CLINICAL STUDY

Subtyping of primary aldosteronism by adrenal vein sampling: effect of acute D₂ receptor dopaminergic blockade on adrenal vein cortisol and chromogranin A levels

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Abstract

Background: Adrenal vein sampling (AVS) is the gold standard for identifying the surgically curable forms of primary aldosteronism. Dopamine modulates adrenocortical steroidogenesis and tonically inhibits aldosterone secretion via D₂ receptor. However, whether it could also affect the release of cortisol and chromogranin A (ChA), which can be used to assess the selectivity of AVS, is unknown. **Objective:** To investigate whether metoclopramide increased the release of cortisol and ChA and could thereby improve assessment of the selectivity at AVS.

Design and methods: We investigated the effect of acute D₂ antagonism with metoclopramide on cortisol and ChA release from the adrenal gland by comparing the adrenal vein and infrarenal inferior vena cava (IVC) hormone levels at baseline and after metoclopramide administration in 34 consecutive patients undergoing AVS.

Results: Metoclopramide increased plasma aldosterone in the IVC ($P < 0.00001$) and in the adrenal vein blood ($P < 0.002$) but failed to increase plasma cortisol concentration or ChA levels. Therefore, it did not increase the selectivity index based on the measurement of either hormone.

Conclusions: This study shows that the release of cortisol and ChA is not subjected to tonic D₂ dopaminergic inhibition. Therefore, these findings lend no evidence for the usefulness of acute metoclopramide administration for enhancing the assessment of the selectivity of blood sampling during AVS with the use of either cortisol or ChA assay.

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Introduction

Primary aldosteronism (PA), a common endocrine cause of arterial hypertension, is surgically curable when lateralization of excessive aldosterone is identified (1, 2). Adrenal vein sampling (AVS) represents the 'gold standard' to this end (2), but notwithstanding recent refinements (3, 4), investigative efforts aimed at optimizing its performance are necessary (2, 5).

Dopamine is held to tonically inhibit aldosterone secretion because the selective D₂ receptor antagonist metoclopramide induces aldosterone release in humans (6, 7). More recent evidence showed that the dopamine acts by blunting Ca²⁺ influx and protein kinase C phosphorylation (8), which suggests that its inhibitory effect involves the early steps of the steroidogenesis and therefore are not confined to aldosterone synthesis, but may involve also cortisol and its precursors. The lack of effect of the D₂ receptor agonists bromocriptine and cabergoline on cortisol production from the adrenocortical cells also suggests a tonic dopaminergic inhibition of cortisol and androstenedione production (9).

Chromogranin A (ChA) is co-localized with catecholamines in chromaffin cells and controls the adrenal medullary release of catecholamines in an autocrine fashion (10). Although regarded as a marker for neuroendocrine tumors, including pheochromocytoma (11), its regulation, secretion rate, and plasma half-life are not known (12).

We recently provided evidence for a step-up of ChA between the adrenal vein and the inferior vena cava (IVC) blood, which was smaller than that of the plasma cortisol concentration (PCC) under baseline conditions (13). However, whether D₂ blockade could be useful to enhance this step-up (13) and therefore can strengthen the identification of surgically curable subtypes of PA (14) remains hitherto unknown.

This study was, therefore, designed to investigate if i) the release of ChA and cortisol is under tonic dopaminergic inhibition and ii) acute D₂ blockade enhances the assessment of the selectivity of AVS by increasing the step-up of ChA and/or cortisol between the IVC and the adrenal vein blood.

Figure 1 Flow chart of the study. AVS was performed with bilateral simultaneous catheterization before and after stimulation with metoclopramide (10 mg i.v.). Blood samples for the measurements of plasma aldosterone concentration (PAC), cortisol (PCC), and chromogranin A (ChA) were obtained from the infrarenal IVC and from the right and left adrenal veins at baseline (t-15 and t0), and again 15 min after metoclopramide stimulation (t15). Baseline and post-metoclopramide values were used for head-to-head comparison.

