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Pastis and hypertension—what is the molecular basis?

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Glycyrrhiza glabra

The therapeutic properties of *Glycyrrhiza glabra* were already known by Egyptians, Greeks, and Romans in antiquity [1]. They used extracts from this plant for a diversity of ailments and as a sweetener. In the modern society it is found in drinks such as Belgian beers, Ouzo, Pernod or Pastis brands. Many chewing gums contain glycyrrhetinic acid. The rationale for adding glycyrrhetinic acid, the active ingredient of liquorice, to chewing gums is the observation that, contrary to glucose, liquorice does not promote bacterial growth and adherence of cariogenic bacteria [2]. In addition liquorice is often added to confectionery. The discovery of the value of liquorice — previously marketed as carbenoxolone, an oleandane derivative of glycyrrhetinic acid — in the treatment of peptic ulcer allowed researchers to establish its adverse effect on salt and water metabolism.

Clinical features and erroneous interpretation

Patients with excessive ingestion of liquorice present with hypokalaemic hypertension in the absence of a renal artery stenosis. The urinary sediment is normal [3]. A metabolic alcalosis is commonly observed. The renin–aldosterone system is suppressed. Serum cortisol and 24-h urinary cortisol levels are within the normal range. When liquorice is prescribed to normal volunteers under experimental conditions a positive sodium balance with an increase in body weight of about 2–3 kg is observed during the initial 10 days. Thereafter sodium intake equals urinary sodium excretion, suggesting escape from the mechanism causing renal sodium retention. A normal urinary potassium excretion in the presence of low potassium concentrations in serum indicates abnormal renal loss of potassium. In Table 1 the rare known causes of low-renin, low-aldosterone hypertension are given.

Taken together the clinical picture of liquorice intake suggests mineralocorticoid excess induced by an agent different from aldosterone. For years it was thought by most clinicians that glycyrrhetinic acid, which has some structural resemblance to aldosterone (Figure 1) accounts for the mineralocorticoid effect of liquorice through the binding of its active component to mineralocorticoid receptors.

Although the structural similarities between aldosterone and glycyrrhetinic acid suggested a direct mineralocorticoid effect due to glycyrrhetinic acid, several observations were not in line with this concept [4–6]. First, the affinity of glycyrrhetinic acid for mineralocorticoid receptors is negligibly low. Secondly, liquorice has no mineralocorticoid effect in adrenalectomized rats or in patients with Addison's disease. Thirdly, the mineralocorticoid effect of glycyrrhetinic acid was restored when liquorice was given together with 11β-hydroxy-glucocorticosteroids to animals or humans without adrenal function, suggesting an interaction between glycyrrhetinic acid and glucocorticoids, rather than a direct effect of glycyrrhetinic acid on renal sodium retention and potassium excretion.

Mechanism of renal sodium retention and potassium loss induced by liquorice

Werder *et al.* [7] and later the group of Maria New [8] observed a patient with low renin, low aldosterone and hypertension. The pattern of cortisol metabolites excreted in urine was abnormal [7,8]. In the late 1980s, Stewart *et al.* showed that the changes in the pathways of adrenal steroid metabolism after liquorice ingestion are similar to those observed in children who exhibit a similar low-renin and low-aldosterone hypertension syndrome [9]. The abnormal pattern of cortisol metabolites, i.e. an increase in the urinary ratio of (tetrahydrocortisol plus 5-allo-tetrahydrocortisol)/tetrahydrocortisone ((THF + 5αTHF)/THE) (Table 1) was compatible with an inhibition of the enzyme shuttling biologically active cortisol into cortisone, a steroid without affinity for glucocorticosteroid or mineralocorticosteroid receptors. Elegant experiments performed by Funder *et al.* [10] revealed that a lower activity of the 11β-hydroxysteroid-dehydrogenase (11βHSD) results in increased cortisol concentrations in cells expressing mineralocorticoid receptors (Figure 2).

