RESEARCH

Copeptin levels increase in response to both insulin-induced hypoglycemia and arginine but not to clonidine: data from GH-stimulation tests

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Abstract

Background: The diagnosis of the polyuria–polydipsia syndrome is challenging. Copeptin is a robust biomarker of arginine vasopressin (AVP) secretion. Arginine, which stimulates growth hormone (GH), has been shown also to stimulate copeptin secretion via unknown mechanisms.
Aim: The aim was to investigate copeptin levels in response to three different GH stimulation tests in patients suspected of GH deficiency.
Methods: In this cross-sectional study, we measured plasma copeptin levels at baseline and at 60, 105, and 150 min in patients undergoing a stimulation test for growth hormone deficiency with either arginine ($n = 16$), clonidine ($n = 8$) or the insulin tolerance test (ITT) ($n = 10$).
Results: In patients undergoing the arginine test, the mean age was 9 years, and 10 years for clonidine. The ITT was only performed in adult patients (>18 years) with a mean age of 49 years. Copeptin level increased significantly from baseline to 60 min after arginine ($P < 0.01$) and ITT ($P < 0.01$). By contrast, copeptin level tended to decrease after clonidine stimulation ($P = 0.14$).
Conclusion: These data support that infusion of arginine increases plasma copeptin levels and reveal a comparable response after an ITT. We hypothesize that the underlying mechanism is abrogation of somatostatin-induced AVP suppression.

Key Words
- copeptin
- arginine
- insulin tolerance test
- clonidine
- pituitary

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Introduction

Disturbances in water homeostasis are highly prevalent and the underlying causes are not always easy to identify. In particular, the differential diagnosis in patients with the polyuria–polydipsia syndrome (PPS) is challenging (1). The polyuria of PPS is characterized by a high output of hypotonic urine (2) and includes four conditions: arginine vasopressin (AVP) deficiency, nephrogenic diabetes insipidus, primary polydipsia, and gestational diabetes insipidus. The water deprivation test is considered the gold standard, but it is cumbersome and the diagnostic performance is poor (with a diagnostic accuracy of only 70%) (3). Measurement of plasma AVP has been suggested as an alternative but analytical difficulties with the assays preclude its use (4). Copeptin, which is a split product of the vasopressin pre-pro-peptide, is cleaved and stored with AVP in equimolar amounts in neurosecretory granules in the posterior pituitary gland. Circulating copeptin levels change in parallel with AVP to osmotic, hemodynamic, and unspecific stress-related stimuli such as nausea (5). In contrast to AVP, copeptin is easy to assay and measurement of copeptin has been recommended for the differential

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diagnosis of PPS (1). Arginine, which is commonly used for diagnosing growth hormone (GH) deficiency (GHD) in children (6, 7), has proven valuable as a stimulant of the posterior pituitary too (1). Arginine-stimulated copeptin measurements is suggested as a safe and expedient test for AVP deficiency (the arginine-stimulated copeptin test has a diagnostic accuracy of 94% (95% CI: 86–97%) (8). However, it should be noted that several copeptin assays are available, which makes the interpretation of absolute copeptin values challenging (9).

Glucagon has also been shown to stimulate copeptin secretion, whereas the ghrelin-receptor agonist, macimorelin, has failed to stimulate copeptin (10). Arginine infused intravenously is assumed to suppress hypothalamic somatostatin release and thereby disinhibit GH release (11), but the mechanisms whereby arginine stimulates copeptin and AVP secretion remain unclear. Hypoglycemia also stimulates GH and copeptin secretion, and the insulin tolerance test (ITT) is used in the diagnosis of GHD in adults (12, 13). Again, the underlying mechanism is considered to involve suppression of hypothalamic somatostatin (14). Furthermore, clonidine is used to diagnose GHD in children (15) and has been reported to inhibit AVP secretion (16).

To further substantiate the ability of arginine to stimulate copeptin secretion and to elucidate the underlying mechanisms, we measured plasma copeptin levels in consecutive patients undergoing a diagnostic test for GH deficiency with either arginine, clonidine, or the ITT.

Methods

Patients at Aarhus University Hospital, who had routinely undergone a GH stimulation test with either arginine, clonidine, or ITT, were included. The patients undergoing either clonidine or arginine stimulation had the following diagnoses: craniopharyngioma (n = 3), glioma (n = 2), medulloblastoma (n = 1) and hypophysitis (n = 1), idiopathic GHD (n = 17). In the ITT group, the underlying diagnoses were adenomas (n = 8), meningioma (n = 1), and hypoplastic pituitary gland (n = 1). The tests were performed from 2018 to 2020. At our institution, arginine and clonidine are routinely used in the diagnosis of GHD in children, whereas the ITT is performed in adults.

