

Comparative studies on fish and bird insulin receptors

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The comparative studies were performed on insulin receptors of erythrocytes and liver plasma membranes in fish (tench and carp) and bird (duck). The Scatchard plots indicated the presence of two pools of binding sites both in fish and duck. These pools show inter-species differences in binding ability and the number of receptors. Specific binding of insulin and the binding affinity are higher in duck than in fish.

For the last few years radioreceptor assays have been used to demonstrate specific insulin receptors in humans and in animal species. The binding of a hormone molecule to its receptor which is at the interface between the external and internal cellular environments [1, 2] is the first and an obligatory step of insulin action on the target tissue. In consequence, this evokes a cascade of intracellular reactions [3]. Thus, investigation of the membrane receptors is crucial for characterization of sensitivity of various cell types to insulin. Out of many cells, erythrocytes serve as a model to investigate binding of insulin, whereas liver plasma membranes or hepatocytes are included in the investigation since liver is the most important target tissue of this hormone [2]. Insulin receptors have been investigated mostly in mammals and there is surprisingly little information available on insulin receptors of other vertebrates, including fish and birds. Moreover, the available results on insulin binding to the different tissues in fish are often contradictory [4-6].

The aim of the present study was to extend the comparative data on the insulin receptors to two fish species (tench and carp) and to one bird species (duck) and to compare erythrocytes and liver in the insulin-receptor interaction.

MATERIALS AND METHODS

Materials. Animals, tench (*Tinca tinca*), carp (*Cyprinus carpio*) and ducks (*Anas domestica*), were obtained from the Fish Farm near Poznań or from the Farm of the Poultry Research and Development Centre in Dworzyska near Poznań. The experiments were performed in late spring.

Preparation of tissues. Erythrocytes were obtained according to the method of Thomopoulos *et al.* [7]. Liver plasma membranes were prepared according to the method of Havranek *et al.* [8]. All procedures were carried out at 4°C.

Radioreceptor assay. In order to investigate insulin binding, 80 pg of ¹²⁵I-labeled porcine hormone (OPiDI Świerk, Poland) and varying amounts of unlabeled insulin (0.02 - 700 nM) were incubated for 16 h at 4°C with erythrocytes or liver plasma membranes (final concentration of proteins 0.5 mg/ml). The nonspecific binding of ¹²⁵I-insulin was determined at 10 μM concentration of unlabeled insulin and subsequently subtracted from each value. Total radioactivity was measured, then liver membranes were centrifuged at 20000 × g for 8 min

¹Abbreviations: HAIR, high affinity insulin receptors; LAIR, low affinity insulin receptors

while erythrocyte suspensions were centrifuged through a layer of dibutyl phthalate (density 1.04) at $1000 \times g$ for 5 min. Next, radioactivity of the pellets was measured.

Dissociation rate constant (K_d) and binding capacity (B_{max}) for insulin were determined by the Scatchard's method [9] using the LIGAND-PC v.3.1 computer program [10].

RESULTS AND DISCUSSION

Erythrocytes as well as plasma liver membranes of fish and duck were able to bind pork insulin. Specific insulin binding to liver plasma membranes was higher in ducks than in fish, however some differences were also noticed between the two fish species; in tench insulin binding was about twice as high as in carp. Also, duck erythrocytes showed significantly higher binding ability when compared to fish red cells. The effect of increasing concentration of unlabeled pork insulin on 125 I-insulin binding to receptors and Scatchard plots are shown

in Figs. 1 and 2. The Scatchard plots were typically curvilinear, suggesting that there were two different classes of receptors: the high affinity and low capacity insulin receptors (HAIR)¹ and low affinity and high capacity insulin receptors (LAIR). The data calculated from Scatchard plots (Table 1) show considerable differences in the equilibrium dissociation constants (K_d) and binding abilities of the two kinds of receptors in erythrocytes and plasma membranes. Taking into account that the investigated species of fish have more membrane receptors than duck, one could expect a higher specific binding of insulin in tench and carp than in duck. However, the results obtained are inconsistent with this supposition since the binding affinity (high K_d) of fish receptors is very low. It should be noticed that the K_d value estimated for HAIR in duck liver membranes is about six times lower than for HAIR in erythrocytes. In tench, the corresponding values are practically the same. This points to remarkably stronger affinity of insulin receptors in duck liver in comparison to the homologous erythro-

