Effect of a natural mineral-rich water on catechol-*O*-methyltransferase function

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Abstract. Catechol-*O*-methyltransferase (COMT) is a magnesium-dependent, catecholamine-metabolizing enzyme, whose impaired activity has been positively associated with cardiovascular diseases, particularly hypertension. Consumption of some natural mineral-rich waters has been shown to exert protective effects on cardiovascular risk factors, eg. by decreasing arterial blood pressure and blood lipids. However, the molecular mechanisms underlying these effects are still poorly understood. So, the aim of this work was to investigate the effect of natural mineral-rich water ingestion upon liver and adrenal glands COMT expression and activity in Wistar Han rats. Over a seven-week period, animals had access to one of the following three drinking solutions: 1) tap water (control group; TW), 2) tap water with added Na^+ (to make the same concentration as in the MW group (TWNaCl group), or 3) natural mineral-rich water [Pedras Salgadas®, which is very rich in bicarbonate, and with higher sodium, calcium and magnesium content than control tap water (MW group)]. COMT expression and activity were determined by RT-PCR and HPLC-ED, respectively. A higher hepatic COMT activity was found in the MW group compared with the TW and TWNaCl groups. On the other hand, adrenal gland COMT mRNA expression decreased in the MW group compared to TW group. In conclusion, the ability of natural mineral-rich waters to increase hepatic COMT activity may eventually explain the positive cardiovascular effects associated with the consumption of some natural mineral-rich waters.

Key words: catechol-*O*-methyltransferase, natural mineral-rich water, cardiovascular health, magnesium

Catechol-*O*-methyltransferase (COMT) is an enzyme that catalyzes the methylation of catechol substrates, and is found widely distributed in mammalian tissues [1, 2]. Although COMT is active in the presence of divalent cations such as copper, manganese, zinc, cadmium, iron and nickel, the most catalytically active form of the enzyme is when it is bound to magnesium [3]. COMT is involved in the metabolism of various compounds including catecholamines, in particular adrenaline and noradrenaline [4], estrogens [5] and xenobiotic catechols [6]. A decrease in COMT activity has been associated with a negative impact on cardiovascular health, i.e. higher systolic blood pressure [7] and waist-to-hip ratio [8], as well as atherosclerosis [9]. Furthermore, spontaneously hypertensive rats, when compared with Wistar-Kyoto rats, not only exhibit lower liver

doi:10.1684/mrh.2014.0369

To cite this article: Bastos P, Araújo JR, Azevedo I, Martins MJ, Ribeiro L. Effect of a natural mineral-rich water on catechol-*O*-methyltransferase function. *Magnes Res* 2014; 27(3): 131-41 doi:10.1684/mrh.2014.0369

membrane-bound COMT expression and activity, but also an attenuation of the ability to methylate catecholamines [10].

Natural mineral-rich waters are an important source of highly bioavailable minerals, contributing to assuring an adequate intake of these elements [11-14]. In fact, in some geographical areas, natural mineral-rich water consumption may provide up to 20-40% of a person's daily magnesium requirements [15, 16]. Moreover, an inverse relationship has been demonstrated between the consumption of natural mineral-rich waters and cardiovascular risk factors [17-20]. In particular, blood pressure was found to decrease in normotensive [19, 20] and mildly hypertensive [21, 22] individuals after ingestion of magnesium, sodium and/or bicarbonate-rich natural mineral waters. In fact, the accompanying anion in the sodium salt has a prime role in the blood pressure effect: unlike sodium chloride, sodium bicarbonate does not increase it [22-24].

Given that the cellular and molecular mechanisms underlying the protective effects of natural mineral-rich waters against cardiovascular risk factors are still poorly understood, and given the opposite association of COMT function with magnesium compared to sodium chloride when considering cardiovascular health, the aim of this study was to evaluate the effect of the ingestion of a natural mineral-rich water (rich in bicarbonate, sodium and magnesium, among other ionic specificities) upon COMT expression and activity in the liver and adrenal glands of Wistar Han rats. Interestingly, in a metabolic syndrome animal model (fructose-fed Sprague-Dawley rats), an eight-week consumption of this natural mineral-rich water prevented the increase in heart rate and plasma triacylglycerols, and delayed the increase in systolic blood pressure, induced by fructose [25].

