Circadian activity of rat kidney enzymes

F. Franciolini, A. Becciolini, V. Casati, D. Cremonini, V. Giachè and S. Porciani

Istituto di Radiologia, Università di Firenze, viale Morgagni, I-50134 Firenze (Italy), 28 August 1978

Summary. Alkaline phosphatase, LAP, β-glucuronidase and cathepsin D activities and protein content of the kidney homogenate did not show any circadian rhythm in animals sacrificed at different hours of the day. The fluctuations of maltase appear modest and not dependent on a light/dark cycle.

Circadian dependence of many biological functions was recently demonstrated in mammals[1-5]. Besides other circadian functions, there appeared modifications of some enzyme activities localized in the brush border of the small intestinal epithelium. In this epithelium, disaccharases and dipeptidases, involved in the 'membrane digestion' process[6], show significant fluctuations during light/dark (L/D) cycle[7-9]. The present experiment was done to ascertain the existence of circadian dependence and the normal levels of similar activities localized in the kidney epithelium. Moreover, some lysosomal enzymes and protein content were assayed.

Materials and methods. 96 female Wistar rats, 12 weeks old and weighing 160–180 g, were kept on a 06.30–18.30 h L/D cycle. They were fed ad libitum with standard diet and allowed free access to water. Any contact with the animals was avoided, except for feeding and cage cleaning twice a week. L/D period was divided into 9 unequal intervals. Groups of 5–16 animals were sacrificed at each interval. When animals per group were numerous, they were sacrificed at subsequent days so that the time of the sacrifice was always less than 1 h for each group.

The kidneys were homogenized at 5% (w/v) with distilled water and after centrifugation at 900 × g, the supernatant was taken for enzyme assays. Maltase (1–4 α-glicosidase)[10], leucinaminopeptidase (LAP)[11], alkaline phosphatase[12], β-glucuronidase[13], cathepsin D[14] and protein content were assayed with the same methods used for intestinal enzymes. All assays were done in duplicate. Student's t-test was used to evaluate statistical significance.

Results. Maltase only presents some oscillations throughout the L/D cycle (figure 1). The activity is lowest in the animals sacrificed at 02.00 and 05.00 h, and reaches a maximum when the rats were killed at 09.30 h. The values at this interval are significantly higher than those reached during the 2nd part of the darkness period ($p < 0.01$). At the other intervals, fluctuations are always modest and have no statistical significance. Alkaline phosphatase and LAP do not show evident modifications at the different intervals. Also lysosomal enzymes (figure 2) do not present marked differences during the L/D cycle, even if cathepsin D was assayed only in some animals. Protein content of the homogenate (figure 2) shows constant values during the whole experiment.

Discussion. The possible circadian dependence of some enzyme activities of the kidney was studied by dividing the L/D cycle into many intervals and using several animals for each group. Previous data[7] (obtained with a small number of intervals and animals) showed the absence of circadian fluctuations in maltase and leucylnaphtylamidase activities.

In the present experiment, maltase - an enzyme localized only in the brush border of epithelial cells of proximal tubules - presents the lowest activity during the night and the highest in the morning. This pattern does not appear significant when using single cosinor method[15] for circadian dependence test.

Alkaline phosphatase and LAP enzymes, which histochemical investigation reveals as localized in the brush border and in other kidney structures, did not show significant fluctuations during the L/D cycle. Similar activities of the small intestine assayed in the same animals (not yet published data) showed a L/D fluctuation with highest activity between 21.00 and 06.00 h, and lowest during the light

