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Alteration in the D-amino acid content of the rat pineal gland under anesthesia

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Summary. In a previous report (Hamase, K. et al., Biochim Biophys Acta 1134: 214–222 (1997)), we showed that the rat pineal gland contains D-leucine (D-Leu) as well as D-aspartic acid (D-Asp). In this communication we report alterations in the content of these D-amino acids during anesthesia. The D-Asp content was significantly increased from 2.8 to 5.0, 4.8 and 5.8 nmol/pineal gland by administration of ether, urethane and pentobarbital, respectively. In contrast, the D-Leu content was decreased by administration of urethane or pentobarbital. The D-Leu content decreased from 4.2 to 2.2 pmol/pineal gland 4 hours after administration of urethane, although the content remained unchanged until 1.5 hours after administration. The content of the L-enantiomers of these amino acids were not affected by anesthesia. The urethane-induced decrease in D-leucine content was almost completely suppressed by a β-agonist, (−)-isoproterenol, whereas the agonist itself had no effect.

Keywords: Amino acids – D-Aspartic acid – D-Leucine – Pineal gland – Sympathetic nervous system – Anesthesia

Introduction

Many studies have demonstrated the existence of various D-amino acids in mammaliam tissues (Hashimoto and Oka, 1997; Imai et al., 1996), such as D-serine (D-Ser), D-aspartic acid (D-Asp), D-alanine and D-proline etc. It is now recognized that these D-amino acids have some as yet unknown physiological functions. In particular, D-Ser is proposed to be an endogenous ligand for the strychnine-insensitive glycine site of the NMDA receptor in the

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central nervous system (Hashimoto and Oka, 1997; Imai et al., 1996; Schell et al., 1995) and D-Asp is thought to participate in regulating the maturation and differentiation of various tissues during development (Hashimoto and Oka, 1997; Imai et al., 1996; Sakai et al., 1997; D'Aniello et al., 1996) or to be a novel messenger in neuronal and neuroendocrine organs (Schell et al., 1997). In our previous studies (Hamase et al., 1997; Imai et al., 1995), we detected several D-amino acids in various regions of the rat brain, and found D-Asp and D-Leu in the pineal gland. The content of D-Asp in the gland was relatively low at 2 weeks of age, increased significantly at 4 to 10 weeks of age, and then gradually decreased up to 36 weeks of age. On the other hand, the content of D-Leu was relatively high until 10 weeks of age and quickly declined thereafter (Hamase et al., 1997). We also immunolocalized D-Asp within the pineal gland (Lee et al., 1997).

In the course of these investigations, we recognized that the contents of D-Asp and D-Leu in the rat pineal gland were altered by administration of certain anesthetics. In this communication, we report the effects of anesthetics (ether, urethane and pentobarbital) on the contents of D-Asp and D-Leu in the rat pineal gland. Modulation of tissue contents of D-amino acids by administration of certain chemicals would provide a clue for understanding the mechanism which regulates tissue concentrations of D-amino acids.

Materials and methods

Materials

D-and L-amino acids and (−)isoproterenol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, ethyl carbamate (urethane) and α-chloralose were products of Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Diethyl ether and pentobarbital were from Kanto Chemical Co., Inc. (Tokyo, Japan) and Dainippon Seiyaku (Osaka, Japan), respectively. Other reagents and solvents were of analytical reagent grade.

Animals

Male Sprague-Dawley rats (6 weeks of age, specific pathogen-free) were purchased from Charles River Japan Inc. (Kanagawa, Japan). The animals were housed under a 12 h light/12 h dark cycle (lights on at 07:00 h) and had free access to food and water.

Anesthesia and sample preparation

Urethane (100 mg urethane and 2.5 mg α-chloralose/ml, at a dose of 1.5 ml/100 g body weight) and pentobarbital (50 mg pentobarbital/ml, at a dose of 1.5 ml/kg) were administered intraperitoneally, and the rats were sacrificed 4 h later. Various other doses of urethane were also administered in some experiments. Ether was inhaled, and the rats were immediately sacrificed. Control rats (not anesthetized) were decapitated. (−)Isoproterenol (5 mg/kg) was administered intraperitoneally 30 min after the administration of urethane, and 3.5 h later the rats were sacrificed. Rats were sacrificed by exsanguination from the abdominal aorta and the pineal gland was quickly removed and homogenized in 500 μl of methanol. Samples for the determination of amino acid contents were prepared as described in our previous report (Hamase et al., 1997). The contents of

D-Asp and D-Leu were determined by a combination of reverse-phase HPLC and HPLC on a Pirkle-type chiral column, as described previously (Hamase et al., 1997).

