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L-tryptophan binding to hepatic nuclei: age and species differences*

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Summary. The present experiments report differences in in vitro nuclear binding affinity for L-tryptophan 1) between livers of young ($6^1/_2$ weeks old) and older (30 weeks old) $NZBWF_1$ mice, but not so in similar aged Swiss mice, and also, 2) in livers of hamsters compared to livers of guinea pigs. In vitro hepatic nuclear specific binding affinity after tube-feeding L-tryptophan (5–20 mg/100 g body weight) to mice 1 h before killing revealed less in young than in older $NZWBF_1$ mice, comparable to the above in vitro assay studies. In vitro nuclear binding affinity for L-tryptophan of livers of hamsters was significantly less than that of livers of guinea pigs or Swiss mice. In general, the degree of stimulatory effect on hepatic protein synthesis, as measured by in vitro [^{14}C]leucine incorporation into protein using microsomes of animals tube-fed L-tryptophan 1 h before killing compared to that of animals tube-fed water, correlated with the basal nuclear specific binding affinity to L-tryptophan of the animals (ages and species) used.

Keywords: Amino acids – L-tryptophan – Hepatic nuclear binding – Age differences – Mice – Hamsters – Guinea pigs

Introduction

In an earlier study (Sidransky and Verney, 1996b), we reported mouse strain differences in the L-tryptophan binding affinity by hepatic nuclei; $NZBWF_1$ mice had a significantly diminished in vitro binding affinity compared to that of other mouse strains (Swiss, DBA, SJLF/J and BALB/C), which had similar binding affinities to that of rats (Sprague-Dawley (Kurl et al., 1987) and Lewis (Sidransky and Verney, 1994)). Earlier we have reported that a tryptophan receptor exists in rat hepatic nuclear envelopes, and that this receptor pro-

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tein has high specificity for L-tryptophan and the binding in saturable, stereospecific, and of high affinity (Kurl et al., 1987, 1988).

In the present report we present data which describes differences in hepatic nuclear binding affinity for L-tryptophan between young and older $NZBWF_1$ mice and between hamsters and guinea pigs. These age-related and species-related alterations, in addition to the earlier reported mouse strain differences (Sidransky and Verney, 1996b) in regard to hepatic nuclear binding affinity for L-tryptophan, suggest that hepatic biochemical and biological responses to the ingestion of L-tryptophan may differ depending on the kind and type of animal selected for study.

Materials and methods

Animals

Male mice of the Swiss strain (Hilltop Lab Animals, Inc., Scottsdale, PA) and of the $NZBWF_1$/J (NZB/BINJ male x NZW/LacJ female) strain (Jackson Laboratory, Bar Harbor, ME), were used in the experiments. Young mice were 6.5 weeks (average) (5–10 weeks range) old and older mice were 30 weeks (average) (29–32 weeks range) old. Male golden syrian hamsters (3.5 months old) were obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN and male guinea pigs (1–2 months old) were obtained from Hilltop Lab Animals, Scottsdale, PA.

The animals were fed Purina laboratory chow (no. 5001; Purina, St. Louis, MO) and maintained in a temperature-controlled room with a 12:12-h light-dark cycle. Before the experiments were begun, the animals were adapted to their quarters for at least 1 wk and then were deprived of food overnight but had free access to water. Animals were killed by decapitation. The protocol for these studies was reviewed and approved by the institutional animal care and use committee.

Chemicals

The [^{3}H]tryptophan used in the experiments was L-[5-^{3}H]tryptophan, 1.15 TBq/mmol, and L-[U-^{14}C]leucine, 12.9 GBq/mmol, obtained from Amersham/Searle (Arlington Heights, IL). L-tryptophan was obtained from US Biochemical (Cleveland, OH).

Preparation and isolation of nuclei

Immediately after the animals were killed, the livers were removed and placed on ice until homogenization was begun (within 15 min). Purified hepatic nuclei were prepared as described by Blobel and Potter (1966).