Study design

In a within-patient study design (Fig. 1), we recruited consecutive patients referred to our Specialized Centre for Hypertension for suspected PA. They met the current indications for AVS (2), they were asked to be on a normal sodium intake (1). Briefly, treatment with mineralocorticoid receptor antagonists was withdrawn at least 6 weeks before the study; treatment with agents that affect the renin–angiotensin–aldosterone system, including diuretics, β -blockers, angiotensin converting enzyme inhibitors, and angiotensin II type 1 receptor antagonists, was withdrawn for at least 2 weeks. A long-acting calcium channel blocker and/or doxazosin were prescribed whenever necessary for minimizing the risks of uncontrolled hypertension (HT). AVS was undertaken after correction of hypokalemia, if present, with K^+ supplementation (4). Procedures were performed according to the principles of the Declaration of Helsinki and the institutional guidelines. A written consent was obtained from all participants.

As proton pump inhibitors raise ChA (15, 16), the exclusion criteria entailed need for such treatment and a concurrent pheochromocytoma, because metoclopramide can trigger hypertensive crises in these patients, besides refusal of the patients to undergo AVS and/or adrenalectomy. Peripheral plasma ChA and urinary free catecholamines, normetanephrine, and metanephrine were systematically measured before AVS to exclude pheochromocytoma.

AVS procedure

AVS was simultaneously performed bilaterally by an experienced radiologist (D M) using a catheter shaped for each adrenal vein (Fig. 1) as described earlier (3). Blood samples for the measurements of plasma aldosterone concentration (PAC), PCC, and ChA were obtained from the infrarenal IVC and simultaneously from the right and left adrenal veins by gravity at baseline (t-15 and t0) and again 15 min after metoclopramide (t15). On the left side, the catheter remained in the vein during the procedure; on the right side, it was withdrawn from the adrenal vein after blood sampling and then repositioned just before collecting the next sample. The selectivity index (SI) was calculated using the PCC or the ChA values as described (4).

Acute D_2 dopaminergic blockade

The administration of metoclopramide (10 mg), given as an i.v. bolus at t0, was used to assess the effect of D_2 antagonism on the adrenal vein and IVC levels of PAC, PCC, and ChA (Fig. 1).

Biochemical measurements

Levels of serum, K^+ , PAC, PCC and plasma renin activity (PRA) were measured as described (1, 13). Normal ranges, intraassay and interassay coefficient of variation, and antibody cross-reactivity for the

Table 1 Clinical and biochemical features of the PA patients and effects of adrenalectomy. The treatment score was calculated as the sum of the number of the drugs. Data is presented as mean $\pm$ s.d., or median (interquartile range), as appropriate.

Variable	Adrenalectomy		P
	Before (n=34)	After (n=20)	
Systolic blood pressure (mmHg)	169 $\pm$ 21	129 $\pm$ 17	0.006
Diastolic blood pressure (mmHg)	100 $\pm$ 16	85 $\pm$ 10	0.016
Serum K^+ levels (mmol/l)	3.0 $\pm$ 0.6	4.2 $\pm$ 0.3	0.001
Supine PRA (ng/ml per h)	0.37 (0.20–0.80)	0.90 (0.50–1.25)	0.03
Supine aldosterone (ng/dl)	16.9 (15.0–26.5)	9.6 (5.9–13.7)	0.002
ARR (ng/dl):(ng/ml per h)	46.8 (26.3–77.6)	8.2 (4.7–43.2)	0.01
24 h urinary Na^+ excretion (mEq)	193 $\pm$ 20	–	
Treatment score	3.0 (2.0–5.0)	2.0 (0–3.0)	0.0001

PRA, plasma renin ratio; ARR, aldosterone to renin (PRA) ratio. To convert ng/dl aldosterone into pmol/l, multiply by 27.76.

Table 2 Plasma cortisol concentrations in infrarenal IVC and in the adrenal veins at baseline (t-15 and t0) and after stimulation with metoclopramide. Data is presented as mean $\pm$ s.d.

