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ORIGINAL CONTRIBUTION

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Production of L-tryptophan-derived catabolites in hepatocytes from streptozotocin-induced diabetic rats

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Abstract *Background* Recently the L-tryptophan (Trp) metabolites such as L-kynurenine(Kyn), L-kinurenic acid, quinolinic acid (QA) and picolinic acid (PA) have been shown physiologically important in central nervous and immune system, and various enzyme activities concerning their production were reported to be affected by insulin-dependent diabetes mellitus. However, the states of these metabolites in diabetes have not been clarified enough yet. *Aim of study* The present study was performed to make clear the states of the productions of L-Kyn, QA, PA and nicotinamide (Nam) in vitro in the hepatocytes prepared from streptozotocin (STZ)-induced diabetic rats using [5-^{3}H]L-Trp. *Methods* The diabetic model rats were made by STZ injection (60 mg/kg) and the hepatocytes isolated from the rats were incubated with [5-^{3}H]L-Trp. The amounts of metabolites derived from L-Trp were determined by the isotope-dilution methods. *Results* The α-amino-β-carboxymuconate-ε-semiarldehyde decarboxylase (ACMSD) mRNA level in the diabetic group was greatly higher than that in the control group. In the STZ-induced diabetes group, the amount of [5-^{3}H]L-Trp converted to tritiated water, L-Kyn or QA were found to be more than 3 times of that in the control group, respectively. The produced amounts of PA and Nam were not significantly different between the diabetic and the control groups. *Conclusions* It is suggested that STZ-diabetes mellitus causes augmentations of both L-Kyn and QA generations but not those of PA and Nam in liver, indicating the possibility that the immune and neuronal systems of insulin dependent diabetes mellitus would be influenced by the increased amounts of LKyn and QA but not by those of PA and Nam.

Key words L-Tryptophan – L-Kynurenine – quinolinic acid – diabetes – hepatocytes

Introduction

L-Tryptophan (LTrp) can be metabolized to several kinds of physiologically active substances such as L-kynurenine (Kyn), L-kinurenic acid, quinolinic acid (QA) and picolinic acid (PA) in addition to nicotinic acid derivatives and serotonine, and the production of these metabolites was thought to be affected by the enzyme activities on the metabolic pathway of L-Trp