Results

During determination of the D-amino acid contents of rat brain regions (Hamase, 1997), we found that the amounts in the pineal gland were altered by the administration of some anesthetic drugs. Table 1 summarizes the contents of D,L-Asp and D,L-Leu in the pineal gland of rats anesthetized with ether, urethane or pentobarbital. The D-Asp content was significantly increased in the rats which received these anesthetics, while the L-Asp concentration was almost the same as the control level. By contrast, the D-Leu content was decreased significantly by administration of urethane. It was also decreased by administration of pentobarbital (not statistically significant), while the L-Leu content was not changed by administration of either anesthetic. Since the D-Leu content of the pineal gland was markedly decreased by urethane anesthesia, time- and dose-dependent changes in the D-Leu content were further investigated. Figure 1A shows the changes in the pineal gland contents of D,L-Leu with time after urethane administration. The D-Leu content of the pineal gland was significantly decreased 4 h after urethane administration and remained constant thereafter, while the content of the L-isomer did not change. Figure 1B shows the effects, 4 h after administration, of various doses of urethane on the pineal contents of D,L-Leu. The D-Leu content was decreased at doses of more than 1.25 ml, whereas the L-Leu content was not changed significantly.

The pineal gland is controlled mainly by the sympathetic nervous system via β-adrenergic receptors (Reiter, 1991). Figure 2 shows the effect of a β-agonist, (−)isoproterenol, on the pineal content of D-Leu. (−)Isoproterenol suppressed the urethane-induced decrease in D-Leu content almost completely, whereas it was not changed by isoproterenol alone.

Table 1. Effect of anesthesia on the D, L-Asp and D, L-Leu contents of the rat pineal gland

	control	ether	urethane	pentobarbital
D-Asp	2.81 ± 0.47 (10)	5.07 ± 0.49 (16)**	4.84 ± 0.59 (8)*	5.82 ± 0.64 (6)**
L-Asp	4.93 ± 0.43 (10)	5.66 ± 0.65 (16)	6.22 ± 0.85 (8)	6.32 ± 0.74 (6)
D-Leu	4.20 ± 0.76 (4)	4.25 ± 0.36 (9)	2.22 ± 0.30 (13)††	2.18 ± 0.67 (4)
L-Leu	551 ± 63 (4)	670 ± 41 (9)	498 ± 56 (13)	888 ± 139 (4)

Control rats were not anesthesized and decapitated. Ether was inhaled to the rats which were immediately sacrificed. Urethane and pentobarbital were administered intraperitoneally and the rats were sacrificed 4 h later. Figures represent means (nmol/pineal gland for Asp and pmol/pineal gland for Leu) ± S.E.M. of 4 to 16 samples. Values in parentheses are the numbers of samples. Control rats were decapitated. Others are as described in Materials and methods.

** $p < 0.01$, * $p < 0.05$, significant increase from value of control; †† $p < 0.01$, significant decrease from value of control.

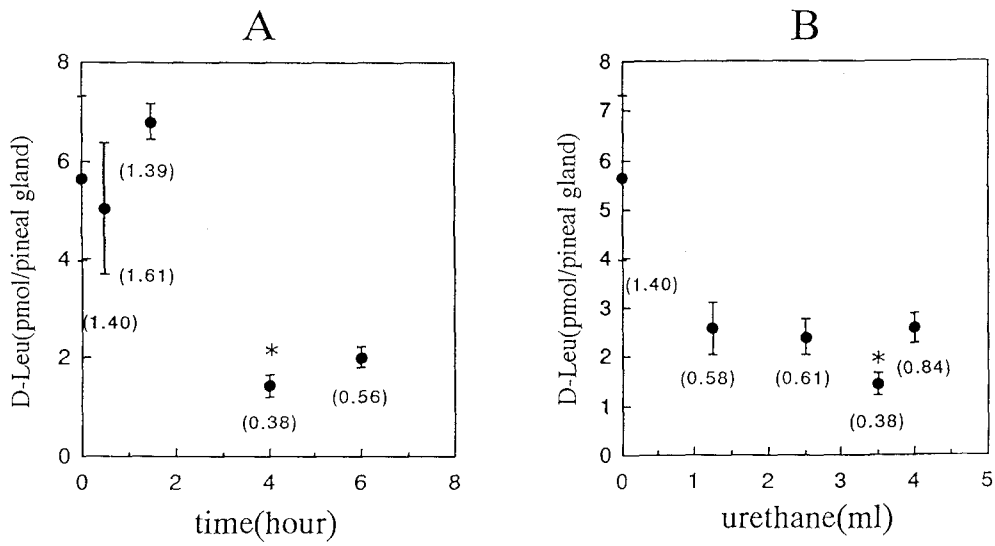

Fig. 1. Time- and dose-dependent changes in the D-Leu content of the rat pineal gland after urethane administration. **A** Urethane was administered intraperitoneally (3.5 ml) and 0.5, 1.5, 4.0, 6.0 h after administration, the pineal glands were removed and their D,L-leucine contents determined. Since ether inhalation does not affect D-Leu content as described in Table 1, ether was inhaled by the control rats (0 h) to minimize pain or discomfort. Details are described in the text. **B** The doses of urethane administered were 1.25, 2.5, 3.5, 4.0 ml, and the pineal glands were removed 4 h after administration. The control rats were etherized and sacrificed as described above. Values represent means ± S.E.M. for 3 samples. Figures in parentheses are D/(L + D) × 100. * $p < 0.05$ vs. control (0 h)