The regional ethics committee system was inquired regarding the present study and did not require a formal approval since it was considered as a method development project. According to Danish legislation, method development projects do not require approval from the ethical committee system.

The arginine test

After an overnight fast, patients received an intravenous infusion with arginine hydrochloride 10% (0.5 g/kg, maximum 30 g). Patients were allowed to consume tap water during the fast and during the test. The infusion was administered over 30 min. Blood samples were collected every 15–30 min for 2.5 h. Three time points similar to those used in previous studies were chosen for measurement of copeptin (8).

The insulin tolerance test

After an overnight fast, the patients received an intravenous bolus of human insulin (0.1 IU/kg) followed by frequent blood sampling for 2.5 h during which water intake was allowed. Three time points similar to those used in previous studies were chosen for measurement of copeptin.

The clonidine test

After an overnight fast, patients received tablets with clonidine hydrochloride (Dixarit®, Boehringer Ingelheim, Ingelheim, Germany) (75 µg/m^2 body surface, maximum 100 µg), which were consumed with tap water. Water ingestion was allowed during the test. Blood samples were collected every 15–30 min for 2.5 h. Three time points similar to those used in previous studies were chosen for measurement of copeptin.

Laboratory measurements

Analysis of plasma copeptin concentration was performed on stored frozen blood samples using a commercial automated immunofluorescence assay (B·R·A·H·M·S KRYPTOR Compact Plus, B·R·A·H·M·S GmbH, Hennigsdorf, Germany). Blood samples from baseline (0 min) and 60, 105, and 150 min after the start of administration of arginine, clonidine, or insulin infusion were analyzed.

Statistical analysis

All analyses and graphics were performed in STATA (SE 17.0). Analysis for statistical significance was not

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performed in the AVP deficiency group due to the low sample size ($n=4$ and $n=2$ for the arginine and ITT group, respectively). Mean values ± S.D. were calculated for baseline and 60 min copeptin. Paired *t*-test was used to compare baseline and peak copeptin levels. For comparisons between two different tests, we used two-way analysis of variance (ANOVA) determining the significance of changes with time and test type. Independent samples *t*-test of the changes in plasma copeptin levels between 0 and 60 min was used for post hoc comparison of the individual tests. Graphs are illustrated with mean copeptin concentration and 95% CIs. $P < 0.05$ were considered significant.

Results

Baseline characteristics

Patients undergoing the arginine test had a mean age was 9 years (S.D. ± 3.7), for clonidine it was 10 years (S.D. ± 2.8). The ITT was only performed in adult patients (>18 years) with a mean age of 49 years (S.D. ± 11.3) (Table 1).

Sixteen patients underwent arginine stimulation of whom four had verified AVP deficiency at the time of the test, and ten patients were diagnosed with GHD.

Ten patients underwent the ITT of whom two had verified AVP deficiency at the time of the test and both of these patients were diagnosed with GHD. In total, six were diagnosed with GHD.

Eight patients were tested with clonidine of whom four were diagnosed with GHD. No patients in this group had AVP deficiency.

Arginine

In patients without AVP deficiency (n = 12), the mean plasma copeptin concentration at baseline was 8.3 pmol/L (S.D. ± 7.5) and increased to 10.3 pmol/L (S.D. ±8.6) ($P=0.01$) at 60 min after infusion of arginine (Fig. 1). This corresponds to 19.4% copeptin increase after 60 min. Patients with AVP deficiency (n = 4) had low baseline copeptin levels with a mean of 1.6 pmol/L without any response to arginine infusion (a mean copeptin level after 60min at 1.4pmol/L) (Fig. 1).

Table 1 Baseline characteristics for patients in each group. Data are presented as sample means ± S.D. or as numbers (*n*).