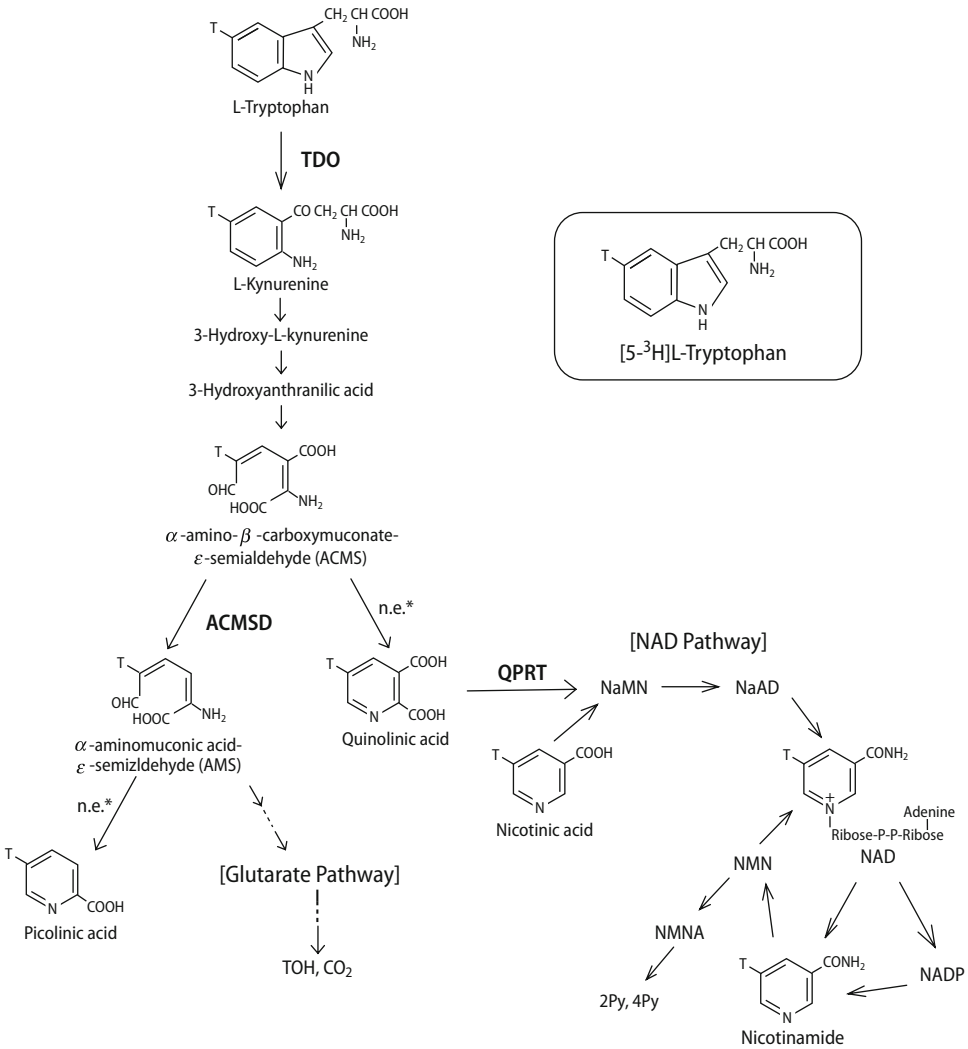

Fig. 1 A metabolic pathway of [5-^{3}H]L-Trp in hepatocytes. *TDO* tryptophan 2,3-dioxygenase, *ACMSD* α-amino-β-carboxymuconate-ε-semiardehyde decarboxylase, *QPRT* quinolinic acid phosphoribosyltransferase, *NaMN* nicotinic acid mononucleotide, *NaAD* nicotinic acid adenine dinucleotide, *NAD* nicotinamide adenine dinucleotide, *NADP* nicotinamide adenine dinucleotide phosphate, *NMN* nicotinamide mononucleotide, *NMNA* N^1-methylnicotinamide, *2-Py* N^1-methyl-2-pyridone-5-carboxamide, *4-Py* N^1-methyl-4-pyridone-3-carboxamide, *NE* nonenzymatic reaction

(Fig. 1). In the insulin dependent diabetic mellitus rats, hepatic activity of α-amino-β-carboxymuconate-ε-semiardehyde decarboxylase (ACMSD) [EC 4.1.1.45] has been shown to be extremely higher than that of normal rats [30], and the amounts of urinary excreted niacin metabolites per L-Trp intake were significantly less than those in the normal rats when they were fed with niacin-free L-Trp containing diet [4]. However, the alteration of the productions of physiologically active L-Trp metabolites in diabetic patients or animals has not been investigated enough up to now. QA has been reported to act as agonist of *N*-methyl-D-aspartate-senstive glutamate receptors and causes the induction of neurotoxicity [18, 24], and this substance was considered to be involved in the pathogenesis of neurodegenerative diseases and convulsive disorders, e.g. AIDS Dementia Complex [9]. On the contrary, it has been considered that L-kynurenic acid play a neuroprotective role as an antagonist of excitatory amino acid receptor [21] and that L-Kyn was also suggested to protect global brain ischaemia after its transport across blood-brain barrier and conversion to kynurenic acid [20]. On the other hand, L-Kyn, QA and PA have been reported to affect the immune system [1, 13, 17]. Moreover, QA was suggested to suppress gluconeogenesis by inhibiting phosphoenolpyruvate carboxykinase [11, 34]. Since insulin dependent diabetes was generally known to be frequently associated with diabetic neuropathy and infectious diseases, it is considered meaningful to examine the production of these biologically active L-Trp metabolites in the diabetes for the elucidation of its pathophysiology. Although the activity of inducible indoleamine 2,3-dioxygenase (IDO) in various tissues including macrophage and dendritic cells is considered to be important for the production of physiologically active L-Trp metabolites which play some roles in immune or nervous systems, the activity of tryptophan 2,3-dioxygenase (TDO) [EC 1.13.11.11] which is a constitutive enzyme existing exclusively in