Binding of [^{3}H]tryptophan to nuclei

Hepatic nuclei of the animals prepared as described above were incubated with L-[5-^{3}H]tryptophan (containing 278 KBq and 0.245 nmol of L-tryptophan per assay) (added last) in the absence or presence of a 2,000-fold excess of unlabeled L-tryptophan (10^{-4} mol/L) at room temperature for 2 h. This concentration was selected based on our earlier findings (Kurl et al., 1968). The nuclei were then washed three times with buffer to remove free and loosely bound radioactivity. After the final wash, the nuclei were suspended in buffer and then radioactivity was measured after adding a scintillation mixture (Opti Fluor, Packard Instrument, Downers Grove, IL). Specific binding of [^{3}H]tryptophan to hepatic nuclei was expressed as cpm per mg nuclear protein (total

binding in absence of unlabeled L-tryptophan minus binding in presence of 2,000-fold excess of unlabeled L-tryptophan).

In vitro protein synthesis

Microsomes prepared from postmitochondrial supernatants of livers of control and experimental animals were used for studies on incorporation in vitro as described earlier (Sidransky et al., 1968). In all assays, cytosols of livers of control (distilled water-treated) animals were used. L-[U-^{14}C]leucine, 18.5 KBq, was added to each incubation tube. Radioactivity in protein was measured using a liquid-scintillation spectrometer (Beckman Instruments, Palo Alto, CA). The protein was determined as described by Lowry et al. (1951).

Statistics

Data were analyzed by Student's t test (Snedecor and Cochran, 1980).

Results

Age-related differences in binding affinity

Young (6.5 weeks old) and older (30 weeks old) male $NZBWF_1$ and Swiss mice were used to study the binding affinities (total and specific) of hepatic nuclei for [^{3}H]tryptophan as measured in vitro. The results are summarized in Table 1. It is apparent that young and older $NZBWF_1$ mice had diminished binding affinities of hepatic nuclei for L-tryptophan compared to those of comparable young and older Swiss mice. However, there appeared to be differences in young and older $NZBWF_1$ mice which revealed that young $NZBWF_1$ mice had a significantly diminished binding affinity compared with that of older $NZBWF_1$ mice. Such a difference was not apparent in young and older Swiss mice.

To determine whether the data obtained in in vitro binding experiments (Table 1) may be applicable to in vivo conditions, we conducted the following experiments. Groups of young and older mice of the Swiss and $NZBWF_1$

Table 1. Comparison of total and specific ^{3}H-tryptophan binding in vitro to hepatic nuclei of young and older Swiss and $NZBWF_1$ mice[a]

Strain and age of mice	Total binding		Specific binding			
	cpm/mg protein	Change (%)	cpm/mg protein	Change (%)	%	Change (%)
Swiss-older	(4) 19,331 ± 970[b]		(4) 13,252 ± 489		(4) 68.7 ± 1.19	
$NZBWF_1$-older	(5) 17,786 ± 1,094	−8.0	(5) 5,998 ± 991[c]	−54.7	(5) 34.8 ± 3.75[c]	−49.3
Swiss-young	(4) 20,514 ± 1,061		(4) 14,148 ± 636		(4) 69.1 ± 1.70	
$NZBWF_1$-young	(7) 10,602 ± 1,286[c,d]	−48.3	(7) 3,001 ± 210[c,e]	−78.8	(7) 29.9 ± 3.13[c]	−56.7

[a] Young mice ($6^1/_2$ weeks old); older mice (30 weeks old). [b] Mean + SEM, number of experiments in parentheses. [c] $P < 0.01$, compared with Swiss strain group. [d] $P < 0.01$, compared with $NZBWF_1$-older group. [e] $0.05 > P > 0.01$, compared with $NZBWF_1$-older group.

strains in two experiments were tube-fed L-tryptophan (5 mg/100 g body weight) 1 h before killing. Hepatic nuclei of each group were prepared and then incubated in vitro with water or with unlabeled L-tryptophan (10^{-4} M) in the presence of ^{3}[H]tryptophan (regular in vitro binding assay). Specific binding for each group was determined from total binding minus non-specific binding. The differences in specific binding in each group of mice between those tube-fed water and those tube-fed L-tryptophan were then assumed to represent in vivo specific binding due to the tube-fed L-tryptophan. Thus, the calculated in vivo specific binding mean values (cpm/mg nuclear protein) were as follows: Swiss, older, 7,546; $NZBWF_1$, older, 3,257 (−56.8%); Swiss, young, 9,437; and $NZBWF_1$, young, 1,029 (−89.1%). These findings are similar to the differences for specific binding found in the in vitro binding experiments where the difference for older mice was −54.7% and for young mice −78.8% (Table 1). These results indicate that the in vitro binding data using hepatic nuclei of older and young Swiss and $NZBWF_1$ mice are representative of what occurs in vivo when L-tryptophan enters the liver through the portal circulation as determined by indirect measurements.