Methods

Reagents

The TriPure™ Isolation Reagent was obtained from Roche Applied Science (Mannheim, Germany). The primers, random hexamers, dNTPs, first-strand buffer, dithiothreitol (DTT), RNase OUT™, Superscript™ II Reverse Transcriptase (RT SSII) and RNase H were purchased from Invitrogen (Carlsbad, CA, USA). The DyNAzyme II™ DNA Polymerase, Mg^{2+} free DyNAzyme Buffer, dNTPs and magnesium chloride ($MgCl_2$) were acquired from Thermo Scientific (Waltham, MA, USA). Agarose, PCR loading buffer, ethidium bromide, 3,4-dihydroxyphenylacetic acid (DOPAC), *S*-adenosyl-L-methionine (SAMe), adrenaline, pargyline and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (St. Louis, MO, USA). Perchloric acid, citric acid, sodium octylsulphate, sodium acetate, dibutylamine and methanol were of HPLC grade and obtained from Sigma (St. Louis, MO, USA). Ethylene glycol bis(b-aminoethyl ether)-*N*,*N*,*N*9,*N*9-tetra-acetic acid (EGTA) was acquired from Merck (Darmstadt, Germany).

Sodium chloride (NaCl), sodium phosphate (Na_2HPO_4), monosodium phosphate (NaH_2PO_4), chloroform, isopropanol, chloroform:isoamyl alcohol (CIA), human serum albumin, Bradford reagent (Coomassie Blue plus methanol plus H_3PO_4), phosphoric acid (H_3PO_4) and $MgCl_2$ were of analytical grade.

The natural mineral-rich water Pedras Salgadas® (MW), which contains a very high level of sodium bicarbonate and has a higher magnesium and calcium content than control tap water, as well as a low chloride-to-sodium ratio, was kindly provided by Unicer Bebidas, S.A., Leça do Balio, Portugal.

Animals, treatment and collection of tissues

Adult male, Wistar Han rats purchased from Charles River Laboratories (Chatillon/Chalaronne, France) were used (weighing 251-343 g at the beginning of the study). The animals were individually housed, in an enriched environment, and in a temperature (20-22°C) and humidity-controlled environment, with a 12-h light/dark cycle. Handling and care of the animals were conducted in conformity with standard guidelines [26]. The animals were randomly divided into three groups, all of which had free access to standard laboratory food (2014 Teklad Global 14% Diet, Harlan Interfauna Ibérica S.A., Barcelona, Spain; 0.1% of sodium, 0.2% of magnesium, 0.3% of chloride and 0.7% of calcium), and one of three different drinking solutions, for seven weeks: a) control rats (TW; n = 6), drinking tap water (with a sodium concentration

of 10.7-12.1 mg/L, chloride concentration of 13.6-16.1 mg/L, magnesium concentration of 3.8-8.2 mg/L and calcium concentration of 26.7-33.2 mg/L); b) sodium chloride rats (TWNaCl; n = 5), drinking tap water with added sodium chloride (to a final concentration of 585.0 mg/L of sodium), and c) natural mineral-rich water rats (MW; n = 6), drinking Pedras Salgadas® (with 1978 mg/L of bicarbonate, 585.0 mg/L of sodium, 29.20 mg/L of chloride, 24.10 mg/L of magnesium and 98.20 mg/L of calcium). [Bicarbonate levels were not determined for tap water.]

At the end of the treatment, animals were deeply anesthetized with sodium pentobarbital (80 mg/kg of body weight), perfused with saline, and the liver and the adrenal glands removed. For determination of COMT gene expression, tissues were immediately frozen in liquid nitrogen and stored at -80°C. For evaluation of COMT activity, tissues were homogenized in 5 mM phosphate buffer at pH 7.8 (5mM Na_2HPO_4 + 5mM NaH_2PO_4) and stored at -20°C.

COMT gene expression

Total RNA was extracted from tissues using the TriPure™ reagent, separated with chloroform, precipitated with isopropanol, and dissolved in DEPC-H_2O. For cDNA synthesis, 5 μg of the extracted RNA were incubated for 5 min at 65°C, in the presence of 50 ng/μL Random Hexamers and 10 mM dNTPs. After 1 min in ice, 4 μL 5× first strand buffer, 0.1M DTT and 40 U/mL RNase OUT™ were added. Following incubation for 2 min at 25°C, a total volume of 20 μL, with 200 units of RT SSII, was incubated in the thermal cycler (Q96 Thermal cycler, Quanta Biotech, Surrey, UK) for 10 min at 25°C, for 50 min at 42°C and 15 min at 70°C. After cDNA synthesis, RNase H was added.

