Hyperprolactinemia, a Tool in Treatment Control of Tetrahydrobiopterin Deficiency: Endocrine Studies in an Affected Girl

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ABSTRACT

Severe tetrahydrobiopterin (BH₄) deficiency is a naturally occurring model of cerebral catecholamine and serotonin shortage. Examination of the stimulated release and physiologic secretion pattern of several hormones in affected individuals permits certain conclusions concerning the involvement of these neurotransmitters in hormone regulation. Treatment, moreover, permits the ranking of the quality of the therapeutic regimens in use according to the degree of hormonal alteration. The 24-h secretion pattern of prolactin, GH, cortisol, and melatonin and the stimulated release of prolactin, GH, TSH, and gonadotropins were studied in an affected girl. Severe hyperprolactinemia with disruption of the pulsatile and circadian secretion pattern was the prevailing feature. The GH physiologic secretion pattern was not affected, but its stimulation was impaired. Melatonin displayed a normal circadian secretion pattern; the rhythm, however, was advanced by several hours. Conventional treatment of BH₄ deficiency, i.e. BH₄, 5-hydroxytryptophan, and l-DOPA/carbidopa (the last named given in three doses per day), suppresses prolactin levels merely for a few hours. l-DOPA/carbidopa given at shorter intervals or, even better, as a slow release preparation, is more effective in suppressing prolactin levels. Our data indicate immense hyperprolactinemia but few other hormonal disturbances in severe BH₄ deficiency. Prolactin secretion may serve as an extremely sensitive marker for the hypothalamic dopamine content under different therapeutic regimens. Treatment with an l-DOPA/carbidopa slow release preparation produces virtually normal prolactin levels. (Pediatr Res 43: 472–477, 1998)

Abbreviations

BH₄, tetrahydrobiopterin
PTPS, 6-pyruvoyltetrahydrobiopterin synthase
HPP, hydroxytryptophan
HVA, homovanillic acid
5HIAA, 5-hydroxyindolacetic acid

BH₄ deficiency, a rare but severe form of phenylketonuria, is characterized by progressive neurologic symptoms despite early detection and treatment with a phenylalanine-restricted diet (1). It is caused by enzyme defects in the biosynthesis or regeneration of BH₄. So far four defects, GTP-cychohydrolylase I, PTPS, dihydropteridine-reductase, and pterin-4a-carbinolamine-dehydrogenase, are known to lead to dysfunction of BH₄-dependent hydroxylases, i.e. phenylalanine-, tyrosine-, and tryptophan-hydroxylase, providing insufficient precursors for serotonin and catecholaminergic neurotransmitters (Fig. 1). BH₄ deficiency occurs in different phenotypes, i.e. severe, mild, and transient form. The severe form of BH₄ deficiency provides a naturally occurring model of cerebral catecholamine and serotonin deficiency (2, 3). PTPS deficiency is the most common form of BH₄ deficiency, with 181 patients registered worldwide (4).

There is considerable evidence for the role of catecholamines and serotonin in the regulation of several neurohormones, but the precise way in which they are involved remains to a great extent unknown (5). However, the role of dopamine as a prolactin-inhibiting factor on both the pituitary and hypothalamic level is well established (6). Also, it is known that the release of growth hormone is stimulated by l-DOPA through a mechanism that involves either norepinephrine- or dopamine-mediated processes. Whether the neurotransmitters mentioned are involved in the physiologic secretion of growth hormone or cortisol is less settled (for review, cf. Refs. 5 and 7). Furthermore, serotonin is a precursor of the pineal hormone melatonin and its shortage has been suggested as a potential cause of melatonin deficiency (8). Therefore, in addition to clinical evidence for endocrine dysfunction, we studied the stimulated release and the spontaneous secretion pattern of several hormones in a girl with severe BH₄ deficiency during the course of her development.