Time	Plasma cortisol levels (μ g/dl)					
	Right adrenal vein	<i>P</i> (right vs IVC)	IVC	<i>P</i> (left vs IVC)	Left adrenal vein	<i>P</i> (right vs left)
Baseline t-15	378.7 $\pm$ 69.6	0.001	18.1 $\pm$ 1.3	0.001	325.9 $\pm$ 49.8	NS
Baseline t0	107.1 $\pm$ 24.7	0.001	16.7 $\pm$ 1.1	0.003	143.2 $\pm$ 40.2	NS
Baseline t-15 vs baseline t0	0.001	—	0.002	—	0.003	—
Metoclopramide	194.1 $\pm$ 60.4	0.006	17.0 $\pm$ 1.1	0.0001	184.2 $\pm$ 37.3	NS
Metoclopramide vs baseline t0	NS	—	NS	—	NS	—

measurements of PAC, PCC, and PRA have already been reported (1). Plasma ChA was measured using a commercial ELISA kit (CGA-RIACT, Cisbio, France); normal ranges are 19–98 μ g/l (or ng/ml) and intra-assay and interassay coefficient of variation are 6 and 8.5% respectively.

Statistical analysis and calculation of power

Results are expressed as mean $\pm$ s.e.m., or median and interquartile range, as appropriate. Because of their skewed distribution, PAC and PRA values were examined after log transformation. A paired *t*-test was used to compare baseline with metoclopramide-stimulated log-transformed variables. Given a sample size of 34 for PCC and 11 for ChA, the study had a 99 and a 96% power to detect a mean difference of 3.4 μ g/dl for PCC and 15 μ g/l for ChA respectively, between baseline (t0) and metoclopramide-stimulated (t15) values, assuming that the common s.d. was 1.1 μ g/dl for PCC and 9 μ g/l for ChA, using a two-sided paired *t*-test at $\alpha=0.05$. Statistical significance was set at $P<0.05$ (two-sided).

Results

Patients and diagnosis

We recruited 34 consecutive patients (age: 50 $\pm$ 12 years; 22 M, 12 F) undergoing AVS with metoclopramide stimulation; concomitant measurements of PCC

and ChA were available in 11 patients. The patients showed an average urinary sodium excretion in the normal range (193 $\pm$ 20 mmEq/24 h, mean $\pm$ s.e.m.). The diagnosis of APA was based on the 'four corner' approach (1). Of the 34 patients, 21 had an APA (median maximum diameter $\sim$ 20 mm, range: 13–25 mm) and 13 patients with a non-lateralized aldosterone secretion suggested idiopathic hyperaldosteronism. Twenty of the patients with an APA underwent adrenalectomy and one patient refused the surgical treatment because of supervening pregnancy. Their main features and the effect of adrenalectomy in the APA patients are shown in Table 1.

All PA patients showed suppressed PRA, elevated PAC, and, therefore, a marked increase in the ARR. After the removal of APA, notwithstanding tapering of the antihypertensive drugs, there was a significant decrease in blood pressure. This was accompanied by a significant decrease of PAC, an increase in PRA, and the normalization of the ARR.

Effect of D₂ receptor blockade

The PCC and ChA levels in the IVC blood were within the normal range in all patients (Tables 2 and 3). At baseline (t-15 and t0), they showed a step-up between the IVC and the adrenal vein blood (PCC: IVC 16.7 $\pm$ 1.1, right: 107.1 $\pm$ 24.7 $P<0.003$, left: 143.2 $\pm$ 40.2 $P<0.003$; ChA: IVC 21.1 $\pm$ 3.0, right: 27.9 $\pm$ 4.7 $P<0.02$, left: 28.6 $\pm$ 3.9 $P<0.009$) in all patients (Fig. 2). This indicates that both hormones are tonically released from the adrenal gland and, therefore, can be

Table 3 Plasma ChA levels in infrarenal IVC and in the adrenal veins, at baseline (t-15 and t0) and after stimulation with metoclopramide. Data is presented as mean $\pm$ s.d.

