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Expression of diamine oxidase (histaminase) in guinea-pig tissues

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Abstract

The expression of mRNA for diamine oxidase (histaminase) and the enzyme activity in guinea-pig tissues were investigated. Reverse transcription-polymerase chain reaction analysis revealed that the message corresponding to the long form present in humans and rats was expressed abundantly in the small intestine and liver. Small but detectable amounts of diamine oxidase mRNA were observed in the kidney, stomach, cerebellum, thalamus + hypothalamus, and cerebral cortex. Northern blot analysis showed that the message (2.8 kb in size) was observed abundantly in the liver and small intestine and was detectable in the kidney and stomach but not in the brain or lung. In situ hybridization showed that diamine oxidase mRNA was localized throughout the liver and epithelial cells of the small intestine. Diamine oxidase activity was detected at various levels in different tissues of the guinea-pig at the following relative abundance: liver > small intestine > lung, kidney > stomach. Histamine dose-dependently induced the contraction of sections of the guinea-pig small intestine, and the pretreatment of the tissue section with aminoguanidine (100 μM), a diamine oxidase inhibitor, but not with *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea (100 μM), an inhibitor of histamine *N*-methyltransferase, shifted the dose–response curve of histamine-induced contraction to lower concentrations. These results suggest that diamine oxidase has a crucial role in the degradation of histamine in the guinea-pig small intestine and probably in the liver.

Keywords: Diamine oxidase (histaminase); Histamine *N*-methyltransferase; mRNA; Expression pattern; Histaminergic signal transmission; Peripheral tissues

1. Introduction

In the mammalian body, histamine is degraded by two enzymes: diamine oxidase (histaminase; EC 1.4.3.6) and histamine *N*-methyltransferase (EC 2.1.1.8) (Schayer, 1959). Diamine oxidase catalyzes the oxidative deamination of histamine to imidazoleacetaldehyde. Histamine *N*-methyltransferase catalyzes the N^{τ}-methylation of histamine to N^{τ}-methylhistamine. These enzymatic inactivation pathways have been suggested to be important both for the prevention of the uptake of ingested histamine from the gastrointestinal tract and for the termination of histaminergic signal transmission in the mammalian body because there is no evidence that histamine is taken up into cytoplasm from the extracellular space via a high affinity mechanism(s). The tissue distribution of diamine oxidase and histamine *N*-methyltransferase appears to be uneven according to the following findings: (i) the diamine oxidase activity is detected predominantly in porcine kidney (Okuyama and Kobayashi, 1961) but not in rat, mouse, guinea-pig, and rabbit brains (Burkard et al., 1963); (ii) the tissue distribution of histamine *N*-methyltransferase activity is uneven in rats, mice, and guinea-pigs (Takemura et al., 1994); and (iii) the expression of mRNAs for diamine oxidase in rats (Lingueglia et al., 1993; Verity and Fuller, 1994) and for histamine *N*-methyltransferase in guinea-pigs (Kitanaka et al., 2001a) varies among the tissues. Therefore, in certain tissues in which histamine has a crucial role in physiological function(s), histamine appears to be inactivated predominantly by either a diamine oxidase or a histamine *N*-methyltransferase pathway. As far as the guinea-pig brain is concerned, histamine *N*-methyltransferase regulates histamine-stimulated phosphoinositide hydrolysis in the cerebellum; the intracellular signal transduction is not governed by diamine oxidase (Kitanaka et al., 2001b). This finding led us to examine in which tissue a diamine oxidase pathway predominantly regulates histamine metabolism in guinea-pigs. In the present study, we identified the guinea-pig liver and small intestine as the tissues in which diamine oxidase is expressed abundantly by Northern blotting, by in situ hybrid-

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ization and by enzyme activity assay. The effects of inhibitors of diamine oxidase and histamine *N*-methyltransferase on histamine-induced contraction of guinea-pig small intestine were also investigated. We also confirmed that diamine oxidase enzymatic activity was undetectable in guinea-pig brain, and found that its mRNA was undetectable by Northern blotting. Using the highly sensitive polymerase chain reaction (PCR) analysis, we could detect very small amounts of diamine oxidase mRNA in certain brain regions.

