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Daily Rhythmic Variations of Renal Molybdenum Hydroxylases in Male Rats

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Abstract: The activities of the xenobiotic metabolizing enzymes, aldehyde oxidase and xanthine oxidase were estimated eight times daily (at equal periods) in partially purified preparations of kidney of rats. Diurnal variation in aldehyde oxidase activity was observed with two substrates (phthalazine and 3-methylisoquinoline). The maximum peak appeared at 0300h whereas the minimum activity occurred at 15h. The differences between the maximum and minimum enzyme activities were extremely significant ($P < 0.0005$). Xanthine oxidase also showed a daily rhythm when xanthine was used as a substrate. The maximum of activity xanthine oxidase occurred at 0300 h whereas the minimum occurred at 1500h. From a statistical point of view, the difference between rhythmic extremes was significant ($P < 0.0005$).

Introduction

Mixed function oxidases or microsomal monooxygenases are involved in the biotransformation of xenobiotic compounds and a wide range of drugs [1-3]. However, molybdenum hdroxylases, aldehyde oxidase (aldehyde: O_2 oxidoreductase EC 1.2.3.1), and xanthine oxidase (xanthine: O_2 oxidoreductase C1.2.3.2) existing in the cytosol can also contribute to this process effectively[4-7]. The activity of this drug – metabolizing enzymes has been found to have temporal variations in diverse species. Three previous studies reported the presence of daily rhythmic variations in hepatic male rat microsomal enzymes[8-10]. Furthermore, the activity of microsomal enzymes showed circadian rhythms in drug metabolism in mice[11]. Lake *et al*.[12]. Noticed a circadian variation of hepatic microsomal enzymes in the golden hamster. As

afterwards stated a diurnal rhythm of molybdenum hydroxylases in guinea pig and hamster was observed [13,14] . Accordingly the aim of the present study was conducted to investigate whether molybdenum hydroxylases in rat exhibit circadian variations in activity or not.

Materials and Methods

Chemicals

Phthalazine was purchased from Aldrich Chemical Company. 3-Methylisoquinoline was supplied by ICN Pharmaceuticals Inc. All other chemicals were analytical reagent grade from BDH.

Animals

Adult, male Wistar rats (220–240g) were obtained from King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats fed ad lib on standard laboratory diet (Oxoid, modified 41B) with free access to water . They were housed in groups of six and maintained in a regime of strictly controlled lighting cycle of 0600–1800h light: 1800–0600h dark. Rats were killed by cervical dislocation; their kidneys were rapidly collected and stored in a deep – freezer at -80°C until required.

Preparation of Renal Enzyme

With some modifications partially purified aldehyde oxidase was prepared from tissue homogenate, according to the method described Johnson *et al*.[15]. as follows: Kidneys were finely chopped and homogenized in 3 volumes of potassium chloride solution (1.15 w/v) containing 10^{-4} M EDTA for 30 second with a polytron homogenizer. The resulting suspension was heated on a water bath at 50-55°C for 15 minutes, and soon cooled to 10°C, then centrifuged at 20,000g for 25 minutes at 4°C using Heraeus Christ 20–3 (LABSCO) centrifuge. Sufficient solid ammonium sulfate was added to the supernatant to give 50% saturation with stirring for 15 minutes at 4°C followed by centrifugation at 3000 g for 15 minutes. The supernatant was discarded and the precipitate was rinsed with distilled water and dissolved in 1.5 ml of 10^{-4} M EDTA solution .The partially purified enzyme was stored in a deep freezer at -80°C and used when required.

Enzyme Assays

The activity of molybdenum hydroxylase fractions was measured spectrophotometrically

using a Varian UV/VIS spectrophotometer, fitted with a thermostatically controlled cell holder maintained at 37°C. The specific activity of aldehyde oxidase was evaluated using two substrates (Phthalazine and 3-methylisoquinoline) as described by Johnson *et. al*.,[16]. The oxidation rate of either phthalazine (1mM) or 3-methylisoquinoline (1 mM) was monitored at 420nm by following potassium ferricyanide reduction. The specific activity of xanthine oxidase was determined using xanthine (50µM) at 295 nm as reported previously by Johnson *et al*.[16,17]. Protein concentration was determined by the Biuret method.