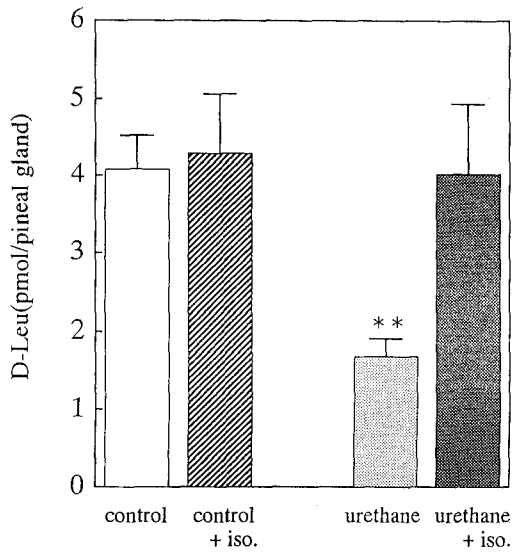

Fig. 2. Effect of (−)isoproterenol on pineal D-Leu content. (−)Isoproterenol was administered (5 mg/kg, i.p.) 30 min after administration of urethane, and the rats were sacrificed 4 h after administration of urethane. The control rats were etherized 3.5 h after administration of saline or isoproterenol, sacrificed and their pineal glands were removed. Since ether does not affect D-Leu content as in Table 1, the control rats inhaled ether to minimize pain and discomfort. Values represent means ± S.E.M. for 5 or 6 samples. ** $p < 0.01$ vs. control (0 h, etherized)

Discussion

In this study, we investigated the effects of anesthesia on the content of D-Asp and D-Leu in the rat pineal gland. The D-Asp content was increased by ether, urethane and pentobarbital anesthesia. Ether increased the content within a few minutes. The D-Leu content was not affected by ether, but was decreased by urethane and pentobarbital, suggesting that the contents of the two D-amino acids are regulated by different mechanisms. The alteration of amino acid levels in the pineal by anesthesia was specific for the D-isomers, since the concentrations of L-amino acids were not changed. In addition, the decrease in D-Leu content caused by urethane was suppressed by a β-agonist, (−)isoproterenol, whereas the agonist itself had no effect. Isoproterenol increased the content of melatonin, a pineal hormone, (from 1.6 ± 0.08 to 7.8 ± 1.0 pmol/pineal gland, n = 6) even under urethane-induced anesthesia. Anesthesia is known to affect sympathetic nervous activity (Matsukawa et al., 1993), which controls the pineal gland via β-adrenergic receptor (Reiter, 1991). However, the mechanism by which these anesthetics affected the pineal contents of these D-amino acids remains unknown.

In the rat pineal gland, D-Asp is present in the cytoplasm of pinealocytes, parenchymal cells in the gland (Lee et al., 1997). The localization of D-Leu within the gland is not known. Details of the origin of D-amino acids in the mammalian body is unclear. *De novo* synthesis of D-amino acids (e.g. D-Ser) has recently been suggested to occur in mammals (Dunlop and Neidle, 1997; Takahashi et al., 1997; Iwama et al., 1997; Wood et al., 1996) along with the existence of the corresponding synthetic enzymes (Dunlop and Neidle, 1997; Takahashi et al., 1997; Iwama et al., 1997; Wood et al., 1996). D-Asp and D-Ser administered intraperitoneally or intravenously, become distributed in various organs including the pineal gland (D'Aniello et al., 1996; Takahashi et al., 1997; Imai et al., 1997). D-Amino acids are not synthesized in primary cultures of rat pinealocytes (Takigawa et al., 1998), suggesting that they originate outside the pineal gland. D-Asp is extensively taken up into cultured rat pinealocytes (Takigawa et al., 1998) as suggested in a recent report (Yatsushiro et al., 1997), while uptake of D-Leu has not yet been studied.

Catabolic enzymes specific for D-amino acids such as D-amino acid oxidase and D-Asp oxidase have been reported (Konno and Yasumura, 1992; Simonic et al., 1997; Setoyama and Miura, 1997). The rat pineal gland is reported to have almost none of these activities (Schell et al., 1997; Horiike et al., 1994). Anesthesia might induce D-amino acid oxidase and repress D-Asp oxidase, which would result in a decrease in the D-Leu content and an increase in the D-Asp content. However, our preliminary experiments failed to demonstrate any induction of D-amino acid oxidase by urethane (data not shown).

Alteration in tissue levels of D-amino acids by drug administration could provide a clue for understanding the mechanism which controls tissue concentration of the amino acids. Clarification of the mechanism should provide valuable insights into the biological roles of D-amino acids in mammals.

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