2. Materials and methods

2.1. Reagents and other materials

The cloning vector pBluescript II-KS(+) was purchased from Stratagene (La Jolla, CA, USA). Nucleotide modifying enzymes were from Takara Shuzo, (Shiga, Japan). [α-^{32}P]-dCTP (specific activity = 111 TBq/mmol) was from NEN Life Science Products (Boston, MA, USA). Peroxidase (type II, derived from horseradish), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid), 1,4-diaminobutane dihydrochloride (putrescine), aminoguanidine hydrochloride, and bovine serum albumin (fraction V) were from Sigma (St. Louis, MO, USA). *S*-[4-(*N*,*N*-dimethylamino)butyl]isothiourea (also known generally as SKF 91488 [Beaven and Shaff, 1979]) was from Tocris Cookson (Ballwin, MO, USA). BigDye terminators for cycle sequencing were from Perkin Elmer-Applied Biosystems Japan (PE-ABI; Urayasu, Japan). The RNA hybridization membranes (GeneScreen Plus) were from NEN Research Products (Boston, MA, USA). Hydrogen peroxide and potassium permanganate solutions were from Wako (Osaka, Japan). All other reagents used were the highest purity commercially available.

2.2. Cloning of the guinea-pig diamine oxidase cDNA fragment

Male Hartley guinea-pigs (4 weeks old, 300–350 g, Japan SLC, Shizuoka, Japan) were killed by decapitation and the tissues were immediately removed. Total RNAs were isolated from the tissues by the acid guanidinium isothiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987). Aliquots of 10 mμ of total RNA were transcribed into cDNA by the method previously described (Kitanaka et al., 2001a). Aliquots (0.5 μl) of cDNA derived from the liver were applied to the PCR mixture containing: 0.25 U of Ex Taq DNA polymerase, 0.2 mM of dNTPs, 1 × buffer supplied by the manufacturer and a primer set designed for diamine oxidase mRNA [20 pmol each; forward primer: 5′-AAC GGG GTG ATG GAG GCC AAG ATG CA-3′, corresponding to the exon between nucleotides 5369 and 5394 of the human diamine oxidase genomic cDNA (Chassande et al., 1994); reverse primer: 5′-CAG TGT CTG GAA GCT GTT CTT GGT GC-3′, corresponding to the exon between nucleotides 6245 and 6270 of the human diamine oxidase genomic cDNA]. PCR was initiated by incubation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 15 min using a GeneAmp PCR System 9700 (PE-ABI). The single band at about 0.2 kb was cut out from the 1% agarose gel after electrophoresis, and cloned into pBluescript II-KS(+). Seven clones were selected at random to exclude the misincorporation and incomplete elongation during PCR and both strands of the cDNAs were analyzed by the cycle sequencing method using BigDye terminators in an automated DNA sequencer (PE-ABI, model 377XL). Nucleotide sequence analysis was performed with the DNASIS-Mac program (Hitachi Software Engineering, Yokohama, Japan). The PCR product had a partial sequence of guinea-pig diamine oxidase mRNA (see Discussion).

2.3. Analyses of diamine oxidase mRNA expression

We investigated the expression of diamine oxidase mRNA by PCR with a primer set based on the nucleotide sequence of guinea-pig diamine oxidase mRNA (20 pmol each; forward primer: 5′-ATG CCA CAG GCT ATG TCC AT-3; reverse primer: 5′-CTG CCA CGT CCA TGT CGA CA-3′) under the same PCR program described above. For RT-PCR analysis of glyceraldehyde 3-phosphate dehydrogenase as a control, we used a primer set [20 pmol each; forward primer: 5′-GGA ATT CAT AGA CAA GAT GGT GAA GG-3′, corresponding to the region between nucleotides 20 and 40 of the rat glyceraldehyde 3-phosphate dehydrogenase cDNA (GenBank accession no. M17701); reverse primer: 5′-GGA ATT CTT ACT CCT TGG AGG CCA TG-3′, corresponding to the region between nucleotides 1014 and 1035 of the rat glyceraldehyde 3-phosphate dehydrogenase cDNA] under the same PCR program described above. Northern blotting was performed by the method described previously (Kitanaka et al., 1994, 2001a,b) with a 132-bp DNA fragment of guinea-pig diamine oxidase and a 973-bp DNA fragment of the guinea-pig glyceraldehyde 3-phosphate dehydrogenase labeled with [α-^{32}P]dCTP by PCR labeling and by the random priming method, respectively. For in situ hybridization, the tissues were removed quickly and used for frozen sections (5 μm). The cRNA probes (sense and antisense) corresponding to the region cloned above were prepared using a digoxigenin RNA Labeling Kit (Boehringer Mannheim, Mannheim, Germany) as described previously (Kitanaka et al., 2001a,b). For hybridization, tissue sections were incubated at 50 °C overnight in hybridization buffer with the probes (Kitanaka et al., 2001a,b).

