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Circadian changes of D-alanine and related compounds in rats and the effect of restricted feeding on their amounts☆

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ABSTRACT

The circadian changes of D-alanine (D-Ala), an intrinsic D-amino acid found in mammals, were investigated in rats with diurnal and nocturnal habits, and the profiles were compared to those of L-Ala, other D-amino acids and several hormones. Determination of D-Ala in the rat plasma, pancreas and anterior pituitary gland was carried out using a sensitive and selective two-dimensional HPLC system combining a micro-ODS column and an enantioselective column after fluorescence derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F). The amount of D-Ala was high during the sleeping period and low during the active period in rats with both diurnal and nocturnal habits, indicating for the first time that the D-Ala is closely related to the activity rhythm of animals. In contrast, L-Ala and other D-amino acids did not show any clear circadian changes. The circadian change of D-Ala inversely correlated with that of the plasma insulin level in rats with both diurnal and nocturnal habits. Considered together with our previous findings that D-Ala is localized in the insulin secreting beta-cells in the rat pancreas, it is strongly suggested that D-Ala has some functional relationships to insulin in mammals.

1. Introduction

D-amino acids are the enantiomers of L-amino acids predominantly observed in living creatures. These D-amino acids were believed to be present only in the lower species such as microorganisms and non-vertebrate marine animals [1,2]. However, recent studies revealed that some D-amino acids, represented by D-serine (D-Ser) and D-aspartic acid (D-Asp), naturally occur in mammals including human beings [3–5]. In the brain, especially in the frontal brain areas such as the cerebrum and hippocampus, large amounts of D-Ser were found [6] and it is now thought to be the neuromodulator of the *N*-methyl-D-aspartate (NMDA) receptor [7–10]. Also, large amounts of D-Asp were observed in various endocrine tissues, and this D-amino acid has been shown to regulate the production and/or secretion of specific hormones in these tissues [11–14]. In addition to D-Ser and D-Asp, several D-amino acids including D-alanine (D-Ala) are reported to be present in mammals [3,15–24], and they are expected to be the candidates of novel physiologically active substances and/or marker molecules of diseases. However, the amounts of these D-amino acids are often too small to precisely determine, and therefore, the physiological functions and the diagnostic values of these D-amino acids have scarcely been clarified mainly due to the lack of sensitive and selective analytical methods.

D-Ala is the first D-amino acid found in mammals using the sera of guinea pigs [25]. Since then, this D-amino acid was determined in the blood (serum or plasma), urine and brain of various mammalian species using enantioselective analytical methods including chiral GC, chiral HPLC, diastereomeric HPLC and enzymatic methods [3,15–19,24,26–32]. In our previous report, we established a sensitive two-dimensional HPLC with fluorescence detection combining a micro-ODS column and an enantioselective column, and the detailed anatomical distribution of D-Ala in mammals has been clarified for the first time, demonstrating that D-Ala is localized to the anterior pituitary gland and pancreas [33]. Especially, in the pancreas, D-Ala is observed only in the insulin secreting beta-cells in the Langerhans islets [34], strongly suggesting that D-Ala has some physiological significance in mammals. We also reported that the amount of D-Ala has a clear circadian change showing a higher amount during the daytime [33], and the clarification of the physiological meanings of this rhythmical D-Ala profile is highly expected.

The aim of the present study is to clarify the physiological meaning and diagnostic value of this circadian D-Ala rhythm in mammals. Circadian changes in bioactive substances are mainly controlled by the environmental light–dark signals or the activity rhythms of

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animals [35]. Therefore, in the present investigation, we changed the activity rhythm of the rats by changing the feeding schedule to have a diurnal habit (rat is an animal with a nocturnal habit under wild conditions [36]), and the amount of D-Ala in these rats have been determined. By comparing the D-Ala rhythm in these rats to that in the normal rats with a nocturnal habit, we found that the D-Ala rhythm is controlled by the activity rhythm of the rats rather than the light–dark change in the environment. The amounts of L-Ala, other typical D-amino acids and various hormones have also been investigated to clarify the relationships between these compounds and D-Ala, and it was revealed that the amount of D-Ala has a clear reverse-correlation to the plasma insulin level.

