Localization of Insulin-Like Growth Factor I (IGF-I) in the Chicken Liver after Fasting and Refeeding: Demonstration by Using Antigen Retrieval Immunohistochemistry

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ABSTRACT. Immunohistochemical localization of insulin-like growth factor-I (IGF-I) was investigated in the liver of fasted and refed chickens by using an antigen retrieval method. The present study is the first one showing the localization of IGF-I in the chicken liver. Immunoreactivity for IGF-I was detected on the paraffin sections of livers from the fed and refed chickens after the treatment with the antigen retrieval agent. A moderate number of cells showing IGF-I immunoreactivity were scattered in the parenchyma of the liver from fed chickens. These cells were relatively large and polygonal in shape and seemed to be hepatocytes. Reaction products were observed as a granular structure in the cytoplasm of IGF-I-immunoreactive hepatocytes. The number of immunoreactive hepatocytes was increased in the liver from refed chickens compared with fed chickens. Diffuse reaction products as well as granular ones were observed throughout the cytoplasm of IGF-I-immunoreactive hepatocytes of livers from refed chickens. There are, however, no regular patterns of the distribution of immunoreactive hepatocytes in the parenchyma of both fed and refed chickens. In the liver of the fasted chickens, clear immunoreactivity for the peptide was not observed. These data show that IGF-I is located in the chicken hepatocytes and influenced by the nutriture.

KEY WORDS: antigen retrieval immunohistochemistry, chicken, insulin-like growth factor-I, liver, nutriture.

There are two distinct molecular forms in insulin-like growth factors, IGF-I and IGF-II. These peptides play important roles in the regulation of metabolism, proliferation and differentiation [19, 25]. IGF-I of chickens has been characterized and shown to consist of 70 amino acids [2]. As in mammals, the growth rate of chickens, which varies widely under various nutritional conditions, is closely related to the plasma IGF-I concentration [9, 10]. Furthermore, co-workers also revealed that changes in plasma IGF-I concentration under various nutritional conditions resulted in the alteration of hepatic IGF-I gene expression in chickens [9–13] as in mammalian species [1, 4, 5, 15, 17, 18, 20, 23, 24, 26, 28].

IGF-I peptide is expressed in many organs; liver, kidney, skeletal muscle and so on [6]. As a few immunohistochemical studies have demonstrated the existence of this peptide in hepatocytes of mammals [3, 7], the liver is thought to be the major synthetic site of IGF-I [22]. These studies have also shown that the IGF-I-immunoreactive cells were distributed in the perivascular or periportal areas of mammalian livers. On the other hand, in avian species, there are virtually no studies investigating the distribution of IGF-I-containing cells in the liver. In addition, the influence of the alteration of nutritional conditions on IGF-I distribution has been also unknown even in mammalian species.

Therefore, the present study using antigen retrieval immunohistochemistry aimed to clarify following two points: 1) the localization of IGF-I-immunoreactive cells in the liver of chickens allowed free access to a normal diet and 2) its change after fasting and refeeding.

MATERIALS AND METHODS

Animals: Single Comb White Leghorn male chickens (n=18) at six weeks of age were used in this study. Birds were kept with a commercial feed and water ad libitum and under the controlled light condition (12L12D). The chickens were divided into three groups consisting of six birds each: control, fasted and refed groups. Birds in the fed control group were kept under the above conditions until the sacrifice. Birds in the fasted group were deprived of their feed for two days and then sacrificed. Refed chickens were allowed free access to the commercial feed after two days of fasting. After two days of refeeding, birds were sacrificed as mentioned below.

Tissue samples: Birds of all groups were perfused with 4% paraformaldehyde in phosphate buffer (pH 7.6) following 0.75% NaCl solution. The left lobe of the liver was immediately removed after perfusion and cut into small blocks with razor blades. Tissue blocks were immersed in the same perfusate at 4°C overnight and embedded in the paraffin wax by the ordinary manner after several washings with phosphate buffer. Paraffin sections were cut at 5 µm thickness and mounted on the silane-coated glass slides.