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HYPERPROLACTINEMIA IN TETRAHYDROBIOPERTIN DEFICIENCY

Figure 1. Schematic representation of the biosynthesis and regeneration of BH4 and of the biosynthesis of neurotransmitters. Known enzyme defects resulting in hyperphenylalaninemia are marked with a bold cross-line. Pathologic metabolites used as specific markers in the differential diagnosis are given in squares. PAH, phenylalanine-4-hydroxylase; GTPCH, GTP cyclohydrolase I; DHPR, dihydropteridine reductase; PCD, pterin-4a-carbinolamine dehydratase; SR, sepiapterin reductase; TH, tyrosine-3-hydroxylase; TPH, tryptophan-5-hydroxylase.

METHODS

Case report. At the age of 5 wk an infant girl was diagnosed as having BH4 deficiency. Immediately after birth at term the patient showed signs of prenatal dystrophy (weight, 2020 g; length, 44 cm). On a routine Guthrie test for phenylketonuria screening, plasma phenylalanine ranged between 1220 and 2400 μmol/L. At the age of 15 d, hyperphenylalaninemia was confirmed by serum amino acid analysis (Table 1). Subsequently, a phenylalanine-restricted diet (20 mg/kg/d) was initiated. At the age of 5 wk the diagnostic test for BH4 deficiency was performed. After an oral load of BH4 (7.5 mg/kg), blood phenylalanine levels decreased from 1255 μmol/L to 90 and 82 μmol/L after 4 and 8 h, whereas tyrosine increased from 60 μmol/L to 173 and 167 μmol/L, respectively. The analysis of the urinary pterins revealed extremely low excretion of biopentin (0.13 mmol/mol creatinine) and very high excretion of neopterin (30.3 mmol/mol creatinine), indicating hyperphenylalaninemia due to BH4 deficiency. Later, reduced PTPS activity in blood cells (0.45 μg U/g Hb; normal, 11–29) was identified as the cause of BH4 deficiency.

Subsequently, the phenylalanine-restricted diet was replaced by normal formula containing 120 mg of phenylalanine/kg/d, and 5 mg/kg BH4 once per d, resulting in blood phenylalanine that was continuously less than 60 μmol/L. However, the girl was unable to establish any social contact, the muscles were hypotonic, but EEG was normal. At the age of 10 wk, neurotransmitter precursors (8–9 mg l-DOPA/kg/d, 0.8–0.9 mg carbidopa/kg/d (Sinemet®), and 4–5 mg of HTP/kg/d in three doses, p.o.) were added to the previous treatment. As a result, social behavior improved but neurologic behavior was still not satisfactory. EEG and sonography of the cranial structures were normal. The child was subsequently seen every month for physical and laboratory examination. The appropriateness of BH4 and neurotransmitter precursor substitution and compliance were investigated via the urinary excretions of the pterins and CSF concentrations of HVA and 5HIAA (Table 1). Somatic development was moderate with height, weight, and head circumference following just below the 3rd centile and with retardation of bone maturation (Fig. 2).

After approval by the child’s parents, initial endocrine examination was performed at the age of 5½ y (see Fig. 3). To possibly suppress the severe hyperprolactinemia observed trough most of the day, the daily amount of l-DOPA/carbidopa (Sinemet®) required was divided into six doses. At age 6½ y the effect of the altered regime of treatment on hormone secretion was examined (see Fig. 4). At the age 7½ a slow release preparation of l-DOPA/carbidopa (Sinemet Depot®) became available, and the therapeutic regimen was switched to Sinemet-Depot® (10 mg l-DOPA/carbidopa and 2.5 mg carbidopa/kg/d) in three doses. Subsequently, the impact of the depot preparation on spontaneous prolactin (see Fig. 5) and melatonin (see Fig. 6) secretion was explored.

At age 12 y the girl had the first clinical signs of puberty (mammary, Tanner II; pubes, Tanner II); activation of the hypothalamus-pituitary-gonadal axis was confirmed by a pu-

Table 1. Levels of amino acids, neurotransmitters, and their metabolites in serum, urine, and CSF and therapeutic regimen in a girl with BH4 deficiency