PCR was performed with 4 μL of the RT product. The PCR master mix (50 μL) contained 25 mM dNTPs, 50 mM $MgCl_2$, 5 μL 10x Mg^{2+}-free DyNAzyme buffer, 50 pM of each primer and 2000 U/mL DyNAzyme II™ DNA Polymerase in Millipore-H_2O. For all of the samples, RT-PCR was performed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene.

The following PCR primers were used: 5'-GGG CTT GGT GAC TAT TTT CTG-3' (COMT forward primer); 5'-CAG GCC ACA TTT CTC CAG-3' (COMT reverse primer); 5'- CAG ACT CCG TCA GTT TCT GCA-3' (GAPDH forward primer); 5'-GAC ATT CAC ATC CTG TTA GC-3' (GAPDH reverse primer).

For both COMT and GADPH, thermocycling consisted of 35 cycles of 45 s at 95°C, 45 s at 57°C and 60 s at 72°C. The predicted sizes of the PCR products were (in bp): 535 for COMT and 682 for GAPDH.

For the visualization of the PCR products, PCR loading buffer 6x and CIA 24:1 were added to 10 μL of each PCR product, which were then run on a 1.6% agarose gel, and visualized with an ultraviolet transilluminator (UVP®, Cambridge, UK) using ethidium bromide staining (final concentration of 0.5 μg/mL). PCR reaction products were recorded using a GelDOC-It™ Imaging System camera with the appropriate filters for UV light (UVP®, Cambridge, UK).

COMT activity determination by high performance liquid chromatography with electrochemical detection (HPLC-ED)

In the liver, COMT activity was evaluated by the enzyme's ability to methylate adrenaline to metanephrine, as previously described [27]. In the adrenal glands, COMT activity was evaluated by the enzyme's ability to methylate DOPAC to homovanillic acid (HVA), this protocol having been specifically developed due to the high concentrations of adrenaline in this tissue. Aliquots of 0.1 mL of tissue homogenates were pre-incubated for 20 min with 1 mL of 5 mM phosphate buffer and then incubated for 5 min with increasing concentrations of adrenaline (0.1-1 mM), for the liver, or for 20 min with increasing concentrations of DOPAC (0.1-1 mM), for the adrenal glands, in the presence of SAMe, in a saturating concentration (500 μM). These incubation media also contained 100 μM pargyline, 100 μM $MgCl_2$ and 1 mM EGTA.

The pre-incubation and incubation periods were carried out at 37°C, under conditions of light protection, with continuous shaking, and without oxygenation. After stopping the reactions with 2 M perchloric acid, the samples were kept at 4°C and used for metanephrine (in the liver homogenates), and HVA (in the adrenal glands homogenates) detection, and quantification by HPLC-ED.

The HPLC system included a pump (Gilson 302; Gilson Medical Electronics, Villiers la Bel,

France) connected to a manometer (Gilson 802C), and a 25 cm-long, 5 μm ODS steel column (Biophase, Bioanalytical Systems, West Lafayette, IN, US). The samples were injected with an automatic injector (Gilson 231), connected to a diluter (Gilson 401). The mobile phase consisted of degassed 0.1 mM citric acid, 0.5 mM sodium octylsulphate, 0.1 mM sodium acetate, 0.17 mM EDTA, 1 mM dibutylamine and 8% (v/v) methanol, adjusted to pH 3.5 with perchloric acid (70%), and pumped at a rate of 1 mL/min. Detection was performed electrochemically with a carbon electrode, an Ag/AgCl electrode and an amperometric detector (Gilson 141). The detector cell was adjusted to 0.75 V. The current was monitored by Gilson 712 HPLC software. The lower limits of detection for HVA and metanephrine were 350 and 1000 fmol, respectively.

Protein determination

The protein content of the tissue homogenates was determined as described by Bradford [28], with human serum albumin as standard.

