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Effect of Fenfluramine on Molybdenum Hydroxylases Activities in Guinea Pigs

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Abstract: The effects of fenfluramine on the activities of molybdenum hydroxylases, aldehyde oxidase and xanthine oxidase, activities in the liver, kidney, small intestine and lung of guinea pigs has been investigated. Pre-treatment of guinea pigs with fenfluramine (10 mg/kg/day) for 7 days resulted in a statistically significant decrease ($p<0.0005$, $p<0.03$) in activity of liver, small intestine and lung aldehyde oxidase using phthalazine, phenanthridine and 3-methylisoquinoline as substrates. Liver and small intestine xanthine oxidase of fenfluramine treated guinea pigs was also found a statistically significant decrease in its activity ($p<0.0005$) using xanthine as a substrate. The Km value for phthalazine which was also a significant decrease ($p<0.005$) and demonstrated regarding hepatic aldehyde oxidase from fenfluramine-treated guinea pig, as compared to the phenanthridine and 3-methylisoquinoline Km values which also revealed no significant change. In addition to that, no significant change to the km value of xanthine was recorded with hepatic xanthine oxidase from fenfluramine-treated guinea pigs.

Key words: Fenfluramine, molybdenum hydroxylases, aldehyde oxidase, xanthine oxidase

INTRODUCTION

Obesity is the main nutritional problem in many countries of the world. It is clear that the obese person, compared to a non-obese one, suffers more and has a variety of illnesses which include; carbohydrate and lipid metabolic abnormalities, the risk of developing diabetes mellitus, hypertension, gallstones, osteoarthritis and cardiovascular disease[1]. Treatment of obesity using anorectic drugs concentrated on the alteration of behavioural metabolic and endocrine modification to reduce body fats. Fenfluramine-as an obesity drug-was generated in the early 1960s. It is a trifluromethyl derivative[2]. Fenfluramine is quickly absorbed from the gastro-intestinal tract. It is widely metabolised and some metabolism has been reported to take place in the liver as well as the gastro-intestinal tract. Primary metabolism implicated de-ethylation to active norfenfluramine. Excretion takes place through urine in the form of unchanged drug and metabolites[3]. The previous studies on the effects of fenfluramine on hormones and enzymes are numerous. Turtle and Burgess[4] showed that fenfluramine causes an increase in growth hormone concentration after intravenous administration of 40 mg followed by infusion of 1 mg/min for 90 min. Treatment with fenfluramine significantly lowered the insulin/growth hormone ratio in obese subjects, but did not affect this ratio in control subjects[5]. Many workers have studied its action using several enzymes[3,6]. The objective of this study was to investigate the effect of fenfluramine on the activity of molybdenum hydroxylases, aldehyde oxidase and xanthine oxidase, in guinea pigs.

MATERIALS AND METHODS

Chemicals: Phenanthridine and phthalazine were purchased from Aldrich Chemical Company, (Gillingham, UK), 3-methyliosquinoline was obtained from ICN Pharmaceuticals Inc. (K and K, Irvine, CA) and xanthine was purchased from Sigma Chemical Company (Poole, UK). Fenfluramine was obtained from Les Laboratories Servier, Gidy-45400 Fluery-Les-Aubrais, France.

Animals: Male Dunkin-Hartley guinea pigs, weighing 450-500 g were obtained from King Fahd Medical Research Centre, Jeddah, Saudi Arabia. The animals were housed in groups of three and allowed food and water *ad lib*. They were kept at a constant temperature of (24°C) and on a 12 h dark-light cycle.

Treatment of guinea pigs: Fenfluramine (10 mg/kg/day) was administered orally (as a solution in drinking water) to male guinea pigs (N=6) for seven days. Control groups (N=6) received water *ad lib*. The animals were then killed by cervical dislocation and different tissues (liver, lung, kidneys and small intestine) were removed then immediately frozen in liquid nitrogen and stored in a deep freezer at-80°C.

Preparation of tissue

Aldehyde oxidase and xanthine oxidase: Partially purified aldehyde oxidase and xanthine oxidase were prepared from tissue homogenate as described by Johnson *et al.*[7] and Al-Tayib[8].