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Phe (μmol/L)</th>
<th>Tyr (μmol/L)</th>
<th>N (mmol/mol cr.)</th>
<th>B (mmol/mol cr.)</th>
<th>N (mmol/L)</th>
<th>B (mmol/L)</th>
<th>Phe (μmol/L)</th>
<th>Tyr (μmol/L)</th>
<th>HVA (nmol/L)</th>
<th>5HIAA (nmol/L)</th>
<th>DOPA/Carbidopa (mg/kg/d)</th>
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<th>BH4</th>
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<tr>
<td>1 &lt;120</td>
<td>13.0</td>
<td>1.0</td>
<td>120 &lt;4</td>
<td>93</td>
<td>8.6/0.8</td>
<td>4.5</td>
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<td>2 141.0</td>
<td>27.0</td>
<td>4.7</td>
<td>144 &lt;1</td>
<td>177</td>
<td>9.0/0.9</td>
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<td>5 48.5</td>
<td>61.8</td>
<td>9.3</td>
<td>133 5.7</td>
<td>460</td>
<td>8.1/0.8</td>
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<td>5½ 46.0</td>
<td>52.0</td>
<td>3.9</td>
<td>123  3.9</td>
<td>97</td>
<td>9.0/0.9</td>
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<td>6½ 51.4</td>
<td>54.2</td>
<td>3.4</td>
<td>87</td>
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N, neopterin; B, biopentin; cr., creatinine; —, not done.

Normal values in urine (mmol/mol cr.): B, 0.5–3.0 (2–12 mo), 0.5–2.7 (>1 y); N, 11–4.0 (2–12 mo), 0.2–1.7 (>1 y). Normal values in CSF (μmol/L): B, 10–30; N, 9–20; 5HIAA, 114–336 (<1 y), 105–299 (2–4 y), 88–178 (5–10 y), 74–163 (11–16 y); HVA, 295–932 (<1 y), 211–871 (2–4 y), 144–801 (5–10 y), 132–551 (11–16 y). Normal values in CSF (μmol/L): Phe, <20; Tyr, <25.
bential response in the LH-releasing hormone test. Despite early onset of treatment, the intellectual development of the girl was impaired. She attended a school for mentally handicapped children. At age 12 y the intelligence quotient explored by Adaptives Intelligenzdiagnosticum (9) was 54 [normal, 100 ± 15 (mean ± SD)], A MRI of the brain was normal.

Endocrine studies. Stimulation tests. Stimulation tests were performed at age 5½ (arginine, Insulintest for GH, thyroid-releasing hormone test for TSH and prolactin), at age 10 and 12¾ y (LH-releasing hormone test), and at age 14½ y (GH-releasing hormone test for GH, Insulintest for cortisol). All stimulation tests were performed according to standard procedures after an overnight fast but after the morning medication.

The 24-h hormone secretion. Hormone secretion profiles were conducted after admission to a research facility at the age of 5¾, 6½, 8¼, and 10¼ y. On the day of the studies the patient was admitted to a research facility between 0700 and 0730 h. A heparinized special needle was inserted into an antecubital vein, and beginning at 0900 h 1- or 2-mL blood samples (depending on the patient’s age) were collected for estimation of prolactin, cortisol, GH, and melatonin. Blood was collected at 15-min intervals for 24 h. Because of the large sample size required for melatonin measurements (2 mL of serum) blood over 2-h intervals was pooled, and its melatonin concentration was estimated. During the investigation on our research facility medication was administered at time points as indicated on the graphs (Figs. 3–6). Blood samples were collected in plastic tubes, and after centrifugation serum was stored at −20°C until assay. During the study the girl was exposed to indoor daylight. Lights were turned off from 2000 to 0700 h. All study periods were finished at 1000 h on the following day.

Analytical methods. Blood phenylalanine was measured by the method of Guthrie (10) or by column chromatography (11), and blood tyrosine by column chromatography (12). The activity of PTPE in erythrocytes was determined according to Shintaku et al. (13). Pterins in urine and cerebrospinal fluid, homovanillic acid and 5-hydroxyindolacetic acid in cerebrospinal fluid were analyzed by automatic HPLC (14, 15). Prolactin, cortisol, GH, TSH, LH, and FSH were determined by commercial available RIA kits. The LIRP 68/40 was used as an LH standard, and the IIIRP 78/549 as an FSH standard. Melatonin levels in serum were measured after extraction by a RIA previously reported in detail (16). Bone age was determined by comparing the left hand and wrist radiographs to the standards of Greulich and Pyle (17).