Statistical analysis of data

Results are expressed as arithmetic means ± SEM. K_m and V_{max} values for COMT were obtained from nonlinear regression analysis using the GraphPad Prism® software [GraphPad version 5.04 (November 6 2010), La Jolla, CA, USA]. The activity of an enzyme is defined by the rate constant K_{enzyme} ($K_{enzyme} = V_{max}/K_m$), indicating that the higher the V_{max} and the lower the K_m, the higher the activity of the enzyme [29]. The statistical significance of the difference between the means of the various groups was evaluated by one-way analysis of variance (ANOVA), followed by the Newman-Keuls test. Differences were considered to be significant when $p < 0.05$.

Results

Animal treatment

No differences were observed, week by week, in body weight or food ingestion between the three groups of animals. Overall, week by week, rats from the MW group ingested more fluid than the TW and TWNaCl rats, with statistical relevance from weeks 2 to 4 (*figure 1*). This was probably due to the organoleptic characteristics of the natural mineral-rich water.

COMT expression

No differences were observed in liver COMT mRNA levels between the three groups of animals (*figures 2A,3A*). However, a different pattern was observed for the adrenal glands, where COMT mRNA values (*figures 2B,3B*) revealed a significant decrease of 46% in rats from the MW group *versus* rats from the TW group.

COMT activity

COMT activity in the liver was evaluated through COMT's ability to methylate adrenaline to metanephrine (*figure 4A*). Natural mineral-rich water ingestion was shown to positively influence enzyme activity, which could be observed through the significant increase, of 32%, in V_{max}, and the tendency to a decrease, of 36%, in K_m *versus* tap water ingestion, and the significant increase, of 58%, in V_{max}, and the tendency to a decrease, of 27%, in K_m *versus* the TWNaCl group. As a whole, these alterations induced an increase of 177% in the K_{enzyme} value in rats from the MW group *versus* rats from the TW and TWNaCl groups (*table 1*). No relevant changes in COMT activity were observed in the TWNaCl *versus* TW rats (*table 1*).

Due to the high levels of adrenaline in the adrenal glands, COMT activity in this organ was evaluated through the enzyme's ability to methylate DOPAC to HVA (*figure 4B*). No significant changes were observed in adrenal COMT activity in any of the three groups. However, adrenal glands from MW rats showed a tendency to an increase in K_{enzyme} (of 29%) in comparison with the TW group, a similar tendency having been observed in TWNaCl treated *versus* TW (of 41%) (*table 1*).

Discussion

To our knowledge, this is the first time the modulation of COMT expression and activity by a natural mineral-rich water has been studied. The natural mineral-rich water used is a