Enzymes assays: Aldehyde oxidase activity was determined spectrophotometri-cally using the method described by Johnson *et al.*[9] with phthalazine (1 mM) and 3-methylisoquinoline (1 mM) at 420 nm, while the oxidation rate of phenanthridine was estimated at 322 nm. Xanthine oxidase activity was evaluated with xanthine (50 μM) at 295 nm[10,11].

Protein concentration was measured by using biuret method[12].

RESULTS AND DISCUSSION

Aldehyde oxidase (EC. 1.2.3.1) and xanthine oxidase (EC. 1.2.3.2) belong to a group of enzymes named "Molybdenum Hydroxylases". These enzymes have very similar properties, however, they differ somewhat in their substrate specificities[13]. These enzymes play an important role in the metabolism of drugs and xenobiotic compounds[14,15]. As well as, Beedham[14], reported that aldehyde oxidase catalyses the oxidation of aldehyde and N-heterocyclic compounds. As a result of this wide specificity, aldehyde oxidase activity was monitored with three different substrates (phthalazine, phenanthridine and 3-methylisoquinoline). Table 1-3 show the specific activities of aldehyde oxidase in the liver, small intestine, kidney and lung tissue of guinea pigs receiving fenfluramine and control guinea pigs. In each substrate, it has been established that the activity of the liver aldehyde oxidase was significantly lower ($p<0.0005$) in the fenfluramine- treated guinea pigs. Moreover, the activity of small intestine aldehyde oxidase was also significantly lower ($p<0.0005$ $p<0.03$) in fenfluramine- treated animals with phthalazine and phenanthridine, respectively. The activity of kidney aldehyde oxidase using phenanthridine was significantly decreased ($p<0.005$) in fenfluramine-treated guinea pigs, whereas the drug had no significant effect on the activity of the enzyme when phthalazine was used. The activity of lung aldehyde oxidase of fenfluramine-treated animals was significantly lower ($p<0.005$ for phenanthridine and $p<0.03$ for phthalazine). Table 3 shows that the activity of the liver aldehyde oxidase was significantly lower ($p<0.0005$) in the fenfluramine-treated guinea pigs using 3-methylisoquinoline and in comparison with the small intestine, kidney and lung enzymes, no activity was detected in these tissues.

Table 1: Effects of fenfluramine administration on guinea pigs aldehyde oxidase activity using phenanthridine as a substrate.

	*Specific activity (μmol/min/mg protein)		
Tissue	Control	Treated	p<°
Liver	0.0139±0.0008	0.0077±0.0006	0.0005
Small intestine	0.0031±0.0007	0.0023±0.0006	0.03
Kidney	0.0072±0.0007	0.0056±0.0006	0.005
Lung	0.0014±0.0001	0.0007±0.0007	0.005

* The values are given as means±SD (N=6)

° The statistical significance (p) of differences between control and fenfluramine-treated guinea pig values are obtained using a two-tailed student's t-test

Table 2: Effects of fenfluramine administration on guinea pigs aldehyde oxidase activity using phthalazine as a substrate.

	*Specific activity (μmol/min/mg protein)		
Tissue	Control	Treated	p<°
Liver	0.17±0.02	0.0643±0.007	0.0005
Small intestine	0.011±0.001	0.0075±0.0009	0.0005
kidney	0.0238±0.005	0.0207±0.003	NS
Lung	0.0048±0.0006	0.0039±0.0005	0.03

* The values are given as means±SD (N=6), NS: non-significant

Table 3: Effects of fenfluramine administration on guinea pigs aldehyde oxidase activity using 3-methylisoquinoline as a substrate.

	* Specific activity (μmol/min/mg protein)		
Tissue	Control	Treated	p<°
Liver	0.0102±0.001	0.0059±0.0004	0.0005
Small intestine	0	0	0
kidney	0	0	0
Lung	0	0	0

* The values are given as means±SD (N=6)

Table 4: Effects of fenfluramine administration on guinea pigs xanthine oxidase activity using xanthine as a substrate.

