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Effects of nicotine and Vitamin E on Carbonic anhydrase activity in some rat tissues *In Vivo* and *In Vitro*

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Abstract

Effects of nicotine, nicotine + vitamin E and nicotine + *Hippophea rhamnoides L.* extract (HRe-1) on muscle, heart, lungs, testicle, kidney, stomach, brain and liver carbonic anhydrase (CA; EC 4.2.1.1.) enzyme activities were investigated *in vivo.* Groups of rats were given nicotine (0.5 mg/kg/day, i.p.), nicotine + vitamin E (75 mg/kg/day, i.g.), nicotine + HRe-1 (250 mg/kg/day, i.g.) and a control group vehicle only. The results showed that nicotine inhibited the heart, lung, stomach and liver CA enzyme activities by ~80% ($p < 0.001$), ~94% ($p < 0.001$), ~47% ($p < 0.001$) and ~81% ($p < 0.001$) respectively, and activated muscle and kidney, but had no effects on the testicle and brain CA activities. Nicotine + vitamin E inhibited the heart and liver CA enzyme activities by ~50% ($p < 0.001$), and ~50% ($p < 0.001$), respectively, and nicotine + vitamin E activated the muscle CA activity. However, nicotine + vitamin E had no effect on lung, testicle, kidney, stomach and brain CA activities. Nicotine + HRe-1 inhibited the heart and stomach CA enzyme activities by ~51% ($p < 0.001$), and ~32% ($p < 0.002$), respectively, and activated the muscle and brain CA activities, but had no effects on the lung, testicle, kidney, and liver CA activities. *In vitro* CA inhibition results for similar experiments correlated well with the *in vivo* experimental results in lungs, testicles, kidney, stomach, brain and liver tissues.

Keywords: *Carbonic anhydrase, inhibition, nicotine, Hippophea rhamnoides*

Introduction

Carbonic anhydrase (CA) (carbonate hydrolyase, EC 4.2.1.1) is member of the zinc metalloenzyme family. CA is a well characterized pH regulatory enzyme in nearly all tissues where it catalyses the reversible hydration of CO_2 to HCO_3^- and H^+. Fourteen different CA isozymes have been described up to the present in higher vertebrates. Among the CA isozymes are cytosolic (such as CA I, CA II, CA III, CA VII), membrane-bound (CA IV, CA IX, CA XII and CA XIV), mitochondrial (CA V), secretory forms (CA VI) and several acatalytic forms (CAVIII, CA X and CA XI). Some of them have also been identified in tumor cells [1–10].

There is much literature related to changes in enzyme activities. A few reports have indicated that some increases and decreases were found in human liver enzyme activity levels such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase [11–14].

Reactive oxygen species (ROS), which are formed in various metabolic processes, lead to damage in molecules such as lipids, proteins and nucleic acids in cells. A number of drugs and chemicals increases the ROS/free radicals ratio in specific organs of the body.

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Many researchers have found that nicotine contributes to ROS production [15,16].

Smoking is common in humans and various studies have shown that nicotine contributes to ROS production [17–19]. Two thirds of the adult population in the United States (U.S.) are addicted to alcohol, and 30% of them are addicted to both cigarettes and alchohol [20–23]. Smoking is a also risk for various cardiovascular diseases and cancer, the major toxic component of cigarette smoke being nicotine [24–28]. In the last decade, Kessler *et al.* found a marked increase in the nicotine ratio in all kinds of cigarettes in the USA [29]. Shaw *et al.* claim that one cigarette decreases lifespan by 11 minutes [30]. Cotinine is the oxidized metabolite of nicotine; it has a long half-life and may take part in vascular diseases [31,32].

Owing to the wide use of cigarettes, we thought it was important to study the effect of nicotine on CA activity and, for this purpose, we investigated the *in vivo* effects of nicotine and nicotine + vitamin E on rat muscle, heart, lungs, testicle, kidney, stomach, brain and liver CA activities. Although the effects of many chemicals and drugs on CA enzyme activity have been investigated [1,33–35] studies with nicotine have not been previously reported.

Materials and methods

Materials

Chemicals were purchased from Sigma Chem. Co or Merck.