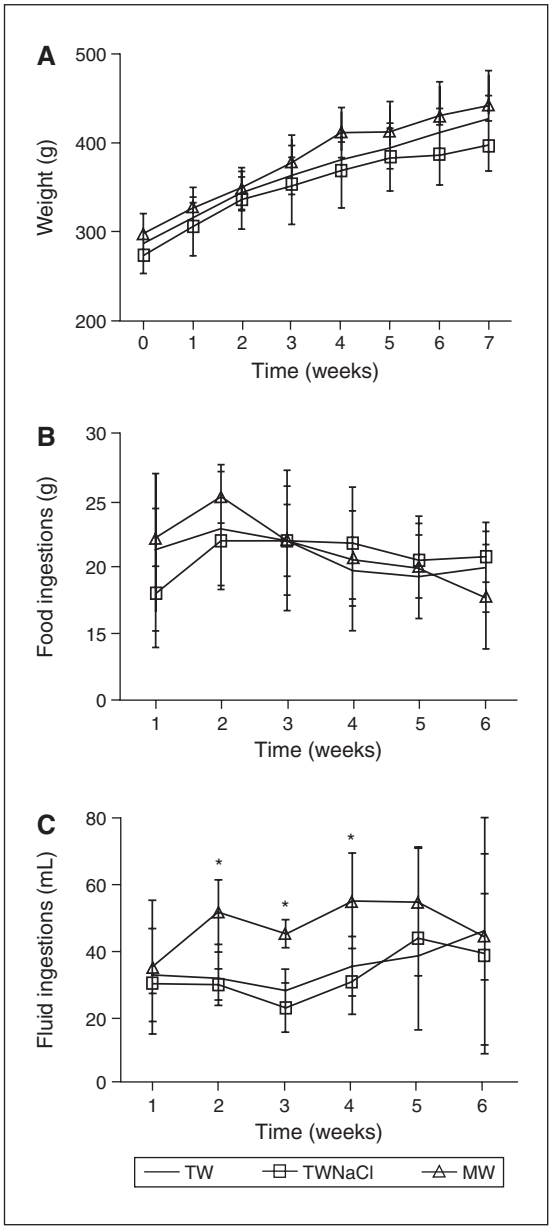

Figure 1. Body weight **(A)**, food **(B)** and fluid **(C)** ingestion in the different groups. The animals had access to one of three different drinking solutions, for seven weeks. Results are given as mean ± SEM. MW: rats drinking a natural mineral-rich water, with 1978 mg/L of bicarbonate, 585.0 mg/L of Na^+, 29.20 mg/L of Cl^-, 24.10 mg/L of Mg^{2+} and 98.20 mg/L of Ca^{2+} (n = 6); TW: control rats drinking tap water, with 10.7-12.1 mg/L of Na^+, 13.6-16.1 mg/mL of Cl^-, 3.8-8.2 mg/L of Mg^{2+} and 26.7-33.2 mg/L of Ca^{2+} (n = 6); TWNaCl: rats drinking tap water, with added sodium chloride to a final concentration of 585.0 mg/L of Na^+ (n = 5). * $p<0.01$ for TW *versus* MW and TWNaCl *versus* MW.

hypersaline, sodium-rich, naturally sparkling water [in conformity with the European Community Council guidelines for natural mineral waters (2009/54/EEC)], very rich in bicarbonate and with a high sodium, calcium and magnesium content, as well as a low chloride-to-sodium ratio. Since sodium chloride has a negative effect on COMT [30, 31], an animal group drinking tap water with added sodium chloride, with a final sodium concentration identical to its concentration in the natural mineral-rich water studied, was included in this work.

As expected, the ingestion of the natural mineral-rich water for seven weeks led to a significant increase in liver COMT activity. Although the natural mineral-rich water studied possesses a specific, high total mineralization content that must be considered, with its alkaline load contributing to better mineral homeostasis (by decreasing mineral renal excretion) [32], and as the ion absorption and transport processes and ion effects may be dependent on the presence of other ions [33], we hypothesized that the higher magnesium content of this water, along with the high bicarbonate content and low chloride-to sodium-ratio, play a role in the modulation of COMT activity in the liver. In line with this, our group previously observed that this natural mineral-rich water increased the magnesium content of the liver from fructose-fed Sprague-Dawley rats [25].

In the rat adrenal glands, a tendency to increased enzyme activity was detected. We hypothesize that the natural mineral-rich water effect on adrenal COMT was most probably similar to that occurring on the liver COMT. However, apparently, the adrenal glands responded with a downregulation of COMT gene expression, and enzyme activity was thus reduced to almost control levels. It would have been interesting to assess the COMT activity and gene expression earlier during treatment in order to clarify this point.

The biological importance of the hepatic COMT modulation by the natural mineral-rich water