Time	Plasma ChA levels (μ g/l)					
	Right adrenal vein	<i>P</i> (right vs IVC)	IVC	<i>P</i> (left vs IVC)	Left adrenal vein	<i>P</i> (right vs left)
Baseline t-15	26.5 $\pm$ 3.8	0.006	22.4 $\pm$ 3.3	0.008	31.6 $\pm$ 4.1	NS
Baseline t0	27.9 $\pm$ 4.7	0.020	21.1 $\pm$ 3.0	0.009	28.6 $\pm$ 3.9	NS
Baseline t-15 vs baseline t0	NS	—	0.022	—	0.039	—
Metoclopramide	28.3 $\pm$ 3.9	0.028	22.4 $\pm$ 3.1	0.011	32.0 $\pm$ 4.5	NS
Metoclopramide vs baseline t0	NS	—	NS	—	NS	—

Figure 2 Effect of acute D_2 receptor blockade with metoclopramide administration on plasma cortisol (PCC, panel A), aldosterone (PAC, panel B), PAC to PCC ratio (panel C), and chromogranin (ChA, panel D) concentrations in the infrarenal inferior vena cava (IVC) and in the right- and left-adrenal vein blood. A significant increase in PCC, PAC, PAC/PCC, and ChA values was observed in each adrenal vein blood compared with IVC. PAC but not PCC significantly increased after metoclopramide stimulation. The selective increase in PAC and not in PCC with acute D_2 receptor dopaminergic blockade is also evident on both sides after correction for the degree of selectivity and adrenal blood dilution in panel C. No significant increase in ChA was found after metoclopramide stimulation.

used to assess the selectivity of catheterization (13). As both PCC (Table 2) the ChA fell, from t-15 to t0 albeit not on the right side (Table 3), the t0 values were used for comparison with the post-metoclopramide values.

Metoclopramide raised PAC in both the IVC and in the adrenal vein blood (Fig. 2, panel A); the increase was more prominent after correction for PCC, e.g. when the values were corrected for the degree of selectivity and dilution (Fig. 2, panel B). In contrast, neither PCC nor ChA levels significantly changed after metoclopramide (Fig. 2, panels C and D).

When results were examined according to the dominant and non-dominant side (Figs 3 and 4), we found that metoclopramide elicited a significant increase in PAC and PAC/PCC values on both the dominant (Fig. 3, panel A) and the non-dominant side (Fig. 3, panel B). At variance PCC and ChA values did not significantly change at any site after metoclopramide stimulation (Fig. 3, panels C and D). Hence, the acute D_2 blockade did not increase the SI, and thus did not facilitate the assessment of AVS selectivity (Fig. 4) using either PCC-SI or ChA-SI.

Discussion

As the assessment of selectivity of adrenal vein catheterization remains challenging (17), this study investigated the hypothesis that cortisol and ChA could be under tonic D_2 receptor-mediated dopaminergic

control (9), and therefore, that metoclopramide could facilitate the ascertainment of the selectivity of AVS by increasing PCC and ChA release, and thus their step-up between the IVC and the adrenal vein blood. In this regard, we recently reported that ChA is tonically released from the adrenal gland (13). Even though the gradient of ChA between the adrenal vein and the IVC blood was smaller (three- to four-fold on the right side and three- to five-fold on the left side) than that of cortisol, this observation suggested that this protein could be used to assess the selectivity of catheterization and to correct for the dilution of the blood sampling. The questions, however, remained whether D_2 receptor blockade could differently stimulate ChA and/or cortisol secretion and whether this stimulation could enhance the assessment of selectivity of AVS by using either measurement or both.

With a high-statistical power, this study shows that metoclopramide did not elicit any detectable change of cortisol and ChA (Fig. 2, panels A and D). Hence, the release of ChA and cortisol, at variance with that of aldosterone and catecholamines, is not subjected to dopamine inhibition acting via D_2 receptor. It might be argued that because the dopaminergic tone is held to be directly related to sodium intake (7), this lack of response to D_2 blockade could be due to sodium depletion. This contention seems, in our view, to be untenable because i) our patients were on a normal sodium intake, as shown by a normal 24 h urinary sodium excretion, and ii) metoclopramide did induce the

Figure 3 Effect of acute D_2 receptor blockade with metoclopramide administration on plasma aldosterone (PAC, panel A), PAC to PCC ratio (panel B), cortisol (PCC, panel C), and chromogranin (ChA, panel D) concentrations in the infrarenal inferior vena cava (IVC) and in the adrenal vein blood of the dominant and non-dominant side. A significant increase in PAC and PAC/PCC values, but not in PCC or ChA, was observed in the adrenal vein blood of the dominant side, compared with the non-dominant side. Acute D_2 receptor blockade with metoclopramide induced a significant increase in PAC and PAC/PCC values in both dominant and non-dominant side, whereas no significant changes were found in either PCC or ChA after metoclopramide stimulation.