2.4. Measurement of diamine oxidase activity

Assay for diamine oxidase was performed by the method of Snyder and Hendley (1968) with slight modifications. Briefly, fresh tissues of the guinea-pigs, mice (ddY strain, about 30 g), and rats (Sprague–Dawley strain, about 200 g) were homogenized in 10 volumes of ice-cold 0.1 M sodium-

potassium phosphate buffer (pH 7.15) with a Teflon/glass homogenizer by 10 up-and-down passages. The homogenates were centrifuged at 12,000 × *g* for 20 min. The supernatants (about 0.5–1.0 mg protein per reaction tube) were incubated in the sodium–potassium phosphate buffer (0.1 M, pH 7.15) containing 13 μg/ml of peroxidase for 10 min with shaking at 37 °C to eliminate endogenous hydrogen peroxide, and then added 0.8 mg/ml of homovanillic acid and 1 mM of putrescine into the reaction tubes for 1-h incubation with shaking at 37 °C. The reaction was stopped by putting the tubes on ice. Under this condition, the reactions were linear up to 90 min in the preliminary experiments. The resultant fluorescence of triplicate samples and one blank for each sample (without putrescine) was determined using a fluorescence spectrophotometer (type F-4500, Hitachi, Japan) at an excitation wavelength of 315 nm and a fluorescence wavelength of 425 nm. The standard hydrogen peroxide solution with the exact molarity calculated by titration with potassium permanganate solution was prepared in all sets of the assays. The protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

2.5. Measurement of histamine-induced contraction of small intestine

Male Hartley guinea-pigs (5–6 weeks old, 350–450 g) were killed by decapitation and the small intestines were immediately removed. The ring sections (15 mm long) were cut from the isolated intestine and mounted in an organ bath filled with 50 ml of Tyrode buffer (mM: NaCl 137, KCl 2.7, $MgCl_2$ 0.5, $CaCl_2$ 1.4, NaH_2PO_4 0.3, $NaHCO_3$ 11.9, D-glucose 5.6) equilibrated with air. The buffer was maintained at 28 °C. The sections of intestine were connected to an isometric Pressure Amp (type PA-011, Gain value = 20 mm Hg/V, Starmedical, Tokyo, Japan) for a continuous recording with a Servo 150 recorder (type SR 6511-1, Graphtec, Kanagawa, Japan). Histamine was added to the organ bath in an accumulative manner to obtain the dose–response curves. For pretreatment of the tissues with inhibitors of histamine-degrading enzymes, aminoguanidine (a diamine oxidase inhibitor [Tamura et al., 1989]) or SKF 91488 (a histamine *N*-methyltransferase inhibitor [Beaven and Shaff, 1979]), each of 100 μM, was applied to the sections of small intestine for 20 min before histamine challenge.

2.6. Experimental guidelines and statistical analysis

All experiments in this study were conducted in strict compliance with institutional and the U.S. National Institutes of Health guidelines, and were approved by the Animal Research Committee of Hyogo College of Medicine.

Results are given as the means ± S.E.M. of *n* observations. The data were statistically evaluated using Student's *t* test. Differences were reported as significant for *P* values less than 0.05.

3. Results

3.1. Expression of diamine oxidase mRNA in the guinea-pig

We examined the expression of diamine oxidase mRNA in the guinea-pig tissues by PCR. As shown in Fig. 1A (first row), diamine oxidase mRNA was detected in the