hepatocytes is thought to catabolize L-Trp in an ordinary life. Besides, ACMSD which has been shown to exist only in kidney, liver and brain catalyzes the decarboxylation of α-amino-β-carboxymuconate-ε-semialdehyde (ACMS), a direct precursor of QA, to α-aminomuconate-ε-semialdehyde (AMS), which enters into TCA cycle via glutarate pathway. Since, hepatic TDO and ACMSD activities have been reported to be influenced by hormones [23] and nutrients [3, 22], and also insulin dependent diabetic mellitus was shown to enhance hepatic but not renal ACMSD activity significantly, it would be interesting to examine the alteration of the production of biologically active L-Trp metabolites especially in the liver from insulin dependent diabetic animals. In the present study, we examined the production of the physiologically active L-Trp metabolites such as L-Kyn, QA, PA and nicotinamide (Nam) from [5-^{3}H]L-Trp in vitro in the hepatocytes prepared from streptozotocin (STZ)-induced diabetic rats and compared with that in normal hepatocytes.

Materials and methods

Chemicals

Reagents were obtained as follows: L-[5-^{3}H]Trp (specific radioactivity 1.15 MBq/nmol) from Moravek Biochemicals Inc. (CA, USA); Dowex 50 W × 8 (200–400 mesh) and Dowex 1 × 2 (200–400 mesh) ion exchange resin from Muromachi Kagaku Kogyo (Japan); activated charcoal from Wako Pure Chemical (Osaka, Japan); cellulose thin-layer plate for chromatography from MERCK (Darmstadt, Germany).

Animals

Male Sprague-Dawley rats (7-week-old) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The rats were housed in an air-conditioned room at 22 ± 1°C with 12 h light and dark cycles. The animals were given a standard diet (AIN93G) and water ad libitum. Diabetes was induced in these rats by intraperitoneal injection of STZ (60 mg/kg B.W.) which was dissolved in 0.05 M citric buffer (pH 4.5, 10 mg/ml) and sterilized. The normal group was injected with the same volume of saline as that of STZ solution used for STZ group. After 13 days, their serum glucose levels were measured using Glucose CII test Wako kit (Wako, Japan), and the STZ-treated rats with more than 400 mg/l of serum glucose were used as the diabetic group and the saline injected rats with normal serum glucose levels as the control group. The hepatocytes were prepared from three diabetic and three normal rats by collagenase perfusion method [31]. The care and treatment of the rats were in accordance with the guidelines in "The Guide for the Care and Use of Laboratory Animals" that was prescribed by the Faculty of Horticulture, Chiba University (Chiba, Japan).

Quantitation of mRNA of the enzymes involved in kynurenine pathway with real-time PCR

Total RNA was isolated using SV total RNA Isolation System (Promega Corp., Madison, WI, USA). One microgram of total RNA was reverse transcripted in a final volume of 10 μl by using first-strand cDNA Synthesis Kit for RT-PCR (AMV; Roch Diagnostics Corp., IN, USA). For real-time PCR, RT solution containing 10 ng cDNA and SYBR Premix Ex Taq II (TaKaRa BIO Inc., Japan) were used as follows: one cycle of 95°C for 10 s, 40 cycles of 5 s at 95°C and 31 s at 60°C, one cycle of 15 s at 95°C, 1 min at 60°C and 15 s at 95°C. The sequences of primers were as follows (forward, reverse): TDO (5′-TCC AGG TCC CTT TCC AGT TG-3′, 5′-GCC TAG CAT CCT GTG CAC CAT-3′), ACMSD (5′-AAC AGCA AGG CAA GGG AGA A-3′, 5′-ACA TGA CGG GAA CTG TGG AAA-3′), quinolinic acid phosphoribosyltransferase (5′-AGA ATG GAT TAC TGG CTT GTT G-3′, 5′-GTG GGA AGC CTA GGT AAA TTT CAG-3′)[19], and GAPDH (5′-GCC AAG TAT GAT GAC ATC AAG AAG-3′, 5′-AGC CCA GGA TGC CCT TTA GT-3′) [26]. Each primers were used at 0.4 μM (final conc.). The real-time PCR was performed with ABI PRISM 7500 system (Applied Biosystems, CA, USA). Calibration curve was made by cDNA with serial dilution as every parameter and demanded mRNA level. The obtained data were normalized by respective GAPDH mRNA levels.