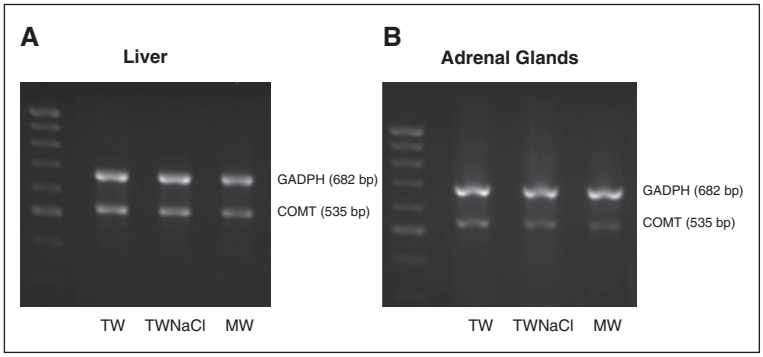

Figure 2. Detection of COMT mRNA by RT-PCR, in the liver **(A)** and adrenal glands **(B)**. For normalization, GAPDH mRNA was detected with specific primers. PCR products were separated by agarose gel electrophoresis, followed by staining with ethidium bromide. The amplified fragments had the estimated size of 535 bp (COMT) and 682 bp (GADPH). MW: rats drinking a natural mineral-rich water, with 1978 mg/L of bicarbonate, 585.0 mg/L of Na^+, 29.20 mg/L of Cl^-, 24.10 mg/L of Mg^{2+} and 98.20 mg/L of Ca^{2+} (n = 6); TW: control rats drinking tap water, with 10.7-12.1 mg/L of Na^+, 13.6-16.1 mg/mL of Cl^-, 3.8-8.2 mg/L of Mg^{2+} and 26.7-33.2 mg/L of Ca^{2+} (n = 6); TWNaCl: rats drinking tap water, with added sodium chloride to a final concentration of 585.0 mg/L of Na^+ (n = 5).

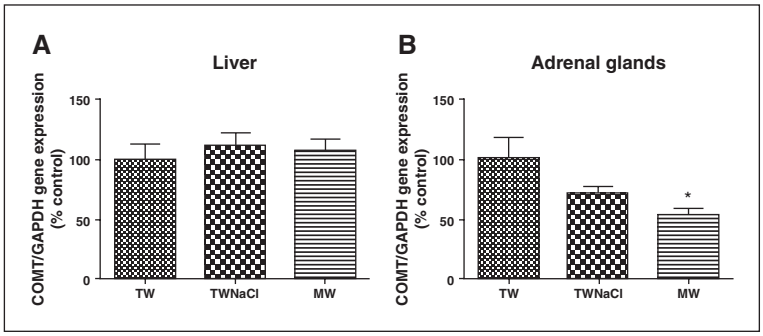

Figure 3. Quantification of COMT mRNA levels in the liver **(A)** and adrenal glands **(B)**. The animals had access to one of three different drinking solutions, for seven weeks. Results are given as mean ± SEM. MW: rats drinking a natural mineral-rich water, with 1978 mg/L of bicarbonate, 585.0 mg/L of Na^+, 29.20 mg/L of Cl^-, 24.10 mg/L of Mg^{2+} and 98.20 mg/L of Ca^{2+} (n = 6); TW: control rats drinking tap water, with 10.7-12.1 mg/L of Na^+, 13.6-16.1 mg/mL of Cl^-, 3.8-8.2 mg/L of Mg^{2+} and 26.7-33.2 mg/L of Ca^{2+} (n = 6); TWNaCl: rats drinking tap water, with added sodium chloride to a final concentration of 585.0 mg/L of Na^+ (n = 5). *$p<0.01$ for TW *versus* MW.

used in this study is shown by the action of the liver in removing circulating catecholamines (57% noradrenaline and 32% adrenaline), and by the large contribution to both normetanephrine (54%) and metanephrine (37%) production from the metabolism of circulating catecholamines [34]. An excess of circulating catecholamines is related to the development of various diseases, for example cardiovascular disorders [35]. Not only are the plasma concentrations of catecholamines in hypertensive subjects 15 to 25% higher than in normotensive ones, but base-line plasma concentrations of noradrenaline also seem to predict an increase in blood pressure [36], being 45% higher in hypertensive compared to healthy individuals [37, 38]. Tsunoda *et al*. [10] observed a decrease in the expression and activity of the hepatic membrane-bound COMT isoform in spontaneously hypertensive rats (SHR), and an increase in the plasma noradrenaline levels, compared