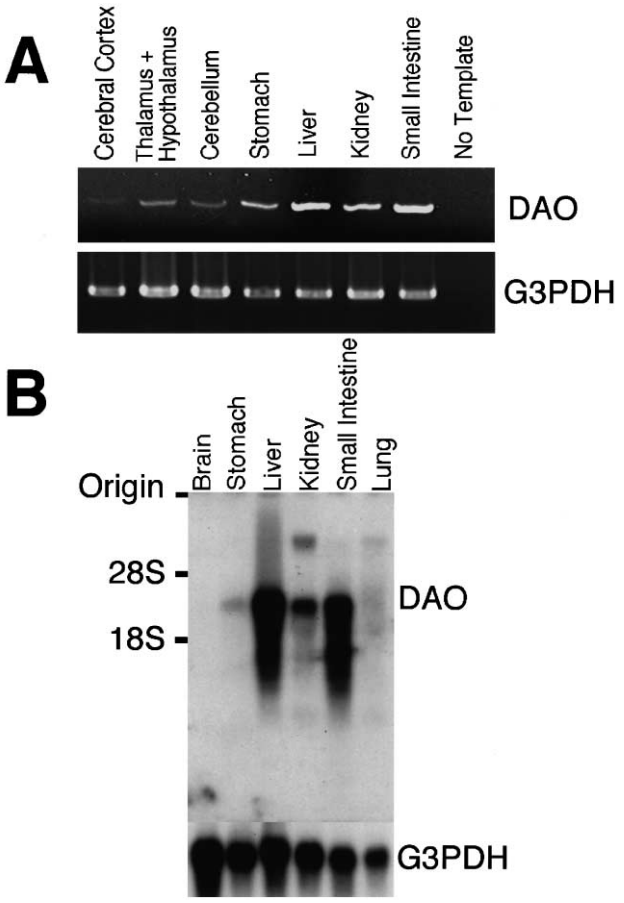

Fig. 1. Diamine oxidase mRNA expression in guinea-pig tissues. (A) RT-PCR analysis. Each cDNA sample, derived from 150 ng of total RNAs, was amplified using the primers described in the text. PCR products of diamine oxidase and glyceraldehyde 3-phosphate dehydrogenase ("G3PDH" in the figure) were loaded into 12% acrylamide gel and 1% agarose gel, respectively, for electrophoresis. DNA fragment of diamine oxidase (132 bp, top) or glyceraldehyde 3-phosphate dehydrogenase (973 bp, bottom; GenBank accession no. AB060340) was ascertained by DNA sequencing. Three independent experiments obtained similar results, and the typical pattern of cDNA fragments is shown. (B) Northern blot analysis. Total RNAs (10 μg/lane) were fractionated by 2.2 M formaldehyde/1.5% agarose gel electrophoresis and transferred onto a nylon membrane with 20 × SSC buffer. The membrane was hybridized with 1×10^5 dpm/ml of a ^{32}P-labeled DNA probe at 42 °C for 17 h. After the hybridization, the membrane was washed with 2 × SSPE/2% SDS buffer at 65 °C for 45 min to reduce the background radioactivity. The membrane was exposed to an X-ray film with an intensifying screen at −80 °C for 1 day (top). Rehybridization of the same membrane with a guinea-pig glyceraldehyde 3-phosphate dehydrogenase probe demonstrated its ubiquitous expression throughout the tissues (bottom). The positions of 28S (4.7 kb) and 18S (1.9 kb) ribosomal RNAs are shown on the left.