Preparation and incubation of rat hepatocytes

The hepatocyte incubation was performed basically according to the methods described by Smith et al. [28]. The cells prepared from each rat were suspended in phosphate-buffered saline (PBS) supplemented with 5 mM glucose and 0.1 mM L-Trp (containing 35 kBq/ml [5-^{3}H]L-Trp) which mimics the blood level of normal rat. Final cell concentration in the cell suspension (total 15 ml) was approximately 0.05 g wet wt./ml in flask gassed with 95% O_2/5% CO_2 (v/v). Incubation was carried out at 37°C for 1 h with shaking at 80 rpm. After incubation, the cell suspension was immediately chilled on ice and centrifuged at 900×*g*, 1 min. The supernatant was considered as "extracellular fluid" and were stored at −20°C until use for the analysis for Trp metabolites as described

below. On the other hand, the precipitate was washed twice with 30 ml of cold PBS without glucose, and homogenized with 8 volumes of 80% methanol containing 0.075 N ammonia. The supernatant after centrifugation was considered as "intracellular extract" and was stored at −20°C until use for analysis. The corresponding unincubated samples were also prepared as the same manner for each hepatocyte group.

Determination of tritiated water generated from [5-^{3}H]L-Trp

As [5-^{3}H]L-Trp was metabolized to CO_2 and tritiated water (TOH) via glutarate pathway in hepatocytes, 0.5 ml of extracellular fluid or 0.2 ml of intracellular extract were passed through both Dowex 50 W × 8 (H^+ form; 17 × 12 mm) and Dowex 1 × 2 (HO^- form; 17 × 12 mm) columns and then washed the columns with 0.7 ml of water, and the TOH in the effluent containing washing water was determined with BECKMAN LS 6500 scintillator system (Beckman Coulter, Inc, CA, USA). The corresponding unincubated samples for individual hepatocyte groups were treated as the same way. The amounts of water generated from L-Trp (A_w) were calculated as described below and expressed as katal (mol s^{-1}) per g wet weight of hepatocytes.

$$A_w = (C_{1ew} - C_{0ew}) \times (C_{Trp})^{-1} \times (0.05)^{-1} + (C_{1iw} - C_{0iw}) \times (C_{Trp}) \times (0.125)^{-1} \times (3600)^{-1}$$

C_{1ew} and C_{0ew}: the radio activities of water in external fluids after 1 or 0 h incubation, respectively (dpm/ml external fluid), 0.05: weight of cells in 1 ml of external fluid (g/ml), C_{1iw} and C_{0iw}: the radio activities of water in internal fluids after 1 or 0 h incubation, respectively (dpm/ml internal fluid), 0.125: weight of cell in 1 ml of internal fluid (g/ml), C_{Trp}: the specific radio activity of L-Trp in the medium (21 dpm/pmol), 3,600: conversion from an hour to a second.

Determination of the amount of L-Trp metabolites

The amount of metabolites derived from L-Trp was determined by the isotope-dilution methods. Generally, the extracellular fluid or intracellular extract was added with fixed amount of relevant non-radioactive metabolite and purified the metabolite to obtain almost constant specific radioactivity. Then the amount of the metabolite produced during 1 h of incubation in the original cell suspension was calculated.