Results and Discussions

The activities of aldehyde oxidase and xanthine oxidase assayed at 3- hours intervals differed markedly over a 24 h period under a controlled lighting cycle, using phthalazine, 3-methylisoquinoline and xanthine. There have been temporal variations in renal aldehyde oxidase activity, using phthalazine and 3-methylisoquinoline as substrates which were monitored indirectly by following the reduction of potassium ferricyanide (an artificial acceptor) at 420 nm. Peaks of the maximum and minimum oxidative activities were evident at 0300 h, 1800 h, 2400 h and 1500 h respectively. The differences between the minimum and any of the maximal peaks were highly significant (P< 0.0005) in both substrates. similar results were obtained with both substrates (Figure 1 and 2).

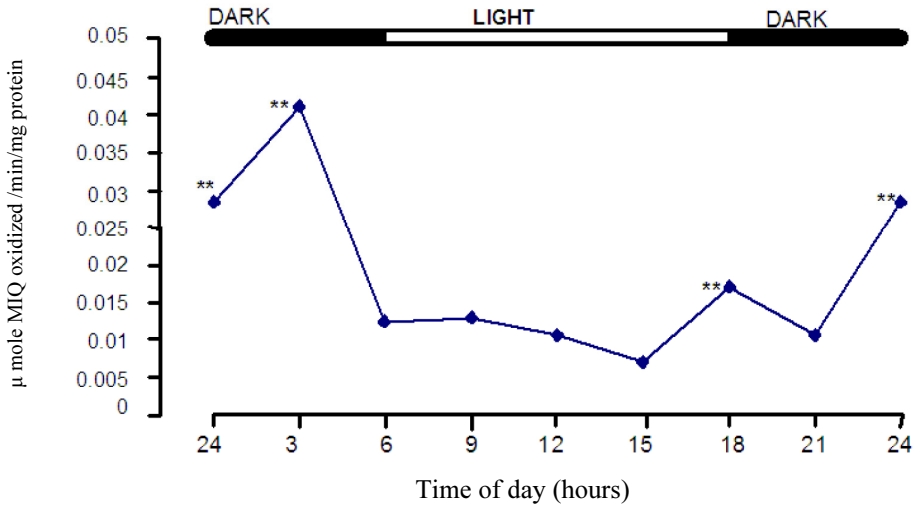

Fig. 1: Daily variation in renal aldehyde oxidase activity .Enzyme activity was measured at 37 °C and expressed as µmol 3Methyliosoquinoline (MIQ) oxidized/min/mg protein . Potassium ferricyanide (1 mM) was used as an electron acceptor . Each point represents the mean of six animals.

(**P < 0.0005) using a two-tailed students t-test.

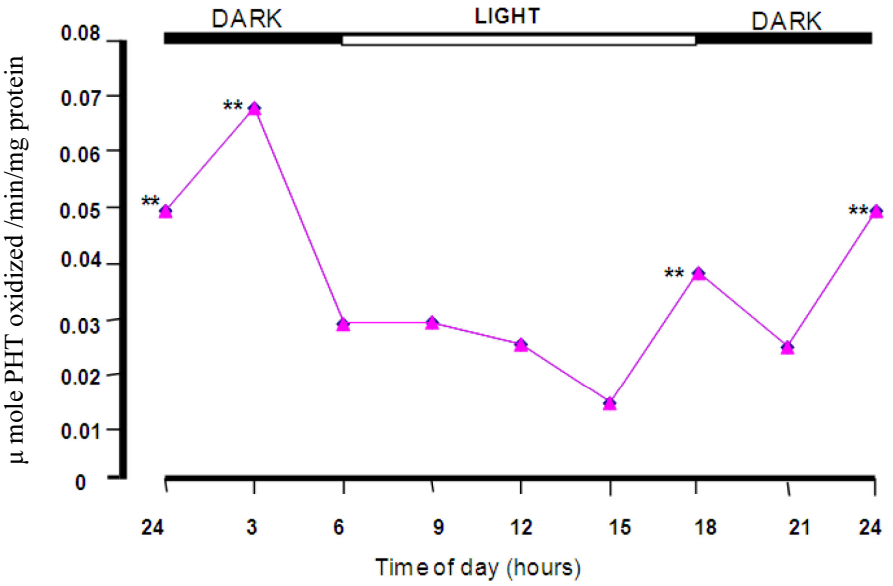

Fig. 2: Daily variation in renal aldehyde oxidase activity .Enzyme activity was measured at 37 °C and expressed as µmol Phthalazine (PHT) oxidized/min/mg protein . Potassium ferricyanide (1 mM) was used as an electron acceptor. Each point represents the mean of six animals.