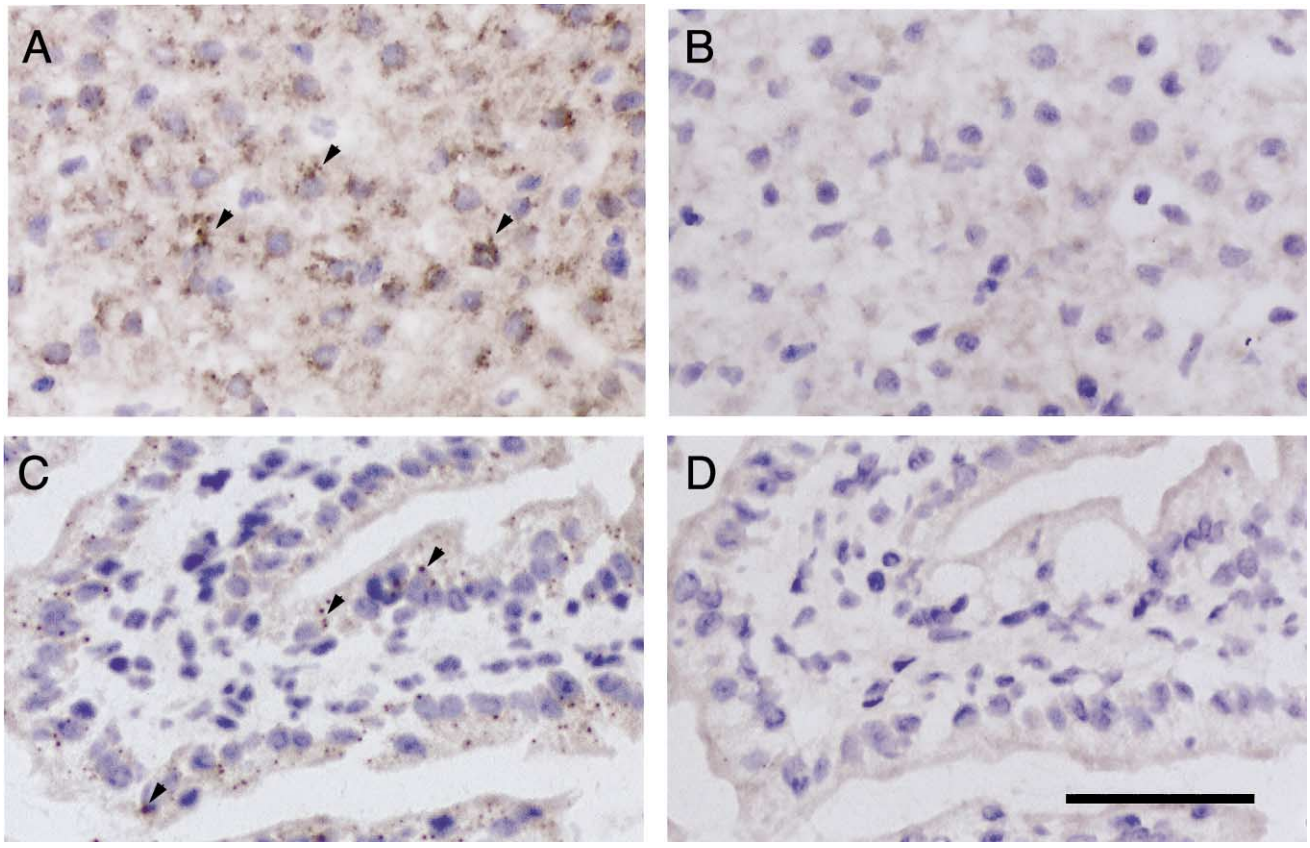

Fig. 2. In situ hybridization analysis of diamine oxidase mRNA expression in the guinea-pig liver (A and B) and small intestine (C and D). The hybridization signal is brown in the picture (A and C), while the blue dots are due to the hematoxylin-eosin staining of cellular nuclei (A–D). Arrowheads (A and C) indicate relatively strong, positive staining. Use of the sense cRNA probe did not show any signal (B and D), compared with antisense (A and C). Bar = 50 μm.

guinea-pig stomach, liver, kidney and small intestine as well as in three brain regions tested (i.e. cerebral cortex, thalamus + hypothalamus and cerebellum), although the relative expression level varied under the same PCR condition (see Materials and methods). Glyceraldehyde 3-phosphate dehydrogenase mRNA was expressed ubiquitously in all samples examined (Fig. 1A, second row). Northern blotting analysis (Fig. 1B) indicated that the 2.8-kb guinea pig diamine oxidase mRNA was expressed at various levels in different tissues at the following relative abundance: liver, small intestine > kidney > stomach. Glyceraldehyde 3-phosphate dehydrogenase mRNA was expressed ubiquitously in all samples (10 μg of total RNA per lane) examined (Fig. 1B).

To visualize the cells expressing diamine oxidase mRNA, we utilized in situ hybridization histochemistry. Diamine oxidase mRNA localized throughout the guinea-pig liver section, mainly in hepatocytes (Fig. 2A, arrow heads). In the guinea-pig small intestine, diamine oxidase mRNA localized mainly in the epithelial cells (Fig. 2C, arrow heads). The use of a sense cRNA probe (negative control) showed no positive signals (Fig. 2B and D) compared with antisense cRNA probe results (Fig. 2A and C).

3.2. Determination of diamine oxidase activity in various tissues of guinea-pigs, mice and rats

In the guinea-pig, diamine oxidase activity was detected at various levels in different tissues at the following relative abundance: liver > small intestine > lung, kidney > stomach (Table 1). In the guinea-pig whole brain, no diamine oxidase activity was detectable. In the mouse, high diamine oxidase

Table 1
Activity of diamine oxidase in various tissues of guinea-pigs, mice and rats

Tissue	Diamine oxidase activity (nmol/mg protein/h)		
	Guinea-pig	Mouse	Rat
Brain	N.D.	N.D.	N.D.
Stomach	0.01 ± 0.01	0.39 ± 0.03	N.D.
Liver	22.73 ± 0.11	0.04 ± 0.04	0.15 ± 0.04
Kidney	0.94 ± 0.02	N.D.	N.D.
Small intestine	10.40 ± 0.11	45.55 ± 0.58	6.79 ± 0.08
Lung	1.39 ± 0.05	N.D.	N.D.

Values are expressed as nmol H_2O_2/mg protein/h, where H_2O_2 was one of the products during catabolism of putrescine by diamine oxidase. Each value represents the mean ± S.E.M. of triplicate determinations of a typical set of experiments. Two other sets of the same experiments showed similar results to that in this table. N.D., not detectable.