Animals

Thirty-two rats (Sprague-Dawley strain, body weight 225 ± 28 g) fed with standard laboratory chow and water, were used in the study. They were randomly divided into 4 groups (8 rats per group) and placed in separate cages during the study. The groups were as follows: Group I: Nicotine (0.5 mg/kg/day, i.p.); Group II: Nicotine (0.5 mg/kg/day, i.p.) + Vitamin E (75 mg/kg/day, i.g.); Group III: Nicotine (0.5 mg/kg/day, i.p.) + HRe-1 (250 mg/kg/day, i.g.); Group IV: Control group (received only the same amount of vehicle, 0.9% NaCl solution, i.p., and corn oil, i.g.). The supplementation period was 3 weeks. Animal experimentations were carried out in an ethical manner following guidelines set by the Ethical Committee of Ataturk University.

Preparation and administration of nicotine

The hydrogen tartrate salt of nicotine (Sigma N-5260) was dissolved in 0.9% NaCl solution to give a 0.15 mg/ml concentration of nicotine and the pH solution was adjusted to 7.4 with 0.1 N NaOH. Nicotine (0.5 mg/kg/day) was administered by intraperitoneal injection to groups 1, 2 and 3 for 3 weeks.

Preparation and administration of vitamin E

Vitamin E (Ephynal 300 capsule, Roche, France) was dissolved in corn oil (30 mg/ml) and administered orally by a stomach tube (approximately 75 mg/kg/day) to group 2 for 3 weeks.

Preparation and administration of Hippophae rhamnoides L. extract

The ripe fresh fruit of *Hippophae rhamnoides L.* were collected from the Tortum area, a town in Erzurum, Turkey. The fruits were removed from the branches, washed with tap water and dried, then crushed in a mortar and mixed. The fruit mash was placed in a glass jar and hexane was added in an equal volume. Forty-eight hours later, the juice was obtained from the mixture by squeezing and centrifuging at 1000 × *g* for 15 min; the clear supernatant was removed by a drip. Hexane was evaporated from the liquid using an evaporator (Büchi, Rotavapor, R110, Switzerland). The *Hippophae rhamnoides L.* extract (HRe-1, 500 mg/ml)) was also mixed with corn oil (1/1, v/v), and administered orally by a stomach tube in 1 mL (250 mg/kg/day) to Group III for 3 weeks.

Sample collection

At the end of the experiment, the animals were anesthetized with ketamine-HCl (Ketalar, 20 mg/kg, i.p.) and killed by exsanguination by cardiac puncture after thoracotomy. Then, each tissue was carefully removed, rinsed in saline and stored at −80°C until homogenization.

Preparation of homogenate

A piece of each tissue (approximately 300 mg) was homogenized by an OMNI TH International, model TH 220 homogenizer (Warrenton, VA 20187 USA) in 20 mM Tris–HCl, pH 7.4 (1/10 weight/volume) on ice for 10 s at the first speed level. Then, the homogenates were centrifuged at 10 000 × *g* for 15 min at 4°C. Aliquots of the supernatant were stored at −80°C until required.

Ammonium sulphate fractionation and dialysis

Ammonium sulphate (20–40%) precipitation was made on the homogenate, the ammonium sulphate being slowly added for completely dissolution. The mixture was centrifuged at 5000 × *g* for 15 min and the precipitate was then dissolved in 20 mM Tris–HCl (pH 7.4) and dialysed at 4°C in 20 mM Tris–HCl (pH 7.4) for 2 h with two changes of buffer.

Thus, partially purified total CA was obtained by ammonium sulphate fractionation and dialysis from tissue homogenates (muscle, heart, lungs, testicle, kidney, stomach, brain and liver tissues).

In Vitro studies

In vitro effects of 20, 40, 60, 80 and 100 μL nicotine (5 mM), nicotine (5 mM) + vitamin E (10 mM) and nicotine (5 mM) + HRe-1 (500 mg/mL) respectively, on the activity of partially purified total CA from tissue homogenates were investigated. Control cuvette activity was accepted as 100%.