2. Experimental

2.1. Materials

The enantiomers of Ala, Asp and Ser were obtained from Nacalai Tesque (Kyoto, Japan) and the derivatizing reagent, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), was purchased from Tokyo Kasei (Tokyo, Japan). *o*-Phthaldialdehyde (OPA) and *N-tert*-butyloxycarbonyl-L-cysteine (Boc-L-Cys) were obtained from Wako (Osaka, Japan) and Novabiochem (Läufelfingen, Switzerland), respectively. Methanol (MeOH) and tetrahydrofuran (THF) of HPLC grade, trifluoroacetic acid (TFA), citric acid monohydrate and boric acid were the products of Wako. Acetonitrile (MeCN) of HPLC grade was from Nacalai Tesque, and water was purified using a Milli-Q gradient A 10 system (Millipore, Bedford, MA, USA). All other reagents were of the highest reagent grade and used without further purification.

2.2. Animals

Male Wistar rats (7–8 weeks of age, specific-pathogen free) were purchased from Kyudo (Kumamoto, Japan) and were housed in the animal center of Kyushu University, Graduate School of Pharmaceutical Sciences, under a 12-h light/12-h dark cycle for at least 1 week (lights on at 07:00 a.m.). The control rats were given free access to food (diet type NMF, Oriental Yeast, Tokyo, Japan) and water. Rats with the diurnal habit were made by restricted feeding (the rats could have free access to water, but had access to food only from 09:00 to 17:00) for 2 weeks. All experiments were performed after the permission (No. PAE04-74, PAE03-40) from the animal care and use committee of the Graduate School of Pharmaceutical Sciences, Kyushu University.

2.3. Sample preparation procedure

The rats were anesthetized with diethyl ether and euthanized by decapitation. The blood was collected from the jugular artery into a heparinized tube (Nippon Becton Dickinson, Tokyo, Japan) and centrifuged at 4500 × *g* at 4 °C for 15 min to obtain the plasma. A part of the plasma was used to determine the amounts of corticosterone and insulin. The anterior pituitary gland, pancreas and pineal gland were quickly excised, and stored at −80 °C after measuring their wet weights. The pineal gland was used to determine the amount of melatonin as described in Section 2.6. The pancreas and plasma samples were homogenized in 20-fold volumes MeOH on ice (200-fold volumes were used for the anterior pituitary gland), and the homogenates were centrifuged at 4500 × *g* for 5 min to obtain the supernatants and used for the determination of the D- and L-amino acids.

2.4. HPLC determination of D- and L-Ala

Determination of D- and L-Ala was carried out as described in a previous report [33] using precolumn fluorescence derivatization with NBD-F and a two-dimensional HPLC system combining a micro-ODS column and an enantioselective column. Briefly, the MeOH supernatants obtained in Section 2.3 (10 μl for the pancreas and plasma, 100 μl for anterior pituitary gland) were evaporated to dryness, and 20 μl of 200 mM sodium-borate buffer (pH 8.0) and 10 μl of 20 mM NBD-F in dry MeCN were added to the residue, then heated at 60 °C for 2 min. To the reaction mixture, 20 μl of 5 vol.% TFA in water was added and 1 μl of the reaction mixture was then injected into the HPLC. The two-dimensional HPLC system consisted of a degasser, two pumps, an injector, a column oven, two fluorescence detectors, two integrators and a column selection unit. The analytical column for the reversed-phase separation was a Mightysil RP-18 GP (100 mm × 1.0 mm I.D., Kanto Chemical, Tokyo, Japan) maintained at 40 °C. The mobile phase was MeCN–THF–TFA–water (10:1:0.02:89, v/v) and the flow rate was 50 μl/min. The enantioselective column was a Sumichiral OA-2500S (250 mm × 4.6 mm I.D., Sumika Chemical Analysis Service, Osaka, Japan) maintained at 40 °C. The mobile phase was 5 mM citric acid in MeOH and the flow rate was 0.8 ml/min. Fluorescence detection of the NBD-amino acids was carried out at 530 nm with excitation at 470 nm.