	Arginine	ITT	Clonidine
Number of patients (*n*)	16	10	8
Gender (*n:* F/M)	6/10	5/5	5/5
Age (years)	8.9 (3.7)	48.6 (11.3)	9.8 (2.8)
BMI (kg/m^2)	16.11 (3.8)	–	16.62 (2.6)
AVP deficiency (*n*)	4	2	0
GH deficiency (*n*)	10	2	0

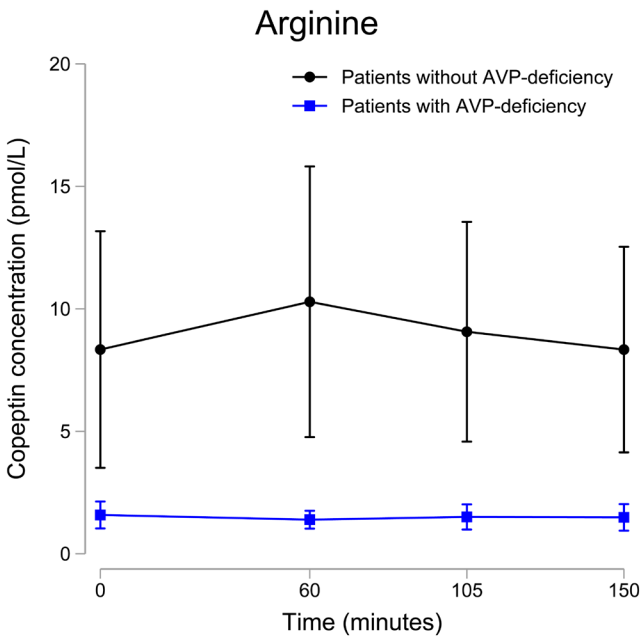

Figure 1
Plasma copeptin concentration during stimulation with arginine for patients with and without AVP deficiency are shown. (Data are shown as mean with 95% CI.)

Insulin tolerance test

The ITT was performed in ten patients. In patients without AVP deficiency (n = 8), the mean copeptin level at baseline was 3.3 pmol/L (S.D. ± 1.2), which increased to 5.7 pmol/L (S.D. ± 2.7) after 60 min ($P=0.01$). This corresponds to a 42.1% copeptin increase after 60 min. Among patients with AVP deficiency (n = 2), plasma copeptin concentrations were low and unresponsive to ITT with a baseline level at 1.9 pmol/L and 2.5pmol/L after 60 min (Fig. 2).

Clonidine

Eight patients, none of whom had AVP deficiency, underwent a clonidine stimulation test. The mean plasma copeptin level at baseline was 6.5 pmol/L (S.D. ± 3.8) with a small nonsignificant decline at 60 min after clonidine (5.5 pmol/L (S.D. ± 3.9, $P=0.14$)) (Fig. 3).

The difference in copeptin level from baseline to 60 min was different across the three test modalities (ANOVA $P < 0.01$) (Table 2). The increase in copeptin concentration from baseline to 60 min was similar for the

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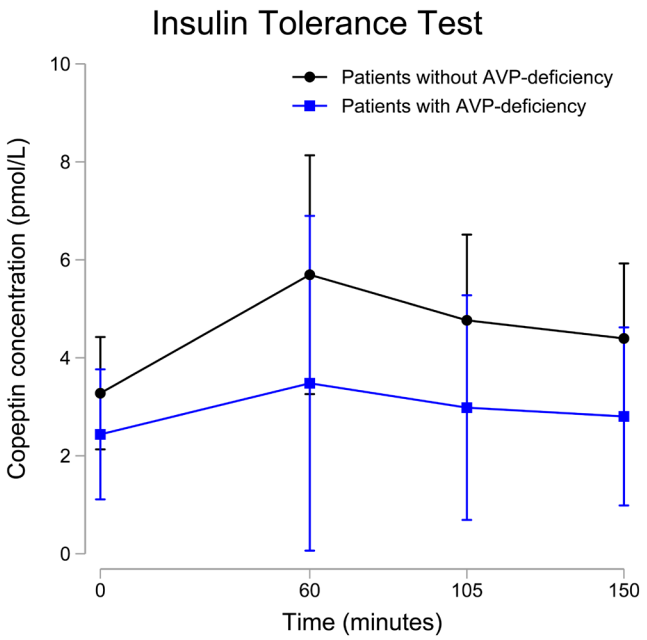

Figure 2
Plasma copeptin concentration during the ITT for patients with and without AVP deficiency. (Data are shown as mean with 95% CI.)

arginine test and the ITT ($P=0.63$) (excluding patients with AVP deficiency). The flat copeptin response to clonidine exposure was significantly different compared to the increase observed after arginine and the ITT (both $P < 0.01$).