Activity determination

Carbonic anhydrase activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson [36]. CO_2– Hydratase activity as an enzyme unit (EU) was calculated by using the equation *to-tc*/*tc* where *t*o and *t*c are the times for pH change of the nonenzymatic and the enzymatic reactions, respectively.

Statistical analysis

One-way ANOVA with post-hoc LSD test was used to compare the group means and $p < 0.05$ was considered statistically significant. SPSS-for windows (version 10.0) was used for statistical analyses.

Results

In this study, the *in vivo* results (Figure 1) showed that nicotine inhibited the heart, lungs, stomach and liver CA enzyme activities by ~80% ($p < 0.001$), ~94% ($p < 0.001$), ~47% ($p < 0.001$) and ~81% ($p < 0.001$) respectively, and activated muscle and kidney but had no effects on the testicle and brain CA activities *in vivo*. Also, nicotine + vitamin E inhibited the heart and liver CA enzyme activities by ~50% ($p < 0.001$), and ~50% ($p < 0.001$), respectively, and activated muscle CA activity, but had no effect on the lungs, testicle, kidney, stomach, and brain CA activities. Also, nicotine + HRe-1 inhibited the heart and stomach CA enzyme activities by ~51% ($p < 0.001$), and ~32% ($p < 0.002$), respectively, activated the muscle and brain CA activities but had no effect on the lungs, testicle, kidney, and liver CA activities. The results of the *in vitro* inhibition studies with nicotine (5 mM), nicotine (5 mM) + vitamin E (10 mM) and nicotine (5 mM) + HRe-1 (500 mg/mL) are shown in Table I.

Discussion

Many chemicals in relatively low dosages affect the metabolism of biota by altering normal enzyme activity, particularly inhibition of a specific enzyme [37], effects which can be systemic and dramatic [38]. For example, a diuretic drug, acetazolamide, inhibits carbonic anhydrase enzyme [39] and two other sulfonamides in dorzolamide and brinzolamide, are topical CA inhibitors [40] used for the treatment of glaucoma.[41–44]

Some studies have shown that nicotine may also affect enzyme activities. For example, inhibition of SOD in kidney and testicles, and catalase in liver, and activation of SOD in liver and catalase in kidney, lung and testicles in nicotine-treated rats has been shown [45].Inhibition of glutathione peroxidase and SOD in erythrocytes, which was tolerated by vitamin E [46] and on the other hand, activation of glutathione peroxidase in brain have been reported in nicotine-treated rats [47]. It has also been shown that smokers have increased lipid peroxidation in their blood [48].

Increased expression of endothelial nitric oxide synthase mRNA due to nicotine [49], inhibition of typtophan hydroxylase due to alcohol and nicotine treatment in rats [50], and activation of the same enzyme in rats treated by nicotine alone (1 mg/kg) have been shown [51]. Some liver enzymes have been activated in rats exposed to cigarette smoke [52] and the expression of some enzymes taking part in energy metabolism have been increased by nicotine in rats [53]. The enzyme, adenylate cyclase, has also been activated in nicotine-treated rats (6 mg/kg) [54].

CA isozymes are important enzymes for body metabolism because they regulate the pH in most tissues. Therefore, in the present study, the investigation of the effects of nicotine, nicotine + vitamin E and nicotine + HRe-1, separately, on some rat tissues total carbonic anhydrase activity were proposed.

Nicotine increased CA activity in muscle and kidney tissues (Figure 1), however, this increase was attenuated in the nicotine + vitamin E group, and was not observed in the nicotine + HRe-1 group.

Nicotine markedly inhibited CA activity in heart, lung, stomach, and liver tissues but this inhibitionory effect was totally eliminated in lung and was attenuated in heart, stomach, and liver tissues in the nicotine + vitamin E group (Figure 1).

The nicotine-induced inhibition of CA activity was totally eliminated in liver and attenuated in heart and stomach tissues in the nicotine + HRe-1 group (Figure 1).