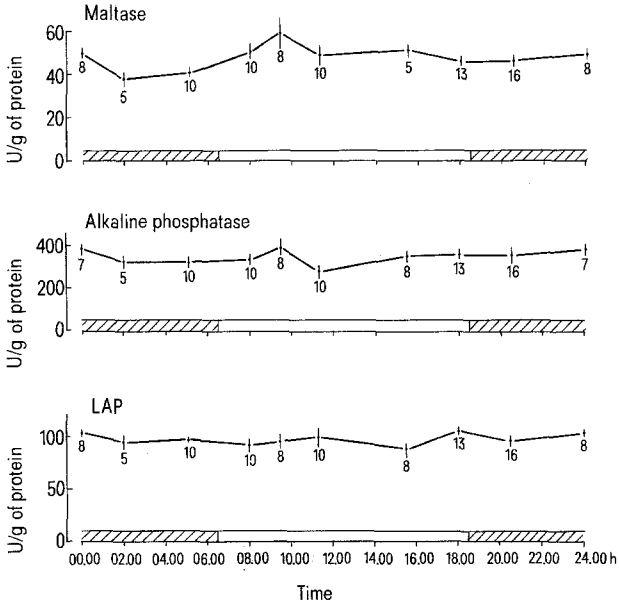


Fig. 1. Maltase, alkaline phosphatase and LAP activities are reported as mean values±SE during the L/D cycle. The number of animals per group is reported under each value.

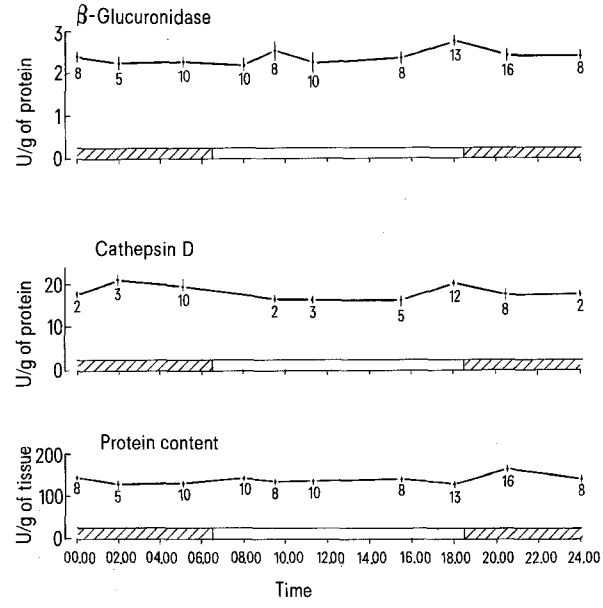


Fig. 2. β-glucuronidase, cathepsin D and protein content are reported as mean values±SE during the L/D cycle. The number of animals is reported under each value.

period. Lysosomal activities and protein content of the kidney, as in the small intestine, did not show circadian fluctuations.

The results show that although some parameters in the kidneys, such as mitotic activity, show circadian dependence[16], others (enzymes playing an important role in tubular absorption) do not show significant fluctuations during the L/D cycle.

1 F. Halberg, A. Rev. Physiol. *31*, 675 (1969).
2 F. Halberg, J. Reinhardt, F.G. Bartter, C.S. Delea, R. Gordon, A. Reinberg, J. Gatha, H. Hofmann, M. Halhuber, R. Gunther, E. Knapp, J.C. Pena and M. Garcia Sainz, Experientia *25*, 107 (1969).
3 E.R. Burns, L.E. Scheving and T.H. Tsai, Science *176*, 71 (1972).
4 B.C. Goodwin, in: Temporal organization in cells. A dynamic theory of cellular processes. Academic Press, New York 1973.
5 L.N. Edmunds and V.P. Cirillo, Int. J. Chronobiol. *2*, 233 (1974).
6 A.M. Ugolev and P. De Laey, Biochim. biophys. Acta *300*, 105 (1973).
7 M. Saito, Biochim. biophys. Acta *286*, 212 (1972).
8 N.R. Stevenson, F. Ferrigni, K. Parnicky, S. Day and J. Fierstein, Biochim. biophys. Acta *406*, 131 (1975).
9 M. Saito, E. Murakami and M. Suda, Biochim. biophys. Acta *421*, 177 (1976).
10 A. Dahlquist, Analyt. Biochem. *7*, 18 (1964).
11 W. Nagel, F. Willing and F.H. Schmidt, Klin. Wschr. *42*, 447 (1964).
12 O.A. Bessey, O.H. Lowry and M.S. Brock, J. biol. Chem. *164*, 321 (1946).
13 I.D. Desai, Can. J. Biochem. *45*, 485 (1969).
14 M. Eggstein and F.M. Kreutz, Klin. Wschr. *33*, 879 (1955).
15 F. Halberg, E.A. Johnson, W. Nelson, W. Runge and R. Sothern, Physiol. Teacher *1*, 1 (1972).
16 T.L. Phillips and G.F. Leong, Cancer Res. *27*, 286 (1963).