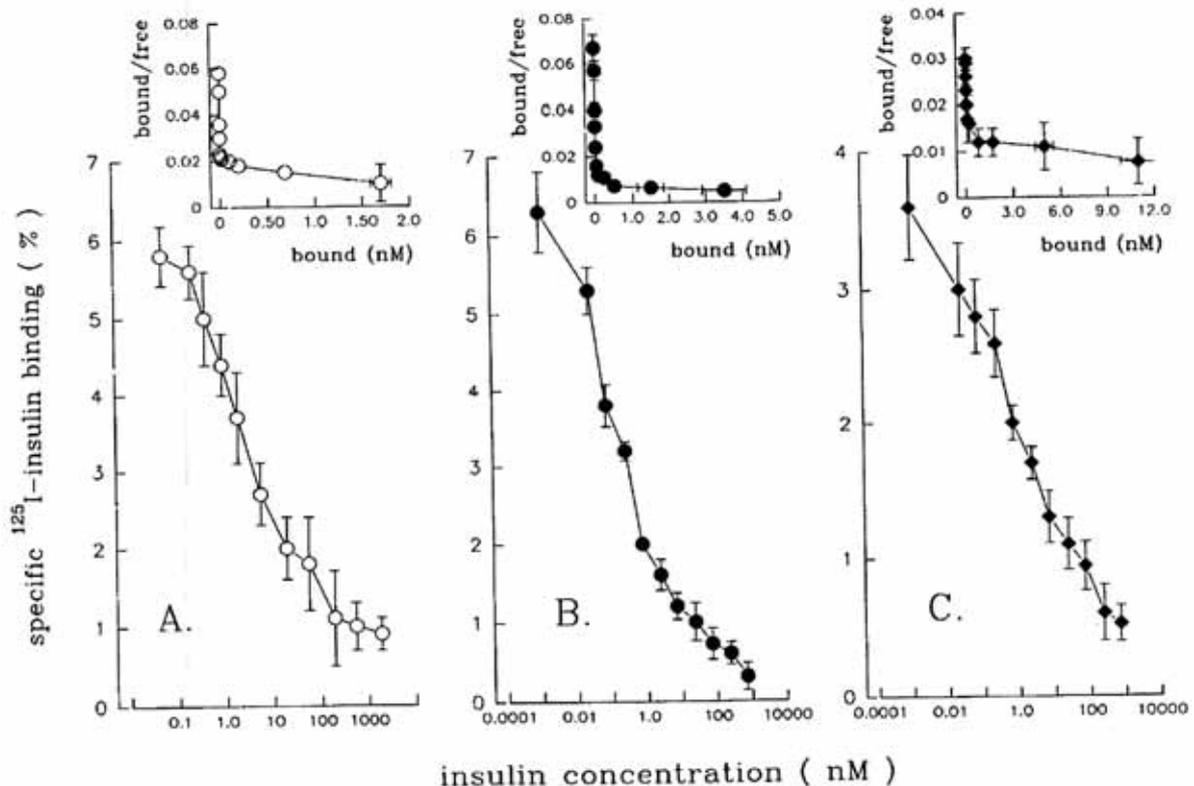


Fig. 1. Insulin binding to tench erythrocytes (A) and liver plasma membranes (B) and carp erythrocytes (C). The data are mean of 6-7 experiments. The insets represent the Scatchard plot of data