Statistical analysis. GH and cortisol profiles were analyzed for pulsatile release by the computer program PULSAR (18), which calculates among others the number of peaks and their average amplitude in a series of hormonal measurements. Cortisol and melatonin profiles were examined for circadian release by the program RHYTHM (19). It examines the original data for the occurrence of circadian secretion pattern and then estimates the best fit sinus wave to the data. Then it determines the acrophase (time of the occurrence of its highest values) and the amplitude (half of the distance between the highest and lowest value) of the fitted sinus wave.

RESULTS

Stimulation tests. GH. GH stimulation with both arginine and insulin resulted in little or no hormone release. With arginine, GH increased from 0.21 to 0.26 pmol/L; similarly insulin administration failed to elevate GH levels. However, the latter procedure had to be interrupted after 60 min because of severe hypoglycemia with blood glucose levels of 1.8 mmol/L. In contrast stimulation with GH-releasing hormone showed an appropriate response from 0.37 to 0.61 pmol/L.

Cortisol. Cortisol responded normally to insulin stimulation with a basal level of 331 nmol/L and a peak level 604 nmol/L.

TSH and prolactin. TSH was normal after thyroid- releasing hormone stimulation, the peak occurred at 30 min and was 8.9 U/L, whereas basal prolactin was already at a level of 60.3 μg/L and revealed a peak level of 85.0 μg/L after 30 min.

Gonadotropins. At age 10 y LH and FSH showed the normal prepubertal response to LH-releasing hormone [for normal values, cf. Frisch et al. (20)]. Both LH and FSH peaked at 60 min with concentrations of 1.6 and 4.9 U/L, respectively. At the age of 12¾ y, however, hormone levels peaked at 30 min (LH 17.7 U/L, FSH 12.7 U/L). The latter response was normal for a pubertal girl and in accordance with her physical development.

Twenty-four hour hormone secretion. The 24-h prolactin measurements. Serum prolactin levels were measured at 15-min intervals for 24 h on three occasions. During the three-
dose l-DOPA/carbidopa regimen, we observed a huge hyperprolactinemia with prolactin levels decreasing only for several hours after l-DOPA/carbidopa administration (Fig. 3, upper panel). After altering the therapeutic regimen to l-DOPA/carbidopa given in six doses per day, prolactin levels dropped into the normal range during the day time, when most of the neurotransmitter was given. However, during the night time, when l-DOPA/carbidopa was withdrawn, prolactin again increased into the high pathologic range (Fig. 4, upper panel). Utilization of a l-DOPA/carbidopa slow release preparation resulted in a better suppression of the undue prolactin elevation with concentrations being in the normal range most of the day and night (Fig. 5). Because of the obvious massive disruption of the normal, physiologic, pulsatile, and circadian prolactin secretion pattern (6, 21) the girl’s 24-h prolactin secretion profiles were not scrutinized further for pulsatile and circadian features.

The 24-h cortisol measurements. On both occasions, at three (Fig. 3, middle panel) and at six (Fig. 4, middle panel) doses of l-DOPA/carbidopa, the girl displayed a normal pulsatile (22, 23) and circadian (24) secretion pattern of cortisol (pulse features: number, 6; amplitude, 342.1 ± 63.4 nmol/L (mean ± SEM) versus number, 12; amplitude, 366.2 ± 55.2; features of circadian rhythm: acrophase, 9:15 h amplitude, 259.3 ± 74.4 versus acrophase, 9:15, amplitude 209.7 ± 66.2).

Figure 3. At 3½ y, 24-h hormone profiles in our patient with severe BH₄ deficiency while on three doses of l-DOPA/carbidopa. Top panel, prolactin profile; middle panel, cortisol profile; bottom panel, GH profile. DOPAC/C 40/4 mg; D, 40 mg of l-DOPA/4 mg of carbidopa; * = occurrence of a hormone pulse; ↓ = occurrence of the acrophase.