Immunohistochemistry: After routine dewaxing and rehydrating, the sections were incubated with 0.05% antigen retrieval agent (citraconic anhydride, Immunosaver®, Nissin EM, Tokyo, Japan) at 98°C for 45 min and rinsed with
phosphate buffered saline (PBS, pH 7.6). Thereafter, horseradish peroxidase (HRP)-labeled streptavidin-biotin method was carried out to detect the localization of IGF-I according to the procedure previously described [8]. Sections pretreated with 10% normal goat serum (No.CL1200, Cedarlane, Canada) were incubated with rabbit anti-IGF-I serum at room temperature overnight. Polyclonal antiserum raised in rabbits against human IGF-I (No.PAA1, diluted to 1:2500, GroPep Pty Ltd, Adelaide, Australia) was generously given from Dr. P. C. Owens (Department of Obstetrics and Gynecology, Adelaide University, Adelaide, SA, Australia). Immunocomplex was visualized with 0.05% 3,3'-diaminobenzidine (DAB) in Tris-HCl buffer (pH 7.6). Sections were counterstained with Mayer's hematoxylin and observed under a light microscope. Some sections were immunostained without the antigen retrieval procedures.

Sections for negative control for staining were incubated with normal rabbit serum instead of the specific primary serum, or without the specific primary serum. No specific reaction was observed on the control sections for the immunostaining.

RESULTS

As shown in Figs. 1–3, immunostaining of IGF-I was enhanced on the sections treated with an antigen retrieval agent compared to sections of routine immunohistochemistry. Clear reaction was not observed on the untreated sections of the liver (Fig. 1a). Specific immunoreaction was detected on the sections treated with the antigen retrieval agent. In the liver from fed controls, a moderate number of cells showing IGF-I immunoreactivity were observed. These cells were large and polygonal in shape and seemed to be hepatocytes. These immunoreactive cells were scattered in the parenchyma and showed no regular patterns of their distribution (Fig. 1b). Reaction products of immunohistochemistry were observed as a granular shape in the cytoplasm of IGF-I-immunoreactive cells in the liver of fed chickens (Fig. 1c, arrows). Regardless of the treatment by the antigen retrieval agent, obvious immunoreactivity for IGF-I was not observed in the liver of fasted chickens (Fig. 2). The number of cells showing immunoreactivity for IGF-I was increased in the liver from refed chickens compared with fed chickens (Fig. 3a). They were also scattered in the parenchyma and indicated no regular distributional pattern as on the sections from fed controls. More intense reaction, however, was detected on sections of the liver from refed chickens than on sections from fed controls. Diffuse reaction products as well as granular ones were observed throughout the cytoplasm of IGF-I-immunoreactive cells of the refed chicken liver (Fig. 3b).

DISCUSSION

This is the first study showing the localization of IGF-I-immunoreactive cells in the chicken liver. Routine immunohistochemistry, however, did not reveal the obvious immunoreactivity for IGF-I. Antigen retrieval process was needed to detect the IGF-I immunoreactivity. This means that the antigenic site for IGF-I might be masked in the process of formaldehyde fixation.

Immunoreactivity for IGF-I was weak on sections from the liver of fed controls even after antigen retrieval process. Two reasons are considered for this. Firstly, we speculate that the amounts of IGF-I peptide are not sufficient to be detected because of very low concentrations of IGF-I in the chicken liver. For example, the concentration of IGF-I peptide in the guinea pig plasma was about 340 ng/ml [23], and in the rat plasma about 430 ng/ml [24]. Interestingly, in young chickens under well-nutritional conditions plasma concentration of IGF-I was approximately 15 ng/ml [10, 14], which is less than a one-tenth of those of mammalian species. Considering these data, it seems that the expression of IGF-I peptide in various chicken tissues would be lower than that in mammals. That may be one of reasons why it is hard to detect IGF-I immunoreactivity in the chicken liver.