Figure 4 Effect of acute D₂ receptor blockade with metoclopramide administration on the selectivity index. No significant changes were found after metoclopramide in the selectivity index calculated with cortisol (PCC SI) or with chromogranin A (ChA SI) either at the dominant or non-dominant side.

expected rise of aldosterone release, indicating that the dopaminergic tone was not blunted. Hence, our finding that ChA release is not under D₂ receptor dopaminergic regulation represents a hitherto unappreciated finding that has some pathophysiological and clinical implications as discussed below.

A potential limitation of this study might be the lack of specificity of metoclopramide as a D₂ receptor dopaminergic blocker as this drug, although initially regarded as the prototype of D₂ receptor-selective antagonists, was thereafter found not to be a pure drug (18, 19). It displays submicromolar affinity for D₂ and D₃ receptors, but micromolar affinity also for D₁, D₄, and D₅. It also showed some affinity (at submicromolar to micromolar concentrations) for the 5-HT₃ and 5-HT₄ serotonin receptors (20, 21). As the normal adrenal cortex, and even more so the APA, can express 5HT₄, which on binding serotonin released from mast cells can elicit aldosterone secretion (22), it might be argued that some of the metoclopramide effects seen in this study occurred via serotonin receptors. We cannot totally exclude this possibility because we could not use for these experiments selective 5-HT₃ and 5-HT₄ blockers. However, metoclopramide did induce the aldosterone increase, which was expected with D₂ receptor dopaminergic blockade, but did not alter cortisol release, which could be anticipated if this hormone was under serotonergic control. It remains controversial whether the 5-HT₄ receptors in the human zona fasciculata play any role in the physiologic control of cortisol secretion: 5-HT was reported to stimulate cortisol much less efficiently than aldosterone secretion *in vitro* and failed to do so *in vivo* (23). The only evidence of 5-HT₄ receptors-mediated cortisol production reported to date pertains to ACTH-independent

macronodular adrenal hyperplasia causing Cushing's syndrome (24), where 5-HT₄ receptors are held to be aberrant. Hence, the failure of acute metoclopramide administration to modify ChA release strongly supports the conclusion that ChA release is not under tonic D₂ receptor-mediated dopaminergic inhibition or at least is unaffected by the metoclopramide dose chosen in this study, which is clinically used.

Considering the crucial role of AVS for the identification of surgically curable subtypes of PA (5, 17), it is worth mentioning that acute D₂ receptor blockade markedly increases aldosterone release in PA patients (7) and, therefore, could enhance the lateralization of aldosterone excess. It is noteworthy that this study by showing that metoclopramide increased the plasma adrenal vein level of aldosterone demonstrates that this hormone is under tonic dopaminergic D₂ receptor-mediated inhibition and confirms previous limited observations (7). Whether this could improve the lateralization of excessive aldosterone to the affected side, and thereby the diagnosis of the surgically curable subtypes of PA, is currently being pursued in a large prospective study.

In conclusion, this study shows that acute D₂ dopaminergic receptor blockade does increase aldosterone but not cortisol and ChA. Hence, metoclopramide could improve the identification of the lateralization of aldosterone excess but not the ascertainment of the selectivity of AVS. The lack of any appreciable increase in ChA with metoclopramide has implications for the work-up of pheochromocytoma and paraganglioma, as it suggests that the acute hypertensive crises induced by abrupt catecholamines release after D₂ receptor blockade, which can even trigger acute coronary syndrome in patients with undetected pheochromocytoma, do not involve enhanced ChA release.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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