Of greater potential relevance than the mechanism of liquorice action in the kidney was the discovery of the biological principle that it is an enzyme which is coexpressed with a receptor, and not the receptor itself, that accounts for the specificity of ligand binding to the receptor [10]. *In vitro* studies with mineralocorticoid receptors had previously shown that the affinity

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Table 1. Differential diagnosis of low-renin, low-aldosterone hypertension of known aetiology

	Glucocorticoid-remediable aldosteronism [16]	Apparent mineralocorticoid excess [3]	Liddle syndrome [17]
Inheritance			
Autosomal	Dominant	Recessive	Dominant
Mutated gene	Aldosterone synthase	11βHSD2	Epithelial sodium channel
Urine			
(THF + 5αTHF)/THE	No	⇑	No
18-oxo-, 18OH-cortisol	⇑	No	No
Response to			
Dexamethasone	+	+	−
Spironolactone	+	+	−
Amiloride	+	+	+
Exogenous form	None	GA	None

11βHSD2, 11β-hydroxysteroid dehydrogenase type 2; GA, glycyrrhetinic acid; THF, tetrahydrocortisol; 5αTHF, 5-allotetrahydrocortisol; THE, tetrahydrocortisone.

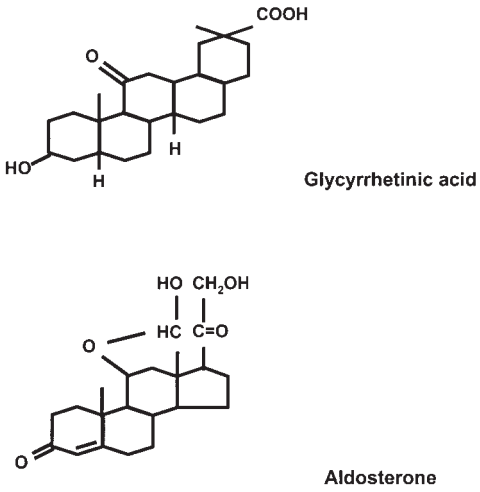

Fig. 1. Chemical structures of aldosterone and glycyrrhetinic acid.

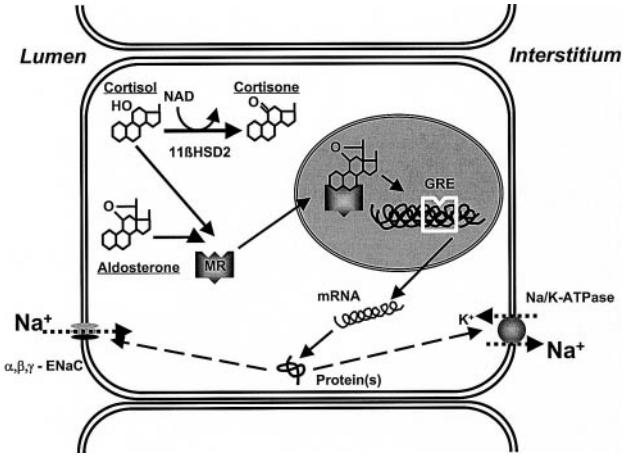

Fig. 2. Schematic representation of mineralocorticoid action in renal cells of the cortical collecting duct. When aldosterone enters the cell, it binds to the mineralocorticoid receptor (MR), thereafter the ligand–receptor complex is translocated into the nucleus. Binding to the glucocorticoid response elements (GRE) increases the transcription of genes which ultimately regulate proteins of the apical epithelial sodium channel (ENaC) and the basolateral sodium–potassium (Na/K) ATPase. The net effect of mineralocorticoid receptor activation is sodium (Na^+) reabsorption and potassium (K^+) excretion. Aldosterone is not the only ligand for mineralocorticoid receptors, since cortisol has an affinity to these receptors similar to aldosterone. Cortisol, however, circulates at a 100–1000-fold higher levels than aldosterone and would therefore occupy the mineralocorticoid receptors. This is not the case because the mineralocorticoid receptor is protected from occupation by cortisol. The gatekeeper which prevents promiscuous access of the glucocorticoid cortisol to the mineralocorticoid receptor is the enzyme 11β-HSD2. The 11β-HSD2 oxidizes cortisol into its receptor-inactive form, cortisone. Glycyrrhetinic acid inhibits the 11β-HSD2 and therefore leads to an unrestricted activation of the mineralocorticoid receptor by cortisol, with increased sodium retention and hypokalaemic hypertension with low-renin and low-aldosterone levels.