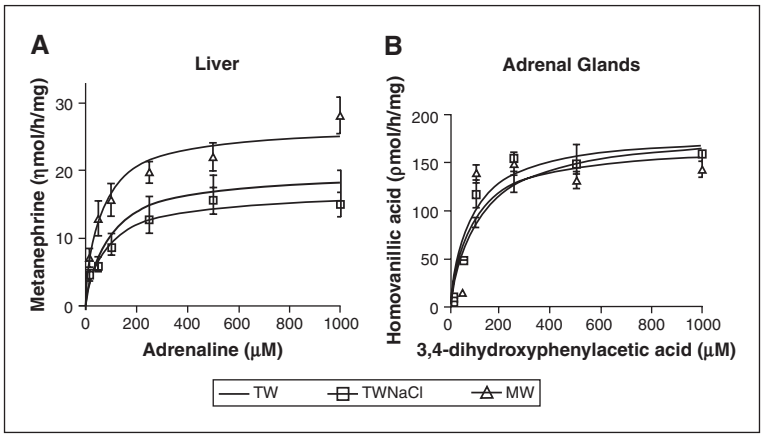

Figure 4. COMT activity in the liver **(A)** and adrenal glands **(B)**. In the liver, determination of activity was based on the enzyme's ability to methylate adrenaline to metanephrine, and in the adrenal glands on the enzyme's ability to methylate 3,4-dihydroxyphenylacetic acid to homovanillic acid. A reaction mixture of the homogenates with 500 μM SAMe, 100 μM pargiline, 100 μM $MgCl_2$ and 1 mM EGTA was incubated with increasing concentrations of adrenaline (0.1-1 mM) for the liver and 3,4-dihydroxyphenylacetic acid (0.1-1 mM) for the adrenal glands. Metanephrine (liver) and homovanillic acid (adrenal glands) were quantified by HPLC-ED. The animals had access to one of three different drinking solutions, for seven weeks. Results are given as mean ± SEM. MW: rats drinking a natural mineral-rich water, with 1978 mg/L of bicarbonate, 585.0 mg/L of Na^+, 29.20 mg/L of Cl^-, 24.10 mg/L of Mg^{2+} and 98.20 mg/L of Ca^{2+} (n = 6); TW: control rats drinking tap water, with 10.7-12.1 mg/L of Na^+, 13.6-16.1 mg/mL of Cl^-, 3.8-8.2 mg/L of Mg^{2+} and 26.7-33.2 mg/L of Ca^{2+} (n = 6); TWNaCl: rats drinking tap water, with added sodium chloride to a final concentration of 585.0 mg/L of Na^+ (n = 5).

Table 1. Kinetic parameters (V_{max}, K_m and K_{enzyme}) of COMT activity in the liver and adrenal glands for the three experimental groups.

		TW[a]	TWNaCl[b]	MW[c]	*p*
Liver	V_{max} (ηmol/h/mg)	19.99 ± 1.36	16.76 ± 1.44	26.46 ± 1.97	TW vs. MW ($p<0.05$) TWNaCl *versus* MW ($p<0.05$)
	K_m (μM)	88.62 ± 22.40	77.28 ± 25.82	56.56 ± 18.03	TWNaCl *versus* MW ($p = 0.09$) TWNaCl *versus* MW ($p = 0.09$)
	K_{enzyme} (ηmol/h/mg/μM)	0.22 ± 0.02	0.22 ± 0.03	0.61 ± 0.22	TWNaCl *versus* MW ($p = 0.18$) TWNaCl *versus* MW ($p = 0.18$)
Adrenal glands	V_{max} (ρmol/h/mg)	182.63 ± 8.43	180.65 ± 9.46	167.08 ± 15.55	TWNaCl *versus* MW ($p = 0.09$)
	K_m (ρM)	113.04 ± 18.04	79.11 ± 15.97	78.81 ± 28.29	TW *versus* TWNaCl ($p = 0.06$)
	K_{enzyme} (ηmol/h/mg/ρM)	1.65 ± 0.07	2.32 ± 0.26	2.13 ± 0.21	TW *versus* TWNaCl ($p = 0.06$) TW *versus* MW ($p = 0.09$)

Values represent mean ± SEM.
[a] Rats drinking tap water, with 10.7-12.1 mg/L of Na^+, 13.6-16.1 mg/mL of Cl^-, 3.8-8.2 mg/L of Mg^{2+} and 26.7-33.2 mg/L of Ca^{2+} (n = 6).
[b] Rats drinking tap water, with added sodium chloride to a final concentration of 585.0 mg/L of Na^+ (n = 4).
[c] Rats drinking a natural mineral-rich water, with 1978 mg/L of bicarbonate, 585.0 mg/L of Na^+, 29.20 mg/L of Cl^-, 24.10 mg/L of Mg^{2+} and 98.20 mg/L of Ca^{2+} (n = 5).

with the control animals, suggesting a lower capacity for catecholamines methylation. Interestingly enough, the intake of this natural mineral-rich water has already been shown to have positive effects on several cardiovascular parameters in metabolic syndrome, including blood pressure and heart rate [25, 39].