Figure 4. At 6½ y, 24-h hormone profiles in our patient with severe BH₄ deficiency while on six doses of l-DOPA/carbidopa. Top panel, prolactin profile; middle panel, cortisol profile; bottom panel, GH profile. DOPAC/C, 20/2 mg; D, 20 mg of l-DOPA/2 mg of carbidopa. * = occurrence of a hormone pulse; ↓ = occurrence of the acrophase.

Figure 5. At 10½ y, 24-h prolactin profile in our patient with severe BH₄ deficiency while on three doses of l-DOPA/carbidopa slow release preparation. DS, l-DOPA/carbidopa slow release preparation.

The 24-h GH measurements. At the age of 5½ y, when the girl received her daily amount of l-DOPA/carbidopa in three doses, evaluation of spontaneous GH profile revealed a normal pulsatile secretion pattern (25) (pulse features: number, 9; amplitude, 0.27 ± 0.09 pmol/L) with four major pulses, three of them shortly after l-DOPA/carbidopa administration and one occurring spontaneously during the night (Fig. 2, lower panel). After changing the therapeutic regimen and giving the daily amount of l-DOPA/carbidopa not in three but in six doses, again a normal 24-h GH secretion profile (25) was observed (pulse features: number, 9; amplitude, 0.4 ± 0.1 pmol/L). Interestingly, l-DOPA/carbidopa administration was
not mandatorily associated with GH release; two medications were not followed by GH pulses and four pulses occurred without l-DOPA/carbidopa administration (Fig. 3, lower panel).

The 24-h melatonin measurements. From serum pooled over 2-h periods for 24 h, the melatonin profile was examined twice, once on six doses of l-DOPA/carbidopa and once on an l-DOPA/carbidopa slow release preparation. On both occasions melatonin levels were in the normal range (16), but the circadian rhythm was advanced with a rather early acrophase (features of circadian rhythm: acrophase, 2200 h; amplitude, 79.6 pmol/L versus acrophase, 2300 h, amplitude, 85.1 pmol/L; Fig. 6). Usually in young adults the melatonin acrophase occurs at 0145 h ± 15.6 min (average clock time ± SEM in min) with an amplitude of 89.4 ± 11.2 pmol/L (average ± SEM) (9).

DISCUSSION

The major finding in our girl with BH₄ deficiency due to severe PTPS deficiency was massive hyperprolactinemia with rigorous disruption of the physiologic prolactin secretion pattern. Previously, in two similar cases elevated prolactin levels were reported (3, 26), whereas a patient with a less severe defect in biotinperin synthesis and evidence for normal brain catecholamine content had normal prolactin levels (27).

In our patient the prolactin profile showed that hyperprolactinemia was treated insufficiently if the daily amount of l-DOPA/carbidopa was given in three doses. However, the same amount of l-DOPA/carbidopa given in smaller doses at shorter intervals was more beneficial in normalizing serum prolactin concentrations and the slow release preparation was even markedly superior. Thus, in severe BH₄ deficiency the daily amount of l-DOPA/carbidopa should be administered at least in small doses, equally spaced over the 24-h period or preferable given as depot preparation.

Inasmuch as serum prolactin is known as an excellent marker of the hypothalamic dopamine content (6), prolactin profiles may compete with measurements of catecholamine metabolites in CSF for evaluation of the therapeutic l-DOPA/carbidopa adjustments in severe BH₄ deficiency. Prolactin measurements can be performed both easily and frequently by routine blood sampling, avoiding the painful procedure of lumbar puncture. Prolactin profiles give information on the brain catecholamine dynamic over a prolonged period compared with the punctual information provide by a single CSF sample. Furthermore, normalization of monoamines in one body compartment, e.g. cerebrospinal fluid is not necessarily associated with normalization throughout the other compartments (3). Thus, serum prolactin profiles may turn out as a useful and easy performable tool in estimating the catecholamine content in the CNS of patients with BH₄ deficiency.