The next series of experiments were concerned with the effects of tube-feeding L-tryptophan (in one experiment 5 mg/100 g body weight and in 3 experiments 20 mg/100 g body weight) for 1 h before killing on in vitro protein synthesis as measured by [^{14}C]leucine incorporation into protein using hepatic microsomes of control and experimental mice of both strains. The results are summarized in Table 2. The results obtained after tube-feeding 5 mg/100 g body weight and 20 mg/100 g body weight L-tryptophan were similar and therefore were combined in Table 2. It is apparent that the effect of administration of L-tryptophan on in vitro [^{14}C]leucine incorporation into protein using microsomes induced a significant increase in older and young Swiss mice but not in older and young $NZBWF_1$ mice. However, the older and young $NZBWF_1$ mice appeared to have essentially little or no increases.

Table 2. In vitro ^{14}C-leucine incorporation into hepatic protein of older and young Swiss and $NZBWF_1$ mice tube-fed water or L-tryptophan

Group	Water	L-Tryptophan treatment[a]	^{14}C-leucine incorporation into hepatic protein %
Swiss – Older	+	−	100
	−	+	171.4 ± 8.6[b,c]
$NZBWF_1$ – Older	+	−	92.7 ± 16.1
	−	+	105.5 ± 18.3
Swiss – Young	+	−	107.5 ± 5.3
	−	+	173.8 ± 7.0[d]
$NZBWF_1$ – Young	+	−	104.0 ± 26.4
	−	+	120.4 ± 30.2

[a] Rats (2 per group) were tube-fed water or L-tryptophan (5–20 mg/100 g body weight) 1 h before killing. [b] Mean ± SEM of 4 experiments. [c] $P < 0.01$, compared with Swiss-older water group. [d] $P < 0.01$, compared with Swiss-young water group.

Species differences in binding affinity

Table 3 summarizes the total and specific binding of [^{3}H]tryptophan to hepatic nuclei in vitro of guinea pigs and hamsters, along with older Swiss and NZBWF$_1$ mice conducted as controls. It is apparent that a species difference exists between guinea pigs (which react similar to Swiss mice) and hamsters (which react similar to NZBWF$_1$ mice) in hepatic nuclear binding affinity to L-tryptophan.

In two experiments we investigated the effects of tube-feeding L-tryptophan (5 mg/100 g body weight) or water to hamsters (overnight fasted) 1 h before killing. Hepatic nuclei of each group were assayed for [^{3}H]tryptophan binding after incubation in the presence or absence of unlabeled L-tryptophan (10^{-4} M). Total and specific binding mean values (cpm/mg protein) respectively, were as follows: control (water tube-fed group, 20,380 and 7,723 and L-tryptophan tube-fed group, 12,520 (−38.6%) and 4.669 (−39.5%). Also, the effect of tube-feeding L-tryptophan on hepatic protein synthesis as measured by in vitro [^{14}C]leucine incorporation into protein by hepatic microsomes revealed an average 24.7% increase in the L-tryptophan over that in the control (water) group.

Discussion

Since mice of the NZBWF$_1$ strain routinely used as a model of systemic lupus erythematous (Hirose et al., 1994; Watanabe et al., 1994) were reported to have a diminished affinity for hepatic specific binding for [^{3}H]2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in comparison to control (C57B/6) mice (Kurl, personal communication), we investigated this strain for [^{3}H]tryptophan binding to hepatic nuclei. Indeed, NZBWF$_1$ mice had a markedly decreased affinity for L-tryptophan binding to hepatic nuclei in vitro compared with other strains (Sidransky and Verney, 1996b). Therefore, when we learned that guinea pigs were TCDD-susceptible and hamsters were TCDD-resistant in relation to TCDD lethality (Unkila et al., 1995), we decided to test these species for specific [^{3}H]tryptophan binding to hepatic nuclei in vitro. The results indicated that the binding affinity for L-tryptophan to hepatic nuclei of guinea pigs were similar to that reported earlier for rats (Kurl et al., 1988) and most strains of mice (Sidransky and Verney, 1996b). However, the binding affinity was markedly diminished in hamsters, similar to that reported earlier for NZBWF$_1$ mice (Sidransky and Verney, 1996b).