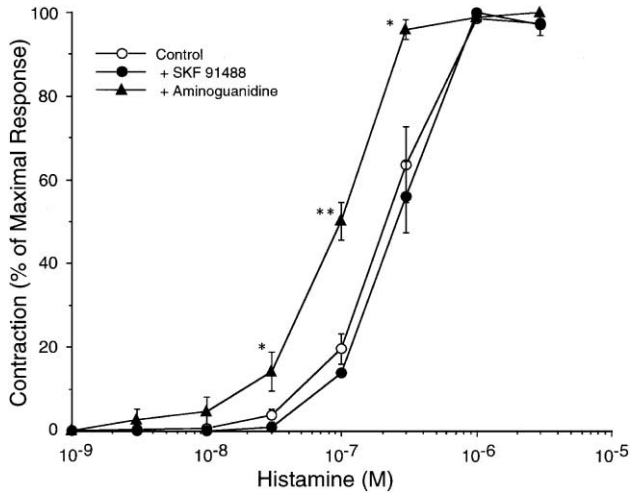

Fig. 3. Effect of aminoguanidine and *S*-[4-(*N*,*N*-dimethylamino)butyl]isothiourea (SKF 91488) on histamine-induced contraction of the guinea-pig small intestine. The ring section (15 mm long) of the guinea-pig small intestine was pretreated with aminoguanidine (100 μM) or SKF 91488 (100 μM) for 20 min, and then histamine was added in an accumulative manner to obtain dose–response curves. Values are the means ± S.E.M. of three independent experiments. * $P<0.05$, ** $P<0.01$, compared with controls (Student's *t* test).

activity was detected in the small intestine. Small but detectable amounts of the activity were also observed in the mouse stomach and liver but not in the brain, kidney or lung (Table 1). Of the rat tissues examined, diamine oxidase activity was detected in the small intestine and liver (Table 1).

3.3. Effects of inhibitors of histamine-metabolizing enzymes on histamine-induced contraction of guinea-pig small intestine

As shown in Fig. 3, histamine dose-dependently induced the contraction of the guinea-pig small intestine with an ED_{50} value of ca. 200 nM. The maximal response was attained by 1 μM histamine. The pretreatment of the tissue section with aminoguanidine (100 μM), a diamine oxidase inhibitor, but not with *S*-[4-(*N*,*N*-dimethylamino)butyl]isothiourea (SKF 91488, 100 μM), an inhibitor of histamine *N*-methyltransferase, significantly shifted the dose–response curve of histamine-induced contraction to lower concentrations (an ED_{50} value of ca. 100 nM; Fig. 3).

4. Discussion

Diamine oxidase mRNA consists of two forms in rats (Lingueglia et al., 1993) and humans (Chassande et al., 1994). As far as the human diamine oxidase gene is concerned, Chassande et al. (1994) reported that the smaller size of transcript might have resulted from an alternative splicing via a putative tissue-specific activation of the internal promoter located in intron 2 of the longer diamine oxidase gene. This is supported by a report of chloramphenicol acetyl transferase assay using intron 2 of the human diamine oxidase gene (Lingueglia et al., 1993). The present PCR primer set for diamine oxidase mRNA (Fig. 1A) corresponded to the region of exons 2 and 3 of the human counterpart (a long form of the transcript). As described in Materials and methods, the PCR product contained 132-bp nucleotides determined by nucleotide sequencing, and its sequence was 81% homologous to the corresponding region of human diamine oxidase mRNA, suggesting that the PCR product was a fragment of guinea-pig diamine oxidase cDNA (GenBank accession no. AB059272). Since the 132-bp nucleotides did not contain any intronic sequence compared with the human diamine oxidase gene (GenBank accession no. X78212), the present PCR clone appeared to be derived from the corresponding putative long form of guinea-pig diamine oxidase mRNA because the short form of the diamine oxidase mRNA has no exon 2-derived sequence (Chassande et al., 1994). Although we designed another primer set corresponding to the short form of the human counterpart to confirm the existence of two forms of diamine oxidase mRNA in guinea-pigs, we could not obtain any amplification with these primers on the cDNA pool derived from the guinea-pig tissues (data not shown). However, this negative result does not imply the absence of the short form of diamine oxidase mRNA expression in guinea pigs, but might be due to heterogeneity in the nucleotide sequences of human and guinea-pig diamine oxidase mRNA.

