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.

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.

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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.

**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

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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 ± s.d., or median (interquartile range), as appropriate.

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

PRA, plasma renin ratio; ARR, aldosterone to renin (PRA) ratio. To convert ng/dl aldosterone into pmol/l, multiply by 27.76.
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 α = 0.05. 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 PAC 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 17.0 ± 1.1, right: 21.1 ± 3.0, left: 28.6 ± 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...
ChA levels significantly changed after metoclopramide acute D2 blockade did not increase the SI, and thus did not significantly change at any site after metoclopramide stimulation (Fig. 3, panel B). At variance PCC and ChA values did not significantly change at any site after 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 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 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 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

**Table 2**

<table>
<thead>
<tr>
<th>IVC</th>
<th>Dominant Non-dominant</th>
</tr>
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<tbody>
<tr>
<td>PAC (µg/l)</td>
<td>100</td>
</tr>
<tr>
<td>PCC (µg/l)</td>
<td>100</td>
</tr>
<tr>
<td>PAC/PCC</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 3**

<table>
<thead>
<tr>
<th>IVC</th>
<th>Dominant Non-dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAC (ng/dl)</td>
<td>100</td>
</tr>
<tr>
<td>PCC (µg/l)</td>
<td>100</td>
</tr>
<tr>
<td>ChA (µg/l)</td>
<td>100</td>
</tr>
</tbody>
</table>

**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 PCC 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.
cells can elicit aldosterone secretion (22), it might be for these experiments selective 5-HT3 and 5-HT4 totally exclude this possibility because we could not use 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 domiant or non-dominant side.

cortisol (PCC SI) or with chromogranin A (ChA SI) either at the cortisol release, which could be anticipated if this receptor dopaminergic blockade, but did not alter aldosterone increase, which was expected with D2 blockers. However, metoclopramide did induce the 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 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 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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