These findings, affecting CA activities by nicotine, nicotine plus vitamin E or HR-1 are in agreement with previous studies. For example, Gumustekin *et al.* have reported that nicotine increased the activity of GSH-Px in the brain while vitamin-E tolerates this effect. Nicotine has also inhibited brain GST enzyme and this activity has also been tolerated by vitamin-E [46]. In addition, Suleyman *et al.* have reported that nicotine inhibits the activities of GSH-Px and SOD of erythrocytes while vitamin-E tolerates these effects [47].

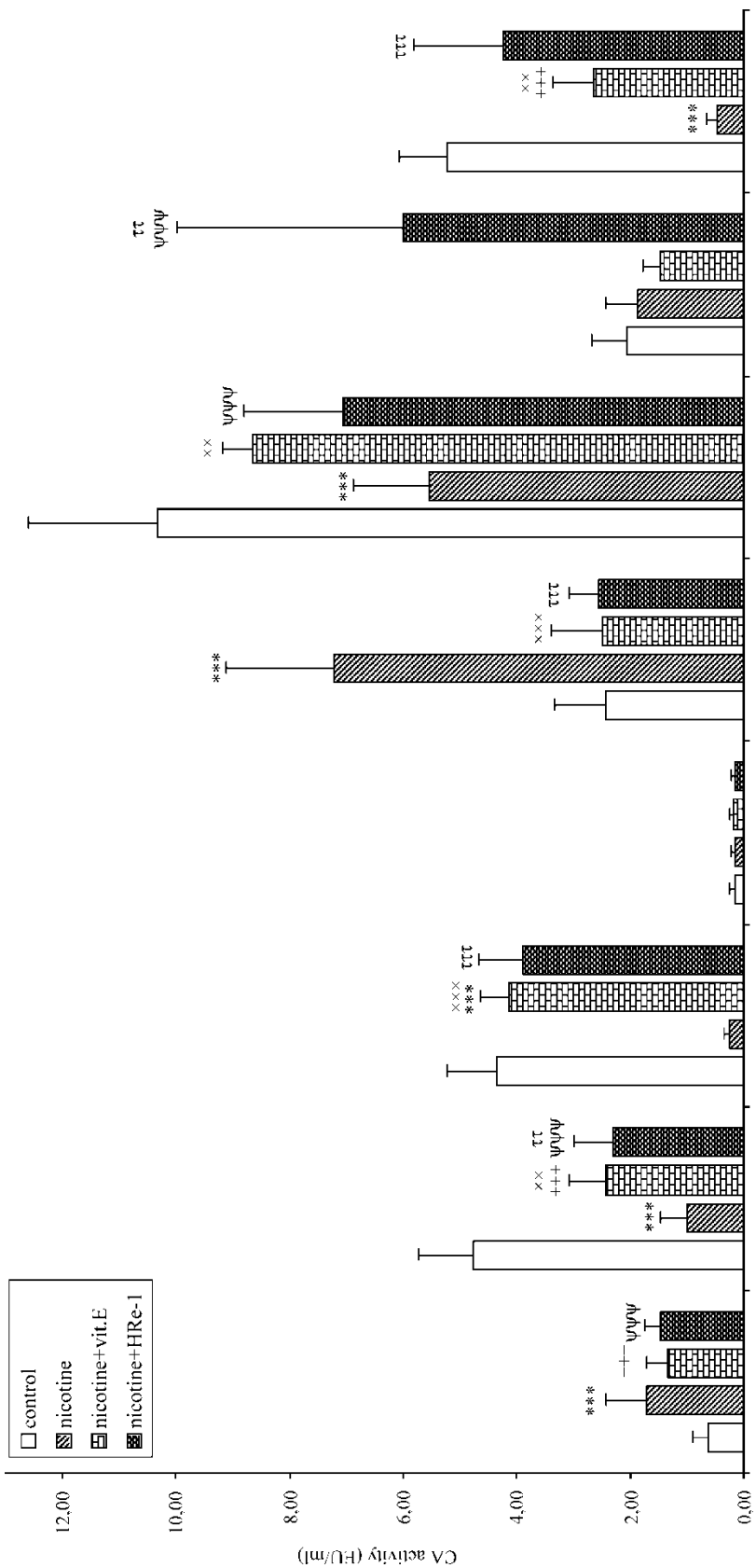

Figure 1. *In vivo* effects of nicotine, nicotine + vitamin E and nicotine + HRe-1 on rat muscle, heart, lungs, testicle, kidney, stomach, brain, and liver tissue carbonic anhydrase enzyme activity (Significant, *nicotine versus control, $^{+}$nicotine + vitamin E versus control, $^{\psi}$nicotine + HRe-1 versus control, ^{x}nicotine + vitamin E versus nicotine, $^{\tau}$nicotine + vitamin E versus nicotine) Significant (**p, $^{++}$p, ^{xx}p and $^{\tau\tau}$p $^{\psi\psi}$p $< .02$; ***p, $^{+++}$p, $^{\psi\psi\psi}$p, ^{xxx}p and $^{\tau\tau\tau}$p $< .001$, n = 6).

Table I. *In vitro* effects of nicotine, nicotine + vitamin E and nicotine + HRe-1 on various rat tissues.

		Tissues							
	Volume(μL)	Muscle (Activity%) EU/mL	Heart (Activity%) EU/mL	Lungs (Activity%) EU/mL	Testicle (Activity%) EU/mL	Kidney (Activity%) EU/mL	Stomach (Activity%) EU/mL	Brain (Activity%) EU/mL	Liver (Activity%)
Nicotine (5 mM)	Control	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	20	105.3	85.7	28.9	102.3	100	67.4	76.4	69.4
	40	108.1	78.5	28.9	89.6	127.9	58.6	55.2	49.4
	60	100	73.2	38.7	80.7	174	58.6	64.5	35.8
	80	100	71.4	28.9	107.1	167	51.3	55.2	21.9
	100	92.2	75.4	28.9	89.6	142.3	38.6	54.4	27.9
Nicotine (5 mM) + Vitamin E (10 mM)	Control	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	20	103.3	67.6	89.4	100	87.8	72	62.5	75.5
	40	105.2	80.9	95.3	89.6	99.4	76.5	58	69
	60	108.1	67.9	87.8	90.7	82.4	76.5	67.6	63.3
	80	105.4	90.6	97.5	107.1	87.9	78.8	53.6	41.3