2.5. HPLC determination of D-Asp and D-Ser

D-Asp and D-Ser in the anterior pituitary gland were determined according to our previous report [31] with slight modifications. The MeOH supernatants obtained in Section 2.3 (50 μl) were evaporated to dryness, and 20 μl of 400 mM sodium-borate buffer (pH 9.0) and 5 μl of a mixed solution of OPA and Boc-L-Cys in MeOH was added. The reaction mixture was stored at room temperature for 2 min, and 5 μl of the solution was then injected into the reversed-phase HPLC system. The analytical column was a TSK-gel ODS-80Ts QA (250 mm × 4.6 mm I.D., Tosoh, Tokyo, Japan) maintained at 40 °C. The mobile phase A was 9 vol.% MeCN in 0.1 M sodium-acetate buffer (pH 6.0), and mobile phase B was 16 vol.% MeCN in 0.1 M sodium-acetate buffer (pH 6.0). The linear gradient from A to B was carried out from 0 to 35 min, followed by the continuous flow of mobile phase B. The flow rate was 1.4 ml/min, and the fluorescence detection was carried out at 443 nm with excitation at 344 nm.

2.6. Determination of melatonin, corticosterone and insulin

Melatonin in the pineal gland was determined using our precolumn oxidation fluorescence HPLC system [37]. Briefly, the pineal gland was homogenized in 400 μl of MeOH on ice, and centrifuged at 4500 × *g* for 5 min. The obtained supernatant (50 μl) was dried, and 40 μl of water, 5 μl of 2 M aqueous Na_2CO_3 and 5 μl of aqueous 1.5 M H_2O_2 were added to the residue. The mixed solution was heated at 100 °C for 30 min, and 1 μl of this solution was injected into the reversed-phase HPLC system. The analytical column was a Capcell pak C18 MG S3 (75 mm × 1.0 mm I.D., Shiseido, Tokyo, Japan) maintained at 40 °C. The mobile phase was MeCN–TFA–water (4:0.01:96, v/v) and the flow rate was 100 μl/min. The fluorescence detection was carried out at 380 nm with excitation at 245 nm. Corticosterone and insulin were determined using commercially available immunoassay kits (Biotrak RPA 548, Amersham Life Science, Tokyo, Japan for corticosterone, and Rat Insulin ELISA KIT, AKRIN-010T, Shibayagi, Gunma, Japan for insulin).

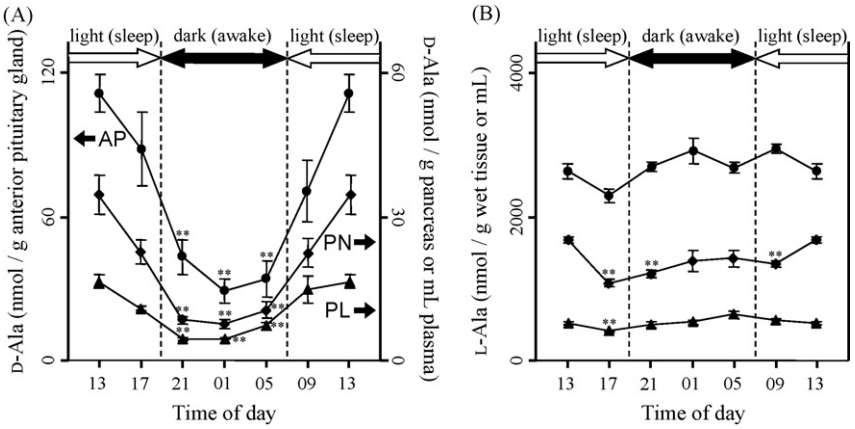

Fig. 1. Circadian changes of (A) D- and (B) L-Ala amounts in the anterior pituitary gland (AP, closed circles), pancreas (PN, closed diamonds) and plasma (PL, closed triangles). The values represent means ± SE of three animals, and the asterisks indicate a significant decrease ($p < 0.01$) from the values at 13:00.