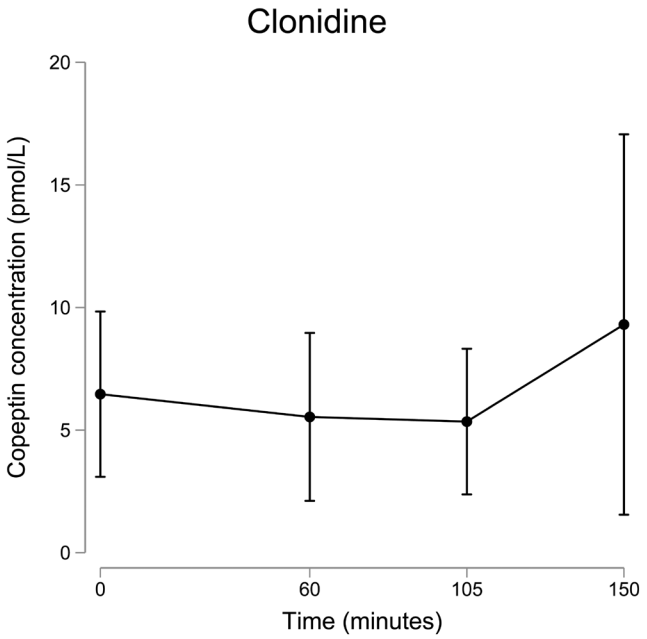

Figure 3
Plasma copeptin concentration during stimulation with clonidine. (Data are shown as mean with 95% CI.)

Patients with a diagnosis of GHD had comparable copeptin levels to patients without GHD (data not shown).

Discussion

In this study, we confirm that arginine infusion induces a significant increase in plasma copeptin levels in children in accordance with previous observations (8, 17). In addition, we report that ITT induces a similar increase in copeptin levels in adults, whereas no increase was observed after clonidine stimulation in children.

Arginine is an amino acid and a recognized GH secretagogue used alone or in combination with growth hormone-releasing hormone (GHRH) for diagnosing GHD in children and adults (6, 18). Surprisingly, it was recently reported that arginine also stimulates copeptin release from the posterior pituitary gland in healthy adults (8). The underlying mechanism is unknown, but it has been suggested to be mediated via nitric oxide (NO), which is synthesized from L-arginine via nitric oxide synthases (NOS) (19). This enzyme is abundantly expressed in the hypothalamus and paraventricular nuclei where the pre-pro-vasopressin molecule is synthesized (20). Experimental studies in rats (21), rabbits (22), and dogs (23) suggest that NO regulates AVP release (24). With regards to GH secretion, several studies fail to support that arginine-induced GH release is NO-dependent (19, 25). Instead, arginine may act by suppressing somatostatin (6), the latter of which is known to suppress both GH and AVP (6, 11, 19, 25, 26, 27). Güllner *et al.* (28) showed a reciprocal relationship between somatostatin and AVP in a rat model of water deprivation. In support of this, Wang *et al.* (27) observed that somatostatin infusion inhibited AVP in a sheep model of intracerebroventricular hemorrhage. Taken together, it is possible that arginine may abrogate somatostatin-induced AVP suppression.

Treatment with lanreotide, a somatostatin agonist has been shown not to impact circulating copeptin levels in patients with autosomal dominant polycystic kidney disease (ADPKD) (29), which may speak against a regulatory effect on somatostatin. However, subcutaneously administered lanreotide is not likely to exert hypothalamic effects and the regulation of vasopressin may also differ in patients with ADPKD.

Our results were obtained in a pediatric population in which the stimulatory effects of arginine on copeptin secretion have not been as thoroughly investigated. Binder *et al.* (17) reported that arginine stimulated copeptin secretion in a pediatric setting but to a lesser

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Table 2 Changes in plasma copeptin concentration in patients without AVP deficiency from baseline to 60 min presented as means ± s.d.

	Arginine	ITT	Clonidine
ΔCopeptin concentration 0 to 60 min (pmol/L)	2.0 (1.9)[a]	2.4 (1.6)[a]	−0.9 (2.3)

[a]$P < 0.01$ compared to clonidine.

extent as compared to adults. In our study, the baseline level of copeptin in the arginine group was 8.3 pmol/L, which is higher than both the stimulated values in the study by Binder and the baseline values observed in a study by Winzeler *et al.* (8). The same assay (BRAHMS KRYPTOR Compact plus) was used in the three studies, thereby minimizing analytical variation as an explanation. The discrepancy could be due to the nature of our study as it was based on samples where the patients had undergone an overnight fast and were allowed to consume water. However, in the absence of osmolality data this is speculative. Emotional stress, which is a strong stimulus of copeptin secretion, may have occurred to a larger degree in our study (30).

