A New Method for Inducing Copious Drinking and the Accompanying Stimulation on the Pars Intermedia of the Mouse Pituitary Gland

Yasuo KOBAYASHI, Toshihiko KUMAZAWA and Makoto TAKEUCHI

Department of Biology, Okayama University Faculty of Science, Okayama, Japan

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Summary. A new method for causing excessive liquid intake was developed as the condition opposite to dehydration, and the effect of this copious drinking for 2, 4 and 6 days on the pars intermedia of the hypophysis was evaluated by quantitative electron microscopy in mice.

Free access to 5% glucose solution and concurrent food deprivation resulted in the development of polydipsic mice. Drinking volume was 18.9±1.1 ml/10g BW on day 2, 20.8±1.2 on day 4 and 24.1±1.3 on day 6, respectively, while remaining at 2.2±0.2ml/10g BW in the control.

Copious drinking was found to elicit secretory activity of the pars intermedia cells. The cytoplasmic volume percentage of rough endoplasmic reticulum (rER) and the numerical density of Golgi granules increased significantly on day 2 reaching a peak on day 4. Contrarily, secretory granules decreased in number, indicating that the granule release exceeded the activated granule formation. The rise in the activity of the gland was followed by a slight fall after 6 days, which was probably due to a malnutritional condition by food deprivation. Neither 20% glucose drinking nor food deprivation for 4 days enhanced the pars intermedia cells. Thus, excessive water intake seems to be a plausible reason for stimulating secretory activity in cells of the pars intermedia of the hypophysis in mice.

The pars intermedia cells of the murine pituitary gland produce at least seven peptides related to opiomelanocortin. They consist of α-melanocyte stimulating hormone (α-MSH), β-MSH, γ-MSH, corticotropin-like intermediate peptide (CLIP), γ-lipotropin, met-enkephalin and β-endorphin (NAKANISHI et al., 1979; EIPER and MAINS, 1980). Stimuli which affect MSH secretion in vitro have been reviewed (HADLEY et al., 1977). The peripheral extrapigmentary effects of MSH have been well documented (O'DONOHUE and DORSA, 1982). The pars intermedia is known to be under the inhibitory control by dopaminergic neurons originating in the arcuate nucleus of the hypothalamus (BAUMGARTEN et al., 1972; BJÖRKLUND et al., 1973; TILDERS and SMELIK, 1977). In addition, dehydration selectively activates the tuberohypophyseal dopaminergic neuronal system in the rat (ALPER et al., 1980). A morphometric ultrastructural study has clearly demonstrated that water deprivation, accompanying hypernatremia, decreases the secretory activity of the pars intermedia in mice (SCHMITT et al., 1982). However, little has been reported in regard to the effect of excessive water intake, as the condition opposite to dehydration, on the pars intermedia of the hypophysis in mammals.
The present study describes a new method for inducing copious drinking and the effect of this novel dipsogenic condition on the secretory activity of the pars intermedia of the mouse pituitary gland.

MATERIALS AND METHODS

1. Inducement of copious drinking in mice
Male mice of the JCL: ICR strain (5-6 weeks of age) were caged individually and divided into four groups, each consisting of seven mice. After a few days of acclimation, the animals were deprived of mouse feed and allowed free access to drinking water of 5% (group I), 10% (group II) and 20% (group III) glucose solution for 7 days. The mice in group IV received only distilled water without a solid diet for 4 days. Liquid intake was measured every day and expressed as volume (ml) per 10 g body weight.

2. Ultrastructural morphometry of the pars intermedia
JCL: ICR mice (5-6 weeks of age) were divided into 6 groups. Group I served as controls; groups II, III and IV received 5% glucose solution as drinking water without mouse feed for 2, 4 and 6 days, respectively. Group V was given 20% glucose solution as drinking water without any solid diet for 4 days. Group VI was deprived of solid food and given distilled water only for 4 days. The drinking liquid was vitaminized with a drop of POPON-S (Shionogi Pharmaceutical Co.) per 100 ml of glucose solution. For morphometric studies, three mice in each group were used.

The animals were sacrificed by decapitation, and their pituitaries were fixed with phosphate buffered 3% glutaraldehyde and postfixed with 1% OsO₄ (pH 7.4) for 1 hr each. After dehydration, the specimens were embedded in an Araldite-Epon mixture. Sections were cut parallel to the sagittal plane of the gland. The mean cell size of pars intermedia cells of each group was estimated on light micrographs (400 x magnification). The total area of the gland was divided by the number of nuclei in the gland. Six thick sections (two sections per animal) were used in each group. Thin sections were contrasted with uranyl acetate and lead citrate, and electron micrographs were taken at an original magnification of 2,500 and optically enlarged at a final magnification of 10,000. From these electron micrographs, 50-60 cells of the pars intermedia in each group were chosen at random, and morphological analyses were made on the volume percentage of the rER (WEIBEL et al., 1966) and the numerical density of secretory granules and of Golgi granules (KOBAYASHI, 1974). The determinations of the parameters were corrected mathematically in such a way that each cell size of the experimental group was multiplied by the ratio of the mean cell size of the control to the mean cell size of the experimental group, and the determinations were recalculated for comparison. The number of Golgi granules was estimated in the same way on the basis of changes in the mean Golgi area of the control to that of the experimental groups. Statistical analysis was based on Student’s t-test.