of the mineralocorticoid receptor for cortisol and aldosterone was of the same magnitude. Since cortisol concentrations are about 100–1000-fold higher than those of aldosterone, cortisol would quantitatively be the most abundant ligand for the mineralocorticoid receptor. By shuttling cortisol to cortisone in aldosterone receptor-expressing tissues, the 11βHSD removes cortisol from the receptor and guarantees its selectivity for aldosterone. In the presence of liquorice the 11βHSD is inhibited and cortisol has free access to the mineralocorticoid receptor, thereby inducing sodium retention, potassium loss, and low-renin, low-aldosterone hypertension (Figure 2).

11β-HSD isoenzymes

Currently two isozymes of 11β-HSD have been cloned. The enzymes share only 14% homology and have different physiological roles, regulation, and tissue distribution. 11βHSD1 acts predominantly as a reductase *in vivo*, is localized in the endoplasmic reticulum membrane with a luminal orientation of the catalytic domain, is NADP-dependent, has a K_m in the micromolar range, and is expressed in most tissues. Its biological relevance is thought to be the catalysis of the

reactivation of cortisone to cortisol, and by that mechanism might regulate access to glucocorticosteroid receptors [3,11–13]. 11βHSD2 on the other hand displays 11β-oxidase activity, is localized in the endoplasmic reticulum membrane with a cytoplasmic orientation of the catalytic domain, is NAD-dependent, has a nanomolar K_m and is preferentially found in tissues expressing mineralocorticoid receptors, including the cortical collecting duct of the kidney [3,12,14]. The pivotal role of 11βHSD2 in excluding endogenous glucocorticoids from the mineralocorticoid receptor is now widely accepted. This assumption is based first, on the observations of the effect of glycyrrhetinic acid on this enzyme, and second, on the studies of the syndrome of apparent mineralocorticoid excess, a disease state that results from a loss of function mutation in 11βHSD2 [3,15]. Phenotypically, the administration of high doses of glycyrrhetinic acid and mutations in 11βHSD2 are identical (Table 1).

Health hazards of liquorice

There is probably a great interindividual and possibly intraindividual variation in the susceptibility to glycyrrhetinic acid. In the most sensitive individuals, regular daily intake of no more than about 100 mg glycyrrhetinic acid, corresponding to 50 g liquorice sweets (assuming a content of 0.2% glycyrrhetinic acid), seems to be enough to produce adverse effects [18]. Most individuals who consume 400 mg glycyrrhetinic acid daily experience adverse effects. Provided glycyrrhetinic acid has no other effects at lower doses the following consideration with respect to health hazards can be made: 100 mg glycyrrhetinic acid per day is the lowest observed adverse effect level. If a safety factor of 10 is considered, a daily intake of 10 mg of glycyrrhetinic acid represents a safe daily dose for healthy adults [15]. In several countries the daily intake of glycyrrhetinic acid was estimated to be 1–10 mg. Since liquorice induces a salt-sensitive type of hypertension, the amount of salt consumed has to be taken into account when the health hazard of glycyrrhetinic acid is analysed. Thus, it is conceivable that even a very low dose of liquorice induces sodium overload in an individual with a high daily sodium chloride consumption, as is the case in modern Western society. Conversely, in ancient societies, where salt intake was restricted, the extracts from the root of *Glycyrrhiza glabra* were welcome therapeutics to overcome disease states requiring renal salt conservation.

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