3. Results and discussion

3.1. Circadian changes of D-Ala amounts in the tissues and plasma of rats

The circadian changes of D-Ala in the anterior pituitary gland, pancreas and plasma have been investigated. The tissues and plasma were obtained from three rats every 4 h (started at 13:00), and the amounts of D- and L-Ala were determined. In both of the tissues and in the plasma, the amounts of D-Ala have clear circadian rhythms, showing higher amounts in the daytime than those in the nighttime (Fig. 1). The amounts of D-Ala during the nighttime (21:00, 01:00, 05:00) are significantly lower than those at 13:00 ($p < 0.01$). It was noted that for all the time periods, the amount of D-Ala is the highest in the anterior pituitary gland, followed by the pancreas, and the plasma. In contrast, the amounts of L-Ala did not show clear circadian changes. Typical chromatograms of the plasma D- and L-Ala (as NBD-derivatives) obtained during the daytime and nighttime are shown in Fig. 2. In the present investigation, we determined a small amount of D-Ala using our sensitive and selective two-dimensional analytical method. The calibration range of the present method is 5–5000 fmol ($r = 1.000$), and within-day and day-to-day precisions of spiked D-Ala to biological samples are 3.9 and 4.8% (RSD), respectively. The lower limit of quantification (LOQ) of the present method is 5 fmol (injection amount), which is sensitive enough to measure D-Ala in all the time periods (the lowest amount of D-Ala in the nighttime plasma is 3.2 nmol/ml (μM); 32 fmol/injection to HPLC). The circadian change of D-Ala in the anterior pituitary gland has already been demonstrated in our previous work [33]; the reported amount was about 30 nmol/g during the nighttime and about 100 nmol/g during the daytime. The values obtained in the present study (28.9–111.3 nmol/g) are in good agreement with those in the previous investigation. In the present report, we indicated that the amounts of D-Ala in the pancreas and in the plasma also show similar circadian changes, and the amounts were the highest in the anterior pituitary gland in all the time periods. The daytime D-Ala amounts in the anterior pituitary gland and in the pancreas were reported to be 7.4 and 2.5 times higher than that in the plasma, respectively [33], which were also in good agreement with the values shown in the present report. These results strongly suggest that the presence of some unspecified mechanisms generating the D-Ala rhythm in the entire body of the rats, co-operating with some D-Ala transport system between the tissues and blood flow, will be clarified in the future.

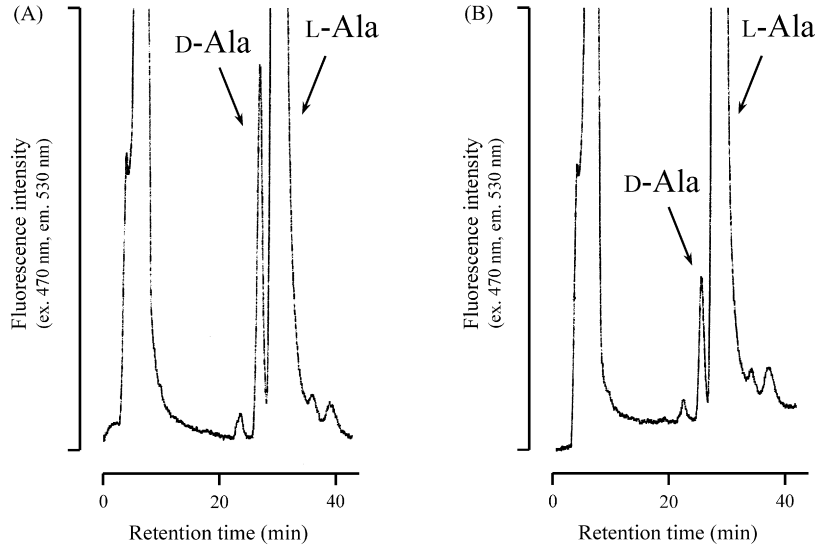

Fig. 2. Chromatograms of NBD-D- and L-Ala in the plasma obtained during the (A) diurnal and (B) nocturnal periods.