(1) l-*Kyn*. Extracellular fluid 2 ml or intracellular extract 0.5 ml were mixed with 4.0 ml of non-radioactive L-Kyn solution (20 mM) and passed through a Dowex 1 × 2 column ($HCOO^-$ form; 17 × 12 mm). The effluent containing washing water, that included L-Kyn together with other contaminants such as Trp and Nam, was then passed through a Dowex 1 × 2 column (HO^- form; 17 × 12 mm) to eliminate contaminants. The adsorbed L-Kyn and L-Trp were eluted with 0.25 N formic acid. L-Kyn in this eluate was adsorbed further on a column of activated charcoal (17 × 30 mm). After washing with water, L-Kyn was eluted with 50% ethanol including 10% ammonia water solution (containing 10% ammonia). Since, the eluate contained L-Kyn together with small amount of L-Trp as contaminants, L-Kyn was further purified by thin layer chromatography (TLC) after lyophilization as described below.

(2) *QA*. Extracellular fluid 2 ml or intracellular extract 0.5 ml were mixed with 4.0 ml of non-radioactive QA solution (20 mM) and passed through a Dowex 50 W × 8 column (H^+ form; 17 × 12 mm). The column was washed with water. The effluent containing QA together with glucose and organic acids, were then passed through an activated charcoal column (17 × 30 mm). After washing with water, QA was eluted with 50% ethanol containing 10% ammonia. QA was further purified repeatedly by TLC after lyophilization.

(3) *PA*. Extracellular fluid 2 ml or intracellular extract 0.5 ml were mixed with 4.0 ml of non-radioactive PA solution (20 mM) and passed through a Dowex 50 W × 8 column (H^+ form; 17 × 25 mm). After the column was washed with water, PA was eluted with 30 ml of 1 N ammonia. The eluate was freeze-dried and re-dissolved in 100 ml of water to pass through a Dowex 1 × 2 column ($HCOO^-$ form; 17 × 25 mm). The column was washed with water and then PA was eluted with 0.01 N formic acid. The eluate was then applied to a column of activated-charcoal (17 × 30 mm). After washing with water, PA was eluted with 50% ethanol containing 10% ammonia. The eluent was lyophilized for further purification by TLC.

(4) *Nam*. Extracellular fluid 2 ml or intracellular extract 0.5 ml were mixed with 4.0 ml of non-radioactive QA solution (20 mM) and passed through a Dowex 1 × 2 column (HO^- form; 17 × 12 mm). The column was then washed with 40 ml of distilled water. The effluent and washing were collected and lyophilized for TLC.

(5) *Further purification of metabolites from* l-*Trp by TLC and quantitative determination of generated metabolites*. Each freeze-dried fractions were

dissolved in water, and further purified using cellulose TLC with 20% KCl. The target spots of metabolites from L-Trp were detected by UV light (254 nm) and extracted with water. This purification process was repeated to obtain constant specific radioactivity (dpm/mmol), in that the concentrations of these metabolites were calculated from the molecular extinction coefficients of L-Kyn (257.2 nm, 7,410), QA (275.6 nm, 4,120), PA (265.2 nm, 7,040) and Nam (262.6 nm, 2,945), respectively. When the specific activity of a metabolite after last purification was almost the same as that immediately before the last purification, the specific activity after last purification was referred to as a constant specific activity. Radioactivity was determined with BECKMAN LS 6500 scintillator system. The specific activity of a metabolite generated from L-Trp for 1 h of incubation (C_m) and the amount of each metabolite derived from L-Trp (A_m) was calculated as follows:

$$C_m = C_{1m} - C_{0m}(\mathrm{dpm/pmol})$$

Where C_{1m} and C_{0m} are the constant specific activities after 1 or 0 h of incubation.

$$A_m = C_m \times M_{add}(C_{Trp})^{-1} W_c^{-1} \times (3600)^{-1}(\mathrm{pkat\,g\,cells^{-1}})$$

M_{add}: The amount of relevant non-radioactive metabolite (80×10^6 (pmol per 2 ml) for extracellular fluid and 80×10^6 (pmol per 0.5 ml))
C_{Trp}: The specific radio activity of L-Trp in the medium (21 dpm/pmol)
W_c: The weight of hepatocytes in 1 ml of sample solution (0.10 (g/2 ml) for extracellular fluid and 0.0556 (g/0.5 ml) for intracellular fluid), 3,600: conversion from an hour to a second

Statistical analysis

Data shows mean ± SE. Data were analyzed by Student's unpaired *t* test between normal and STZ-diabetic groups. Differences with $P < 0.05$ were considered significant.