**P < 0.0005 .

Holmes [18]. Ohkubo *et al.*[19] and Beedham *et al.*[20] had been reported the presence of isozymes of aldehyde oxidase in mouse, rat and guinea pig. However, the present study pointed out that the two substrates have the same fluctuation activity. The results obtained by biochemical reactions suggest that these compounds may be used as substrates for the same isoenzymes of aldehyde oxidase. The activity of the other renal molybdenum hydroxylase, xanthine oxidase, was assayed using xanthine as a substrate and oxygen as the electron acceptor at 295nm. The maximum daily activity of xanthine oxidase peak emerged at 0300hr and intermediate peak activity occurred at 2400h, 0600h and 1800h. Whereas the minimum activity appeared at 1500h. (Figure 3). The difference between the minimum and any of the peak maxima were significant (P<0.005). Since 1968, there have been many more reports regarding daily rhythms for microsomal monooxygenases in several animal species [8-12]. Their findings support the results of the present study, which have exhibited daily variations in molybdenum hydroxylase activity. Furthermore, a work carried out by Beedham *et al.*,[13] found that the activity of aldehyde oxidase and xanthine oxidase showed daily variation in guinea pig. Their results were similar to those of our study, hence providing further support to the above mentioned daily variations. These diurnal rhythms have been attributed to many factors, including

changes in coricosteroids, light dark-schedule and dietary protein [8,9,21].

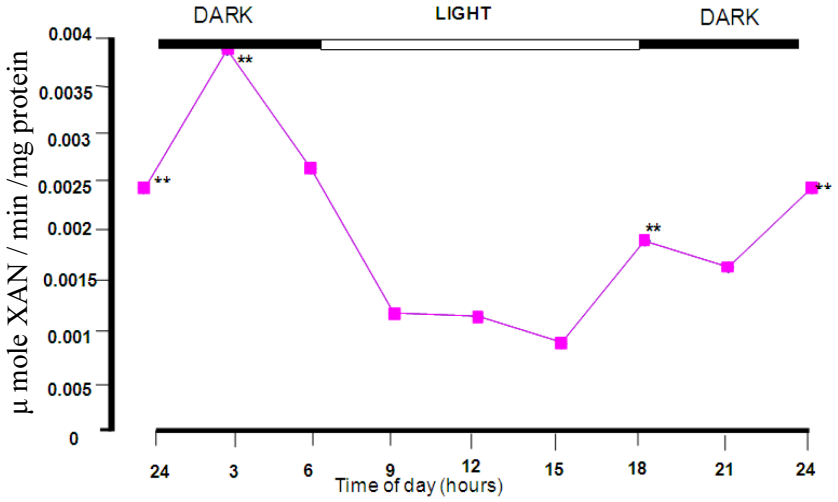

Fig. 3: Daily variation in renal xanthine oxidase activity .Enzyme activity was measured at 37 °C and expressed as µmol Xanthine (XAN) oxidized/min/mg protein .Oxygen was used as an electron acceptor. Each point represents the mean of six animals.

*P<0.005 , **P < 0.0005

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التغيرات النَظْمية اليومية في إنزيمات هِدرُ كسيلازات مُوليِبدينم الكلوية لذكور الجرذان

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المستخلص: قُيِّمت فاعليات الإنزيمات المُسْتقِلبة الحيوية الغريبة: ألدهيد أكسيداز, والزنتين أكسيداز, ثمان مرات يوميا (بينها فترات زمنية فاصلة متساوية), في مستحضرات مُنقاة جزئياً، أخذت من كُلى الجرذان. لقد لوحظ التغير النهاري في نشاط ألدهيد أكسيداز باستخدام مادتين وسيطتين هما الثالازين و٣– ميتيل اِيزوكوينولين.

ظهر النشاط الأعظم الأساسي للإنزيم الأنف الذكر، في الساعة (٠٣٠٠), في حين لوحظ النشاط الأصغري لهذا الإنزيم في الساعة (١٥).

كانت الفروق بين النشاطات الإنزيمية الأعظمية والأصغرية معنوية جداً من الناحية الإحصائية ($p<0.0005$). أظهر إنزيم الزنتين أكسيداز أيضًا؛ نَظْمًا يوميًا عندما استخدم الزنتين كمادة وسيطة .من جهة ثانية؛ لوحظ النشاط الأعظم للزنتين أكسيداز في الساعة (٣:٠٠) في حين حدث النشاط الأصغري في الساعة (١٥:٠٠).

لقد كانت الفروق بين الدرجات القصوى النظمية من وجهة نظر إحصائية؛ معنوية ($p<0.0005$).