Diethylmesoxalate hydrate, a new irreversible inhibitor of cholinesterases

G. Voss and R. Neumann

Agrochemicals Division, Ciba-Geigy Ltd, CH-4002 Basel (Switzerland), 18 August 1978

Summary. Bovine erythrocyte acetylcholinesterase and human plasma cholinesterase are irreversibly inhibited by diethylmesoxalate hydrate, the inhibition potency being comparable to that of certain insecticidal organophosphates and carbamates. Insect cholinesterases, however, appear to be much less affected by diethylmesoxalate hydrate. The compound was also found to inhibit the hydrolysis of paraoxon by rabbit plasma A-esterase, but in a reversible mode.

The inhibition of cholinesterases represents a very important and successful principle for insecticidal activity. Although this biochemical mode of action does not a priori lead to selective insect control agents, the pesticide industry has succeeded in identifying and developing a number of organophosphates and carbamates with a favourable mammal/insect toxicity ratio. New types of cholinesterase inhibiting compounds are, therefore, of great potential value.

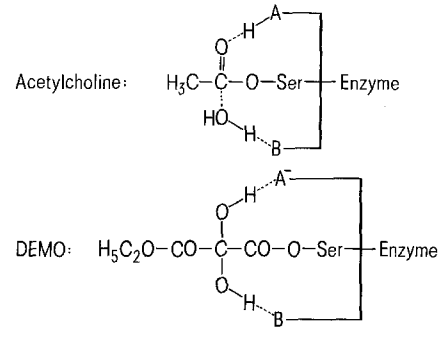
The hydrate of diethylmesoxalate (DEMO), $H_5C_2-O-CO-C(OH)_2-CO-OC_2H_5$, is an example for a new irreversible cholinesterase inhibitor. The inhibition characteristics of DEMO and its diisopropyl analogue (DIPMO) have been evaluated in some detail and will be described in the present communication.

Materials and methods. The following enzyme preparations and inhibitors were used: acetylcholinesterase (AChE) from bovine erythrocytes and electric eel (Sigma), from houseflies (homogenates of fly-heads), from *Spodoptera littoralis* (homogenates of first instar larvae); human plasma cholinesterase (ChE) and rabbit plasma A-esterase; diethylmesoxalate (Fluka), diisopropylmesoxalate (synthesized according to reference[1]); monocrotophos (dimethyl cis-1-methyl-2-methylcarbamoylvinyl phosphate), dioxacarb (2-(1,3-dioxolan-2-yl) phenyl-N-methyl carbamate) and paraoxon (diethyl 4-nitrophenyl phosphate) (Ciba-Geigy, analytical standards). An automated procedure[2] was used for most of the cholinesterase inhibition experiments.

Results. DEMO and DIPMO were found to inactivate cholinesterases from several sources in an irreversible mode, i.e. inhibition was found to be time-dependent similar to carbamates and organophosphates. Expressed in terms of I_{50}-values, the inhibition potencies of DEMO and DIPMO towards bovine AChE and human ChE were equal to or greater than those of dioxacarb and monocrotophos (table). Eel and housefly AChE were less readily inhibited by the mesoxalates, and AChE from *Spodoptera littoralis* was almost insensitive to DEMO and DIPMO.

Irreversibly inhibited cholinesterases, i.e. the carbamylated or phosphorylated enzymes, reactivate slowly. It was therefore interesting to see how fast the inhibition by DEMO would reverse. With bovine AChE at 37 °C and pH 8.0, the reactivation constant 'k_3' was found to be 0.017 min^{-1}. This figure is between the rate constants determined for the reactivation of dimethylphosphorylated and N-methylcarbamylated AChE. DEMO inhibited housefly AChE, however, was more rapidly reactivated, k_3 being 0.092 min^{-1}.

DEMO, by topical application, is only marginally toxic to insects. 3rd instar larvae of *Spodoptera littoralis* were not at all affected at a dosage of 1 mg/larva, and houseflies tolerated 10 µg/fly without showing symptoms. Houseflies



Binding of acyl serine to 2 catalytic groups (A and B) of a cholinesterase.