitary leading to hemorrhage in the anterior lobe. In addition, increased blood volume due to the stimulated vasopressin release would occur in mice treated with hypertonic solution. Further, hypertonic solution induced dehydration might cause conglutination of erythrocytes in the blood vessels followed by stasis of the blood stream in the anterior...
lobe of the pituitary. Subsequent hemorrhagic infarct and diapedesis are likely to be involved in this bleeding. The dark-red color of the anterior pituitary and structural integrity of the walls of the capillaries filled with conglutinated erythrocytes, at least at the onset of bleeding, seem to support this hypothesis. However, these assumptions are not applicable to neonatal mice because they are tolerant of hypertonic solutions at the doses which produce pituitary hemorrhage in adult mice. This may partly relate to the fact that hypophysial portal vessels establish the adult condition 40 days after birth in the rat (Glydon, 1957).

In the present study the cavity of Rathke's pouch is considered to be not merely a residual lumen, but a site that plays a significant role in providing for accumulation and digestion of extraneous cells from the bleeding anterior pituitary. A stack of excess basal lamina in the granulation tissue is one of the characteristics of the repair phase of the anterior pituitary. Fine structural similarities are found in the Call-Exner bodies in growing follicles from the ovary (Bloom and Fawcett, 1975). The precise role of the excess basal lamina in the process of recovery from pituitary hemorrhage remains to be studied.

References