Results

The serum glucose levels of STZ-induced diabetic and control rats

The serum glucose levels of the experimental animals used for the present experiment were 5.36 ± 0.48 and 27.50 ± 2.22 (mmol/l) in control and diabetic groups, respectively, and significant difference was observed between them ($P < 0.001$).

Effect of STZ-induced diabetes on the mRNA expression of the enzymes in L-Trp metabolism

The mRNA levels of the three enzymes, TDO, ACMSD and quinolinic acid phosphoribosyltransferase, involved in L-Trp metabolism were shown in Fig. 2. TDO mRNA level in the diabetic rats was tended to be higher than that in the control rats but significant difference was not observed between these groups. The ACMSD mRNA level in the diabetic group was approximately 13-fold higher than that in the control group, while quinolinic acid phosphoribosyltransferase mRNA level in diabetic group was approximately

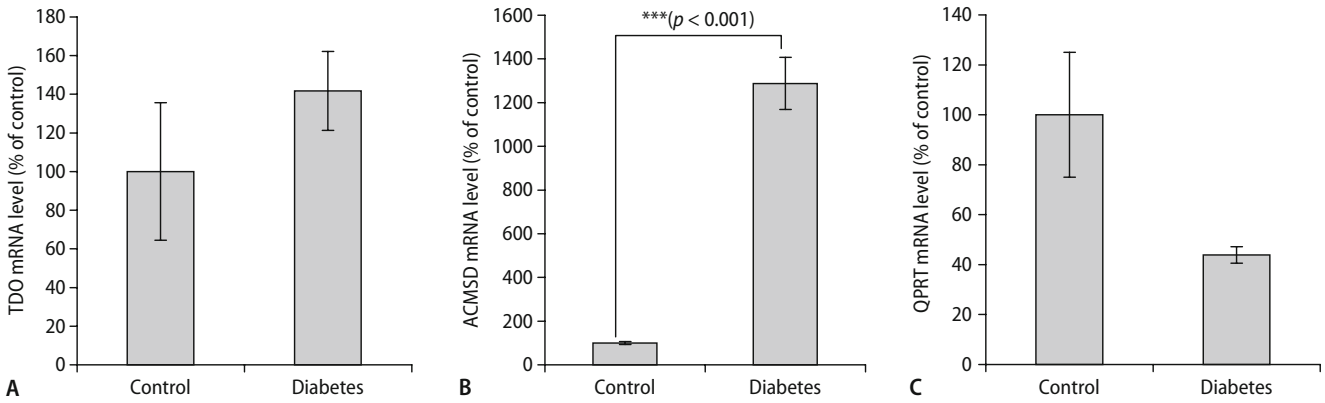

Fig. 2 The mRNA levels of TDO, ACMSD and QPRT in hepatocytes. Total RNA was isolated from hepatocytes prepared by collagenase perfusion method and analyzed for the levels of mRNA for TDO (**a**), ACMSD (**b**) and QPRT; quinolinic acid phosphoribosyltransferase (**c**) by realtime-PCR as described in the text. Values are mean ± SE ($n = 3$) expressed as the relative value to the mean of the respective control value. ***$P < 0.001$, significantly different from control values by Student's *t* test

1/2-fold of that in the control group but this difference was not significant.

The amounts of water (TOH) generated from [5-^{3}H]L-Trp in hepatocytes

The amounts of TOH produced from [5-^{3}H]L-Trp during 1 h of incubation at 37°C was 72.43 ± 14.68 and 217.53 ± 23.16 (pkat g cells^{-1}) in control group and diabetes group, respectively. There was a significant difference between them ($P < 0.01$). These values are the sum of those in extracellular fluid and intracellular extract. In the STZ-induced diabetic group, the amount of [5-^{3}H]L-Trp converted to TOH was found to be more than 3 times of that in the control group.