Our earlier study with different strains of mice (Sidransky and Verney, 1996b) as well as the present study with guinea pigs and hamsters demonstrated altered binding affinity for L-tryptophan by hepatic nuclei of certain strains or species. This altered nuclear specific binding affinity seems to correlate with responses (binding or lethal toxicity) to TCDD by certain strains of mice (NZBWF$_1$) (Kurl, personal communication) or by certain species (guinea pigs (Schwetz et al., 1973) vs hamsters (Olson et al., 1980)). Although Unkila et al. (1995) described that guinea pigs (TCDD susceptible) did not reveal changes in plasma free and total tryptophan levels after low doses of

TCDD and that hamster (TCDD resistant) showed increased levels after high doses of TCDD, they could not conclude that changes in tryptophan metabolism explained the wide interspecies differences in toxic response to acute lethality of TCDD. However, the findings by Unkila et al. (1994) using Long-Evans (TCDD-susceptible) and Han/Wistar (TCDD-resistant) rats were different in that plasma tryptophan levels were increased in Long-Evans but not in Han/Wistar rats in response to the lethality due to TCDD. Our current data regarding hepatic nuclear specific binding of L-tryptophan raise the possibility that a correlation between binding affinity with L-tryptophan and TCDD effects may exist. However, preliminary findings with in vitro [^{3}H]tryptophan binding assays have failed to reveal that the presence of TCDD in vitro competes with L-tryptophan binding to rat hepatic nuclei. Whether L-tryptophan may compete with TCDD or metabolite binding to its receptor is as yet not known.

In view of our earlier studies (Sidransky, 1985), we have concluded that L-tryptophan, unlike other single, indispensable amino acids, has unique biological and biochemical actions in the liver. Our recent studies dealing with the specific nuclear binding of L-tryptophan indicate that a variety of factors, strain of animals (Sidransky and Verney, 1996b), age of animals (Table 1), and species of animals (Table 3), can influence the basal specific binding affinity. In general, animals which have a decreased binding affinity of hepatic nuclei for L-tryptophan show little or no stimulatory response (hepatic protein synthesis) to the administration of L-tryptophan. The overall consequences of the above cited variations need further clarification. Evidence that the specific binding affinity of hepatic nuclei for L-tryptophan is associated with or related to L-tryptophan's stimulatory effect on hepatic protein synthesis comes from earlier studies. We have demonstrated that the compounds, such as DL-β-(1-naphthyl) alanine and L-alanine, which compete for nuclear tryptophan receptor binding but alone do not affect hepatic protein synthesis, can when administered together with L-tryptophan act to negate L-tryptophan's stimulatory effect on hepatic protein synthesis (Sidransky et al., 1990, 1992b; Sidransky and Verney, 1996a).

In earlier studies (Sidransky et al., 1992a; 1994), we reported that L-tryptophan implicated in the eosinophilia-myalgia syndrome or a contaminant, 3-phenylamino-L-alanine (PAA), affected (diminished) hepatic nuclear

Table 3. Comparison of total and specific ^{3}H-tryptophan binding in vitro to hepatic nuclei of guinea pigs and hamsters

Species	Total binding	Specific binding	
	cpm/mg protein	cpm/mg protein	%
Guinea pig	(2) 18,702 ± 489[a]	10,859 ± 702	58.1 ± 2.2
Hamster	(4) 20,773 ± 635	8,007 ± 282[b]	38.7 ± 1.9[c]
Mouse – Swiss	(3) 20,744 ± 1,355	13,795 ± 590	66.6 ± 1.5
Mouse – $NZBWF_1$	(3) 20,008 ± 1,098	6,406 ± 414[c]	32.1 ± 2.0[c]

[a] Number of experiments in parentheses. Mean ± SEM. [b] $0.05 > P > 0.01$. [c] $P < 0.01$.

specific tryptophan binding affinity in rats. Whether this biologic effect may in any way be implicated in the pathogenesis of the eosinophilia-myalgia syndrome is highly conjectural. Nonetheless, such an effect in a selected animal model where the hepatic nuclear specific tryptophan binding affinity is normally diminished may be effective in inducing pathologic alterations. The present study provides information pertaining to established variations in binding affinities in certain species and strains which may be utilized to obtain vital data which should be helpful in understanding how L-tryptophan may act in health and in disease states.

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