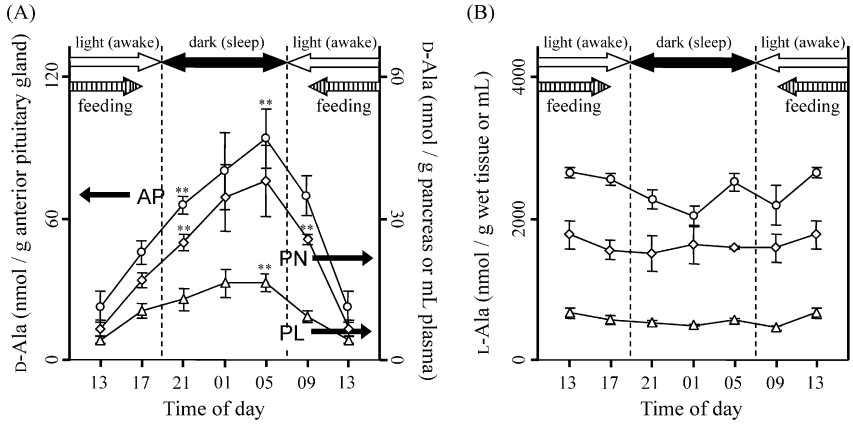

Fig. 3. Circadian changes of (A) D- and (B) L-Ala amounts in the anterior pituitary gland (AP, open circles), pancreas (PN, open diamonds) and plasma (PL, open triangles) under restricted feeding conditions. The values represent means ± SE of three animals, and the asterisks indicate a significant increase ($p < 0.01$) from the values at 13:00.

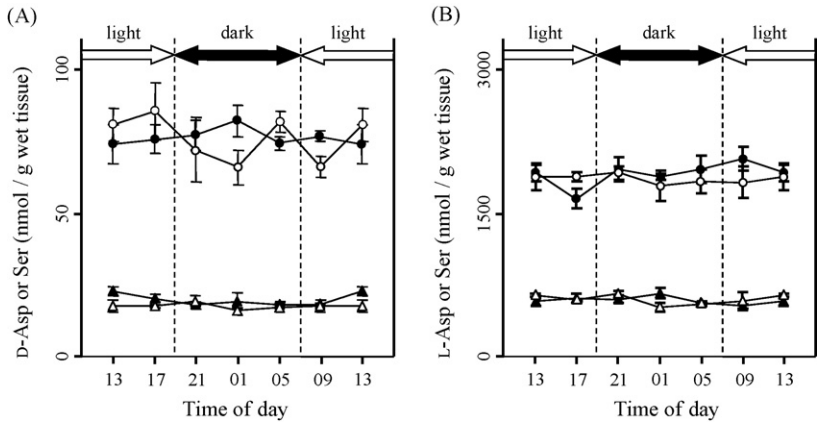

Fig. 4. (A and B) Circadian changes of D,L-Asp (circles) and D,L-Ser (triangles) in the rat anterior pituitary gland. Closed symbols indicate the values for the normal rats and open symbols indicate those of the food-restricted rats. The values represent means ± SE of three animals.

3.2. Circadian changes of D-Ala amounts in tissues and plasma of rats grown under restricted feeding conditions

In order to delineate the mechanism controlling the circadian D-Ala rhythm, we investigated the amounts of D-Ala in the rats with diurnal habits, showing an activity rhythm different from that of the wild type animals. These rats with diurnal habits were made by restricted feeding for 2 weeks (had access to food only during the daytime, 09:00–17:00), and the reversal of their habits were checked by observation of their activity. These rats were inclined to sleep during the nighttime and to be awake during the daytime. The amounts of D-Ala in the anterior pituitary gland, pancreas and plasma are shown in Fig. 3. Similar to the results shown in Fig. 1, the amounts of D-Ala were highest in the anterior pituitary gland for all the time periods, followed by the pancreas and the plasma, while the circadian D-Ala profiles were clearly different from those in Fig. 1. In both the tissues and in the plasma, higher amounts of D-Ala were observed during the nighttime, versus those during the daytime. Compared to the values at 13:00, the amounts of D-Ala during the nighttime (21:00, 01:00, 05:00) are significantly higher (p values of all points are lower than 0.031, only the points of the p values lower than 0.01 are shown by the asterisks in Fig. 3). Under the present experimental conditions, the environmental light–dark conditions remained usual, and only the activity rhythms of the animals were changed by the restricted feeding. The present results strongly suggest that the D-Ala rhythm is closely related to physiological activities of the animals.