RESULTS

1. Copious drinking in mice
Substitution of glucose solution for drinking water and simultaneous food deprivation caused a very significant increase in liquid intake in mice. Among the three concen-
trations of 5%, 10% and 20% glucose solutions tested, the isotonic 5% solution was the most effective to induce excessive water intake in mice. The mean volume of drinking was 10.4 ±1.0 ml/10g body weight on day 1, 18.9±1.1 ml /10g on day 2, 20.8±1.2 ml/10g on day 4 and 24.1±1.3 ml/10g on day 6, while remaining at 2.2±0.2 ml/10g in the control (Fig. 1). With increasing concentrations of glucose, reciprocally decreased drinking, but still at higher levels, was observed (Fig. 1). Body weight loss was 17.1% on day 2, 22.2% on day 4 and 25.1% on day 6, respectively (N=7), in food-deprived mice given 5% glucose solution.

2. Effects of excessive intake of 5% glucose solution on cells of the pars intermedia of the pituitary in food-deprived mice

Excessive liquid intake caused marked hyperactivity in cells of the pars intermedia, showing a peak on day 4 (Fig. 2, 3). Three parameters of fine structures of the glandular cells were applied to assess the cytological activity; 1) cytoplasmic percentage of the rER, 2) the numerical density of Golgi granules and 3) the numerical density of secretory granules in the cytoplasm. Copious drinking of 5% glucose solution provoked a significant increase in the percent of cytoplasm of the rER and in the numerical density

Fig. 2. Pars intermedia cell of normal mouse. Note numerous secretory granules, sparse rough endoplasmic reticulum (ER) and small Golgi apparatus (G). ×8,900

Fig. 1. Daily intake of 5%, 10% and 20% glucose solution for 7 days (N=7) and of distilled water (D.W.) for 4 days (N=6) in food-deprived mice (ml/10g body weight ±SEM).
of Golgi granules by day 2, reaching a peak by day 4 (Fig. 4). The release of secretory granules exceeded the granule formation, resulting in a decrease in the number of secretory granules (Fig. 4). This active phase was followed by a slight fall, but remained higher than the control, even after 6 days of treatment (Fig. 4).

Fig. 3. Pars intermedia cell of mouse exhibiting copious drinking on day 4. Note fewer secretory granules, well developed endoplasmic reticulum (ER) and prominent Golgi apparatus (G). ×8,900

Fig. 4. The percentage of the cytoplasm occupied by the rough endoplasmic reticulum (left), the numerical density of immature Golgi granules (center) and of secretory granules (right) of pars intermedia cells of mice given 5% glucose solution for 6 days. *: P < 0.01, **: P < 0.001.
3. Effects of 20% glucose solution intake on the pars intermedia of the pituitary in food-deprived mice

If glucose ingestion is responsible for the activation of the pars intermedia cells, the administration of hypertonic glucose solution (20%) would cause a similar or more heightened activity of the gland. The results indicated, however, no significant differences in the three ultrastructural parameters on day 4 of the treatment (Fig. 5).

4. Effects of food deprivation on the pars intermedia of the pituitary

Water intake (DW) of the food-deprived mice was less than that of the normal control (Fig. 1), and the conditions of these food-deprived mice deteriorated considerably by day 4 (body weight loss 33%). Although Golgi granules appeared to be at a similar level to those of the control, a significant decrease in the percent of cytoplasmic volume of r-ER and a marked increase in the storage of secretory granules were obvious after 4 days of food deprivation (Fig. 5).

DISCUSSION

The present study indicated that the pars intermedia of the mouse pituitary gland responded actively to excessive liquid intake, a new experimental condition which was induced by the administration of isotonic glucose solution to food-deprived mice. In this method, drinking was approximately 100% of body weight (v/w) by day 1 and 200% (v/w) by day 4. Polyurea was obvious in these mice. There was a reciprocal relationship between the volume of drinking and the concentration of glucose solutions tested (Fig. 1). Total glucose ingestion was roughly comparable among these three groups being administered 5%, 10% and 20% glucose solutions. Thus, water intake is likely to be a passive influx associated with glucose ingestion as an energy source. Further hematological studies and urinalyses are needed to explain this dipsogenic status. The activity of brain angiotensin II, a potent stimulator of the thirst center (Fitzsimons, 1972) is also unknown.
The present method for inducing copious drinking has been developed in order to obtain a condition opposite to dehydration. Dehydration activates the tuberohypophyseal dopaminergic system (Alper et al., 1980) which exerts an inhibitory control on the pars intermedia of the hypophysis (Baumgarten et al., 1972; Björklund et al., 1973; Tilders and Smelik, 1977). Therefore, excessive water intake could be expected to elicit the pars intermedia of the murine pituitary. As was expected, the pars intermedia showed cytological signs of heightened activity in response to copious drinking. The three parameters of ultrastructural morphometry clearly showed a very significant hypersecretion of the pars intermedia (Fig. 4). However, the activated phase of the gland was short in this procedure, showing a peak on day 4. The following fall in the activity of the gland on day 6 seems to be due to a malnutritional condition caused by food deprivation. In fact, food deprivation and DW drinking for 4 days resulted in the lowered activity of the pars intermedia. Glucose ingestion is excluded from the causality since 20% glucose solution intake had no effect on the gland.

If copious drinking in the present study reflects an condition opposite to dehydration, there is a possibility that the attenuation of dopaminergic inhibitory control may have activated the pars intermedia cells. In addition, water deprivation causes marked hypernatremia to coincide with a decreased activity of the pars intermedia in mice (Schmitt et al., 1982). Accordingly, excessive water intake may cause hyponatremia through a presumable condition of water intoxication. Indeed, dietary sodium depletion induced hyponatremia stimulates the pars intermedia of the mouse pituitary (Schmitt et al., 1982; Kobayashi, 1974; Kobayashi and Takema, 1976). Either hyponatremia or impairment of dopaminergic inhibition or both together are likely to be plausible hypotheses which account for the stimulating effect of copious drinking on the pars intermedia of the hypophysis in mice.

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