The amounts of Trp metabolites generated from [5-^{3}H]L-Trp in hepatocytes

The amounts of L-Kyn, QA, PA and Nam derived from [5-^{3}H]L-Trp during 1 h of incubation were shown in Table 1. The amounts of L-Kyn produced and released in extracellular media in diabetic hepatocytes were approximately threefold higher than that in the control. However, those amounts in the intracellular extracts in both groups were negligible. The amount of QA in extracellular fluid in the STZ-treated group was also approximately threefold higher than that in the control. On the contrary, that in the intracellular extract of the STZ-treated group was approximately 1/3 of that in the control, although there was no significant difference between these groups. PA was produced only small amounts in intracellular extract of both groups but was negligible in the extracellular medium of both groups. The amounts of Nam produced from [5-^{3}H]L-Trp were also revealed to be small and negligible in the extracellular solution in the STZ-treated group.

Discussion

This investigation performed as a part of the study to decide whether the biologically active L-Trp metabolites would be involved in the pathophysiological state of insulin dependent diabetes, and the generations of biologically active L-Trp metabolites in the hepatocytes from the STZ-treated rats were compared with those in the cells from the control rats. Although L-Trp can be metabolized by various pathways other than kynurenine pathway such as serotonin, tryptamine or indole synthesis pathways, the major catabolism of L-Trp in hepatocytes is considered to be conducted via kynurenine pathway [27]. Therefore, enzymatic release of ^{3}H from [5-^{3}H]L-Trp by other pathways was thought to be negligible. However, as the slight release of ^{3}H from substrate and the exchange of hydrogen atoms in metabolites for tritium in water should occur spontaneously while keeping, the radioactivity of these ^{3}H was subtracted from the values for individual samples as described in Methods.

The threefold elevation of the amount of L-Trp converted to water in diabetic hepatocytes was considered to be caused by the increased amount of L-Trp metabolized through kynurenine-pathway followed by glutarate-pathway that is initiated by TDO reaction. The present results for TOH production in diabetic hepatocytes were partially consistent with the previous report, in that the activity of TDO was threefold greater and the TOH production from L-Trp was sevenfold higher in diabetic hepatocytes than those in the control [29]. In the present study, the TDO mRNA level in diabetic hepatocytes tended to be higher than that in the control group, implying the elevation of TDO activity. The inconsistency between present results and the report might be caused by the difference in the condition of the experiments such as food and circadian rhythm of the rats [32].

The level of QA in the extracellular fluid was unexpectedly higher in STZ-diabetic group than in

Table 1 Quantity of metabolites generated from the L-[5-^{3}H]Trp during incubation (pkat·g cells^{-1})

	Extracellular fluid		Intracellular extract	
	Control	Diabetes	Control	Diabetes
[^{3}H]L-Kyn	9.76 ± 1.48 (13.5%)	26.46 ± 7.15(12.2%)	ND	ND
[^{3}H]QA	29.03 ± 0.58 (40.1%)	80.01 ± 8.15*** (36.8%)	4.77 ± 0.36 (6.6%)	1.63 ± 1.11 (0.75%)
[^{3}H]PA	ND	ND	0.19 ± 0.01 (0.26%)	0.18 ± 0.02 (0.08%)
[^{3}H]Nam	0.67 ± 0.28 (0.93%)	ND	0.19 ± 0.13 (0.26%)	0.13 ± 0.01 (0.06%)

Values are mean ± SE, $n = 3$. The values were calculated as described in the text. The value in the parenthesis is the ratios (%) against the value of the respective TOH generated from L-Trp shown in the Results
ND not detected (<0.03 pkat·g cells^{-1})
*Significantly different from control value (***$P < 0.001$)