Despite its fundamental role in noradrenaline and adrenaline metabolism, COMT has other important metabolic functions. In the liver, the main metabolic organ, and where COMT expression and activity reach the highest levels [40], an increase in enzyme activity may confer a protective role as regards other compounds by increasing the capacity for the *O*-methylation of active and/or toxic catechols, thus preventing their oxidative metabolization, and generation of free radicals [6]. A higher ingestion of natural mineral-rich waters would eventually, by that way, not only act preventively in carcinogenic processes related to a reduction in COMT activity [41], but would also compensate for the competitive inhibition created by flavonoids, catechins and exogenous catecholic compounds [42, 43] on the *O*-methylation of endogenous catechols. This latter, protective effect could be even more significant in females who, compared with males, present low COMT activity and expression [44], and in whom COMT activity, through its role in the inactivation of estradiol metabolites, is suggested to play a part in the etiology of breast and endometrial cancers [45, 46].

Adrenaline is a powerful hormone with profound effects, and its metabolism in the adrenals themselves assumes high importance since 91% of the circulating metanephrine [34] comes directly from these glands. In contrast with the liver, where the metanephrine and the normetanephrine produced by COMT are further metabolized by MAO, the metanephrine in the adrenal glands does not constitute a substrate for further reactions, allowing it to exert a possible down-regulation effect upon the COMT gene. Recently, it has been proposed that metanephrine is a hormone and not merely an adrenaline metabolite [47].

Tissue-specific differences in COMT regulation have already been described [44]. Interestingly, in SHR compared to Wistar-Kyoto rats, variations in membrane-bound COMT expression and activity were only observed in the liver, with these groups of rats presenting maintenance of enzyme expression and activity in kidneys, erythrocytes and adrenal glands [10, 48]. Hirano and colleagues [30, 31] observed a suppressor effect of a salt-rich water (8% NaCl) on the activity of the membrane-bound COMT of the liver, kidneys and cerebral cortex in Dahl salt-sensitive rats, with these animals exhibiting a greater release of noradrenaline and dopamine. In our experiment, both treated groups (TWNaCl and MW) had access to drinking solutions with a Na^+ concentration of 585.0 mg/L, bicarbonate being the major anion present in the natural mineral-rich water and chloride presenting a higher concentration in the TWNaCl group. Our results show that the Na^+ concentration did not influence liver COMT activity, as no differences were observed in enzyme activity between TWNaCl and TW groups (*figure 4A*, *table 1*). Bicarbonate has been reported to modify sodium effects on blood pressure [19, 20, 22-24]. As to the effect of the increased concentration of Na^+ on adrenal COMT, although no significant difference was registered, there was a tendency to an increase in COMT activity in comparison with the TW group. This may have been an indirect effect, but we have no data to discuss this further at this time.

This study presents some limitations, since we are unable to ascribe the observed effects to the presence, in the natural mineral-rich water, of a specific ion or a specific mixture of ions (magnesium/magnesium plus bicarbonate), or to the low chloride-to-sodium ratio or both. Additionally, other ions/components with biological activity that have so far not been reported to affect COMT, but were yet present in both waters, could have influenced the outcome.

In conclusion, the results of this study showed that consumption of a natural mineral-rich water increased the activity of COMT in the liver. A putative similar effect on COMT in the adrenal glands led to a decrease of COMT mRNA levels that maintained the enzyme activity in these glands near to the control values. As the liver is the main contributor to the removal and metabolism of circulating catecholamines, the effect of natural mineral-rich water consumption, through an increase in hepatic COMT activity, would eventually reduce circulating catecholamine levels and thus contribute to the prevention of the development of cardiac pathologies related to high levels of these amines. The higher COMT activity may also promote an increase in the *O*-methylation of catecholestrogens, whose high concentrations are involved in the initiation of the carcinogenic process.

Disclosure

This work was supported financially by FCT (Fundação para a Ciência e Tecnologia, PEst-OE/SAU/UI0038/2011) through the Centro de Farmacologia e Biopatalogia Química, Faculty of Medicine, University of Porto, which integrates the Department of Biochemistry (U38/FCT) and the Faculty of Medicine, University of Porto. Conflicts of interest: none.

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