	100	103.3	67.9	100	105	91.5	74.9	62.5	41.3
Nicotine (5 mM) + HRe-1 (500 mg/mL)	Control	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
	20	100	92.2	79.4	81.4	81.3	71.5	97.2	75.5
	40	100	78.2	87.9	91.3	89.8	76.5	80.8	63.3
	60	100	76	79.4	100	93.7	78.8	97.2	48.8
	80	100	78.2	97.8	101.1	81.3	78.8	80.8	41.3
	100	95.6	93.5	99.4	84	100	94.5	80.8	31.4

As seen in Table I, nicotine remarkably inhibited total CA activity in lung tissue and this inhibition was eliminated by vitamin E and HRe-1. While nicotine inhibited total CA activity in stomach and liver tissues, this inhibition was partially eliminated by vitamin E and HRe-1. In heart and brain tissues, nicotine inhibited total CA activity and this inhibition could not be eliminated by vitamin E but was by HRe-1. On the other hand, nicotine activated total CA activity in kidney tissue and this activation was prevented by vitamin E and HRe-1. However, total CA activities in muscle and testicle were not affected by nicotine vitamin E and HRe-1.

In vitro CA inhibition results correlate well with *in vivo* experimental results in lungs, testicles, kidney, stomach, brain and liver tissues. There was a partial agreement between *in vitro* and *in vivo* results in heart tissue. However, *in vitro* and *in vivo* results did not correlate in muscle tissue.

Although it is difficult to envisage how nicotine, vitamine E and HRe-1 modifies the CA activity in various tissues *in vivo* and *in vitro*, the inhibition of CA activity by nicotine may result from the binding of the pyridine nitrogen to zinc of CA in heart, lung, stomach, brain and liver [55]. On the other hand, facilitating proton transfer may be responsible for the increased CA activity, which may be a different isoenzyme of CA, in kidney tissue [55].

According to the results of this study, vitamin E or HRe-1 totally eliminates or attenuates the adverse effects of nicotine on CA activity in muscle, kidney, heart, lung, stomach and liver tissues.

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