Our patient exhibited an insufficient GH response to both arginine and insulin, although 24-h GH secretion analysis revealed a normal pulsatile secretion pattern with spontaneously occurring GH pulses on two different occasions. Furthermore, growth rate was nearly normal in our patient, paralleling the 3rd centile, a growth pattern often encountered in severe or chronic illness. The insufficient GH release after both arginine and insulin might be due to a nonresponsiveness to both agents. In an evaluation of GH stimulation tests, Weldon et al. (28) observed a proportion of 14.8% of nonresponders after arginine infusion and 15.4% after insulin-induced hypoglycemia. An alternative explanation is that dopamine plays a role as a mediator in the provocation of GH release with arginine or insulin. Thus, impairment of the endogenous l-DOPA/dopamine axis would trigger insufficient GH responses. The normal growth and the normal 24-h GH secretion pattern underline this hypothesis as well.

Interestingly, oral l-DOPA/carbidopa administration during the course of treatment did not result in GH release. Several explanations seem suitable. First, the lack of association between l-DOPA/carbidopa administration and GH release could be due to the adaptation to prolonged l-DOPA/carbidopa medication. According to the literature further GH response to dopaminergic stimulation is prevented after its repeated usage (29). Second, simple nonresponsiveness to l-DOPA would be conceivable. Schönberger et al. (30) found a proportion of 16% of nonresponders after a bolus of 250 mg of l-DOPA and 25 mg of carbidopa. Third, the lack of stimulation could be a problem of dosage. The daily amount of l-DOPA of approximately 10 mg/kg of body weight was divided into three or six dosages or administered thrice as a slow release preparation. Thus, the bolus given might have been too low for eliciting a pharmacologic response.

The physiologic spontaneous GH and cortisol secretion pattern was not altered in our patient. GH was secreted in several discrete pulses over 24 h, which were inconstantly connected with the administration of medication. Similarly, cortisol was released in frequent pulses that were superimposed on the

Figure 6. At 6½ y (top) and 8½ y (bottom), 24-h melatonin profiles in our patient with severe BH₄ deficiency while on six doses of l-DOPA/carbidopa (top panel) or three doses of l-DOPA/carbidopa slow release preparation (DS; bottom panel). DOPAC, l-DOPA/carbidopa; DOPAC, 20 μg; D, 20 mg of l-DOPA/2 mg of carbidopa. ↓ = occurrence of the acrophase.
normal circadian basal pattern. Because our patient represents a natural occurring model for severe catecholamine and serotonin deficiency, the data may indicate that the catecholamine system and serotonin are not crucial for the spontaneous release of GH and cortisol in man. An alternative explanation of the data would be that even low concentrations of brain catecholamines and serotonin suffice for maintenance of physiologic secretion of GH and cortisol but not for prolactin. [For discussion, cf. McCann and Krulich (5) and Wheeler and Styne (7)].

Finally the normal circadian melatonin secretion pattern was preserved in our patient, but its nocturnal elevation was advanced for 2 h. The normal melatonin levels observed, particularly the high concentrations during night time, indicate provision of sufficient melanin precursors to produce enough of the pineal hormone. This is remarkable because melatonin is manufactured from tryptophan by a four-step process which includes the conversion of tryptophan by the BH_{4}-dependent hydroxylase to 5-hydroxytryptophan and then to serotonin (8). The advance of the melatonin acrophase—an advance of the cortisol acrophase was not noticed—may be the result of l-DOPA/carbidopa administration, because a similar alteration occurred in l-DOPA/carbidopa-treated but not in untreated parkinsonian patients (9). Alternatively, it may reflect the altered life style of children, who go to bed earlier than do adults. Environmental factors such as sleep/wake, light/darkness are well known for their phase-setting capabilities of circadian rhythms (31).

In conclusion, severe BH_{4} deficiency is associated with serious hyperprolactinemia. The use of a slow release l-DOPA/carbidopa preparation renders near normoprolactinemic conditions. Serum prolactin profiles may represent a useful tool for monitoring the metabolic control in these patients. Because severe BH_{4} deficiency represents a useful model for catecholamine and serotonin deficiency, our patients indicate that even much reduced concentrations of these neurotransmitters are associated with a normal spontaneous GH, cortisol, or melatonin secretion pattern in man.

Note added to proof. Clinical, biochemical, and DNA analysis data of the patient (ID #48) are available via BIODEF database at: http://www.univzh.ch/~blau/biodef1.html.

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