3.3. Circadian changes of D-Asp and D-Ser amounts in the rat anterior pituitary gland

The rat pituitary gland has also been reported to contain D-Asp and D-Ser [19]. Therefore, we investigated the circadian profiles of the D-Asp and D-Ser amounts as well as those of their L-enantiomers in rats with both the nocturnal and diurnal habits. As shown in Fig. 4, the amounts of both D- and L-enantiomers of Asp and Ser do not have clear circadian changes and the profiles are quite similar in both rat groups. The typical chromatogram is shown in Fig. 5. Until

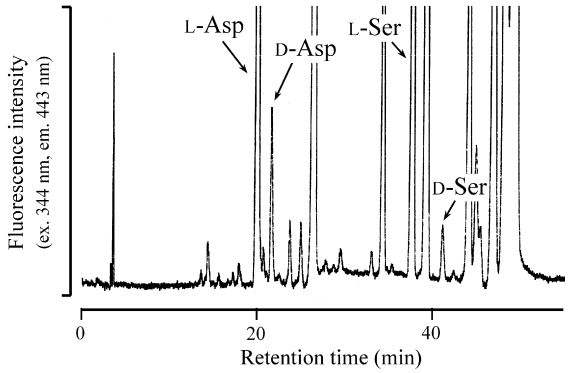

Fig. 5. Chromatogram of OPA derivatives for D,L-Asp and D,L-Ser in rat anterior pituitary gland. HPLC conditions are described in the text.

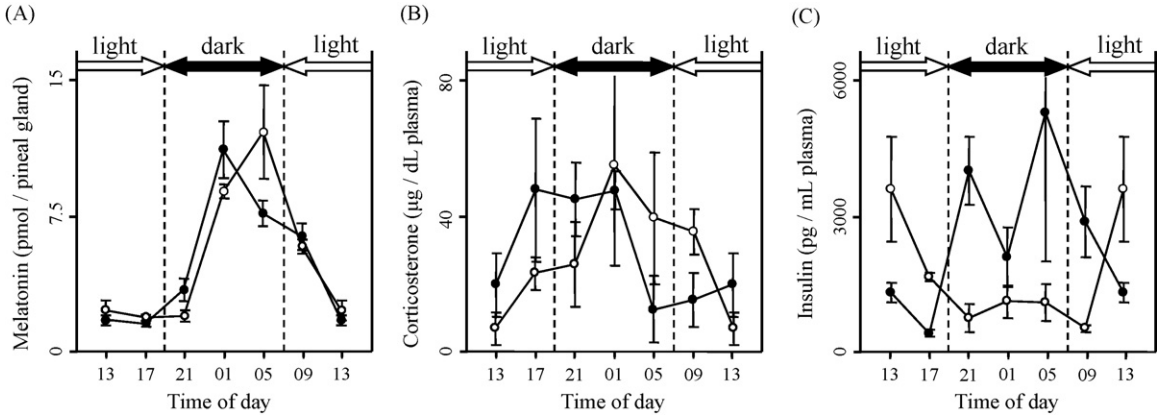

Fig. 6. Circadian changes of (A) melatonin, (B) corticosterone and (C) insulin. Closed symbols indicate the values for the normal rats and open symbols indicate those of the food-restricted rats. The values represent means ± SE of three animals.

now, the amounts of D-Asp and D-Ser in the rat pituitary gland was reported to be 81–190 and 12–24 nmol/g, respectively [19,38–41], and the present values are consistent with those reports. Concerning the circadian changes, the amount of D-Asp in the rat pituitary gland was reported to have a circadian change showing a higher amount during the nighttime in the wild type rats with the nocturnal habits [41]. However, in the present investigation, both D-Asp and D-Ser do not show any circadian changes; this inconsistency might due to the difference in the environmental conditions or ages of the animals, which should be addressed in the future. Concerning the circadian changes of D-Ala in the anterior pituitary gland, we carried out 3 individual trials (rats of 6 weeks of age [33], 9 weeks of age [33] and the present investigation), and clear circadian changes were observed with no exception. In addition, the circadian profile was obviously reversed by the restricted feeding in the present study. These results clearly indicate that D-Ala is regulated differently in mammalian tissues compared to D-Asp and D-Ser.