Based on the nucleotide sequence of 132-bp PCR fragment of guinea-pig diamine oxidase cDNA, we designed additional PCR primers to detect tissue expression. As shown in Fig. 1A, diamine oxidase mRNA (long form) was expressed abundantly in the liver and small intestine of the guinea-pig. In the peripheral tissues, the kidney and stomach also expressed diamine oxidase mRNA but the amount was lower than that of the liver and small intestine. Very small but detectable amounts of diamine oxidase mRNA were observed in the cerebral cortex, thalamus + hypothalamus and cerebellum under the present PCR conditions. It is of interest because a previous study reported no diamine oxidase activity in normal rat, mouse, guinea-pig and rabbit brains (Burkard et al., 1963; Lortie et al., 1996). By Northern blot analysis using the 132-bp PCR cDNA fragment as a probe (Fig. 1B), an abundant message of 2.8 kb in size was detected only in the liver and small intestine and a weak but positive signal was also observed in the kidney and stomach. The finding from Northern blotting (Fig. 1B) agrees with that of the PCR analysis (Fig. 1A) in terms of the most abundant expression. In rats, the long form of the diamine oxidase mRNA (2.7 kb in size) was reported to be expressed in the liver, small intestine, placenta and thymus (Lingueglia et al., 1993). In the guinea-pig kidney and lung, the signal at about 9 kb was observed (Fig. 1B), but it appeared to be due to non-specific hybridization because no signal was detected at around 9 kb in rats (Lingueglia et al., 1993) and in mice, humans and pigs (Barbry et al., 1990). Since our probe contained only about 20 nucleotides corresponding to exon 3 of human diamine

oxidase mRNA, there is a possibility that Northern blot failed to detect the apparent diversity (long and short forms) of diamine oxidase mRNA in the guinea-pig (Fig. 1B).

To clarify whether the relative activity of diamine oxidase among tissue is associated with the expression level of diamine oxidase mRNA in the guinea-pig, we investigated the diamine oxidase activity by fluorescence spectrophotometry. As expected from the result shown in Fig. 1, the high activity of diamine oxidase (putrescine as a substrate for diamine oxidase) was observed in the liver and small intestine of the guinea-pig (Table 1). Small but detectable amounts of diamine oxidase activity were observed in the lung and kidney, but the brain and stomach had little or undetectable diamine oxidase activity (Table 1). This observation suggests that diamine oxidase is an important element for the degradation of histamine in the guinea-pig liver and small intestine but not in the brain. In mice, the diamine oxidase activity was high in the small intestine and low or undetectable in the stomach, liver or brain (Table 1). In rats, the small intestine contained the highest activity, which was apparently lower than the activity in the guinea-pig and mouse small intestine; marginal activity was detected in the liver. This tissue distribution of diamine oxidase activity is in good agreement with the distribution of mRNA as reported previously (Linguegliaet al., 1993; Verity and Fuller, 1994). Thus, our results (Fig. 1 and Table 1) and others (Lingueglia et al., 1993; Verity and Fuller, 1994) indicate (i) that there are considerable species difference in the tissue distribution pattern in both diamine oxidase enzymatic activity and its mRNA expression and (ii) that the diamine oxidase activity that we observed in the guinea-pig tissues is attributed to the enzyme that corresponds to the long form of diamine oxidase mRNA present in rats and humans.

Next, we visualized the exact localization of diamine oxidase mRNA in the guinea-pig liver and small intestine, where the expression was abundant, by in situ hybridization histochemistry. As shown in Fig. 2A, a positive signal was observed throughout the liver section, mainly in hepatocytes. The positive signal was also observed mainly in the epithelial cells of the small intestine (Fig. 2C), but not in the myenteric plexus neurons or the brain regions such as frontal cortex, hippocampus, striatum, thalamus, hypothalamus, cerebellum, or medulla oblongata (data not shown). In the guinea-pig brain, we conclude that the expression level of diamine oxidase is too low to be detected directly by Northern blotting analysis, in situ hybridization histochemistry or enzyme activity measurement.

To examine the possibility that diamine oxidase participates in peripheral signal transduction, we examined the histamine-induced contraction of the small intestine under the application of aminoguanidine, a diamine oxidase inhibitor (Tamura et al., 1989), in comparison with SKF 91488, a histamine *N*-methyltransferase inhibitor (Beaven and Shaff, 1979). The response to histamine was abolished completely by 0.3 μM diphenhydramine (data not shown), suggesting that the receptor involved may be histamine H_1 receptor. The pretreatment of the small intestine with aminoguanidine (100 μM) shifted the dose–response curve significantly to lower concentrations with the same maximal contraction; SKF 91488, however, had no effect on the histamine-induced contraction (Fig. 3). We concluded, from these results, that diamine oxidase but not histamine *N*-methyltransferase appears to play a major role in the guinea-pig small intestine.