	* Specific activity (μmol/min/mg protein)		
Tissue	Control	Treated	p<°
Liver	0.0014±0.0002	0.0005±0.0002	0.0005
Small intestine	0.0084±0.0004	0.0057±0.0006	0.0005
kidney	0	0	0
Lung	0	0	0

* The values are given as means±SD (N=6)

Xanthine oxidase activity in the liver and small intestine of control and fenfluramine-treated guinea pigs was obtainable using xanthine as a substrate. Both hepatic and small intestine xanthine oxidase activities were significantly lower ($p<0.0005$) in fenfluramine-treated guinea pigs (Table 4).

For all these substrates the Vmax values with enzymes from fenfluramine-treated guinea pigs were also significantly lower ($p<0.05$) than that obtained with control enzymes (Table 5). However, it has been recorded that the Km values only for phthalazine and xanthine were significantly decreased ($p<0.005$, $p<0.0005$, respectively) in regard to enzymes from fenfluramine-treated guinea pigs. The obtained results revealed the presence of different effects of fenfluramine on aldehyde oxidase and explained the differences between the Km values of

Table 5: Kinetic constants for aldehyde oxidase and xanthine oxidase from control and treated guinea pigs.

	Km (M)		Vmax (μmol/min/mg protein)	
Substrate	Control (N=3)	Treated (N=3)	Control (N=3)	Treated (N=3)
Phenanthridine	1.8×10^{-5}	1.75×10^{-5}	0.0323	0.0249**
Phthalazine	5.6×10^{-5}	3.3×10^{-5}***	0.1448	0.06**
3-methylisoquinoline	3.4×10^{-5}	2.9×10^{-5}	0.0130	0.006****
Xanthine	7.2×10^{-5}	2.4×10^{-5}****	0.0101	0.0033*

As compared with matched control guinea pigs, * $p<0.03$; ** $p<0.05$; *** $p<0.005$; **** $p<0.0005$ (Student's t-test)

phthalazine, phenanthridine and 3-methylisoquinoline. In this connection, this phenomenon also gave an indication regarding the presence of isozymes of aldehyde oxidase and this is consistent with the results demonstrating that different aldehyde oxidase isozymes are present in human, baboon, rabbit, guinea pigs, rat and mouse[16-19].

In the present investigation, fenfluramine (10^{-3} M) was tested with aldehyde oxidase from control guinea pigs. However, no reaction was observed, either as a substrate or as an inhibitor of aldehyde oxidase catalysed oxidation of phenanthridine. Molybdenum hydroxylases were found to be controlled by a number of factors such as: genetic determinants, hormonal influences and induction[20-23].

Concerning the correlation between testosterone and these oxidases, it was demonstrated that testosterone was shown to induce aldehyde oxidase and xanthine oxidase in mice and rats[21,22,24]. On the other hand, it was detected that castration did apparently inhibit the increase in these enzymes[22]. A few studies pointed out that xenobiotics were demonstrated to cause an increase in the activity of aldehyde oxidase and xanthine oxidase in liver of rabbit using phthalazine or1-phthalazinone[9]. The activities of the key enzymes of glucolytic pathway such as hexokinase, 6-phosphofructo-1-kinase were significantly reduced after treatment of rats with fenfluramine and many reports indicated that fenfluramine exerts its action through its effect on serotonin[3,25,26]. Present findings support the previous mentioned data, however, on the basis of the present study, it could be concluded that fenfluramine has a good effect on aldehyde oxidase and xanthine oxidase through melatonin action and proved to be satisfactory. This suggestion is reinforced by two unequivocal pieces of evidence. The first evidence is termed serotonin (5-hydroxytryptamine) which acts as precursor of melatonin (N-acetyl-5-methoxytryptamine) in terms of function (biochemistry)[27-30] and the second one was reported by Beedham *et al.*[11] and Al-Tayib[31], which demonstrated that guinea pig aldehyde oxidase and xanthine oxidase activities were increased after melatonin treatment.

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