3.4. Circadian changes of melatonin, corticosterone and insulin amounts in the rats

As the typical hormones having clear circadian changes, melatonin, corticosterone and insulin in the same rats were also determined and the effects of restricted feeding were investigated. As shown in Fig. 6, all of these hormones show clear circadian rhythms. Under the normal conditions of nocturnal habits, high levels of melatonin and insulin was observed during the nighttime, and high levels of corticosterone was observed in the evening. Under the food restricted conditions (diurnal habit), only slight changes were observed in the circadian profiles of melatonin and corticosterone, while insulin showed a totally different and almost reversal circadian rhythm in the food restricted rats. The change in the circadian profile is similar to that of D-Ala, and the amount of insulin in each rat is inversely related to the amount of D-Ala. Considered together that a high amount of D-Ala is present in the pancreas [33] and the evident fact that the cellular localization is similar to that of insulin secreting beta-cells [34], it is quite possible that D-Ala has some functions regarding insulin regulation, and clarification of this function would be strongly recommended.

4. Conclusion

In the present investigation, we determined D-Ala by a sensitive 2D-HPLC system, and revealed that the circadian changes of D-Ala are closely related to the activity rhythms of animals using the rats with a diurnal habit made by restricted feeding. The profile of D-Ala is also related to the plasma insulin level in an inverse manner. These results suggest that D-Ala might have some physiological function in connection with the activity rhythm and insulin rhythm in mammals, e.g., regulation of the blood glucose level, and a detailed study of its function is highly recommended.

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References

[1] J.J. Corrigan, Science 164 (1969) 142.
[2] N. Fujii, T. Saito, Chem. Rec. 4 (2004) 267.
[3] K. Hamase, A. Morikawa, K. Zaitsu, J. Chromatogr. B 781 (2002) 73.
[4] S.A. Fuchs, R. Berger, L.W.J. Klomp, T.J. de Koning, Mol. Genet. Metab. 85 (2005) 168.
[5] R. Konno, H. Brückner, A. D'Aniello, G. Fisher, N. Fujii, H. Homma (Eds.), D-Amino Acids: A New Frontier in Amino Acids and Protein Research—Practical Methods and Protocols, Nova Science Publishers, New York, NY, 2007.
[6] A. Hashimoto, T. Nishikawa, T. Hayashi, N. Fujii, K. Harada, T. Oka, K. Takahashi, FEBS Lett. 296 (1992) 33.
[7] J.P. Mothet, A.T. Parent, H. Wolosker, R.O. Brady Jr., D.J. Linden, C.D. Ferris, M.A. Rogawski, S.H. Snyder, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 4926.
[8] S.H. Snyder, P.M. Kim, Neurochem. Res. 25 (2000) 553.
[9] A. Hashimoto, T. Oka, Prog. Neurobiol. 52 (1997) 325.
[10] T. Nishikawa, Biol. Pharm. Bull. 28 (2005) 1561.
[11] H. Homma, Viva Origino 30 (2002) 204.
[12] Y. Takigawa, H. Homma, J.-A. Lee, T. Fukushima, T. Santa, T. Iwatsubo, K. Imai, Biochem. Biophys. Res. Commun. 248 (1998) 641.
[13] Y. Nagata, H. Homma, J.-A. Lee, K. Imai, FEBS Lett. 444 (1999) 160.
[14] G. D'Aniello, A. Tolino, A. D'Aniello, F. Errico, G.H. Fisher, M.M.D. Fiore, Endocrinology 141 (2000) 3862.
[15] Y. Nagata, R. Masui, T. Akino, Experientia 48 (1992) 986.
[16] D.W. Armstrong, M.P. Gasper, S.H. Lee, N. Ercal, J. Zukowski, Amino Acids 5 (1993) 299.
[17] A. Dossena, G. Galaverna, R. Corradini, R. Marchelli, J. Chromatogr. A 653 (1993) 229.
[18] H. Brückner, A. Schieber, Biomed. Chromatogr. 15 (2001) 166.
[19] K. Hamase, H. Homma, Y. Takigawa, T. Fukushima, T. Santa, K. Imai, Biochim. Biophys. Acta 1334 (1997) 214.
[20] T. Inoue, K. Hamase, A. Morikawa, K. Zaitsu, J. Chromatogr. B 744 (2000) 213.
[21] H. Zhao, K. Hamase, A. Morikawa, Z. Qiu, K. Zaitsu, J. Chromatogr. B 810 (2004) 245.
[22] K. Hamase, S. Takagi, A. Morikawa, R. Konno, A. Niwa, K. Zaitsu, Anal. Bioanal. Chem. 386 (2006) 705.

