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 (A VS) 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.
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 Briefly, treatment with mineralocorticoid receptor antagonists was withdrawn at least 6 weeks before the study; treatment with agents that affect the renin–angiotensin–aldosterone system, including diuretics, β-blockers, angiotensin converting enzyme inhibitors, and angiotensin II type 1 receptor antagonists, was withdrawn for at least 2 weeks.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), 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.

Acute D₂ dopaminergic blockade

The administration of metoclopramide (10 mg), given as an i.v. bolus at t0, was used to assess the effect of D₂ 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
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 μg/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 ± 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 mg/dl for PCC and 15 mg/l for ChA respectively, between baseline (t0) and metoclopramide-stimulated (t15) values, assuming that the common S.D. was 1.1 mg/dl for PCC and 9 mg/l for ChA, using a two-sided paired t-test at a Z0.05. Statistical significance was set at P!0.05 (two-sided).

Results

Patients and diagnosis

We recruited 34 consecutive patients (age: 50 ± 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 ± 20 mmEq/24 h, mean ± 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 ~ 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 ± 1.1, right: 107.1 ± 24.7 P < 0.003, left: 143.2 ± 40.2 P < 0.003; ChA: IVC 21.1 ± 3.0, right: 28.3 ± 4.7 P < 0.02, left: 28.6 ± 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>
<thead>
<tr>
<th>Time</th>
<th>Right adrenal vein</th>
<th>P (right vs IVC)</th>
<th>IVC</th>
<th>P (left vs IVC)</th>
<th>Left adrenal vein</th>
<th>P (right vs left)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline t-15</td>
<td>378.7 ± 69.6</td>
<td>0.001</td>
<td>18.1 ± 1.3</td>
<td>0.001</td>
<td>325.9 ± 49.8</td>
<td>NS</td>
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<tr>
<td>Baseline t0</td>
<td>107.1 ± 24.7</td>
<td>0.001</td>
<td>16.7 ± 1.1</td>
<td>0.003</td>
<td>143.2 ± 40.2</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline t-15 vs baseline t0</td>
<td>0.001</td>
<td>–</td>
<td>0.002</td>
<td>–</td>
<td>0.003</td>
<td>–</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>194.1 ± 60.4</td>
<td>0.006</td>
<td>17.0 ± 1.1</td>
<td>0.0001</td>
<td>184.2 ± 37.3</td>
<td>NS</td>
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<tr>
<td>Metoclopramide vs baseline t0</td>
<td>NS</td>
<td>–</td>
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<th>Left adrenal vein</th>
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<tbody>
<tr>
<td>Baseline t-15</td>
<td>26.5 ± 3.8</td>
<td>0.006</td>
<td>22.4 ± 3.3</td>
<td>0.008</td>
<td>31.6 ± 4.1</td>
<td>NS</td>
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<tr>
<td>Baseline t0</td>
<td>27.9 ± 4.7</td>
<td>0.020</td>
<td>21.1 ± 3.0</td>
<td>0.009</td>
<td>28.6 ± 3.9</td>
<td>NS</td>
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<tr>
<td>Baseline t-15 vs baseline t0</td>
<td>NS</td>
<td>–</td>
<td>0.022</td>
<td>–</td>
<td>0.039</td>
<td>–</td>
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<tr>
<td>Metoclopramide</td>
<td>28.3 ± 3.9</td>
<td>0.028</td>
<td>22.4 ± 3.1</td>
<td>0.011</td>
<td>32.0 ± 4.5</td>
<td>NS</td>
</tr>
<tr>
<td>Metoclopramide vs baseline t0</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</table>
ChA levels significantly changed after metoclopramide acute D2 blockade did not increase the SI, and thus did dominate (Fig. 3, panel A) and the non-dominant side increase in PAC and PAC/PCC values on both the found that metoclopramide elicited a significant dominance of (Fig. 3, panel B). At variance PCC and ChA values did increase in PAC and not in PAC with acute D2 receptor stimulation. The selective increase in PAC and not in PAC with acute D2 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 PAC, 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 D2 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 D2 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 D2 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 D2 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 D2 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

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**Figure 2** Effect of acute D2 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 PAC with acute D2 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.

**Figure 3** Effect of acute D2 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 D2 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.

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this study occurred via serotonin receptors. We cannot
argued that some of the metoclopramide effects seen in
molar to micromolar concentrations) for the 5-HT3 and
5-HT4, which on binding serotonin released from mast
cells can elicit aldosterone secretion (22), it might be
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 impli-
cations 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 submicro-
molar 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 serotoninergic 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 pro-
duction 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 identifi-
cation 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 aldoster-
one 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 block-
ade, 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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