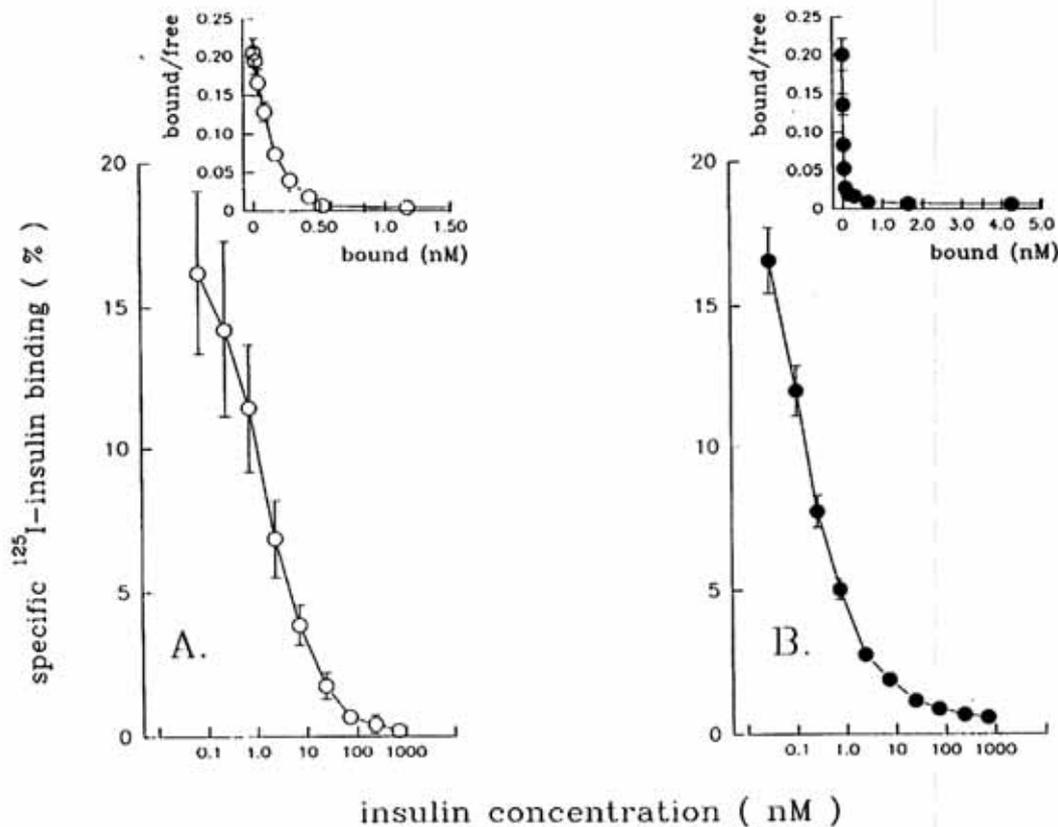


Fig. 2. Insulin binding to erythrocytes (A) and liver plasma membranes (B) of a duck. The data are mean of 6 experiments. The insets represent the Scatchard plot of data

cytes, and considerably higher sensitivity of liver membrane receptors for insulin at physiological concentration in blood. On the other hand, a high LAIR to HAIR ratio both in liver plasma membranes and erythrocytes indicates the occurrence of a large pool of receptors which can bind insulin present in blood at high concentration.

Binding of insulin to its receptors in various tissues of numerous mammals is well documented but only scanty data are available on characteristics of insulin receptors of lower vertebrates, including bony fish and birds. Data were reported on the receptors in skeletal muscles and hepatocytes of rainbow trout and chicken [4, 11, 12], liver plasma membranes of scorpiotfish [13], stingray [14], chick [4, 15, 16] and pigeon [17], in brain and cardiac muscle of lamprey [18], and in erythrocytes of hagfish [4], brown trout [5], and turkey [4, 19, 20]. However, most of the presented results include neither mathematical calculation of the number and binding affinity of the receptors nor differentiation into HAIR and LAIR receptors.

Thus, these results do not permit comparative characterization of insulin receptors.

Our data on binding sites of tench erythrocytes are consistent with those given by Muggeo *et al.* [4] for trout (11.6 pmol per 10^9 red cells) but the estimated by us HAIR / LAIR ratio and the receptors affinities add to the information on fish erythrocytes. Also, the affinity and the number of HAIR in the carp and tench liver membranes are in the range reported for coho salmon [21] (40 - 400 fmol/mg protein, K_d 1.1 - 8.3 nM). However, there are pronounced differences in the LAIR receptors; the membranes of carp and tench liver show markedly greater number of this class of receptors and higher K_d than that of salmon (550 - 1700 fmol/mg protein, K_d 26 - 63 nM).