We recorded a significant increase in copeptin levels after the ITT, which is compatible with previous data showing an increase in AVP levels in response to ITT (31, 32). Hypoglycemia has also been reported to stimulate copeptin secretion in a pediatric population (33). The underlying mechanism is unknown, but it has previously been shown that insulin-induced hypoglycemia does not change plasma osmolality (31, 32). Sodium is the most potent solute known to stimulate AVP (34), yet the increased sodium level second to glucose uptake is not considered to contribute significantly to the rise in AVP (31). Thus, the observed effect of insulin-induced hypoglycemia is believed to be primarily mediated by a nonosmotic stimulus. Hypoglycemia as a stimulator of GH-release has been thoroughly investigated and considered to be caused by inhibition of glucose-mediated hypothalamic somatostatin release (14). This is supported by the discovery that the stimulatory effect of glucose on somatostatin release is reversed by pyridostigmine, which suppresses somatostatin release from the hypothalamus (35). Chiodera *et al.* performed a study in which they infused somatostatin during insulin-induced hypoglycemia in man (36) and showed a significant reduction of the plasma AVP response during somatostatin infusion. This supports the hypothesis that somatostatin suppresses AVP release and, by inference, that the increase in copeptin levels in response to both arginine and the ITT involves suppression of hypothalamic somatostatin secretion.

The interplay between hypoglycemia and NO is ambiguous since one study suggests that NO suppresses AVP during hypoglycemia (37), although it is generally assumed that NO increases when the glucose supply to the brain is limited (38). It thus seems less likely that NO contributes to AVP regulation during physiological hypoglycemia, since AVP and NO both increase in response to hypoglycemia.

A recent study by Atila *et al.* (10) suggests that the rapid drop in glucose level itself upon both insulin and glucagon administration cause the rise in copeptin levels. However, copeptin has been found to mirror cortisol levels in response to stress, myocardial infarction, and other acute illnesses, and copeptin has been proposed as a prognostic marker in such cases (39). AVP is known to potentiate corticotropin-releasing hormone (CRH)-induced adrenocorticotropic hormone secretion and as such can be regarded as a stress hormone. Taken together, the copeptin increase in response to ITT-induced hypoglycemia could be regarded as an appropriate stress response.

Clonidine is an ∝2-adrenergic agonist, which is used for diagnosing GH deficiency in children via stimulation of GHRH from the hypothalamus (40). However, other studies suggest that the effect of clonidine on GH release also involves hypothalamic somatostatin suppression (15). In our study, clonidine did not induce a significant increase in plasma copeptin levels, and experimental data even suggest an inhibitory effect of clonidine on AVP secretion (16, 41). This is contrary to both arginine and ITT that stimulate both GH and AVP. The mechanisms whereby clonidine may suppress AVP release have been suggested to involve a reduction of presynaptic noradrenalin release (16, 42, 43).

In addition to arginine, clonidine, and hypoglycemia, copeptin in response to other GH secretagogues has been studied (10, 44). Glucagon, which is used for diagnosing GHD in adults and children (45, 46), also stimulates copeptin (44), which likely is caused by the changes in glucose levels. Macimorelin, a synthetic ghrelin receptor agonist, does not seem to stimulate copeptin (47) and the same appears true for ghrelin itself (48).

Our study has the strength that several potential modulators of copeptin were investigated, but our study

also has several limitations. First, the conditions before and during the tests were not standardized according to fluid intake and patients were allowed to ingest water before and after the test. This may have suppressed AVP secretion and reduced the stimulatory response to the tests. Second, the patients from the three groups differed in baseline copeptin levels and age, which make comparisons between the groups difficult. Third, the data derived from patients undergoing GH stimulation tests, which are likely to reduce the generalizability of the results.

In summary, the present data support that infused arginine increases plasma copeptin levels and that a comparable effect is observed after an ITT. This could indicate a common stimulatory mechanism, which may involve abrogation of somatostatin-induced AVP suppression, but further mechanistic studies are needed. More real-world data are also welcomed to substantiate the usefulness of arginine-stimulated copeptin measurements for the diagnosis of AVP deficiency.

Declaration of interest

The first author, Stankovic J., has received a speaker's fee from OTSUKA Pharmaceuticals. Other authors declare they have no financial interest.

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Author contribution statement

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