[23] K. Hamase, A. Morikawa, T. Ohgusu, W. Lindner, K. Zaitsu, J. Chromatogr. A 1143 (2007) 105.
[24] K. Hamase, Chem. Pharm. Bull. 55 (2007) 503.
[25] P.D. Hoeprich, J. Biol. Chem. 240 (1965) 1654.
[26] G.H. Fisher, A. D'Aniello, A. Vetere, L. Padula, G.P. Cusano, E.H. Man, Brain Res. Bull. 26 (1991) 983.
[27] Y. Nagata, K. Yamamoto, T. Shimojo, R. Konno, Y. Yasumura, T. Akino, Biochim. Biophys. Acta 1115 (1992) 208.
[28] A. Hashimoto, T. Nishikawa, R. Konno, A. Niwa, Y. Yasumura, T. Oka, K. Takahashi, Neurosci. Lett. 152 (1993) 33.
[29] R. Konno, T. Oowada, A. Ozaki, T. Iida, A. Niwa, Y. Yasumura, T. Mizutani, Am. J. Physiol. 265 (1993) G699.
[30] H. Brückner, S. Haasmann, M. Langer, T. Westhauser, R. Wittner, H. Godel, J. Chromatogr. A 666 (1994) 259.
[31] A. Morikawa, K. Hamase, T. Inoue, R. Konno, A. Niwa, K. Zaitsu, J. Chromatogr. B 757 (2001) 119.
[32] A. Morikawa, K. Hamase, T. Inoue, R. Konno, K. Zaitsu, Amino Acids 32 (2007) 13.
[33] A. Morikawa, K. Hamase, K. Zaitsu, Anal. Biochem. 312 (2003) 66.
[34] A. Morikawa, K. Hamase, T. Ohgusu, S. Etoh, H. Tanaka, I. Koshiishi, Y. Shoyama, K. Zaitsu, Biochem. Biophys. Res. Commun. 355 (2007) 872.
[35] F. Halberg, Annu. Rev. Physiol. 31 (1969) 675.
[36] C.M. Lambert, D.R. Weaver, J. Biol. Rhythms 21 (2006) 77.
[37] K. Hamase, J. Hirano, Y. Kosai, T. Tomita, K. Zaitsu, J. Chromatogr. B 811 (2004) 237.
[38] D.S. Dunlop, A. Neidle, D. McHale, D.M. Dunlop, A. Lajtha, Biochem. Biophys. Res. Commun. 141 (1986) 27.
[39] A. Hashimoto, T. Oka, T. Nishikawa, Eur. J. Neurosci. 7 (1995) 1657.
[40] A. D'Aniello, A.D. Cosmo, C.D. Cristo, L. Annunziato, L. Petrucelli, G. Fisher, Life Sci. 59 (1996) 97.
[41] A. D'Aniello, M.M.D. Fiore, G.H. Fisher, A. Milone, A. Seleni, S. D'Aniello, A.F. Perna, D. Ingrosso, FASEB J. 14 (2000) 699.