One fundamental function of diamine oxidase in the small intestine is to prevent the uptake of histamine and diamines from the intestines for control of intestinal and blood concentrations, as diamine oxidase inhibition with antimycobacterial drugs and concomitant fish intake may result in anaphylactoid reaction (Taylor, 1986). Also, in rats, there is evidence that intestinal diamine oxidase prevents the enteric uptake of diamines (Nilsson et al., 1996). The tissue distribution pattern suggests that the diamine oxidase activity in the small intestine and liver of the guinea-pig (Dave and Sachdev, 1967; Schmutzler et al., 1974) and in the small intestine of the mouse and rat is likely a mechanism to control the histamine concentration in the blood in these animals. In this context, the location of the expression of diamine oxidase which is predominant in the mucosal epithelium (Fig. 2C) is reasonable. Although it was previously reported that histamine *N*-methyltransferase is also expressed in the epithelial cells of the guinea-pig small intestine (Kitanaka et al., 2001a), a histamine *N*-methyltransferase inhibitor SKF 91488 had no effect on histamine-induced contraction of the small intestine sections (Fig. 3). This absence of an effect of histamine *N*-methyltransferase-blockade may be caused by the low enzymatic activity of histamine *N*-methyltransferase in the guinea-pig jejunum (Takemura et al., 1994) being only 1/5 of diamine oxidase activity (Table 1). We conclude that diamine oxidase appears to play a physiological role in preventing ingested histamine from penetrating through the small intestine.

It was also reported that histamine-containing nerve fibers are present in the small intestine (Panula et al., 1985). No mRNA for diamine oxidase was, however, detected in the enteric nervous system, whereas histamine *N*-methyltransferase mRNA has been visualized in the myenteric plexus (Kitanaka et al., 2001a). Thus, histamine *N*-methyltransferase may play a role in the modulation of histaminergic neurotransmission in the guinea-pig small intestine. We are now interested in elucidating the exact localization of the activity of individual histamine-degrading enzymes in the subcellular fractions for better understanding of the relations between the expression pattern and physiological relevance of these enzymes in mammals.

The physiological importance of diamine oxidase in the guinea-pig liver is still unclear. One explanation is that histamine released from enterochromaffin-like cells in the gastric glands might not be inactivated completely by histamine *N*-methyltransferase expressed in that organ (Lönroth et al., 1989) and diamine oxidase in the liver may metabolize the substance. A second possibility is that hepatic diamine oxidase is involved in the inactivation of histamine

and polyamines absorbed from the small intestine. In the mice lacking histidine decarboxylase, diamine oxidase in the small intestine is not adequate for preventing histamine from being taken up from the small intestine (H. Ohtsu et al., personal communication). As shown in Table 1, diamine oxidase activity in the small intestine is highest in the mouse followed by the guinea-pig and the rat. Therefore, histamine may be taken up from the small intestine and transported via the portal vein in these two latter animals. Since the guinea-pig is highly sensitive to histamine, it would be crucial to remove histamine from the blood before it enters the systemic blood flow, and so diamine oxidase in the liver might serve as a second barrier. In the mouse, histamine *N*-methyltransferase activity is high in the liver (Takemura et al., 1994) and this might be a barrier mechanism in this species. In the rat, no histamine *N*-methyltransferase activity and very low diamine oxidase activity were detected in the liver (Takemura et al., 1994; Table 1), but, as this animal is less sensitive to histamine than the guinea-pig or mouse, histamine might be gradually removed from systemic blood by large amounts of histamine *N*-methyltransferase in the kidney (Takemura et al., 1994). A third possibility is that diamine oxidase in the liver of the guinea-pig might regulate the blood concentration of histamine, because the highest activity of diamine oxidase was found in this organ (Table 1), whereas only low activity of histamine *N*-methyltransferase was seen in the tissues except the brain. These possibilities require further clarification.

Taken together, we examined the expression pattern of diamine oxidase in terms of the mRNA level and of the enzyme activity in guinea-pig tissues, and showed that diamine oxidase had a very restricted expression. Although histamine *N*-methyltransferase, another histamine-degrading enzyme, is predominant in the central nervous system, diamine oxidase appears to have a crucial role in the degradation of histamine in the peripheral tissues such as the small intestine and liver.

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