Secondly, the existing pattern of IGF-I was different from that of other hormones in endocrine cells. There have been only a few studies showing the immunohistochemical localization of IGF-I in the liver even of mammals [3, 7], although many studies have indicated the expression of this peptide in the liver by use of ribonuclease protection assay or biochemical assay. It is well known that insulin having a similar structure to IGF-I is packed in secretory granules of pancreatic B cells [28]. No ultrastructural observations, however, indicate the existence of secretory granules in the hepatocytes. Synthesized IGF-I may be diffused in the cytoplasm of hepatocytes. Such a manner of peptide existence in hepatocytes may be related to the weakness of the immunostaining for IGF-I in the chicken liver.

Bastetti et al. [3] investigated the localization of IGF-I in the liver of rats, pigs and calves, and showed that relatively many hepatocytes in the perivenous region were IGF-I-immunoreactive in pigs and rats, and those in the periporal region in calves. In the present study, IGF-I immunoreactivity in the chicken liver was observed in large and polygonal cells which seemed to be hepatocytes. O’Neill et al. [21] have demonstrated that cultured chicken hepatocytes produce IGF-I. So IGF-I-immunoreactive cells in the present study may be regarded as hepatocytes. Immunoreactive hepatocytes were scattered in the liver parenchyma and showed no specific distributional pattern. Moreover, the number of IGF-I-immunoreactive hepatocytes was fewer than that in mammalian liver [3], which may also reflect the low level of plasma IGF-I concentration in the chicken. These results suggest that there is obviously a species difference in the distributional pattern and the number of the IGF-I-containing cells in the liver between avian and mammalian species.

Many studies in mammalian species have demonstrated that nutritional conditions influenced the tissue and plasma IGF-I concentration [1, 4, 5, 17, 18, 20, 23, 24, 26, 28]. It is well recognized that the alteration in plasma IGF-I concentration of the chicken is also very sensitive and parallel to
Fig. 1. Immunohistochemical localization of IGF-I in the liver from control (fed) chickens. a) Specific immunoreactivity is not observed on the section without the antigen retrieval treatment. b) A moderate number of hepatocytes show obvious immunoreactivity for IGF-I on the sections treated with the antigen retrieval agent. c) High-powered view of IGF-I-immunoreactive hepatocytes. Reaction products showing the granular shape (arrows) are found in the cytoplasm of IGF-I-immunoreactive hepatocytes.

Fig. 2. Obvious immunoreactivity for IGF-I is not observed on the section of the liver from fasted chickens even after the treatment with the antigen retrieval agent.

Fig. 3. Immunohistochemical localization of IGF-I in the liver from refed chickens. a) Many hepatocytes are found showing immunoreactivity for IGF-I. b) High-powered view of IGF-I-immunoreactive hepatocytes. Cytoplasm of IGF-I-immunoreactive hepatocytes (arrows) is filled with reaction products.
changes in nutritional conditions [10]. Plasma concentration of IGF-I increased with elevating dietary protein levels from deficiency to the requirement level [10], and above that level, it decreased significantly [9]. Food restriction and fasting also decreased plasma IGF-I concentration and refeeding recovered it to the level of fed chickens [10], which is in good agreement with other studies [12, 16].

In the present study, we demonstrated the distributional pattern of IGF-I peptide in the liver of layer chickens after fasting and refeeding. Immunohistochemistry after antigen retrieval treatment indicated the increase of the number and intensity of immunoreaction to IGF-I in the refed chickens and the reduction of those in the fasted ones. These results may reflect the IGF-I concentration in the plasma. As co-workers have previously reported, plasma IGF-I concentration in fasted young chickens followed by refeeding was higher than that in control chickens allowed free access to the control diet [13]. Moreover, IGF-I mRNA level in the liver of layer chickens after refeeding recovered it to the level of fed chickens [10], and fasting also decreased plasma IGF-I concentration and that level, it decreased significantly [9]. Food restriction and fasting also decreased plasma IGF-I mRNA concentration according to nutritional conditions [10]. The increase in the number and the staining intensity of IGF-I-immunoreactive hepatocytes after refeeding seemed to result in the high plasma concentration of IGF-I and the high tissue level of IGF-I-mRNA in refed chickens compared to the fed control.

REFERENCES