Insulin receptors were quantified in liver membranes and red cells of turkey and chicken. In chicken membranes total capacity of the receptors amounted to 0.415 pmol/mg protein [4]. According to Kemmler *et al.* [16] it is about 25 fmol HAIR/mg protein with K_d 0.22 nM and 0.5 pmol LAIR/mg protein with K_d 29

Table 1

The Scatchard analysis of the insulin receptors of fish and bird erythrocytes and liver plasma membranes.
The results presented are mean of 6 - 7 determinations \pm SEM. K_d , dissociation rate constant; B_{max} , binding capacity; HAIR, high affinity insulin receptor; LAIR, low affinity insulin receptor

	K_d (mol/l)	B_{max}
	<i>Erythrocytes</i>	(mol/10 ⁹ cells)
Tench		
HAIR	$0.85 \pm 0.18 \times 10^{-9}$	$24.2 \pm 1.2 \times 10^{-15}$
LAIR	$1.98 \pm 0.89 \times 10^{-6}$	$10.2 \pm 8.0 \times 10^{-12}$
Duck		
HAIR	$0.60 \pm 0.15 \times 10^{-9}$	$55.9 \pm 12.0 \times 10^{-15}$
LAIR	$12.2 \pm 3.5 \times 10^{-9}$	$0.22 \pm 0.04 \times 10^{-12}$
	<i>Liver membranes</i>	(mol/mg protein)
Tench		
HAIR	$1.08 \pm 0.34 \times 10^{-9}$	$100.0 \pm 10.0 \times 10^{-15}$
LAIR	$0.54 \pm 0.23 \times 10^{-6}$	$14.3 \pm 4.7 \times 10^{-12}$
Carp		
HAIR	$1.08 \pm 0.14 \times 10^{-9}$	$68.3 \pm 8.5 \times 10^{-15}$
LAIR	$0.52 \pm 0.14 \times 10^{-6}$	$15.2 \pm 4.9 \times 10^{-12}$
Duck		
HAIR	$0.10 \pm 0.01 \times 10^{-9}$	$48.5 \pm 6.0 \times 10^{-15}$
LAIR	$0.07 \pm 0.03 \times 10^{-6}$	$3.0 \pm 1.3 \times 10^{-12}$

nM [16]. Duck liver membranes show twice as much HAIR as in chicken and six times more LAIR. Moreover, pronounced differences between receptor affinities in birds should be noted. The affinity of the duck liver membrane HAIR is twice as high as in chicken (Table 1). Also, chicken and turkey reveal four and twenty times more insulin receptors, respectively, in red cells (0.9 and 5.0 pmol/10⁹ cells) than in duck erythrocytes. Taking into consideration that binding of insulin is a result of the number and affinity of the receptors, it must be pointed out that duck liver membranes are more sensitive to insulin at low concentration and are definitively able to bind more insulin at high concentration.

It is more difficult to compare the ability of bird erythrocytes to bind insulin, because of the lack of information on the affinities of avian receptors.

Basing on the data obtained with other vertebrate species, it can be concluded that the tench erythrocytes show an average number of HAIR

and a high number of LAIR, whereas duck exhibits rather high number of HAIR and a low number of LAIR. In man, the number of HAIR amounts to 1.7 fmol and that of LAIR to 18.3 fmol per 10⁹ red cells [22]. In frog the total number of receptors is extremely high (8.0 pmol/10⁹ cells) [4]. In our previous experiments we found 19.6 and 44.2 fmol HAIR and 3.1 and 13.1 pmol LAIR per 10⁹ red cells in pig and rabbit, respectively. It is of interest that cow erythrocytes did not reveal any insulin binding [23].

Similarly, differences occur both in the number and affinity of the receptors in liver plasma membranes originating from different species. The number of HAIR receptors varies from 1.7 - 11.3 in frog to 300 fmol/mg protein in guinea pig and that of LAIR receptors from 432 in frog to 5600 fmol/mg protein in rat [16, 21, 24, 26]. Thus the number of both kinds of receptors found in our experiments for tench, carp and duck represents, among the results reported for various animals an average number.

The highest affinity so far found was revealed for HAIR in duck (K_d 0.1 nM). In chicken this value is at least twice as high [16, 24]. The carp, tench and coho salmon [24] receptors bind insulin very poorly (K_d 1.08 nM and 1.1 - 1.8 nM). Similarly, the dissociation rate constants of HAIR receptors is very low in mouse - 1.7 nM [24], but similar in other mammals: 0.24 - 0.7 in rat [24, 25], 0.3 in guinea pig and 0.21 in calf [16, 24]. The K_d value of LAIR receptors in duck (70 nM) is about twice as high as in guinea pig, chicken and calf [16, 21], whereas these values in carp and tench are remarkably higher (540 - 520 nM) than in other investigated species.

It can be concluded that only full biochemical and molecular characterization of insulin receptors representing different animal species will allow to elucidate conservation of the receptor structures during evolution.

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