control group (Table 1), in spite of the 13-fold high level of ACMSD mRNA in diabetic group. On the other hand, the value in the cellular extract from the diabetic group was tended to be lower than that in the control extract. The hepatic ACMSD activity has been reported to increase in STZ-induced diabetic rats [10, 16] as the manner positively correlated with the ACMSD mRNA levels [30]. Thus, the ACMSD activity of the diabetic hepatocytes in the present experiment was supposed to be considerably high. As an increase in ACMSD activity was expected to reduce the non-enzymatic conversion of aminocarboxymuconate (ACMS), a metabolite of L-Trp, to QA, the ratio of QA against that of water derived from L-Trp could be speculated to be low in diabetic group. However, the ratios were almost the same between diabetic and control groups, and the QA level in diabetic group was approximately 3 times of that in the control (Table 1). According to the report of Smith et al. [29], generation of QA in diabetic hepatocytes was varied by the L-Trp level in the medium. So, the concentration of ACMS in the cells might affect the results, although the true reason is unclear at present. In the present experiment, because the quinolinic acid phosphoribosyltransferase mRNA expression tended to be low in the diabetic hepatocytes, there is a possibility that the QA generated in diabetic hepatocytes would not convert easily to nicotinate mononucleotide and further metabolites in NAD pathway as compared with that in control group. Moreover, because the concentration of intracellular QA tended to be low in diabetic group, its release into the extracellular fluid could be greater than that in control group. Besides, it is supposed that the intracellular low QA level in diabetic hepatocytes would be physiologically important, because QA was shown to inhibit gluconeogenesis [14, 15]. The generated amount of L-Kyn in the extracellular fluid tended to be high level in diabetic group like QA, but its ratios against water derived from L-Trp in both groups were the almost same. However, the levels of intracellular L-Kyn were less than detectable lower limit in both groups, suggesting that substantial amount of L-Kyn was released immediately without accumulation in hepatocytes. The small amounts of PA derived from L-Trp were detected only in the intracellular extracts of diabetic and control hepatocytes but not in extracellular fluid. So, PA produced in hepatocytes might not be released easily into the extracellular fluid. The amounts of Nam derived from L-Trp were as low as those of PA and was not detectable in the extracellular fluid of diabetic hepatocytes.

Since there is a report that plasma tryptophan level in the diabetic rats is approximately 30% lower than that in normal rats in spite of the large food intake in the former [12], the balance between supply of L-Trp and its catabolism should be declined toward latter even under normal food. Therefore, the amounts of L-Trp metabolites produced in diabetic rats in vivo might be greater according to the increase of the L-Trp supply from their diets, although it could not be simply estimated from the present results.

The results of the present experiment indicated that the concentration of L-Kyn and QA derived from L-Trp in the extracellular fluid were higher in diabetic hepatocytes than those in the control hepatocytes. It was reported that L-Kyn suppressed the production of Interferon-γ in natural killer T cells [17] and the multiplication of CD4(+)T cells, CD8(+)T cells or natural killer cells [6]. Besides, the quinolinic acid was shown to repress the production of interleukin-10 [13] and cause the apoptosis in Th1 cells [5]. Therefore, the elevated production of these metabolites in hepatocytes in the insulin dependent diabetic mellitus was deduced to modulate their immune system. On the other hand, it is demonstrated that L-Kyn in blood is able to pass the blood-brain barrier [7] and converts to kynurenic acid, which could moderate overstimulation by QA or glutamic acid the agonists of N-methyl-D-aspartate receptor in nerve cells. Therefore, blood L-Kyn level would possibly affect central nervous system. While QA in blood was reported to be taken up into the brain in the diabetes [8], but not in normal animal, and cause a nervous overstimulation. Although Nam was shown to inhibit poly(ADP-ribose)polymerase activity [25] and also affect proinflammatory cytokines in vitro [33], its production in hepatocytes was found to be negligible in relation to immunological point of view. PA was also shown to affect the function of macrophage in vitro [2], but the effect of its production in hepatocytes could be also negligible as a modulator of immune system.

In conclusion, the increased production of both L-Kyn and QA, in the hepatocytes of STZ-induced diabetic rats suggests the possibility that these bioactive L-Trp metabolites, but not PA and Nam, would affect the pathophysiological state of insulin dependent diabetes mellitus, especially in immune and neuronal systems. This study was focused on the L-Trp metabolism in hepatocytes of diabetic rats. However, further in vivo examination is necessary to elucidate the physiological role of L-Trp metabolites in diabetes.

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